EXHIBIT 2009

IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

INSTITUTE FOR ENVIRONMENTAL HEALTH, INC.,	)	
Plaintiff,	)	
	)	C.A. No. 23-826-JHS
v.	)	
NATIONAL BEEF PACKING COMPANY, LLC,	)	
Defendant.	)	

DEFENDANT NATIONAL BEEF PACKING COMPANY, LLC'S INITIAL INVALIDITY CONTENTIONS

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DATED: October 3, 2024

Pursuant to paragraph 6(d) of the Proposed Scheduling Order filed May 16, 2024 (D.I. 24-1) and the District of Delaware's Default Standard of Discovery paragraph 4(d), defendant National Beef Packing Company, LLC ("National Beef") hereby serves its Invalidity Contentions ("Contentions") for each Asserted Claim for U.S. Patent Nos. 7,534,584 (the "'584 Patent"); 8,822,143 (the "'143 Patent"); 9,637,771 (the "'771 Patent"); and 9,845,486 (the "'486 Patent) (collectively, the "Asserted Patents") on plaintiff Institute for Environmental Health, Inc. ("IEH"). The "Asserted Claims," as identified by IEH in its Disclosure of Asserted Claims and Infringement Contentions served August 16, 2024, are:

Patent	Claims
'584 Patent	1-6, 10-15, 17-24, 27-33
'143 Patent	1-7, 11-16, 18-24, 29-36
'771 Patent	1-11, 15-20, 22-31, 37-38, 41-44, 48-49
'486 Patent	1, 4-9, 17, 19

For avoidance of doubt, National Beef raises the following inconsistencies in IEH's identification of Asserted Claims versus the claims included in its infringement charts and states how National Beef has treated these inconsistencies in its Invalidity Contentions. With respect to the '584 Patent, IEH improperly used the original claim language for claim 2, 32, and 33 instead of the amended claim language following reexamination proceedings. National Beef has charted the as-amended claim language for claims 2, 32, and 33. Additionally, IEH has also improperly asserted claim 24, which was cancelled during reexamination proceedings. With respect to the '143 Patent, IEH charted the pre-certificate of correction version of claim limitation 1(c). National Beef has charted the post-certificate of correction version of claim limitation 1(c). With

respect to the ’771 Patent, IEH did not chart claim 25, despite identifying the claim in its Asserted Claims, and mislabeled claim 26, using the language of claim 26 for claim 25 and the language of claim 27 for claim 26. National Beef has included in its Invalidity Contentions claims 25, 26, and 27 with the appropriate language. With respect to the ’486 Patent, IEH did not identify claim 10 in its list of Asserted Claims, but charted claim 10. For avoidance of doubt, National Beef will include claim 10 as an Asserted Claim. Given IEH’s errors in its infringement contentions (National Beef has requested, by letter dated September 30, 2024, correction of the identified errors and incomplete infringement contentions), National Beef reserves the right to supplement and/or amend its Invalidity Contentions based upon these errors and inconsistencies.

For each claim asserted in each Asserted Patent (the “Asserted Claim(s)”), National Beef: (i) identifies each currently known item of prior art that either anticipates or renders obvious each claim; (ii) submits charts for illustrative prior art references identifying where each claim limitation is disclosed and/or rendered obvious; and (iii) identifies grounds of invalidity based on indefiniteness and/or written description. The related invalidating prior art references are being produced herewith.

I. RESERVATION OF RIGHTS

National Beef’s investigation is ongoing, and these Contentions are based on National Beef’s current knowledge and understanding of the Asserted Patents as well as IEH’s current infringement contentions, the prior art, and other information. To the extent IEH modifies, supplements, or amends its Infringement Contentions, National Beef reserves the right to likewise modify, supplement, or amend its Invalidity Contentions. Because National Beef’s investigation regarding the invalidity of the Asserted Claims is ongoing, certain defenses, including, for example, non-patentable subject matter under 35 U.S.C. § 101, knowledge or use

by others under pre-AIA or AIA 35 U.S.C. § 102(a), public use and/or on-sale bar under pre-AIA 35 U.S.C. § 102(b), derivation or prior inventorship under pre-AIA 35 U.S.C. §§ 102(f)/(g), inequitable conduct, unenforceability, and estoppel, etc., may only become apparent as additional information becomes available. National Beef has not yet had the opportunity to fully conduct discovery regarding any invalidity or unenforceability defenses. To the extent that during discovery any evidence is produced that supports a contention that any Asserted Patent is invalid or unenforceable due to inequitable conduct during prosecution or for any other reason, National Beef reserves all rights to amend and/or supplement these Contentions to include such invalidity or unenforceability contentions. National Beef further reserves the right to assert that the Asserted Claims are invalid under 35 U.S.C. § 102(f) if discovery reveals that the named inventor or inventors of the Asserted Patents did not invent (either alone or in conjunction with others) the subject matter recited in the Asserted Claims. If National Beef asserts invalidity under 35 U.S.C. § 102(f), National Beef will provide amended Invalidity Contentions.

These Contentions are necessarily preliminary and are provided without prejudice to National Beef's rights. National Beef reserves the right to amend or supplement these Contentions based on further investigation, developments during discovery, evaluation of the prior art or additional prior art that may be discovered, admissions, any other reasonable basis, and as permitted by the Court's orders, the Local Rules, and/or the Federal Rules of Civil Procedure.

The Court has not yet construed any claim terms and additional bases or revised bases for invalidity may become relevant based on those constructions, or the parties' positions taken during the claim construction process. Accordingly, National Beef reserves the right to revise and/or supplement these Contentions as discovery proceeds and after the Court construes the

Asserted Claims. In addition, nothing in these Contentions constitutes an admission concerning the proper construction of the Asserted Claims. National Beef reserves all rights to propose any constructions based upon the patents and prosecution histories (including positions taken in IPRs) in view of one of skill in the art and to rebut IEH's claim construction positions once known. Moreover, National Beef reserves the right to supplement and/or amend these Contentions based on any findings as to the priority date of the Asserted Claims, and/or positions that IEH or its expert witnesses may take concerning claim interpretation, infringement, and/or invalidity issues.

Invalidating prior art references not included in this disclosure, whether known or unknown to National Beef, may become relevant. In particular, National Beef is currently unaware of the extent, if any, to which IEH will contend that limitations of the Asserted Claims are not disclosed in the prior art that National Beef identifies, or will contend that any of the identified references do not qualify as prior art under 35 U.S.C. § 102. The identification of any patents as prior art shall be deemed to refer to the application that was submitted for the same and to include identification of any foreign counterpart patents. To the extent that such an issue arises, National Beef reserves the right to identify additional teachings in the same references or in other references that anticipate or would have rendered the addition of the allegedly missing limitation obvious.

National Beef's claim charts cite to exemplary teachings and disclosures of the prior art as applied to elements of the Asserted Claims. Persons of ordinary skill in the art view prior art in context, including, additional publications, literature, and/or products. Accordingly, the cited portions are examples, and National Beef reserves the right to rely on uncited portions of references and on other publications and expert testimony as aids in understanding and

interpreting references and their disclosures. Where National Beef cites a particular figure in a reference, the citation should be understood to encompass the caption and any other description of the figure. National Beef further reserves the right to rely on uncited portions of the prior art references, other publications, and testimony to establish bases for combinations of certain cited references that render the Asserted Claims obvious. Further, for any combination, National Beef reserves the right to also rely on information generally known to those skilled in the art and/or common sense.

In the attached claim charts, the identified passages in the references are provided both to demonstrate anticipation and to show how the identified disclosure would render the claim obvious alone or in combination, for example, with any of the other cited references. To the extent any reference does not explicitly disclose an element, the reference anticipates or renders obvious the claim(s) because the element is inherently disclosed or would have been obvious to a person of ordinary skill in the art ("POSITA"). By mapping the claim language to the references, National Beef does not imply or admit that the claim language satisfies any applicable legal requirement. For all the reasons stated above and herein, National Beef reserves the right to supplement the charts, and the tables below, as appropriate.

Citations from the listed references are not a ratification or acceptance of the manner in which IEH applies particular claim elements to the elements and functions of the accused instrumentalities. The citations are instead intended to demonstrate that, if certain claim elements are applied against the prior art in the manner as IEH applies them in its infringement contentions, then certain prior art discloses those claim elements to the same extent. The prior art may also disclose these same claim elements if the claim elements are applied differently than in IEH's infringement contentions. Nothing in these Contentions is in any way an admission that

IEH's infringement contentions correctly describe the scope of the Asserted Patents or that National Beef infringes any Asserted Claim.

National Beef incorporates, and may rely on, applicant admitted prior art (AAPA), all prior art references cited in the Asserted Patents (as well as any related patents and applications), and its prosecution history (including *Inter Partes* Reviews ("IPRs"), Inter Partes Reexaminations ("IPXs"), or *Ex Parte* Reexaminations ("EPXs"), if any), whether the related patents and applications were filed in the United States or in a foreign country. National Beef further incorporates, and may rely on, other references disclosed herein regarding motivation to combine, as well as background references. In addition, National Beef incorporates by reference any expert reports, invalidity contentions, identified prior art, invalidity claim charts or defense disclosed at any date by any party to any other litigation or USPTO/PTAB proceeding involving the Asserted Patents or any related patent. Furthermore, nothing stated herein shall be treated as an admission or suggestion that any of National Beef's accused microbiological testing methods meet any limitation of any Asserted Claim. National Beef denies that it infringes any valid claim of the Asserted Patents.

II. MATERIALS INCORPORATED BY REFERENCE

National Beef hereby incorporates by reference, as if fully set forth herein, all documents filed in any IPR, IPX, or EPX before the Patent Trial and Appeal Board or U.S. Patent and Trademark Office involving any of the Asserted Patents, as well as any patents related to the Asserted Patents, whether filed previously or at any time in the future.

National Beef hereby further incorporates by reference, as if set forth fully herein, all invalidity contentions, including amendments and/or supplements thereto, served by any defendant (or declaratory judgment plaintiff) or respondent in any proceeding in which IEH

asserted or will assert one or more of the Asserted Patents and/or their family members. The incorporated materials include, but are not limited to, all invalidity contentions, claim charts, and underlying prior art references included in any such proceeding. Any such incorporated materials pertaining to a particular claim limitation apply here to any Asserted Claims with an identical or similar claim limitation. Without limitation, National Beef expressly incorporates by reference the invalidity contentions, claim charts, and underlying prior art references in connection with the following proceedings:

- Inter Partes Reexamination Proceeding No. 95/001,294, USPTO April 5, 2010 (the '584 Patent);
- *National Beef Packing Company, LLC v. Institute for Environmental Health, Inc.*, IPR2024-00183, PTAB July 16, 2024 (the '584 Patent);
- *National Beef Packing Company, LLC v. Institute for Environmental Health, Inc.*, IPR2024-00185, PTAB July 16, 2024 (the '143 Patent);
- *National Beef Packing Company, LLC v. Institute for Environmental Health, Inc.*, IPR2024-00186, PTAB July 16, 2024 (the '771 Patent);
- *National Beef Packing Company, LLC v. Institute for Environmental Health, Inc.*, IPR2024-01156, PTAB July 31, 2024 (the '486 Patent).

National Beef further notes that the obviousness arguments as well as the evidence of motivation to combine and expectation of success identified in these proceedings also apply to the identified references in these Contentions.

National Beef further incorporates its First Amended Answer and Counterclaims (D.I. No. 40) and briefings related to inequitable conduct (D.I. No. 47).

III. PRIOR ART

National Beef identifies the prior art references that anticipate and/or render obvious each of the Asserted Claims below and in Exhibits A1-A9, B1-B9, C1-C9, and D1-D6, which are hereby incorporated by reference as if fully set forth herein.

To the extent that prior art identified by National Beef as anticipating is found not to anticipate, the prior art establishes that the claimed subject matter would have been obvious to a POSITA at the time of the alleged invention. Even if a particular claim element is not expressly found in one of the charted references, those charted references render the relevant Asserted Claims obvious without the need to combine with any other references, having in mind the information that generally would have been known to or understood by a POSITA, common sense, the Admitted Prior Art, and/or routine innovation. Accordingly, National Beef discloses that each cited anticipatory reference renders obvious the Asserted Claims as shown in the charts referenced above. In addition, the charted references in combination with one or more of the other charted references render obvious the Asserted Claims.

As explained in these Contentions, it would have been obvious to a POSITA at the time of the alleged invention of the Asserted Claims to combine the teachings from the various references cited for each of the Asserted Patents so as to practice the Asserted Claims. In addition to the specific combinations of prior art and the specific combinations of groups of prior art disclosed herein, National Beef reserves the right to rely on any other combination of prior art references disclosed in these Contentions. National Beef further reserves the right to rely upon combinations disclosed within the prosecution histories of the references cited in these Contentions. These obviousness combinations reflect National Beef's present understanding of the potential scope of the claims that IEH appears to be advocating and should not be construed as National Beef's acquiescence to IEH's interpretation of the Asserted Claims.

The U.S. Supreme Court decision in *KSR Int'l Co. v. Teleflex Inc.*, 127 S. Ct. 1727, 1739 (2007) ("KSR"), held that a claimed invention can be obvious even if there is no explicit, written teaching, suggestion, or motivation for combining the prior art to produce that invention.

Accordingly, no showing of an express motivation to combine is required to combine the teachings of the references disclosed in Exhibits A1-A9, B1-B9, C1-C9, and D1-D6, as each combination of art would have no unexpected results, and at most would have simply represented a known alternative to one of ordinary skill in the art. *See id.,* at 1739–40 (rejecting the Federal Circuit's "rigid" application of the teaching, suggestion, or motivation to combine test, and instead espousing an "expansive and flexible" approach). The Supreme Court in *KSR* held that a POSITA is "a person of ordinary creativity, not an automaton," and "in many cases a person of ordinary skill in the art will be able to fit the teachings of multiple patents together like pieces of a puzzle." *Id.* at 1742. "In determining whether the subject matter of a patent claim is obvious, neither the particular motivation nor the avowed purpose of the patentee controls. What matters is the objective reach of the claim." *Id.* at 1741–42. "Under the correct analysis, any need or problem known in the field of endeavor at the time of invention and addressed by the patent can provide a reason for combining the elements in the manner claimed." *Id.* at 1742. In particular, the Supreme Court emphasized the principle that "[t]he combination of familiar elements according to known methods is likely to be obvious when it does no more than yield predictable results." *Id.* at 1739. A key inquiry is whether the "improvement is more than the predictable use of prior art elements according to their established functions." *Id.* at 1740.

The rationale to combine or modify the teachings of prior art references is significantly stronger when, as here, the references seek to solve the same problem, come from the same field, and correspond well. In *In re Inland Steel Co.*, 265 F.3d 1354 (Fed. Cir. 2001), the Federal Circuit allowed two references to be combined as invalidating art when "[the prior art] focus[es] on the same problem that the . . . patent addresses. . . . Moreover, both [prior art references]

come from the same field. . . . Finally, the solutions to the identified problems found in the two references correspond well." *Id.* at 1362.

One or more combinations of the prior art references identified herein would have been obvious because the teachings of these references would have been combined using: known methods to yield predictable results; known techniques in the same way; a simple substitution of one known, equivalent element for another to obtain predictable results; or a teaching, suggestion, or motivation in the prior art generally.

In addition, it would have been obvious to try combining the teachings of the prior art references identified herein and in the accompanying exhibits because there were only a finite number of predictable solutions or because known work in one field of endeavor prompted variations based on predictable design incentives or market forces either in the same field or a different one. In addition, the combination of prior art references identified herein and in the accompanying exhibits would have been obvious because the combinations represent known potential options with a reasonable expectation of success.

Additional evidence that there would have been a motivation to combine the teachings of the prior art references identified in the attached exhibits includes the interrelated teachings of multiple prior art references; the existence of a known problem for which there was an obvious solution encompassed by the Asserted Claims; the existence of a known need or problem in the field of the endeavor at the time of the invention(s); and the background knowledge that would have been possessed by a person having ordinary skill in the art. Thus, a skilled artisan seeking to solve this problem would look to these cited references in combination.

Accordingly, the motivation to combine the teachings of the prior art references disclosed herein is found in the references themselves and: (1) the nature of the problem being solved; (2)

the express, implied and inherent teachings of the prior art; (3) the knowledge of persons of ordinary skill in the art; (4) the fact that the prior art is generally directed towards relevant methods and systems; or (5) the predictable results obtained in combining the different elements of the prior art.

Furthermore, a POSITA at the time of the alleged invention would have had reasons to combine or modify the teachings of one or more of the references listed and charted in the attached Exhibits (Exhibits A1-A9, B1-B9, C1-C9, and D1-D6) in light of the knowledge of a POSITA at the time of the invention and information in the prior art cited herein. The reasons or motivation to combine the teachings of the prior art would include, for example, the fact that the prior art for the Asserted Claims is in the same field, and one of ordinary skill in the art implementing a device, system, or method in accordance with the Asserted Claims would have been motivated to investigate the various related existing devices, systems, methods, publications or patents identified in these Contentions to address particular needs. Indeed, the claimed subject matter required nothing more than combining prior art elements according to known methods to yield predictable results. One of ordinary skill in the art would have been motivated to combine the teachings of the prior art, in that it required only simple substitutions of one known element for another to obtain predictable results.

To the extent any piece of prior art refers to or discusses other pieces of prior art, either expressly or inherently, it would have been obvious to combine the teachings of those pieces of prior art for that reason. In addition, design incentives and other market forces would have prompted those combinations and modifications. Furthermore, prior art references may arise from common assignees or multiple companies operating within the relevant subject matter. The motivation to combine the teachings of references includes the common objectives and subject

matter of the identified references. Accordingly, the teachings of the individual prior art references, combined with the industry knowledge of a POSITA at the time of the alleged invention of the Asserted Patents, would have rendered obvious the Asserted Claims.

To the extent IEH challenges a combination of prior art with respect to a particular element, National Beef reserves all rights to supplement these Contentions to further specify the reasons to combine the teachings of the prior art. National Beef may rely on cited or uncited portions of the prior art, other documents, and/or expert testimony to establish that a POSITA would have been motivated to modify or combine the teachings of the prior art so as to render the Asserted Claims invalid as obvious.

Whenever the Exhibits (Exhibits A1-A9, B1-B9, C1-C9, and D1-D6) include a figure, diagram, table, or drawing, all of the text associated with that figure, diagram, table, or drawing is included by reference (even if that text is not explicitly cited in the exhibit). Similarly, if an exhibit includes the text associated with a figure, diagram, table, or drawing, the described figure, diagram, or drawing is included by reference (even if that figure, diagram, table, or drawing is not explicitly cited).

The tables below provide the identity of prior art patents, patent publications, and non-patent literature that anticipate and/or render obvious the Asserted Claims of the Asserted Patents.

A. Patents and Patent Publications

Patent / Publication No.	Priority Date	Grant / Publication Date	Abbreviation	Bates No.
EP 1,253,203 A1	April 25, 2001	October 30, 2002	Nagar	NB_00010539 - 10550
WO 01/12853 A1	August 13, 1999	February 22, 2001	Karlsen	NB_00010795 - 10850
WO 94/21816	March 25, 1993	September 29, 1994	Hird	NB_00010851 - 10874

B. Non-Patent Literature

Author	Title	Publication Date	Abbreviation(s)	Bates No.
M. K. Rayman et al.	ICMSF methods studies. XIII. An international comparative study of the MPN procedure and the Anderson–Baird-Parker direct plating method for the enumeration of *Escherichia coli* biotype I in raw meats	November 1979	Rayman	NB_00010740 - 10746
U.S. Department of Agriculture Food and Safety Inspection Service	Pathogen Reduction; Hazard Analysis and Critical Control Point (HACCP) Systems	July 25, 1996	PR / HACCP	NB_00010551 - 10735
Judith M. Anderson; A. C. Baird-Parker	A Rapid and Direct Plate Method for Enumerating *Escherichia Coli* biotype I in Food	October 1975	Anderson	NB_00007297 - 7303
U.S. National Research Council Food and Nutrition Board Committee on Food Protection Subcommittee on Microbiological Criteria	An Evaluation of the Role of Microbiological Criteria for Foods and Food Ingredients	May 29, 1985	NRC	NB_00010510 - 10538
U.S. Department of Agriculture Food Safety and Inspection Service (FSIS)	Microbiology Laboratory Guidebook	January 1998	MLG / MLG 1998	NB_00010321 – 10378
U.S. Department of Agriculture	Microbiology Laboratory Guidebook	January 1998	MLG / MLG 1998	NB_00010379 - 10396

Food Safety and Inspection Service (FSIS)				
U.S. Department of Agriculture Food Safety and Inspection Service (FSIS)	Microbiology Laboratory Guidebook	April 9, 2003	MLG / MLG 2003	NB_00010397 - 10457
U.S. Department of Agriculture Food Safety and Inspection Service (FSIS)	Microbiology Laboratory Guidebook	April 10, 2003	MLG / MLG 2003	NB_00010397 - 10457
U.S. Department of Agriculture Food Safety and Inspection Service (FSIS)	Microbiology Laboratory Guidebook	July 7, 2003	MLG / MLG 2003	NB_00010397 - 10457
U.S. Department of Agriculture Food Safety and Inspection Service (FSIS)	Microbiology Laboratory Guidebook	February 2, 2004	MLG / MLG 2004	NB_00010397 - 10457
International Commission on Microbiological Specifications for Foods (ICMSF)	Microorganisms in Foods 2: Sampling for microbiological analysis: Principles and specific applications	1986	ICMSF 2 / ICMSF 1986	NB_00010013 - 10144
International Commission on Microbiological Specifications for Foods (ICMSF)	Microorganisms in Foods 7: Microbiological Testing in Food Safety Management	2002	ICMSF / ICMSF 7 / ICMSF 2002	NB_00010145 - 10242
U.S. Food & Drug Administration	Bacteriological Analytical Manual	1996	BAM 1996	NB_00007304
U.S. Food & Drug Administration	Bacteriological Analytical Manual	June 2003	BAM 2003	NB_00007305

Ira J. Mehlman	Coliforms, Fecal Coliforms, Escherichia Coli and Enteropathogenic E. Coli, in Compendium of Methods for The Microbiological Examination of Foods	1984	Mehlman	NB_00010461 - 10486
T. Yoohividhya & G. H. Fleet	An Evaluation of the A-1 Most Probable Number and the Anderson and Baird-Parker Plate Count Methods for Enumerating Escherichia coli in the Sydney Rock Oyster, Crassostrea commercialis	June 1981	Yoohividhya	NB_00010902 - 10911
Eystein Skjerve et al.	Detection of Listeria monocytogenes in Foods by Immunomagnetic Separation	November 1990	Skjerve	NB_00010768 - 10771
P. Duncanson et al.	Application of an automated immunomagnetic separation-enzyme immunoassay for the detection of Salmonella spp. During an outbreak associated with a retail premises	2003	Duncanson	NB_00007505 - 7509
Barbara A. Mitchell et al.	Use of Immunomagnetic Capture on Beads to Recover Listeria from Environmental Samples	1994	Mitchell	NB_00010500 - 10502
Arun K. Bhunia	Biosensors and Bio-Based Methods for the Separation and Detection of Foodborne Pathogens, in Advances in Food	2008	Bhunia	NB_00007416 - 7459

	and Nutrition Research			
David Gombas et al.	Survey of Listeria monocytogenes in Ready-to-Eat Foods	2003	Gombas	NB_00009970 - 9980
R. Holbrook et al.	Modified direct plate method for counting Escherichia coli in foods	1980	Holbrook	NB_00009981 - 9982
Denise Hughes et al.	Salmonella in Foods—A New Enrichment Procedure for Use with the TECRA Salmonella Visual Immunoassay: Collaborative Study	1999	Hughes 1999	NB_00009983 - 9996
Denise Hughes et al.	Salmonella in Foods: New Enrichment Procedure for TECRA Salmonella Visual Immunoassay Using a Single RV(R10) Only, TT Only, or Dual RV(R10) and TT Selective Enrichment Broths (AOAC Official Method 998.09): Collaborative Study	2003	Hughes 2003	NB_00009997 - 10012
W.R. Price et al.	Salmonella Testing of Pooled PreEnrichment Broth Cultures for Screening Multiple Food Samples	April 1972	Price	NB_00010736 - 10739
Diana Stewart et al.	Comparison of Assurance Gold Salmonella EIA, BAX for Screening/Salmonella, and GENE-TRAK Salmonella DLP Rapid Assays for Detection of Salmonella in Alfalfa Sprouts and Sprout Irrigation Water	2002	Stewart	NB_00010772 - 10780

Pina Fratamico et al.	Rapid isolation of Escherichia coli O157:H7 from enrichment cultures of foods using an immunomagnetic separation method	1992	Fratamico	NB_00009945 - 9953
Eva D'Haese & Hans Nelis	Rapid Detection of Single Cell Bacteria as a Novel Approach in Food Microbiology	2002	D'Haese	NB_00007500 - 7504
Jean-Yves D'Aoust	Update on Preenrichment and Selective Enrichment Conditions for Detection of Salmonella in Foods	1981	D'Aoust	NB_00007493 - 7499
Siragusa, GR et al.	Isolation of Listeria spp. From Feces of Feedlot Cattle	1993	Siragusa	NB_00010912 - 10916
U.S. Department of Agriculture Food Safety and Inspection Service (FSIS)	FSIS Directive 10.010.1	March 31, 2004	FSIS Directive	NB_00007515 - 7542
Thaddeus Midura & Raymond Bryant	Sampling Plans, Sample Collection, Shipment, and Preparation for Analysis, in Compendium of Methods for the Microbiological Examination of Foods	2001	Midura	NB_00010487 - 10499
J.H. Silliker & D.A. Gabis	ICMSF methods studies. Comparison of analytical schemes for detection of Salmonella in dried foods	1973	Silliker	NB_00010763 - 10767
D.A. Gabis & J.H. Silliker	CMSF methods studies. II. Comparison of analytical schemes for detection of	1974	Gabis	NB_00009954 - 9960

	Salmonella in high moisture foods			
Basil Jarvis	Errors Associated with Dilution Count and Presence/Absence Methods, in Statistical Aspects of the Microbiological Analysis of Foods	1989	Jarvis	NB_00010252 - 10283
Madhu Badoni et al.	Development and testing of a prototype automatic trim sampler	February 7, 2017	Badoni	NB_00007396 - 7401
Tommy Wheeler & Terrance Arthur	Novel Continuous and Manual Sampling Methods for Beef Trim Microbiological Testing	September 7, 2018	Wheeler	NB_00010882 - 10890
J. of AOAC	Changes in Official Methods of Analysis Made at the Eightieth Annual Meeting, October 10-13, 1966	February 1967	JAOAC	NB_00010243 - 10251
H. Wang & A. N. Sharpe	An immune-capturing and concentrating procedure for Escherichia coli O157:H7 and its detection by epifluorescence microscopy	1998	Wang	NB_00010875 - 10881
O.M. Cloak et al.	Development of a Surface Adhesion Immunofluorescent Technique for the rapid detection of Salmonella spp. From meat and poultry	1999	Cloak	NB_00007478 - 7485
Lawrence Restaino et al.	A 5-h screening and 24-h confirmation procedure for detecting Escherichia coli O157:H7 in beef using direct epifluorescent microscopy and	1997	Restaino 1997	NB_00010747 - 10750

	immunomagnetic separation			
Lawrence Restaino et al.	Antibody-Direct Epifluorescent Filter Technique and Immunomagnetic Separation for 10-h Screening and 24-h Confirmation of Escherichia coli O157:H7 in Beef	1996	Restaino 1996	NB_00010751 - 10754
Mary L. Tortorello & Diana S. Steward	Antibody-Direct Epifluorescent Filter Technique for Rapid, Direct Enumeration of Escherichia coli O157:H7 in Beef	1994	Tortorello 1994	NB_00010781 - 10787
Mary L. Tortorello et al.	Comparison of Antibody-Direct Epifluorescent Filter Technique with the Most Probable Number Procedure for Rapid Enumeration of Listeria in Fresh Vegetables	1997	Tortorello 1997	NB_00010788 - 10794
Life Technologies	ATHATRIX Pooling System for Salmonella Species, AAOAC ® Performance Tested (SM) Cert. No. 090203C	November 1, 2021	AOAC Certificate No. 090203C	NB_00010315 - 10320
Life Technologies	ATHATRIX Pooling System for Listeria spp., AAOAC ® Performance Tested (SM) Cert. No. 090201B	August 11, 2022	AOAC Certificate No. 090201B	NB_00010310 - 10314
Life Technologies	Pathatrix® 5-Pooling Listeria spp. Kit Linked to Selective Agar Plates, Life User Guide	August 14, 2012	Life Technologies PATHATRIX User Guide	NB_00010284 - 10309

Matrix MicroScience	PATHATRIX Listeria species Test, AAOAC ® Performance Tested (SM) Cert. No. 090201	September 5, 2002	AOAC Certificate No. 090201	NB_00007295
Matrix MicroScience	ATHATRIX System for Salmonella Species, AAOAC ® Performance Tested (SM) Cert. No. 090203	September 9, 2002	AOAC Certificate No. 090203	NB_00007296
Matrix MicroScience	Press release – A novel "PATHATRIX" Pooling Strategy" from Matrix MicroScience has gained AOAC-RI approvals for its Salmonella and Listeria product ranges	June 2005	Matrix MicroScience Press Release	NB_00010458 - 10460
Diane J. Fagerberg & John S. Avens	Enrichment and Plating Methodology for Salmonella Detection in Food. A Review	1976	Fagerberg	NB_00007543 - 7561
Patrick W. Benoit & Darrell W. Donahue	Methods for Rapid Separation and Concentration of Bacteria in Food that Bypass Time-Consuming Cultural Enrichment	2003	Benoit	NB_00007402 - 7415
Elizabeth Borch et al.	Bacterial spoilage of meat and cured meat products	1996	Borch	NB_00007460 - 7477
C. O. Gill	Meat Spoilage and Evaluation of the Potential Storage Life of Fresh Meat	1983	Gill	NB_00009961 - 9969
Kofitsyo S. Cudjoe et al.	IMS: a new selective enrichment technique for detection of Salmonella in foods	1994	Cudjoe	NB_00007486 - 7492
J.A. Scanga et al.	Microbiological contamination of raw	2000	Scanga	NB_00010755 - 10762

	beef trimmings and ground beef			
N.R. Mullane et al.	Detection of Enterobacter sakazakii in Dried Infant Milk Formula by Cationic-Magnetic-Bead Capture	2006	Mullane	NB_00010503 - 10509
Robert O. Elder et al.	Correlation of enterohemorrhagic Escherichia coli 0157 prevalence in feces, hides, and carcasses of beef cattle during processing	2000	Elder	NB_00007510 - 7514
USPTO	Inter Partes Reexamination Proceeding No. 95/001,294	April 5, 2010	IPR No. 95/001,294	NB_00007562 - 9944
Vivian C.H. Wu et al.,	Rapid Protocol (5.25H) for the Detection of Escherichia Coli 0157:H7 in Raw Ground Beef by an Immuno-capture System (PATHATRIX) in Combination with COLORTRIX and CT-SMAC	2004	Wu	NB_00010891 - 10901

C. Applicant Admitted Prior Art ("AAPA") and POSITA Knowledge

Applicant Admitted Prior Art (AAPA)			
#	**Cite**	**Summary**	**Step**
1	'584 Patent 1:40-45; '771 Patent 1:59-65; '143 Patent 1:52-57	Sampling plans are used by food producers to test beef and ready-to-eat ("RTE") products for microorganisms.	'584 Patent 1(a); '771 Patent 1(a); '143 Patent 1(a)

2	'584 Patent 12:29-33; '771 Patent 12:63-66; '143 Patent 12:36-39	Under FSIS guidelines, producers of **RTE** "meat and poultry products may adapt an **'end product' sampling program** as verification that their product is free of" *L. monocytogenes*.	'584 Patent 1(a); '771 Patent 1(a); '143 Patent 1(a)
3	'584 Patent 11:12-16; '771 Patent 11:37-41; '143 Patent 11:18-22	A **lot** for trim testing of E. coli O157:H7 in the beef industry may consist of **five separate 'combos,'** each combo being a single production unit weighing ~2,000 lbs.	'584 Patent 1(a); '771 Patent 1(a); '143 Patent 1(a)
4	'584 Patent 12:44-55; '771 Patent 13:10-22; '143 Patent 12:50-61	Producers **define** and produce single-unit **lots** to maximize information available for investigating failures, e.g. possible definitions of lots include "**all of the product by a particular production line in a particular period of time**" or "a volume of product such as a pallet of packed boxes produced on a particular production line, or a production area in the plant, etc.	'584 Patent 1(a); '771 Patent 1(a); '143 Patent 1(a)
5	'584 Patent 12:34-43; '771 Patent 12:67-13:9; '143 Patent 12:40-49	**[F]requency** of end product sampling is **selected** by the producer/establishment, based on **valid sampling statistics."** "[**S]amples are collected at random times over a production shift** (e.g., from 'cleanup to cleanup'), and from several product lines. The **samples** are **combined to form a composite sample,** and **enrichment buffer is added to the composite sample** to allow" microorganisms "to grow and multiply under the favorable conditions of **enrichment.**	'584 Patent 1(a)-(d); '771 Patent 1(a)-(d); '143 Patent 1(a)-(d)

6	'584 Patent 1:62-2:16; '771 Patent 2:15-36; '143 Patent 2:11-19	**Sampling plans** under ICMSF **guidelines** involve combining **several samples representing production lots** into a **combined production lot** prior to sampling. "For example, a typical plan for microbial pathogen detection calls for taking more than one sample, for instance ten (10) to **sixty (60) sample pieces** from a given **sample lot (combined production lot),** to form **a composite sample for testing.**	'584 Patent 1(a) and 1(d); '771 Patent 1(a) and 1(d); '143 Patent 1(a) and (d)
7	'584 Patent 2:46-58; '771 Patent 3:1-12; '143 Patent 2:58-3:3	"[O]nce a **composite sample** is constructed . . . it is then **enriched** for the presence of a given pathogen/microbe by addition of appropriate amounts of enrichment media" and incubated. Testing is "by art recognized methods."	'584 Patent 1(c) and 1(d); '771 Patent 1(c)-(e); '143 Patent 1(c)-(e)
8	'486 Patent 8:24-25	Prior art methods, as summarized herein, under the 'Background' section, are based on 1:10 (wt./vol.) dilutions of the sample.	'486 Patent
9	'486 Patent 2:10-42; *see also id.,* 1:58-2:9, 2:49-5:34, 13:54-14:32.	"[f]or the detection of *Escherichia coli O157:*H7 in food, **25 g of ground beef** is **incubated** in **225 ml of selective enrichment medium** for 16 to 18 h at 37° C. with agitation . . . . **Enzyme-linked immunosorbent assay (ELISA) is used to detect the pathogen from the enriched culture."**	'486 Patent Claim 1
10	'486 Patent 2:10-32; *see also id.,* 1:58-2:9, 4:35-44	*Escherichia coli.* The assay for the detection of *E. coli* in **citrus juices,** as described in the Bacteriological Analytical manual (BAM) (United States Food & Drug	'486 Patent Claim 11

		Administration, and Center for Food Safety & Applied Nutrition, Bacteriological Analytical Manual, Chapter 4, Enumeration of *Escherichia coli* and the Coliform Bacteria, September 2002), is carried out in duplicate. Basically, a **10-mL portion of juice** is aseptically inoculated into **90 mL of Universal Pre-enrichment Broth** (UPEB) and **incubated** at 35° C. for 24 h. After enrichment, **the sample is mixed and 1 mL** is transferred from each UPEB enrichment broth into **9 mL of EC broth** containing a CC disc, and EC/CC broth tubes are **incubated** at 44.5° C. in a circulating water bath for 24 h. Preferably, positive (tube inoculated with a MUG (+) *E. coli* strain) and negative (tube inoculated with *K. pneumoniae)* controls are included. The tubes are checked in the dark and under long wave UV light. **The presence of blue fluorescence in either tube is indicative that *E. coli* is present in the sample.**	

IV. INVALIDITY OF THE ASSERTED PATENTS

A. The ’584 Patent

1) Priority Date

The ’584 Patent was filed on August 8, 2005, and claims priority to Provisional Application No. 60/599,473 filed August 6, 2004. The priority date of the patent is pre-AIA 35 U.S.C. §§ 101 et seq.

2) Identification of Prior Art

National Beef identifies the following prior art references that anticipate and/or render obvious one or more of the Asserted Claims of the '584 Patent under 35 U.S.C. §§ 102 and/or 103. Invalidity claim charts for the Asserted Claims are attached hereto as Exhibits A1–A9.

- David Gombas et al., Survey of Listeria monocytogenes in Ready-to-Eat Foods, 66 J. FOOD PROTECTION 559–569 (2003);
- INT'L COMM'N ON MICROBIOLOGICAL SPEC'S FOR FOODS (ICMSF), MICROORGANISMS IN FOODS 7: MICROBIOLOGICAL TESTING IN FOOD SAFETY MANAGEMENT (Kluwer Academic/Plenum Publishers, 2002);
- INT'L COMM'N ON MICROBIOLOGICAL SPEC'S FOR FOODS (ICMSF), MICROORGANISMS IN FOODS 2 SAMPLING FOR MICROBIOLOGICAL ANALYSIS: PRINCIPLES AND SPECIFIC APPLICATIONS (Univ. Toronto Press 2nd ed. 1986);
- CTR. FOR FOOD SAFETY & APPLIED NUTRITION, U.S. FOOD & DRUG ADMIN., BACTERIOLOGICAL ANALYTICAL MANUAL Table of Contents, Introduction, About the Bacteriological Analytical Manual, Ch. 1, 4, 4A, 5, 10 (2003), https://web.archive.org/web/20030417133033/http://www.cfsan.fda.gov:80/~EBAM/BAM-TOC.HTML (as appeared on Apr. 17, 2003).
- Denise Hughes et al., *Salmonella in Foods—A New Enrichment Procedure for Use with the TECRA Salmonella Visual Immunoassay: Collaborative Study*, 82 J. AOAC INT'L 634–647 (1999);
- W.R. Price et al., *Salmonella Testing of Pooled PreEnrichment Broth Cultures for Screening Multiple Food Samples*, 23 APPLIED MICROBIOLOGY 679–682 (1972);
- Diana Stewart et al., *Comparison of Assurance Gold Salmonella EIA, BAX for Screening/Salmonella, and GENE-TRAK Salmonella DLP Rapid Assays for Detection of Salmonella in Alfalfa Sprouts and Sprout Irrigation Water*, 85 J. AOAC INT'L 395–403 (2002);
- Pina Fratamico et al., *Rapid isolation of Escherichia coli O157:H7 from enrichment cultures of foods using an immunomagnetic separation method*, 9 FOOD MICROBIOLOGY 105–113 (1992);
- Eva D'Haese & Hans Nelis, *Rapid Detection of Single Cell Bacteria as a Novel Approach in Food Microbiology*, 85 J. AOAC INT'L 979–983 (2002);
- Jean-Yves D'Aoust, *Update on Preenrichment and Selective Enrichment Conditions for Detection of Salmonella in Foods*, 44 J. FOOD PROTECTION 369–374, 393 (1981);

- Siragusa, GR et al, *Isolation of Listeria spp. From Feces of Feedlot Cattle*, J. OF FOOD PROTECTION 56(2):102-105 (1993);

- Denise Hughes et al., *Salmonella in Foods: New Enrichment Procedure for TECRA Salmonella Visual Immunoassay Using a Single RV(R10) Only, TT Only, or Dual RV(R10) and TT Selective Enrichment Broths (AOAC Official Method 998.09): Collaborative Study*, 86 J. AOAC INT'L, VOL. 775–790 (2003);

- Microbiological Testing Program and Other Verification Activities for Escherichia coli O157:H7 in Raw Ground Beef Products and Raw Ground Beef Components and Beef Patty Components, FSIS Directive 10.010.1, Rev. 1 (Mar. 31, 2004) (to be implemented on May 17, 2004);

- Thaddeus Midura & Raymond Bryant, *Sampling Plans, Sample Collection, Shipment, and Preparation for Analysis*, *in* COMPENDIUM OF METHODS FOR THE MICROBIOLOGICAL EXAMINATION OF FOODS 13-23 (American Public Health Association, 4th ed. 2001);

- J.H. Silliker & D.A. Gabis, *ICMSF methods studies. Comparison of analytical schemes for detection of Salmonella in dried foods*, 19 CAN. J. MICROBIOLOGY 475–479 (1973);

- D.A. Gabis & J.H. Silliker, *ICMSF methods studies. II. Comparison of analytical schemes for detection of Salmonella in high moisture foods*, 20 CAN. J. MICROBIOLOGY 663–669 (1974);

- Basil Jarvis, *Errors Associated with Dilution Count and Presence/Absence Methods, in* STATISTICAL ASPECTS OF THE MICROBIOLOGICAL ANALYSIS OF FOODS 117-146 (Elsevier Science Publishers, 1989);

- FOOD SAFETY AND INSPECTION SERVICE (FSIS), U.S. DEPT. OF AGRIC., MICROBIOLOGY LABORATORY GUIDEBOOK Ch. 8 (3rd ed. 1998);

- Madhu Badoni et al., *Development and testing of a prototype automatic trim sampler*, 77 FOOD CONTROL 82–87 (2017);

- Tommy Wheeler & Terrance Arthur, *Novel Continuous and Manual Sampling Methods for Beef Trim Microbiological Testing*, 81 J. FOOD PROTECTION 1605–1613 (2018);

- CTR. FOR FOOD SAFETY & APPLIED NUTRITION, U.S. FOOD & DRUG ADMIN., BACTERIOLOGICAL ANALYTICAL MANUAL Ch. 10, § 10.04 (1969);

- *Changes in Official Methods of Analysis Made at the Eightieth Annual Meeting, October 10-13*, *1966*, 50 J. of the AOAC 231–239 (Feb. 1967);

- Assurance® GDS Salmonella Tq Data Sheet from MilliporeSigma; and

- H. Wang & A. N. Sharpe, *An immune-capturing and concentrating procedure for Escherichia coli O157:H7 and its detection by epifluorescence microscopy*, 15 FOOD MICROBIOLOGY 559-565 (1998);

- O.M. Cloak et al., *Development of a Surface Adhesion Immunofluorescent Technique for the rapid detection of Salmonella spp. From meat and poultry*, 86 J. OF APPLIED MICROBIOLOGY 583-590 (1999);

- Lawrence Restaino et al., *A 5-h screening and 24-h confirmation procedure for detecting Escherichia coli O157:H7 in beef using direct epifluorescent microscopy and immunomagnetic separation*, 24 LETTERS IN APPLIED MICROBIOLOGY 401-404 (1997);

- Lawrence Restaino et al., *Antibody-Direct Epifluorescent Filter Technique and Immunomagnetic Separation for 10-h Screening and 24-h Confirmation of Escherichia coli O157:H7 in Beef*, 59 J. OF FOOD PROTECTION 1072-1075 (1996);

- Mary L. Tortorello & Diana S. Steward, *Antibody-Direct Epifluorescent Filter Technique for Rapid, Direct Enumeration of Escherichia coli O157:H7 in Beef*, 60 APPLIED AND ENVIRONMENTAL MICROBIOLOGY 3553-3559 (1994);

- Mary L. Tortorello et al., *Comparison of Antibody-Direct Epifluorescent Filter Technique with the Most Probable Number Procedure for Rapid Enumeration of Listeria in Fresh Vegetables*, 80 J. OF AOAC INT'L 1208-1214 (1997);

- Life Technologies, *PATHATRIX Pooling System for Salmonella Species*, AAOAC ® Performance Tested (SM) Cert. No. 090203C (Nov. 1, 2021);

- Life Technologies, *PATHATRIX Pooling System for Listeria spp.*, AAOAC ® Performance Tested (SM) Cert. No. 090201B (Nov. 11, 2022);

- Pathatrix® 5-Pooling Listeria spp. Kit Linked to Selective Agar Plates, LIFE USER GUIDE APL250P, MAN0006909 1-24 (Aug. 14, 2012);

- Matrix MicroScience, Ltd., *PATHATRIX System for Salmonella Species*, AAOAC ® Performance Tested (SM) Cert. No. 090203 (Sept. 9, 2002);

- Matrix MicroScience, Ltd., *PATHATRIX Listeria species Test*, AAOAC ® Performance Tested (SM) Cert. No. 090201 (Sept. 5, 2002);

- Vivian C.H. Wu et al., *Rapid Protocol (5.25H) for the Detection of Escherichia Coli 0157:H7 in Raw Ground Beef by an Immuno-capture System (PATHATRIX) in Combination with COLORTRIX and CT-SMAC*, 12 J. OF RAPID METHODS AND AUTOMATION IN MICROBIOLOGY 57-67 (2004);

- Press Release, Matrix Microscience, A Novel "PATHATRIX" Pooling Strategy" from Matrix MicroScience has gained AOAC-RI approvals for its Salmonella and Listeria product ranges (June 2005), www.matrixmsci.com;

- Diane J. Fagerberg & John S. Avens, *Enrichment and Plating Methodology for Salmonella Detection in Food. A Review,* 39 J. MILK FOOD TECHNOL. 628-646 (1976);

- Patrick W. Benoit & Darrell W. Donahue, *Methods for Rapid Separation and Concentration of Bacteria in Food that Bypass Time-Consuming Cultural Enrichment*, 66 J. OF FOOD PROTECTION 1935-1948 (2003);

- Elizabeth Borch et al., *Bacterial spoilage of meat and cured meat products*, 33 INT'L J. OF FOOD MICROBIOLOGY 103-120 (1996);

- C. O. Gill, *Meat Spoilage and Evaluation of the Potential Storage Life of Fresh Meat*, 46 J. OF FOOD PROTECTION 444-452 (1983);

- Kofitsyo S. Cudjoe et al., *IMS: a new selective enrichment technique for detection of Salmonella in foods*, 23 INT'L J. OF FOOD MICROBIOLOGY 159-165 (1994);

- J.A. Scanga et al., *Microbiological contamination of raw beef trimmings and ground beef*, 56 MEAT SCIENCE 145-152 (2000);

- N.R. Mullane et al., *Detection of Enterobacter sakazakii in Dried Infant Milk Formula by Cationic-Magnetic-Bead Capture*, APPLIED AND ENVIRONMENTAL MICROBIOLOGY 6325-6330 (2006);

- Robert O. Elder et al., *Correlation of enterohemorrhagic Escherichia coli 0157 prevalence in feces, hides, and carcasses of beef cattle during processing*, 97 PNAS 2999-3003 (2000);

- Inter Partes Reexamination Proceeding No. 95/001,294, USPTO April 5, 2010 (the '584 Patent); and

- Applicant Admitted Prior Art.

3) Anticipation

As set forth in the accompanying claim charts, Exhibits A5-A8, the Asserted Claims of the '584 Patent are anticipated under 35 U.S.C. § 102 (including Sections 102(a), 102(b), 102(e), and/or 102(g)) by Silliker, Gabis, Gombas, and Price. The prior art's associated claim chart sets forth where in the prior art reference each element of the Asserted Claims are found. To the extent that

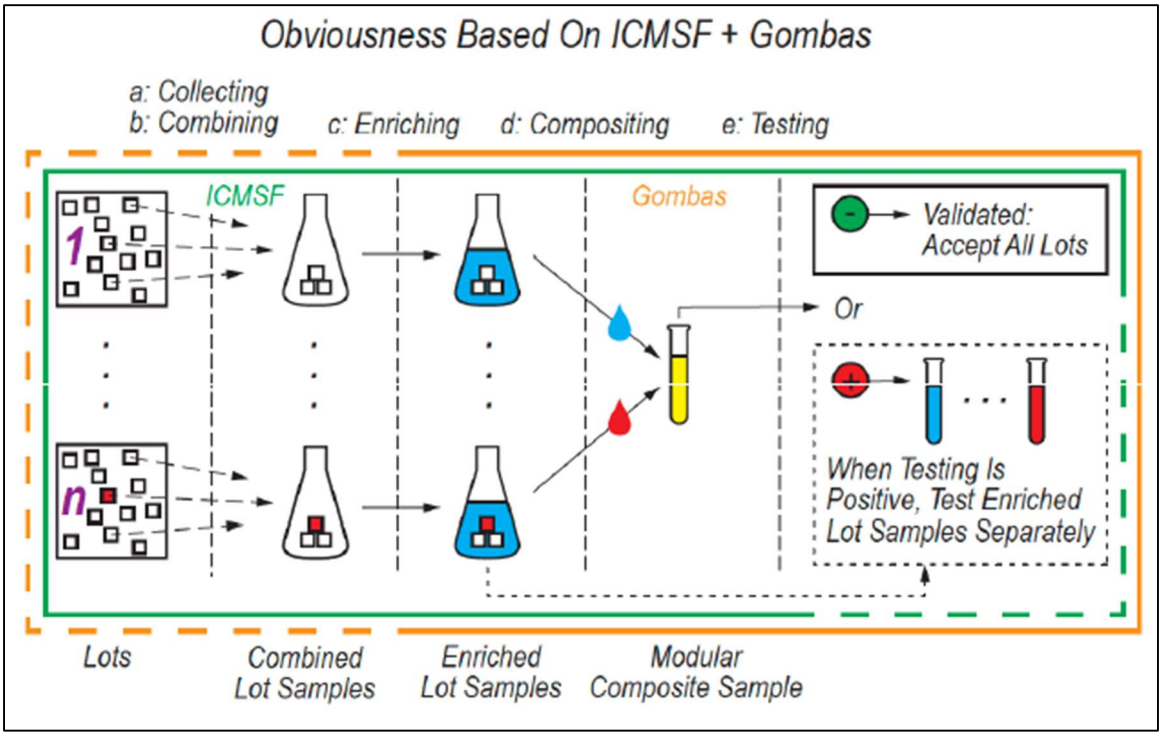
arguments or contentions within the claim charts conflict with, or are incompatible with, arguments or contentions that have been incorporated by reference, they are to be considered in the alternative.

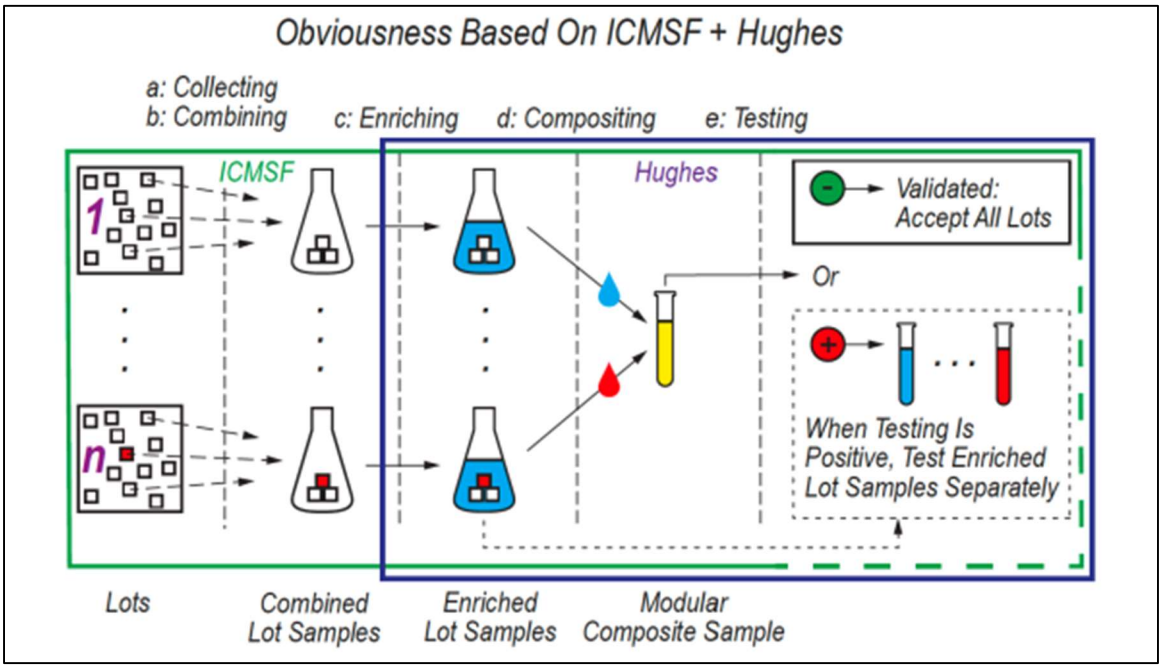
4) Obviousness

Each prior art reference identified herein, either alone or in combination with other identified references, renders the Asserted Claims invalid as obvious. In addition, each identified reference in the claim charts may be combined with: (1) information known to persons skilled in the art at the time of the alleged invention, and/or (2) any other reference identified herein. As detailed below and in the claim charts, the combination of elements and all the subject matter of the Asserted Claims were well-known in the food safety industry long before the earliest priority date of the '584 Patent. Therefore, the Asserted Claims are invalid.

To the extent it would have been necessary to combine prior art references, and to the extent the motivation to combine those references is necessary, it would have been found in the knowledge of one skilled in the art, as well as the nature of the problem to be solved. The motivation to combine references can also be found in the microbiological testing fields, and other similar fields, including in the prior art identified herein and as a matter of common sense and routine methods. Therefore, such references would have been highly relevant to people of ordinary skill.

For example, ICMSF, in combination with Gombas or Hughes, as disclosed below:





Therefore, to the extent the Asserted Claims are not anticipated, they would have been obvious to one of ordinary skill in the art at the relevant time period.

5) 35 U.S.C. § 101

National Beef contends that the Asserted Claims of the '584 Patent are invalid under 35 U.S.C. § 101 because the claims are not directed to patentable subject matter. A claim is patent-eligible if it recites a "new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof." 35 U.S.C. § 101. In *Alice*, the Supreme Court reaffirmed the two-step framework used to "distinguish[] patents that claim laws of nature, natural phenomena, and abstract ideas from those that claim patent-eligible applications of those concepts." *Alice Corp. Pty., Ltd. v. CLS Bank Int'l*, 573 U.S. 208, 217 (2014). This framework requires determining (1) if the claim is directed to ineligible subject matter such as an abstract idea, and, if so, (2) whether the remainder of the claim recites sufficient inventive elements that amount to "significantly more" than the abstract idea identified in Step 1. *Id*. The Asserted Claims are invalid under Section 101 because the claims are directed to nothing more than a basic concept of sampling, testing and validating test lots.

As one example, following the IPR proceedings for the '584 Patent, the patentee amended independent claims 1 and 17 to include "operationally-linked" test lots of beef (claim 1) or meat or poultry (claim 17). This so-called improvement on the prior art is nothing more than a basic human organizational concept and abstract idea which involves grouping together related production samples and tests to make the groups easier to track or to perform certain testing methods. There is nothing new or useful about operationally-linked methods of sampling, testing, and validating test lots, as it is a natural way in which to organize similar testing groups. Applying *Alice* step two, none of what remains recites anything inventive or new. All of the steps for wet pooling and compositing portions obtained from test lots are all well-understood, routine, and conventional.

National Beef's investigation regarding the invalidity of the Asserted Claims under 35 U.S.C. § 101 is ongoing. National Beef reserves the right to supplement or amend its invalidity contentions under 35 U.S.C. § 101 as the case progresses and in light of further investigation and discovery, claim construction proceedings, and/or review and analysis of expert witnesses. National Beef will disclose expert testimony in accordance with the applicable case schedule.

6) 35 U.S.C. § 112

National Beef identifies the following Asserted Claims that are — at least as apparently construed by IEH as the basis for its infringement contentions — invalid pursuant to 35 U.S.C. § 112 as lacking a sufficient written description, or indefinite.

Limitation	Claim(s) where limitation appears
"plurality"	1, 13-15, 17
"substantially uniform"	1, 17
"operationally-linked" / "operationally-linked product specimens"	1, 2, 17
"suitable"	1, 4, 5, 17, 23
"lot(s)" / "test lot" / "lot group"	1, 2, 4, 6, 10-15, 17-18, 22, 24, 31
"statistical-based sampling plan"	1, 17,
"target microbial agent or organism"	1, 4, 17, 19-21, 28
"a number of portions"	1, 13, 17
"portion"	1, 4, 12-15, 17, 18, 27, 31
"particular microbial agent or organism"	1, 17
"modular composite sample"	1, 3, 6, 12, 17, 30
"the more sensitive protocol"	1, 6, 17, 30

"production assemblage"	10

A more detailed basis for National Beef's written description and/or indefiniteness defenses will be set forth in National Beef's claim construction briefs or expert reports on invalidity, to be served in accordance with the parties' Proposed Scheduling Order (D.I. 24-1) or in accordance with any such scheduling order as may later be entered by the Court. National Beef specifically reserves the right to amend and/or supplement these Contentions based on a failure to comply with the requirements of 35 U.S.C. § 112.

B. The '143 Patent

1) Priority Date

The '143 Patent was filed on March 4, 2013, and is a continuation of Application No. 12/360,796 filed on January 27, 2009, and issued as U.S. Patent No. 8,389,233, which is a continuation of Application No. 11/199,871 filed on August 8, 2005, and issued as the '584 Patent, which claims priority to Provisional Application No. 60/599,473 filed August 6, 2004. The priority date of the patent is pre-AIA 35 U.S.C. §§ 101 et seq.

2) Identification of Prior Art

National Beef identifies the following prior art references that anticipate and/or render obvious one or more of the Asserted Claims of the '143 Patent under 35 U.S.C. §§ 102 and/or 103. The invalidity claim charts are attached hereto as Exhibits C1–C9.

- David Gombas et al., Survey of Listeria monocytogenes in Ready-to-Eat Foods, 66 J. FOOD PROTECTION 559–569 (2003);
- INT'L COMM'N ON MICROBIOLOGICAL SPEC'S FOR FOODS (ICMSF), MICROORGANISMS IN FOODS 7: MICROBIOLOGICAL TESTING IN FOOD SAFETY MANAGEMENT (Kluwer Academic/Plenum Publishers, 2002);

- INT'L COMM'N ON MICROBIOLOGICAL SPEC'S FOR FOODS (ICMSF), MICROORGANISMS IN FOODS 2 SAMPLING FOR MICROBIOLOGICAL ANALYSIS: PRINCIPLES AND SPECIFIC APPLICATIONS (Univ. Toronto Press 2nd ed. 1986);

- CTR. FOR FOOD SAFETY & APPLIED NUTRITION, U.S. FOOD & DRUG ADMIN., BACTERIOLOGICAL ANALYTICAL MANUAL Table of Contents, Introduction, About the Bacteriological Analytical Manual, Ch. 1, 4, 4A, 5, 10 (2003), https://web.archive.org/web/20030417133033/http://www.cfsan.fda.gov:80/~EBAM/BAM-TOC.HTML (as appeared on Apr. 17, 2003).

- Denise Hughes et al., *Salmonella in Foods—A New Enrichment Procedure for Use with the TECRA Salmonella Visual Immunoassay: Collaborative Study*, 82 J. AOAC INT'L 634–647 (1999);

- W.R. Price et al., *Salmonella Testing of Pooled PreEnrichment Broth Cultures for Screening Multiple Food Samples*, 23 APPLIED MICROBIOLOGY 679–682 (1972);

- Diana Stewart et al., *Comparison of Assurance Gold Salmonella EIA, BAX for Screening/Salmonella, and GENE-TRAK Salmonella DLP Rapid Assays for Detection of Salmonella in Alfalfa Sprouts and Sprout Irrigation Water*, 85 J. AOAC INT'L 395–403 (2002);

- Pina Fratamico et al., *Rapid isolation of Escherichia coli O157:H7 from enrichment cultures of foods using an immunomagnetic separation method*, 9 FOOD MICROBIOLOGY 105–113 (1992);

- Eva D'Haese & Hans Nelis, *Rapid Detection of Single Cell Bacteria as a Novel Approach in Food Microbiology*, 85 J. AOAC INT'L 979–983 (2002);

- Jean-Yves D'Aoust, *Update on Preenrichment and Selective Enrichment Conditions for Detection of Salmonella in Foods*, 44 J. FOOD PROTECTION 369–374, 393 (1981);

- Siragusa, GR et al, *Isolation of Listeria spp. From Feces of Feedlot Cattle*, J. OF FOOD PROTECTION 56(2):102-105 (1993);

- Denise Hughes et al., *Salmonella in Foods: New Enrichment Procedure for TECRA Salmonella Visual Immunoassay Using a Single RV(R10) Only, TT Only, or Dual RV(R10) and TT Selective Enrichment Broths (AOAC Official Method 998.09): Collaborative Study*, 86 J. AOAC INT'L, VOL. 775–790 (2003);

- Microbiological Testing Program and Other Verification Activities for Escherichia coli O157:H7 in Raw Ground Beef Products and Raw Ground Beef Components and Beef Patty Components, FSIS Directive 10.010.1, Rev. 1 (Mar. 31, 2004) (to be implemented on May 17, 2004);

- Thaddeus Midura & Raymond Bryant, *Sampling Plans, Sample Collection, Shipment, and Preparation for Analysis*, *in* COMPENDIUM OF METHODS FOR THE MICROBIOLOGICAL EXAMINATION OF FOODS 13-23 (American Public Health Association, 4th ed. 2001);

- J.H. Silliker & D.A. Gabis, *ICMSF methods studies. Comparison of analytical schemes for detection of Salmonella in dried foods*, 19 CAN. J. MICROBIOLOGY 475–479 (1973);

- D.A. Gabis & J.H. Silliker, *ICMSF methods studies. II. Comparison of analytical schemes for detection of Salmonella in high moisture foods*, 20 CAN. J. MICROBIOLOGY 663–669 (1974);

- Basil Jarvis, *Errors Associated with Dilution Count and Presence/Absence Methods, in* STATISTICAL ASPECTS OF THE MICROBIOLOGICAL ANALYSIS OF FOODS 117-146 (Elsevier Science Publishers, 1989);

- FOOD SAFETY AND INSPECTION SERVICE (FSIS), U.S. DEPT. OF AGRIC., MICROBIOLOGY LABORATORY GUIDEBOOK Ch. 8 (3rd ed. 1998);

- Madhu Badoni et al., *Development and testing of a prototype automatic trim sampler*, 77 FOOD CONTROL 82–87 (2017);

- Tommy Wheeler & Terrance Arthur, *Novel Continuous and Manual Sampling Methods for Beef Trim Microbiological Testing*, 81 J. FOOD PROTECTION 1605–1613 (2018);

- CTR. FOR FOOD SAFETY & APPLIED NUTRITION, U.S. FOOD & DRUG ADMIN., BACTERIOLOGICAL ANALYTICAL MANUAL Ch. 10, § 10.04 (1969);

- *Changes in Official Methods of Analysis Made at the Eightieth Annual Meeting, October 10-13*, *1966*, 50 J. of the AOAC 231–239 (Feb. 1967);

- Assurance® GDS Salmonella Tq Data Sheet from MilliporeSigma; and

- H. Wang & A. N. Sharpe, *An immune-capturing and concentrating procedure for Escherichia coli O157:H7 and its detection by epifluorescence microscopy*, 15 FOOD MICROBIOLOGY 559-565 (1998);

- O.M. Cloak et al., *Development of a Surface Adhesion Immunofluorescent Technique for the rapid detection of Salmonella spp. From meat and poultry*, 86 J. OF APPLIED MICROBIOLOGY 583-590 (1999);

- Lawrence Restaino et al., *A 5-h screening and 24-h confirmation procedure for detecting Escherichia coli O157:H7 in beef using direct epifluorescent microscopy and immunomagnetic separation*, 24 LETTERS IN APPLIED MICROBIOLOGY 401-404 (1997);

- Lawrence Restaino et al., *Antibody-Direct Epifluorescent Filter Technique and Immunomagnetic Separation for 10-h Screening and 24-h Confirmation of Escherichia coli O157:H7 in Beef*, 59 J. OF FOOD PROTECTION 1072-1075 (1996);

- Mary L. Tortorello & Diana S. Steward, *Antibody-Direct Epifluorescent Filter Technique for Rapid, Direct Enumeration of Escherichia coli O157:H7 in Beef*, 60 APPLIED AND ENVIRONMENTAL MICROBIOLOGY 3553-3559 (1994);

- Mary L. Tortorello et al., *Comparison of Antibody-Direct Epifluorescent Filter Technique with the Most Probable Number Procedure for Rapid Enumeration of Listeria in Fresh Vegetables*, 80 J. OF AOAC INT'L 1208-1214 (1997);

- Life Technologies, *PATHATRIX Pooling System for Salmonella Species*, AAOAC ® Performance Tested (SM) Cert. No. 090203C (Nov. 1, 2021);

- Life Technologies, *PATHATRIX Pooling System for Listeria spp.*, AAOAC ® Performance Tested (SM) Cert. No. 090201B (Nov. 11, 2022);

- Pathatrix® 5-Pooling Listeria spp. Kit Linked to Selective Agar Plates, LIFE USER GUIDE APL250P, MAN0006909 1-24 (Aug. 14, 2012);

- Matrix MicroScience, Ltd., *PATHATRIX System for Salmonella Species*, AAOAC ® Performance Tested (SM) Cert. No. 090203 (Sept. 9, 2002);

- Matrix MicroScience, Ltd., *PATHATRIX Listeria species Test*, AAOAC ® Performance Tested (SM) Cert. No. 090201 (Sept. 5, 2002);

- Vivian C.H. Wu et al., *Rapid Protocol (5.25H) for the Detection of Escherichia Coli 0157:H7 in Raw Ground Beef by an Immuno-capture System (PATHATRIX) in Combination with COLORTRIX and CT-SMAC*, 12 J. OF RAPID METHODS AND AUTOMATION IN MICROBIOLOGY 57-67 (2004);

- Press Release, Matrix Microscience, A Novel "PATHATRIX" Pooling Strategy" from Matrix MicroScience has gained AOAC-RI approvals for its Salmonella and Listeria product ranges (June 2005), www.matrixmsci.com;

- Diane J. Fagerberg & John S. Avens, *Enrichment and Plating Methodology for Salmonella Detection in Food. A Review,* 39 J. MILK FOOD TECHNOL. 628-646 (1976);

- Patrick W. Benoit & Darrell W. Donahue, *Methods for Rapid Separation and Concentration of Bacteria in Food that Bypass Time-Consuming Cultural Enrichment*, 66 J. OF FOOD PROTECTION 1935-1948 (2003);

- Elizabeth Borch et al., *Bacterial spoilage of meat and cured meat products*, 33 INT'L J. OF FOOD MICROBIOLOGY 103-120 (1996);

- C. O. Gill, *Meat Spoilage and Evaluation of the Potential Storage Life of Fresh Meat*, 46 J. OF FOOD PROTECTION 444-452 (1983);

- Kofitsyo S. Cudjoe et al., *IMS: a new selective enrichment technique for detection of Salmonella in foods*, 23 INT'L J. OF FOOD MICROBIOLOGY 159-165 (1994);

- J.A. Scanga et al., *Microbiological contamination of raw beef trimmings and ground beef*, 56 MEAT SCIENCE 145-152 (2000);

- N.R. Mullane et al., *Detection of Enterobacter sakazakii in Dried Infant Milk Formula by Cationic-Magnetic-Bead Capture*, APPLIED AND ENVIRONMENTAL MICROBIOLOGY 6325-6330 (2006);

- Robert O. Elder et al., *Correlation of enterohemorrhagic Escherichia coli 0157 prevalence in feces, hides, and carcasses of beef cattle during processing*, 97 PNAS 2999-3003 (2000);

- Inter Partes Reexamination Proceeding No. 95/001,294, USPTO April 5, 2010 (the '584 Patent); and

- Applicant Admitted Prior Art.

3) Invalidity based on inequitable conduct before the U.S. Patent and Trademark Office

National Beef contends that the Asserted Claims are invalid based on inequitable conduct during the prosecution of the '143 Patent. During prosecution, IEH failed to disclose relevant and material documents, such as office actions, responses, and third-party comments from the co-pending '584 reexamination proceeding that would have impacted the patentability of the Asserted Claims. Even though the Examiner found that the claims of the '143 Patent were not patentably distinct from the claims of the '584 Patent, the Examiner allowed the claims of the '143 Patent while the claims of the '584 Patent stood rejected after multiple reviews and decisions. IEH was further aware of the overlap and similarity between the '584 Patent and the '143 Patent because it agreed to file a terminal disclaimer in order to avoid rejection based upon nonstatutory double patenting. IEH was forced to narrow the scope of the '584 Patent by amending its claims, but did not do so with the '143 Patent, even though it was aware that the

claims of the ’143 Patent would be susceptible to similar issues. The failure to disclose pertinent documents from the ’584 reexamination proceeding was done in violation of IEH’s and its attorneys’ duty of candor under 37 C.F.R. § 1.56 and constitutes inequitable conduct. National Beef hereby incorporates by reference the prior art references, responses and third-party comments from Inter Partes Reexamination Proceeding No. 90/001,294, USPTO April 5, 2010 (the ’584 Patent).

4) Anticipation

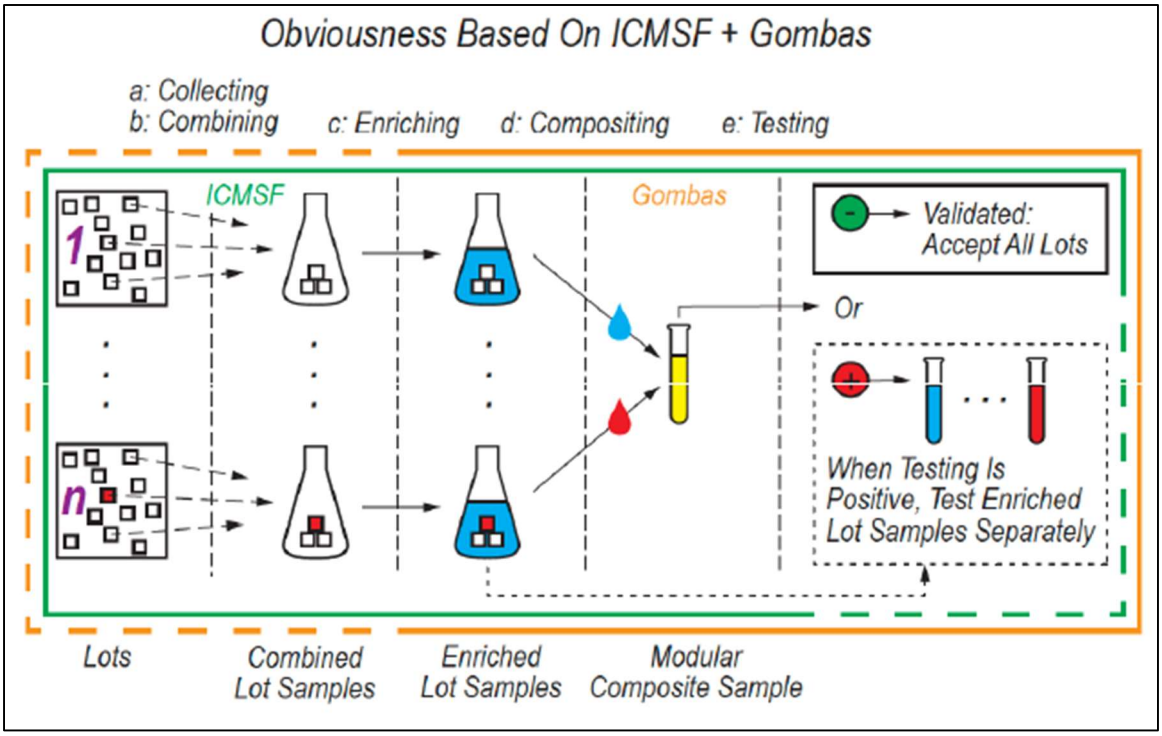
As set forth in the accompanying claim charts, Exhibits C5-C8, the Asserted Claims of the ’143 Patent are anticipated under 35 U.S.C. § 102 (including Sections 102(a), 102(b), 102(e), and/or 102(g)) by Silliker, Gabis, Gombas, and Price. The prior art’s associated claim chart sets forth where in the prior art reference each element of the Asserted Claims are found. To the extent that arguments or contentions within the claim charts conflict with, or are incompatible with, arguments or contentions that have been incorporated by reference, they are to be considered in the alternative.

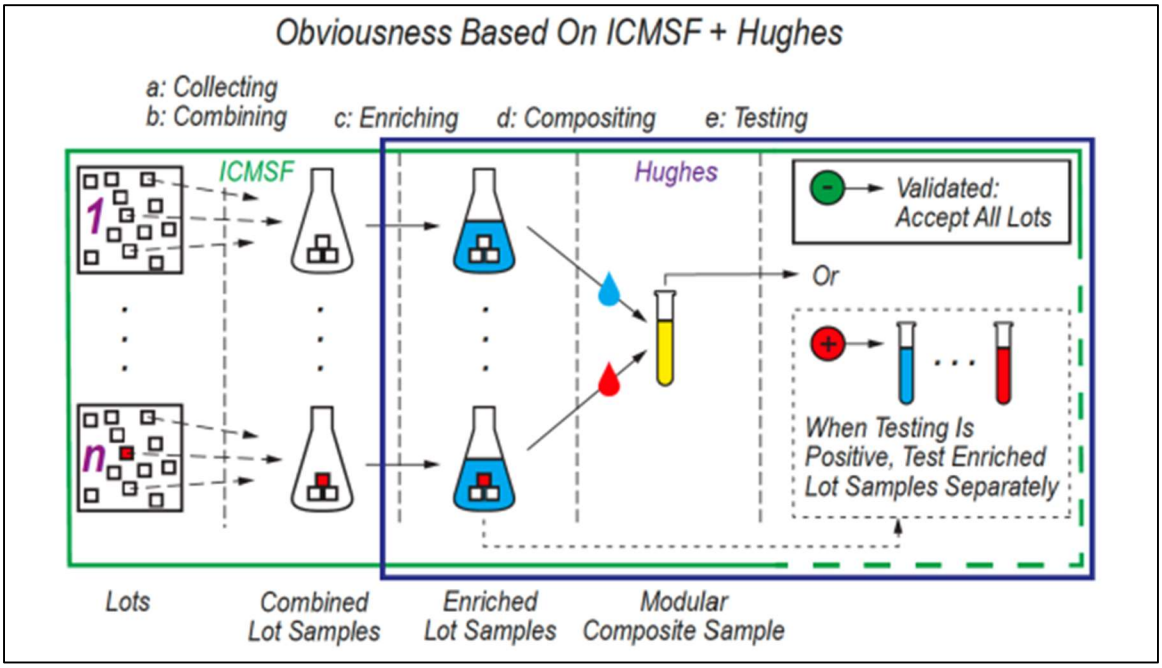
5) Obviousness

Each prior art reference identified herein, either alone or in combination with other identified references, renders the Asserted Claims invalid as obvious. In addition, each identified reference in the claim charts may be combined with: (1) information known to persons skilled in the art at the time of the alleged invention, and/or (2) any other reference identified herein. As detailed below and in the claim charts, the combination of elements and all the subject matter of the Asserted Claims were well-known in the food safety industry long before the earliest priority date of the ’143 Patent. Therefore, the Asserted Claims are invalid.

To the extent it would have been necessary to combine prior art references, and to the extent the motivation to combine those references is necessary, it would have been found in the knowledge of one skilled in the art, as well as the nature of the problem to be solved. The motivation to combine references can also be found in the microbiological testing fields, and other similar fields, including in the prior art identified herein and as a matter of common sense and routine methods. Therefore, such references would have been highly relevant to people of ordinary skill.

For example, ICMSF, in combination with Gombas or Hughes, as disclosed below:





Therefore, to the extent the Asserted Claims are not anticipated, they would have been obvious to one of ordinary skill in the art at the relevant time

6) 35 U.S.C. § 101

National Beef contends that the Asserted Claims of the '143 Patent are invalid under 35 U.S.C. § 101 because the claims are not directed to patentable subject matter. A claim is patent-eligible if it recites a "new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof." 35 U.S.C. § 101. In *Alice*, the Supreme Court reaffirmed the two-step framework used to "distinguish[] patents that claim laws of nature, natural phenomena, and abstract ideas from those that claim patent-eligible applications of those concepts." *Alice Corp. Pty., Ltd. v. CLS Bank Int'l*, 573 U.S. 208, 217 (2014). This framework requires determining (1) if the claim is directed to ineligible subject matter such as an abstract idea, and, if so, (2) whether the remainder of the claim recites sufficient inventive elements that amount to "significantly more" than the abstract idea identified in Step 1. *Id*. The Asserted

Claims are invalid under Section 101 because the claims are directed to nothing more than a basic concept of sampling, testing and validating test lots.

"Operationally-linked" appears in claims 2 and 3 of the '143 Patent. This so-called improvement on the prior art is nothing more than a basic human organizational concept and abstract idea which involves grouping together related production samples and tests to make the groups easier to track or to perform certain testing methods. There is nothing new or useful about operationally-linked methods of sampling, testing, and validating test lots, as it is a natural way in which to organize similar testing groups. Applying *Alice* step two, none of what remains recites anything inventive or new. All of the steps for wet pooling and compositing portions obtained from test lots are all well-understood, routine, and conventional.

National Beef's investigation regarding the invalidity of the Asserted Claims under 35 U.S.C. § 101 is ongoing. National Beef reserves the right to supplement or amend its invalidity contentions under 35 U.S.C. § 101 as the case progresses and in light of further investigation and discovery, claim construction proceedings, and/or review and analysis of expert witnesses. National Beef will disclose expert testimony in accordance with the applicable case schedule.

7) 35 U.S.C. § 112

National Beef identifies the following Asserted Claims that are — at least as apparently construed by IEH as the basis for its infringement contentions — invalid pursuant to 35 U.S.C. § 112 as lacking a sufficient written description, or indefinite.

Limitation	Claim(s) where limitation appears
"operationally-linked" / "operationally-linked … specimen"	2, 3
"suitable"	1, 5, 6, 23, 35

"independent" / "multiple independent samples"	1, 14-16, 35
"lot(s)" / "test lot" / "production lot samples"	1-3, 5, 7, 11-16, 18, 22, 24, 35-36.
"target microbe(s)" / "non-target microbe"	1, 5-6, 19-21, 30, 32, 35
"multiple separate test lots"/ "separate test lots" / "single unit product/production lot(s)"	1, 2, 14-17, 24, 35-38
"portion(s)"	1, 5, 18, 22, 29, 35, 36
"particular corresponding separate test lot" / "particular corresponding separate single-unit production lot"	1, 35
"modular composite sample"	1, 4-7, 13, 23, 32, 35
"the/a more sensitive protocol"	7, 32
"production assemblage"	11

A more detailed basis for National Beef's written description and/or indefiniteness defenses will be set forth in National Beef's claim construction briefs or expert reports on invalidity, to be served in accordance with the parties' Proposed Scheduling Order (D.I. 24-1) or in accordance with any such scheduling order as may later be entered by the Court. National Beef specifically reserves the right to amend and/or supplement these Contentions based on a failure to comply with the requirements of 35 U.S.C. § 112.

C. The ’771 Patent

1) Priority Date

The ’771 Patent was filed on August 18, 2014, and is a continuation of Application No. 13/784,606 filed on March 4, 2013, and issued as the ’143 Patent, which is a continuation of Application No. 12/360,796 filed on January 27, 2009, and issued as U.S. Patent No. 8,389,233, which is a continuation of Application No. 11/199,871 filed on August 8, 2005, and issued as the ’584 Patent, which claims priority to Provisional Application No. 60/599,473 filed August 6, 2004. The priority date of the patent is pre-AIA 35 U.S.C. §§ 101 et seq.

2) Identification of Prior Art

National Beef identifies the following prior art references that anticipate and/or render obvious one or more of the Asserted Claims of the ’771 Patent under 35 U.S.C. §§ 102 and/or 103. The invalidity claim charts are attached hereto as Exhibits B1 – B9.

- David Gombas et al., Survey of Listeria monocytogenes in Ready-to-Eat Foods, 66 J. FOOD PROTECTION 559–569 (2003);
- INT’L COMM’N ON MICROBIOLOGICAL SPEC’S FOR FOODS (ICMSF), MICROORGANISMS IN FOODS 7: MICROBIOLOGICAL TESTING IN FOOD SAFETY MANAGEMENT (Kluwer Academic/Plenum Publishers, 2002);
- INT’L COMM’N ON MICROBIOLOGICAL SPEC’S FOR FOODS (ICMSF), MICROORGANISMS IN FOODS 2 SAMPLING FOR MICROBIOLOGICAL ANALYSIS: PRINCIPLES AND SPECIFIC APPLICATIONS (Univ. Toronto Press 2nd ed. 1986);
- CTR. FOR FOOD SAFETY & APPLIED NUTRITION, U.S. FOOD & DRUG ADMIN., BACTERIOLOGICAL ANALYTICAL MANUAL Table of Contents, Introduction, About the Bacteriological Analytical Manual, Ch. 1, 4, 4A, 5, 10 (2003), https://web.archive.org/web/20030417133033/http://www.cfsan.fda.gov:80/~EBAM/BAM-TOC.HTML (as appeared on Apr. 17, 2003).
- Denise Hughes et al., *Salmonella in Foods—A New Enrichment Procedure for Use with the TECRA Salmonella Visual Immunoassay: Collaborative Study*, 82 J. AOAC INT’L 634–647 (1999);
- W.R. Price et al., *Salmonella Testing of Pooled PreEnrichment Broth Cultures for Screening Multiple Food Samples*, 23 APPLIED MICROBIOLOGY 679–682 (1972);

- Diana Stewart et al., *Comparison of Assurance Gold Salmonella EIA, BAX for Screening/Salmonella, and GENE-TRAK Salmonella DLP Rapid Assays for Detection of Salmonella in Alfalfa Sprouts and Sprout Irrigation Water*, 85 J. AOAC INT'L 395–403 (2002);

- Pina Fratamico et al., *Rapid isolation of Escherichia coli O157:H7 from enrichment cultures of foods using an immunomagnetic separation method*, 9 FOOD MICROBIOLOGY 105–113 (1992);

- Eva D'Haese & Hans Nelis, *Rapid Detection of Single Cell Bacteria as a Novel Approach in Food Microbiology*, 85 J. AOAC INT'L 979–983 (2002);

- Jean-Yves D'Aoust, *Update on Preenrichment and Selective Enrichment Conditions for Detection of Salmonella in Foods*, 44 J. FOOD PROTECTION 369–374, 393 (1981);

- Siragusa, GR et al, *Isolation of Listeria spp. From Feces of Feedlot Cattle*, J. OF FOOD PROTECTION 56(2):102-105 (1993);

- Denise Hughes et al., *Salmonella in Foods: New Enrichment Procedure for TECRA Salmonella Visual Immunoassay Using a Single RV(R10) Only, TT Only, or Dual RV(R10) and TT Selective Enrichment Broths (AOAC Official Method 998.09): Collaborative Study*, 86 J. AOAC INT'L, VOL. 775–790 (2003);

- Microbiological Testing Program and Other Verification Activities for Escherichia coli O157:H7 in Raw Ground Beef Products and Raw Ground Beef Components and Beef Patty Components, FSIS Directive 10.010.1, Rev. 1 (Mar. 31, 2004) (to be implemented on May 17, 2004);

- Thaddeus Midura & Raymond Bryant, *Sampling Plans, Sample Collection, Shipment, and Preparation for Analysis*, *in* COMPENDIUM OF METHODS FOR THE MICROBIOLOGICAL EXAMINATION OF FOODS 13-23 (American Public Health Association, 4th ed. 2001);

- J.H. Silliker & D.A. Gabis, *ICMSF methods studies. Comparison of analytical schemes for detection of Salmonella in dried foods*, 19 CAN. J. MICROBIOLOGY 475–479 (1973);

- D.A. Gabis & J.H. Silliker, *ICMSF methods studies. II. Comparison of analytical schemes for detection of Salmonella in high moisture foods*, 20 CAN. J. MICROBIOLOGY 663–669 (1974);

- Basil Jarvis, *Errors Associated with Dilution Count and Presence/Absence Methods, in* STATISTICAL ASPECTS OF THE MICROBIOLOGICAL ANALYSIS OF FOODS 117-146 (Elsevier Science Publishers, 1989);

- FOOD SAFETY AND INSPECTION SERVICE (FSIS), U.S. DEPT. OF AGRIC., MICROBIOLOGY LABORATORY GUIDEBOOK Ch. 8 (3rd ed. 1998);

- Madhu Badoni et al., *Development and testing of a prototype automatic trim sampler*, 77 FOOD CONTROL 82–87 (2017);

- Tommy Wheeler & Terrance Arthur, *Novel Continuous and Manual Sampling Methods for Beef Trim Microbiological Testing*, 81 J. FOOD PROTECTION 1605–1613 (2018);

- CTR. FOR FOOD SAFETY & APPLIED NUTRITION, U.S. FOOD & DRUG ADMIN., BACTERIOLOGICAL ANALYTICAL MANUAL Ch. 10, § 10.04 (1969);

- *Changes in Official Methods of Analysis Made at the Eightieth Annual Meeting, October 10-13*, *1966*, 50 J. of the AOAC 231–239 (Feb. 1967);

- Assurance® GDS Salmonella Tq Data Sheet from MilliporeSigma; and

- H. Wang & A. N. Sharpe, *An immune-capturing and concentrating procedure for Escherichia coli O157:H7 and its detection by epifluorescence microscopy*, 15 FOOD MICROBIOLOGY 559-565 (1998);

- O.M. Cloak et al., *Development of a Surface Adhesion Immunofluorescent Technique for the rapid detection of Salmonella spp. From meat and poultry*, 86 J. OF APPLIED MICROBIOLOGY 583-590 (1999);

- Lawrence Restaino et al., *A 5-h screening and 24-h confirmation procedure for detecting Escherichia coli O157:H7 in beef using direct epifluorescent microscopy and immunomagnetic separation*, 24 LETTERS IN APPLIED MICROBIOLOGY 401-404 (1997);

- Lawrence Restaino et al., *Antibody-Direct Epifluorescent Filter Technique and Immunomagnetic Separation for 10-h Screening and 24-h Confirmation of Escherichia coli O157:H7 in Beef*, 59 J. OF FOOD PROTECTION 1072-1075 (1996);

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3) Invalidity based on inequitable conduct before the U.S. Patent and Trademark Office

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4) Anticipation

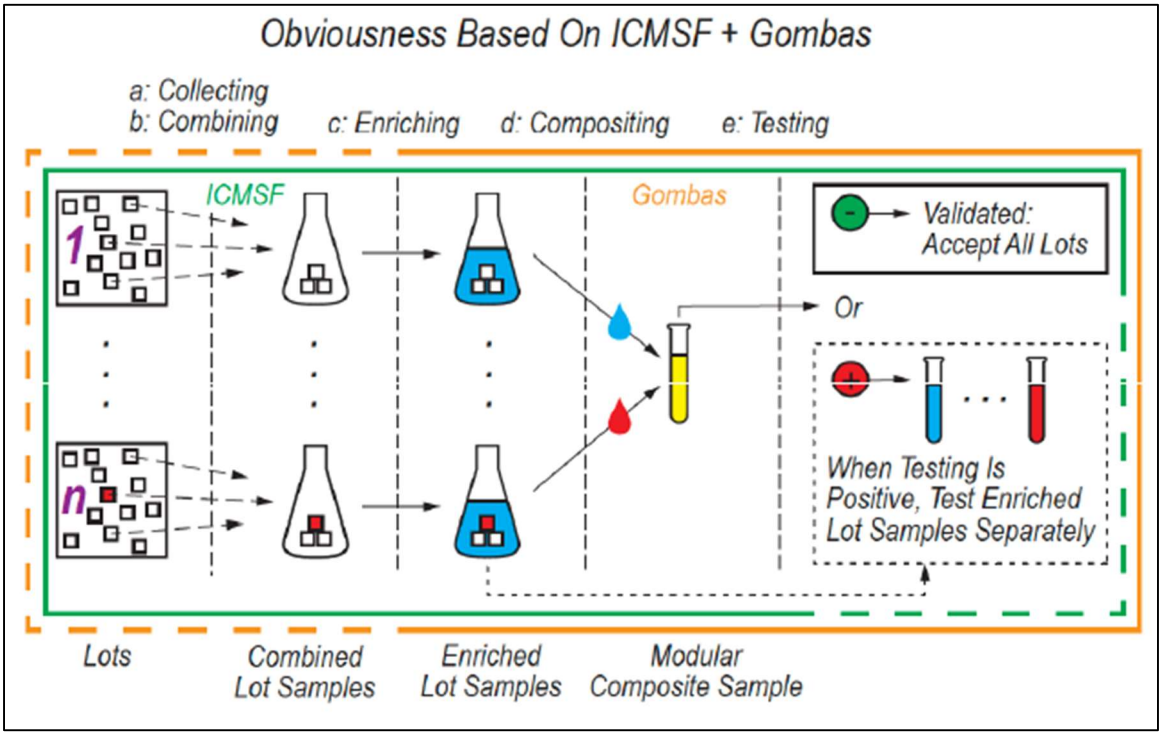
As set forth in the accompanying claim charts, Exhibits B5-B8, the Asserted Claims of the '771 Patent are anticipated under 35 U.S.C. § 102 (including Sections 102(a), 102(b), 102(e), and/or 102(g)) by Silliker, Gabis, Gombas, and Price. The prior art's associated claim chart sets forth where in the prior art reference each element of the Asserted Claims are found. To the extent that arguments or contentions within the claim charts conflict with, or are incompatible with, arguments or contentions that have been incorporated by reference, they are to be considered in the alternative.

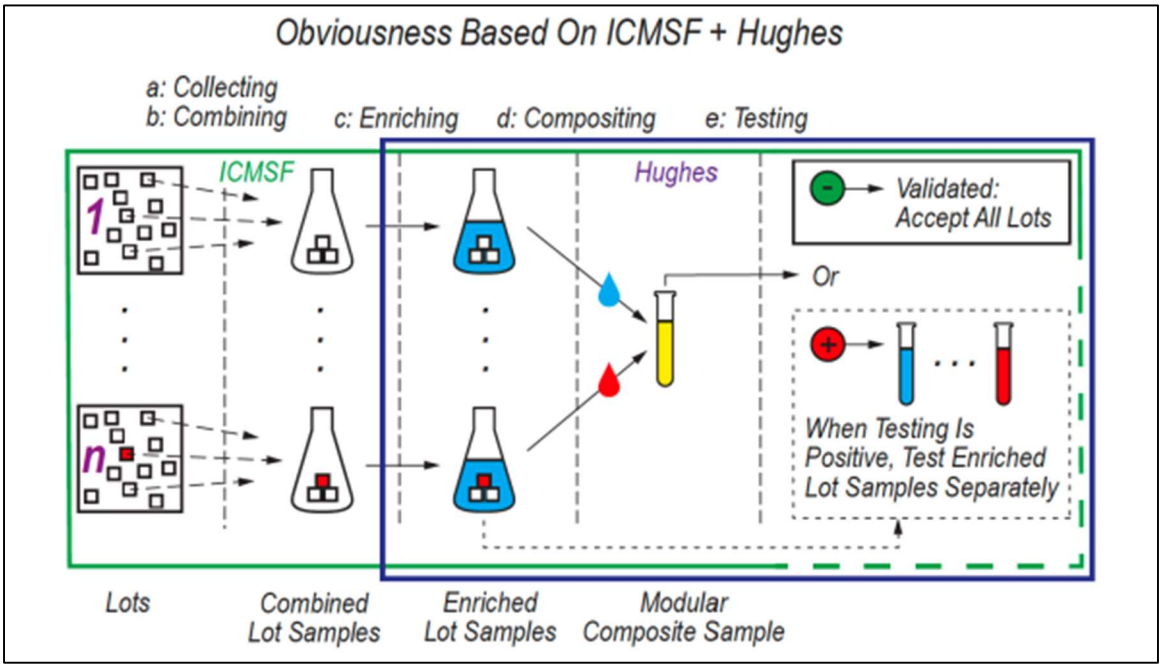
5) Obviousness

Each prior art reference identified herein, either alone or in combination with other identified references, renders the Asserted Claims invalid as obvious. In addition, each identified reference in the claim charts may be combined with: (1) information known to persons skilled in the art at the time of the alleged invention, and/or (2) any other reference identified herein. As detailed below and in the claim charts, the combination of elements and all the subject matter of the Asserted Claims were well-known in the food safety industry long before the earliest priority date of the '771 Patent. Therefore, the Asserted Claims are invalid.

To the extent it would have been necessary to combine prior art references, and to the extent the motivation to combine those references is necessary, it would have been found in the knowledge of one skilled in the art, as well as the nature of the problem to be solved. The motivation to combine references can also be found in the microbiological testing fields, and other similar fields, including in the prior art identified herein and as a matter of common sense and routine methods. Therefore, such references would have been highly relevant to people of ordinary skill.

For example, ICMSF, in combination with Gombas or Hughes, as disclosed below:





Therefore, to the extent the Asserted Claims are not anticipated, they would have been obvious to one of ordinary skill in the art at the relevant time

6) 35 U.S.C. § 101

National Beef contends that the Asserted Claims of the '143 Patent are invalid under 35 U.S.C. § 101 because the claims are not directed to patentable subject matter. A claim is patent-eligible if it recites a "new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof." 35 U.S.C. § 101. In *Alice*, the Supreme Court reaffirmed the two-step framework used to "distinguish[] patents that claim laws of nature, natural phenomena, and abstract ideas from those that claim patent-eligible applications of those concepts." *Alice Corp. Pty., Ltd. v. CLS Bank Int'l*, 573 U.S. 208, 217 (2014). This framework requires determining (1) if the claim is directed to ineligible subject matter such as an abstract idea, and, if so, (2) whether the remainder of the claim recites sufficient inventive elements that amount to "significantly more" than the abstract idea identified in Step 1. *Id*. The Asserted Claims are invalid under Section 101 because the claims are directed to nothing more than a basic concept of sampling, testing and validating test lots.

"Operationally-linked" appears in claims 2 and 3 of the '771 Patent. This so-called improvement on the prior art is nothing more than a basic human organizational concept and abstract idea which involves grouping together related production samples and tests to make the groups easier to track or to perform certain testing methods. There is nothing new or useful about operationally-linked methods of sampling, testing, and validating test lots, as it is a natural way in which to organize similar testing groups. Applying *Alice* step two, none of what remains recites anything inventive or new. All of the steps for wet pooling and compositing portions obtained from test lots are all well-understood, routine, and conventional.

National Beef's investigation regarding the invalidity of the Asserted Claims under 35 U.S.C. § 101 is ongoing. National Beef reserves the right to supplement or amend its invalidity

contentions under 35 U.S.C. § 101 as the case progresses and in light of further investigation and discovery, claim construction proceedings, and/or review and analysis of expert witnesses. National Beef will disclose expert testimony in accordance with the applicable case schedule.

7) 35 U.S.C. § 112

National Beef identifies the following Asserted Claims that are — at least as apparently construed by IEH as the basis for its infringement contentions — invalid pursuant to 35 U.S.C. § 112 as lacking a sufficient written description, or indefinite.

Limitation	Claim(s) where limitation appears
"independent"/ "multiple independent samples"	1, 18-20, 37
"operationally-linked" / "operationally-linked … specimen"	2, 3
"suitable"	1, 6, 8, 27, 37
"lot" / "single production lot" / "lot sample" / "lot group" / "test lot"	1, 2, 3, 6, 10, 15-20, 22, 26, 28, 37, 38, 41
"separate"	1, 2, 3, 6, 10, 16-20, 22, 26, 28, 37-38, 41
"target microbe(s)" / "non-target microbe"	1, 6-8, 11, 23-25, 37, 48-49
"portion(s)"	1, 4, 6, 22, 26, 38
"particular corresponding separate lot"	1
"modular composite sample"	1, 5, 6, 8, 10-11, 17, 27
"more sensitive protocol"	10, 11
"production assemblage"	15

A more detailed basis for National Beef's written description and/or indefiniteness defenses will be set forth in National Beef's claim construction briefs or expert reports on invalidity, to be served in accordance with the parties' Proposed Scheduling Order (D.I. 24-1) or in accordance with any such scheduling order as may later be entered by the Court. National Beef specifically reserves the right to amend and/or supplement these Contentions based on a failure to comply with the requirements of 35 U.S.C. § 112.

D. The '486 Patent

1) Priority Date

The '486 Patent was filed on January 17, 2009, and is a continuation of Application No. 10/848,253 filed on May 17, 2004, and issued as U.S. Patent No. 7,531,163, which claims priority to Provisional Application No. 60/470,975 filed May 16, 2003. The priority date of the patent is pre-AIA 35 U.S.C. §§ 101 et seq.

2) Identification of Prior Art

National Beef identifies the following prior art references that anticipate and/or render obvious one or more of the Asserted Claims of the '486 Patent under 35 U.S.C. §§ 102 and/or 103. The invalidity claim charts are attached hereto as Exhibits D1 – D6.

- M. K. Rayman et al., *ICMSF methods studies. XIII. An international comparative study of the MPN procedure and the Anderson–Baird-Parker direct plating method for the enumeration of Escherichia coli biotype I in raw meats*, 25 CAN. J. OF MICROBIOLOGY 1321 (1979);
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- Applicant Admitted Prior Art.

3) Anticipation

As set forth in the accompanying claim charts, Exhibits D5 and D6, Asserted Claim 1 of the '486 Patent is anticipated under 35 U.S.C. § 102 (including Sections 102(a), 102(b), 102(e), and/or 102(g)) by Rayman and Anderson. The prior art's associated claim chart sets forth where in the prior art reference each element of the Asserted Claims are found. To the extent that arguments or contentions within the claim charts conflict with, or are incompatible with, arguments or contentions that have been incorporated by reference, they are to be considered in the alternative.

4) Obviousness

Each prior art reference identified herein, either alone or in combination with other identified references, renders the Asserted Claims invalid as obvious. In addition, each identified reference in the claim charts may be combined with: (1) information known to persons skilled in the art at the time of the alleged invention, and/or (2) any other reference identified herein. As detailed below and in the claim charts, the combination of elements and all the subject matter of the Asserted Claims were well-known in the food safety industry long before the earliest priority date of the '486 Patent. Therefore, the Asserted Claims are invalid.

To the extent it would have been necessary to combine prior art references, and to the extent the motivation to combine those references is necessary, it would have been found in the knowledge of one skilled in the art, as well as the nature of the problem to be solved. The motivation to combine references can also be found in the pathogen testing fields, and other similar fields, including in the prior art identified herein and as a matter of common sense and routine methods. Therefore, such references would have been highly relevant to people of ordinary skill.

Therefore, to the extent the Asserted Claims are not anticipated, they would have been obvious to one of ordinary skill in the art at the relevant time

5) 35 U.S.C. § 112

National Beef identifies the following Asserted Claims that are — at least as apparently construed by IEH as the basis for its infringement contentions — invalid pursuant to 35 U.S.C. § 112 as lacking a sufficient written description, or indefinite.

Limitation	Claim(s) where limitation appears
"diluting . . . between about 1.01 and 1:5 (wt./vol.)"	1
"diluting . . . between about 1.01 and 1:0.5 (wt./vol.)"	2
"diluting . . . between about 1.01 and 1:0.3 (wt./vol.)"	3
"suitable"	1, 6
"or lesser dilution"	1
"particular"	1, 4, 6-8, 19
"test sample"	1
"standard lot-unit sample"	9
"portion"	1
"optimal temperature" / "optimal growth"	1, 4, 5
"and combinations thereof"	6, 7, 19
"enrichment-detection protocols"	17

A more detailed basis for National Beef's written description and/or indefiniteness defenses will be set forth in National Beef's claim construction briefs or expert reports on invalidity, to be served in accordance with the parties' Proposed Scheduling Order (D.I. 24-1) or in accordance with any such scheduling order as may later be entered by the Court. National Beef specifically reserves the right to amend and/or supplement these Contentions based on a failure to comply with the requirements of 35 U.S.C. § 112.

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CERTIFICATE OF SERVICE

I hereby certify that on October 3, 2024, true and correct copies of the foregoing document were served, via e-mail, on the following:

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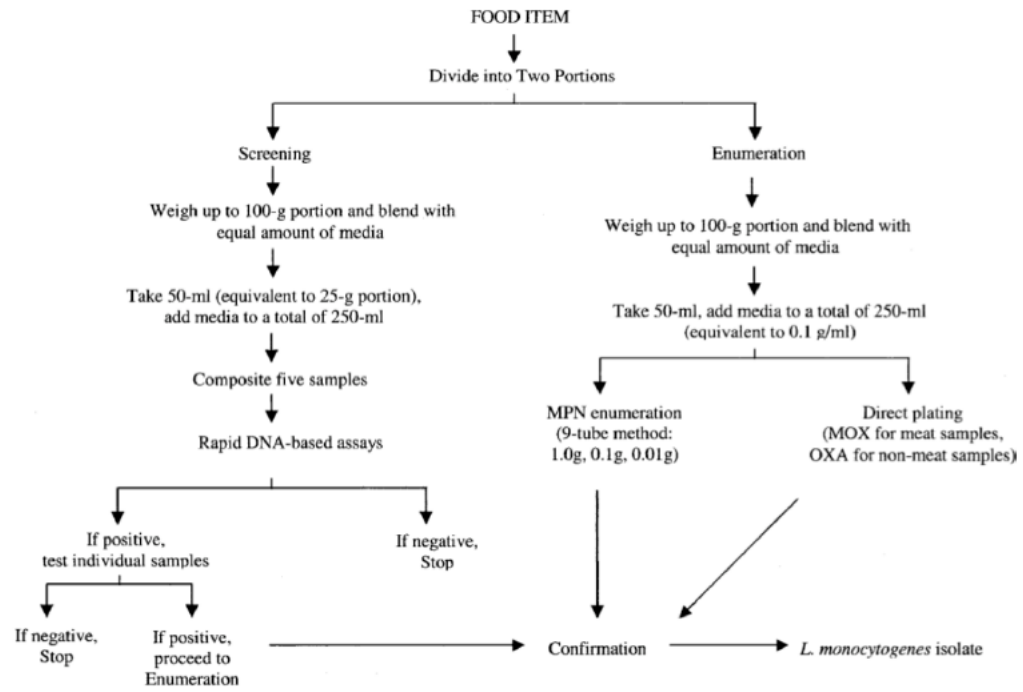
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EXHIBIT A1

Invalidity of U.S. Patent No. 7,534,584 in light of ICMSF & Gombas

National Beef denies that any of its microbiological testing methods infringe any claim of U.S. Patent No. 7,534,584 ("the '584 Patent"). If the claims of the '584 Patent were construed so broadly as to cover the microbiological testing methods used by National Beef, they would be invalid at least in view of INT'L COMM'N ON MICROBIOLOGICAL SPEC'S FOR FOODS (ICMSF), MICROORGANISMS IN FOODS 7: MICROBIOLOGICAL TESTING IN FOOD SAFETY MANAGEMENT (Kluwer Academic/Plenum Publishers, 2002) ("ICMSF") and David Gombas et al., *Survey of Listeria monocytogenes in Ready-to-Eat Foods*, 66 J. FOOD PROTECTION 559–569 (2003) ("Gombas"), which anticipates and/or discloses the limitations of the '584 Patent under at least pre-AIA 35 U.S.C. § 102(a), pre-AIA 35 U.S.C. § 102(b), pre-AIA 35 U.S.C. §§ 102(f)/(g), and/or pre-AIA 35 U.S.C. § 103. To the extent that ICMSF and/or Gombas do not expressly disclose one or more limitations of the asserted claims, as they would have to be construed to support IEH's claim of infringement, they discloses the limitations alone or in combination with one or more additional prior art references and/or the knowledge of one of ordinary skill in the art.

Claim 1	**Disclosure of ICMSF and Gombas**
[1pre] A method of sampling, testing and validating a plurality of operationally-linked test lots of beef for release into commerce, comprising:	To the extent the preamble is limiting, as discussed herein, ICMSF, alone or in combination with Gombas, discloses a method of sampling, testing and validating a plurality of operationally-linked test lots of beef for release into commerce. ICMSF includes many other references to testing lots of beef or poultry, two of the most common types of meat in commerce. ICMSF, 114 (§ 6.3), 184–191 (§§ 10.2–10.6), 225–226 (§ 12.1), 285 (§ 16.1), 313–330 (Chapter 17). ICMSF is relevant to any kind of food product including beef or poultry. Gombas also discloses testing and validating a plurality of operationally-linked test lots of beef for release into commerce. Gombas describes sampling from ready to eat ("RTE") products: All products were to be sliced (either presliced by manufacturers or sliced by the retail stores)" including beef bologna and "products made from pork or poultry." Gombas, 562.

	 FIGURE 1. *Detection and enumeration of* L. monocytogenes *in RTE food samples.* Gombas, 563 (Figure 1).
[1a] a) collecting a plurality of portions from each of a plurality of separate operationally-linked test lots of beef, each to be validated for release into commerce, the test lots each comprising an assemblage of one or more specimens from which the portions are collected, wherein each test lot is separately sampled to conform to a statistical-based sampling plan specifying, for a particular microbial agent or organism, a number of	ICMSF, alone or in combination with Gombas, discloses collecting a plurality of portions from each of a plurality of separate operationally-linked test lots of beef, each to be validated for release into commerce, the test lots each comprising an assemblage of one or more specimens from which the portions are collected, wherein each test lot is separately sampled to conform to a statistical-based sampling plan specifying, for a particular microbial agent or organism, a number of portions to be taken sufficient to provide for validation and release of the test lot into commerce. "Many different types of samples are collected and submitted to the laboratory for analysis. Some are sample units from lots or consignments of food or ingredients for

portions to be taken sufficient to provide for validation and release of the test lot into commerce;	lot acceptance determination. . . . A sample unit is a small portion of the lot in which the distribution of both numbers and types of microorganisms should be representative of the whole lot." ICMSF, 255. The lots may be beef or poultry. *Id.*, 184-91, 226, 230. Thus, ICMSF discloses collecting a plurality of samples or sample units (other synonyms include portions, specimens, and sub-samples) from lots of beef or poultry. ICMSF describes collecting from a lot or lots: "If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." *Id.*, 119. Additionally, ICMSF indicates a consignment may be "a very large quantity of food" and may be "composed of several lots representing, for example, different days of production from the same plant[.]" *Id.*, 119-120; *see also* AAPA #3-#5.

6.11 WHAT IS A REPRESENTATIVE SAMPLE?

A *representative sample* reflects, as far as is possible, the composition of the lot from which it is drawn.

How then should a representative sample be drawn? It is important to avoid bias and to draw a sufficient number of sample units to confidently make a judgment about a lot. Sampling at random is the universally recognized way of avoiding bias and is more reliable than consciously trying to draw the sample units from the various parts of a lot. The units (cartons or containers, particular weights of solid, or volumes of liquid) are drawn by using random numbers. There is, of course, no guarantee that a random sample has characteristics identical with those of the lot, but the randomness of sampling is the basis for calculation of the probability that a sample will give a biased result.

It is also possible, and may be desirable, to use a *stratified random sampling* approach, drawing a random sample of a given number of sample units from each *stratum* (e.g., each sublot or carton). The distribution of sample units on strata should correspond to the distribution of lot units on strata. This means that if each stratum contains the same quantity as every other one, the number of sample units per stratum should be the same. Otherwise, they should differ just as the proportions of the lot contained in the strata differ. Stratification is a device for handling known sources of variation and may be used where one has prior knowledge that the consignment is potentially not of uniform quality. A consignment might not be of uniform quality if portions of it are shipped in different vehicles or in different holds of a vessel, or if it is known that it is really composed of several lots representing, for example, different days of production from the same plant or different plants of the same company. The results for different strata should be assessed separately and then pooled if they appear to be homogeneous.

The calculation of acceptance probabilities is based on the assumption that random samples have been drawn and analyzed. Where physical or practical constraints prevent random sampling, such calculated probabilities may not be valid. Their reliability depends on how close reality approaches the ideal situation of random sampling. This fact should be borne in mind when subsequently interpreting results.

ICMSF, 119-20 (annotated).

6.10 WHAT IS A LOT?

Ideally, a lot is a quantity of food or food units produced and handled under uniform conditions. Under certain circumstances, it has been shown that the logarithms of the counts from a batch of food are likely to be normally distributed (Baird-Parker, 1980; Kilsby & Baird-Parker, 1983 [their Table 2]; Ingram & Roberts, 1976). The implication is that there is homogeneity within a lot, which, when considering microbial levels and distributions, rarely occurs in practice. In most instances, the distribution of microorganisms within lots of food is heterogeneous. Even in food factories, this heterogeneity makes the interpretation of sample results difficult, and it becomes even more so in circumstances where a lot is less well defined, such as in a consignment of a very large quantity of food.

It is therefore helpful if suppliers can be persuaded to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load).

If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment.

A lot should be composed of food produced with as little variation as possible for a given process or commodity. Because of the uncertainties in identifying a lot commercially, however, the use of the word *lot* in this book is usually in its statistical sense, as a collection of units of a product, the acceptability of which is determined by examining a sample drawn from it.

ICMSF, 118-19 (annotated).

	The ICMSF has defined a lot as follows: In the commercial sense, a lot is a quantity of food supposedly produced under identical conditions, all packages of which would normally bear a lot number that identifies the production during a particular time interval, and usually from a particular line, retort, or other critical processing unit. Statistically, a lot is considered as a collection of units of a product from which a sample is drawn to determine acceptability of the lot (ICMSF, 1986). In this example, the maximum size of a lot consists of all the ground beef produced on common equipment from cleanup to cleanup. Smaller lots could be established (e.g., hourly). ICMSF, 327 (annotated). This definition also reflects the common practice referred to in the '584 Patent, wherein "[p]referably, the producer defines and produces these single-unit lots in such a fashion as to maximize the information available[.]" '584 Patent, 12:46–48 (Example II). This corresponds with the specification of the '584 Patent which reflects the common practice of producers defining what constitutes a lot grouped for traceability purposes. '584 Patent, 2:60-3:3, 11:12-16, and 12:46-49. Thus, ICMSF discloses collecting a plurality of portions from separate lots. ICMSF describes a lot as "a collection of units of a product." IMCSF, 119. ICMSF further indicates a lot has been defined as: "a quantity of food supposedly produced under identical conditions, all packages of which would normally bear a lot number that identifies the production during a particular time interval, and usually from a particular line, retort, or other critical processing unit. Statistically, a lot is considered as a collection of units of a product from which a sample is drawn to determine acceptability of the lot[.]" ICMSF, 327. ICMSF advocates use of a "statistically based sampling plan." *Id.*, 113-15, 119-21, 320, 327-28. In the sampling plans, "*n* is the number of sample units chosen separately and independently, and . . . *c* is the maximum allowable number of sample

	units yielding a positive result." *Id.*, 115. ICMSF discloses a wide range for the number of sample units (portions) for a particular microbial agent or organism. For example, ICMSF discusses collecting 15, 30, 60 or 100 sample units from a lot. *Id.*, 320, 326. **6.5 THE SAMPLING PLAN** The sample units so drawn will, after examination, yield results that will be compared with certain criteria to reach a decision as to whether the entire lot should be accepted or rejected (see section 6.9 for explanation of rejection). The particular choice of sampling procedure and the decision criteria make up the *sampling plan*. Proper drawing of samples is essential if the decision making criteria of the sampling plan are to give unbiased results. By sampling randomly, we are able to reduce the risk of making biased decisions. A simple example of a sampling plan follows. Take 10 sample units of a food from a lot and subject them to appropriate laboratory procedure to test for the presence or absence of a microorganism. If two or fewer of the 10 sample units show presence of the organism, that is, give a positive result, then the whole lot of food is acceptable (relative to this organism). But if three or more give a positive result, the whole lot is to be rejected. This plan is described by the use of two terms $n = 10$ and $c = 2$, where n is the number of sample units chosen separately and independently, and where c is the maximum allowable number of sample units yielding a positive result. ICMSF, 115 (annotated).

	6.1 INTRODUCTION It is important to recognize that the management of food safety using the approaches outlined in the first five chapters, based on controlling hazards through Good Hygienic Practices (GHP) and Hazard Analysis Critical Control Point (HACCP), is much more effective than trying to ensure safety by lot acceptance through end-product testing. This chapter discusses the concepts of probability and sampling that determine the limitations of end-product testing. Those concepts form the basis of the design of statistically based sampling plans (Chapter 7) and the establishment of cases (Chapter 8). In Chapters 6 and 7, it is assumed that the microbiological test will give an absolutely accurate result, with no false positives or false negatives. This is not a realistic assumption, and problems arising from sampling, sample transport and preparation for analysis, and the imprecision of microbiological methods are addressed in Chapters 10 and 12 and elsewhere in the text. ICMSF, 113 (annotated); *see also id.*, 115–21 (§§ 6.4–6.13), 319–20 (§ 17.2.3.3), and 326–30 (§§ 17.5.3.1–17.6). ICMSF discusses the number of portions to be taken sufficient for validation of a particular microorganism including *Salmonella* (*id.*, 276 (§ 15.3.3); *see also id.*, 282 (§ 15.6.3); *Listeria* (*id.*, 309 (§ 16.6.3)), and *E. coli* (*id.*, 318–21 (§§ 17.2.3.2–17.2.3.4), 326 (§ 17.5.3.1)). IEH's AAPA discloses statistical-based sampling plans taking 10-60 sample units. '584 Patent, 5:57-6:6 (AAPA #1, #2, #5, and #6). Determining meat is safe for consumption so it is "commercially possible to market" the meat, is the well-understood point of testing meat and is taught by ICMSF. ICMSF, 185-89. Moreover, the recitation of a lot being validated for "release . . . into commerce" is not limiting. Prosecution History for Reexamination Control No. 95/011,294. "Operationally-linked" is simply grouping like items, which is also disclosed by ICMSF. "Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole

	consignment." ICMSF, 119. It is therefore helpful if suppliers can be persuaded to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load). If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment. *Id.* (annotated).
[1b] b) combining the collected conforming plurality of portions corresponding to each of the separate test lots to provide a corresponding set of separate test lot samples, wherein each separate test lot sample is attributed to a particular corresponding separate test lot;	ICMSF, alone or in combination with Gombas, discloses combining the collected conforming plurality of portions corresponding to each of the separate test lots to provide a corresponding set of separate test lot samples, wherein each separate test lot sample is attributed to a particular corresponding separate test lot. ICMSF states: "To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units of the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. . . . This finding suggests that considerable reductions may be made in the cost of laboratory analysis by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, n, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort." ICMSF, 187.

	To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. A laboratory investigation undertaken for ICMSF (Silliker & Gabis, 1973) indicated that different sized analytical units drawn from a single well-mixed sample unit of dried food give very similar results. A subsequent investigation (Gabis & Silliker, 1974) using foods of high moisture content (including eggs, poultry meat, meat, and meat products) showed comparable reliability in the use of composite analytical units; for example, examination of three composites of 20 × 25 g analytical units gave the same result as examination of all 60 analytical units individually (the total amount examined being 1500 g in both instances). This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n*, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort. *Id*.

10.5.1 Conceivable Alternative Plans

As indicated above, for an infectious agent such as *Salmonella*, there are strong reasons for adopting sampling plans in which $c = 0$:

1. It is philosophically objectionable to some people to accept a lot containing a recognized pathogen, regardless of how the food is to be used in a manufacturing process or by the consumer.
2. There is the very great technical advantage of combining many analytical units for bacteriological examination in systems with $c = 0$, because a single positive decides the outcome.
3. The plan with $c = 1$ (or more) would always require the examination of (many) more analytical units, for equal probability of acceptance, than a plan with $c = 0$.
4. Even if the indicated probabilities of plans with $c = 0$ versus $c = 1$ are the same, their implications are not. For example, when a lot is accepted with the plan $n = 60$, $c = 0$, it is possible (though not certain) that the lot may not be contaminated at all. But using the plan $n = 95$, $c = 1$ and accepting one positive, it is known that the lot certainly is contaminated (though the probability that a lot with 5% of analytical units contaminated would be accepted is in both instances $P_a = 0.05$).

Id., 188 (annotated). ICMSF also provides an example of "[u]sing a sampling plan involving n = 15 (25 g analytical units) and a composite of 375 g." *Id.*, 195 (§ 10.7.2). ICMSF also states "[f]or qualitative analyses, 25 g analytical units are frequently analyzed individually or as composites of 100 g, 200 g, or more." *Id.*, 232 (§ 12.5.2).

The ICMSF incorporates two references, Silliker & Gabis (1973) and Gabis & Silliker (1974). Silliker notes that "[c]ompositing of multiple samples (lumping) and wet compositing of pre-enrichment cultures have been tested as economical approaches to *Salmonella* control." Silliker, 475.

Silliker further notes that "[b]oth approaches are being used widely by the food industry in the United States." *Id.* Gabis discloses application of the Silliker teachings to testing meat.

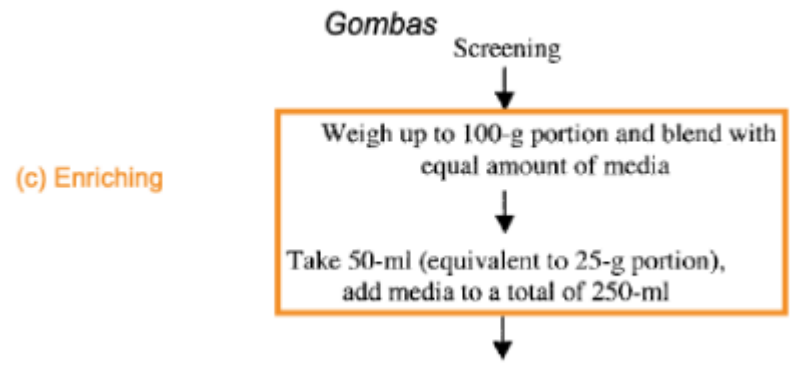
	To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. A laboratory investigation undertaken for ICMSF (Silliker & Gabis, 1973) indicated that different sized analytical units drawn from a single well-mixed sample unit of dried food give very similar results. A subsequent investigation (Gabis & Silliker, 1974) using foods of high moisture content (including eggs, poultry meat, meat, and meat products) showed comparable reliability in the use of composite analytical units; for example, examination of three composites of 20 × 25 g analytical units gave the same result as examination of all 60 analytical units individually (the total amount examined being 1500 g in both instances). This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n*, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort. ICMSF, 187. "There is the very great technical advantage of combining many analytical units for bacteriological examination." *Id*., 188. ICMSF also describes composites prepared by combining "15 (25 g analytical units)" and "composites of 100 g, 200 g, or more." *Id.,* 195, 232; *see also* AAPA #5. ICMSF states, "All steps up to the analysis must be controlled . . . to allow reliable traceability." ICMSF, 227. ICMSF indicates lot samples are traceable to a particular corresponding lot: "It is therefore helpful . . . to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a

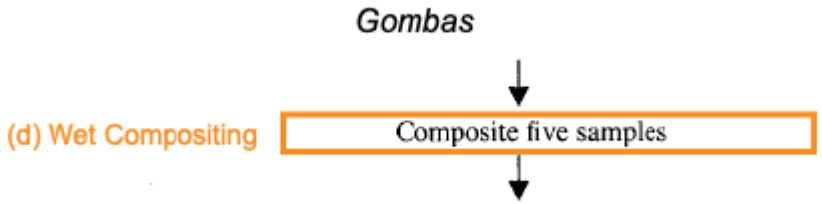
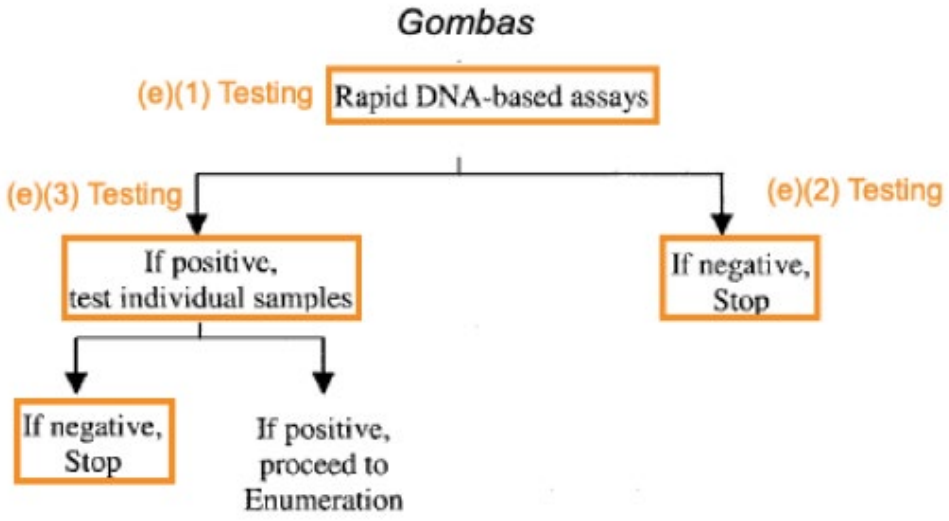
	batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load). . . . Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119. Gombas also discloses this element. Gombas states "samples were assigned codes" and product information was recorded for the luncheon meats including "sampling location." Gombas, 563. Gombas inherently requires tracking the individual samples that contributed to the composite. *See, e.g.*, Gombas, 564 ("If a composite tested positive, samples in the composite were tested individually by Gene-Trak assay with the PBS from the second mLCA plate or by the BAX assay with the secondary enrichment broths. Individual samples that tested negative were not tested further If an individual sample tested positive, the retained portion was subjected to an enumeration assay.") Thus, Gombas also discloses that each of the separate composited lot samples is attributed to a particular corresponding separate lot.
[1c] c) incubating the set of separate test lot samples under conditions suitable to allow levels of a target microbial agent or organism that is present in one or more of the separate test lot samples to reach detectable levels and become uniform, or substantially uniform, throughout the respective one or more separate test lot samples, to provide a set of separate test lot samples enriched for the target microbial agent or organism;	ICMSF, alone or in combination with Gombas, discloses incubating the set of separate test lot samples under conditions suitable to allow levels of a target microbial agent or organism that is present in one or more of the separate test lot samples to reach detectable levels and become uniform, or substantially uniform, throughout the respective one or more separate test lot samples, to provide a set of separate test lot samples enriched for the target microbial agent or organism. ICMSF states: "To reduce laboratory effort . . . it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment." ICMSF, 187. Also, ICMSF describes preparing composites of analytical units then diluting "for qualitative (enrichment)" analysis. *Id.,* 231-32; *see also* AAPA #5, #7.

	For practical reasons, however, analytical units of 10 g up to 50 g are typical for quantitative methods and are diluted 1:10. For qualitative analyses, 25 g analytical units are frequently analyzed individually or as composites of 100 g, 200 g, or more. ICMSF, 232 (annotated). Preparation of the first dilution, either for qualitative (enrichment) or quantitative (direct) analyses, can have a marked impact on the microbial population in the sample. This is often due to changes in the physico-chemical characteristics of the suspension as compared to the food matrix. Where the food does not confer protection, the composition of the diluent is important to prevent or minimize shock/injury to the microorganisms that can result from rapid changes in the ionic concentrations. ICMSF, 232 (annotated). ICMSF also describes strategic use of non-selective enrichment to resuscitate microorganisms and selective enrichments for growing microorganisms. *Id.*, 232–33 (§ 12.6); *see e.g.*, *id.,* 233 ("use of nonselective medium . . . to enhance recovery of injured or stressed cells."). ICMSF § 12.6 also describes optimizing the incubation period. S*ee e.g.*, *id.,* 232. It was well known long before the priority date to use non-selective enrichments after dry compositing and prior to wet compositing as evidenced by Price, Silliker, Gabis, and MLG. A POSITA would also know to use selective enrichments after dry compositing and prior to wet compositing as disclosed by ICMSF and Gombas. A POSITA would find that incubating so microorganisms are detectable and at least substantially uniform in the selective enrichment broth was considered the standard practice in official methods because the methods otherwise fail. For example, D'Aoust states "standard methodology recommends incubation of selective enrichment broths." D'Aoust, 372. Gombas also discloses incubating "approximately half (up to 100 g) of a sample was aseptically transferred to a sterile stomacher bag and blended with an equal amount of enrichment broth. . . . For northern California samples, demi-Fraser broth was

	used as the enrichment broth for all products. After blending, 50 g of the homogenate was added to 200 ml of enrichment broth and stomached for 1 min. This procedure resulted in a detection sensitivity equivalent to that of the current regulatory methods (i.e., 1 CFU/25 g). The initial sample-blending step was performed to account for the potential heterogeneous distribution of *L. monocytogenes* in the sample. The 250-ml enrichment was incubated at 35°C for 24 ± 2 h." Gombas, 563; *see also id.,* 564. Gombas further indicates that: "For each California sample, 0.1 ml of the demi-Fraser enrichment culture was transferred to 10 ml of morpholinepropanesulfonic acid-*Listeria* enrichment broth and incubated at 35°C for 20 to 24 h. After the secondary enrichment, 1 ml of the broth culture was used for screening with the BAX assay as described below." *Id.,* 564. Gombas avoids potential heterogenous distribution and ensures the target microorganism is at least substantially uniform in a sample by blending the sample and the enrichment broth in the stomacher bag to yield a homogenate. *Id.,* 563.

	Sample screening. Each sample was divided into two portions for screening and enumeration. For screening, approximately half (up to 100 g) of a sample was aseptically transferred to a sterile stomacher bag and blended with an equal amount of enrichment broth. For Maryland samples, University of Vermont broth 2 (UVM-2 broth) was used as the enrichment broth for luncheon meats, smoked seafood, and seafood salads; phosphate-buffered *Listeria* enrichment broth was used for the other product types. For northern California samples, demi-Fraser broth was used as the enrichment broth for all products. After blending, 50 g of the homogenate was added to 200 ml of enrichment broth and stomached for 1 min. This procedure resulted in a detection sensitivity equivalent to that of the current regulatory methods (i.e., 1 CFU/25 g). The initial sample-blending step was performed to account for the potential heterogeneous distribution of *L. monocytogenes* in the sample. The 250-ml enrichment was incubated at 35°C for 24 ± 2 h. Gombas, 563 (annotated). A POSITA would find that Gombas utilized conventional steps to avoid a "potential heterogeneous distribution." Gombas, 563. A POSITA would also find that blending a sample in a stomacher bag as done by Gombas enables vigorous mixing of the sample and the enrichment broth and achieves a homogenous mixture referred to as a homogenate. *Id.* Gombas further ensures that detectable levels and uniformity are achieved because "[a]fter blending, 50 g of the homogenate was added to 200 ml of enrichment broth and stomached for 1 min" and "[t]he 250-ml enrichment was incubated at 35°C for 24 ± 2 h." *Id.* Achievement of detectable levels and uniformity is also evidenced by Gombas concluding the "procedure resulted in a detection sensitivity equivalent to that of current regulatory methods." *Id.*

	 Gombas, 563 (Figure 1 (annotated) showing an enriching step).
[1d] d) removing portions of each enriched separate test lot sample, and combining the removed portions to provide a modular composite sample; and	ICMSF describes compositing the enriched lot samples: "it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. . . . This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n,* with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort." ICMSF, 187. *See also* AAPA #5-#7. Additionally, Gombas discloses wet compositing of enriched samples: "Up to five like-product samples were composited and screened. . . . For California samples, five 1-ml samples of secondary enrichment broths were composited." Gombas, 564. Gombas's Figure 1 depicts wet compositing as shown in the excerpt below:

	 Gombas, 563 (Figure 1 (annotated) showing a wet compositing step).
[1e.1] e) testing of the modular composite sample, using a suitable detection assay, for the target microbial agent or organism,	ICMSF describes testing enrichment cultures of composite samples with suitable detection assays and "presence/absence tests". ICMSF 187, 233. Suitable detection assays were established in Silliker and Gabis as discussed in ICMSF. Gombas provides a complete framework for testing, and a POSITA would have been motivated to modify ICMSF based on Gombas's framework:  Gombas, 563 (Figure 1 (annotated) showing the testing step).

	Gombas also discloses a suitable detection assay: "For California samples, . . . the 5-ml composite was tested by the BAX assay according to the manufacturer's procedures." Gombas, 564. These assay types were "art recognized." '584 Patent, 2:54-58 (AAPA #7).
[1e.2] wherein when such testing is negative all of said separate test lots are validated for release into commerce, and	ICMSF describes correlating lots in a consignment with lot samples so a negative or positive test result enables a lot to be validated or rejected. ICMSF, 118-19. ICMSF describes sampling plans in which "c is the maximum allowable number of sample units yielding a positive result." ICMSF, 115. For many scenarios, the requirement is c = 0, which indicates the testing is negative. *Id.*, 118, 183-90, 309, 325, 326. A negative test result (*id.*, 113-15, 325, 326) is the expected and desired outcome to determine meat is not contaminated and is safe for consumption (*id.*, 184-89) such that a lot is validated and it is "commercially possible to market" (*id.*, 185) the meat.

6.10 WHAT IS A LOT?

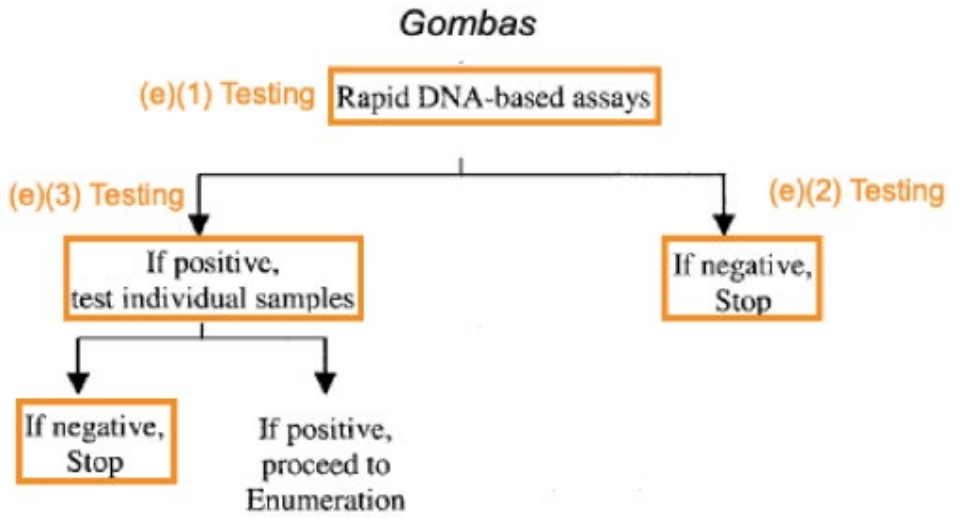
Ideally, a lot is a quantity of food or food units produced and handled under uniform conditions. Under certain circumstances, it has been shown that the logarithms of the counts from a batch of food are likely to be normally distributed (Baird-Parker, 1980; Kilsby & Baird-Parker, 1983 [their Table 2]; Ingram & Roberts, 1976). The implication is that there is homogeneity within a lot, which, when considering microbial levels and distributions, rarely occurs in practice. In most instances, the distribution of microorganisms within lots of food is heterogeneous. Even in food factories, this heterogeneity makes the interpretation of sample results difficult, and it becomes even more so in circumstances where a lot is less well defined, such as in a consignment of a very large quantity of food.

It is therefore helpful if suppliers can be persuaded to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load).

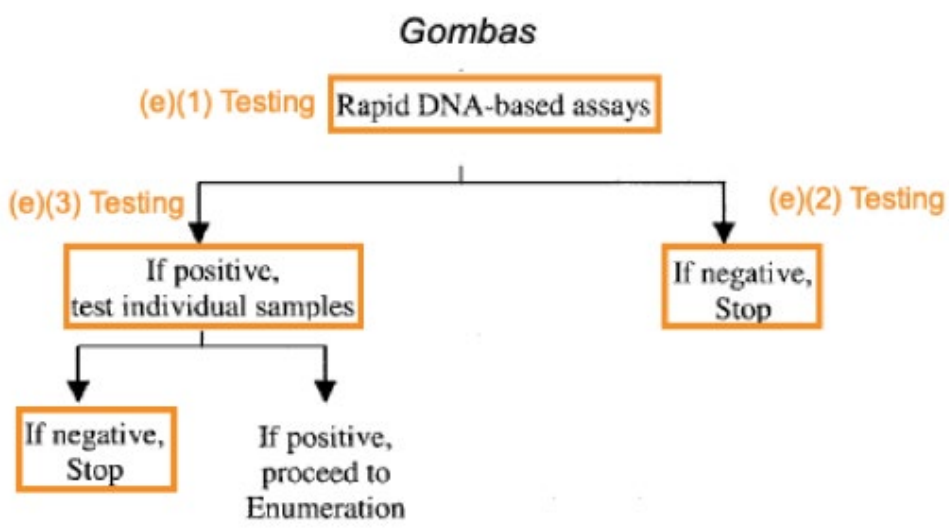
If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment.

A lot should be composed of food produced with as little variation as possible for a given process or commodity. Because of the uncertainties in identifying a lot commercially, however, the use of the word *lot* in this book is usually in its statistical sense, as a collection of units of a product, the acceptability of which is determined by examining a sample drawn from it.

ICMSF, 118-19 (annotated).

	To the extent ICMSF is considered not to disclose some aspect of step (e)(2), Gombas indicates a negative test result is the end of the process: "If a composite tested negative, all samples in the composite were considered negative (in 25 g) and were not tested further." Gombas, 564. In the combination, a POSITA would find that a negative test result equates to being validated.  Gombas, 563 (Figure 1 (annotated) showing "if negative, stop.").
[1e.3] wherein when such testing is positive, each of the individual separate test lots are validated for release into commerce, according to the statistical-based sampling plan, by further testing of a portion of the respective enriched separate test lot sample using the same or a more sensitive protocol	The combination of ICMSF and Gombas describes retaining a portion of the enriched lot samples (steps (b)-(d)). "Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119.

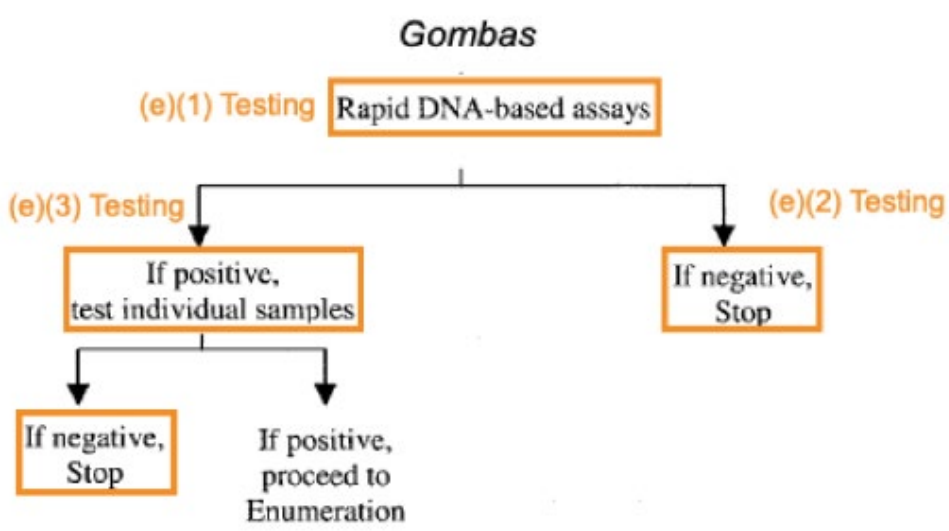
and obtaining a negative test result for each test lot,	"There is often a desire to retest retained samples." ICMSF, 191. If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment. ICMSF, 119 (annotated). ICMSF discloses validating for release into commerce. ICMSF, 113–15 (§§ 6.2–6.6), 118–19 (§§ 6.9–6.10), 184–85 (§ 10.2), 325–27 (§§ 17.5.3–17.5.3.4); *see also id.*, 186–89 (§ 10.4–10.6) according to a statistically-based sampling plan, 113 (§ 6.1), 115–18 (§§ 6.4–6.8), 184 (§ 10.2), 319–21 (§§ 17.2.3.3–17.2.3.4), 326–30 (§§ 17.5.3.1–17.6), as described regarding steps (a) and (e)(2). "If a composite tested positive, samples in the composite were tested individually . . . by the BAX assay with the secondary enrichment broths. Individual samples that tested negative were not tested further." Gombas, 564.



Gombas, 563 (Figure 1 (annotated) showing testing procedures for further testing).

"Individual samples that tested negative were not tested further. If an individual sample tested positive, the retained portion was subjected to an enumeration assay." Gombas, 564.

The combination teaches that after the composited samples test positive, then the individual samples used to form the composited sample are individually tested with the same protocol to determine which of the separate composited lot samples is positive for the target microbe(s).

[1e.4] wherein the enrichment in step c), prior to removing portions in step d), is sufficient to enable said validation of individual separate test lots with no requirement for further enrichment thereof, and with no requirement for further sampling in a).	 Gombas, 563 (Figure 1 (annotated) showing testing procedures for further testing). A POSITA would understand that ICMSF and Gombas disclose selective enrichment so there is no requirement for further enrichment. Gombas explicitly indicates portions of the enrichment cultures are retained for further testing; thus there is no need for further sampling in Gombas. "Individual samples that tested negative were not tested further. If an individual sample tested positive, the retained portion was subjected to an enumeration assay." Gombas, 564.
Claim 2	**Disclosure of ICMSF and Gombas**
The method of claim 1, wherein each of the test lots is a single-unit production lot, comprised of operationally-linked product specimens.	ICMSF, alone or in combination with Gombas, discloses the method of claim 1, upon which this claim relies. See above re Claim 1. ICMSF, alone or in combination with Gombas, further discloses wherein each of the test lots is a single-unit production lot, comprised of operationally-linked product specimens. The specification of the '584 Patent indicates a test lot is a single-unit production lot

	('584 Patent, 5:21-32) such as a single combo, bin. pallet, or segment of production ('584 Patent, 7:28-36 (AAPA #3 and #4). ICMSF teaches collecting from "individual production batches as lots" and "[a] lot is considered as a collection of units." ICMSF, 119, 327. "Operationally-linked" is simply grouping like items, which is also disclosed by ICMSF. "Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119.
Claim 3	**Disclosure of ICMSF, Gombas, and Stewart**
The method of claim 1, further comprising incubating the modular composite sample of d), prior to testing in e).	ICMSF, alone or in combination with Gombas, discloses the method of claim 1, upon which this claim relies. See above re Claim 1. ICMSF and Gombas, in combination with Stewart, discloses the method of claim 1, further comprising incubating the modular composite sample of d), prior to testing in e). Stewart discloses that after the selective enrichment in Rappaport-Vassiliadis (RV) broth and tetrathionate (TT) broths, the two selective enrichments were combined into a single sample for further incubation and analysis. "[P]ost-enrichment by transfer and combination of 0.5 mL RV and 1 mL TT into a single tube containing 10 mL prewarmed trypticase soy broth supplemented with novobiocin (TSB + n) at 10 µg/mL with incubation for 16–20 h at 42°C." Stewart, 398.
Claim 4	**Disclosure of ICMSF and Gombas**
The method of claim 1, wherein incubating each separate test lot sample in c) comprises incubating each separate test lot sample with enrichment medium suitable to enrich for the target microbial agent or organism, and wherein removing in d) comprises removing a	ICMSF, alone or in combination with Gombas, discloses the method of claim 1, upon which this claim relies. See above re Claim 1. ICMSF, alone or in combination with Gombas, further discloses wherein incubating each separate test lot sample in c) comprises incubating each separate test lot sample with enrichment medium suitable to enrich for the target microbial agent or organism, and wherein removing in d)

portion of the enrichment medium from each enriched separate test lot sample and combining the removed portions to provide a modular composite sample.	comprises removing a portion of the enrichment medium from each enriched separate test lot sample and combining the removed portions to provide a modular composite sample. ICMSF describes the well-known benefits of combining enrichment cultures from analytical units from lots in a consignment. To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. A laboratory investigation undertaken for ICMSF (Silliker & Gabis, 1973) indicated that different sized analytical units drawn from a single well-mixed sample unit of dried food give very similar results. A subsequent investigation (Gabis & Silliker, 1974) using foods of high moisture content (including eggs, poultry meat, meat, and meat products) showed comparable reliability in the use of composite analytical units; for example, examination of three composites of 20 × 25 g analytical units gave the same result as examination of all 60 analytical units individually (the total amount examined being 1500 g in both instances). This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n*, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort. ICMSF, 187. Gombas describes incubating an enriched test lot sample. “The 250-ml enrichment was incubated at 35°C for 24 ± 2 h.” Gombas, 563.
Claim 5	**Disclosure of ICMSF and Gombas**
The method of claim 1, wherein testing of the modular composite sample, using a suitable detection assay further comprises a	ICMSF, alone or in combination with Gombas, discloses the method of claim 1, upon which this claim relies. See above re Claim 1. ICMSF, alone or in combination with Gombas, further discloses wherein testing of the modular composite sample, using a suitable detection assay further comprises a concentration step to concentrate

concentration step to concentrate the microbial agent or organism.	the microbial agent or organism. ICMSF discloses the use of "immunomagnetic beads to concentrate cells." ICMSF, 318. This was a well-known concentration technique before the priority date. Fratamico discusses the rapid isolation of target microorganisms using an immunomagnetic separation method. Fratamico,105. Fratamico discloses that use of immunomagnetic beads resulted in "an increase in the level of *E. coli* B1409 in the sample," as well as "an increase in the number of organisms recovered." *Id.*, 107. Additionally, Fratamico provides detailed instructions for preparing and using immunomagnetic bead separation to concentrate a target microbial agent or organism. Fratamico developed "[a] method employing immunomagnetic beads" to concentrate and thereby improve the isolation and detection of E. coli. *Id.* For example, Fratamico was able to use immunomagnetic beads to isolate *E. coli* strains from enrichment cultures of raw ground beef "when the initial inoculum level was 1 cfu g-1." *Id.*, 109. Fratamico further teaches that "[t]he percentage of bacteria recovered increased with increasing amounts of beads." *Id.*, 108. Consequently, Fratamico "demonstrates that immunomagnetic separation [using immunomagnetic beads] is a simple and rapid technique" for concentrating a microbe for isolation and detection. *Id.*, 109. These teachings of Fratamico would have motivated a POSITA to modify the combination of ICMSF and Gombas and ICMSF and Hughes to use immunomagnetic beads to concentrate microbes in a modular composite sample to benefit from accurate tests. Use of immunomagnetic beads was already known in the art for concentrating during testing beef samples. "Tu et al. (78) detected pathogenic *E. coli* O157:H7 in beef hamburger with the use of IMS and ATP measurement. After 6 h of enrichment at 37°C, inoculated beef samples (0.9 ml each) were filtered to remove large particles prior to 10 min of incubation with ca. 10^5 magnetic beads coated with anti–*E. coli* O157:H7 (Dynabeads, Dynal Inc., Oslo, Norway). Following incubation, the

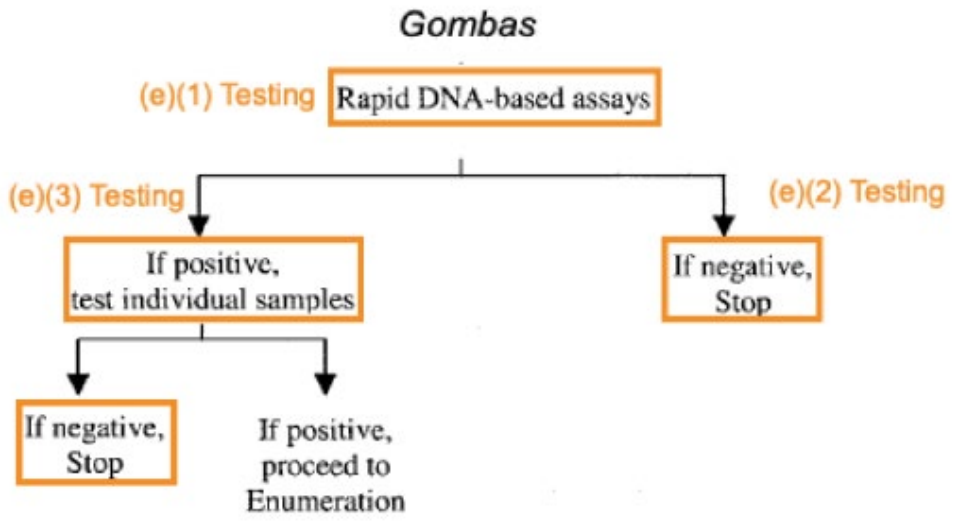
	magnetic beads were collected with a Dynal magnetic concentrator and resuspended in 0.9 ml of Trypticase soy broth for ATP analysis." Benoit, 1941. "Hudson et al. (26) used IMS to directly separate and concentrate *L. monocytogenes* from ham samples prior to detection by PCR. IMS was used on the basis of the commercial availability of anti-*Listeria* magnetic beads (Dynal Inc.) to avoid time-consuming cultural enrichment steps and to remove PCR inhibitors." Benoit, 1941-42. Cudjoe investigates the use of immunomagnetic beads in pooling methods. In a study designed to evaluate the performance of Dynabeads® Anti-Salmonella, immunomagnetic separation (IMS) followed by plating (IMS-Plating) proved far superior to the conventional ISO *Salmonella* methodology and the Modified Semi-solid Rappaport-Vassiliadis (MSRV) method. *Salmonella* species were isolated and detected from 135 out of the 180 diverse poultry samples by IMS analysis as against 98 by the conventional method and 33 by the MSRV technique. All results were confirmed biochemically and serologically. It appeared from the data generated that some of the salmonellae isolated using IMS were greatly inhibited in Rappaport-Vassiliadis enrichment broth at 42°C and to a lesser extent in selenite cystine broth at 37°C. A moderately selective plating medium like XLD proved to be better in isolating these possible sensitive wild-type strains of salmonellae than the more selective BGA. Cudjoe, Abstract.

	Samples. One hundred and eighty (180) diverse poultry samples, consisting of 40 pools of cloacal/faecal swabs (5 swabs/pool; 5 pools samples/flock) from eight different flocks, 10 raw chicken breast meat samples, 10 pooled egg shell samples, 60 raw chicken liver samples and 60 chicken neck skins sampled from the slaughter line, were used in this study. All samples were obtained from a poultry slaughter and processing plant in the Netherlands. A pool of five swab samples was pre-enriched in 10 ml of buffered peptone water (BPW; Difco Laboratories, Detroit, Michigan, USA) at 37°C for 16–20 h, whilst 25 g of each of the other samples above, were pre-enriched similarly in 225 ml of BPW and examined as detailed below. Cudjoe, 160.
Claim 6	**Disclosure of ICMSF and Gombas**
The method of claim 1, wherein testing in e) of the modular composite sample is positive, and wherein the individual separate test lot samples are further tested, such further testing comprises the more sensitive protocol.	ICMSF, alone or in combination with Gombas, discloses the method of claim 1, upon which this claim relies. See above re Claim 1. ICMSF, alone or in combination with Gombas, further discloses wherein testing in e) of the modular composite sample is positive, and wherein the individual separate test lot samples are further tested, such further testing comprises the more sensitive protocol. ICMSF discloses the use of "immunomagnetic beads to concentrate cells." ICMSF, 318. This was a well-known concentration technique before the priority date. Gombas discloses retesting using the same protocol. Gombas discusses the use of "biochemical confirmation" to confirm testing results. Gombas, 565. A POSITA would have recognized the concentration step could be employed to provide a more sensitive protocol for further testing. *See, e.g.*, D'Haese. A POSITA would understand that retesting the non-pooled, singular samples is an inherently more sensitive process because the sample is not diluted with other samples in the testing pool. Therefore, the retesting of a singular sample is more concentrated and therefore, more sensitive. *See also, generally*, Jarvis.

	Fratamico discusses the rapid isolation of target microorganisms using an immunomagnetic separation method. Fratamico, 105. Fratamico discloses that use of immunomagnetic beads resulted in "an increase in the level of E. coli B1409 in the sample," as well as "an increase in the number of organisms recovered." *Id.*, 107. Additionally, Fratamico provides detailed instructions for preparing and using immunomagnetic bead separation to concentrate a target microbial agent or organism. Fratamico developed "[a] method employing immunomagnetic beads" to concentrate and thereby improve the isolation and detection of *E. coli*. *Id.* For example, Fratamico was able to use immunomagnetic beads to isolate *E. coli* strains from enrichment cultures of raw ground beef "when the initial inoculum level was 1 cfu g-1." *Id.*, 109. Fratamico further teaches that "[t]he percentage of bacteria recovered increased with increasing amounts of beads." *Id.*, 108. Consequently, Fratamico "demonstrates that immunomagnetic separation [using immunomagnetic beads] is a simple and rapid technique" for concentrating a microbe for isolation and detection. *Id.*, 109. These teachings of Fratamico would have motivated a POSITA to modify the combination of ICMSF and Gombas and ICMSF and Hughes to use immunomagnetic beads to concentrate microbes in a modular composite sample to benefit from accurate tests. Use of immunomagnetic beads was already known in the art for concentrating during testing beef samples. "Tu et al. (78) detected pathogenic *E. coli* O157:H7 in beef hamburger with the use of IMS and ATP measurement. After 6 h of enrichment at 37°C, inoculated beef samples (0.9 ml each) were filtered to remove large particles prior to 10 min of incubation with ca. 10^5 magnetic beads coated with anti–*E. coli* O157:H7 (Dynabeads, Dynal Inc., Oslo, Norway). Following incubation, the magnetic beads were collected with a Dynal magnetic concentrator and resuspended in 0.9 ml of Trypticase soy broth for ATP analysis." Benoit, 1941.

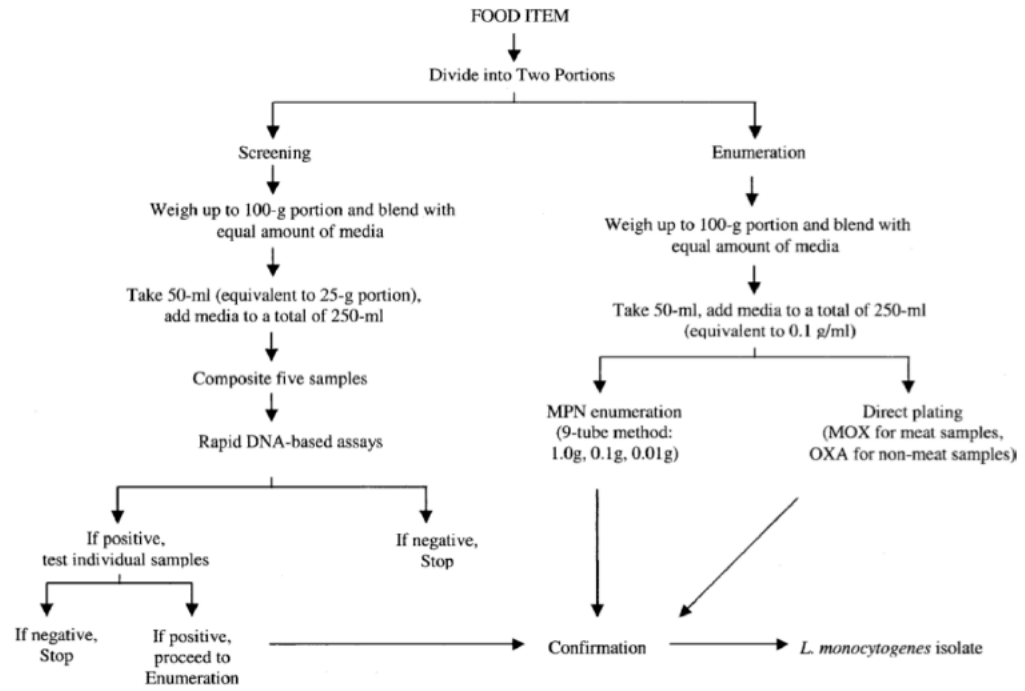
	"Hudson et al. (26) used IMS to directly separate and concentrate L. monocytogenes from ham samples prior to detection by PCR. IMS was used on the basis of the commercial availability of anti-Listeria magnetic beads (Dynal Inc.) to avoid time-consuming cultural enrichment steps and to remove PCR inhibitors." Benoit, 1941-42. Cudjoe investigates the use of immunomagnetic beads in pooling methods. In a study designed to evaluate the performance of Dynabeads® Anti-Salmonella, immunomagnetic separation (IMS) followed by plating (IMS-Plating) proved far superior to the conventional ISO *Salmonella* methodology and the Modified Semi-solid Rappaport-Vassiliadis (MSRV) method. *Salmonella* species were isolated and detected from 135 out of the 180 diverse poultry samples by IMS analysis as against 98 by the conventional method and 33 by the MSRV technique. All results were confirmed biochemically and serologically. It appeared from the data generated that some of the salmonellae isolated using IMS were greatly inhibited in Rappaport-Vassiliadis enrichment broth at 42°C and to a lesser extent in selenite cystine broth at 37°C. A moderately selective plating medium like XLD proved to be better in isolating these possible sensitive wild-type strains of salmonellae than the more selective BGA. Cudjoe, Abstract. *Samples*. One hundred and eighty (180) diverse poultry samples, consisting of 40 pools of cloacal/faecal swabs (5 swabs/pool; 5 pools samples/flock) from eight different flocks, 10 raw chicken breast meat samples, 10 pooled egg shell samples, 60 raw chicken liver samples and 60 chicken neck skins sampled from the slaughter line, were used in this study. All samples were obtained from a poultry slaughter and processing plant in the Netherlands. A pool of five swab samples was pre-enriched in 10 ml of buffered peptone water (BPW; Difco Laboratories, Detroit, Michigan, USA) at 37°C for 16–20 h, whilst 25 g of each of the other samples above, were pre-enriched similarly in 225 ml of BPW and examined as detailed below. Cudjoe, 160.

Claim 10	Disclosure of ICMSF and Gombas
The method of claim 1, wherein the test lot is selected from the lot group consisting of a single combo, single bin, single pallet, and a production assemblage.	ICMSF, alone or in combination with Gombas, discloses the method of claim 1, upon which this claim relies. See above re Claim 1. ICMSF, alone or in combination with Gombas, further discloses wherein the test lot is selected from the lot group consisting of a single combo, single bin, single pallet, and a production assemblage. The specification of the '584 Patent indicates a test lot is a single-unit production lot ('584 Patent, 5:21-32) such as a single combo, bin. pallet, or segment of production ('584 Patent, 7:28-36 (AAPA #3 and #4). ICMSF teaches collecting from "individual production batches as lots" (ICMSF, 119) and "[a] lot is considered as a collection of units." ICMSF, 327. "Operationally-linked" is simply grouping like items, which is also disclosed by ICMSF. "Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119.
Claim 11	**Disclosure of ICMSF and Gombas**
The method of claim 1, wherein the number of separate test lots is from 3 to 10.	ICMSF, alone or in combination with Gombas, discloses the method of claim 1, upon which this claim relies. See above re Claim 1. ICMSF, alone or in combination with Gombas, further discloses wherein the number of separate test lots is from 3 to 10. ICMSF indicates a consignment may be "composed of several lots." ICMSF, 119-20. "Several" lots includes at least three lots. Additionally, Gombas tests five lots. *See, e.g.* Gombas, Fig. 1 ("composite five samples"), *id.*, 564 ("up to five like-product samples were composited and screened.").
Claim 12	**Disclosure of ICMSF and Gombas**

The method of claim 1, further comprising, where testing of the modular composite sample is positive, determining which individual separate test lots are positive, application of remedial measures to said separate positive test lots, and validating by further testing of a portion of the respective remediated separate test lots and obtaining a negative test result.	ICMSF, alone or in combination with Gombas, discloses the method of claim 1, upon which this claim relies. See above re Claim 1. ICMSF, alone or in combination with Gombas, further discloses where testing of the modular composite sample is positive, determining which individual separate test lots are positive, application of remedial measures to said separate positive test lots, and validating by further testing of a portion of the respective remediated separate test lots and obtaining a negative test result.  Gombas, 563 (Figure 1 (annotated) showing testing procedures for further testing). A POSITA understands the purpose of testing for pathogens and other organisms in food is to determine if a food is safe for entry into commerce to be sold for consumption. One having ordinary skill in the art understands that if a sample tests positive, a remediation step, like cooking, needs to be completed before it can be sold into commerce to comply with USDA guidelines.
Claim 13	**Disclosure of ICMSF and Gombas**

The method of claim 1, wherein, in a), separately collecting said plurality of portions from each of said plurality of separate test lots comprises separately collecting from each separate test lot a number of portions from 5 to 100.	ICMSF, alone or in combination with Gombas, discloses the method of claim 1, upon which this claim relies. See above re Claim 1. ICMSF, alone or in combination with Gombas, further discloses wherein, in a), separately collecting said plurality of portions from each of said plurality of separate test lots comprises separately collecting from each separate test lot a number of portions from 5 to 100. ICMSF teaches collecting 5, 30, 60, 95, or 100 portions. ICMSF, 184-90, 276, 282, 309, 320, 326-30. Gombas also teaches collecting 25, 50, or 100 samples. "After culling unacceptable samples from luncheon meats and deli salads, 100 samples were selected for testing. For the other product categories, 50 samples were selected for the first several weeks of sampling, and 25 samples were selected each week thereafter for testing." Gombas, 563. *See also* MPEP § 2144.05.
Claim 14	**Disclosure of ICMSF and Gombas**
The method of claim 1, wherein, in a), separately collecting said plurality of portions from each of said plurality of separate test lots comprises separately collecting from each separate test lot 30 to 60 portions.	ICMSF, alone or in combination with Gombas, discloses the method of claim 1, upon which this claim relies. See above re Claim 1. ICMSF, alone or in combination with Gombas, further discloses wherein, wherein, in a), separately collecting said plurality of portions from each of said plurality of separate test lots comprises separately collecting from each separate test lot 30 to 60 portions. ICMSF teaches collecting 5, 30, 60, 95, or 100 portions. ICMSF, 184-90, 276, 282, 309, 320, 326-30. Gombas also teaches collecting 25, 50, or 100 samples. "After culling unacceptable samples from luncheon meats and deli salads, 100 samples were selected for testing. For the other product categories, 50 samples were selected for the first several weeks of sampling, and 25 samples were selected each week thereafter for testing." Gombas, 563. *See also* MPEP § 2144.05.

Claim 15	Disclosure of ICMSF and Gombas
The method of claim 1, wherein, in a), separately collecting said plurality of portions from each of said plurality of separate test lots comprises separately collecting 30 portions from each separate test lot.	ICMSF, alone or in combination with Gombas, discloses the method of claim 1, upon which this claim relies. See above re Claim 1. ICMSF, alone or in combination with Gombas, further discloses wherein, in a), separately collecting said plurality of portions from each of said plurality of separate test lots comprises separately collecting 30 portions from each separate test lot. ICMSF teaches collecting 5, 30, 60, 95, or 100 portions. ICMSF, 184-90, 276, 282, 309, 320, 326-30. Gombas also teaches collecting 30 samples. "In a week when < 100 or <25 samples were purchased (owing to product shortages in stores), all acceptable samples were tested." Gombas, 563. *See also* MPEP § 2144.05.
Claim 17	**Disclosure of ICMSF and Gombas**
[17pre] A method of sampling, testing and validating a plurality of operationally-linked test lots of meat or poultry for release into commerce, comprising:	To the extent the preamble is limiting, as discussed herein, ICMSF, alone or in combination with Gombas, discloses a method of sampling, testing and validating a plurality of operationally-linked test lots of beef for release into commerce. ICMSF includes many other references to testing lots of beef or poultry, two of the most common types of meat in commerce. ICMSF, 114 (§ 6.3), 184–191 (§§ 10.2–10.6), 225–226 (§ 12.1), 285 (§ 16.1), 313–330 (Chapter 17). ICMSF is relevant to any kind of food product including beef or poultry. Gombas also discloses testing and validating a plurality of operationally-linked test lots of beef for release into commerce. Gombas describes sampling from ready to eat ("RTE") products: All products were to be sliced (either presliced by manufacturers or sliced by the retail stores)" including beef bologna and "products made from pork or poultry." Gombas, 562.

	 FIGURE 1. *Detection and enumeration of* L. monocytogenes *in RTE food samples.* Gombas, 563 (Figure 1).
[17a] a) collecting a plurality of portions from each of a plurality of operationally-linked single-unit production lots of meat or poultry, the single-unit production lots each comprising an assemblage of one or more production specimens from which the portions are collected, wherein each single-unit production lot is separately sampled to conform to a statistical-based sampling plan specifying, for a particular microbial agent or	"Many different types of samples are collected and submitted to the laboratory for analysis. Some are sample units from lots or consignments of food or ingredients for lot acceptance determination. . . . A sample unit is a small portion of the lot in which the distribution of both numbers and types of microorganisms should be representative of the whole lot." ICMSF, 255. The lots may be beef or poultry. *Id.*, 184-91, 226, 230. Thus, ICMSF discloses collecting a plurality of samples or sample units (other synonyms include portions, specimens, and sub-samples) from lots of beef or

organism, a number of portions to be taken sufficient to provide for validation and release of the single-unit production lot into commerce;	poultry. ICMSF describes collecting from a lot or lots: "If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." *Id.*, 119. Additionally, ICMSF indicates a consignment may be "a very large quantity of food" and may be "composed of several lots, representing for example, different days of production from the same plant." *Id.*, 119, 120; *see also* AAPA #3-#5.

6.11 WHAT IS A REPRESENTATIVE SAMPLE?

A *representative sample* reflects, as far as is possible, the composition of the lot from which it is drawn.

How then should a representative sample be drawn? It is important to avoid bias and to draw a sufficient number of sample units to confidently make a judgment about a lot. Sampling at random is the universally recognized way of avoiding bias and is more reliable than consciously trying to draw the sample units from the various parts of a lot. The units (cartons or containers, particular weights of solid, or volumes of liquid) are drawn by using random numbers. There is, of course, no guarantee that a random sample has characteristics identical with those of the lot, but the randomness of sampling is the basis for calculation of the probability that a sample will give a biased result.

It is also possible, and may be desirable, to use a *stratified random sampling* approach, drawing a random sample of a given number of sample units from each *stratum* (e.g., each sublot or carton). The distribution of sample units on strata should correspond to the distribution of lot units on strata. This means that if each stratum contains the same quantity as every other one, the number of sample units per stratum should be the same. Otherwise, they should differ just as the proportions of the lot contained in the strata differ. Stratification is a device for handling known sources of variation and may be used where one has prior knowledge that the consignment is potentially not of uniform quality. A consignment might not be of uniform quality if portions of it are shipped in different vehicles or in different holds of a vessel, or if it is known that it is really composed of several lots representing, for example, different days of production from the same plant or different plants of the same company. The results for different strata should be assessed separately and then pooled if they appear to be homogeneous.

The calculation of acceptance probabilities is based on the assumption that random samples have been drawn and analyzed. Where physical or practical constraints prevent random sampling, such calculated probabilities may not be valid. Their reliability depends on how close reality approaches the ideal situation of random sampling. This fact should be borne in mind when subsequently interpreting results.

ICMSF, 119-20 (annotated).

6.10 WHAT IS A LOT?

Ideally, a lot is a quantity of food or food units produced and handled under uniform conditions. Under certain circumstances, it has been shown that the logarithms of the counts from a batch of food are likely to be normally distributed (Baird-Parker, 1980; Kilsby & Baird-Parker, 1983 [their Table 2]; Ingram & Roberts, 1976). The implication is that there is homogeneity within a lot, which, when considering microbial levels and distributions, rarely occurs in practice. In most instances, the distribution of microorganisms within lots of food is heterogeneous. Even in food factories, this heterogeneity makes the interpretation of sample results difficult, and it becomes even more so in circumstances where a lot is less well defined, such as in a consignment of a very large quantity of food.

It is therefore helpful if suppliers can be persuaded to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load).

If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment.

A lot should be composed of food produced with as little variation as possible for a given process or commodity. Because of the uncertainties in identifying a lot commercially, however, the use of the word *lot* in this book is usually in its statistical sense, as a collection of units of a product, the acceptability of which is determined by examining a sample drawn from it.

ICMSF, 118-19 (annotated).

	The ICMSF has defined a lot as follows: In the commercial sense, a lot is a quantity of food supposedly produced under identical conditions, all packages of which would normally bear a lot number that identifies the production during a particular time interval, and usually from a particular line, retort, or other critical processing unit. Statistically, a lot is considered as a collection of units of a product from which a sample is drawn to determine acceptability of the lot (ICMSF, 1986). In this example, the maximum size of a lot consists of all the ground beef produced on common equipment from cleanup to cleanup. Smaller lots could be established (e.g., hourly). ICMSF, 327 (annotated). This definition also reflects the common practice referred to in the '584 Patent, wherein "[p]referably, the producer defines and produces these single-unit lots in such a fashion as to maximize the information available." '584 Patent at 12:46–48 (Example II). This corresponds with the specification of the '584 Patent which reflects the common practice of producers defining what constitutes a lot grouped for traceability purposes. '584 Patent, 2:60-3:3, 11:12-16 (AAPA #3), and 12:46-49. Thus, ICMSF discloses collecting a plurality of portions from separate lots. ICMSF describes a lot as "a collection of units of a product." IMCSF, 119. ICMSF further indicates a lot has been defined as: "a quantity of food supposedly produced under identical conditions, all packages of which would normally bear a lot number that identifies the production during a particular time interval, and usually from a particular line, retort, or other critical processing unit. Statistically, a lot is considered as a collection of units of a product from which a sample is drawn to determine acceptability of the lot." ICMSF, 327. ICMSF advocates use of a "statistically based sampling plan." *Id.*, 113-15, 119-21, 320, 327, 328. In the sampling plans, "*n* is the number of sample units chosen separately and independently, and . . . *c* is the maximum allowable number of sample

	units yielding a positive result." *Id.*, 115. ICMSF discloses a wide range for the number of sample units (portions) for a particular microbial agent or organism. For example, ICMSF discusses collecting 15, 30, 60 or 100 sample units from a lot. *Id.*, 320, 326. **6.5 THE SAMPLING PLAN** The sample units so drawn will, after examination, yield results that will be compared with certain criteria to reach a decision as to whether the entire lot should be accepted or rejected (see section 6.9 for explanation of rejection). The particular choice of sampling procedure and the decision criteria make up the *sampling plan*. Proper drawing of samples is essential if the decision making criteria of the sampling plan are to give unbiased results. By sampling randomly, we are able to reduce the risk of making biased decisions. A simple example of a sampling plan follows. Take 10 sample units of a food from a lot and subject them to appropriate laboratory procedure to test for the presence or absence of a microorganism. If two or fewer of the 10 sample units show presence of the organism, that is, give a positive result, then the whole lot of food is acceptable (relative to this organism). But if three or more give a positive result, the whole lot is to be rejected. This plan is described by the use of two terms $n = 10$ and $c = 2$, where n is the number of sample units chosen separately and independently, and where c is the maximum allowable number of sample units yielding a positive result. ICMSF, 115 (annotated).

	6.1 INTRODUCTION It is important to recognize that the management of food safety using the approaches outlined in the first five chapters, based on controlling hazards through Good Hygienic Practices (GHP) and Hazard Analysis Critical Control Point (HACCP), is much more effective than trying to ensure safety by lot acceptance through end-product testing. This chapter discusses the concepts of probability and sampling that determine the limitations of end-product testing. Those concepts form the basis of the design of statistically based sampling plans (Chapter 7) and the establishment of cases (Chapter 8). In Chapters 6 and 7, it is assumed that the microbiological test will give an absolutely accurate result, with no false positives or false negatives. This is not a realistic assumption, and problems arising from sampling, sample transport and preparation for analysis, and the imprecision of microbiological methods are addressed in Chapters 10 and 12 and elsewhere in the text. ICMSF, 113 (annotated); *see also id.* at 115–21 (§§ 6.4–6.13), 319–20 (§ 17.2.3.3), and 326–30 (§§ 17.5.3.1–17.6). ICMSF discusses the number of portions to be taken sufficient for validation of a particular microorganism including *Salmonella* (*id.*, 276 (§ 15.3.3); *see also id.*, 282 (§ 15.6.3)), *Listeria* (*id.* at 309 (§ 16.6.3)), and *E. coli* (*id.*, 318–21 (§§ 17.2.3.2–17.2.3.4), 326 (§ 17.5.3.1)). IEH's AAPA discloses statistical-based sampling plans taking 10-60 sample units. '584 Patent, 5:57-6:6 (AAPA #1, #2, #5, and #6). Determining meat is safe for consumption so it is "commercially possible to market" the meat, is the well-understood point of testing meat and is taught by ICMSF. ICMSF, 185-89. Moreover, the recitation of a lot being validated for "release . . . into commerce" is not limiting. Prosecution History for Reexamination Control No. 95/011,294. "Operationally-linked" is simply grouping like items, which is also disclosed by ICMSF. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole

	consignment." ICMSF, 119. It is therefore helpful if suppliers can be persuaded to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load). If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment. ICMSF, 119 (annotated).
[17b] b) combining the collected conforming plurality of product portions corresponding to each of the separate single-unit production lots to provide a corresponding set of separate single-unit production lot samples, wherein each separate single-unit production lot sample is attributed to a particular corresponding separate single-unit production lot;	ICMSF states: "To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units of the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. . . . This finding suggests that considerable reductions may be made in the cost of laboratory analysis by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, n, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort." ICMSF, 187.

	To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. A laboratory investigation undertaken for ICMSF (Silliker & Gabis, 1973) indicated that different sized analytical units drawn from a single well-mixed sample unit of dried food give very similar results. A subsequent investigation (Gabis & Silliker, 1974) using foods of high moisture content (including eggs, poultry meat, meat, and meat products) showed comparable reliability in the use of composite analytical units; for example, examination of three composites of 20 × 25 g analytical units gave the same result as examination of all 60 analytical units individually (the total amount examined being 1500 g in both instances). This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n*, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort. ICMSF, 187.

10.5.1 Conceivable Alternative Plans

As indicated above, for an infectious agent such as *Salmonella*, there are strong reasons for adopting sampling plans in which $c = 0$:

1. It is philosophically objectionable to some people to accept a lot containing a recognized pathogen, regardless of how the food is to be used in a manufacturing process or by the consumer.
2. There is the very great technical advantage of combining many analytical units for bacteriological examination in systems with $c = 0$, because a single positive decides the outcome.
3. The plan with $c = 1$ (or more) would always require the examination of (many) more analytical units, for equal probability of acceptance, than a plan with $c = 0$.
4. Even if the indicated probabilities of plans with $c = 0$ versus $c = 1$ are the same, their implications are not. For example, when a lot is accepted with the plan $n = 60$, $c = 0$, it is possible (though not certain) that the lot may not be contaminated at all. But using the plan $n = 95$, $c = 1$ and accepting one positive, it is known that the lot certainly is contaminated (though the probability that a lot with 5% of analytical units contaminated would be accepted is in both instances $P_a = 0.05$).

ICMSF, 188 (annotated). ICMSF also provides an example of "[u]sing a sampling plan involving n = 15 (25 g analytical units) and a composite of 375 g." *Id.* at 195 (§ 10.7.2). ICMSF also states "[f]or qualitative analyses, 25 g analytical units are frequently analyzed individually or as composites of 100 g, 200 g, or more." *Id.* at 232 (§ 12.5.2).

The ICMSF incorporates two references, Silliker & Gabis (1973) and Gabis & Silliker (1974). Silliker notes that "[c]ompositing of multiple samples (lumping) and wet compositing of pre-enrichment cultures have been tested as economical approaches to *Salmonella* control." Silliker, 475.

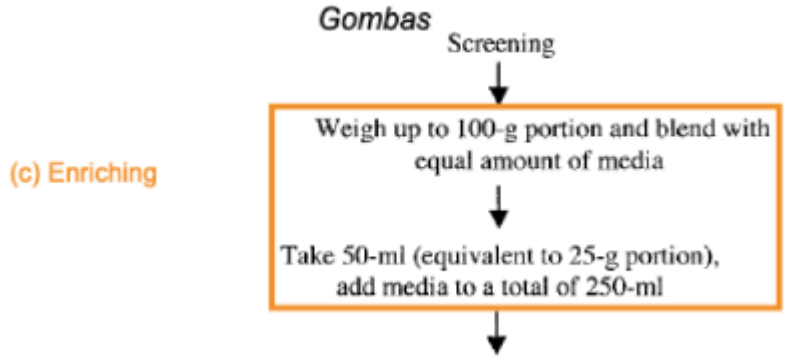
Silliker further notes that "[b]oth approaches are being used widely by the food industry in the United States." *Id*. Gabis discloses application of the Silliker teachings to testing meat. Gabis.

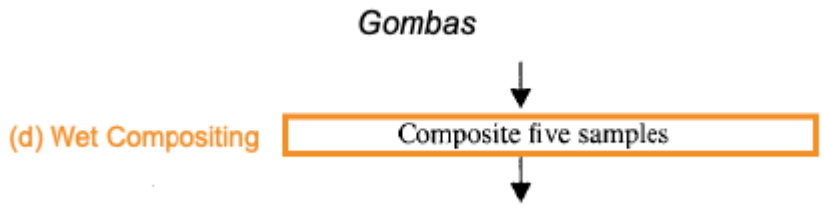
	To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. A laboratory investigation undertaken for ICMSF (Silliker & Gabis, 1973) indicated that different sized analytical units drawn from a single well-mixed sample unit of dried food give very similar results. A subsequent investigation (Gabis & Silliker, 1974) using foods of high moisture content (including eggs, poultry meat, meat, and meat products) showed comparable reliability in the use of composite analytical units; for example, examination of three composites of 20 × 25 g analytical units gave the same result as examination of all 60 analytical units individually (the total amount examined being 1500 g in both instances). This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n*, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort. "There is the very great technical advantage of combining many analytical units for bacteriological examination." ICMSF, 188. ICMSF also describes composites prepared by combining "15 (25 g analytical units)" and "composites of 100 g, 200 g, or more." *Id.,* 195, 232; *see* AAPA #5. ICMSF states, "All steps up to the analysis must be controlled . . . to allow reliable traceability." ICMSF, 227. ICMSF indicates lot samples are traceable to a particular corresponding lot: "It is therefore helpful . . . to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load). . . . Treating the individual production

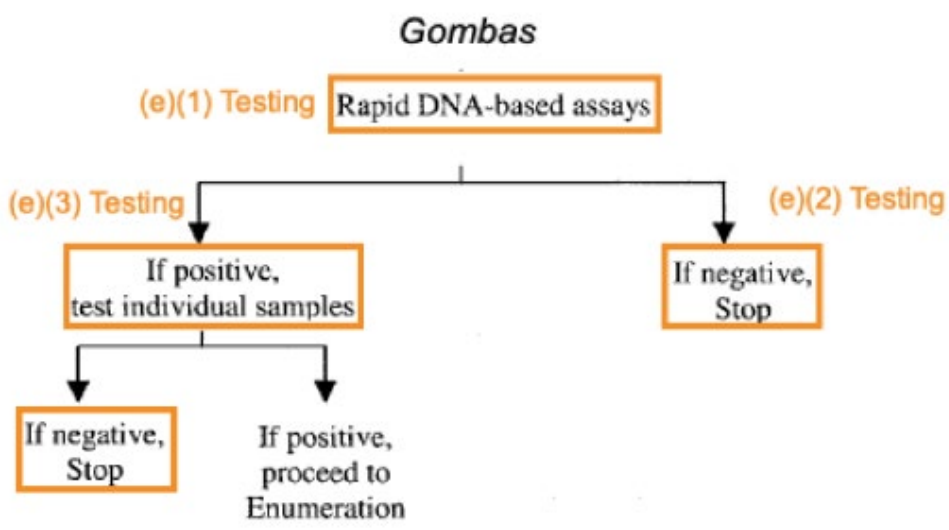
	batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119. Gombas also discloses this element. Gombas states "samples were assigned codes" ad product information was recorded for the luncheon meats including "sampling location." Gombas, 563. A POSITA would find Gombas' system for testing individual samples, which composited samples test positive, inherently requires tracking the individual samples that contributed to the composite. *See, e.g.*, Gombas, 564 ("If a composite tested positive, samples in the composite were tested individually by Gene-Trak assay with the PBS from the second mLCA plate or by the BAX assay with the secondary enrichment broths. Individual samples that tested negative were not tested further If an individual sample tested positive, the retained portion was subjected to an enumeration assay.") Thus, Gombas also discloses that each of the separate composited lot samples is attributed to a particular corresponding separate lot.
[17c] c) incubating the set of separate single-unit production lot samples under conditions suitable to allow levels of a target microbial agent or organism that is present in one or more of the separate single-unit production lot samples to reach detectable levels and become uniform, or substantially uniform, throughout the respective one or more separate single-unit production lot samples, to provide a set of separate single-unit production lot samples enriched for the target microbial agent or organism;	ICMSF states: To reduce laboratory effort . . . it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. ICMSF, 187. Also, ICMSF describes preparing composites of analytical units then diluting "for qualitative (enrichment)" analysis. *Id.,* 231-32; *see also* AAPA #5, #7. For practical reasons, however, analytical units of 10 g up to 50 g are typical for quantitative methods and are diluted 1:10. For qualitative analyses, 25 g analytical units are frequently analyzed individually or as composites of 100 g, 200 g, or more. ICMSF at 232 (annotated).

	Preparation of the first dilution, either for qualitative (enrichment) or quantitative (direct) analyses, can have a marked impact on the microbial population in the sample. This is often due to changes in the physico-chemical characteristics of the suspension as compared to the food matrix. Where the food does not confer protection, the composition of the diluent is important to prevent or minimize shock/injury to the microorganisms that can result from rapid changes in the ionic concentrations. ICMSF at 232 (annotated). ICMSF also describes strategic use of non-selective enrichment to resuscitate microorganisms and selective enrichments for growing microorganisms. *Id.* at 232–33 (§ 12.6) ("use of nonselective medium . . . to enhance recovery of injured or stressed cells."). ICMSF at § 12.6 also describes optimizing the incubation period. S*ee e.g.*, *id.,* 232. It was well known long before the priority date to use non-selective enrichments after dry compositing and prior to wet compositing as evidenced by Price, Silliker, Gabis, and MLG. A POSITA would also know to use selective enrichments after dry compositing and prior to wet compositing as disclosed by ICMSF and Gombas. A POSITA would find that incubating so microorganisms are detectable and at least substantially uniform in the selective enrichment broth was considered the standard practice in official methods because the methods otherwise fail. For example, D'Aoust states "standard methodology recommends incubation of selective enrichment broths." D'Aoust, 372. Gombas also discloses incubating: [A]pproximately half (up to 100 g) of a sample was aseptically transferred to a sterile stomacher bag and blended with an equal amount of enrichment broth. . . . For northern California samples, demi-Fraser broth was used as the enrichment broth for all products. After blending, 50 g of the homogenate was added to 200 ml of enrichment broth and stomached for 1 min. This procedure resulted in a detection sensitivity equivalent to that of the current regulatory methods (i.e., 1 CFU/25 g). The initial sample-blending step was performed to account for the potential heterogeneous distribution of *L. monocytogenes* in the sample. The 250-ml enrichment was incubated at 35°C for 24

	± 2 h." Gombas, 563; *see also id.,* 564. Gombas further indicates that: "For each California sample, 0.1 ml of the demi-Fraser enrichment culture was transferred to 10 ml of morpholinepropanesulfonic acid-*Listeria* enrichment broth and incubated at 35°C for 20 to 24 h. After the secondary enrichment, 1 ml of the broth culture was used for screening with the BAX assay as described below." *Id.,* 564. Gombas avoids potential heterogenous distribution and ensures the target microorganism is at least substantially uniform in a sample by blending the sample and the enrichment broth in the stomacher bag to yield a homogenate. *Id.,* 563. **Sample screening.** Each sample was divided into two portions for screening and enumeration. For screening, approximately half (up to 100 g) of a sample was aseptically transferred to a sterile stomacher bag and blended with an equal amount of enrichment broth. For Maryland samples, University of Vermont broth 2 (UVM-2 broth) was used as the enrichment broth for luncheon meats, smoked seafood, and seafood salads; phosphate-buffered *Listeria* enrichment broth was used for the other product types. For northern California samples, demi-Fraser broth was used as the enrichment broth for all products. After blending, 50 g of the homogenate was added to 200 ml of enrichment broth and stomached for 1 min. This procedure resulted in a detection sensitivity equivalent to that of the current regulatory methods (i.e., 1 CFU/25 g). The initial sample-blending step was performed to account for the potential heterogeneous distribution of *L. monocytogenes* in the sample. The 250-ml enrichment was incubated at 35°C for 24 ± 2 h. Gombas, 563 (annotated). A POSITA would find that Gombas utilized conventional steps to avoid a "potential

	heterogeneous distribution." Gombas, 563. A POSITA would also find that blending a sample in a stomacher bag as done by Gombas enables vigorous mixing of the sample and the enrichment broth and achieves a homogenous mixture referred to as a homogenate. *Id.* Gombas further ensures that detectable levels and uniformity are achieved because "[a]fter blending, 50 g of the homogenate was added to 200 ml of enrichment broth and stomached for 1 min" and "[t]he 250-ml enrichment was incubated at 35°C for 24 ± 2 h." *Id.* Achievement of detectable levels and uniformity is also evidenced by Gombas concluding the "procedure resulted in a detection sensitivity equivalent to that of current regulatory methods." *Id.*  *Id.*, (Figure 1 (annotated) showing an enriching step).
[17d] d) removing portions of each enriched separate single-unit production lot sample, and combining the removed portions to provide a modular composite sample; and	ICMSF describes compositing the enriched lot samples: "it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. . . . This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n,* with corresponding increase in the stringency of examination, without correspondingly increasing

	laboratory effort." ICMSF, 187. *See also* AAPA #5-#7. Additionally, Gombas discloses wet compositing of enriched samples: "Up to five like-product samples were composited and screened. . . . For California samples, five 1-ml samples of secondary enrichment broths were composited." Gombas, 564. Gombas's Figure 1 depicts wet compositing as shown in the excerpt below:  Gombas, 563 (Figure 1 (annotated) showing a wet compositing step).
[17e.1] e) testing of the modular composite sample, using a suitable detection assay, for the target microbial agent or organism,	ICMSF describes testing enrichment cultures of composite samples with suitable detection assays (ICMSF, 187) and "presence/absence tests" (ICMSF, 233). Suitable detection assays were established in Silliker and Gabis as discussed in ICMSF. Gombas provides a complete framework for testing, and a POSITA would have been motivated to modify ICMSF based on Gombas's framework:

	 Gombas, 563 (Figure 1 (annotated) showing the testing step). Gombas also discloses a suitable detection assay: "For California samples, . . . the 5-ml composite was tested by the BAX assay according to the manufacturer's procedures." Gombas, 564. These assay types were "art recognized." '584 Patent, 2:54-58 (AAPA #7).
[17e.2] wherein when such testing is negative all of said separate single-unit production lot samples are validated for release into commerce, and	ICMSF describes correlating lots in a consignment with lot samples so a negative or positive test result enables a lot to be validated or rejected. ICMSF, 118-19. ICMSF describes sampling plans in which "c is the maximum allowable number of sample units yielding a positive result." ICMSF, 115. For many scenarios, the requirement is c = 0, which indicates the testing is negative. *Id.*, 118, 183-90, 309, 325, 326. A negative test result (*id.*, 113-15, 325, 326) is the expected and desired outcome to determine meat is not contaminated and is safe for consumption (*id.*, 184-89) such that a lot is validated and it is "commercially possible to market" (*id.*, 185) the meat.

6.10 WHAT IS A LOT?

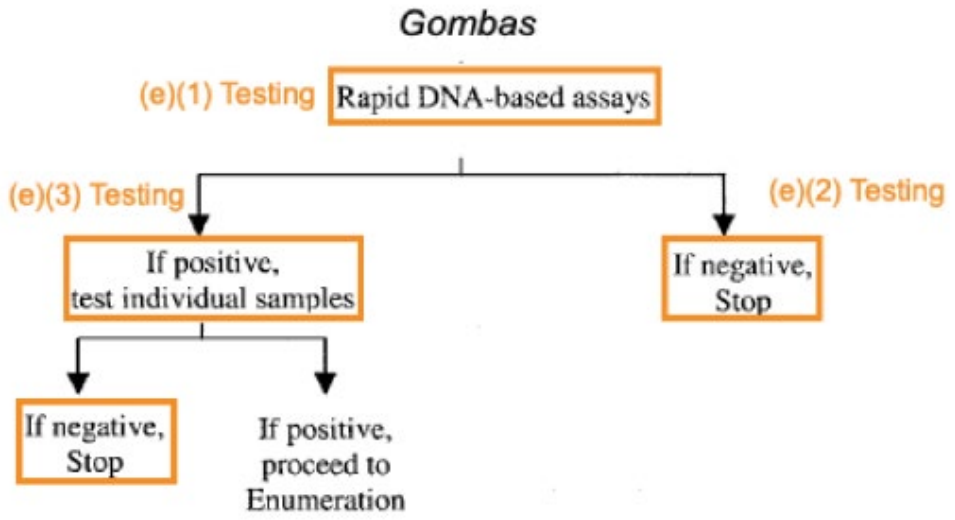
Ideally, a lot is a quantity of food or food units produced and handled under uniform conditions. Under certain circumstances, it has been shown that the logarithms of the counts from a batch of food are likely to be normally distributed (Baird-Parker, 1980; Kilsby & Baird-Parker, 1983 [their Table 2]; Ingram & Roberts, 1976). The implication is that there is homogeneity within a lot, which, when considering microbial levels and distributions, rarely occurs in practice. In most instances, the distribution of microorganisms within lots of food is heterogeneous. Even in food factories, this heterogeneity makes the interpretation of sample results difficult, and it becomes even more so in circumstances where a lot is less well defined, such as in a consignment of a very large quantity of food.

It is therefore helpful if suppliers can be persuaded to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load).

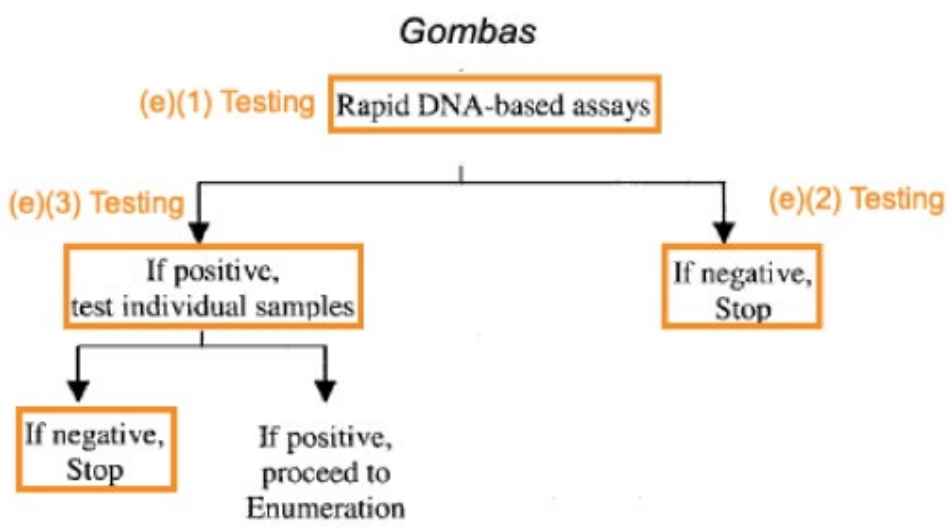
If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment.

A lot should be composed of food produced with as little variation as possible for a given process or commodity. Because of the uncertainties in identifying a lot commercially, however, the use of the word *lot* in this book is usually in its statistical sense, as a collection of units of a product, the acceptability of which is determined by examining a sample drawn from it.

ICMSF, 118-19 (annotated).

	To the extent ICMSF is considered not to disclose some aspect of step (e)(2), Gombas indicates a negative test result is the end of the process: "If a composite tested negative, all samples in the composite were considered negative (in 25 g) and were not tested further." Gombas, 564. A POSITA would find that in the combination, a negative test result equates to being validated.  Gombas, 563 (Figure 1 (annotated) showing "if negative, stop.").
[17e.3] wherein when such testing is positive, individual separate single-unit production lots are validated for release into commerce, according to the statistical-based sampling plan, by further testing of a portion of the respective enriched separate single-unit production lot sample using the same or a more sensitive protocol and obtaining a	The combination of ICMSF and Gombas describes retaining a portion of the enriched lot samples (steps (b)-(d)). "Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119.

negative test result for each single-unit production lot,	"There is often a desire to retest retained samples." ICMSF, 191. If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment. ICMSF, 119 (annotated). ICMSF discloses validating for release into commerce (ICMSF, 113–15 (§§ 6.2–6.6), 118–19 (§§ 6.9–6.10), 184–85 (§ 10.2), 325–27 (§§ 17.5.3–17.5.3.4); *see also id.*, 186–89 (§ 10.4–10.6)) according to a statistically-based sampling plan (*id.*, 113 (§ 6.1); *see also id.*, 115–18 (§§ 6.4–6.8), 184 (§ 10.2), 319–21 (§§ 17.2.3.3–17.2.3.4), 326–30 (§§ 17.5.3.1–17.6)), as described regarding steps (a) and (e)(2). "If a composite tested positive, samples in the composite were tested individually . . . by the BAX assay with the secondary enrichment broths. Individual samples that tested negative were not tested further." Gombas, 564.



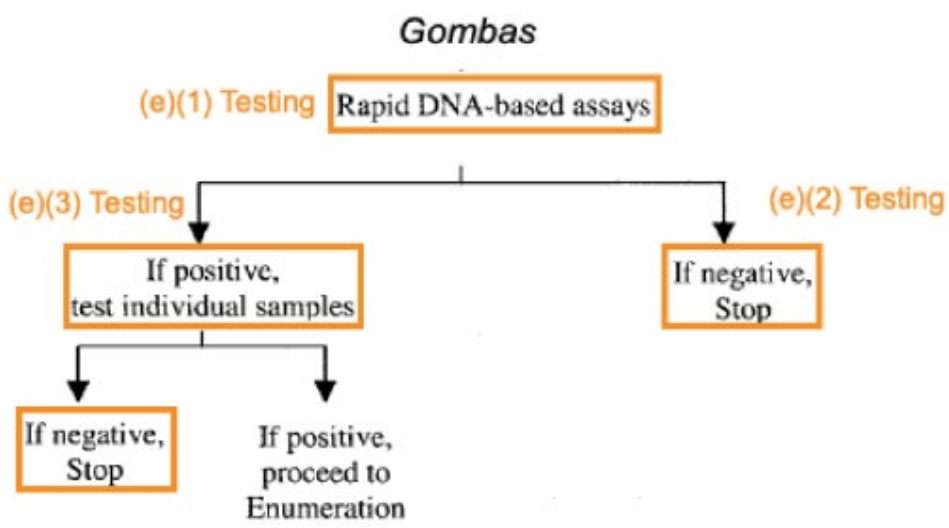
Gombas, 563 (Figure 1 (annotated) showing testing procedures for further testing).

"Individual samples that tested negative were not tested further. If an individual sample tested positive, the retained portion was subjected to an enumeration assay." Gombas, 564.

The combination teaches that after the composited samples test positive, then the individual samples used to form the composited sample are individually tested with the same protocol to determine which of the separate composited lot samples is positive for the target microbe(s).

A POSITA would have recognized the concentration step could be employed to provide a more sensitive protocol for further testing.

A POSITA would understand that retesting the non-pooled, singular samples is an inherently more sensitive process because the sample is not diluted with other samples in the testing pool. Therefore, the retesting of a singular sample is more concentrated and therefore, more sensitive. *See also, generally*, Jarvis.

[17e.4] wherein the enrichment in step c), prior to removing portions in step d), is sufficient to enable said validation of individual separate single-unit production lots with no requirement for further enrichment thereof, and with no requirement for further sampling in a).	 Gombas, 563 (Figure 1 (annotated) showing testing procedures for further testing). ICMSF and Gombas disclose selective enrichment so there is no requirement for further enrichment. Gombas explicitly indicates portions of the enrichment cultures are retained for further testing; thus there is no need for further sampling in Gombas. "Individual samples that tested negative were not tested further. If an individual sample tested positive, the retained portion was subjected to an enumeration assay." Gombas, 564.
Claim 18	**Disclosure of ICMSF and Gombas**
The method of claim 1, wherein removing portions in step d) is removing equal portions, aseptically, so as to preclude contamination of the enriched separate test lot samples.	ICMSF, alone or in combination with Gombas, discloses the method of claim 1, upon which this claim relies. See above re Claim 1. ICMSF, alone or in combination with Gombas, further discloses wherein removing portions in step d) is removing equal portions, aseptically, so as to preclude contamination of the enriched separate test lot samples.

	ICMSF describes aseptically withdrawing and weighing sample units into the appropriate diluent. ICMSF, 231, 232. Gombas indicates "a sample was aseptically transferred to a sterile stomacher bag and blended with an equal amount of enrichment broth." Gombas, 563. Gombas further indicates "five 1-ml samples of secondary enrichment broths were composited." *Id.*, 564. Moreover, a POSITA would consider this to be standard practice. A POSITA would have aseptically removed equal volume portions of each enriched separate test lot sample.
Claim 19	**Disclosure of ICMSF and Gombas**
The method of claim 1, wherein the target microbial agent or organism comprises at least one selected from the group consisting of *Listeria*, *Salmonella* and *Escherichia coli.*	ICMSF, alone or in combination with Gombas, discloses the method of claim 1, upon which this claim relies. See above re Claim 1. ICMSF, alone or in combination with Gombas, further discloses wherein the target microbial agent or organism comprises at least one selected from the group consisting of *Listeria*, *Salmonella* and *Escherichia coli* ICMSF describes testing for *Listeria* (ICMSF, 285-312), *Salmonella* (*id.*, 273-84), and *Escherichia coli* (*id.*, 313-32). Gombas focuses on *Listeria*. Gombas, 559.
Claim 20	**Disclosure of ICMSF and Gombas**
The method of claim 1, wherein the target microbial agent or organism comprises at least one selected from the group consisting of *Listeria* spp., *Listeria monocytogenes*, *Salmonella* spp., *Escherichia coli* O157:H7, coliforms and fecal coliforms.	ICMSF, alone or in combination with Gombas, discloses the method of claim 1, upon which this claim relies. See above re Claim 1. ICMSF, alone or in combination with Gombas, further discloses wherein the target microbial agent or organism comprises at least one selected from the group consisting of *Listeria* spp., *Listeria monocytogenes*, *Salmonella* spp., *Escherichia coli* O157:H7, coliforms and fecal coliforms.

	ICMSF describes testing for *Listeria* (ICMSF, 285-312), *Salmonella* (*id.*, 273-84), and *Escherichia coli* (*id.*, 313-32). Gombas focuses on *Listeria*. Gombas, 559.
Claim 21	**Disclosure of ICMSF and Gombas**
The method of claim 1, wherein the target microbial agent or organism comprises a spoilage organism.	ICMSF, alone or in combination with Gombas, discloses the method of claim 1, upon which this claim relies. See above re Claim 1. ICMSF, alone or in combination with Gombas, further discloses wherein the target microbial agent or organism comprises a spoilage organism. A POSITA would find it obvious to apply the teachings of ICMSF and Gombas to target spoilage organisms. "Examination of every piece of whole meat is clearly impractical, hence any sampling procedure for estimating shelf-life would involve taking enough samples to give some statistical assurance of the keeping qualities of meat." Gill, 449. Borch is titled "Bacterial spoilage of meat and cured meat products." Borch. The reference discusses spoilage organisms in meat: Lactic acid bacteria are the major bacterial group associated with the spoilage of refrigerated vacuum- or MA-packaged cooked, cured meat products (Blickstad and Molin, 1983b; Shaw and Harding, 1989; von Holy et al., 1991). At the time of spoilage some products contain a 'pure' culture of only one species, while in others a mixture of *Lactobacillus* spp. and *Leuconostoc* spp. was found (Kant-Muermans, unpublished results). The great diversity of bacteria isolated from spoiled meat products is confirmed by several studies (Korkeala and Mäkelä, 1989; von Holy et al., 1991; Yang and Ray, 1994). The genus/species of lactic acid bacteria responsible for spoilage, depend on the product composition (product-related flora) as well as the manufacturing site (house-related flora; Korkeala and Mäkelä, 1989).

	Borch, 113. “Lactic acid bacteria spoil refrigerated meat products by causing defects such as sour off-flavours, discolouration, gas production, slime production and decrease in PH.” Borch, 114; *see also, id.*, 115-17 (Section 4 Analysis of Spoilage). It is understood in the art that aerobic plate counts (“APC”) can test for spoilage organisms. *See. e.g.*, ICMSF 1986, 122 (“tests to be made (e.g., APC, *Salmonella*, Clostridium perfringens"), 132 (“However, aerobic plate counts (APC) can be used to monitor Good Commercial Practice (GCP), and criteria based on such examinations are a valuable aid to in-plant quality control.”).

TABLE 19

Sampling plans and recommended microbiological limits for raw meat[a]

Product[b]	Test	Method reference[c]	Case	Plan class	n	c	Limit per cm^2 or gram m	M
Carcass meat, before chilling	APC	118	1	3	5	3	10^5	10^6
Carcass meat, chilled	APC	118	1	3	5	3	10^6	10^7
Edible offal, chilled	APC	118	1	3	5	3	10^6	10^7
Carcass meat, frozen	APC	118	1	3	5	3	5×10^5	10^7
Boneless meat, frozen (beef, veal, pork, mutton)	APC	118	1	3	5	3	5×10^5	10^7
Comminuted meat, frozen	APC	118	1	3	5	3	10^6	10^7
Edible offal, frozen	APC	118	1	3	5	3	5×10^5	10^7

a **Not for use at port of entry** but for in-plant quality control. Refer to Section B for appropriate applications.
b Unfrozen carcasses and primal cuts, swab counts per cm^2; other meats and meat products, per gram.
c Refers to page number in ICMSF, 1978.

ICMSF 1986, 133 (Table 19 (annotated)).

Although for “quality control,” a POSITA would understand that "quality control" means testing for spoilage organisms could also be accomplished by this well-known method. It is disclosed within the prior art during the production lifecycle of beef production. ICMSF 1986, 134 (“The FAOIWHO Working Group (FAQ/WHO, 1979) considered that the only point at which APC could be used for chilled meat, to evaluate the hygienic conditions under which it was produced, was at the slaughterhouse, and because of its perishable nature it would not be possible to set

	APC limits for use in guidelines or specifications for chilled meats at port of entry."), 153 ("Monitoring for APC, E. coli, and Staph. aureus would give some indication of inadequate processing (heating, curing, smoking), postprocess contamination, or storage at temperatures above those recommended for frozen or refrigerated distribution and storage.") E. TEST PROCEDURES (a) *Aerobic plate counts* Comminute, blend, or stomach the various portions of meat composing the sample unit and withdraw the analytical unit. Combine the swabs to form an analytical unit, or treat each separately. Prepare dilutions and perform the APC test according to the directions given in ICMSF, 1978, p. 118. Spread plates are preferable to pour plates when examining raw meats. The temperature used for incubation of plates should be related to the material being examined. To estimate numbers of mesophilic contaminants on freshly dressed carcasses it is advisable to incubate plates at 35 or 37°C. For meat that has been held under chilled conditions, plates should be incubated at 25°C to include psychrotrophs and thus give a better indication of the microbial growth that has occurred. Counts at 20°C give similar results after a longer incubation time. ICMSF 1986, 136 (annotated). *See also*, Bhunia 4 ("Bacteria-specific generic rapid methods such ATP-based luminescence and total plate count (APC) are also needed for monitoring cleaning and hygienic practices employed in a processing plant."); Wheeler, 1606 (use of APC in a beef-processing plant); PR/HAACP, 38849 ("Various alternative indicator

	organisms were suggested, including generic E. coli (biotype I), total plate counts, Enterobacteriaceae, Total Viable Counts (TVC), and Aerobic Plate Counts (APC).”).
Claim 22	**Disclosure of ICMSF and Gombas**
The method of claim 1, wherein removing portions of each enriched separate test lot sample comprises removing equal volume portions of each enriched separate test lot sample.	ICMSF, alone or in combination with Gombas, discloses the method of claim 1, upon which this claim relies. See above re Claim 1. ICMSF, alone or in combination with Gombas, further discloses wherein removing portions of each enriched separate test lot sample comprises removing equal volume portions of each enriched separate test lot sample. ICMSF describes aseptically withdrawing and weighing sample units into the appropriate diluent. ICMSF, 231, 232. Gombas indicates “a sample was aseptically transferred to a sterile stomacher bag and blended with an equal amount of enrichment broth.” Gombas, 563. Gombas further indicates “five 1-ml samples of secondary enrichment broths were composited.” *Id.*, 564. Moreover, a POSITA would consider this to be standard practice. A POSITA would have aseptically removed equal volume portions of each enriched separate test lot sample.
Claim 23	**Disclosure of ICMSF and Gombas**
The method of claim 1, wherein testing of the modular composite sample, using a suitable detection assay comprises use of at least one of a DNA-based method, an immunochemistry-based method, and a biosensor-based method.	ICMSF, alone or in combination with Gombas, discloses the method of claim 1, upon which this claim relies. See above re Claim 1. ICMSF, alone or in combination with Gombas, further discloses wherein testing of the modular composite sample, using a suitable detection assay comprises use of at least one of a DNA-based method, an immunochemistry-based method, and a biosensor-based method. Gombas discloses use of DNA-based assays. “ The presence and levels of *L monocytogenes* in the samples were determined by rapid DNA-based assays in combination with culture methods.” Gombas, 559 (abstract).

Claim 24	Disclosure of ICMSF and Gombas
The method of claim 1, wherein the separate test lots are production lots of a meat or poultry product, comprising an assemblage of one or more meat or poultry specimens, respectively.	Claim 24 was cancelled during reexamination. IEH cannot assert a cancelled claim against National Beef. As such National Beef does not substantively address this claim.
Claim 27	**Disclosure of ICMSF and Gombas**
The method of claim 2, wherein product specimens are synonymous with product portions.	ICMSF, alone or in combination with Gombas, discloses the method of claim 2, upon which this claim relies. See above re Claim 2. ICMSF, alone or in combination with Gombas, further discloses wherein product specimens are synonymous with product portions. ICMSF teaches collecting portions or samples, which were known synonyms for product portions or specimens. ICMSF states that "[a] sample unit is a small portion of the lot[.]" ICMSF, 225. **12.1 INTRODUCTION** Many different types of samples are collected and submitted to the laboratory for analysis. Some are sample units from lots or consignments of foods or ingredients for lot acceptance determination. Others may be for investigational purposes to assess control of the environment, investigate the source of a problem, or validate a process. Some may have legal implications relative to a lawsuit or for regulatory compliance. This chapter briefly discusses some of the major factors that should be considered when collecting sample units, shipping them to a laboratory, preparing them for analysis, and analyzing them. A sample unit is a small portion of the lot in which the distribution of both numbers and types of microorganisms should be representative of the whole lot. This requirement is only completely fulfilled when the food and processing conditions result in a homogeneous distribution of microorganisms. This only occurs in well-agitated fluid products. In general, the distribution of microorganisms is presumed to be random. ICMSF, 225 (annotated).
Claim 28	**Disclosure of ICMSF and Gombas**

The method of claim 4, wherein the medium comprises a selective agent that will inhibit a competing non-target microbial agent or organism.	ICMSF, alone or in combination with Gombas, discloses the method of claim 4, upon which this claim relies. See above re Claim 4. ICMSF, alone or in combination with Gombas and secondary prior art references, further discloses wherein the medium comprises a selective agent that will inhibit a competing non-target microbial agent or organism. Additionally, Claim 28 recites what a POSITA would consider the well-known property of selective enrichment, inhibition of a competing non-target microorganism. DAoust describes "selective enrichment [that] inhibits growth of non-salmonellae and facilitates isolation of Salmonella on differential plating media." D'Aoust, 369. A POSITA would have been motivated to use an enrichment medium comprising a selective agent that inhibits a non-target agent or organisms as described by D'Aoust, for the test lot samples described by ICMSF and Gombas, to ensure an accurate test. A POSITA would understand that incubating samples at different temperature ranges for different target microorganisms would, effectively, inhibit the growth of other organisms. "Incubation temperature markedly affects productivity of selective enrichment broths by suppressing growth of competing microorganisms but not *Salmonella*." D'Aoust, 372. "This may be achieved by utilization of enrichment media that through various mechanisms may favor and enhance growth and multiplication of salmonellae while other gram-negative organisms such as coliforms, Proteus, and Pseudomonas are suppressed or inhibited." Fagerberg, 630.
Claim 29	**Disclosure of ICMSF and Gombas**
The method of claim 5, wherein the concentration step comprises at least one of	ICMSF, alone or in combination with Gombas, discloses the method of claim 5, upon which this claim relies. See above re Claim 5. ICMSF, alone or in combination

immunomagnetic bead separation, and affinity chromatography.	with Gombas, further discloses wherein the concentration step comprises at least one of immunomagnetic bead separation, and affinity chromatography. ICMSF discloses the use of "immunomagnetic beads to concentrate cells." ICMSF, 318. A POSITA would consider this a well-known concentration technique before the priority date. Fratamico discusses the rapid isolation of target microorganisms using an immunomagnetic separation method. Fratamico, 105. Fratamico discloses that use of immunomagnetic beads resulted in "an increase in the level of E. coli B1409 in the sample," as well as "an increase in the number of organisms recovered." *Id.*, 107. Additionally, Fratamico provides detailed instructions for preparing and using immunomagnetic bead separation to concentrate a target microbial agent or organism. Fratamico developed "[a] method employing immunomagnetic beads" to concentrate and thereby improve the isolation and detection of *E. coli. Id.* For example, Fratamico was able to use immunomagnetic beads to isolate *E. coli* strains from enrichment cultures of raw ground beef "when the initial inoculum level was 1 cfu g-1." *Id.*, 109. Fratamico further teaches that "[t]he percentage of bacteria recovered increased with increasing amounts of beads." *Id.*, 108. Consequently, Fratamico "demonstrates that immunomagnetic separation [using immunomagnetic beads] is a simple and rapid technique" for concentrating a microbe for isolation and detection. *Id.*, 109. These teachings of Fratamico would have motivated a POSA to modify the combination of ICMSF and Gombas and ICMSF and Hughes to use immunomagnetic beads to concentrate microbes in a modular composite sample to benefit from accurate tests. Use of immunomagnetic beads was already known in the art for concentrating during testing beef samples. "Tu et al. (78) detected pathogenic *E. coli* O157:H7 in beef hamburger with the use of IMS and ATP measurement. After 6 h of enrichment at 37°C, inoculated beef samples (0.9 ml each) were filtered to remove large particles

	prior to 10 min of incubation with ca. 10^5 magnetic beads coated with anti–*E. coli* O157:H7 (Dynabeads, Dynal Inc., Oslo, Norway). Following incubation, the magnetic beads were collected with a Dynal magnetic concentrator and resuspended in 0.9 ml of Trypticase soy broth for ATP analysis." Benoit, 1941. "Hudson et al. (26) used IMS to directly separate and concentrate L. monocytogenes from ham samples prior to detection by PCR. IMS was used on the basis of the commercial availability of anti-Listeria magnetic beads (Dynal Inc.) to avoid time-consuming cultural enrichment steps and to remove PCR inhibitors." *Id*. Cudjoe investigates the use of immunomagnetic beads in pooling methods. In a study designed to evaluate the performance of Dynabeads® Anti-Salmonella, immunomagnetic separation (IMS) followed by plating (IMS-Plating) proved far superior to the conventional ISO *Salmonella* methodology and the Modified Semi-solid Rappaport-Vassiliadis (MSRV) method. *Salmonella* species were isolated and detected from 135 out of the 180 diverse poultry samples by IMS analysis as against 98 by the conventional method and 33 by the MSRV technique. All results were confirmed biochemically and serologically. It appeared from the data generated that some of the salmonellae isolated using IMS were greatly inhibited in Rappaport-Vassiliadis enrichment broth at 42°C and to a lesser extent in selenite cystine broth at 37°C. A moderately selective plating medium like XLD proved to be better in isolating these possible sensitive wild-type strains of salmonellae than the more selective BGA. Cudjoe, Abstract.

	Samples. One hundred and eighty (180) diverse poultry samples, consisting of 40 pools of cloacal/faecal swabs (5 swabs/pool; 5 pools samples/flock) from eight different flocks, 10 raw chicken breast meat samples, 10 pooled egg shell samples, 60 raw chicken liver samples and 60 chicken neck skins sampled from the slaughter line, were used in this study. All samples were obtained from a poultry slaughter and processing plant in the Netherlands. A pool of five swab samples was pre-enriched in 10 ml of buffered peptone water (BPW; Difco Laboratories, Detroit, Michigan, USA) at 37°C for 16–20 h, whilst 25 g of each of the other samples above, were pre-enriched similarly in 225 ml of BPW and examined as detailed below. Cudjoe, 160.
Claim 30	**Disclosure of ICMSF and Gombas**
The method of claim 6, wherein the more sensitive protocol further comprises a concentration step to concentrate the microbial agent or organism, in combination with the same test protocol that is used to test the modular composite sample.	ICMSF, alone or in combination with Gombas, discloses the method of claim 6, upon which this claim relies. See above re Claim 6. ICMSF, alone or in combination with Gombas, further discloses wherein the more sensitive protocol further comprises a concentration step to concentrate the microbial agent or organism, in combination with the same test protocol that is used to test the modular composite sample. ICMSF discloses the use of "immunomagnetic beads to concentrate cells." ICMSF, 318. A POSITA would consider this a well-known technique before the priority date. Gombas discloses retesting using the same protocol. Gombas discusses the use of "biochemical confirmation" to confirm testing results. Gombas, 565. A POSITA would have recognized the concentration step could be employed to provide a more sensitive protocol for further testing. A POSITA would understand that retesting the non-pooled, singular samples is an inherently more sensitive process because the sample is not diluted with other

	samples in the testing pool. Therefore, the retesting of a singular sample is more concentrated and therefore, more sensitive. *See also, generally*, Jarvis. Fratamico discusses the rapid isolation of target microorganisms using an immunomagnetic separation method. Fratamico, 105. Fratamico discloses that use of immunomagnetic beads resulted in "an increase in the level of E. coli B1409 in the sample," as well as "an increase in the number of organisms recovered." *Id.*, 107. Additionally, Fratamico provides detailed instructions for preparing and using immunomagnetic bead separation to concentrate a target microbial agent or organism. Fratamico developed "[a] method employing immunomagnetic beads" to concentrate and thereby improve the isolation and detection of E. coli. *Id.* For example, Fratamico was able to use immunomagnetic beads to isolate *E. coli* strains from enrichment cultures of raw ground beef "when the initial inoculum level was 1 cfu g-1." *Id.*, 109. Fratamico further teaches that "[t]he percentage of bacteria recovered increased with increasing amounts of beads." *Id.*, 108. Consequently, Fratamico "demonstrates that immunomagnetic separation [using immunomagnetic beads] is a simple and rapid technique" for concentrating a microbe for isolation and detection. *Id.*, 109. These teachings of Fratamico would have motivated a POSITA to modify the combination of ICMSF and Gombas and ICMSF and Hughes to use immunomagnetic beads to concentrate microbes in a modular composite sample to benefit from accurate tests. Use of immunomagnetic beads was already known in the art for concentrating during testing beef samples. "Tu et al. (78) detected pathogenic *E. coli* O157:H7 in beef hamburger with the use of IMS and ATP measurement. After 6 h of enrichment at 37°C, inoculated beef samples (0.9 ml each) were filtered to remove large particles prior to 10 min of incubation with ca. 10^5 magnetic beads coated with anti–*E. coli* O157:H7 (Dynabeads, Dynal Inc., Oslo, Norway). Following incubation, the

	magnetic beads were collected with a Dynal magnetic concentrator and resuspended in 0.9 ml of Trypticase soy broth for ATP analysis." Benoit, 1941. "Hudson et al. (26) used IMS to directly separate and concentrate L. monocytogenes from ham samples prior to detection by PCR. IMS was used on the basis of the commercial availability of anti-Listeria magnetic beads (Dynal Inc.) to avoid time-consuming cultural enrichment steps and to remove PCR inhibitors." Benoit, 1941-42. Cudjoe investigates the use of immunomagnetic beads in pooling methods. In a study designed to evaluate the performance of Dynabeads® Anti-Salmonella, immunomagnetic separation (IMS) followed by plating (IMS-Plating) proved far superior to the conventional ISO *Salmonella* methodology and the Modified Semi-solid Rappaport-Vassiliadis (MSRV) method. *Salmonella* species were isolated and detected from 135 out of the 180 diverse poultry samples by IMS analysis as against 98 by the conventional method and 33 by the MSRV technique. All results were confirmed biochemically and serologically. It appeared from the data generated that some of the salmonellae isolated using IMS were greatly inhibited in Rappaport-Vassiliadis enrichment broth at 42°C and to a lesser extent in selenite cystine broth at 37°C. A moderately selective plating medium like XLD proved to be better in isolating these possible sensitive wild-type strains of salmonellae than the more selective BGA. Cudjoe, Abstract.

	Samples. One hundred and eighty (180) diverse poultry samples, consisting of 40 pools of cloacal/faecal swabs (5 swabs/pool; 5 pools samples/flock) from eight different flocks, 10 raw chicken breast meat samples, 10 pooled egg shell samples, 60 raw chicken liver samples and 60 chicken neck skins sampled from the slaughter line, were used in this study. All samples were obtained from a poultry slaughter and processing plant in the Netherlands. A pool of five swab samples was pre-enriched in 10 ml of buffered peptone water (BPW; Difco Laboratories, Detroit, Michigan, USA) at 37°C for 16–20 h, whilst 25 g of each of the other samples above, were pre-enriched similarly in 225 ml of BPW and examined as detailed below. Cudjoe, 160.
Claim 31	**Disclosure of ICMSF and Gombas**
The method of claim 17, wherein removing portions in step d) is removing equal portions, aseptically, so as to preclude contamination of the enriched separate single-unit production lot samples.	ICMSF, alone or in combination with Gombas, discloses the method of claim 17, upon which this claim relies. See above re Claim 17. ICMSF, alone or in combination with Gombas, further discloses wherein removing portions in step d) is removing equal portions, aseptically, so as to preclude contamination of the enriched separate single-unit production lot samples. ICMSF describes aseptically withdrawing and weighing sample units into the appropriate diluent. ICMSF, 231, 232. Gombas indicates “a sample was aseptically transferred to a sterile stomacher bag and blended with an equal amount of enrichment broth.” Gombas, 563. Gombas further indicates “five 1-ml samples of secondary enrichment broths were composited.” *Id.*, 564. Moreover, a POSITA would consider this to be standard practice. A POSITA would have aseptically removed equal volume portions of each enriched separate test lot sample.

Claim 32	Disclosure of ICMSF and Gombas
The method of claim 1, wherein the beef specimens are meat trim pieces.	ICMSF, alone or in combination with Gombas, discloses the method of claim 1, upon which this claim relies. See above re Claim 1. ICMSF, alone or in combination with Gombas, further discloses wherein the beef specimens are meat trim pieces. ICMSF indicates "[t]rimmings to be used for ground beef could be pretested for E. coli O157:H7 by various sampling plans." ICMSF, 323. ICMSF indicates "[r]esearch has validated that up to fifteen 25 g analytical units of ground beef or trimmings can be composited without significant loss in E. coli O157:H7 detection sensitivity." *Id.*, 327 *see also id.*, 226, 314, 320, and 324. ICMSF includes many other references to testing lots of beef or poultry, two of the most common types of meat in commerce. *Id.* at 114 (§ 6.3), 184–191 (§§ 10.2–10.6), 225–226 (§ 12.1), 285 (§ 16.1), 313–330 (Chapter 17). ICMSF is relevant to any kind of food product including beef or poultry.
Claim 33	**Disclosure of ICMSF and Gombas**
The method of claim 17, wherein the meat is that of beef, pork, sheep or bison.	ICMSF, alone or in combination with Gombas, discloses the method of claim 17, upon which this claim relies. See above re Claim 17. ICMSF, alone or in combination with Gombas, further discloses, wherein the meat is that of beef, pork, sheep or bison ICMSF indicates "[t]rimmings to be used for ground beef could be pretested for E. coli O157:H7 by various sampling plans." ICMSF, 323. ICMSF indicates "[r]esearch has validated that up to fifteen 25 g analytical units of ground beef or trimmings can be composited without significant loss in E. coli O157:H7 detection sensitivity." *Id.*, 327 *see also id.*, 226, 314, 320, and 324. ICMSF includes many other references to testing lots of beef or poultry, two of the most common types of meat in commerce. *Id.*, 114 (§ 6.3), 184–191 (§§ 10.2–10.6),

	225–226 (§ 12.1), 285 (§ 16.1), 313–330 (Chapter 17). ICMSF is relevant to any kind of food product including beef or poultry.

EXHIBIT A2

Invalidity of U.S. Patent No. 7,534,584 in light of ICMSF & Hughes

National Beef denies that any of its microbiological testing methods infringe any claim of U.S. Patent No. 7,534,584 ("the '584 Patent"). If the claims of the '584 Patent were construed so broadly as to cover the microbiological testing methods used by National Beef, they would be invalid at least in view of INT'L COMM'N ON MICROBIOLOGICAL SPEC'S FOR FOODS (ICMSF), MICROORGANISMS IN FOODS 7: MICROBIOLOGICAL TESTING IN FOOD SAFETY MANAGEMENT (Kluwer Academic/Plenum Publishers, 2002) ("ICMSF") and Denise Hughes et al., Salmonella in Foods—A New Enrichment Procedure for Use with the TECRA Salmonella Visual Immunoassay: Collaborative Study, 82 J. AOAC INT'L 634–647 (1999) ("Hughes"), which anticipates and/or discloses the limitations of the '584 Patent under at least pre-AIA 35 U.S.C. § 102(a), pre-AIA 35 U.S.C. § 102(b), pre-AIA 35 U.S.C. §§ 102(f)/(g), and/or pre-AIA 35 U.S.C. § 103. To the extent that ICMSF and/or Hughes do not expressly disclose one or more limitations of the asserted claims, as they would have to be construed to support IEH's claim of infringement, they discloses the limitations alone or in combination with one or more additional prior art references and/or the knowledge of one of ordinary skill in the art.

Claim 1	Disclosure of ICMSF and Hughes
[1pre] A method of sampling, testing and validating a plurality of operationally-linked test lots of beef for release into commerce, comprising:	To the extent the preamble is limiting, as discussed herein, ICMSF, alone or in combination with Hughes, discloses a method of sampling, testing and validating a plurality of operationally-linked test lots of beef for release into commerce. ICMSF includes many other references to testing lots of beef or poultry, two of the most common types of meat in commerce. *Id.*, 114 (§ 6.3), 184–191 (§§ 10.2–10.6), 225–226 (§ 12.1), 285 (§ 16.1), 313–330 (Chapter 17). ICMSF is relevant to any kind of food product including beef. Hughes discusses turkey (Hughes, Abstract), and a POSITA would be motivated to combine the teachings of ICMSF and Hughes as they both test meat for release into commerce. Hughes discloses testing turkey for Salmonella. "Three food types (. . . and raw turkey) were analyzed in the United States." Hughes, 634, 645, Table 8.

	Table 8 shows results from 12 collaborators for naturally contaminated raw turkey. For the 180 samples tested, using confirmed data, there was one false negative at the low level for the TECRA method. There were 3 false negatives at the low level and 3 false negatives at the high level for the cultural method. The incidence of false-positive results was very high for the cultural method, with a total of 41 false positives, compared with 5 for the TECRA method. It should, however, be taken into account that the comparison was with the method in BAM 7th Ed., not the current 8th Ed. The method for raw flesh food in the BAM 8th Ed. uses elevated temperature with RV and TT broth, which would be more selective. No significant differences ($p > 0.05$) were observed for the pairwise comparison of the proportion of positive samples for the TECRA method with that for the reference method. Hughes, 646. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Moreover, the recitation of a lot being validated for "release . . . into commerce" is not limiting. Prosecution History for Reexamination Control No. 95/011,294 of the '584 Patent.
[1a] a) collecting a plurality of portions from each of a plurality of separate operationally-	ICMSF, alone or in combination with Hughes, discloses collecting a plurality of portions from each of a plurality of separate operationally-linked test lots of beef,

linked test lots of beef, each to be validated for release into commerce, the test lots each comprising an assemblage of one or more specimens from which the portions are collected, wherein each test lot is separately sampled to conform to a statistical-based sampling plan specifying, for a particular microbial agent or organism, a number of portions to be taken sufficient to provide for validation and release of the test lot into commerce;	each to be validated for release into commerce, the test lots each comprising an assemblage of one or more specimens from which the portions are collected, wherein each test lot is separately sampled to conform to a statistical-based sampling plan specifying, for a particular microbial agent or organism, a number of portions to be taken sufficient to provide for validation and release of the test lot into commerce. "Many different types of samples are collected and submitted to the laboratory for analysis. Some are sample units from lots or consignments of food or ingredients for lot acceptance determination. . . . A sample unit is a small portion of the lot in which the distribution of both numbers and types of microorganisms should be representative of the whole lot." ICMSF, 255. The lots may be beef or poultry. *Id.*, 184-91, 226, 230. Thus, ICMSF discloses collecting a plurality of samples or sample units (other synonyms include portions, specimens, and sub-samples) from lots of beef or poultry. ICMSF describes collecting from a lot or lots: "If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." *Id.*, 119. Additionally, ICMSF indicates a consignment may be "a very large quantity of food" and may be "composed of several lots, representing for example, different days of production from the same plant." *Id.*, 119, 120; *see also* AAPA #3-#5.

6.11 WHAT IS A REPRESENTATIVE SAMPLE?

A *representative sample* reflects, as far as is possible, the composition of the lot from which it is drawn.

How then should a representative sample be drawn? It is important to avoid bias and to draw a sufficient number of sample units to confidently make a judgment about a lot. Sampling at random is the universally recognized way of avoiding bias and is more reliable than consciously trying to draw the sample units from the various parts of a lot. The units (cartons or containers, particular weights of solid, or volumes of liquid) are drawn by using random numbers. There is, of course, no guarantee that a random sample has characteristics identical with those of the lot, but the randomness of sampling is the basis for calculation of the probability that a sample will give a biased result.

It is also possible, and may be desirable, to use a *stratified random sampling* approach, drawing a random sample of a given number of sample units from each *stratum* (e.g., each sublot or carton). The distribution of sample units on strata should correspond to the distribution of lot units on strata. This means that if each stratum contains the same quantity as every other one, the number of sample units per stratum should be the same. Otherwise, they should differ just as the proportions of the lot contained in the strata differ. Stratification is a device for handling known sources of variation and may be used where one has prior knowledge that the consignment is potentially not of uniform quality. A consignment might not be of uniform quality if portions of it are shipped in different vehicles or in different holds of a vessel, or if it is known that it is really composed of several lots representing, for example, different days of production from the same plant or different plants of the same company. The results for different strata should be assessed separately and then pooled if they appear to be homogeneous.

The calculation of acceptance probabilities is based on the assumption that random samples have been drawn and analyzed. Where physical or practical constraints prevent random sampling, such calculated probabilities may not be valid. Their reliability depends on how close reality approaches the ideal situation of random sampling. This fact should be borne in mind when subsequently interpreting results.

ICMSF, 119-20 (annotated).

6.10 WHAT IS A LOT?

Ideally, a lot is a quantity of food or food units produced and handled under uniform conditions. Under certain circumstances, it has been shown that the logarithms of the counts from a batch of food are likely to be normally distributed (Baird-Parker, 1980; Kilsby & Baird-Parker, 1983 [their Table 2]; Ingram & Roberts, 1976). The implication is that there is homogeneity within a lot, which, when considering microbial levels and distributions, rarely occurs in practice. In most instances, the distribution of microorganisms within lots of food is heterogeneous. Even in food factories, this heterogeneity makes the interpretation of sample results difficult, and it becomes even more so in circumstances where a lot is less well defined, such as in a consignment of a very large quantity of food.

It is therefore helpful if suppliers can be persuaded to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load).

If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment.

A lot should be composed of food produced with as little variation as possible for a given process or commodity. Because of the uncertainties in identifying a lot commercially, however, the use of the word *lot* in this book is usually in its statistical sense, as a collection of units of a product, the acceptability of which is determined by examining a sample drawn from it.

ICMSF, 118-19 (annotated).

	The ICMSF has defined a lot as follows: In the commercial sense, a lot is a quantity of food supposedly produced under identical conditions, all packages of which would normally bear a lot number that identifies the production during a particular time interval, and usually from a particular line, retort, or other critical processing unit. Statistically, a lot is considered as a collection of units of a product from which a sample is drawn to determine acceptability of the lot (ICMSF, 1986). In this example, the maximum size of a lot consists of all the ground beef produced on common equipment from cleanup to cleanup. Smaller lots could be established (e.g., hourly). ICMSF, 327 (annotated). This definition also reflects the common practice referred to in the '584 Patent, wherein "[p]referably, the producer defines and produces these single-unit lots in such a fashion as to maximize the information available[.]" '584 Patent, 12:46–48 (Example II). This corresponds with the specification of the '584 Patent which reflects the common practice of producers defining what constitutes a lot grouped for traceability purposes. '584 Patent, 2:60-3:3, 11:12-16 (AAPA #3), and 12:46-49. Thus, ICMSF discloses collecting a plurality of portions from separate lots. ICMSF describes a lot as "a collection of units of a product." IMCSF, 119. ICMSF further indicates a lot has been defined as: "a quantity of food supposedly produced under identical conditions, all packages of which would normally bear a lot number that identifies the production during a particular time interval, and usually from a particular line, retort, or other critical processing unit. Statistically, a lot is considered as a collection of units of a product from which a sample is drawn to determine acceptability of the lot." ICMSF, 327. ICMSF advocates use of a "statistically based sampling plan." *Id.*, 113-15, 119-21, 320, 327, 328. In the sampling plans, "*n* is the number of sample units chosen separately and independently, and . . . *c* is the maximum allowable number of sample

	units yielding a positive result." *Id.*, 115. ICMSF discloses a wide range for the number of sample units (portions) for a particular microbial agent or organism. For example, ICMSF discusses collecting 15, 30, 60 or 100 sample units from a lot. *Id.*, 320, 326. **6.5 THE SAMPLING PLAN** The sample units so drawn will, after examination, yield results that will be compared with certain criteria to reach a decision as to whether the entire lot should be accepted or rejected (see section 6.9 for explanation of rejection). The particular choice of sampling procedure and the decision criteria make up the *sampling plan*. Proper drawing of samples is essential if the decision making criteria of the sampling plan are to give unbiased results. By sampling randomly, we are able to reduce the risk of making biased decisions. A simple example of a sampling plan follows. Take 10 sample units of a food from a lot and subject them to appropriate laboratory procedure to test for the presence or absence of a microorganism. If two or fewer of the 10 sample units show presence of the organism, that is, give a positive result, then the whole lot of food is acceptable (relative to this organism). But if three or more give a positive result, the whole lot is to be rejected. This plan is described by the use of two terms $n = 10$ and $c = 2$, where n is the number of sample units chosen separately and independently, and where c is the maximum allowable number of sample units yielding a positive result. ICMSF, 115 (annotated).

	6.1 INTRODUCTION It is important to recognize that the management of food safety using the approaches outlined in the first five chapters, based on controlling hazards through Good Hygienic Practices (GHP) and Hazard Analysis Critical Control Point (HACCP), is much more effective than trying to ensure safety by lot acceptance through end-product testing. This chapter discusses the concepts of probability and sampling that determine the limitations of end-product testing. Those concepts form the basis of the design of statistically based sampling plans (Chapter 7) and the establishment of cases (Chapter 8). In Chapters 6 and 7, it is assumed that the microbiological test will give an absolutely accurate result, with no false positives or false negatives. This is not a realistic assumption, and problems arising from sampling, sample transport and preparation for analysis, and the imprecision of microbiological methods are addressed in Chapters 10 and 12 and elsewhere in the text. ICMSF, 113 (annotated); *see also id.*, 115–21 (§§ 6.4–6.13), 319–20 (§ 17.2.3.3), and 326–30 (§§ 17.5.3.1–17.6). ICMSF discusses the number of portions to be taken sufficient for validation of a particular microorganism including *Salmonella* (*id.*, 276 (§ 15.3.3), 282 (§ 15.6.3)); *Listeria* (*id.*, 309 (§ 16.6.3)), and *E. coli* (*id.*, 318–21 (§§ 17.2.3.2–17.2.3.4), 326 (§ 17.5.3.1)). IEH's AAPA discloses statistical-based sampling plans taking 10-60 sample units. '584 Patent, 5:57-6:6 (AAPA #1, #2, #5, and #6). Determining meat is safe for consumption so it is "commercially possible to market" the meat, is the well-understood point of testing meat and is taught by ICMSF. ICMSF, 185-89. Moreover, the recitation of a lot being validated for "release . . . into commerce" is not limiting. Prosecution History for Reexamination Control No. 95/011,294 of the '584 Patent. "Operationally-linked" is simply grouping like items, which is also disclosed by ICMSF. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment."

	ICMSF, 119. It is therefore helpful if suppliers can be persuaded to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load). If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment. ICMSF, 119 (annotated).
[1b] b) combining the collected conforming plurality of portions corresponding to each of the separate test lots to provide a corresponding set of separate test lot samples, wherein each separate test lot sample is attributed to a particular corresponding separate test lot;	ICMSF, alone or in combination with Hughes, discloses combining the collected conforming plurality of portions corresponding to each of the separate test lots to provide a corresponding set of separate test lot samples, wherein each separate test lot sample is attributed to a particular corresponding separate test lot. ICMSF states: "To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units of the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. . . . This finding suggests that considerable reductions may be made in the cost of laboratory analysis by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, n, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort." ICMSF, 187.

	To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. A laboratory investigation undertaken for ICMSF (Silliker & Gabis, 1973) indicated that different sized analytical units drawn from a single well-mixed sample unit of dried food give very similar results. A subsequent investigation (Gabis & Silliker, 1974) using foods of high moisture content (including eggs, poultry meat, meat, and meat products) showed comparable reliability in the use of composite analytical units; for example, examination of three composites of 20 × 25 g analytical units gave the same result as examination of all 60 analytical units individually (the total amount examined being 1500 g in both instances). This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n*, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort. ICMSF, 187 (annotated).

10.5.1 Conceivable Alternative Plans

As indicated above, for an infectious agent such as *Salmonella*, there are strong reasons for adopting sampling plans in which $c = 0$:

1. It is philosophically objectionable to some people to accept a lot containing a recognized pathogen, regardless of how the food is to be used in a manufacturing process or by the consumer.
2. There is the very great technical advantage of combining many analytical units for bacteriological examination in systems with $c = 0$, because a single positive decides the outcome.
3. The plan with $c = 1$ (or more) would always require the examination of (many) more analytical units, for equal probability of acceptance, than a plan with $c = 0$.
4. Even if the indicated probabilities of plans with $c = 0$ versus $c = 1$ are the same, their implications are not. For example, when a lot is accepted with the plan $n = 60$, $c = 0$, it is possible (though not certain) that the lot may not be contaminated at all. But using the plan $n = 95$, $c = 1$ and accepting one positive, it is known that the lot certainly is contaminated (though the probability that a lot with 5% of analytical units contaminated would be accepted is in both instances $P_a = 0.05$).

ICMSF, 188 (annotated). ICMSF also provides an example of "[u]sing a sampling plan involving n = 15 (25 g analytical units) and a composite of 375 g." *Id.*, 195 (§ 10.7.2). ICMSF also states "[f]or qualitative analyses, 25 g analytical units are frequently analyzed individually or as composites of 100 g, 200 g, or more." *Id.*, 232 (§ 12.5.2).

The ICMSF incorporates two references, Silliker & Gabis (1973) and Gabis & Silliker (1974). Silliker notes that "[c]ompositing of multiple samples (lumping) and wet compositing of pre-enrichment cultures have been tested as economical approaches to *Salmonella* control." Silliker, 475.

Silliker further notes that "[b]oth approaches are being used widely by the food industry in the United States." *Id.*.

	To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. A laboratory investigation undertaken for ICMSF (Silliker & Gabis, 1973) indicated that different sized analytical units drawn from a single well-mixed sample unit of dried food give very similar results. A subsequent investigation (Gabis & Silliker, 1974) using foods of high moisture content (including eggs, poultry meat, meat, and meat products) showed comparable reliability in the use of composite analytical units; for example, examination of three composites of 20 × 25 g analytical units gave the same result as examination of all 60 analytical units individually (the total amount examined being 1500 g in both instances). This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n*, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort. "There is the very great technical advantage of combining many analytical units for bacteriological examination." ICMSF, 188. ICMSF also describes composites prepared by combining "15 (25 g analytical units)" and "composites of 100 g, 200 g, or more." *Id.,* 195, 232; *see also* AAPA #5. ICMSF states, "All steps up to the analysis must be controlled . . . to allow reliable traceability." ICMSF, 227. ICMSF indicates lot samples are traceable to a particular corresponding lot: "It is therefore helpful . . . to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load). . . . Treating the individual production

	batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119.
[1c] c) incubating the set of separate test lot samples under conditions suitable to allow levels of a target microbial agent or organism that is present in one or more of the separate test lot samples to reach detectable levels and become uniform, or substantially uniform, throughout the respective one or more separate test lot samples, to provide a set of separate test lot samples enriched for the target microbial agent or organism;	ICMSF, alone or in combination with Hughes, discloses incubating the set of separate test lot samples under conditions suitable to allow levels of a target microbial agent or organism that is present in one or more of the separate test lot samples to reach detectable levels and become uniform, or substantially uniform, throughout the respective one or more separate test lot samples, to provide a set of separate test lot samples enriched for the target microbial agent or organism. ICMSF states: To reduce laboratory effort . . . it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. ICMSF, 187. Also, ICMSF describes preparing composites of analytical units then diluting "for qualitative (enrichment)" analysis. *Id.,* 231-32; *see also* AAPA #5, #7. For practical reasons, however, analytical units of 10 g up to 50 g are typical for quantitative methods and are diluted 1:10. For qualitative analyses, 25 g analytical units are frequently analyzed individually or as composites of 100 g, 200 g, or more. ICMSF, 232 (annotated). Preparation of the first dilution, either for qualitative (enrichment) or quantitative (direct) analyses, can have a marked impact on the microbial population in the sample. This is often due to changes in the physico-chemical characteristics of the suspension as compared to the food matrix. Where the food does not confer protection, the composition of the diluent is important to prevent or minimize shock/injury to the microorganisms that can result from rapid changes in the ionic concentrations. ICMSF, 232 (annotated). ICMSF also describes strategic use of non-selective enrichment to resuscitate microorganisms and selective enrichments for growing microorganisms. *Id.*, 232–33

	(§ 12.6); *see e.g.*, *id.,* 233 ("use of nonselective medium . . . to enhance recovery of injured or stressed cells."). ICMSF § 12.6 also describes optimizing the incubation period. S*ee e.g.*, *id.,* 232. It was well known long before the priority date to use non-selective enrichments after dry compositing and prior to wet compositing as evidenced by Price, Silliker, Gabis, and MLG. A POSITA would also know to use selective enrichments after dry compositing and prior to wet compositing as disclosed by ICMSF and Hughes. A POSITA would find that incubating so microorganisms are detectable and at least substantially uniform in the selective enrichment broth was considered the standard practice in official methods because the methods otherwise fail. For example, D'Aoust states "standard methodology recommends incubation of selective enrichment broths." D'Aoust, 372. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638.

	(a) *Pre-enrichment.*—Pre-enrich product in noninhibitory broth to initiate growth of *Salmonella* spp. Methods used may vary with product and should be performed as indicated in **967.26A** or BAM, 7th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, chapter 5, section C. Pre-enrichment broths should be incubated at 35°–37°C. (b) *Selective enrichment.*—Transfer 0.1 mL incubated pre-enrichment mixtures to Rappaport-Vassiliadis R10 broth (9.9 mL) and 1 mL to TT broth (9 mL). For all foods other than raw foods or foods having a high microbial load, incubate 6–8 h at 41°–43°C. Selective enrichments of raw foods or foods having a high microbial load must be incubated 16–20 h at 41°–43°C. An air incubator is suitable for this purpose. Hughes, 636 (F. Preparation of Sample). Hughes instructs that, after pre-enrichment, portions of the "incubated pre-enrichment mixtures" should be transferred to two separate selective enrichment broths, Rappaport-Vassiliadis R10 broth and TT broth, and incubated for 16-20 hours at 41°-43°C. Hughes, 636, 638. Additionally, Hughes instructs that these selective broths should be removed from incubation and mixed by hand or by Vortex mixer. Hughes, 638. Hughes further instructs to then remove 1 mL from the TT tube and 1 mL from the Rappaport-Vassiliadis R10 tube and transfer to separate, sterile M broths. *Id.,* 636, 638. The separate M broths are then incubated ("16-20 h at 35°-37°C" for "all foods other than raw foods or foods having a high microbial load" or "6 h at 35°-37°C" for "raw foods or foods having a high microbial load"). *Id.* A POSITA would find that the long incubation at an elevated temperature and mixing ensures the target microbial agent or organism reaches detectable levels and

	becomes substantially uniform throughout the separate lot samples.
[1d] d) removing portions of each enriched separate test lot sample, and combining the removed portions to provide a modular composite sample; and	ICMSF describes compositing the enriched lot samples: "it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. . . . This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n,* with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort." ICMSF, 187. *See also* AAPA #5-#7. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Hughes also discloses a method of removing portions of each enriched separate test lot sample, and combining the removed portions to provide a modular composite sample. Hughes instructs to "[c]ombine equal volumes (0.5-1 mL) from each M broth tube in a clean screw-cap tube." Hughes, 638.

	that has been warmed to 35°–37°C. Also remove 1 mL from Rappaport-Vassiliadis R10 tube and transfer to a separate tube of prewarmed M broth (10 mL). For all foods other than raw foods or foods having a high microbial load, incubate M broth tubes 16–20 h at 35°–37°C and return selective enrichment broth tubes to 41°–43°C incubator and incubate for additional 16–18 h. For raw foods or foods having a high microbial load, incubate M broth tubes 6 h at 35°–37°C and return selective enrichment broth tubes to 41°–43°C and incubate for an additional 6 h. (**d**) *Preparation of sample for EIA analysis.*—Remove M broth tubes from incubation and mix tubes by hand or Vortex mixer. Combine equal volumes (0.5–1 mL) from each M broth tube in a clean screw-cap tube and heat in boiling H_2O bath or in flowing steam 15 min. Refrigerate (2°–8°C) remaining M broth and TT and Rappaport-Vassiliadis R10 tubes from (**c**) for culture confirmation of any EIA-positive samples (*see* **967.26B, 967.26C, 967.27** and **967.28**). Cool heated M broths to 25°–37°C prior to analysis. Hughes, 638. Hughes 2003 confirms the pooling of enrichments. Hughes 2003, 775-76.
[1e.1] e) testing of the modular composite sample, using a suitable detection assay, for the target microbial agent or organism,	ICMSF describes testing enrichment cultures of composite samples with suitable detection assays (ICMSF, 187) and "presence/absence tests" (ICMSF, 233). Suitable detection assays were established in Silliker and Gabis as discussed in ICMSF. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of

	sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Hughes states testing of the composite sample is "by an enzyme immunoassay (EIA) method." Hughes, 635, 638. These assay types were "art recognized." '584 Patent, 2:54-58 (AAPA #7).
[1e.2] wherein when such testing is negative all of said separate test lots are validated for release into commerce, and	ICMSF describes correlating lots in a consignment with lot samples so a negative or positive test result enables a lot to be validated or rejected. ICMSF, 118-19. ICMSF describes sampling plans in which "c is the maximum allowable number of sample units yielding a positive result." ICMSF, 115. For many scenarios, the requirement is c = 0, which indicates the testing is negative. *Id.*, 118, 183-90, 309, 325, 326. A negative test result (*id.*, 113-15, 325, 326) is the expected and desired outcome to determine meat is not contaminated and is safe for consumption (*id.*, 184-89) such that a lot is validated and it is "commercially possible to market" (*id.*, 185) the meat.

6.10 WHAT IS A LOT?

Ideally, a lot is a quantity of food or food units produced and handled under uniform conditions. Under certain circumstances, it has been shown that the logarithms of the counts from a batch of food are likely to be normally distributed (Baird-Parker, 1980; Kilsby & Baird-Parker, 1983 [their Table 2]; Ingram & Roberts, 1976). The implication is that there is homogeneity within a lot, which, when considering microbial levels and distributions, rarely occurs in practice. In most instances, the distribution of microorganisms within lots of food is heterogeneous. Even in food factories, this heterogeneity makes the interpretation of sample results difficult, and it becomes even more so in circumstances where a lot is less well defined, such as in a consignment of a very large quantity of food.

It is therefore helpful if suppliers can be persuaded to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load).

If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment.

A lot should be composed of food produced with as little variation as possible for a given process or commodity. Because of the uncertainties in identifying a lot commercially, however, the use of the word *lot* in this book is usually in its statistical sense, as a collection of units of a product, the acceptability of which is determined by examining a sample drawn from it.

ICMSF, 118-19 (annotated).

	Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Because Hughes explains the steps to be taken when testing is positive (*see* Claim 1 step (e)(3), below), a POSITA would also expect negative testing results. Hughes, 638. Determining meat is safe for consumption so it may be released into commerce is the well-understood point of testing meat. Moreover, the recitation of a lot being validated for "release . . . into commerce" is not limiting. Prosecution History for Reexamination Control No. 95/011,294 of the '584 Patent.
[1e.3] wherein when such testing is positive, each of the individual separate test lots are validated for release into commerce, according to the statistical-based sampling plan, by further testing of a portion of the respective enriched separate test lot sample using the same or a more sensitive protocol and obtaining a negative test result for each test lot,	The combination of ICMSF and Hughes describes retaining a portion of the enriched lot samples (steps (b)-(d)). "Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119. "There is often a desire to retest retained samples." ICMSF, 191.

	If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment. ICMSF, 119 (annotated). ICMSF discloses validating for release into commerce (ICMSF, 113–15 (§§ 6.2–6.6), 118–19 (§§ 6.9–6.10), 184–85 (§ 10.2), 325–27 (§§ 17.5.3–17.5.3.4); *see also id.*, 186–89 (§ 10.4–10.6)) according to a statistically-based sampling plan (*id.*, 113 (§ 6.1), 115–18 (§§ 6.4–6.8), 184 (§ 10.2), 319–21 (§§ 17.2.3.3–17.2.3.4), 326–30 (§§ 17.5.3.1–17.6)), as described regarding steps (a) and (e)(2). Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Hughes explicitly teaches retention of the enriched test lot samples for further testing after a positive result. Hughes provides instructions to refrigerate the "remaining M broth and TT and Rappaport-Vassiliadis R10 tubes [that were enriched] for culture confirmation of any EIA-positive samples." Hughes, 638. Hughes refers to the results as the "confirmed positive samples" (Hughes, 635) and also states that diagnostic reagents are "necessary for culture confirmation of presumptive EIA tests." Hughes, 636. Hughes indicates "[e]nrichment broths and M broths from samples positive by an enzyme immunoassay (EIA) method must be streaked on selective media[.]" *Id.*, 635.

	Culture confirmation using streaking is more sensitive than the EIA method, as was commonly understood and confirmed by the teachings of D'Haese. A POSITA would have recognized the concentration step could be employed to provide a more sensitive protocol for further testing. A POSITA would understand that retesting the non-pooled, singular samples is an inherently more sensitive process because the sample is not diluted with other samples in the testing pool. Therefore, the retesting of a singular sample is more concentrated and therefore, more sensitive. *See also, generally*, Jarvis. Each sample was analyzed by the BAM method (7th Ed.) and the TSVIA method using the enrichment protocol described in section F of the method, *Preparation of Sample*. Equal portions (0.5–1 mL) from each of the 2M broths arising from the 2 selective-enrichment broths were combined in a small screw-capped tube and heat treated in boiling water for 15 min prior to performing the TSVIA. Confirmation for the immunoassay was by streaking from the M broth culture onto selective agar, as specified in the BAM reference culture method. Hughes, 635. Moreover, the recitation of a lot being validated for "release . . . into commerce" is not limiting. Prosecution History for Reexamination Control No. 95/011,294 of the '584 Patent. A POSITA would understand that retesting the non-pooled, singular samples is an inherently more sensitive process because the sample is not diluted with other samples in the testing pool. Therefore, the retesting of a singular sample is more

	concentrated and therefore, more sensitive. *See also, generally*, Jarvis.
[1e.4] wherein the enrichment in step c), prior to removing portions in step d), is sufficient to enable said validation of individual separate test lots with no requirement for further enrichment thereof, and with no requirement for further sampling in a).	Hughes explicitly indicates portions of the enrichment cultures are retained for further testing; thus Hughes has no need for further sampling. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. ICMSF and Hughes disclose selective enrichment so further enrichment is unnecessary. Also, as discussed regarding step (e)(3), Hughes explicitly indicates portions of the enrichment cultures are retained for further testing; thus Hughes has no need for further sampling. The same would be expected for enrichment cultures in ICMSF because retaining samples at each process stage was considered standard practice for a POSITA. in flowing steam 15 min. Refrigerate (2°–8°C) remaining M broth and TT and Rappaport-Vassiliadis R10 tubes from (c) for culture confirmation of any EIA-positive samples (*see* **967.26B, 967.26C, 967.27** and **967.28**). Cool heated M broths to 25°–37°C prior to analysis. Hughes, 638 (annotated). Thus, the negative limitation of Claim 1 step (e)(4) is disclosed by and is obvious based on the combination of ICSMF and Hughes. *Id.*
Claim 2	**Disclosure of ICMSF and Hughes**

The method of claim 1, wherein each of the test lots is a single-unit production lot, comprised of operationally-linked product specimens.	ICMSF, alone or in combination with Hughes, discloses the method of claim 1, upon which this claim relies. See above re Claim 1. ICMSF, alone or in combination with Hughes, further discloses wherein each of the test lots is a single-unit production lot, comprised of operationally-linked product specimens. The specification of the '584 Patent indicates a test lot is a single-unit production lot ('584 Patent, 5:21-32) such as a single combo, bin. pallet, or segment of production ('584 Patent, 7:28-36; *see also* AAPA #3 and #4). ICMSF teaches collecting from "individual production batches as lots" (ICMSF, 119) and "[a] lot is considered as a collection of units" (ICMSF, 327). "Operationally-linked" is simply grouping like items, which is also disclosed by ICMSF. "Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119.
Claim 3	**Disclosure of ICMSF, Hughes, and Stewart**
The method of claim 1, further comprising incubating the modular composite sample of d), prior to testing in e).	ICMSF, alone or in combination with Hughes, discloses the method of claim 1, upon which this claim relies. See above re Claim 1. ICMSF, alone or in combination with Hughes, further discloses, further comprising incubating the modular composite sample of d), prior to testing in e). Hughes describes incubating an enriched test lot sample. Hughes, 636, 638. Claim 1 is disclosed by ICMSF and Hughes. Stewart discloses that after the selective enrichment in Rappaport-Vassiliadis (RV) broth and tetrathionate (TT) broths, the two selective enrichments were combined into a single sample for further incubation and analysis. "[P]ost-enrichment by transfer and combination of 0.5 mL RV and 1 mL TT into a single tube containing

	10 mL prewarmed trypticase soy broth supplemented with novobiocin (TSB + n) at 10 µg/mL with incubation for 16–20 h at 42°C." Stewart, 398. Hughes also discloses an incubation protocol. "For the previous AOAC studies on TSVIA, a cultural protocol using selective enrichment in selenite cystine (SC) and tetrathionate (TT) broths at 35°C was used. However, there has been a recent trend to use elevated incubation temperatures and to replace SC broth with the Rappaport-Vassiliadis (RV) medium (2)." Hughes, 634. A POSITA would have been motivated to incubate the pooled modular composite sample of Gombas or Hughes, as taught by Stewart, for reliable testing results.
Claim 4	**Disclosure of ICMSF and Hughes**
The method of claim 1, wherein incubating each separate test lot sample in c) comprises incubating each separate test lot sample with enrichment medium suitable to enrich for the target microbial agent or organism, and wherein removing in d) comprises removing a portion of the enrichment medium from each enriched separate test lot sample and combining the removed portions to provide a modular composite sample.	ICMSF, alone or in combination with Hughes, discloses the method of claim 1, upon which this claim relies. See above re Claim 1. ICMSF, alone or in combination with Hughes, further discloses wherein incubating each separate test lot sample in c) comprises incubating each separate test lot sample with enrichment medium suitable to enrich for the target microbial agent or organism, and wherein removing in d) comprises removing a portion of the enrichment medium from each enriched separate test lot sample and combining the removed portions to provide a modular composite sample. ICMSF describes the well-known benefits of combining enrichment cultures from analytical units from lots in a consignment.

	To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. A laboratory investigation undertaken for ICMSF (Silliker & Gabis, 1973) indicated that different sized analytical units drawn from a single well-mixed sample unit of dried food give very similar results. A subsequent investigation (Gabis & Silliker, 1974) using foods of high moisture content (including eggs, poultry meat, meat, and meat products) showed comparable reliability in the use of composite analytical units; for example, examination of three composites of 20 × 25 g analytical units gave the same result as examination of all 60 analytical units individually (the total amount examined being 1500 g in both instances). This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n*, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort. ICMSF, 187. Hughes describes incubating an enriches test lot sample. Hughes 636, 638.
Claim 5	**Disclosure of ICMSF and Hughes**
The method of claim 1, wherein testing of the modular composite sample, using a suitable detection assay further comprises a concentration step to concentrate the microbial agent or organism.	ICMSF, alone or in combination with Hughes, discloses the method of claim 1, upon which this claim relies. See above re Claim 1. ICMSF, alone or in combination with Hughes, further discloses wherein testing of the modular composite sample, using a suitable detection assay further comprises a concentration step to concentrate the microbial agent or organism. ICMSF discloses the use of "immunomagnetic beads to concentrate cells." ICMSF, 318. A POSITA would consider this a well-known technique before the priority date. Fratamico discusses the rapid isolation of target microorganisms using an immunomagnetic separation method. Fratamico, 105. Fratamico discloses that use of immunomagnetic beads resulted in "an increase in the level of E. coli B1409 in the sample," as well as "an increase in the number of organisms recovered." *Id.*, 107.

	Additionally, Fratamico provides detailed instructions for preparing and using immunomagnetic bead separation to concentrate a target microbial agent or organism. Fratamico developed "[a] method employing immunomagnetic beads" to concentrate and thereby improve the isolation and detection of E. coli. *Id.* For example, Fratamico was able to use immunomagnetic beads to isolate *E. coli* strains from enrichment cultures of raw ground beef "when the initial inoculum level was 1 cfu g-1." *Id.*, 109. Fratamico further teaches that "[t]he percentage of bacteria recovered increased with increasing amounts of beads." *Id.*, 108. Consequently, Fratamico "demonstrates that immunomagnetic separation [using immunomagnetic beads] is a simple and rapid technique" for concentrating a microbe for isolation and detection. *Id.*, 109. These teachings of Fratamico would have motivated a POSA to modify the combination of ICMSF and Gombas and ICMSF and Hughes to use immunomagnetic beads to concentrate microbes in a modular composite sample to benefit from accurate tests. Use of immunomagnetic beads was already known in the art for concentrating during testing beef samples. "Tu et al. (78) detected pathogenic *E. coli* O157:H7 in beef hamburger with the use of IMS and ATP measurement. After 6 h of enrichment at 37°C, inoculated beef samples (0.9 ml each) were filtered to remove large particles prior to 10 min of incubation with ca. 10^5 magnetic beads coated with anti–*E. coli* O157:H7 (Dynabeads, Dynal Inc., Oslo, Norway). Following incubation, the magnetic beads were collected with a Dynal magnetic concentrator and resuspended in 0.9 ml of Trypticase soy broth for ATP analysis." Benoit, 1941. "Hudson et al. (26) used IMS to directly separate and concentrate L. monocytogenes from ham samples prior to detection by PCR. IMS was used on the basis of the commercial availability of anti-Listeria magnetic beads (Dynal Inc.) to avoid time-

	consuming cultural enrichment steps and to remove PCR inhibitors." Benoit, 1941-42. Cudjoe investigates the use of immunomagnetic beads in pooling methods. In a study designed to evaluate the performance of Dynabeads® Anti-Salmonella, immunomagnetic separation (IMS) followed by plating (IMS-Plating) proved far superior to the conventional ISO *Salmonella* methodology and the Modified Semi-solid Rappaport-Vassiliadis (MSRV) method. *Salmonella* species were isolated and detected from 135 out of the 180 diverse poultry samples by IMS analysis as against 98 by the conventional method and 33 by the MSRV technique. All results were confirmed biochemically and serologically. It appeared from the data generated that some of the salmonellae isolated using IMS were greatly inhibited in Rappaport-Vassiliadis enrichment broth at 42°C and to a lesser extent in selenite cystine broth at 37°C. A moderately selective plating medium like XLD proved to be better in isolating these possible sensitive wild-type strains of salmonellae than the more selective BGA. Cudjoe, Abstract. *Samples*. One hundred and eighty (180) diverse poultry samples, consisting of 40 pools of cloacal/faecal swabs (5 swabs/pool; 5 pools samples/flock) from eight different flocks, 10 raw chicken breast meat samples, 10 pooled egg shell samples, 60 raw chicken liver samples and 60 chicken neck skins sampled from the slaughter line, were used in this study. All samples were obtained from a poultry slaughter and processing plant in the Netherlands. A pool of five swab samples was pre-enriched in 10 ml of buffered peptone water (BPW; Difco Laboratories, Detroit, Michigan, USA) at 37°C for 16–20 h, whilst 25 g of each of the other samples above, were pre-enriched similarly in 225 ml of BPW and examined as detailed below. Cudjoe, 160.
Claim 6	**Disclosure of ICMSF and Hughes**

The method of claim 1, wherein testing in e) of the modular composite sample is positive, and wherein the individual separate test lot samples are further tested, such further testing comprises the more sensitive protocol.	ICMSF, alone or in combination with Hughes, discloses the method of claim 1, upon which this claim relies. See above re Claim 1. ICMSF, alone or in combination with Hughes, further discloses wherein testing in e) of the modular composite sample is positive, and wherein the individual separate test lot samples are further tested, such further testing comprises the more sensitive protocol. ICMSF discloses the use of "immunomagnetic beads to concentrate cells." ICMSF, 318. A POSITA would consider this a well-known technique before the priority date. Hughes uses an EIA method followed by a more sensitive protocol—streaking on selective media: Each sample was analyzed by the BAM method (7th Ed.) and the TSVIA method using the enrichment protocol described in section F of the method, *Preparation of Sample*. Equal portions (0.5–1 mL) from each of the 2M broths arising from the 2 selective-enrichment broths were combined in a small screw-capped tube and heat treated in boiling water for 15 min prior to performing the TSVIA. Confirmation for the immunoassay was by streaking from the M broth culture onto selective agar, as specified in the BAM reference culture method. Hughes, 635. A POSITA would find that modifying Hughes based on D'Haese ensures a more sensitive test is used for further testing. A POSITA would have recognized the concentration step could be employed to provide a more sensitive protocol for further testing.

	A POSITA would understand that retesting the non-pooled, singular samples is an inherently more sensitive process because the sample is not diluted with other samples in the testing pool. Therefore, the retesting of a singular sample is more concentrated and therefore, more sensitive. *See also, generally*, Jarvis. Fratamico discusses the rapid isolation of target microorganisms using an immunomagnetic separation method. Fratamico, 105. Fratamico discloses that use of immunomagnetic beads resulted in "an increase in the level of E. coli B1409 in the sample," as well as "an increase in the number of organisms recovered." *Id.*, 107. Additionally, Fratamico provides detailed instructions for preparing and using immunomagnetic bead separation to concentrate a target microbial agent or organism. Fratamico developed "[a] method employing immunomagnetic beads" to concentrate and thereby improve the isolation and detection of E. coli. *Id.* For example, Fratamico was able to use immunomagnetic beads to isolate *E. coli* strains from enrichment cultures of raw ground beef "when the initial inoculum level was 1 cfu g-1." *Id.*, 109. Fratamico further teaches that "[t]he percentage of bacteria recovered increased with increasing amounts of beads." *Id.*, 108. Consequently, Fratamico "demonstrates that immunomagnetic separation [using immunomagnetic beads] is a simple and rapid technique" for concentrating a microbe for isolation and detection. *Id.*, 109. These teachings of Fratamico would have motivated a POSA to modify the combination of ICMSF and Gombas and ICMSF and Hughes to use immunomagnetic beads to concentrate microbes in a modular composite sample to benefit from accurate tests. Use of immunomagnetic beads was already known in the art for concentrating during testing beef samples. "Tu et al. (78) detected pathogenic *E. coli* O157:H7 in beef hamburger with the use of IMS and ATP measurement. After 6 h of enrichment at 37°C, inoculated beef samples (0.9 ml each) were filtered to remove large particles prior to 10 min of incubation with ca. 10^5 magnetic beads coated with anti–*E. coli*

	O157:H7 (Dynabeads, Dynal Inc., Oslo, Norway). Following incubation, the magnetic beads were collected with a Dynal magnetic concentrator and resuspended in 0.9 ml of Trypticase soy broth for ATP analysis." Benoit, 1941. "Hudson et al. (26) used IMS to directly separate and concentrate L. monocytogenes from ham samples prior to detection by PCR. IMS was used on the basis of the commercial availability of anti-Listeria magnetic beads (Dynal Inc.) to avoid time-consuming cultural enrichment steps and to remove PCR inhibitors." Benoit, 1941-42. Cudjoe investigates the use of immunomagnetic beads in pooling methods. In a study designed to evaluate the performance of Dynabeads® Anti-Salmonella, immunomagnetic separation (IMS) followed by plating (IMS-Plating) proved far superior to the conventional ISO *Salmonella* methodology and the Modified Semi-solid Rappaport-Vassiliadis (MSRV) method. *Salmonella* species were isolated and detected from 135 out of the 180 diverse poultry samples by IMS analysis as against 98 by the conventional method and 33 by the MSRV technique. All results were confirmed biochemically and serologically. It appeared from the data generated that some of the salmonellae isolated using IMS were greatly inhibited in Rappaport-Vassiliadis enrichment broth at 42°C and to a lesser extent in selenite cystine broth at 37°C. A moderately selective plating medium like XLD proved to be better in isolating these possible sensitive wild-type strains of salmonellae than the more selective BGA. Cudjoe, Abstract.

	Samples. One hundred and eighty (180) diverse poultry samples, consisting of 40 pools of cloacal/faecal swabs (5 swabs/pool; 5 pools samples/flock) from eight different flocks, 10 raw chicken breast meat samples, 10 pooled egg shell samples, 60 raw chicken liver samples and 60 chicken neck skins sampled from the slaughter line, were used in this study. All samples were obtained from a poultry slaughter and processing plant in the Netherlands. A pool of five swab samples was pre-enriched in 10 ml of buffered peptone water (BPW; Difco Laboratories, Detroit, Michigan, USA) at 37°C for 16–20 h, whilst 25 g of each of the other samples above, were pre-enriched similarly in 225 ml of BPW and examined as detailed below. Cudjoe, 160.
Claim 10	**Disclosure of ICMSF and Hughes**
The method of claim 1, wherein the test lot is selected from the lot group consisting of a single combo, single bin, single pallet, and a production assemblage.	ICMSF, alone or in combination with Hughes, discloses the method of claim 1, upon which this claim relies. See above re Claim 1. ICMSF, alone or in combination with Hughes, further discloses wherein the test lot is selected from the lot group consisting of a single combo, single bin, single pallet, and a production assemblage. The specification of the '584 Patent indicates a test lot is a single-unit production lot ('584 Patent, 5:21-32) such as a single combo, bin. pallet, or segment of production ('584 Patent, 7:28-36; *see also* AAPA #3 and #4). ICMSF teaches collecting from "individual production batches as lots" (ICMSF, 119) and "[a] lot is considered as a collection of units." ICMSF, 327. "Operationally-linked" is simply grouping like items, which is also disclosed by ICMSF. "Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119.
Claim 11	**Disclosure of ICMSF and Hughes**

The method of claim 1, wherein the number of separate test lots is from 3 to 10.	ICMSF, alone or in combination with Hughes, discloses the method of claim 1, upon which this claim relies. See above re Claim 1. ICMSF, alone or in combination with Hughes, further discloses wherein the number of separate test lots is from 3 to 10. ICMSF indicates a consignment may be "composed of several lots." ICMSF, 119-20. "Several" lots includes at least three lots.
Claim 12	**Disclosure of ICMSF and Hughes**
The method of claim 1, further comprising, where testing of the modular composite sample is positive, determining which individual separate test lots are positive, application of remedial measures to said separate positive test lots, and validating by further testing of a portion of the respective remediated separate test lots and obtaining a negative test result.	ICMSF, alone or in combination with Hughes, discloses the method of claim 1, upon which this claim relies. See above re Claim 1. ICMSF, alone or in combination with Hughes, further discloses further comprising, where testing of the modular composite sample is positive, determining which individual separate test lots are positive, application of remedial measures to said separate positive test lots, and validating by further testing of a portion of the respective remediated separate test lots and obtaining a negative test result. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638.

	Each sample was analyzed by the BAM method (7th Ed.) and the TSVIA method using the enrichment protocol described in section F of the method, *Preparation of Sample*. Equal portions (0.5–1 mL) from each of the 2M broths arising from the 2 selective-enrichment broths were combined in a small screw-capped tube and heat treated in boiling water for 15 min prior to performing the TSVIA. Confirmation for the immunoassay was by streaking from the M broth culture onto selective agar, as specified in the BAM reference culture method. Hughes, 635. A POSITA understands the purpose of testing for pathogens and other organisms in food is to determine if a food is safe for entry into commerce to be sold for consumption. One having ordinary skill in the art understands that if a sample tests positive, a remediation step, like cooking, needs to be completed before it can be sold into commerce to comply with USDA guidelines.
Claim 13	**Disclosure of ICMSF and Hughes**
The method of claim 1, wherein, in a), separately collecting said plurality of portions from each of said plurality of separate test lots comprises separately collecting from each separate test lot a number of portions from 5 to 100.	ICMSF, alone or in combination with Hughes, discloses the method of claim 1, upon which this claim relies. See above re Claim 1. ICMSF, alone or in combination with Hughes, further discloses wherein, in a), separately collecting said plurality of portions from each of said plurality of separate test lots comprises separately collecting from each separate test lot a number of portions from 5 to 100. ICMSF teaches collecting 5, 30, 60, 95, or 100 portions. ICMSF, 184-90, 276, 282, 309, 320, 326-30. *See also* MPEP § 2144.05.

Claim 14	Disclosure of ICMSF and Hughes
The method of claim 1, wherein, in a), separately collecting said plurality of portions from each of said plurality of separate test lots comprises separately collecting from each separate test lot 30 to 60 portions.	ICMSF, alone or in combination with Hughes, discloses the method of claim 1, upon which this claim relies. See above re Claim 1. ICMSF, alone or in combination with Hughes, further discloses wherein, in a), separately collecting said plurality of portions from each of said plurality of separate test lots comprises separately collecting from each separate test lot 30 to 60 portions. ICMSF teaches collecting 5, 30, 60, 95, or 100 portions. ICMSF, 184-90, 276, 282, 309, 320, 326-30. *See also* MPEP § 2144.05.
Claim 15	**Disclosure of ICMSF and Hughes**
The method of claim 1, wherein, in a), separately collecting said plurality of portions from each of said plurality of separate test lots comprises separately collecting 30 portions from each separate test lot.	ICMSF, alone or in combination with Hughes, discloses the method of claim 1, upon which this claim relies. See above re Claim 1. ICMSF, alone or in combination with Hughes, further discloses wherein, in a), separately collecting said plurality of portions from each of said plurality of separate test lots comprises separately collecting 30 portions from each separate test lot. ICMSF teaches collecting 5, 30, 60, 95, or 100 portions. ICMSF, 184-90, 276, 282, 309, 320, 326-30. *See also* MPEP § 2144.05.
Claim 17	**Disclosure of ICMSF and Hughes**
[17pre] A method of sampling, testing and validating a plurality of operationally-linked test lots of meat or poultry for release into commerce, comprising:	To the extent the preamble is limiting, ss discussed herein, ICMSF, alone or in combination with Hughes, discloses a method of sampling, testing and validating a plurality of operationally-linked test lots of meat or poultry for release into commerce. ICMSF includes many other references to testing lots of beef or poultry, two of the most common types of meat in commerce. *Id.*, 114 (§ 6.3), 184–191 (§§ 10.2–10.6),

	225–226 (§ 12.1), 285 (§ 16.1), 313–330 (Chapter 17). ICMSF is relevant to any kind of food product including beef. Hughes discusses turkey (Hughes, Abstract), and a POSITA would be motivated to combine the teachings of ICMSF and Hughes as they both test meat for release into commerce. Hughes discloses testing turkey for Salmonella. "Three food types (. . . and raw turkey) were analyzed in the United States." Hughes, 634, 645, Table 8. Table 8 shows results from 12 collaborators for naturally contaminated raw turkey. For the 180 samples tested, using confirmed data, there was one false negative at the low level for the TECRA method. There were 3 false negatives at the low level and 3 false negatives at the high level for the cultural method. The incidence of false-positive results was very high for the cultural method, with a total of 41 false positives, compared with 5 for the TECRA method. It should, however, be taken into account that the comparison was with the method in BAM 7th Ed., not the current 8th Ed. The method for raw flesh food in the BAM 8th Ed. uses elevated temperature with RV and TT broth, which would be more selective. No significant differences ($p > 0.05$) were observed for the pairwise comparison of the proportion of positive samples for the TECRA method with that for the reference method. Hughes, 646. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite

	sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Moreover, the recitation of a lot being validated for "release . . . into commerce" is not limiting. Prosecution History for Reexamination Control No. 95/011,294 of the '584 Patent.
[17a] a) collecting a plurality of portions from each of a plurality of operationally-linked single-unit production lots of meat or poultry, the single-unit production lots each comprising an assemblage of one or more production specimens from which the portions are collected, wherein each single-unit production lot is separately sampled to conform to a statistical-based sampling plan specifying, for a particular microbial agent or organism, a number of portions to be taken sufficient to provide for validation and release of the single-unit production lot into commerce;	ICMSF, alone or in combination with Hughes, discloses collecting a plurality of portions from each of a plurality of operationally-linked single-unit production lots of meat or poultry, the single-unit production lots each comprising an assemblage of one or more production specimens from which the portions are collected, wherein each single-unit production lot is separately sampled to conform to a statistical-based sampling plan specifying, for a particular microbial agent or organism, a number of portions to be taken sufficient to provide for validation and release of the single-unit production lot into commerce. "Many different types of samples are collected and submitted to the laboratory for analysis. Some are sample units from lots or consignments of food or ingredients for lot acceptance determination. . . . A sample unit is a small portion of the lot in which the distribution of both numbers and types of microorganisms should be representative of the whole lot." ICMSF, 255. The lots may be beef or poultry. *Id.*, 184-91, 226, 230. Thus, ICMSF discloses collecting a plurality of samples or sample units (other synonyms include portions, specimens, and sub-samples) from lots of beef or poultry. ICMSF describes collecting from a lot or lots: "If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits

	more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." *Id.*, 119. Additionally, ICMSF indicates a consignment may be "a very large quantity of food" and may be "composed of several lots, representing for example, different days of production from the same plant." *Id.*, 119, 120; *see* AAPA #3-#5.

6.11 WHAT IS A REPRESENTATIVE SAMPLE?

A *representative sample* reflects, as far as is possible, the composition of the lot from which it is drawn.

How then should a representative sample be drawn? It is important to avoid bias and to draw a sufficient number of sample units to confidently make a judgment about a lot. Sampling at random is the universally recognized way of avoiding bias and is more reliable than consciously trying to draw the sample units from the various parts of a lot. The units (cartons or containers, particular weights of solid, or volumes of liquid) are drawn by using random numbers. There is, of course, no guarantee that a random sample has characteristics identical with those of the lot, but the randomness of sampling is the basis for calculation of the probability that a sample will give a biased result.

It is also possible, and may be desirable, to use a *stratified random sampling* approach, drawing a random sample of a given number of sample units from each *stratum* (e.g., each sublot or carton). The distribution of sample units on strata should correspond to the distribution of lot units on strata. This means that if each stratum contains the same quantity as every other one, the number of sample units per stratum should be the same. Otherwise, they should differ just as the proportions of the lot contained in the strata differ. Stratification is a device for handling known sources of variation and may be used where one has prior knowledge that the consignment is potentially not of uniform quality. A consignment might not be of uniform quality if portions of it are shipped in different vehicles or in different holds of a vessel, or if it is known that it is really composed of several lots representing, for example, different days of production from the same plant or different plants of the same company. The results for different strata should be assessed separately and then pooled if they appear to be homogeneous.

The calculation of acceptance probabilities is based on the assumption that random samples have been drawn and analyzed. Where physical or practical constraints prevent random sampling, such calculated probabilities may not be valid. Their reliability depends on how close reality approaches the ideal situation of random sampling. This fact should be borne in mind when subsequently interpreting results.

ICMSF, 119-20 (annotated).

6.10 WHAT IS A LOT?

Ideally, a lot is a quantity of food or food units produced and handled under uniform conditions. Under certain circumstances, it has been shown that the logarithms of the counts from a batch of food are likely to be normally distributed (Baird-Parker, 1980; Kilsby & Baird-Parker, 1983 [their Table 2]; Ingram & Roberts, 1976). The implication is that there is homogeneity within a lot, which, when considering microbial levels and distributions, rarely occurs in practice. In most instances, the distribution of microorganisms within lots of food is heterogeneous. Even in food factories, this heterogeneity makes the interpretation of sample results difficult, and it becomes even more so in circumstances where a lot is less well defined, such as in a consignment of a very large quantity of food.

It is therefore helpful if suppliers can be persuaded to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load).

If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment.

A lot should be composed of food produced with as little variation as possible for a given process or commodity. Because of the uncertainties in identifying a lot commercially, however, the use of the word *lot* in this book is usually in its statistical sense, as a collection of units of a product, the acceptability of which is determined by examining a sample drawn from it.

ICMSF, 118-19 (annotated).

	The ICMSF has defined a lot as follows: In the commercial sense, a lot is a quantity of food supposedly produced under identical conditions, all packages of which would normally bear a lot number that identifies the production during a particular time interval, and usually from a particular line, retort, or other critical processing unit. Statistically, a lot is considered as a collection of units of a product from which a sample is drawn to determine acceptability of the lot (ICMSF, 1986). In this example, the maximum size of a lot consists of all the ground beef produced on common equipment from cleanup to cleanup. Smaller lots could be established (e.g., hourly). ICMSF, 327 (annotated). This definition also reflects the common practice referred to in the '584 Patent, wherein "[p]referably, the producer defines and produces these single-unit lots in such a fashion as to maximize the information available[.]" '584 Patent,12:46–48 (Example II). This corresponds with the specification of IEH's Patents which reflects the common practice of producers defining what constitutes a lot grouped for traceability purposes. '584 Patent, 2:60-3:3, 11:12-16 (AAPA #3), 12:46-49. Thus, ICMSF discloses collecting a plurality of portions from separate lots. ICMSF describes a lot as "a collection of units of a product." IMCSF, 119. ICMSF further indicates a lot has been defined as: "a quantity of food supposedly produced under identical conditions, all packages of which would normally bear a lot number that identifies the production during a particular time interval, and usually from a particular line, retort, or other critical processing unit. Statistically, a lot is considered as a collection of units of a product from which a sample is drawn to determine acceptability of the lot." ICMSF, 327. ICMSF advocates use of a "statistically based sampling plan." *Id.*, 113-15, 119-21, 320, 327, 328. In the sampling plans, "*n* is the number of sample units chosen separately and independently, and . . . *c* is the maximum allowable number of sample

	units yielding a positive result." *Id.*, 115. ICMSF discloses a wide range for the number of sample units (portions) for a particular microbial agent or organism. For example, ICMSF discusses collecting 15, 30, 60 or 100 sample units from a lot. *Id.*, 320, 326. **6.5 THE SAMPLING PLAN** The sample units so drawn will, after examination, yield results that will be compared with certain criteria to reach a decision as to whether the entire lot should be accepted or rejected (see section 6.9 for explanation of rejection). The particular choice of sampling procedure and the decision criteria make up the *sampling plan*. Proper drawing of samples is essential if the decision making criteria of the sampling plan are to give unbiased results. By sampling randomly, we are able to reduce the risk of making biased decisions. A simple example of a sampling plan follows. Take 10 sample units of a food from a lot and subject them to appropriate laboratory procedure to test for the presence or absence of a microorganism. If two or fewer of the 10 sample units show presence of the organism, that is, give a positive result, then the whole lot of food is acceptable (relative to this organism). But if three or more give a positive result, the whole lot is to be rejected. This plan is described by the use of two terms $n = 10$ and $c = 2$, where n is the number of sample units chosen separately and independently, and where c is the maximum allowable number of sample units yielding a positive result. ICMSF, 115 (annotated).

	6.1 INTRODUCTION It is important to recognize that the management of food safety using the approaches outlined in the first five chapters, based on controlling hazards through Good Hygienic Practices (GHP) and Hazard Analysis Critical Control Point (HACCP), is much more effective than trying to ensure safety by lot acceptance through end-product testing. This chapter discusses the concepts of probability and sampling that determine the limitations of end-product testing. Those concepts form the basis of the design of statistically based sampling plans (Chapter 7) and the establishment of cases (Chapter 8). In Chapters 6 and 7, it is assumed that the microbiological test will give an absolutely accurate result, with no false positives or false negatives. This is not a realistic assumption, and problems arising from sampling, sample transport and preparation for analysis, and the imprecision of microbiological methods are addressed in Chapters 10 and 12 and elsewhere in the text. ICMSF, 113 (annotated); *see also id.*, 115–21 (§§ 6.4–6.13), 319–20 (§ 17.2.3.3), and 326–30 (§§ 17.5.3.1–17.6). ICMSF discusses the number of portions to be taken sufficient for validation of a particular microorganism including *Salmonella* (*id.*, 276 (§ 15.3.3); *see also id.*, 282 (§ 15.6.3)); *Listeria* (*id.*, 309 (§ 16.6.3)), and *E. coli* (*id.*, 318–21 (§§ 17.2.3.2–17.2.3.4), 326 (§ 17.5.3.1)). IEH's AAPA discloses statistical-based sampling plans taking 10-60 sample units. '584 Patent, 5:57-6:6; *see* AAPA #1, #2, #5, and #6. Determining meat is safe for consumption so it is "commercially possible to market" the meat, is the well-understood point of testing meat and is taught by ICMSF. ICMSF, 185-89. Moreover, the recitation of a lot being validated for "release . . . into commerce" is not limiting. Prosecution History for Reexamination Control No. 95/011,294 of the '584 Patent. "Operationally-linked" is simply grouping like items, which is also disclosed by ICMSF. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole

	consignment." ICMSF, 119. It is therefore helpful if suppliers can be persuaded to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load). If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment. ICMSF, 119 (annotated).
[17b] b) combining the collected conforming plurality of product portions corresponding to each of the separate single-unit production lots to provide a corresponding set of separate single-unit production lot samples, wherein each separate single-unit production lot sample is attributed to a particular corresponding separate single-unit production lot;	ICMSF, alone or in combination with Hughes, discloses combining the collected conforming plurality of product portions corresponding to each of the separate single-unit production lots to provide a corresponding set of separate single-unit production lot samples, wherein each separate single-unit production lot sample is attributed to a particular corresponding separate single-unit production lot. ICMSF states: "To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units of the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. . . . This finding suggests that considerable reductions may be made in the cost of laboratory analysis by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, n, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort." ICMSF, 187.

	To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. A laboratory investigation undertaken for ICMSF (Silliker & Gabis, 1973) indicated that different sized analytical units drawn from a single well-mixed sample unit of dried food give very similar results. A subsequent investigation (Gabis & Silliker, 1974) using foods of high moisture content (including eggs, poultry meat, meat, and meat products) showed comparable reliability in the use of composite analytical units; for example, examination of three composites of 20 × 25 g analytical units gave the same result as examination of all 60 analytical units individually (the total amount examined being 1500 g in both instances). This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n*, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort. ICMSF, 187.

10.5.1 Conceivable Alternative Plans

As indicated above, for an infectious agent such as *Salmonella*, there are strong reasons for adopting sampling plans in which $c = 0$:

1. It is philosophically objectionable to some people to accept a lot containing a recognized pathogen, regardless of how the food is to be used in a manufacturing process or by the consumer.
2. There is the very great technical advantage of combining many analytical units for bacteriological examination in systems with $c = 0$, because a single positive decides the outcome.
3. The plan with $c = 1$ (or more) would always require the examination of (many) more analytical units, for equal probability of acceptance, than a plan with $c = 0$.
4. Even if the indicated probabilities of plans with $c = 0$ versus $c = 1$ are the same, their implications are not. For example, when a lot is accepted with the plan $n = 60$, $c = 0$, it is possible (though not certain) that the lot may not be contaminated at all. But using the plan $n = 95$, $c = 1$ and accepting one positive, it is known that the lot certainly is contaminated (though the probability that a lot with 5% of analytical units contaminated would be accepted is in both instances $P_a = 0.05$).

ICMSF, 188 (annotated). ICMSF also provides an example of "[u]sing a sampling plan involving n = 15 (25 g analytical units) and a composite of 375 g." *Id.*, 195 (§ 10.7.2). ICMSF also states "[f]or qualitative analyses, 25 g analytical units are frequently analyzed individually or as composites of 100 g, 200 g, or more." *Id.*, 232 (§ 12.5.2).

The ICMSF incorporates two references, Silliker & Gabis (1973) and Gabis & Silliker (1974). Silliker notes that "[c]ompositing of multiple samples (lumping) and wet compositing of pre-enrichment cultures have been tested as economical approaches to *Salmonella* control." Silliker, 475.

Silliker further notes that "[b]oth approaches are being used widely by the food industry in the United States." *Id.*

	To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. A laboratory investigation undertaken for ICMSF (Silliker & Gabis, 1973) indicated that different sized analytical units drawn from a single well-mixed sample unit of dried food give very similar results. A subsequent investigation (Gabis & Silliker, 1974) using foods of high moisture content (including eggs, poultry meat, meat, and meat products) showed comparable reliability in the use of composite analytical units; for example, examination of three composites of 20 × 25 g analytical units gave the same result as examination of all 60 analytical units individually (the total amount examined being 1500 g in both instances). This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n*, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort. "There is the very great technical advantage of combining many analytical units for bacteriological examination." ICMSF, 188. ICMSF also describes composites prepared by combining "15 (25 g analytical units)" and "composites of 100 g, 200 g, or more." *Id.,* 195, 232; *see* AAPA #5. ICMSF states, "All steps up to the analysis must be controlled . . . to allow reliable traceability." ICMSF, 227. ICMSF indicates lot samples are traceable to a particular corresponding lot: "It is therefore helpful . . . to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load). . . . Treating the individual production

	batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119.
[17c] c) incubating the set of separate single-unit production lot samples under conditions suitable to allow levels of a target microbial agent or organism that is present in one or more of the separate single-unit production lot samples to reach detectable levels and become uniform, or substantially uniform, throughout the respective one or more separate single-unit production lot samples, to provide a set of separate single-unit production lot samples enriched for the target microbial agent or organism;	ICMSF, alone or in combination with Hughes, discloses incubating the set of separate single-unit production lot samples under conditions suitable to allow levels of a target microbial agent or organism that is present in one or more of the separate single-unit production lot samples to reach detectable levels and become uniform, or substantially uniform, throughout the respective one or more separate single-unit production lot samples, to provide a set of separate single-unit production lot samples enriched for the target microbial agent or organism. ICMSF states: To reduce laboratory effort . . . it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. ICMSF, 187. Also, ICMSF describes preparing composites of analytical units then diluting "for qualitative (enrichment)" analysis. *Id.,* 231-32; *see also* AAPA #5, #7. For practical reasons, however, analytical units of 10 g up to 50 g are typical for quantitative methods and are diluted 1:10. For qualitative analyses, 25 g analytical units are frequently analyzed individually or as composites of 100 g, 200 g, or more. ICMSF, 232 (annotated). Preparation of the first dilution, either for qualitative (enrichment) or quantitative (direct) analyses, can have a marked impact on the microbial population in the sample. This is often due to changes in the physico-chemical characteristics of the suspension as compared to the food matrix. Where the food does not confer protection, the composition of the diluent is important to prevent or minimize shock/injury to the microorganisms that can result from rapid changes in the ionic concentrations. ICMSF, 232 (annotated). ICMSF also describes strategic use of non-selective enrichment to resuscitate

	microorganisms and selective enrichments for growing microorganisms. *Id.*, 232–33 (§ 12.6); *see e.g.*, *id.,* 233 ("use of nonselective medium . . . to enhance recovery of injured or stressed cells."). ICMSF, § 12.6 also describes optimizing the incubation period. S*ee e.g.*, *id.,* 232. It was well known long before the priority date to use non-selective enrichments after dry compositing and prior to wet compositing as evidenced by Price, Silliker, Gabis, and MLG. A POSITA would also know to use selective enrichments after dry compositing and prior to wet compositing as disclosed by ICMSF and Hughes. A POSITA would find that incubating so microorganisms are detectable and at least substantially uniform in the selective enrichment broth was considered the standard practice in official methods because the methods otherwise fail. For example, D'Aoust states "standard methodology recommends incubation of selective enrichment broths." D'Aoust, 372. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638.

	(a) *Pre-enrichment.*—Pre-enrich product in noninhibitory broth to initiate growth of *Salmonella* spp. Methods used may vary with product and should be performed as indicated in **967.26A** or BAM, 7th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, chapter 5, section C. Pre-enrichment broths should be incubated at 35°–37°C. (b) *Selective enrichment.*—Transfer 0.1 mL incubated pre-enrichment mixtures to Rappaport-Vassiliadis R10 broth (9.9 mL) and 1 mL to TT broth (9 mL). For all foods other than raw foods or foods having a high microbial load, incubate 6–8 h at 41°–43°C. Selective enrichments of raw foods or foods having a high microbial load must be incubated 16–20 h at 41°–43°C. An air incubator is suitable for this purpose. Hughes, 636. Hughes instructs that, after pre-enrichment, portions of the "incubated pre-enrichment mixtures" should be transferred to two separate selective enrichment broths, Rappaport-Vassiliadis R10 broth and TT broth, and incubated for 16-20 hours at 41°-43°C. Hughes, 636, 638. Additionally, Hughes instructs that these selective broths should be removed from incubation and mixed by hand or by Vortex mixer. Hughes, 638. Hughes further instructs to then remove 1 mL from the TT tube and 1 mL from the Rappaport-Vassiliadis R10 tube and transfer to separate, sterile M broths. *Id.,* 636, 638. The separate M broths are then incubated ("16-20 h at 35°-37°C" for "all foods other than raw foods or foods having a high microbial load" or "6 h at 35°-37°C" for "raw foods or foods having a high microbial load"). *Id.*, 638. A POSITA would find that the long incubation at an elevated temperature and mixing ensures the target microbial agent or organism reaches detectable levels and

	becomes substantially uniform throughout the separate lot samples.
[17d] d) removing portions of each enriched separate single-unit production lot sample, and combining the removed portions to provide a modular composite sample; and	ICMSF describes compositing the enriched lot samples: "it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. . . . This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n,* with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort." ICMSF, 187. *See also* AAPA #5-#7. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Hughes also discloses a method of removing portions of each enriched separate test lot sample, and combining the removed portions to provide a modular composite sample. Hughes instructs to "[c]ombine equal volumes (0.5-1 mL) from each M broth tube in a clean screw-cap tube." Hughes, 638.

	that has been warmed to 35°–37°C. Also remove 1 mL from Rappaport-Vassiliadis R10 tube and transfer to a separate tube of prewarmed M broth (10 mL). For all foods other than raw foods or foods having a high microbial load, incubate M broth tubes 16–20 h at 35°–37°C and return selective enrichment broth tubes to 41°–43°C incubator and incubate for additional 16–18 h. For raw foods or foods having a high microbial load, incubate M broth tubes 6 h at 35°–37°C and return selective enrichment broth tubes to 41°–43°C and incubate for an additional 6 h. (**d**) *Preparation of sample for EIA analysis.*—Remove M broth tubes from incubation and mix tubes by hand or Vortex mixer. Combine equal volumes (0.5–1 mL) from each M broth tube in a clean screw-cap tube and heat in boiling H_2O bath or in flowing steam 15 min. Refrigerate (2°–8°C) remaining M broth and TT and Rappaport-Vassiliadis R10 tubes from (**c**) for culture confirmation of any EIA-positive samples (*see* **967.26B, 967.26C, 967.27** and **967.28**). Cool heated M broths to 25°–37°C prior to analysis. Hughes, 638. Hughes 2003 confirms the pooling of enrichments. Hughes 2003, 775-76.
[17e.1] e) testing of the modular composite sample, using a suitable detection assay, for the target microbial agent or organism,	ICMSF describes testing enrichment cultures of composite samples with suitable detection assays (ICMSF, 187) and "presence/absence tests" (ICMSF, 233). Suitable detection assays were established in Silliker and Gabis as discussed in ICMSF. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of

	sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Hughes states testing of the composite sample is "by an enzyme immunoassay (EIA) method." Hughes, 635, 638. These assay types were "art recognized." '584 Patent, 2:54-58 (AAPA #7).
[17e.2] wherein when such testing is negative all of said separate single-unit production lot samples are validated for release into commerce, and	ICMSF describes correlating lots in a consignment with lot samples so a negative or positive test result enables a lot to be validated or rejected. ICMSF, 118-19. ICMSF describes sampling plans in which "c is the maximum allowable number of sample units yielding a positive result." ICMSF, 115. For many scenarios, the requirement is c = 0, which indicates the testing is negative. *Id.*, 118, 183-90, 309, 325, 326. A negative test result (*id.*, 113-15, 325, 326) is the expected and desired outcome to determine meat is not contaminated and is safe for consumption (*id.*, 184-89) such that a lot is validated and it is "commercially possible to market" (*id.*, 185) the meat.

6.10 WHAT IS A LOT?

Ideally, a lot is a quantity of food or food units produced and handled under uniform conditions. Under certain circumstances, it has been shown that the logarithms of the counts from a batch of food are likely to be normally distributed (Baird-Parker, 1980; Kilsby & Baird-Parker, 1983 [their Table 2]; Ingram & Roberts, 1976). The implication is that there is homogeneity within a lot, which, when considering microbial levels and distributions, rarely occurs in practice. In most instances, the distribution of microorganisms within lots of food is heterogeneous. Even in food factories, this heterogeneity makes the interpretation of sample results difficult, and it becomes even more so in circumstances where a lot is less well defined, such as in a consignment of a very large quantity of food.

It is therefore helpful if suppliers can be persuaded to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load).

If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment.

A lot should be composed of food produced with as little variation as possible for a given process or commodity. Because of the uncertainties in identifying a lot commercially, however, the use of the word *lot* in this book is usually in its statistical sense, as a collection of units of a product, the acceptability of which is determined by examining a sample drawn from it.

ICMSF, 118-19 (annotated).

	Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Because Hughes explains the steps to be taken when testing is positive (*see* ie.3), a POSITA would also expect negative testing results. Hughes, 638. Determining meat is safe for consumption so it may be released into commerce is the well-understood point of testing meat. Moreover, the recitation of a lot being validated for "release . . . into commerce" is not limiting. Prosecution History for Reexamination Control No. 95/011,294 of the '584 Patent.
[17e.3] wherein when such testing is positive, individual separate single-unit production lots are validated for release into commerce, according to the statistical-based sampling plan, by further testing of a portion of the respective enriched separate single-unit production lot sample using the same or a more sensitive protocol and obtaining a negative test result for each single-unit production lot,	The combination of ICMSF and Hughes describes retaining a portion of the enriched lot samples (steps (b)-(d)). "Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119. "There is often a desire to retest retained samples." ICMSF, 191. If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment.

	ICMSF, 119 (annotated). ICMSF discloses validating for release into commerce (ICMSF, 113–15 (§§ 6.2–6.6), 118–19 (§§ 6.9–6.10), 184–85 (§ 10.2), 325–27 (§§ 17.5.3–17.5.3.4); *see also id.*, 186–89 (§ 10.4–10.6)) according to a statistically-based sampling plan (*id.*, 113 (§ 6.1); *see also id.*, 115–18 (§§ 6.4–6.8), 184 (§ 10.2), 319–21 (§§ 17.2.3.3–17.2.3.4), 326–30 (§§ 17.5.3.1–17.6), as described regarding steps (a) and (e)(2). Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Hughes explicitly teaches retention of the enriched test lot samples for further testing after a positive result. Hughes provides instructions to refrigerate the "remaining M broth and TT and Rappaport-Vassiliadis R10 tubes [that were enriched] for culture confirmation of any EIA-positive samples." Hughes, 638. Hughes refers to the results as the "confirmed positive samples" (Hughes, 635) and also states that diagnostic reagents are "necessary for culture confirmation of presumptive EIA tests." Hughes, 636. Hughes indicates "[e]nrichment broths and M broths from samples positive by an enzyme immunoassay (EIA) method must be streaked on selective media." *Id.*, 635. Culture confirmation using streaking is more sensitive than the EIA method, as was commonly understood and confirmed by the teachings of D'Haese. *See, e.g.*, D'Haese.

	Each sample was analyzed by the BAM method (7th Ed.) and the TSVIA method using the enrichment protocol described in section F of the method, *Preparation of Sample*. Equal portions (0.5–1 mL) from each of the 2M broths arising from the 2 selective-enrichment broths were combined in a small screw-capped tube and heat treated in boiling water for 15 min prior to performing the TSVIA. Confirmation for the immunoassay was by streaking from the M broth culture onto selective agar, as specified in the BAM reference culture method. Hughes, 635. Moreover, the recitation of a lot being validated for "release . . . into commerce" is not limiting. Prosecution History for Reexamination Control No. 95/011,294 (Six Parts).
[17e.4] wherein the enrichment in step c), prior to removing portions in step d), is sufficient to enable said validation of individual separate single-unit production lots with no requirement for further enrichment thereof, and with no requirement for further sampling in a).	Hughes explicitly indicates portions of the enrichment cultures are retained for further testing; thus Hughes has no need for further sampling. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. ICMSF and Hughes disclose selective enrichment so further enrichment is unnecessary. Also, as discussed regarding step (e)(3), Hughes explicitly indicates portions of the enrichment cultures are retained for further testing; thus Hughes has no need for further sampling. The same would be expected for enrichment cultures in

	ICMSF because retaining samples at each process stage was considered standard practice for a POSITA. in flowing steam 15 min. Refrigerate (2°–8°C) remaining M broth and TT and Rappaport-Vassiliadis R10 tubes from (c) for culture confirmation of any EIA-positive samples (*see* **967.26B, 967.26C, 967.27** and **967.28**). Cool heated M broths to 25°–37°C prior to analysis. Hughes, 638. Thus, the negative limitation of step (e)(4) is disclosed by and is obvious based on the combination of ICSMF and Hughes. *Id.*
Claim 18	**Disclosure of ICMSF and Hughes**
The method of claim 1, wherein removing portions in step d) is removing equal portions, aseptically, so as to preclude contamination of the enriched separate test lot samples.	ICMSF, alone or in combination with Hughes, discloses the method of claim 1, upon which this claim relies. See above re Claim 1. ICMSF, alone or in combination with Hughes, further discloses wherein removing portions in step d) is removing equal portions, aseptically, so as to preclude contamination of the enriched separate test lot samples. ICMSF describes aseptically withdrawing and weighing sample units into the appropriate diluent. ICMSF, 231, 232. Hughes instructs that after enrichment "[c]ombine equal volumes (0.5-1 mL) from each M broth tube in a clean screw-cap tube[.]" Hughes, 638. Moreover, a POSITA would consider this to be standard practice. A POSITA would have aseptically removed equal volume portions of each enriched separate test lot sample.

Claim 19	**Disclosure of ICMSF and Hughes**
The method of claim 1, wherein the target microbial agent or organism comprises at least one selected from the group consisting of *Listeria*, *Salmonella* and *Escherichia coli.*	ICMSF, alone or in combination with Hughes, discloses the method of claim 1, upon which this claim relies. See above re Claim 1. ICMSF, alone or in combination with Hughes, further discloses wherein the target microbial agent or organism comprises at least one selected from the group consisting of *Listeria*, *Salmonella* and *Escherichia coli.* ICMSF describes testing for *Listeria* (ICMSF, 285-312), *Salmonella* (*id.*, 273-84), and *Escherichia coli* (*id.*, 313-32). Hughes focuses on Salmonella. Hughes, 634.
Claim 20	**Disclosure of ICMSF and Hughes**
The method of claim 1, wherein the target microbial agent or organism comprises at least one selected from the group consisting of *Listeria* spp., *Listeria monocytogenes*, *Salmonella* spp., *Escherichia coli* O157:H7, coliforms and fecal coliforms.	ICMSF, alone or in combination with Hughes, discloses the method of claim 1, upon which this claim relies. See above re Claim 1. ICMSF, alone or in combination with Hughes, further discloses wherein the target microbial agent or organism comprises at least one selected from the group consisting of *Listeria* spp., *Listeria monocytogenes*, *Salmonella* spp., *Escherichia coli* O157:H7, coliforms and fecal coliforms. ICMSF describes testing for *Listeria* (ICMSF, 285-312), *Salmonella* (*id.*, 273-84), and *Escherichia coli* (*id.*, 313-32). Hughes focuses on Salmonella. Hughes, 634.
Claim 21	**Disclosure of ICMSF and Hughes**
The method of claim 1, wherein the target microbial agent or organism comprises a spoilage organism.	ICMSF, alone or in combination with Hughes, discloses the method of claim 1, upon which this claim relies. See above re Claim 1. ICMSF, alone or in combination with Hughes and other secondary prior art, further discloses wherein the target microbial agent or organism comprises a spoilage organism.

	A POSITA would find it obvious to apply the teachings of ICMSF and Hughes to target spoilage organisms. "Examination of every piece of whole meat is clearly impractical, hence any sampling procedure for estimating shelf-life would involve taking enough samples to give some statistical assurance of the keeping qualities of meat." Gill, 449. Borch is titled "Bacterial spoilage of meat and cured meat products." Borch. The reference discusses spoilage organisms in meat: Lactic acid bacteria are the major bacterial group associated with the spoilage of refrigerated vacuum- or MA-packaged cooked, cured meat products (Blickstad and Molin, 1983b; Shaw and Harding, 1989; von Holy et al., 1991). At the time of spoilage some products contain a 'pure' culture of only one species, while in others a mixture of *Lactobacillus* spp. and *Leuconostoc* spp. was found (Kant-Muermans, unpublished results). The great diversity of bacteria isolated from spoiled meat products is confirmed by several studies (Korkeala and Mäkelä, 1989; von Holy et al., 1991; Yang and Ray, 1994). The genus/species of lactic acid bacteria responsible for spoilage, depend on the product composition (product-related flora) as well as the manufacturing site (house-related flora; Korkeala and Mäkelä, 1989). Borch, 113. "Lactic acid bacteria spoil refrigerated meat products by causing defects such as sour off-flavours, discolouration, gas production, slime production and decrease in PH." Borch, 114; *see also, id.*, 115-17 (Section 4 Analysis of Spoilage). It is understood in the art that aerobic plate counts ("APC") can test for spoilage organisms. *See. e.g.*, ICMSF 1986, 122 ("tests to be made (e.g., APC, *Salmonella*, Clostridium perfringens"); *id.* at 132 ("However, aerobic plate counts (APC) can be used to monitor Good Commercial Practice (GCP), and criteria based on such examinations are a valuable aid to in-plant quality control.").

TABLE 19

Sampling plans and recommended microbiological limits for raw meat[a]

Product[b]	Test	Method reference[c]	Case	Plan class	n	c	Limit per cm² or gram m	M
Carcass meat, before chilling	APC	118	1	3	5	3	10^5	10^6
Carcass meat, chilled	APC	118	1	3	5	3	10^6	10^7
Edible offal, chilled	APC	118	1	3	5	3	10^6	10^7
Carcass meat, frozen	APC	118	1	3	5	3	5×10^5	10^7
Boneless meat, frozen (beef, veal, pork, mutton)	APC	118	1	3	5	3	5×10^5	10^7
Comminuted meat, frozen	APC	118	1	3	5	3	10^6	10^7
Edible offal, frozen	APC	118	1	3	5	3	5×10^5	10^7

a **Not for use at port of entry** but for in-plant quality control. Refer to Section B for appropriate applications.
b Unfrozen carcasses and primal cuts, swab counts per cm²; other meats and meat products, per gram.
c Refers to page number in ICMSF, 1978.

ICMSF 1986, 133 (Table 19 (annotated)).

Although for “quality control,” a POSITA would understand that "quality control" means testing for spoilage organisms could also be accomplished by this well-known method. It is disclosed within the prior art during the production lifecycle of beef production. ICMSF 1986, 134 (“The FAOIWHO Working Group (FAQ/WHO, 1979) considered that the only point at which APC could be used for chilled meat, to evaluate the hygienic conditions under which it was produced, was at the slaughterhouse, and because of its perishable nature it would not be possible to set

APC limits for use in guidelines or specifications for chilled meats at port of entry."); *see also, id.*, 153 ("Monitoring for APC, E. coli, and Staph. aureus would give some indication of inadequate processing (heating, curing, smoking), postprocess contamination, or storage at temperatures above those recommended for frozen or refrigerated distribution and storage.")

E. TEST PROCEDURES

(a) *Aerobic plate counts*

Comminute, blend, or stomach the various portions of meat composing the sample unit and withdraw the analytical unit. Combine the swabs to form an analytical unit, or treat each separately. Prepare dilutions and perform the APC test according to the directions given in ICMSF, 1978, p. 118. Spread plates are preferable to pour plates when examining raw meats.

The temperature used for incubation of plates should be related to the material being examined. To estimate numbers of mesophilic contaminants on freshly dressed carcasses it is advisable to incubate plates at 35 or 37°C. For meat that has been held under chilled conditions, plates should be incubated at 25°C to include psychrotrophs and thus give a better indication of the microbial growth that has occurred. Counts at 20°C give similar results after a longer incubation time.

ICMSF 1986, 136 (annotated).

See also, Bhunia 4 ("Bacteria-specific generic rapid methods such ATP-based luminescence and total plate count (APC) are also needed for monitoring cleaning and hygienic practices employed in a processing plant."); Wheeler, 1606 (use of APC in a beef-processing plant); PR/HAACP, 38849 ("Various alternative indicator

	organisms were suggested, including generic E. coli (biotype I), total plate counts, Enterobacteriaceae, Total Viable Counts (TVC), and Aerobic Plate Counts (APC).”).
Claim 22	**Disclosure of ICMSF and Hughes**
The method of claim 1, wherein removing portions of each enriched separate test lot sample comprises removing equal volume portions of each enriched separate test lot sample.	ICMSF, alone or in combination with Hughes, discloses the method of claim 1, upon which this claim relies. See above re Claim 1. ICMSF, alone or in combination with Hughes, further discloses wherein removing portions of each enriched separate test lot sample comprises removing equal volume portions of each enriched separate test lot sample. ICMSF describes aseptically withdrawing and weighing sample units into the appropriate diluent. ICMSF, 231, 232. Hughes instructs that after enrichment “[c]ombine equal volumes (0.5-1 mL) from each M broth tube in a clean screw-cap tube[.]” Hughes, 638. Moreover, a POSITA would consider this to be standard practice. A POSITA would have aseptically removed equal volume portions of each enriched separate test lot sample.
Claim 23	**Disclosure of ICMSF and Hughes**
The method of claim 1, wherein testing of the modular composite sample, using a suitable detection assay comprises use of at least one of a DNA-based method, an immunochemistry-based method, and a biosensor-based method.	ICMSF, alone or in combination with Hughes, discloses the method of claim 1, upon which this claim relies. See above re Claim 1. ICMSF, alone or in combination with Hughes, further discloses wherein testing of the modular composite sample, using a suitable detection assay comprises use of at least one of a DNA-based method, an immunochemistry-based method, and a biosensor-based method. Hughes discloses use of “an enzyme immunoassay (EIA) method,” which is an immunochemistry-based method. Hughes, 635.

	Each sample was analyzed by the BAM method (7th Ed.) and the TSVIA method using the enrichment protocol described in section F of the method, *Preparation of Sample*. Equal portions (0.5–1 mL) from each of the 2M broths arising from the 2 selective-enrichment broths were combined in a small screw-capped tube and heat treated in boiling water for 15 min prior to performing the TSVIA. Confirmation for the immunoassay was by streaking from the M broth culture onto selective agar, as specified in the BAM reference culture method. Hughes, 635.
Claim 24	**Disclosure of ICMSF and Hughes**
The method of claim 1, wherein the separate test lots are production lots of a meat or poultry product, comprising an assemblage of one or more meat or poultry specimens, respectively.	Claim 24 was cancelled during reexamination. IEH cannot assert a cancelled claim against National Beef. As such, National Beef does not substantively address this claim.
Claim 27	**Disclosure of ICMSF and Hughes**
The method of claim 2, wherein product specimens are synonymous with product portions.	ICMSF, alone or in combination with Hughes, discloses the method of claim 2, upon which this claim relies. See above re Claim 2. ICMSF, alone or in combination with Hughes, further discloses wherein product specimens are synonymous with product portions.

	ICMSF teaches collecting portions or samples, which were known synonyms for product portions or specimens. ICMSF states that "[a] sample unit is a small portion of the lot." ICMSF, 225. **12.1 INTRODUCTION** Many different types of samples are collected and submitted to the laboratory for analysis. Some are sample units from lots or consignments of foods or ingredients for lot acceptance determination. Others may be for investigational purposes to assess control of the environment, investigate the source of a problem, or validate a process. Some may have legal implications relative to a lawsuit or for regulatory compliance. This chapter briefly discusses some of the major factors that should be considered when collecting sample units, shipping them to a laboratory, preparing them for analysis, and analyzing them. A sample unit is a small portion of the lot in which the distribution of both numbers and types of microorganisms should be representative of the whole lot. This requirement is only completely fulfilled when the food and processing conditions result in a homogeneous distribution of microorganisms. This only occurs in well-agitated fluid products. In general, the distribution of microorganisms is presumed to be random. ICMSF at 225 (annotated).
Claim 28	**Disclosure of ICMSF and Hughes**
The method of claim 4, wherein the medium comprises a selective agent that will inhibit a competing non-target microbial agent or organism.	ICMSF, alone or in combination with Hughes, discloses the method of claim 4, upon which this claim relies. See above re Claim 4. ICMSF, alone or in combination with Hughes and other secondary prior art, further discloses wherein the medium comprises a selective agent that will inhibit a competing non-target microbial agent or organism. Additionally, Claim 28 recites what a POSITA would consider the well-known property of selective enrichment, inhibition of a competing non-target microorganism. D'Aoust describes "selective enrichment [that] inhibits growth of non-salmonellae and facilitates isolation of Salmonella on differential plating media." D'Aoust, 369.

	A POSITA would have been motivated to use an enrichment medium comprising a selective agent that inhibits a non-target agent or organisms as described by D'Aoust, for the test lot samples described by ICMSF and Hughes, to ensure an accurate test. A POSITA would understand that incubating samples at different temperature ranges for different target microorganisms would, effectively, inhibit the growth of other organisms. "Incubation temperature markedly affects productivity of selective enrichment broths by suppressing growth of competing microorganisms but not Salmonella." D'Aoust, 372. "This may be achieved by utilization of enrichment media that through various mechanisms may favor and enhance growth and multiplication of salmonellae while other gram-negative organisms such as coliforms, Proteus, and Pseudomonas are suppressed or inhibited." Fagerberg, 630.
Claim 29	**Disclosure of ICMSF and Hughes**
The method of claim 5, wherein the concentration step comprises at least one of immunomagnetic bead separation, and affinity chromatography.	ICMSF, alone or in combination with Hughes, discloses the method of claim 5, upon which this claim relies. See above re Claim 5. ICMSF, alone or in combination with Hughes and other secondary prior art, further discloses wherein the concentration step comprises at least one of immunomagnetic bead separation, and affinity chromatography. ICMSF discloses the use of "immunomagnetic beads to concentrate cells." ICMSF, 318. A POSITA would consider this a well-known technique before the priority date. Fratamico discusses the rapid isolation of target microorganisms using an immunomagnetic separation method. Fratamico, 105. Fratamico discloses that use of immunomagnetic beads resulted in "an increase in the level of E. coli B1409 in the sample," as well as "an increase in the number of organisms recovered." *Id.*, 107. Additionally, Fratamico provides detailed instructions for preparing and using

	immunomagnetic bead separation to concentrate a target microbial agent or organism. Fratamico developed "[a] method employing immunomagnetic beads" to concentrate and thereby improve the isolation and detection of E. coli. *Id.* For example, Fratamico was able to use immunomagnetic beads to isolate *E. coli* strains from enrichment cultures of raw ground beef "when the initial inoculum level was 1 cfu g-1." *Id.*, 109. Fratamico further teaches that "[t]he percentage of bacteria recovered increased with increasing amounts of beads." *Id.*, 108. Consequently, Fratamico "demonstrates that immunomagnetic separation [using immunomagnetic beads] is a simple and rapid technique" for concentrating a microbe for isolation and detection. *Id.*, 109. These teachings of Fratamico would have motivated a POSA to modify the combination of ICMSF and Gombas and ICMSF and Hughes to use immunomagnetic beads to concentrate microbes in a modular composite sample to benefit from accurate tests. Use of immunomagnetic beads was already known in the art for concentrating during testing beef samples. "Tu et al. (78) detected pathogenic *E. coli* O157:H7 in beef hamburger with the use of IMS and ATP measurement. After 6 h of enrichment at 37°C, inoculated beef samples (0.9 ml each) were filtered to remove large particles prior to 10 min of incubation with ca. 10^5 magnetic beads coated with anti–*E. coli* O157:H7 (Dynabeads, Dynal Inc., Oslo, Norway). Following incubation, the magnetic beads were collected with a Dynal magnetic concentrator and resuspended in 0.9 ml of Trypticase soy broth for ATP analysis." Benoit, 1941. "Hudson et al. (26) used IMS to directly separate and concentrate L. monocytogenes from ham samples prior to detection by PCR. IMS was used on the basis of the commercial availability of anti-Listeria magnetic beads (Dynal Inc.) to avoid time-consuming cultural enrichment steps and to remove PCR inhibitors." Benoit, 1941-42.

	Cudjoe investigates the use of immunomagnetic beads in pooling methods. In a study designed to evaluate the performance of Dynabeads® Anti-Salmonella, immunomagnetic separation (IMS) followed by plating (IMS-Plating) proved far superior to the conventional ISO *Salmonella* methodology and the Modified Semi-solid Rappaport-Vassiliadis (MSRV) method. *Salmonella* species were isolated and detected from 135 out of the 180 diverse poultry samples by IMS analysis as against 98 by the conventional method and 33 by the MSRV technique. All results were confirmed biochemically and serologically. It appeared from the data generated that some of the salmonellae isolated using IMS were greatly inhibited in Rappaport-Vassiliadis enrichment broth at 42°C and to a lesser extent in selenite cystine broth at 37°C. A moderately selective plating medium like XLD proved to be better in isolating these possible sensitive wild-type strains of salmonellae than the more selective BGA. Cudjoe, Abstract. *Samples*. One hundred and eighty (180) diverse poultry samples, consisting of 40 pools of cloacal/faecal swabs (5 swabs/pool; 5 pools samples/flock) from eight different flocks, 10 raw chicken breast meat samples, 10 pooled egg shell samples, 60 raw chicken liver samples and 60 chicken neck skins sampled from the slaughter line, were used in this study. All samples were obtained from a poultry slaughter and processing plant in the Netherlands. A pool of five swab samples was pre-enriched in 10 ml of buffered peptone water (BPW; Difco Laboratories, Detroit, Michigan, USA) at 37°C for 16–20 h, whilst 25 g of each of the other samples above, were pre-enriched similarly in 225 ml of BPW and examined as detailed below. Cudjoe, 160.
Claim 30	**Disclosure of ICMSF and Hughes**
The method of claim 6, wherein the more sensitive protocol further comprises a concentration step to concentrate the microbial agent or organism, in combination	ICMSF, alone or in combination with Hughes, discloses the method of claim 6, upon which this claim relies. See above re Claim 6. ICMSF, alone or in combination with Hughes and other secondary prior art, further discloses wherein the more sensitive protocol further comprises a concentration step to concentrate the microbial agent or

with the same test protocol that is used to test the modular composite sample.	organism, in combination with the same test protocol that is used to test the modular composite sample. ICMSF discloses the use of "immunomagnetic beads to concentrate cells." ICMSF, 318. A POSITA would consider this a well-known technique before the priority date. Hughes uses an EIA method followed by a more sensitive protocol—streaking on selective media. Hughes, 635. Each sample was analyzed by the BAM method (7th Ed.) and the TSVIA method using the enrichment protocol described in section F of the method, *Preparation of Sample*. Equal portions (0.5–1 mL) from each of the 2M broths arising from the 2 selective-enrichment broths were combined in a small screw-capped tube and heat treated in boiling water for 15 min prior to performing the TSVIA. Confirmation for the immunoassay was by streaking from the M broth culture onto selective agar, as specified in the BAM reference culture method. Hughes, 635. A POSITA would find that modifying Hughes based on D'Haese ensures a more sensitive test is used for further testing. A POSITA would have recognized the concentration step could be employed to provide a more sensitive protocol for further testing. A POSITA would understand that retesting the non-pooled, singular samples is an inherently more sensitive process because the sample is not diluted with other samples in the testing pool. Therefore, the retesting of a singular sample is more concentrated and therefore, more sensitive. *See also, generally*, Jarvis.

	Fratamico discusses the rapid isolation of target microorganisms using an immunomagnetic separation method. Fratamico, 105. Fratamico discloses that use of immunomagnetic beads resulted in "an increase in the level of E. coli B1409 in the sample," as well as "an increase in the number of organisms recovered." *Id.*, 107. Additionally, Fratamico provides detailed instructions for preparing and using immunomagnetic bead separation to concentrate a target microbial agent or organism. Fratamico developed "[a] method employing immunomagnetic beads" to concentrate and thereby improve the isolation and detection of E. coli. *Id.* For example, Fratamico was able to use immunomagnetic beads to isolate *E. coli* strains from enrichment cultures of raw ground beef "when the initial inoculum level was 1 cfu g-1." *Id.*, 109. Fratamico further teaches that "[t]he percentage of bacteria recovered increased with increasing amounts of beads." *Id.*, 108. Consequently, Fratamico "demonstrates that immunomagnetic separation [using immunomagnetic beads] is a simple and rapid technique" for concentrating a microbe for isolation and detection. *Id.*, 109. These teachings of Fratamico would have motivated a POSITA to modify the combination of ICMSF and Gombas and ICMSF and Hughes to use immunomagnetic beads to concentrate microbes in a modular composite sample to benefit from accurate tests. Use of immunomagnetic beads was already known in the art for concentrating during testing beef samples. "Tu et al. (78) detected pathogenic E. coli O157:H7 in beef hamburger with the use of IMS and ATP measurement. After 6 h of enrichment at 37°C, inoculated beef samples (0.9 ml each) were filtered to remove large particles prior to 10 min of incubation with ca. 10^5 magnetic beads coated with anti–*E. coli* O157:H7 (Dynabeads, Dynal Inc., Oslo, Norway). Following incubation, the magnetic beads were collected with a Dynal magnetic concentrator and resuspended in 0.9 ml of Trypticase soy broth for ATP analysis." Benoit, 1941.

	"Hudson et al. (26) used IMS to directly separate and concentrate L. monocytogenes from ham samples prior to detection by PCR. IMS was used on the basis of the commercial availability of anti-Listeria magnetic beads (Dynal Inc.) to avoid time-consuming cultural enrichment steps and to remove PCR inhibitors." Benoit, 1941-42. Cudjoe investigates the use of immunomagnetic beads in pooling methods. In a study designed to evaluate the performance of Dynabeads® Anti-Salmonella, immunomagnetic separation (IMS) followed by plating (IMS-Plating) proved far superior to the conventional ISO *Salmonella* methodology and the Modified Semi-solid Rappaport-Vassiliadis (MSRV) method. *Salmonella* species were isolated and detected from 135 out of the 180 diverse poultry samples by IMS analysis as against 98 by the conventional method and 33 by the MSRV technique. All results were confirmed biochemically and serologically. It appeared from the data generated that some of the salmonellae isolated using IMS were greatly inhibited in Rappaport-Vassiliadis enrichment broth at 42°C and to a lesser extent in selenite cystine broth at 37°C. A moderately selective plating medium like XLD proved to be better in isolating these possible sensitive wild-type strains of salmonellae than the more selective BGA. Cudjoe, Abstract. *Samples*. One hundred and eighty (180) diverse poultry samples, consisting of 40 pools of cloacal/faecal swabs (5 swabs/pool; 5 pools samples/flock) from eight different flocks, 10 raw chicken breast meat samples, 10 pooled egg shell samples, 60 raw chicken liver samples and 60 chicken neck skins sampled from the slaughter line, were used in this study. All samples were obtained from a poultry slaughter and processing plant in the Netherlands. A pool of five swab samples was pre-enriched in 10 ml of buffered peptone water (BPW; Difco Laboratories, Detroit, Michigan, USA) at 37°C for 16–20 h, whilst 25 g of each of the other samples above, were pre-enriched similarly in 225 ml of BPW and examined as detailed below. Cudjoe, 160.

Claim 31	Disclosure of ICMSF and Hughes
The method of claim 17, wherein removing portions in step d) is removing equal portions, aseptically, so as to preclude contamination of the enriched separate single-unit production lot samples.	ICMSF, alone or in combination with Hughes, discloses the method of claim 17, upon which this claim relies. See above re Claim 17. ICMSF, alone or in combination with Hughes, further discloses wherein removing portions in step d) is removing equal portions, aseptically, so as to preclude contamination of the enriched separate single-unit production lot samples. ICMSF describes aseptically withdrawing and weighing sample units into the appropriate diluent. ICMSF, 231, 232. Hughes instructs that after enrichment "[c]ombine equal volumes (0.5-1 mL) from each M broth tube in a clean screw-cap tube[.]" Hughes, 638. Moreover, a POSITA would consider this to be standard practice. A POSITA would have aseptically removed equal volume portions of each enriched separate test lot sample.
Claim 32	**Disclosure of ICMSF and Hughes**
The method of claim 1, wherein the beef specimens are meat trim pieces.	ICMSF, alone or in combination with Hughes, discloses the method of claim 1, upon which this claim relies. See above re Claim 1. ICMSF, alone or in combination with Hughes, further discloses wherein the beef specimens are meat trim pieces ICMSF indicates "[t]rimmings to be used for ground beef could be pretested for E. coli O157:H7 by various sampling plans." ICMSF, 323. ICMSF indicates "[r]esearch has validated that up to fifteen 25 g analytical units of ground beef or trimmings can be composited without significant loss in E. coli O157:H7 detection sensitivity." *Id.*, 327 *see also id.*, 226, 314, 320, and 324. ICMSF includes many other references to testing lots of beef or poultry, two of the most common types of meat in commerce. *Id.,* 114 (§ 6.3), 184–191 (§§ 10.2–10.6),

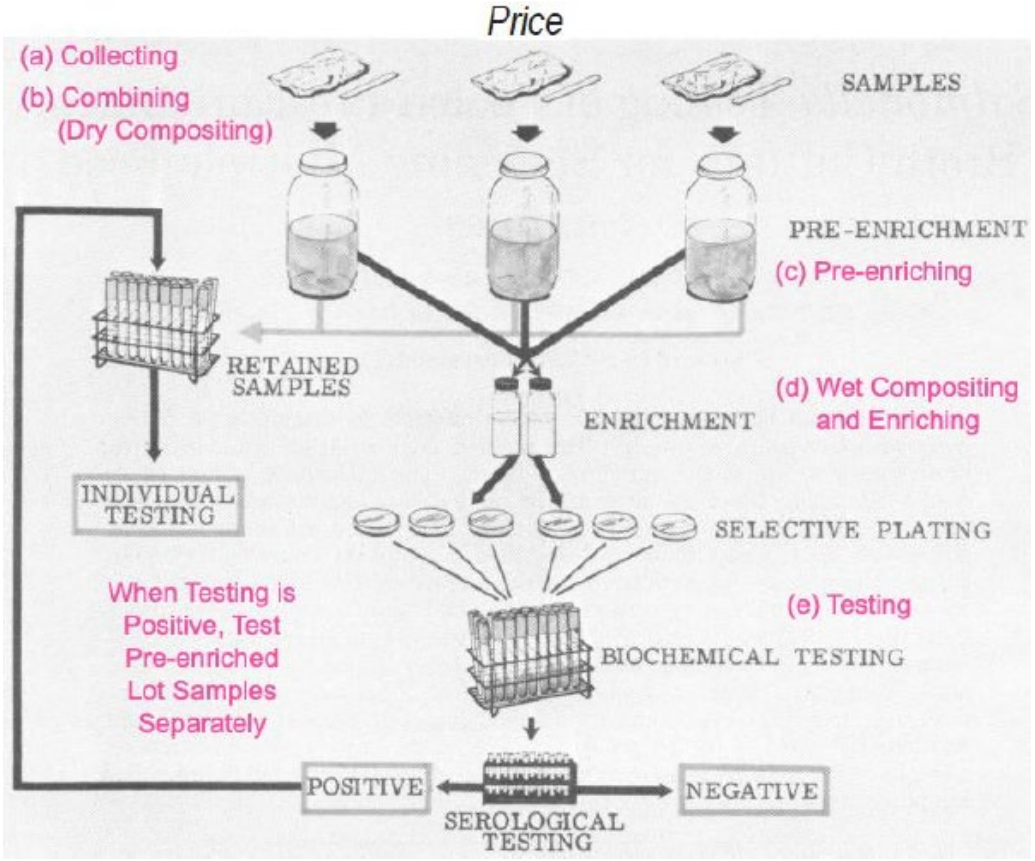
	225–226 (§ 12.1), 285 (§ 16.1), 313–330 (Chapter 17). ICMSF is relevant to any kind of food product including beef or poultry.
Claim 33	**Disclosure of ICMSF and Hughes**
The method of claim 17, wherein the meat is that of beef, pork, sheep or bison.	ICMSF, alone or in combination with Hughes, discloses the method of claim 17, upon which this claim relies. See above re Claim 17. ICMSF, alone or in combination with Hughes, further discloses wherein the meat is that of beef, pork, sheep or bison. ICMSF indicates "[t]rimmings to be used for ground beef could be pretested for E. coli O157:H7 by various sampling plans." ICMSF, 323. ICMSF indicates "[r]esearch has validated that up to fifteen 25 g analytical units of ground beef or trimmings can be composited without significant loss in E. coli O157:H7 detection sensitivity." *Id.*, 327 *see also id.*, 226, 314, 320, and 324. ICMSF includes many other references to testing lots of beef or poultry, two of the most common types of meat in commerce. *Id.* at 114 (§ 6.3), 184–191 (§§ 10.2–10.6), 225–226 (§ 12.1), 285 (§ 16.1), 313–330 (Chapter 17). ICMSF is relevant to any kind of food product including beef or poultry.

EXHIBIT A3

Invalidity of U.S. Patent No. 7,534,584 in light of Price, ICMSF, and Hughes

National Beef denies that any of its microbiological testing methods infringe any claim of U.S. Patent No. 7,534,584 ("the '584 Patent"). If the claims of the '584 Patent were construed so broadly as to cover the microbiological testing methods used by National Beef, they would be invalid at least in view of W.R. Price et al., *Salmonella Testing of Pooled PreEnrichment Broth Cultures for Screening Multiple Food Samples*, 23 APPLIED MICROBIOLOGY 679–682 (1972) ("Price"); INT'L COMM'N ON MICROBIOLOGICAL SPEC'S FOR FOODS (ICMSF), MICROORGANISMS IN FOODS 7: MICROBIOLOGICAL TESTING IN FOOD SAFETY MANAGEMENT (Kluwer Academic/Plenum Publishers, 2002) ("ICMSF"); and Denise Hughes et al., *Salmonella in Foods—A New Enrichment Procedure for Use with the TECRA Salmonella Visual Immunoassay: Collaborative Study*, 82 J. AOAC INT'L 634–647 (1999) ("Hughes") , which anticipates and/or discloses the limitations of the '584 Patent under at least pre-AIA 35 U.S.C. § 102(a), pre-AIA 35 U.S.C. § 102(b), pre-AIA 35 U.S.C. §§ 102(f)/(g), and/or pre-AIA 35 U.S.C. § 103. To the extent that Price, ICMSF and/or Gombas do not expressly disclose one or more limitations of the asserted claims, as they would have to be construed to support IEH's claim of infringement, they discloses the limitations alone or in combination with one or more additional prior art references and/or the knowledge of one of ordinary skill in the art.

Claim 1	**Disclosure of Price, ICMSF, and Hughes**
[1pre] A method of sampling, testing and validating a plurality of operationally-linked test lots of beef for release into commerce, comprising:	To the extent the preamble is limiting, as discussed herein, Price, alone or in combination with ICMSF and Hughes, discloses a method of sampling, testing and validating a plurality of operationally-linked test lots of beef for release into commerce. Price established that "pre-enrichment broths can be pooled for analysis without loss in sensitivity to *Salmonella* in the individual samples." Price, 679. Price tested dry food materials inoculated (purposefully contaminated) with *Salmonella. Id.*



Price, 680 (Figure 1 (annotated)).

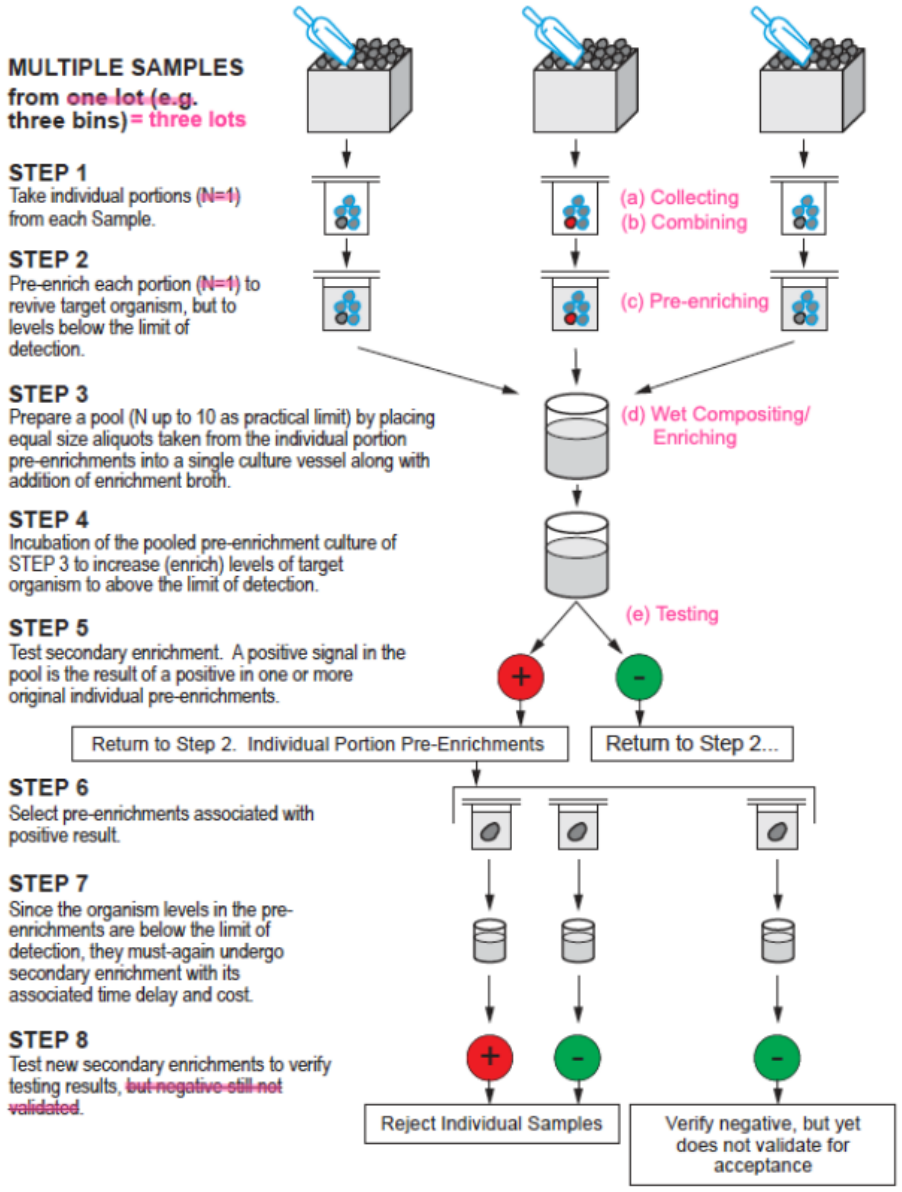
A POSITA would find it obvious to apply the teachings of Price to beef.

ICMSF includes many other references to testing lots of beef or poultry, two of the most common types of meat in commerce. *Id.*, 114 (§ 6.3), 184–191 (§§ 10.2–10.6), 225–226 (§ 12.1), 285 (§ 16.1), 313–330 (Chapter 17). ICMSF is relevant to any kind of food product including beef.

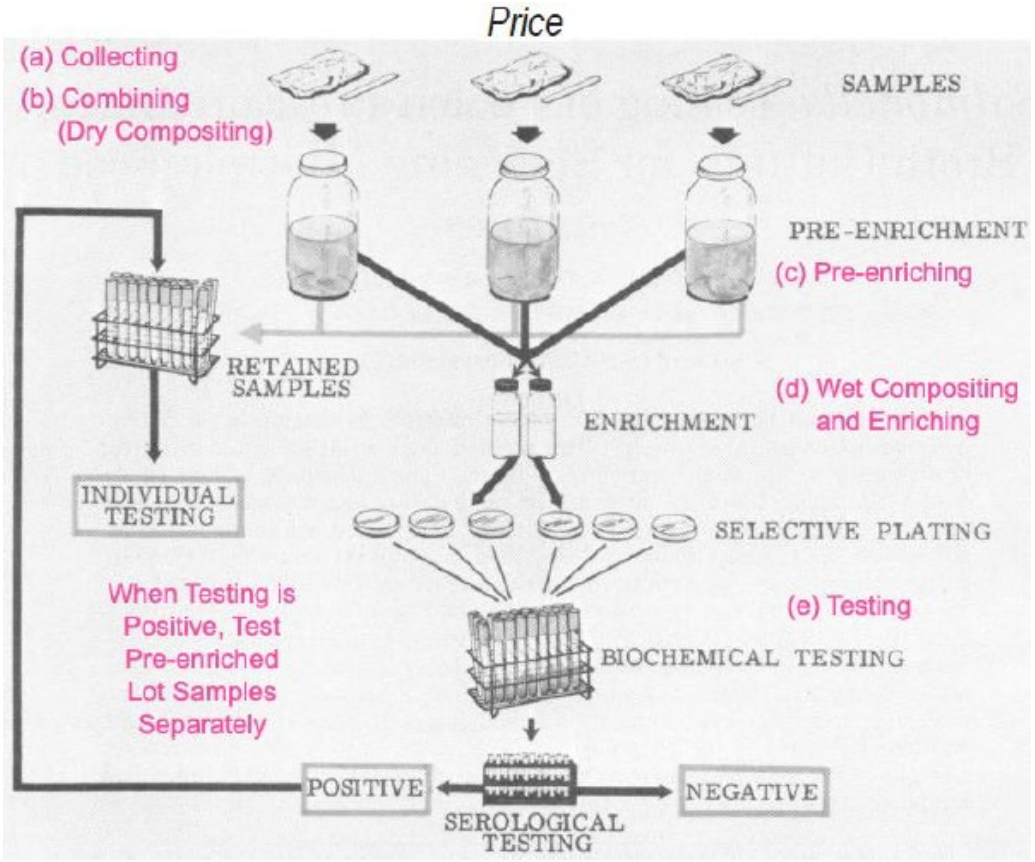
	Hughes discusses turkey (Hughes, Abstract), and a POSITA would be motivated to combine the teachings of ICMSF and Hughes as they both test meat for release into commerce. Hughes discloses testing turkey for *Salmonella*. "Three food types (. . . and raw turkey) were analyzed in the United States." Hughes, 634, 645, Table 8. Table 8 shows results from 12 collaborators for naturally contaminated raw turkey. For the 180 samples tested, using confirmed data, there was one false negative at the low level for the TECRA method. There were 3 false negatives at the low level and 3 false negatives at the high level for the cultural method. The incidence of false-positive results was very high for the cultural method, with a total of 41 false positives, compared with 5 for the TECRA method. It should, however, be taken into account that the comparison was with the method in BAM 7th Ed., not the current 8th Ed. The method for raw flesh food in the BAM 8th Ed. uses elevated temperature with RV and TT broth, which would be more selective. No significant differences ($p > 0.05$) were observed for the pairwise comparison of the proportion of positive samples for the TECRA method with that for the reference method. Hughes, 646. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638.

	Moreover, the recitation of a lot being validated for "release . . . into commerce" is not limiting. Prosecution History for Reexamination Control No. 95/011,294 (Six Parts).
[1a] a) collecting a plurality of portions from each of a plurality of separate operationally-linked test lots of beef, each to be validated for release into commerce, the test lots each comprising an assemblage of one or more specimens from which the portions are collected, wherein each test lot is separately sampled to conform to a statistical-based sampling plan specifying, for a particular microbial agent or organism, a number of portions to be taken sufficient to provide for validation and release of the test lot into commerce;	Price, alone or in combination with ICMSF and Hughes, discloses collecting a plurality of portions from each of a plurality of separate operationally-linked test lots of beef, each to be validated for release into commerce, the test lots each comprising an assemblage of one or more specimens from which the portions are collected, wherein each test lot is separately sampled to conform to a statistical-based sampling plan specifying, for a particular microbial agent or organism, a number of portions to be taken sufficient to provide for validation and release of the test lot into commerce. Price's teachings for collecting a plurality of portions are based on standard practices as described in BAM 1969 (BAM 1969, 1 § 10.04) which cites to JAOAC 1967 (JAOAC, 234). Price, 679, 682. ***Salmonella* analyses were carried out as described in the *Bacteriological Analytical Manual* (BAM) (2) except for the pooling of pre-enrichment broths described in the following paragraph.** Price, 679. IEH's diagram of Price from Prosecution History for Reexamination Control No. 95/001,294 of the '584 Patent (reproduced below) shows step 1 corresponds to IEH's step (a), with instructions to "[t]ake individual portions . . . from each Sample."

Figure 3: CITED PRIOR ART (PRICE et al, 1972)



	Prosecution History for Reexamination Control No. 95/001,294, 298 (annotated). IEH's diagram also indicates multiple samples are taken from three bins, which is analogous to three lots rather than a single lot because each bin is an assemblage of specimens. The purpose of Jarvis and Price was to determine whether pooling samples reduced the ability to detect contaminated food lots. It would have been commonsense to combine samples from the same bin and subject them to enrichment, since each bin represents a discrete unit of the production run and results obtained from the enriched sample could be attributed to the bin from which the sample was obtained. Prosecution History for Reexamination Control No. 95/001,294, at 1575 (First Board Decision of January 20, 2015 at 16). Additionally, the specification describes a bin of dry food materials (almonds), like those in Price, as one example of a test lot/single unit of production, similar to a combo of beef. '584 Patent, 5:33-38. Price discloses testing of "Dried egg albumen, cocoa, and nonfat dry milk" (Price, 679) and "wheat flour, coconut, and cottonseed flour" (Price, 681).



Price, 680 (Figure 1 (annotated)).

A POSITA would find it obvious to apply the teachings of Price to beef.

	A method has been described for testing multiple food samples for *Salmonella* without loss in sensitivity. The method pools multiple pre-enrichment broth cultures into single enrichment broths. The subsequent stages of the *Salmonella* analysis are not altered. The method was found applicable to several dry food materials including nonfat dry milk, dried egg albumin, cocoa, cottonseed flour, wheat flour, and shredded coconut. As many as 25 pre-enrichment broth cultures were pooled without apparent loss in the sensitivity of *Salmonella* detection as compared to individual sample analysis. The procedure offers a simple, yet effective, way to increase sample capacity in the *Salmonella* testing of foods, particularly where a large proportion of samples ordinarily is negative. It also permits small portions of pre-enrichment broth cultures to be retained for subsequent individual analysis if positive tests are found. *Salmonella* testing of pooled pre-enrichment broths provides increased consumer protection for a given amount of analytical effort as compared to individual sample analysis. Price, 679 (Abstract). A POSITA would have replaced Price's collection and combination of dry food with ICMSF's collection and combination of portions from a consignment of meat lots in conformance to a statistical-based sampling plan for release into commerce. "Many different types of samples are collected and submitted to the laboratory for analysis. Some are sample units from lots or consignments of food or ingredients for lot acceptance determination. . . . A sample unit is a small portion of the lot in which the distribution of both numbers and types of microorganisms should be representative of the whole lot." ICMSF, 255. The lots may be beef or poultry. *Id.*, 184-91, 226, 230. Thus, ICMSF discloses collecting a plurality of samples or sample units (other synonyms include portions, specimens, and sub-samples) from lots of beef or poultry. ICMSF describes collecting from a lot or lots:

	"If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." *Id.*, 119. Additionally, ICMSF indicates a consignment may be "a very large quantity of food" and may be "composed of several lots, representing for example, different days of production from the same plant." *Id.*, 119, 120; *see also* AAPA #3-#5.

6.11 WHAT IS A REPRESENTATIVE SAMPLE?

A *representative sample* reflects, as far as is possible, the composition of the lot from which it is drawn.

How then should a representative sample be drawn? It is important to avoid bias and to draw a sufficient number of sample units to confidently make a judgment about a lot. Sampling at random is the universally recognized way of avoiding bias and is more reliable than consciously trying to draw the sample units from the various parts of a lot. The units (cartons or containers, particular weights of solid, or volumes of liquid) are drawn by using random numbers. There is, of course, no guarantee that a random sample has characteristics identical with those of the lot, but the randomness of sampling is the basis for calculation of the probability that a sample will give a biased result.

It is also possible, and may be desirable, to use a *stratified random sampling* approach, drawing a random sample of a given number of sample units from each *stratum* (e.g., each sublot or carton). The distribution of sample units on strata should correspond to the distribution of lot units on strata. This means that if each stratum contains the same quantity as every other one, the number of sample units per stratum should be the same. Otherwise, they should differ just as the proportions of the lot contained in the strata differ. Stratification is a device for handling known sources of variation and may be used where one has prior knowledge that the consignment is potentially not of uniform quality. A consignment might not be of uniform quality if portions of it are shipped in different vehicles or in different holds of a vessel, or if it is known that it is really composed of several lots representing, for example, different days of production from the same plant or different plants of the same company. The results for different strata should be assessed separately and then pooled if they appear to be homogeneous.

The calculation of acceptance probabilities is based on the assumption that random samples have been drawn and analyzed. Where physical or practical constraints prevent random sampling, such calculated probabilities may not be valid. Their reliability depends on how close reality approaches the ideal situation of random sampling. This fact should be borne in mind when subsequently interpreting results.

ICMSF, 119-20 (annotated).

6.10 WHAT IS A LOT?

Ideally, a lot is a quantity of food or food units produced and handled under uniform conditions. Under certain circumstances, it has been shown that the logarithms of the counts from a batch of food are likely to be normally distributed (Baird-Parker, 1980; Kilsby & Baird-Parker, 1983 [their Table 2]; Ingram & Roberts, 1976). The implication is that there is homogeneity within a lot, which, when considering microbial levels and distributions, rarely occurs in practice. In most instances, the distribution of microorganisms within lots of food is heterogeneous. Even in food factories, this heterogeneity makes the interpretation of sample results difficult, and it becomes even more so in circumstances where a lot is less well defined, such as in a consignment of a very large quantity of food.

It is therefore helpful if suppliers can be persuaded to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load).

If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment.

A lot should be composed of food produced with as little variation as possible for a given process or commodity. Because of the uncertainties in identifying a lot commercially, however, the use of the word *lot* in this book is usually in its statistical sense, as a collection of units of a product, the acceptability of which is determined by examining a sample drawn from it.

ICMSF, 118-19 (annotated).

	The ICMSF has defined a lot as follows: In the commercial sense, a lot is a quantity of food supposedly produced under identical conditions, all packages of which would normally bear a lot number that identifies the production during a particular time interval, and usually from a particular line, retort, or other critical processing unit. Statistically, a lot is considered as a collection of units of a product from which a sample is drawn to determine acceptability of the lot (ICMSF, 1986). In this example, the maximum size of a lot consists of all the ground beef produced on common equipment from cleanup to cleanup. Smaller lots could be established (e.g., hourly). ICMSF, 327 (annotated). This definition also reflects the common practice referred to in the '584 Patent at Example II, wherein "[p]referably, the producer defines and produces these single-unit lots in such a fashion as to maximize the information available[.]" '584 Patent,12:46–48. This corresponds with the specification of the '584 Patent which reflects the common practice of producers defining what constitutes a lot grouped for traceability purposes. '584 Patent, 2:60-3:3, 11:12-16 (AAPA #3), 12:46-49. Thus, ICMSF discloses collecting a plurality of portions from separate lots. ICMSF describes a lot as "a collection of units of a product." IMCSF, 119. ICMSF further indicates a lot has been defined as: "a quantity of food supposedly produced under identical conditions, all packages of which would normally bear a lot number that identifies the production during a particular time interval, and usually from a particular line, retort, or other critical processing unit. Statistically, a lot is considered as a collection of units of a product from which a sample is drawn to determine acceptability of the lot." ICMSF, 327. ICMSF advocates use of a "statistically based sampling plan." *Id.*, 113-15, 119-21, 320, 327, 328. In the sampling plans, "*n* is the number of sample units chosen separately and independently, and . . . *c* is the maximum allowable number of sample

	units yielding a positive result." *Id.*, 115. ICMSF discloses a wide range for the number of sample units (portions) for a particular microbial agent or organism. For example, ICMSF discusses collecting 15, 30, 60 or 100 sample units from a lot. *Id.*, 320, 326. **6.5 THE SAMPLING PLAN** The sample units so drawn will, after examination, yield results that will be compared with certain criteria to reach a decision as to whether the entire lot should be accepted or rejected (see section 6.9 for explanation of rejection). The particular choice of sampling procedure and the decision criteria make up the *sampling plan*. Proper drawing of samples is essential if the decision making criteria of the sampling plan are to give unbiased results. By sampling randomly, we are able to reduce the risk of making biased decisions. A simple example of a sampling plan follows. Take 10 sample units of a food from a lot and subject them to appropriate laboratory procedure to test for the presence or absence of a microorganism. If two or fewer of the 10 sample units show presence of the organism, that is, give a positive result, then the whole lot of food is acceptable (relative to this organism). But if three or more give a positive result, the whole lot is to be rejected. This plan is described by the use of two terms $n = 10$ and $c = 2$, where n is the number of sample units chosen separately and independently, and where c is the maximum allowable number of sample units yielding a positive result. ICMSF, 115 (annotated).

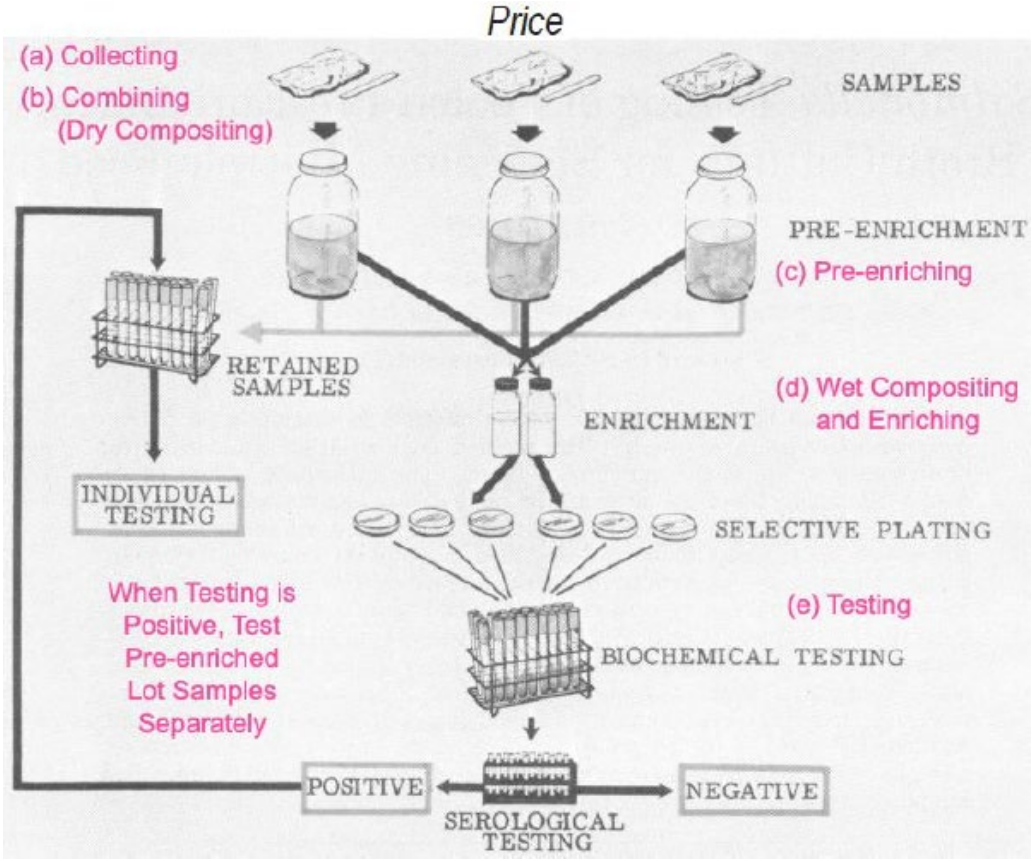
	6.1 INTRODUCTION It is important to recognize that the management of food safety using the approaches outlined in the first five chapters, based on controlling hazards through Good Hygienic Practices (GHP) and Hazard Analysis Critical Control Point (HACCP), is much more effective than trying to ensure safety by lot acceptance through end-product testing. This chapter discusses the concepts of probability and sampling that determine the limitations of end-product testing. Those concepts form the basis of the design of statistically based sampling plans (Chapter 7) and the establishment of cases (Chapter 8). In Chapters 6 and 7, it is assumed that the microbiological test will give an absolutely accurate result, with no false positives or false negatives. This is not a realistic assumption, and problems arising from sampling, sample transport and preparation for analysis, and the imprecision of microbiological methods are addressed in Chapters 10 and 12 and elsewhere in the text. ICMSF, 113 (annotated); *see also id.*, 115–21 (§§ 6.4–6.13), 319–20 (§ 17.2.3.3), and 326–30 (§§ 17.5.3.1–17.6). ICMSF discusses the number of portions to be taken sufficient for validation of a particular microorganism including *Salmonella* (*id.*, 276 (§ 15.3.3); *see also id.*, 282 (§ 15.6.3)); *Listeria* (*id.*, 309 (§ 16.6.3)), and *E. coli* (*id.*, 318–21 (§§ 17.2.3.2–17.2.3.4), 326 (§ 17.5.3.1)). IEH's AAPA discloses statistical-based sampling plans taking 10-60 sample units. '584 Patent, 5:57-6:6; *see* AAPA #1, #2, #5, and #6. Determining whether meat is safe for consumption so it may be released into commerce is a well-understood purpose of testing meat. *See* ICMSF, 185-89. Moreover, the recitation of a lot being validated for "release . . . into commerce" is not limiting. Prosecution History for Reexamination Control No. 95/011,294 of the '584 Patent. "Operationally-linked" is simply grouping like items, which is also disclosed by ICMSF. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment."

	ICMSF, 119. It is therefore helpful if suppliers can be persuaded to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load). If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment. ICMSF, 119 (annotated).
[1b] b) combining the collected conforming plurality of portions corresponding to each of the separate test lots to provide a corresponding set of separate test lot samples, wherein each separate test lot sample is attributed to a particular corresponding separate test lot;	Price, alone or in combination with ICMSF and Hughes, discloses combining the collected conforming plurality of portions corresponding to each of the separate test lots to provide a corresponding set of separate test lot samples, wherein each separate test lot sample is attributed to a particular corresponding separate test lot. Like ICMSF, the test lot samples in Price are attributable to a particular lot. For example, Price's abstract states that the procedure "permits small portions of pre-enrichment broth cultures to be retained for subsequent individual analysis if positive tests are found," as shown below:

	A method has been described for testing multiple food samples for *Salmonella* without loss in sensitivity. The method pools multiple pre-enrichment broth cultures into single enrichment broths. The subsequent stages of the *Salmonella* analysis are not altered. The method was found applicable to several dry food materials including nonfat dry milk, dried egg albumin, cocoa, cottonseed flour, wheat flour, and shredded coconut. As many as 25 pre-enrichment broth cultures were pooled without apparent loss in the sensitivity of *Salmonella* detection as compared to individual sample analysis. The procedure offers a simple, yet effective, way to increase sample capacity in the *Salmonella* testing of foods, particularly where a large proportion of samples ordinarily is negative. It also permits small portions of pre-enrichment broth cultures to be retained for subsequent individual analysis if positive tests are found. *Salmonella* testing of pooled pre-enrichment broths provides increased consumer protection for a given amount of analytical effort as compared to individual sample analysis. Price, 679 (Abstract). Price further describes this retention step as enabling "individual tests on the retained sample" to "be made to determine which sample or samples contributed *Salmonella* to the pool" as shown below:

	MATERIALS AND METHODS *Salmonella* analyses were carried out as described in the *Bacteriological Analytical Manual* (BAM) (2) except for the pooling of pre-enrichment broths described in the following paragraph. The procedure for *Salmonella* testing of pooled pre-enrichment broths is illustrated in Fig. 1. Samples are individually pre-enriched followed by transfer from multiple pre-enrichments to single selenite and tetrathionate broths at the enrichment stage. During pooling, a portion of each pre-enrichment is transferred to a sterile culture tube and retained at 4 C for later reference to the individual samples. If the *Salmonella* test of the pooled pre-enrichment broths is positive, individual tests on the retained samples can be made to determine which sample or samples contributed *Salmonella* to the pool. *Id.* (annotated). A POSITA would understand Price to implicitly disclose combining the collected confirming plurality of portions based on the inherent mixing involved with dry food materials.

	Salmonella-positive test samples were prepared by blending the dry test food with one of three dry inocula. Inoculum 1 was a dry enzyme drain cleaner which had been found to contain multiple *Salmonella* serotypes including E_1, z_{10}; G, z_{29} (*S. cubana*); and C_1, g complex. Inoculum 2 was a freeze-dried, skim milk suspension of serotypes *S. anatum*, *S. binza*, *S. tennessee*, *S. worthington*, *S. cubana*, *S. braenderup*, and a B_1, g complex. Inoculum 3 consisted of four laboratory isolates of serotypes G, z_{29} (*S. cubana*); E_1, b; E_1, l complex; and C_1, l complex grown in a sterile aqueous suspension of cottonseed flour and freeze dried. *Id.*



Price, 680 (Figure 1 (annotated)).

A POSITA would have replaced Price's collection and combination of dry food with ICMSF's collection and combination of portions from a consignment of meat lots in conformance to a statistical-based sampling plan for release into commerce.

ICMSF states: "To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units of the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. . . . This finding suggests that considerable reductions may

	be made in the cost of laboratory analysis by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, n, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort." ICMSF, 187. To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. A laboratory investigation undertaken for ICMSF (Silliker & Gabis, 1973) indicated that different sized analytical units drawn from a single well-mixed sample unit of dried food give very similar results. A subsequent investigation (Gabis & Silliker, 1974) using foods of high moisture content (including eggs, poultry meat, meat, and meat products) showed comparable reliability in the use of composite analytical units; for example, examination of three composites of 20 × 25 g analytical units gave the same result as examination of all 60 analytical units individually (the total amount examined being 1500 g in both instances). This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n*, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort. ICMSF, 187.

10.5.1 Conceivable Alternative Plans

As indicated above, for an infectious agent such as *Salmonella*, there are strong reasons for adopting sampling plans in which $c = 0$:

1. It is philosophically objectionable to some people to accept a lot containing a recognized pathogen, regardless of how the food is to be used in a manufacturing process or by the consumer.
2. There is the very great technical advantage of combining many analytical units for bacteriological examination in systems with $c = 0$, because a single positive decides the outcome.
3. The plan with $c = 1$ (or more) would always require the examination of (many) more analytical units, for equal probability of acceptance, than a plan with $c = 0$.
4. Even if the indicated probabilities of plans with $c = 0$ versus $c = 1$ are the same, their implications are not. For example, when a lot is accepted with the plan $n = 60$, $c = 0$, it is possible (though not certain) that the lot may not be contaminated at all. But using the plan $n = 95$, $c = 1$ and accepting one positive, it is known that the lot certainly is contaminated (though the probability that a lot with 5% of analytical units contaminated would be accepted is in both instances $P_a = 0.05$).

ICMSF, 188 (annotated). ICMSF also provides an example of "[u]sing a sampling plan involving n = 15 (25 g analytical units) and a composite of 375 g." *Id.*, 195 (§ 10.7.2). ICMSF also states "[f]or qualitative analyses, 25 g analytical units are frequently analyzed individually or as composites of 100 g, 200 g, or more." *Id.*, 232 (§ 12.5.2).

The ICMSF incorporates two references, Silliker & Gabis (1973) and Gabis & Silliker (1974). Silliker notes that "[c]ompositing of multiple samples (lumping) and wet compositing of pre-enrichment cultures have been tested as economical approaches to *Salmonella* control." Silliker, 475.

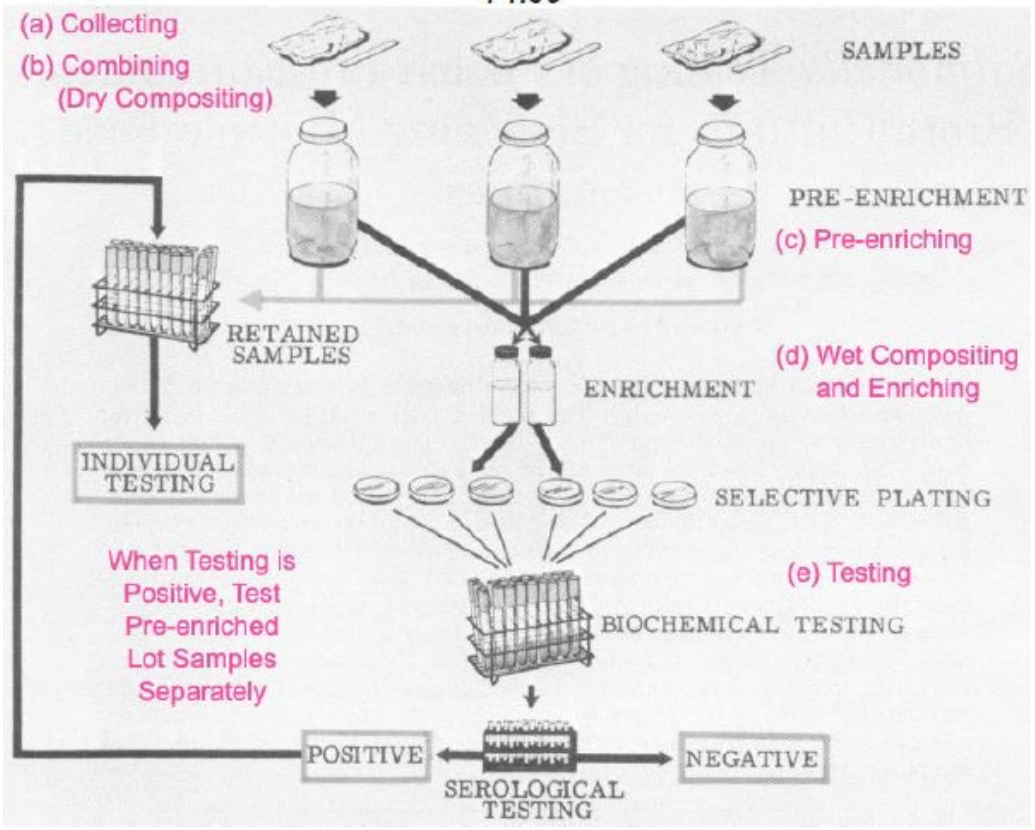
Silliker further notes that "[b]oth approaches are being used widely by the food industry in the United States." *Id.*

	"There is the very great technical advantage of combining many analytical units for bacteriological examination." ICMSF, 188. ICMSF also describes composites prepared by combining "15 (25 g analytical units)" and "composites of 100 g, 200 g, or more." *Id.,* 195, 232; *see also* AAPA #5. ICMSF states, "All steps up to the analysis must be controlled . . . to allow reliable traceability." ICMSF, 227. ICMSF indicates lot samples are traceable to a particular corresponding lot: "It is therefore helpful . . . to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load). . . . Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119.
[1c] c) incubating the set of separate test lot samples under conditions suitable to allow levels of a target microbial agent or organism that is present in one or more of the separate test lot samples to reach detectable levels and become uniform, or substantially uniform, throughout the respective one or more separate test lot samples, to provide a set of separate test lot samples enriched for the target microbial agent or organism;	Price, alone or in combination with ICMSF and Hughes, discloses incubating the set of separate test lot samples under conditions suitable to allow levels of a target microbial agent or organism that is present in one or more of the separate test lot samples to reach detectable levels and become uniform, or substantially uniform, throughout the respective one or more separate test lot samples, to provide a set of separate test lot samples enriched for the target microbial agent or organism. Price states "[i]n the multiple pooling of pre-enrichment broth cultures . . . , 1-ml quantities of cultures were transferred, generally, to a volume of enrichment broth." Price, 682. Price discloses "[s]amples are individually pre-enriched followed by transfer from

multiple pre-enrichments to single selenite and tetrathionate broths at the enrichment stage." Price, 679. These pre-enrichment and enrichment steps inherently disclose an incubation period in which levels of the target microbial agent, in this case, *Salmonella*, grow to detectable levels for testing.

Price also states "[a]lternatively, problems with interfering organisms may warrant the use of direct selective enrichments." *Id.* Based on this statement, the Board noted "there is a suggestion in Price (at 682) . . . of direct enrichment." Prosecution History for Reexamination Control No. 95/001,294, 1576.

Price



	Price, 680 (Figure 1 (annotated)). Price states "[a]lternatively, problems with interfering organisms may warrant the use of direct selective enrichments" and further mentions "a reduction in the incubation time of the pre-enrichment cultures." Price inherently discloses an incubation period of the pre-enrichment samples. **Whether pooled pre-enrichments are less sensitive than individual tests in the presence of interfering organisms probably is not known. For the present, we would recommend that, if numerous non-*Salmonella* colonies that resemble *Salmonella* on selective agar media are encountered in the pooled test, the retained pre-enrichment cultures should be tested individually. Alternatively, problems with interfering organisms may warrant the use of direct selective enrichments, two selective enrichments, or possibly even a reduction in the incubation time of the pre-enrichment cultures.** Price, 682 (annotated). A POSITA would have replaced Price's pre-enriching and compositing pre-enrichments with teachings of ICMSF and Hughes for enriching lot samples, removing portions of each enriched lot sample, and combining the portions to provide a composite sample. Also, instead of retaining portions of pre-enrichments like Price, a POSITA would have retained portions of enriched lot samples as taught by ICMSF and Hughes. ICMSF states: To reduce laboratory effort . . . it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which

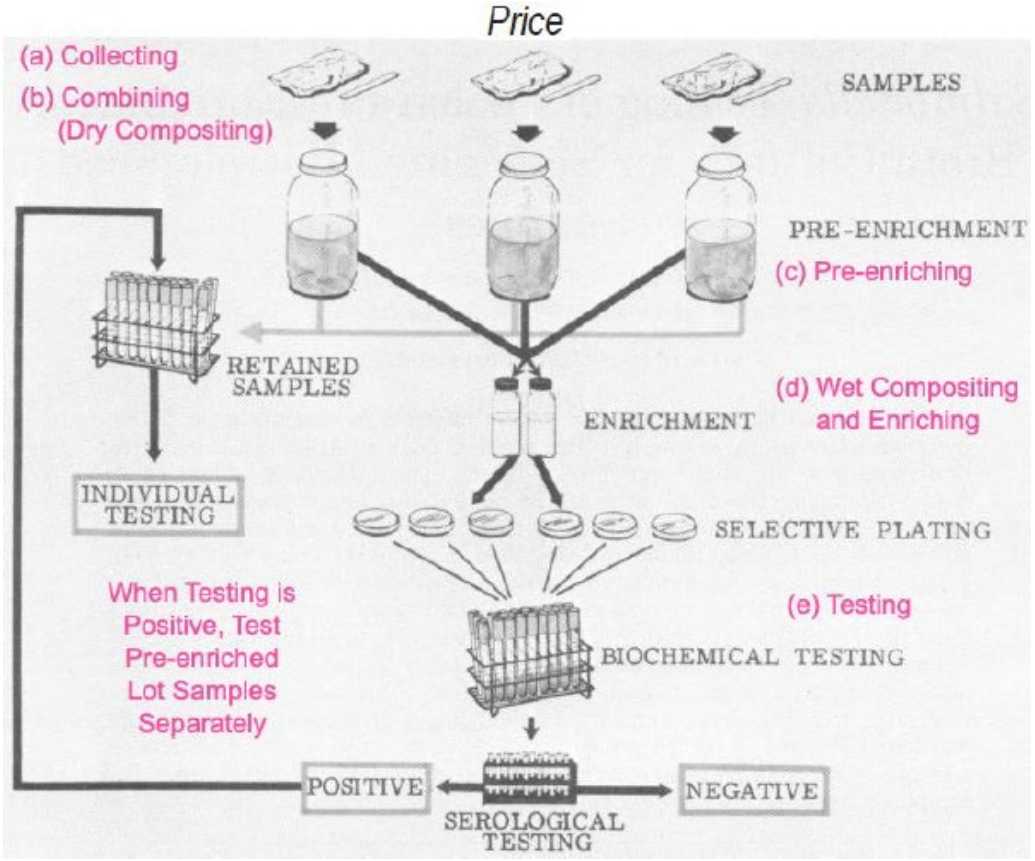
	the presence of a single positive rejects the consignment. ICMSF, 187. Also, ICMSF describes preparing composites of analytical units then diluting "for qualitative (enrichment)" analysis. *Id.,* 231-32; *see also* AAPA #5, #7. For practical reasons, however, analytical units of 10 g up to 50 g are typical for quantitative methods and are diluted 1:10. For qualitative analyses, 25 g analytical units are frequently analyzed individually or as composites of 100 g, 200 g, or more. ICMSF, 232 (annotated). Preparation of the first dilution, either for qualitative (enrichment) or quantitative (direct) analyses, can have a marked impact on the microbial population in the sample. This is often due to changes in the physico-chemical characteristics of the suspension as compared to the food matrix. Where the food does not confer protection, the composition of the diluent is important to prevent or minimize shock/injury to the microorganisms that can result from rapid changes in the ionic concentrations. ICMSF, 232 (annotated). ICMSF also describes strategic use of non-selective enrichment to resuscitate microorganisms and selective enrichments for growing microorganisms. *Id.*, 232–33 (§ 12.6); *see e.g.*, *id.,* 233 ("use of nonselective medium . . . to enhance recovery of injured or stressed cells."). ICMSF, § 12.6 also describes optimizing the incubation period. S*ee e.g.*, *id.,* 232. It was well known long before the priority date to use non-selective enrichments after dry compositing and prior to wet compositing as evidenced by Price, Silliker, Gabis, and MLG. A POSITA would also know to use selective enrichments after dry compositing and prior to wet compositing as disclosed by ICMSF and Hughes. A POSITA would find that incubating so microorganisms are detectable and at least substantially uniform in the selective enrichment broth was considered the standard practice in official methods because the methods otherwise fail. For example, D'Aoust states "standard methodology recommends incubation of selective

	enrichment broths." D'Aoust, 372. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. **(a)** *Pre-enrichment.*—Pre-enrich product in noninhibitory broth to initiate growth of *Salmonella* spp. Methods used may vary with product and should be performed as indicated in **967.26A** or BAM, 7th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, chapter 5, section C. Pre-enrichment broths should be incubated at 35°–37°C. **(b)** *Selective enrichment.*—Transfer 0.1 mL incubated pre-enrichment mixtures to Rappaport-Vassiliadis R10 broth (9.9 mL) and 1 mL to TT broth (9 mL). For all foods other than raw foods or foods having a high microbial load, incubate 6–8 h at 41°–43°C. Selective enrichments of raw foods or foods having a high microbial load must be incubated 16–20 h at 41°–43°C. An air incubator is suitable for this purpose. Hughes, 636. Hughes instructs that, after pre-enrichment, portions of the "incubated pre-enrichment mixtures" should be transferred to two separate selective enrichment broths, Rappaport-Vassiliadis R10 broth and TT broth, and incubated for 16-20 hours at 41°-43°C. Hughes, 636, 638. Additionally, Hughes instructs that these selective broths should be removed from

	incubation and mixed by hand or by Vortex mixer. Hughes, 638. Hughes further instructs to then remove 1 mL from the TT tube and 1 mL from the Rappaport-Vassiliadis R10 tube and transfer to separate, sterile M broths. *Id.,* 636, 638. The separate M broths are then incubated ("16-20 h at 35°-37°C" for "all foods other than raw foods or foods having a high microbial load" or "6 h at 35°-37°C" for "raw foods or foods having a high microbial load"). *Id.*, 638. A POSITA would find that the long incubation at an elevated temperature and mixing ensures the target microbial agent or organism reaches detectable levels and becomes substantially uniform throughout the separate lot samples.
[1d] d) removing portions of each enriched separate test lot sample, and combining the removed portions to provide a modular composite sample; and	Price, alone or in combination with ICMSF and Hughes, discloses removing portions of each enriched separate test lot sample, and combining the removed portions to provide a modular composite sample. Price discloses wet compositing as "[s]amples are individually pre-enriched followed by transfer from multiple pre-enrichments to single selenite and tetrathionate broths at the enrichment stage." Price, 679.

	MATERIALS AND METHODS *Salmonella* analyses were carried out as described in the *Bacteriological Analytical Manual* (BAM) (2) except for the pooling of pre-enrichment broths described in the following paragraph. The procedure for *Salmonella* testing of pooled pre-enrichment broths is illustrated in Fig. 1. Samples are individually pre-enriched followed by transfer from multiple pre-enrichments to single selenite and tetrathionate broths at the enrichment stage. During pooling, a portion of each pre-enrichment is transferred to a sterile culture tube and retained at 4 C for later reference to the individual samples. If the *Salmonella* test of the pooled pre-enrichment broths is positive, individual tests on the retained samples can be made to determine which sample or samples contributed *Salmonella* to the pool. Price 679 (annotated).

	Pooled samples versus pooled pre-enrichment broths. Silliker (4) reported a loss in sensitivity when several samples were pooled in a large container of pre-enrichment broth as compared to individual sample analysis. This is not surprising since, to be detected, the desiccated and debilitated *Salmonella* cells in a dried food sample must grow out in pre-enrichment broth in competition with other bacteria in the sample. If many food samples are pooled at the pre-enrichment stage, the salmonellae face competition from the flora of all the samples in the pool. If, on the other hand, the samples are individually pre-enriched, the actively growing salmonellae might be expected to compete more favorably with organisms introduced from other samples when pooled at the enrichment stage. In addition, pooling pre-enrichment broths avoids the hazards and inconvenience of handling very large flasks of culture. Price, 681 (discussing Silliker).

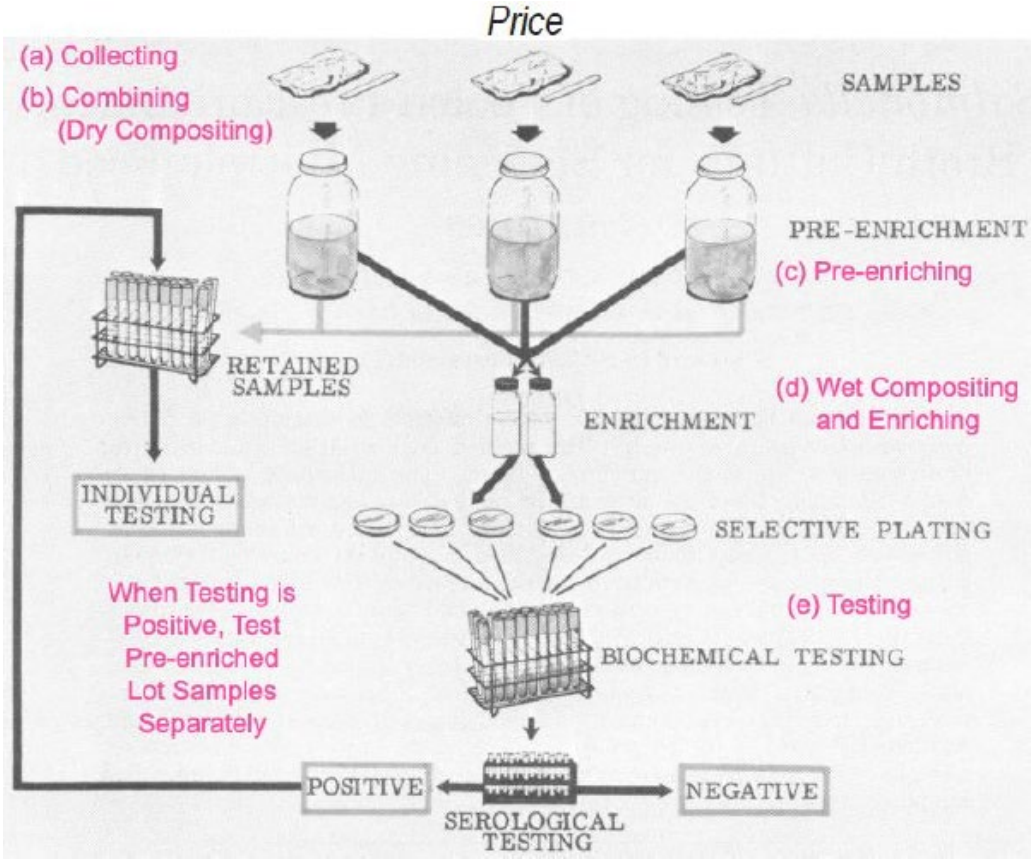


Price, 680 (Figure 1 (annotated)). Price's Figure 1 indicates that selective plating, biochemical testing, and serological testing are used.

A POSITA would have replaced Price's pre-enriching and compositing pre-enrichments with teachings of ICMSF and Hughes for enriching lot samples, removing portions of each enriched lot sample, and combining the portions to provide a composite sample. Also, instead of retaining portions of pre-enrichments like Price, a POSITA would have retained portions of enriched lot samples as taught by ICMSF and Hughes.

	ICMSF describes compositing the enriched lot samples: "it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. . . . This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n,* with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort." ICMSF, 187. *See also* AAPA #5-#7. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Hughes also discloses a method of removing portions of each enriched separate test lot sample, and combining the removed portions to provide a modular composite sample. Hughes instructs to "[c]ombine equal volumes (0.5-1 mL) from each M broth tube in a clean screw-cap tube." Hughes, 638.

	that has been warmed to 35°–37°C. Also remove 1 mL from Rappaport-Vassiliadis R10 tube and transfer to a separate tube of prewarmed M broth (10 mL). For all foods other than raw foods or foods having a high microbial load, incubate M broth tubes 16–20 h at 35°–37°C and return selective enrichment broth tubes to 41°–43°C incubator and incubate for additional 16–18 h. For raw foods or foods having a high microbial load, incubate M broth tubes 6 h at 35°–37°C and return selective enrichment broth tubes to 41°–43°C and incubate for an additional 6 h. (**d**) *Preparation of sample for EIA analysis.*—Remove M broth tubes from incubation and mix tubes by hand or Vortex mixer. Combine equal volumes (0.5–1 mL) from each M broth tube in a clean screw-cap tube and heat in boiling H_2O bath or in flowing steam 15 min. Refrigerate (2°–8°C) remaining M broth and TT and Rappaport-Vassiliadis R10 tubes from (**c**) for culture confirmation of any EIA-positive samples (*see* **967.26B, 967.26C, 967.27** and **967.28**). Cool heated M broths to 25°–37°C prior to analysis. Hughes, 638. Hughes 2003 confirms the pooling of enrichments. Hughes 2003, 775-76.
[1e.1] e) testing of the modular composite sample, using a suitable detection assay, for the target microbial agent or organism,	Price, alone or in combination with ICMSF and Hughes, discloses testing of the modular composite sample, using a suitable detection assay, for the target microbial agent or organism.



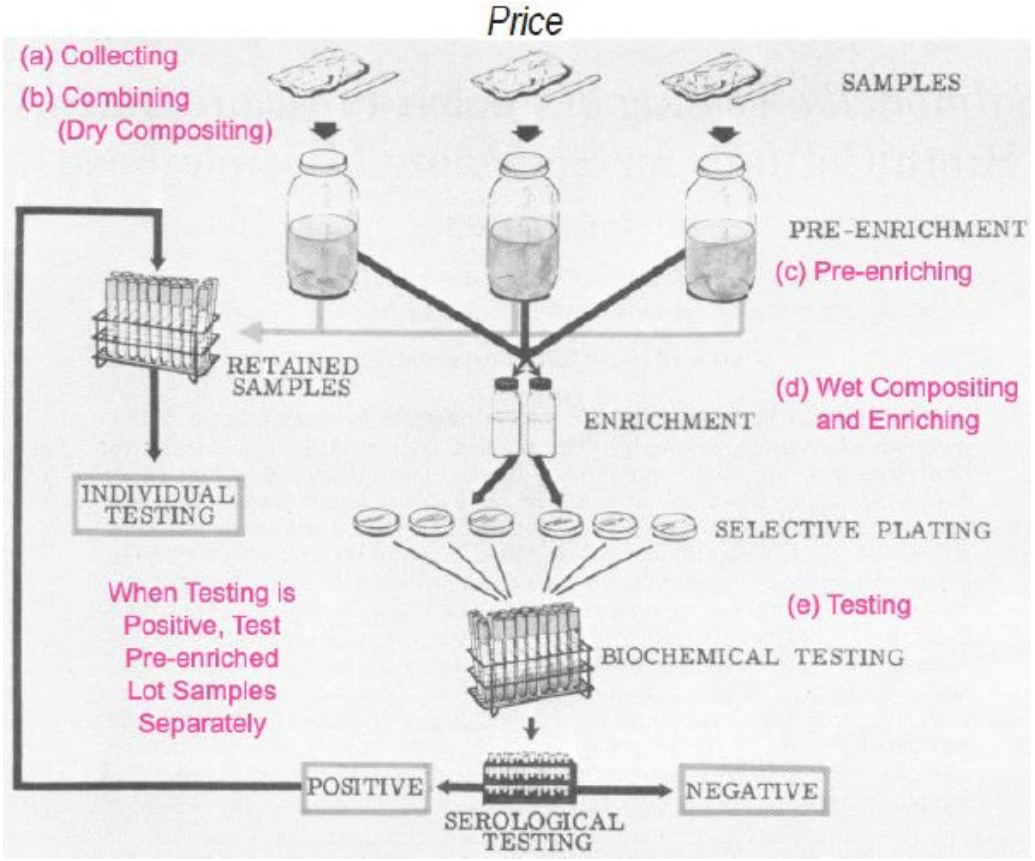
Price, 680 (Figure 1 (annotated)).

Price teaches testing the composite sample using a detection assay for *Salmonella*. Price, 680 (Figure 1).

Price states "a test procedure that gives a single negative answer for many negative samples offers an important saving in the analytical effort required for quality control":

	Positive test results are relatively rare among the many samples of food ingredients and products which are analyzed for *Salmonella*. Therefore, a test procedure that gives a single negative answer for many negative samples offers an important saving in the analytical effort required for quality control. These studies support the conclusion that *Salmonella* pre-enrichment broths can be pooled for analysis without loss in sensitivity to *Salmonella* in the individual samples. Price, 679 (annotated). Price indicates that "positive test results are relatively rare." *Id.* A POSITA would have replaced Price's steps of testing a composite sample from the pre-enriched lot samples to obtain a negative result and further testing retained portions of the pre-enriched lot samples with the combined teachings of ICMSF and Hughes. These combined teachings provide for testing a composite made from the selectively enriched lot samples to obtain a negative result and further testing retained portions of the enriched lot samples after a positive test of the composite by a more sensitive protocol. ICMSF describes testing enrichment cultures of composite samples with suitable detection assays (ICMSF, 187) and "presence/absence tests" (ICMSF, 233). Suitable detection assays were established in Silliker and Gabis as discussed in ICMSF. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture

	confirmation of any EIA-positive samples." *Id.,* 638. Hughes states testing of the composite sample is "by an enzyme immunoassay (EIA) method." Hughes, 635, 638. These assay types were "art recognized." '584 Patent, 2:54-58 (AAPA #7).
[1e.2] wherein when such testing is negative all of said separate test lots are validated for release into commerce, and	Price, alone or in combination with ICMSF and Hughes, discloses wherein when such testing is negative all of said separate test lots are validated for release into commerce.



Price, 680 (Figure 1 (annotated)).

Price states "a test procedure that gives a single negative answer for many negative samples offers an important saving in the analytical effort required for quality control." Price, 679.

A POSITA would have replaced Price's steps of testing a composite sample from the pre-enriched lot samples to obtain a negative result and further testing retained portions of the pre-enriched lot samples with the combined teachings of ICMSF and Hughes. These combined teachings provide for testing a composite made from the

	selectively enriched lot samples to obtain a negative result and further testing retained portions of the enriched lot samples after a positive test of the composite by a more sensitive protocol. ICMSF describes correlating lots in a consignment with lot samples so a negative or positive test result enables a lot to be validated or rejected. ICMSF, 118-19. ICMSF describes sampling plans in which "c is the maximum allowable number of sample units yielding a positive result." ICMSF, 115. For many scenarios, the requirement is c = 0, which indicates the testing is negative. *Id.*, 118, 183-90, 309, 325, 326. A negative test result (*id.*, 113-15, 325, 326) is the expected and desired outcome to determine meat is not contaminated and is safe for consumption (*id.*, 184-89) such that a lot is validated and it is "commercially possible to market" (*id.*, 185) the meat.

6.10 WHAT IS A LOT?

Ideally, a lot is a quantity of food or food units produced and handled under uniform conditions. Under certain circumstances, it has been shown that the logarithms of the counts from a batch of food are likely to be normally distributed (Baird-Parker, 1980; Kilsby & Baird-Parker, 1983 [their Table 2]; Ingram & Roberts, 1976). The implication is that there is homogeneity within a lot, which, when considering microbial levels and distributions, rarely occurs in practice. In most instances, the distribution of microorganisms within lots of food is heterogeneous. Even in food factories, this heterogeneity makes the interpretation of sample results difficult, and it becomes even more so in circumstances where a lot is less well defined, such as in a consignment of a very large quantity of food.

It is therefore helpful if suppliers can be persuaded to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load).

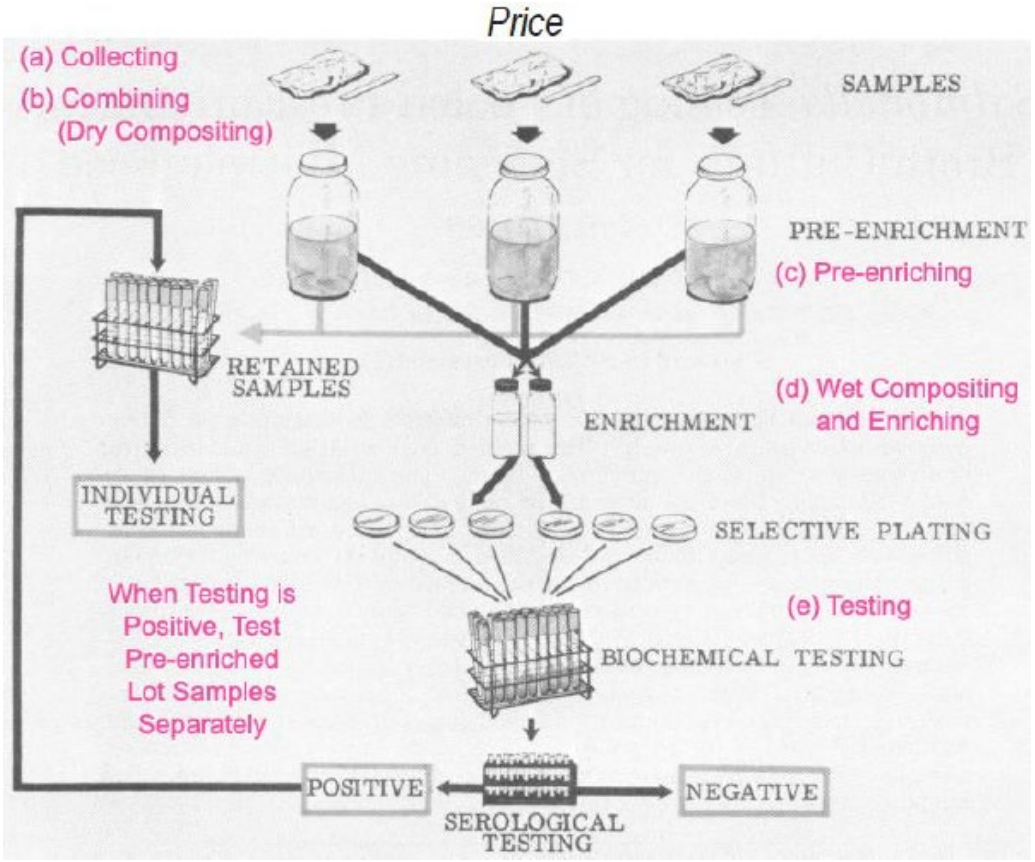
If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment.

A lot should be composed of food produced with as little variation as possible for a given process or commodity. Because of the uncertainties in identifying a lot commercially, however, the use of the word *lot* in this book is usually in its statistical sense, as a collection of units of a product, the acceptability of which is determined by examining a sample drawn from it.

ICMSF, 118-19 (annotated).

	Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Because Hughes explains the steps to be taken when testing is positive (*see* step (e)(3)*,* a POSITA would also expect negative testing results. Hughes, 638. Determining whether meat is safe for consumption so it may be released into commerce is a well-understood purpose of testing meat. Moreover, the recitation of a lot being validated for "release . . . into commerce" is not limiting. Prosecution History for Reexamination Control No. 95/011,294.
[1e.3] wherein when such testing is positive, each of the individual separate test lots are validated for release into commerce, according to the statistical-based sampling plan, by further testing of a portion of the respective enriched separate test lot sample using the same or a more sensitive protocol and obtaining a negative test result for each test lot,	Price, alone or in combination with ICMSF and Hughes, discloses wherein when such testing is positive, each of the individual separate test lots are validated for release into commerce, according to the statistical-based sampling plan, by further testing of a portion of the respective enriched separate test lot sample using the same or a more sensitive protocol and obtaining a negative test result for each test lot. The combination of Price, ICMSF, and Hughes describes retaining a portion of the enriched lot samples (steps (b)-(d)). Price indicates "a portion of each pre-enrichment is . . . retained . . . for later reference to the individual samples" and "individual tests on the retained samples can be made to determine which sample or samples contributed *Salmonella* to the pool." Price, 679. Price states, "If the *Salmonella* test of the pooled preenrichment broths is positive, individual tests on the retained samples can be made to determine which sample or samples contributed *Salmonella* to the pool." *Id.,* 679. Price discloses "the pooling of broth cultures permits small portions of each individual culture to be retained for subsequent analysis if a positive test is detected following enrichment, thus maintaining the individuality of each test sample." Price,

681.



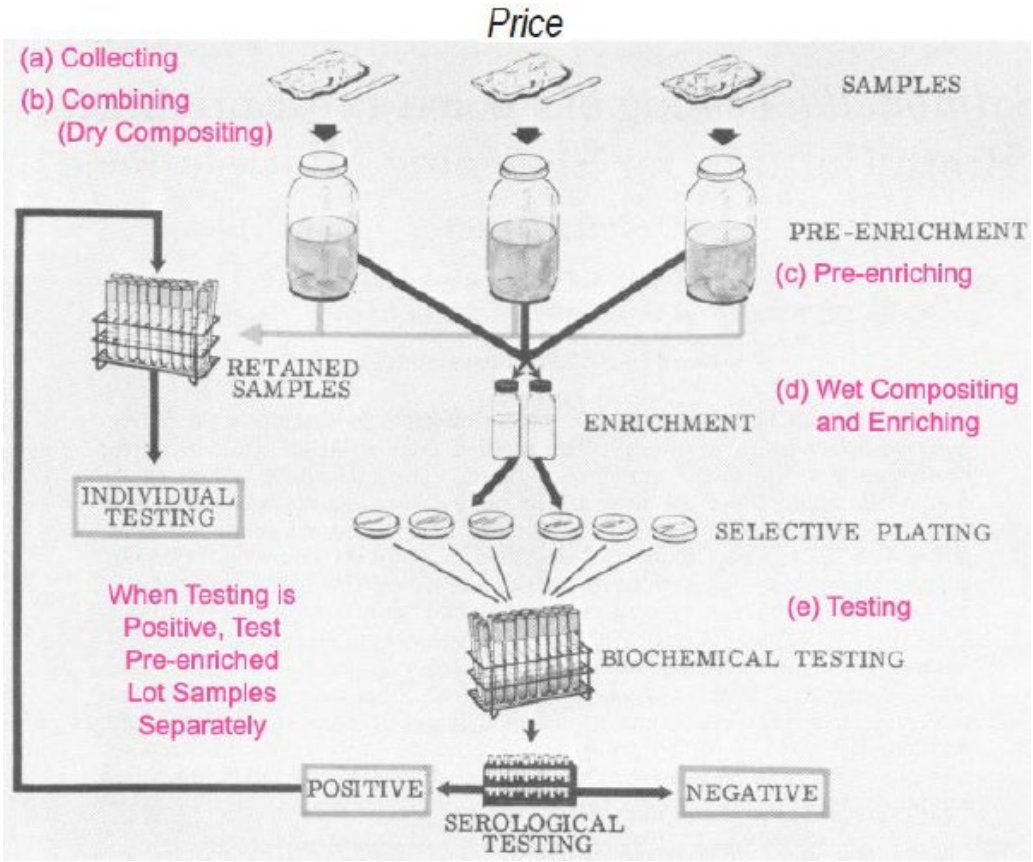
Price, 680 (Figure 1 (annotated)).

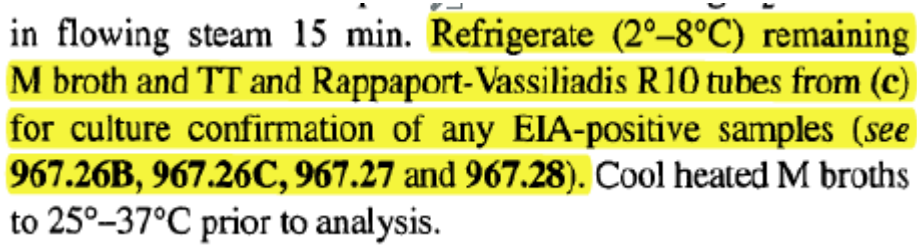
Price states "[i]f the *Salmonella* test of the pooled pre-enrichment broths is positive, individual tests on the retained samples can be made to determine which sample or samples contributed *Salmonella* to the pool":

	MATERIALS AND METHODS *Salmonella* analyses were carried out as described in the *Bacteriological Analytical Manual* (BAM) (2) except for the pooling of pre-enrichment broths described in the following paragraph. The procedure for *Salmonella* testing of pooled pre-enrichment broths is illustrated in Fig. 1. Samples are individually pre-enriched followed by transfer from multiple pre-enrichments to single selenite and tetrathionate broths at the enrichment stage. During pooling, a portion of each pre-enrichment is transferred to a sterile culture tube and retained at 4 C for later reference to the individual samples. If the *Salmonella* test of the pooled pre-enrichment broths is positive, individual tests on the retained samples can be made to determine which sample or samples contributed *Salmonella* to the pool. Price, 679 (annotated). A POSITA would have considered retention of samples that had been selectively enriched as an option. A POSITA would have replaced Price's steps of testing a composite sample from the pre-enriched lot samples to obtain a negative result and further testing retained portions of the pre-enriched lot samples with the combined teachings of ICMSF and Hughes. These combined teachings provide for testing a composite made from the selectively enriched lot samples to obtain a negative result and further testing retained portions of the enriched lot samples after a positive test of the composite by a more sensitive protocol. "Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119.

	"There is often a desire to retest retained samples." ICMSF, 191. If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment. ICMSF, 119 (annotated). ICMSF discloses validating for release into commerce (ICMSF, 113–15 (§§ 6.2–6.6), 118–19 (§§ 6.9–6.10), 184–85 (§ 10.2), 325–27 (§§ 17.5.3–17.5.3.4); *see also id.*, 186–89 (§ 10.4–10.6)) according to a statistically-based sampling plan (*id.*, 113 (§ 6.1); *see also id.*, 115–18 (§§ 6.4–6.8), 184 (§ 10.2), 319–21 (§§ 17.2.3.3–17.2.3.4), 326–30 (§§ 17.5.3.1–17.6), as described regarding steps (a) and (e)(2). Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Hughes explicitly teaches retention of the enriched test lot samples for further testing after a positive result. Hughes provides instructions to refrigerate the "remaining M broth and TT and Rappaport-Vassiliadis R10 tubes [that were enriched] for culture confirmation of any EIA-positive samples." Hughes, 638. Hughes refers to the results as the "confirmed positive samples" (Hughes, 635) and also states that diagnostic reagents are "necessary for culture confirmation of presumptive EIA tests." Hughes, 636. Hughes indicates "[e]nrichment broths and M broths from samples positive by an

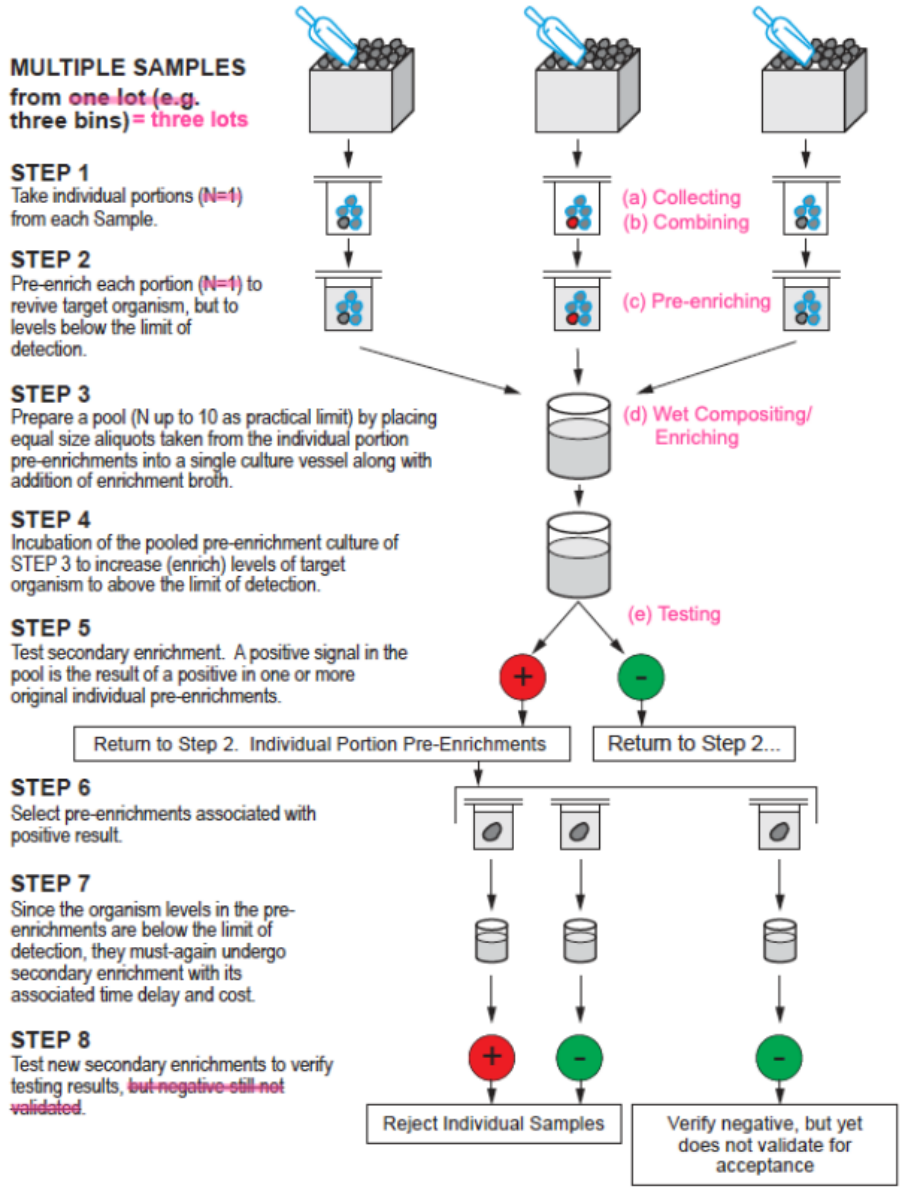
	enzyme immunoassay (EIA) method must be streaked on selective media." *Id.*, 635. Culture confirmation using streaking is more sensitive than the EIA method, as was commonly understood and confirmed by the teachings of D'Haese. *See, e.g.*, D'Haese. Each sample was analyzed by the BAM method (7th Ed.) and the TSVIA method using the enrichment protocol described in section F of the method, *Preparation of Sample*. Equal portions (0.5–1 mL) from each of the 2M broths arising from the 2 selective-enrichment broths were combined in a small screw-capped tube and heat treated in boiling water for 15 min prior to performing the TSVIA. Confirmation for the immunoassay was by streaking from the M broth culture onto selective agar, as specified in the BAM reference culture method. Hughes, 635. Moreover, the recitation of a lot being validated for "release . . . into commerce" is not limiting. Prosecution History for Reexamination Control No. 95/011,294. A POSITA would have recognized the concentration step could be employed to provide a more sensitive protocol for further testing. A POSITA would understand that retesting the non-pooled, singular samples is an inherently more sensitive process because the sample is not diluted with other samples in the testing pool. Therefore, the retesting of a singular sample is more concentrated and therefore, more sensitive. *See also, generally*, Jarvis.
[1e.4] wherein the enrichment in step c), prior to removing portions in step d), is sufficient	Price, alone or in combination with ICMSF and Hughes, discloses wherein the enrichment in step c), prior to removing portions in step d), is sufficient to enable

to enable said validation of individual separate test lots with no requirement for further enrichment thereof, and with no requirement for further sampling in a).	said validation of individual separate test lots with no requirement for further enrichment thereof, and with no requirement for further sampling in a).  Price, 680 (Figure 1 (annotated)). ICMSF and Hughes disclose selectively enriching prior to removing portions, thus a POSITA would consider the enrichment sufficient to enable validation of the lots without further enrichment or sampling. Hughes explicitly indicates portions of the enrichment cultures are retained for

	further testing; thus Hughes has no need for further sampling. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. ICMSF and Hughes disclose selective enrichment so further enrichment is unnecessary. Also, as discussed regarding step (e)(3), Hughes explicitly indicates portions of the enrichment cultures are retained for further testing; thus Hughes has no need for further sampling. The same would be expected for enrichment cultures in ICMSF because retaining samples at each process stage was considered standard practice for a POSITA.  in flowing steam 15 min. Refrigerate (2°–8°C) remaining M broth and TT and Rappaport-Vassiliadis R10 tubes from (c) for culture confirmation of any EIA-positive samples (*see* **967.26B, 967.26C, 967.27** and **967.28**). Cool heated M broths to 25°–37°C prior to analysis. Hughes, 638 (annotated). Thus, the negative limitation of step (e)(4) is disclosed by and is obvious based on the combination of ICSMF and Hughes. *Id.*
Claim 2	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 1, wherein each of the test lots is a single-unit production lot, comprised of operationally-linked product specimens.	Price, alone or in combination with ICMSF and/or Hughes, discloses the method of claim 1, upon which this claim relies. See above re Claim 1. Price, alone or in combination with ICMSF and/or Hughes, further discloses wherein each of the test lots is a single-unit production lot, comprised of operationally-linked product

	specimens.

Figure 3: CITED PRIOR ART (PRICE et al, 1972)



	Prosecution History for Reexamination Control No. 95/001,294, 298 (annotated). IEH's diagram also indicates multiple samples are taken from three bins, which is analogous to three lots rather than a single lot because each bin is an assemblage of specimens. The specification of the '584 Patent indicates a test lot is a single-unit production lot ('584 Patent, 5:21-32) such as a single combo, bin. pallet, or segment of production ('584 Patent, 7:28-36; *see also* AAPA #3 and #4). ICMSF teaches collecting from "individual production batches as lots" (ICMSF, 119) and "[a] lot is considered as a collection of units" (ICMSF, 327). "Operationally-linked" is simply grouping like items, which is also disclosed by ICMSF. "Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119.
Claim 3	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 1, further comprising incubating the modular composite sample of d), prior to testing in e).	Price, alone or in combination with ICMSF and/or Hughes, discloses the method of claim 1, upon which this claim relies. See above re Claim 1. Price, alone or in combination with ICMSF and/or Hughes, further discloses further comprising incubating the modular composite sample of d), prior to testing in e). Price discloses "[s]amples are individually pre-enriched followed by transfer from multiple pre-enrichments to single selenite and tetrathionate broths at the enrichment stage." Price, 679. These pre-enrichment and enrichment steps (after the samples are composited) inherently disclose an incubation period in which levels of the target microbial agent, in this case, *Salmonella*, grow to detectable levels for testing. Price states "[a]lternatively, problems with interfering organisms may warrant the use of direct selective enrichments" and further mentions "a reduction in the

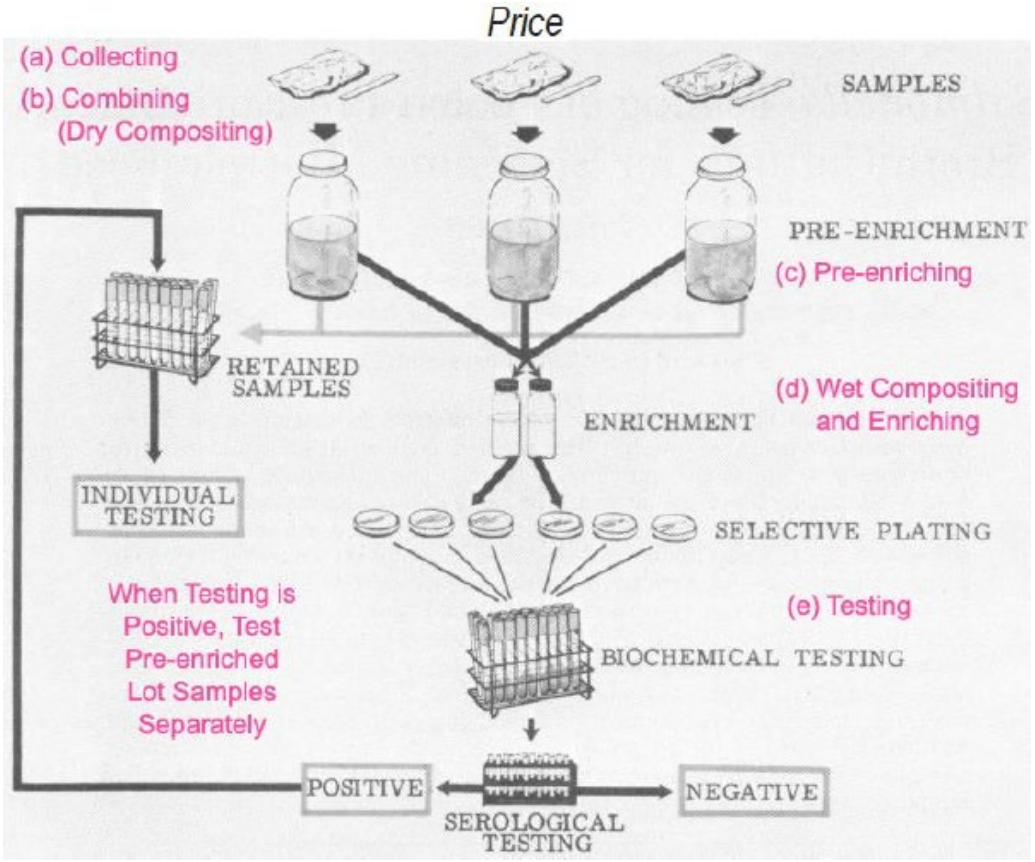
	incubation time of the pre-enrichment cultures." Price inherently discloses an incubation period of the pre-enrichment samples. **Whether pooled pre-enrichments are less sensitive than individual tests in the presence of interfering organisms probably is not known. For the present, we would recommend that, if numerous non-*Salmonella* colonies that resemble *Salmonella* on selective agar media are encountered in the pooled test, the retained pre-enrichment cultures should be tested individually. Alternatively, problems with interfering organisms may warrant the use of direct selective enrichments, two selective enrichments, or possibly even a reduction in the incubation time of the pre-enrichment cultures.** Price, 682 (annotated). Hughes describes incubating an enriched test lot sample. Hughes, 636, 638. Stewart discloses that after the selective enrichment in Rappaport-Vassiliadis (RV) broth and tetrathionate (TT) broths, the two selective enrichments were combined into a single sample for further incubation and analysis. "[P]ost-enrichment by transfer and combination of 0.5 mL RV and 1 mL TT into a single tube containing 10 mL prewarmed trypticase soy broth supplemented with novobiocin (TSB + n) at 10 µg/mL with incubation for 16–20 h at 42°C." Stewart, 398. Hughes also discloses an incubation protocol. "For the previous AOAC studies on TSVIA, a cultural protocol using selective enrichment in selenite cystine (SC) and tetrathionate (TT) broths at 35°C was used. However, there has been a recent trend

	to use elevated incubation temperatures and to replace SC broth with the Rappaport-Vassiliadis (RV) medium (2).” Hughes, 634. A POSITA would have been motivated to incubate the pooled modular composite sample of Gombas or Hughes, as taught by Stewart, for reliable testing results.
Claim 4	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 1, wherein incubating each separate test lot sample in c) comprises incubating each separate test lot sample with enrichment medium suitable to enrich for the target microbial agent or organism, and wherein removing in d) comprises removing a portion of the enrichment medium from each enriched separate test lot sample and combining the removed portions to provide a modular composite sample.	Price, in combination with ICMSF and Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. Price, in combination with ICMSF and Hughes, further discloses incubating each separate test lot sample in c) comprises incubating each separate test lot sample with enrichment medium suitable to enrich for the target microbial agent or organism, and wherein removing in d) comprises removing a portion of the enrichment medium from each enriched separate test lot sample and combining the removed portions to provide a modular composite sample. Price discloses “[s]amples are individually pre-enriched followed by transfer from multiple pre-enrichments to single selenite and tetrathionate broths at the enrichment stage.” Price, 679. The pre-enrichment step inherently discloses an incubation period for each individual sample in which levels of the target microbial agent, in this case, *Salmonella*, grow to detectable levels for testing. The enriched sample is then transferred and combined with other samples. Price states “[a]lternatively, problems with interfering organisms may warrant the use of direct selective enrichments” and further mentions “a reduction in the incubation time of the pre-enrichment cultures.” Price inherently discloses an incubation period of the pre-enrichment samples.

	Whether pooled pre-enrichments are less sensitive than individual tests in the presence of interfering organisms probably is not known. For the present, we would recommend that, if numerous non-*Salmonella* colonies that resemble *Salmonella* on selective agar media are encountered in the pooled test, the retained pre-enrichment cultures should be tested individually. Alternatively, problems with interfering organisms may warrant the use of direct selective enrichments, two selective enrichments, or possibly even a reduction in the incubation time of the pre-enrichment cultures. Price, 682 (annotated). Price discloses wet compositing as "[s]amples are individually pre-enriched followed by transfer from multiple pre-enrichments to single selenite and tetrathionate broths at the enrichment stage." Price, 679.

	MATERIALS AND METHODS *Salmonella* analyses were carried out as described in the *Bacteriological Analytical Manual* (BAM) (2) except for the pooling of pre-enrichment broths described in the following paragraph. The procedure for *Salmonella* testing of pooled pre-enrichment broths is illustrated in Fig. 1. Samples are individually pre-enriched followed by transfer from multiple pre-enrichments to single selenite and tetrathionate broths at the enrichment stage. During pooling, a portion of each pre-enrichment is transferred to a sterile culture tube and retained at 4 C for later reference to the individual samples. If the *Salmonella* test of the pooled pre-enrichment broths is positive, individual tests on the retained samples can be made to determine which sample or samples contributed *Salmonella* to the pool. Price 679 (annotated).

	Pooled samples versus pooled pre-enrichment broths. Silliker (4) reported a loss in sensitivity when several samples were pooled in a large container of pre-enrichment broth as compared to individual sample analysis. This is not surprising since, to be detected, the desiccated and debilitated *Salmonella* cells in a dried food sample must grow out in pre-enrichment broth in competition with other bacteria in the sample. If many food samples are pooled at the pre-enrichment stage, the salmonellae face competition from the flora of all the samples in the pool. If, on the other hand, the samples are individually pre-enriched, the actively growing salmonellae might be expected to compete more favorably with organisms introduced from other samples when pooled at the enrichment stage. In addition, pooling pre-enrichment broths avoids the hazards and inconvenience of handling very large flasks of culture. Price, 681 (discussing Silliker).



Price, 680 (Figure 1 (annotated)). Price's Figure 1 indicates that selective plating, biochemical testing, and serological testing are used.

ICMSF describes the well-known benefits of combining enrichment cultures from analytical units from lots in a consignment.

	To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. A laboratory investigation undertaken for ICMSF (Silliker & Gabis, 1973) indicated that different sized analytical units drawn from a single well-mixed sample unit of dried food give very similar results. A subsequent investigation (Gabis & Silliker, 1974) using foods of high moisture content (including eggs, poultry meat, meat, and meat products) showed comparable reliability in the use of composite analytical units; for example, examination of three composites of 20 × 25 g analytical units gave the same result as examination of all 60 analytical units individually (the total amount examined being 1500 g in both instances). This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n*, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort. ICMSF, 187. Hughes describes incubating an enriches test lot sample. Hughes 636, 638.
Claim 5	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 1, wherein testing of the modular composite sample, using a suitable detection assay further comprises a concentration step to concentrate the microbial agent or organism.	Price, in combination with ICMSF and Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. Price, in combination with ICMSF, Hughes and other secondary prior art, further discloses testing of the modular composite sample, using a suitable detection assay further comprises a concentration step to concentrate the microbial agent or organism. Price discloses an enrichment stage after compositing where "[s]amples are individually pre-enriched followed by transfer from multiple pre-enrichments to single selenite and tetrathionate broths at the enrichment stage." Price, 679.

	A POSITA would understand that a detection assay that includes enrichment could further comprise a concentration step. Enrichment for the microbial agent or organism increases its concentration. ICMSF discloses the use of "immunomagnetic beads to concentrate cells." ICMSF, 318. A POSITA would consider this a well-known technique before the priority date. Fratamico discusses the rapid isolation of target microorganisms using an immunomagnetic separation method. Fratamico, 105. Fratamico discloses that use of immunomagnetic beads resulted in "an increase in the level of E. coli B1409 in the sample," as well as "an increase in the number of organisms recovered." *Id.*, 107. Additionally, Fratamico provides detailed instructions for preparing and using immunomagnetic bead separation to concentrate a target microbial agent or organism. Fratamico developed "[a] method employing immunomagnetic beads" to concentrate and thereby improve the isolation and detection of E. coli. *Id.* For example, Fratamico was able to use immunomagnetic beads to isolate *E. coli* strains from enrichment cultures of raw ground beef "when the initial inoculum level was 1 cfu g-1." *Id.*, 109. Fratamico further teaches that "[t]he percentage of bacteria recovered increased with increasing amounts of beads." *Id.*, 108. Consequently, Fratamico "demonstrates that immunomagnetic separation [using immunomagnetic beads] is a simple and rapid technique" for concentrating a microbe for isolation and detection. *Id.*, 109. These teachings of Fratamico would have motivated a POSITA to modify the combination of ICMSF and Gombas and ICMSF and Hughes to use immunomagnetic beads to concentrate microbes in a modular composite sample to benefit from accurate tests. Use of immunomagnetic beads was already known in the art for concentrating during testing beef samples. "Tu et al. (78) detected pathogenic E. coli O157:H7 in beef hamburger with the use of IMS and ATP measurement. After 6 h of enrichment at

	37°C, inoculated beef samples (0.9 ml each) were filtered to remove large particles prior to 10 min of incubation with ca. 10^5 magnetic beads coated with anti–*E. coli* O157:H7 (Dynabeads, Dynal Inc., Oslo, Norway). Following incubation, the magnetic beads were collected with a Dynal magnetic concentrator and resuspended in 0.9 ml of Trypticase soy broth for ATP analysis." Benoit, 1941. "Hudson et al. (26) used IMS to directly separate and concentrate L. monocytogenes from ham samples prior to detection by PCR. IMS was used on the basis of the commercial availability of anti-Listeria magnetic beads (Dynal Inc.) to avoid time-consuming cultural enrichment steps and to remove PCR inhibitors." Benoit, 1941-42. Cudjoe investigates the use of immunomagnetic beads in pooling methods. In a study designed to evaluate the performance of Dynabeads® Anti-Salmonella, immunomagnetic separation (IMS) followed by plating (IMS-Plating) proved far superior to the conventional ISO *Salmonella* methodology and the Modified Semi-solid Rappaport-Vassiliadis (MSRV) method. *Salmonella* species were isolated and detected from 135 out of the 180 diverse poultry samples by IMS analysis as against 98 by the conventional method and 33 by the MSRV technique. All results were confirmed biochemically and serologically. It appeared from the data generated that some of the salmonellae isolated using IMS were greatly inhibited in Rappaport-Vassiliadis enrichment broth at 42°C and to a lesser extent in selenite cystine broth at 37°C. A moderately selective plating medium like XLD proved to be better in isolating these possible sensitive wild-type strains of salmonellae than the more selective BGA. Cudjoe, Abstract.

	Samples. One hundred and eighty (180) diverse poultry samples, consisting of 40 pools of cloacal/faecal swabs (5 swabs/pool; 5 pools samples/flock) from eight different flocks, 10 raw chicken breast meat samples, 10 pooled egg shell samples, 60 raw chicken liver samples and 60 chicken neck skins sampled from the slaughter line, were used in this study. All samples were obtained from a poultry slaughter and processing plant in the Netherlands. A pool of five swab samples was pre-enriched in 10 ml of buffered peptone water (BPW; Difco Laboratories, Detroit, Michigan, USA) at 37°C for 16–20 h, whilst 25 g of each of the other samples above, were pre-enriched similarly in 225 ml of BPW and examined as detailed below. Cudjoe, 160.
Claim 6	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 1, wherein testing in e) of the modular composite sample is positive, and wherein the individual separate test lot samples are further tested, such further testing comprises the more sensitive protocol.	Price, in combination with ICMSF and Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. Price, in combination with ICMSF, Hughes and other secondary prior art, further discloses wherein testing in e) of the modular composite sample is positive, and wherein the individual separate test lot samples are further tested, such further testing comprises the more sensitive protocol. ICMSF discloses the use of "immunomagnetic beads to concentrate cells." ICMSF, 318. A POSITA would consider this a well-known technique before the priority date. Hughes uses an EIA method followed by a more sensitive protocol—streaking on selective media. Hughes, 635.

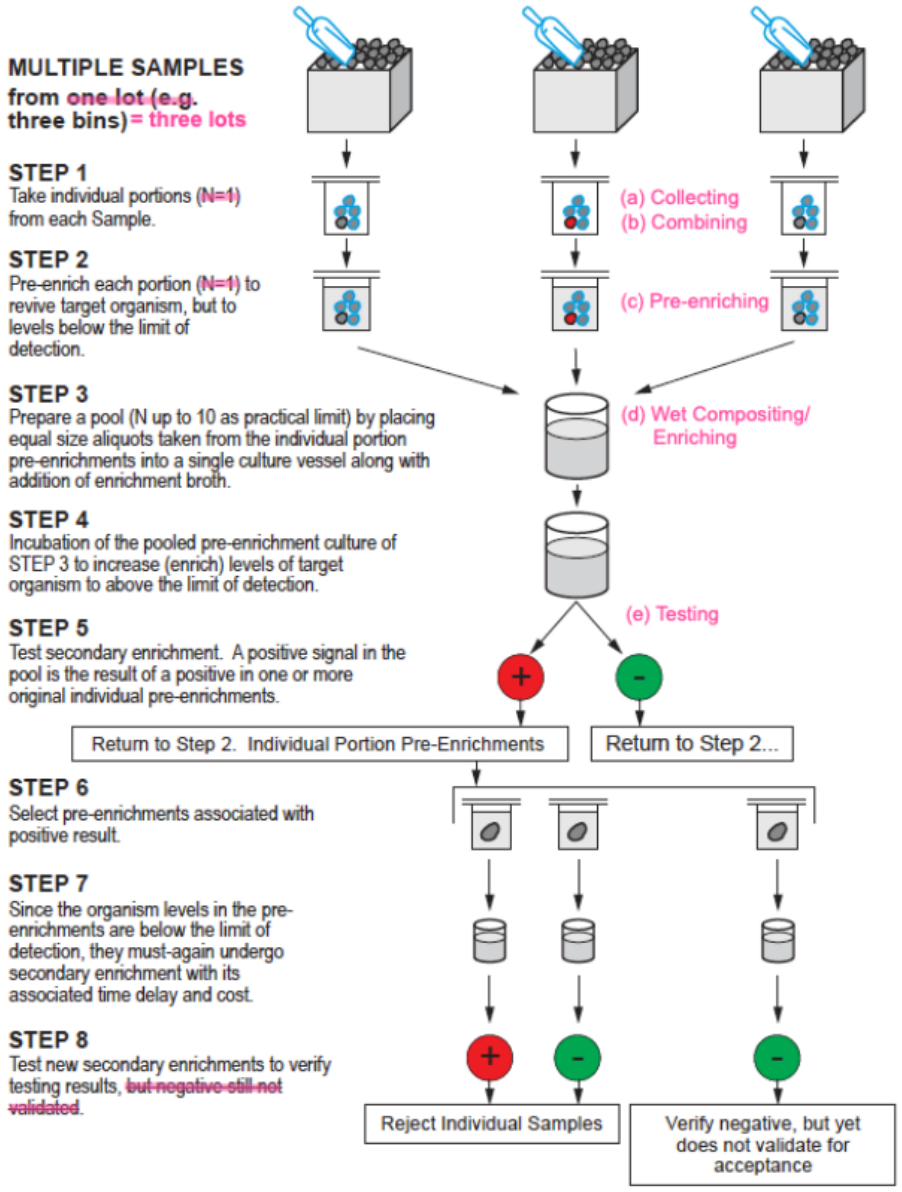
	Each sample was analyzed by the BAM method (7th Ed.) and the TSVIA method using the enrichment protocol described in section **F** of the method, *Preparation of Sample*. Equal portions (0.5–1 mL) from each of the 2M broths arising from the 2 selective-enrichment broths were combined in a small screw-capped tube and heat treated in boiling water for 15 min prior to performing the TSVIA. Confirmation for the immunoassay was by streaking from the M broth culture onto selective agar, as specified in the BAM reference culture method. Hughes, 635. A POSITA would find that modifying Hughes based on D'Haese ensures a more sensitive test is used for further testing. A POSITA would have recognized the concentration step could be employed to provide a more sensitive protocol for further testing. A POSITA would understand that retesting the non-pooled, singular samples is an inherently more sensitive process because the sample is not diluted with other samples in the testing pool. Therefore, the retesting of a singular sample is more concentrated and therefore, more sensitive. *See also, generally*, Jarvis. Fratamico discusses the rapid isolation of target microorganisms using an immunomagnetic separation method. Fratamico, 105. Fratamico discloses that use of immunomagnetic beads resulted in "an increase in the level of E. coli B1409 in the sample," as well as "an increase in the number of organisms recovered." *Id.*, 107. Additionally, Fratamico provides detailed instructions for preparing and using immunomagnetic bead separation to concentrate a target microbial agent or organism.

	Fratamico developed "[a] method employing immunomagnetic beads" to concentrate and thereby improve the isolation and detection of E. coli. *Id.* For example, Fratamico was able to use immunomagnetic beads to isolate *E. coli* strains from enrichment cultures of raw ground beef "when the initial inoculum level was 1 cfu g-1." *Id.*, 109. Fratamico further teaches that "[t]he percentage of bacteria recovered increased with increasing amounts of beads." *Id.*, 108. Consequently, Fratamico "demonstrates that immunomagnetic separation [using immunomagnetic beads] is a simple and rapid technique" for concentrating a microbe for isolation and detection. *Id.*, 109. These teachings of Fratamico would have motivated a POSITA to modify the combination of ICMSF and Gombas and ICMSF and Hughes to use immunomagnetic beads to concentrate microbes in a modular composite sample to benefit from accurate tests. Use of immunomagnetic beads was already known in the art for concentrating during testing beef samples. "Tu et al. (78) detected pathogenic E. coli O157:H7 in beef hamburger with the use of IMS and ATP measurement. After 6 h of enrichment at 37°C, inoculated beef samples (0.9 ml each) were filtered to remove large particles prior to 10 min of incubation with ca. 10^5 magnetic beads coated with anti–*E. coli* O157:H7 (Dynabeads, Dynal Inc., Oslo, Norway). Following incubation, the magnetic beads were collected with a Dynal magnetic concentrator and resuspended in 0.9 ml of Trypticase soy broth for ATP analysis." Benoit, 1941. "Hudson et al. (26) used IMS to directly separate and concentrate L. monocytogenes from ham samples prior to detection by PCR. IMS was used on the basis of the commercial availability of anti-Listeria magnetic beads (Dynal Inc.) to avoid time-consuming cultural enrichment steps and to remove PCR inhibitors." Benoit, 1941-42. Cudjoe investigates the use of immunomagnetic beads in pooling methods.

	In a study designed to evaluate the performance of Dynabeads® Anti-Salmonella, immunomagnetic separation (IMS) followed by plating (IMS-Plating) proved far superior to the conventional ISO *Salmonella* methodology and the Modified Semi-solid Rappaport-Vassiliadis (MSRV) method. *Salmonella* species were isolated and detected from 135 out of the 180 diverse poultry samples by IMS analysis as against 98 by the conventional method and 33 by the MSRV technique. All results were confirmed biochemically and serologically. It appeared from the data generated that some of the salmonellae isolated using IMS were greatly inhibited in Rappaport-Vassiliadis enrichment broth at 42°C and to a lesser extent in selenite cystine broth at 37°C. A moderately selective plating medium like XLD proved to be better in isolating these possible sensitive wild-type strains of salmonellae than the more selective BGA. Cudjoe, Abstract. *Samples*. One hundred and eighty (180) diverse poultry samples, consisting of 40 pools of cloacal/faecal swabs (5 swabs/pool; 5 pools samples/flock) from eight different flocks, 10 raw chicken breast meat samples, 10 pooled egg shell samples, 60 raw chicken liver samples and 60 chicken neck skins sampled from the slaughter line, were used in this study. All samples were obtained from a poultry slaughter and processing plant in the Netherlands. A pool of five swab samples was pre-enriched in 10 ml of buffered peptone water (BPW; Difco Laboratories, Detroit, Michigan, USA) at 37°C for 16–20 h, whilst 25 g of each of the other samples above, were pre-enriched similarly in 225 ml of BPW and examined as detailed below. Cudjoe, 160. A POSITA would understand that retesting the individual test lot samples is an inherently more sensitive process because the sample is not diluted with other samples in the testing pool. Therefore, the retesting of a singular sample is more concentrated and therefore, more sensitive. *See also, generally*, Jarvis.
Claim 10	**Disclosure of Price, ICMSF, and Hughes**

The method of claim 1, wherein the test lot is selected from the lot group consisting of a single combo, single bin, single pallet, and a production assemblage.	Price, in combination with ICMSF and Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. Price, in combination with ICMSF and Hughes, further discloses wherein the test lot is selected from the lot group consisting of a single combo, single bin, single pallet, and a production assemblage. The specification of the '584 Patent indicates a test lot is a single-unit production lot ('584 Patent, 5:21-32) such as a single combo, bin. pallet, or segment of production ('584 Patent, 7:28-36; *see also* AAPA #3 and #4). ICMSF teaches collecting from "individual production batches as lots" (ICMSF, 119) and "[a] lot is considered as a collection of units." ICMSF, 327. "Operationally-linked" is simply grouping like items, which is also disclosed by ICMSF. "Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119.
Claim 11	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 1, wherein the number of separate test lots is from 3 to 10.	Price, in combination with ICMSF and Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. Price, in combination with ICMSF and Hughes, further discloses wherein the number of separate test lots is from 3 to 10 ICMSF indicates a consignment may be "composed of several lots." ICMSF, 119-20. "Several" lots includes at least three lots.

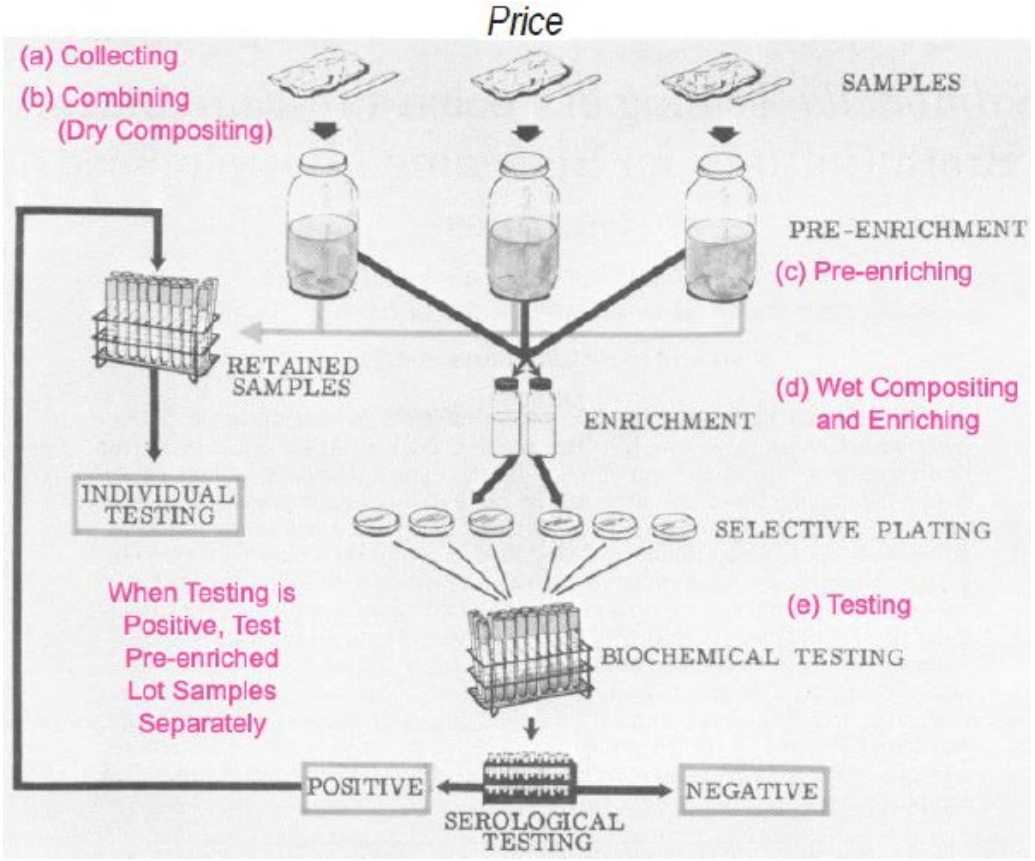
Figure 3: CITED PRIOR ART (PRICE et al, 1972)



	Prosecution History for Reexamination Control No. 95/001,294, 298 (annotated). IEH's diagram also indicates multiple samples are taken from three bins, which is analogous to three lots rather than a single lot because each bin is an assemblage of specimens.
Claim 12	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 1, further comprising, where testing of the modular composite sample is positive, determining which individual separate test lots are positive, application of remedial measures to said separate positive test lots, and validating by further testing of a portion of the respective remediated separate test lots and obtaining a negative test result.	Price, in combination with ICMSF and Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. Price, in combination with ICMSF and Hughes, further discloses further comprising, where testing of the modular composite sample is positive, determining which individual separate test lots are positive, application of remedial measures to said separate positive test lots, and validating by further testing of a portion of the respective remediated separate test lots and obtaining a negative test result. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638.

	Each sample was analyzed by the BAM method (7th Ed.) and the TSVIA method using the enrichment protocol described in section F of the method, *Preparation of Sample*. Equal portions (0.5–1 mL) from each of the 2M broths arising from the 2 selective-enrichment broths were combined in a small screw-capped tube and heat treated in boiling water for 15 min prior to performing the TSVIA. Confirmation for the immunoassay was by streaking from the M broth culture onto selective agar, as specified in the BAM reference culture method. Hughes, 635. A POSITA understands the purpose of testing for pathogens and other organisms in food is to determine if a food is safe for entry into commerce to be sold for consumption. One having ordinary skill in the art understands that if a sample tests positive, a remediation step, like cooking, needs to be completed before it can be sold into commerce to comply with USDA guidelines.
Claim 13	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 1, wherein, in a), separately collecting said plurality of portions from each of said plurality of separate test lots comprises separately collecting from each separate test lot a number of portions from 5 to 100.	Price, in combination with ICMSF and Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. Price, in combination with ICMSF and Hughes, further discloses wherein, in a), separately collecting said plurality of portions from each of said plurality of separate test lots comprises separately collecting from each separate test lot a number of portions from 5 to 100. ICMSF teaches collecting 5, 30, 60, 95, or 100 portions. ICMSF, 184-90, 276, 282, 309, 320, 326-30. *See also* MPEP § 2144.05.

Claim 14	Disclosure of Price, ICMSF, and Hughes
The method of claim 1, wherein, in a), separately collecting said plurality of portions from each of said plurality of separate test lots comprises separately collecting from each separate test lot 30 to 60 portions.	Price, in combination with ICMSF and Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. Price, in combination with ICMSF and Hughes, further discloses wherein, in a), separately collecting said plurality of portions from each of said plurality of separate test lots comprises separately collecting from each separate test lot 30 to 60 portions. ICMSF teaches collecting 5, 30, 60, 95, or 100 portions. ICMSF, 184-90, 276, 282, 309, 320, 326-30. *See also* MPEP § 2144.05.
Claim 15	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 1, wherein, in a), separately collecting said plurality of portions from each of said plurality of separate test lots comprises separately collecting 30 portions from each separate test lot.	Price, in combination with ICMSF and Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. Price, in combination with ICMSF and Hughes, further discloses wherein, in a), separately collecting said plurality of portions from each of said plurality of separate test lots comprises separately collecting 30 portions from each separate test lot. ICMSF teaches collecting 5, 30, 60, 95, or 100 portions. ICMSF, 184-90, 276, 282, 309, 320, 326-30. *See also* MPEP § 2144.05.
Claim 17	**Disclosure of Price, ICMSF, and Hughes**
[17pre] A method of sampling, testing and validating a plurality of operationally-linked test lots of meat or poultry for release into commerce, comprising:	To the extent the preamble is limiting, as discussed herein, Price, alone or in combination with ICMSF and Hughes, discloses a method of sampling, testing and validating a plurality of operationally-linked test lots of meat or poultry for release into commerce. Price established that "pre-enrichment broths can be pooled for analysis without loss in sensitivity to *Salmonella* in the individual samples." Price, 679. Price tested dry food materials inoculated (purposefully contaminated) with *Salmonella. Id.*



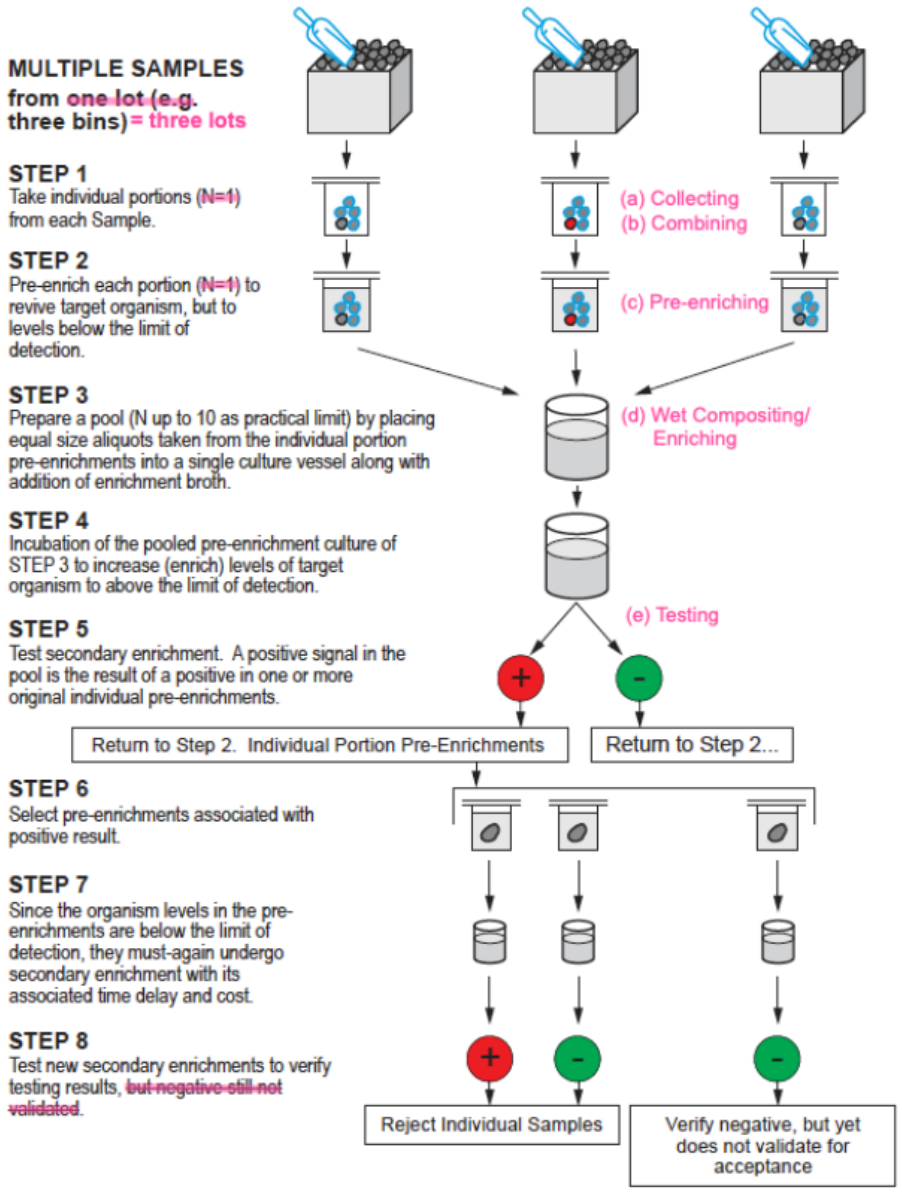
Price, 680 (Figure 1 (annotated)).

ICMSF includes many other references to testing lots of beef or poultry, two of the most common types of meat in commerce. *Id.*, 114 (§ 6.3), 184–191 (§§ 10.2–10.6), 225–226 (§ 12.1), 285 (§ 16.1), 313–330 (Chapter 17). ICMSF is relevant to any kind of food product including beef.

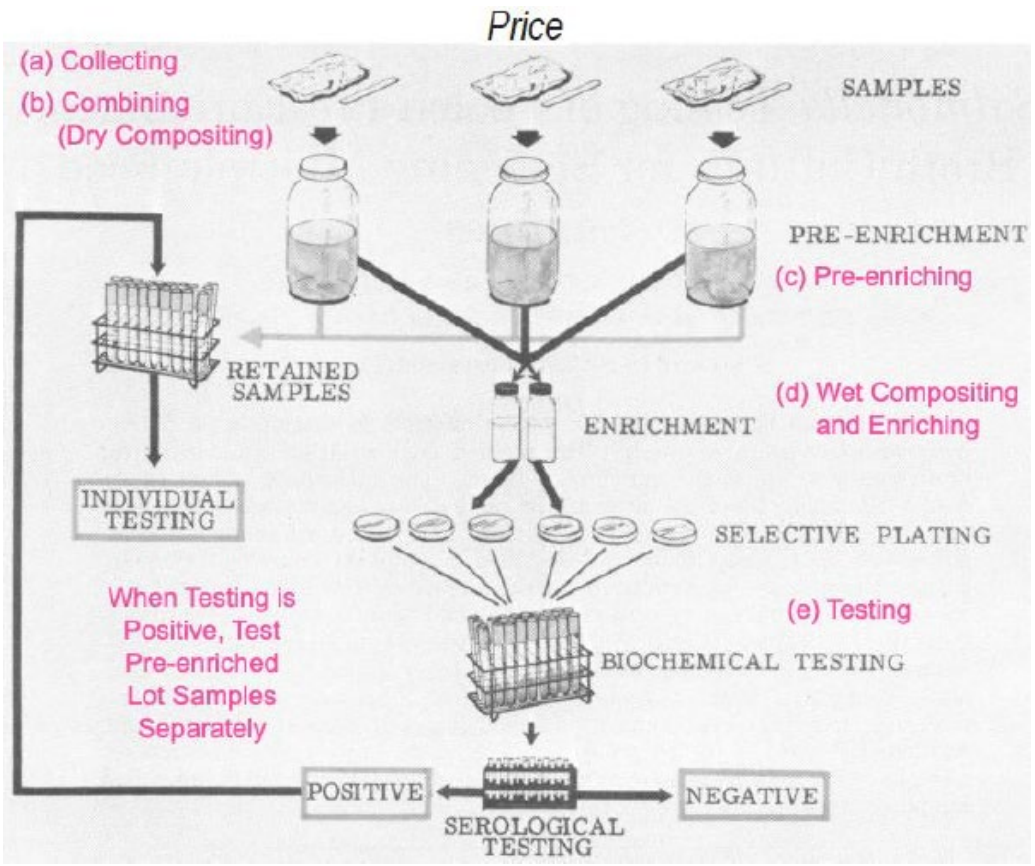
	Hughes discusses turkey (Hughes, Abstract), and a POSITA would be motivated to combine the teachings of ICMSF and Hughes as they both test meat for release into commerce. Hughes discloses testing turkey for *Salmonella*. "Three food types (. . . and raw turkey) were analyzed in the United States." Hughes, 634, 645, Table 8. Table 8 shows results from 12 collaborators for naturally contaminated raw turkey. For the 180 samples tested, using confirmed data, there was one false negative at the low level for the TECRA method. There were 3 false negatives at the low level and 3 false negatives at the high level for the cultural method. The incidence of false-positive results was very high for the cultural method, with a total of 41 false positives, compared with 5 for the TECRA method. It should, however, be taken into account that the comparison was with the method in BAM 7th Ed., not the current 8th Ed. The method for raw flesh food in the BAM 8th Ed. uses elevated temperature with RV and TT broth, which would be more selective. No significant differences ($p > 0.05$) were observed for the pairwise comparison of the proportion of positive samples for the TECRA method with that for the reference method. Hughes, 646. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638.

	Moreover, the recitation of a lot being validated for "release . . . into commerce" is not limiting. Prosecution History for Reexamination Control No. 95/011,294 (Six Parts).
[17a] a) collecting a plurality of portions from each of a plurality of operationally-linked single-unit production lots of meat or poultry, the single-unit production lots each comprising an assemblage of one or more production specimens from which the portions are collected, wherein each single-unit production lot is separately sampled to conform to a statistical-based sampling plan specifying, for a particular microbial agent or organism, a number of portions to be taken sufficient to provide for validation and release of the single-unit production lot into commerce;	Price, alone or in combination with ICMSF and Hughes, discloses collecting a plurality of portions from each of a plurality of operationally-linked single-unit production lots of meat or poultry, the single-unit production lots each comprising an assemblage of one or more production specimens from which the portions are collected, wherein each single-unit production lot is separately sampled to conform to a statistical-based sampling plan specifying, for a particular microbial agent or organism, a number of portions to be taken sufficient to provide for validation and release of the single-unit production lot into commerce. Price's teachings for collecting a plurality of portions are based on standard practices as described in BAM 1969 (BAM 1969, 1 § 10.04) which cites to JAOAC 1967 (JAOAC, 234). Price, 679, 682. ***Salmonella* analyses were carried out as described in the *Bacteriological Analytical Manual* (BAM) (2) except for the pooling of pre-enrichment broths described in the following paragraph.** Price, 679. IEH's diagram of Price from Prosecution History for Reexamination Control No. 95/001,294 (reproduced below) shows step 1 corresponds to IEH's step (a), with instructions to "[t]ake individual portions . . . from each Sample."

Figure 3: CITED PRIOR ART (PRICE et al, 1972)



	Prosecution History for Reexamination Control No. 95/001,294, 298 (annotated). IEH's diagram also indicates multiple samples are taken from three bins, which is analogous to three lots rather than a single lot because each bin is an assemblage of specimens. The purpose of Jarvis and Price was to determine whether pooling samples reduced the ability to detect contaminated food lots. It would have been commonsense to combine samples from the same bin and subject them to enrichment, since each bin represents a discrete unit of the production run and results obtained from the enriched sample could be attributed to the bin from which the sample was obtained. Prosecution History for Reexamination Control No. 95/001,294, at 1575 (First Board Decision of January 20, 2015 at 16). Additionally, the specification describes a bin of dry food materials (almonds), like those in Price, as one example of a test lot/single unit of production, like a combo of beef. '584 Patent, 5:33-38. Price discloses testing of "Dried egg albumen, cocoa, and nonfat dry milk" (Price, 679) and "wheat flour, coconut, and cottonseed flour" (Price, 681).



Price, 680 (Figure 1 (annotated)).

	A method has been described for testing multiple food samples for *Salmonella* without loss in sensitivity. The method pools multiple pre-enrichment broth cultures into single enrichment broths. The subsequent stages of the *Salmonella* analysis are not altered. The method was found applicable to several dry food materials including nonfat dry milk, dried egg albumin, cocoa, cottonseed flour, wheat flour, and shredded coconut. As many as 25 pre-enrichment broth cultures were pooled without apparent loss in the sensitivity of *Salmonella* detection as compared to individual sample analysis. The procedure offers a simple, yet effective, way to increase sample capacity in the *Salmonella* testing of foods, particularly where a large proportion of samples ordinarily is negative. It also permits small portions of pre-enrichment broth cultures to be retained for subsequent individual analysis if positive tests are found. *Salmonella* testing of pooled pre-enrichment broths provides increased consumer protection for a given amount of analytical effort as compared to individual sample analysis. Price, 679 (annotated). A POSITA would have replaced Price's collection and combination of dry food with ICMSF's collection and combination of portions from a consignment of meat lots in conformance to a statistical-based sampling plan for release into commerce. A POSITA would find it obvious to apply the teachings of Price to beef. "Many different types of samples are collected and submitted to the laboratory for analysis. Some are sample units from lots or consignments of food or ingredients for lot acceptance determination. . . . A sample unit is a small portion of the lot in which the distribution of both numbers and types of microorganisms should be representative of the whole lot." ICMSF, 255. The lots may be beef or poultry. *Id.*, 184-91, 226, 230. Thus, ICMSF discloses collecting a plurality of samples or sample units (other synonyms include portions, specimens, and sub-samples) from lots of beef or poultry.

	ICMSF describes collecting from a lot or lots: "If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." *Id.*, 119. Additionally, ICMSF indicates a consignment may be "a very large quantity of food" and may be "composed of several lots, representing for example, different days of production from the same plant." *Id.*, 119, 120; *see also* AAPA #3-#5.

6.11 WHAT IS A REPRESENTATIVE SAMPLE?

A *representative sample* reflects, as far as is possible, the composition of the lot from which it is drawn.

How then should a representative sample be drawn? It is important to avoid bias and to draw a sufficient number of sample units to confidently make a judgment about a lot. Sampling at random is the universally recognized way of avoiding bias and is more reliable than consciously trying to draw the sample units from the various parts of a lot. The units (cartons or containers, particular weights of solid, or volumes of liquid) are drawn by using random numbers. There is, of course, no guarantee that a random sample has characteristics identical with those of the lot, but the randomness of sampling is the basis for calculation of the probability that a sample will give a biased result.

It is also possible, and may be desirable, to use a *stratified random sampling* approach, drawing a random sample of a given number of sample units from each *stratum* (e.g., each sublot or carton). The distribution of sample units on strata should correspond to the distribution of lot units on strata. This means that if each stratum contains the same quantity as every other one, the number of sample units per stratum should be the same. Otherwise, they should differ just as the proportions of the lot contained in the strata differ. Stratification is a device for handling known sources of variation and may be used where one has prior knowledge that the consignment is potentially not of uniform quality. A consignment might not be of uniform quality if portions of it are shipped in different vehicles or in different holds of a vessel, or if it is known that it is really composed of several lots representing, for example, different days of production from the same plant or different plants of the same company. The results for different strata should be assessed separately and then pooled if they appear to be homogeneous.

The calculation of acceptance probabilities is based on the assumption that random samples have been drawn and analyzed. Where physical or practical constraints prevent random sampling, such calculated probabilities may not be valid. Their reliability depends on how close reality approaches the ideal situation of random sampling. This fact should be borne in mind when subsequently interpreting results.

ICMSF, 119-20 (annotated).

6.10 WHAT IS A LOT?

Ideally, a lot is a quantity of food or food units produced and handled under uniform conditions. Under certain circumstances, it has been shown that the logarithms of the counts from a batch of food are likely to be normally distributed (Baird-Parker, 1980; Kilsby & Baird-Parker, 1983 [their Table 2]; Ingram & Roberts, 1976). The implication is that there is homogeneity within a lot, which, when considering microbial levels and distributions, rarely occurs in practice. In most instances, the distribution of microorganisms within lots of food is heterogeneous. Even in food factories, this heterogeneity makes the interpretation of sample results difficult, and it becomes even more so in circumstances where a lot is less well defined, such as in a consignment of a very large quantity of food.

It is therefore helpful if suppliers can be persuaded to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load).

If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment.

A lot should be composed of food produced with as little variation as possible for a given process or commodity. Because of the uncertainties in identifying a lot commercially, however, the use of the word *lot* in this book is usually in its statistical sense, as a collection of units of a product, the acceptability of which is determined by examining a sample drawn from it.

ICMSF, 118-19 (annotated).

	The ICMSF has defined a lot as follows: In the commercial sense, a lot is a quantity of food supposedly produced under identical conditions, all packages of which would normally bear a lot number that identifies the production during a particular time interval, and usually from a particular line, retort, or other critical processing unit. Statistically, a lot is considered as a collection of units of a product from which a sample is drawn to determine acceptability of the lot (ICMSF, 1986). In this example, the maximum size of a lot consists of all the ground beef produced on common equipment from cleanup to cleanup. Smaller lots could be established (e.g., hourly). ICMSF, 327 (annotated). This definition also reflects the common practice referred to in the '584 Patent at Example II, wherein "[p]referably, the producer defines and produces these single-unit lots in such a fashion as to maximize the information available[.]" '584 Patent,12:46–48. This corresponds with the specification of the '584 Patent which reflects the common practice of producers defining what constitutes a lot grouped for traceability purposes. '584 Patent, 2:60-3:3, 11:12-16 (AAPA #3), 12:46-49. Thus, ICMSF discloses collecting a plurality of portions from separate lots. ICMSF describes a lot as "a collection of units of a product." IMCSF, 119. ICMSF further indicates a lot has been defined as: "a quantity of food supposedly produced under identical conditions, all packages of which would normally bear a lot number that identifies the production during a particular time interval, and usually from a particular line, retort, or other critical processing unit. Statistically, a lot is considered as a collection of units of a product from which a sample is drawn to determine acceptability of the lot." ICMSF, 327. ICMSF advocates use of a "statistically based sampling plan." *Id.*, 113-15, 119-21, 320, 327, 328. In the sampling plans, "*n* is the number of sample units chosen separately and independently, and . . . *c* is the maximum allowable number of sample

	units yielding a positive result." *Id.*, 115. ICMSF discloses a wide range for the number of sample units (portions) for a particular microbial agent or organism. For example, ICMSF discusses collecting 15, 30, 60 or 100 sample units from a lot. *Id.*, 320, 326. **6.5 THE SAMPLING PLAN** The sample units so drawn will, after examination, yield results that will be compared with certain criteria to reach a decision as to whether the entire lot should be accepted or rejected (see section 6.9 for explanation of rejection). The particular choice of sampling procedure and the decision criteria make up the *sampling plan*. Proper drawing of samples is essential if the decision making criteria of the sampling plan are to give unbiased results. By sampling randomly, we are able to reduce the risk of making biased decisions. A simple example of a sampling plan follows. Take 10 sample units of a food from a lot and subject them to appropriate laboratory procedure to test for the presence or absence of a microorganism. If two or fewer of the 10 sample units show presence of the organism, that is, give a positive result, then the whole lot of food is acceptable (relative to this organism). But if three or more give a positive result, the whole lot is to be rejected. This plan is described by the use of two terms $n = 10$ and $c = 2$, where n is the number of sample units chosen separately and independently, and where c is the maximum allowable number of sample units yielding a positive result. ICMSF, 115 (annotated).

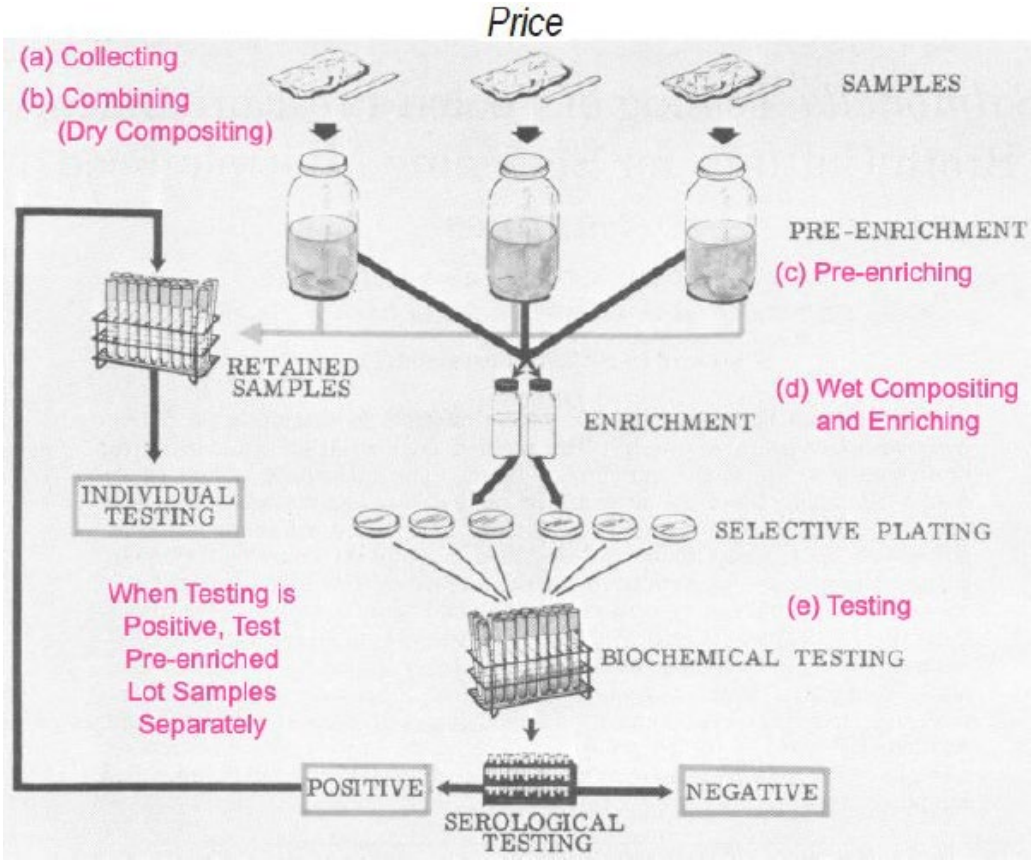
	6.1 INTRODUCTION It is important to recognize that the management of food safety using the approaches outlined in the first five chapters, based on controlling hazards through Good Hygienic Practices (GHP) and Hazard Analysis Critical Control Point (HACCP), is much more effective than trying to ensure safety by lot acceptance through end-product testing. This chapter discusses the concepts of probability and sampling that determine the limitations of end-product testing. Those concepts form the basis of the design of statistically based sampling plans (Chapter 7) and the establishment of cases (Chapter 8). In Chapters 6 and 7, it is assumed that the microbiological test will give an absolutely accurate result, with no false positives or false negatives. This is not a realistic assumption, and problems arising from sampling, sample transport and preparation for analysis, and the imprecision of microbiological methods are addressed in Chapters 10 and 12 and elsewhere in the text. ICMSF, 113 (annotated); *see also id.*, 115–21 (§§ 6.4–6.13), 319–20 (§ 17.2.3.3), and 326–30 (§§ 17.5.3.1–17.6). ICMSF discusses the number of portions to be taken sufficient for validation of a particular microorganism including *Salmonella* (*id.*, 276 (§ 15.3.3); *see also id.*, 282 (§ 15.6.3)); *Listeria* (*id.*, 309 (§ 16.6.3)), and *E. coli* (*id.*, 318–21 (§§ 17.2.3.2–17.2.3.4), 326 (§ 17.5.3.1)). IEH's AAPA discloses statistical-based sampling plans taking 10-60 sample units. '584 Patent, 5:57-6:6; *see* AAPA #1, #2, #5, and #6. Determining whether meat is safe for consumption so it may be released into commerce is a well-understood purpose of testing meat. *See* ICMSF, 185-89. Moreover, the recitation of a lot being validated for "release . . . into commerce" is not limiting. Prosecution History for Reexamination Control No. 95/011,294. "Operationally-linked" is simply grouping like items, which is also disclosed by ICMSF. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119.

	It is therefore helpful if suppliers can be persuaded to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load). If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment. ICMSF, 119 (annotated).
[17b] b) combining the collected conforming plurality of product portions corresponding to each of the separate single-unit production lots to provide a corresponding set of separate single-unit production lot samples, wherein each separate single-unit production lot sample is attributed to a particular corresponding separate single-unit production lot;	Price, alone or in combination with ICMSF and Hughes, discloses combining the collected conforming plurality of product portions corresponding to each of the separate single-unit production lots to provide a corresponding set of separate single-unit production lot samples, wherein each separate single-unit production lot sample is attributed to a particular corresponding separate single-unit production lot. Like ICMSF, the test lot samples in Price are attributable to a particular lot. For example, Price's abstract states that the procedure "permits small portions of pre-enrichment broth cultures to be retained for subsequent individual analysis if positive tests are found," as shown below:

	A method has been described for testing multiple food samples for *Salmonella* without loss in sensitivity. The method pools multiple pre-enrichment broth cultures into single enrichment broths. The subsequent stages of the *Salmonella* analysis are not altered. The method was found applicable to several dry food materials including nonfat dry milk, dried egg albumin, cocoa, cottonseed flour, wheat flour, and shredded coconut. As many as 25 pre-enrichment broth cultures were pooled without apparent loss in the sensitivity of *Salmonella* detection as compared to individual sample analysis. The procedure offers a simple, yet effective, way to increase sample capacity in the *Salmonella* testing of foods, particularly where a large proportion of samples ordinarily is negative. It also permits small portions of pre-enrichment broth cultures to be retained for subsequent individual analysis if positive tests are found. *Salmonella* testing of pooled pre-enrichment broths provides increased consumer protection for a given amount of analytical effort as compared to individual sample analysis. Price, 679 (annotated). Price further describes this retention step as enabling "individual tests on the retained sample" to "be made to determine which sample or samples contributed *Salmonella* to the pool" as shown below:

	MATERIALS AND METHODS *Salmonella* analyses were carried out as described in the *Bacteriological Analytical Manual* (BAM) (2) except for the pooling of pre-enrichment broths described in the following paragraph. The procedure for *Salmonella* testing of pooled pre-enrichment broths is illustrated in Fig. 1. Samples are individually pre-enriched followed by transfer from multiple pre-enrichments to single selenite and tetrathionate broths at the enrichment stage. During pooling, a portion of each pre-enrichment is transferred to a sterile culture tube and retained at 4 C for later reference to the individual samples. If the *Salmonella* test of the pooled pre-enrichment broths is positive, individual tests on the retained samples can be made to determine which sample or samples contributed *Salmonella* to the pool. *Id.* (annotated). A POSITA would understand Price to implicitly disclose combining the collected confirming plurality of portions based on the inherent mixing involved with dry food materials.

	Salmonella-positive test samples were prepared by blending the dry test food with one of three dry inocula. Inoculum 1 was a dry enzyme drain cleaner which had been found to contain multiple *Salmonella* serotypes including E_1, z_{10}; G, z_{29} (*S. cubana*); and C_1, g complex. Inoculum 2 was a freeze-dried, skim milk suspension of serotypes *S. anatum*, *S. binza*, *S. tennessee*, *S. worthington*, *S. cubana*, *S. braenderup*, and a B_1, g complex. Inoculum 3 consisted of four laboratory isolates of serotypes G, z_{29} (*S. cubana*); E_1, b; E_1, l complex; and C_1, l complex grown in a sterile aqueous suspension of cottonseed flour and freeze dried. Price, 679.



Price, 680 (Figure 1 (annotated)).

A POSITA would have replaced Price's collection and combination of dry food with ICMSF's collection and combination of portions from a consignment of meat lots in conformance to a statistical-based sampling plan for release into commerce.

ICMSF states: "To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units of the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. . . . This finding suggests that considerable reductions may

	be made in the cost of laboratory analysis by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, n, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort." ICMSF, 187. To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. A laboratory investigation undertaken for ICMSF (Silliker & Gabis, 1973) indicated that different sized analytical units drawn from a single well-mixed sample unit of dried food give very similar results. A subsequent investigation (Gabis & Silliker, 1974) using foods of high moisture content (including eggs, poultry meat, meat, and meat products) showed comparable reliability in the use of composite analytical units; for example, examination of three composites of 20 × 25 g analytical units gave the same result as examination of all 60 analytical units individually (the total amount examined being 1500 g in both instances). This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n*, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort. ICMSF, 187 (annotated).

10.5.1 Conceivable Alternative Plans

As indicated above, for an infectious agent such as *Salmonella*, there are strong reasons for adopting sampling plans in which $c = 0$:

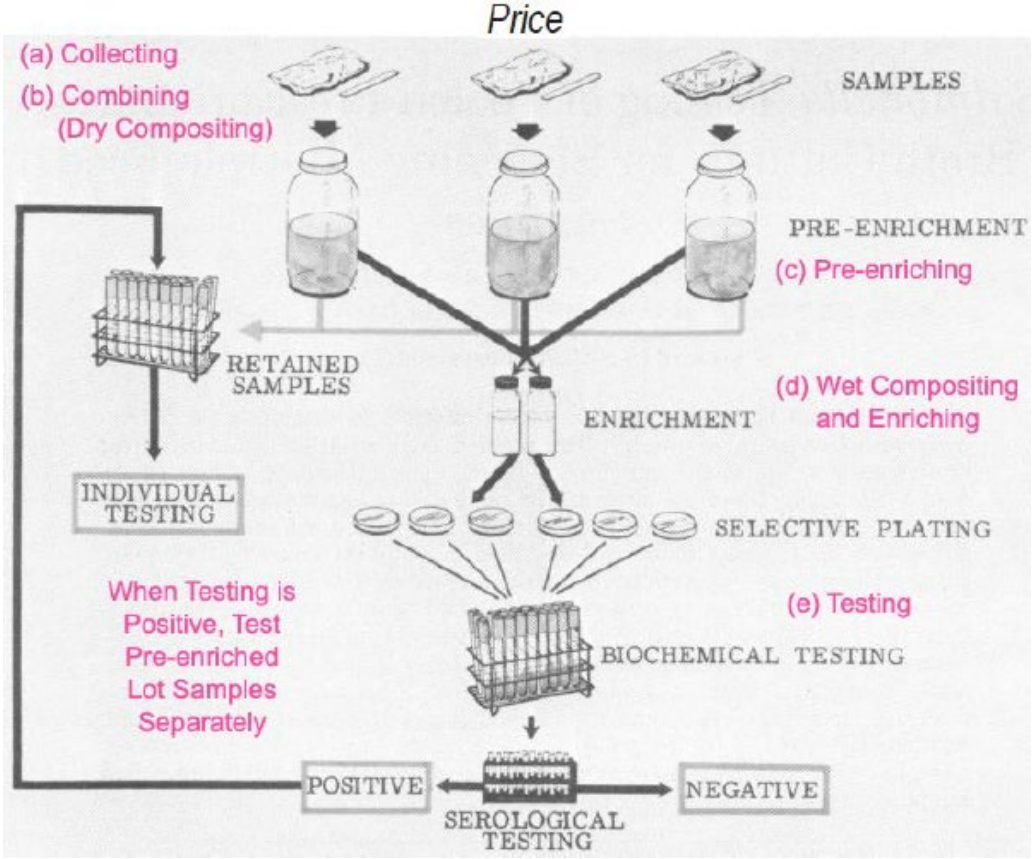
1. It is philosophically objectionable to some people to accept a lot containing a recognized pathogen, regardless of how the food is to be used in a manufacturing process or by the consumer.
2. There is the very great technical advantage of combining many analytical units for bacteriological examination in systems with $c = 0$, because a single positive decides the outcome.
3. The plan with $c = 1$ (or more) would always require the examination of (many) more analytical units, for equal probability of acceptance, than a plan with $c = 0$.
4. Even if the indicated probabilities of plans with $c = 0$ versus $c = 1$ are the same, their implications are not. For example, when a lot is accepted with the plan $n = 60$, $c = 0$, it is possible (though not certain) that the lot may not be contaminated at all. But using the plan $n = 95$, $c = 1$ and accepting one positive, it is known that the lot certainly is contaminated (though the probability that a lot with 5% of analytical units contaminated would be accepted is in both instances $P_a = 0.05$).

ICMSF, 188 (annotated). ICMSF also provides an example of "[u]sing a sampling plan involving n = 15 (25 g analytical units) and a composite of 375 g." *Id.*, 195 (§ 10.7.2). ICMSF also states "[f]or qualitative analyses, 25 g analytical units are frequently analyzed individually or as composites of 100 g, 200 g, or more." *Id.*, 232 (§ 12.5.2).

The ICMSF incorporates two references, Silliker & Gabis (1973) and Gabis & Silliker (1974). Silliker notes that "[c]ompositing of multiple samples (lumping) and wet compositing of pre-enrichment cultures have been tested as economical approaches to *Salmonella* control." Silliker, 475.

Silliker further notes that "[b]oth approaches are being used widely by the food industry in the United States." *Id.*

	"There is the very great technical advantage of combining many analytical units for bacteriological examination." ICMSF, 188. ICMSF also describes composites prepared by combining "15 (25 g analytical units)" and "composites of 100 g, 200 g, or more." *Id.,* 195, 232; *see* AAPA #5. ICMSF states, "All steps up to the analysis must be controlled . . . to allow reliable traceability." ICMSF, 227. ICMSF indicates lot samples are traceable to a particular corresponding lot: "It is therefore helpful . . . to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load). . . . Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119.
[17c] c) incubating the set of separate single-unit production lot samples under conditions suitable to allow levels of a target microbial agent or organism that is present in one or more of the separate single-unit production lot samples to reach detectable levels and become uniform, or substantially uniform, throughout the respective one or more separate single-unit production lot samples, to provide a set of separate single-unit	Price, alone or in combination with ICMSF and Hughes, incubating the set of separate single-unit production lot samples under conditions suitable to allow levels of a target microbial agent or organism that is present in one or more of the separate single-unit production lot samples to reach detectable levels and become uniform, or substantially uniform, throughout the respective one or more separate single-unit production lot samples, to provide a set of separate single-unit production lot samples enriched for the target microbial agent or organism. Price states "[i]n the multiple pooling of pre-enrichment broth cultures . . . , 1-ml quantities of cultures were transferred, generally, to a volume of enrichment broth." Price, 682.

production lot samples enriched for the target microbial agent or organism;	Price also states "[a]lternatively, problems with interfering organisms may warrant the use of direct selective enrichments." *Id.* Based on this statement, the Board noted "there is a suggestion in Price (at 682) . . . of direct enrichment." Prosecution History for Reexamination Control No. 95/001,294, 1576.  Price, 680 (Figure 1 (annotated)). Price states "[a]lternatively, problems with interfering organisms may warrant the use of direct selective enrichments":

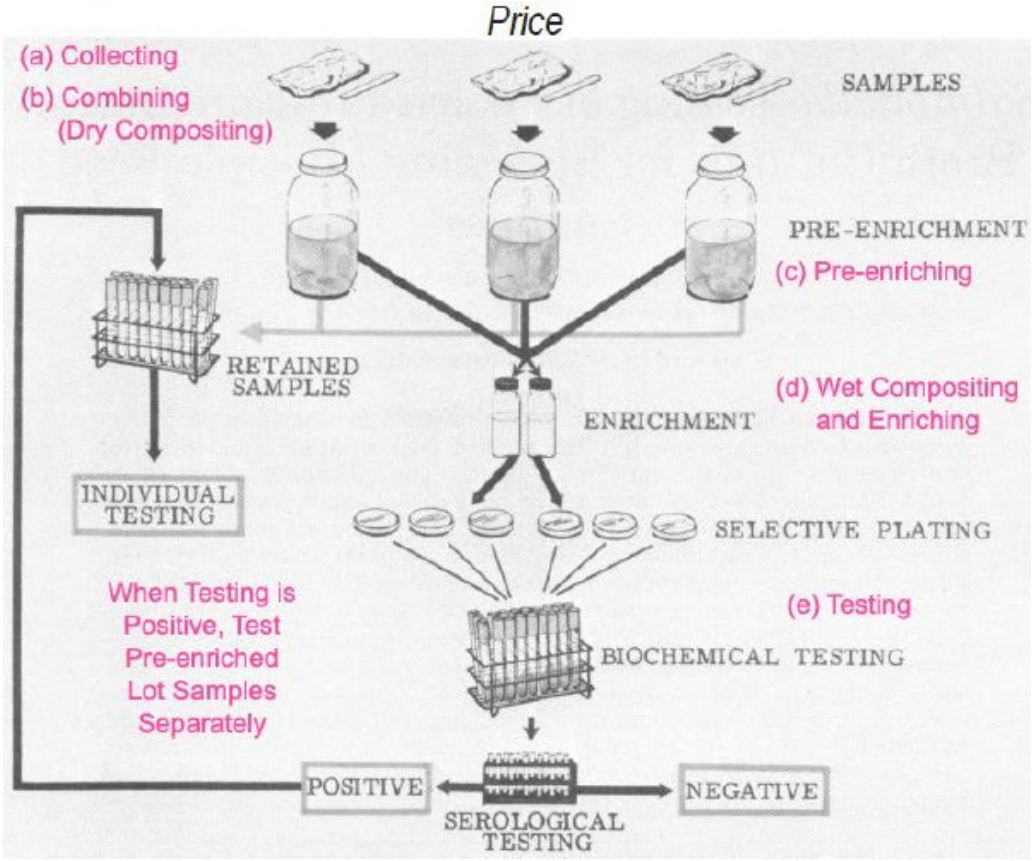
	Whether pooled pre-enrichments are less sensitive than individual tests in the presence of interfering organisms probably is not known. For the present, we would recommend that, if numerous non-*Salmonella* colonies that resemble *Salmonella* on selective agar media are encountered in the pooled test, the retained pre-enrichment cultures should be tested individually. Alternatively, problems with interfering organisms may warrant the use of direct selective enrichments, two selective enrichments, or possibly even a reduction in the incubation time of the pre-enrichment cultures. Price, 682 (annotated). A POSITA would have replaced Price's pre-enriching and compositing pre-enrichments with teachings of ICMSF and Hughes for enriching lot samples, removing portions of each enriched lot sample, and combining the portions to provide a composite sample. Also, instead of retaining portions of pre-enrichments like Price, a POSITA would have retained portions of enriched lot samples as taught by ICMSF and Hughes. ICMSF states: To reduce laboratory effort . . . it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. ICMSF, 187. Also, ICMSF describes preparing composites of analytical units then diluting "for qualitative (enrichment)" analysis. *Id.,* 231-32; *see also* AAPA #5, #7.

	For practical reasons, however, analytical units of 10 g up to 50 g are typical for quantitative methods and are diluted 1:10. For qualitative analyses, 25 g analytical units are frequently analyzed individually or as composites of 100 g, 200 g, or more. ICMSF, 232 (annotated). Preparation of the first dilution, either for qualitative (enrichment) or quantitative (direct) analyses, can have a marked impact on the microbial population in the sample. This is often due to changes in the physico-chemical characteristics of the suspension as compared to the food matrix. Where the food does not confer protection, the composition of the diluent is important to prevent or minimize shock/injury to the microorganisms that can result from rapid changes in the ionic concentrations. ICMSF, 232 (annotated). ICMSF also describes strategic use of non-selective enrichment to resuscitate microorganisms and selective enrichments for growing microorganisms. *Id.*, 232–33 (§ 12.6); *see e.g.*, *id.,* 233 ("use of nonselective medium . . . to enhance recovery of injured or stressed cells."). ICMSF, § 12.6 also describes optimizing the incubation period. S*ee e.g.*, *id.,* 232. It was well known long before the priority date to use non-selective enrichments after dry compositing and prior to wet compositing as evidenced by Price, Silliker, Gabis, and MLG. A POSITA would also know to use selective enrichments after dry compositing and prior to wet compositing as disclosed by ICMSF and Hughes. A POSITA would find that incubating so microorganisms are detectable and at least substantially uniform in the selective enrichment broth was considered the standard practice in official methods because the methods otherwise fail. For example, D'Aoust states "standard methodology recommends incubation of selective enrichment broths." D'Aoust, 372. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section

	further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. (a) *Pre-enrichment.*—Pre-enrich product in noninhibitory broth to initiate growth of *Salmonella* spp. Methods used may vary with product and should be performed as indicated in **967.26A** or BAM, 7th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, chapter 5, section C. Pre-enrichment broths should be incubated at 35°–37°C. (b) *Selective enrichment.*—Transfer 0.1 mL incubated pre-enrichment mixtures to Rappaport-Vassiliadis R10 broth (9.9 mL) and 1 mL to TT broth (9 mL). For all foods other than raw foods or foods having a high microbial load, incubate 6–8 h at 41°–43°C. Selective enrichments of raw foods or foods having a high microbial load must be incubated 16–20 h at 41°–43°C. An air incubator is suitable for this purpose. Hughes, 636. Hughes instructs that, after pre-enrichment, portions of the "incubated pre-enrichment mixtures" should be transferred to two separate selective enrichment broths, Rappaport-Vassiliadis R10 broth and TT broth, and incubated for 16-20 hours at 41°-43°C. Hughes, 636, 638. Additionally, Hughes instructs that these selective broths should be removed from incubation and mixed by hand or by Vortex mixer. Hughes, 638. Hughes further instructs to then remove 1 mL from the TT tube and 1 mL from the Rappaport-Vassiliadis R10 tube and transfer to separate, sterile M broths. *Id.,* 636, 638. The separate M broths are then incubated ("16-20 h at 35°-37°C" for "all foods other than raw foods or foods having a high microbial load" or "6 h at 35°-37°C" for

	"raw foods or foods having a high microbial load"). *Id.*, 638. A POSITA would find that the long incubation at an elevated temperature and mixing ensures the target microbial agent or organism reaches detectable levels and becomes substantially uniform throughout the separate lot samples.
[17d] d) removing portions of each enriched separate single-unit production lot sample, and combining the removed portions to provide a modular composite sample; and	Price, alone or in combination with ICMSF and Hughes, removing portions of each enriched separate single-unit production lot sample, and combining the removed portions to provide a modular composite sample. Price discloses wet compositing as "[s]amples are individually pre-enriched followed by transfer from multiple pre-enrichments to single selenite and tetrathionate broths at the enrichment stage." Price, 679. **MATERIALS AND METHODS** ***Salmonella*** **analyses were carried out as described in the *Bacteriological Analytical Manual* (BAM) (2) except for the pooling of pre-enrichment broths described in the following paragraph.** **The procedure for *Salmonella* testing of pooled pre-enrichment broths is illustrated in Fig. 1. Samples are individually pre-enriched followed by transfer from multiple pre-enrichments to single selenite and tetrathionate broths at the enrichment stage. During pooling, a portion of each pre-enrichment is transferred to a sterile culture tube and retained at 4 C for later reference to the individual samples. If the *Salmonella* test of the pooled pre-enrichment broths is positive, individual tests on the retained samples can be made to determine which sample or samples contributed *Salmonella* to the pool.** Price 679 (annotated).

	Pooled samples versus pooled pre-enrichment broths. Silliker (4) reported a loss in sensitivity when several samples were pooled in a large container of pre-enrichment broth as compared to individual sample analysis. This is not surprising since, to be detected, the desiccated and debilitated *Salmonella* cells in a dried food sample must grow out in pre-enrichment broth in competition with other bacteria in the sample. If many food samples are pooled at the pre-enrichment stage, the salmonellae face competition from the flora of all the samples in the pool. If, on the other hand, the samples are individually pre-enriched, the actively growing salmonellae might be expected to compete more favorably with organisms introduced from other samples when pooled at the enrichment stage. In addition, pooling pre-enrichment broths avoids the hazards and inconvenience of handling very large flasks of culture. Price, 681 (discussing Silliker).

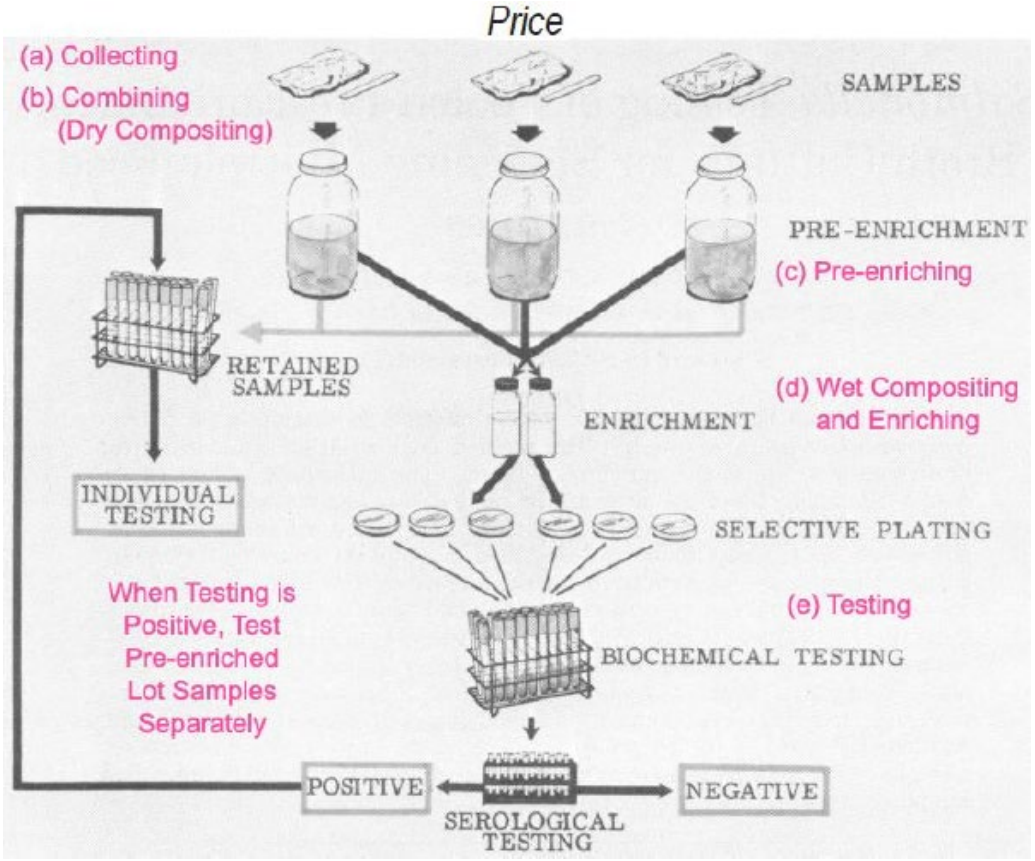


Price, 680 (Figure 1 (annotated)). Price's Figure 1 indicates that selective plating, biochemical testing, and serological testing are used.

A POSITA would have replaced Price's pre-enriching and compositing pre-enrichments with teachings of ICMSF and Hughes for enriching lot samples, removing portions of each enriched lot sample, and combining the portions to provide a composite sample. Also, instead of retaining portions of pre-enrichments like Price, a POSITA would have retained portions of enriched lot samples as taught by ICMSF and Hughes.

	ICMSF describes compositing the enriched lot samples: "it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. . . . This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n,* with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort." ICMSF, 187. *See also* AAPA #5-#7. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Hughes also discloses a method of removing portions of each enriched separate test lot sample, and combining the removed portions to provide a modular composite sample. Hughes instructs to "[c]ombine equal volumes (0.5-1 mL) from each M broth tube in a clean screw-cap tube." Hughes, 638.

	that has been warmed to 35°–37°C. Also remove 1 mL from Rappaport-Vassiliadis R10 tube and transfer to a separate tube of prewarmed M broth (10 mL). For all foods other than raw foods or foods having a high microbial load, incubate M broth tubes 16–20 h at 35°–37°C and return selective enrichment broth tubes to 41°–43°C incubator and incubate for additional 16–18 h. For raw foods or foods having a high microbial load, incubate M broth tubes 6 h at 35°–37°C and return selective enrichment broth tubes to 41°–43°C and incubate for an additional 6 h. (**d**) *Preparation of sample for EIA analysis.*—Remove M broth tubes from incubation and mix tubes by hand or Vortex mixer. Combine equal volumes (0.5–1 mL) from each M broth tube in a clean screw-cap tube and heat in boiling H_2O bath or in flowing steam 15 min. Refrigerate (2°–8°C) remaining M broth and TT and Rappaport-Vassiliadis R10 tubes from (**c**) for culture confirmation of any EIA-positive samples (*see* **967.26B, 967.26C, 967.27** and **967.28**). Cool heated M broths to 25°–37°C prior to analysis. Hughes, 638. Hughes 2003 confirms the pooling of enrichments. Hughes 2003, 775-76.
[17e.1] e) testing of the modular composite sample, using a suitable detection assay, for the target microbial agent or organism,	Price, alone or in combination with ICMSF and Hughes, discloses testing of the modular composite sample, using a suitable detection assay, for the target microbial agent or organism.



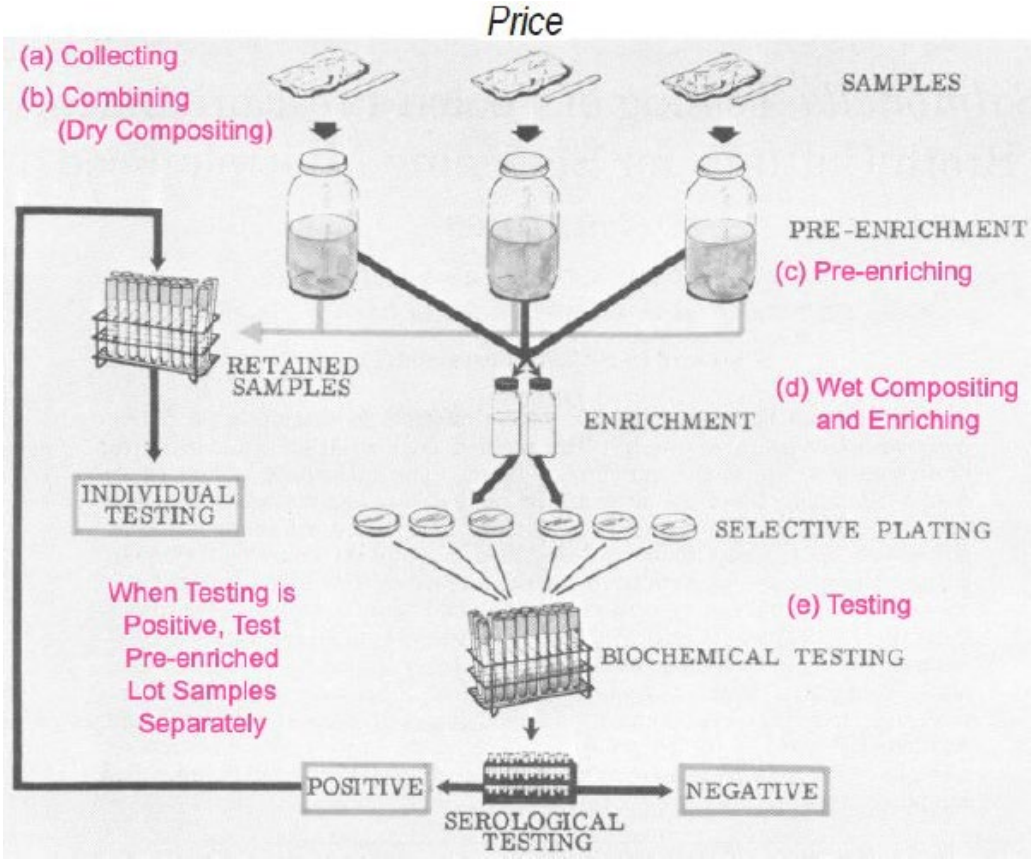
Price, 680 (Figure 1 (annotated)).

Price teaches testing the composite sample using a detection assay for *Salmonella*. Price, 680 (Figure 1).

Price states "a test procedure that gives a single negative answer for many negative samples offers an important saving in the analytical effort required for quality control":

	Positive test results are relatively rare among the many samples of food ingredients and products which are analyzed for *Salmonella*. Therefore, a test procedure that gives a single negative answer for many negative samples offers an important saving in the analytical effort required for quality control. These studies support the conclusion that *Salmonella* pre-enrichment broths can be pooled for analysis without loss in sensitivity to *Salmonella* in the individual samples. Price, 679 (annotated). Price indicates that "positive test results are relatively rare." *Id.* A POSITA would have replaced Price's steps of testing a composite sample from the pre-enriched lot samples to obtain a negative result and further testing retained portions of the pre-enriched lot samples with the combined teachings of ICMSF and Hughes. These combined teachings provide for testing a composite made from the selectively enriched lot samples to obtain a negative result and further testing retained portions of the enriched lot samples after a positive test of the composite by a more sensitive protocol. ICMSF describes testing enrichment cultures of composite samples with suitable detection assays (ICMSF, 187) and "presence/absence tests" (ICMSF, 233). Suitable detection assays were established in Silliker and Gabis as discussed in ICMSF. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture

	confirmation of any EIA-positive samples." *Id.,* 638. Hughes states testing of the composite sample is "by an enzyme immunoassay (EIA) method." Hughes, 635, 638. These assay types were "art recognized." '584 Patent, 2:54-58 (AAPA #7).
[17e.2] wherein when such testing is negative all of said separate single-unit production lot samples are validated for release into commerce, and	Price, alone or in combination with ICMSF and Hughes, wherein when such testing is negative all of said separate single-unit production lot samples are validated for release into commerce.



Price, 680 (Figure 1 (annotated)).

Price states "a test procedure that gives a single negative answer for many negative samples offers an important saving in the analytical effort required for quality control." Price, 679.

A POSITA would have replaced Price's steps of testing a composite sample from the pre-enriched lot samples to obtain a negative result and further testing retained portions of the pre-enriched lot samples with the combined teachings of ICMSF and Hughes. These combined teachings provide for testing a composite made from the

	selectively enriched lot samples to obtain a negative result and further testing retained portions of the enriched lot samples after a positive test of the composite by a more sensitive protocol. ICMSF describes correlating lots in a consignment with lot samples so a negative or positive test result enables a lot to be validated or rejected. ICMSF, 118-19. ICMSF describes sampling plans in which "c is the maximum allowable number of sample units yielding a positive result." ICMSF, 115. For many scenarios, the requirement is c = 0, which indicates the testing is negative. *Id.*, 118, 183-90, 309, 325, 326. A negative test result (*id.*, 113-15, 325, 326) is the expected and desired outcome to determine meat is not contaminated and is safe for consumption (*id.*, 184-89) such that a lot is validated and it is "commercially possible to market" (*id.*, 185) the meat.

6.10 WHAT IS A LOT?

Ideally, a lot is a quantity of food or food units produced and handled under uniform conditions. Under certain circumstances, it has been shown that the logarithms of the counts from a batch of food are likely to be normally distributed (Baird-Parker, 1980; Kilsby & Baird-Parker, 1983 [their Table 2]; Ingram & Roberts, 1976). The implication is that there is homogeneity within a lot, which, when considering microbial levels and distributions, rarely occurs in practice. In most instances, the distribution of microorganisms within lots of food is heterogeneous. Even in food factories, this heterogeneity makes the interpretation of sample results difficult, and it becomes even more so in circumstances where a lot is less well defined, such as in a consignment of a very large quantity of food.

It is therefore helpful if suppliers can be persuaded to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load).

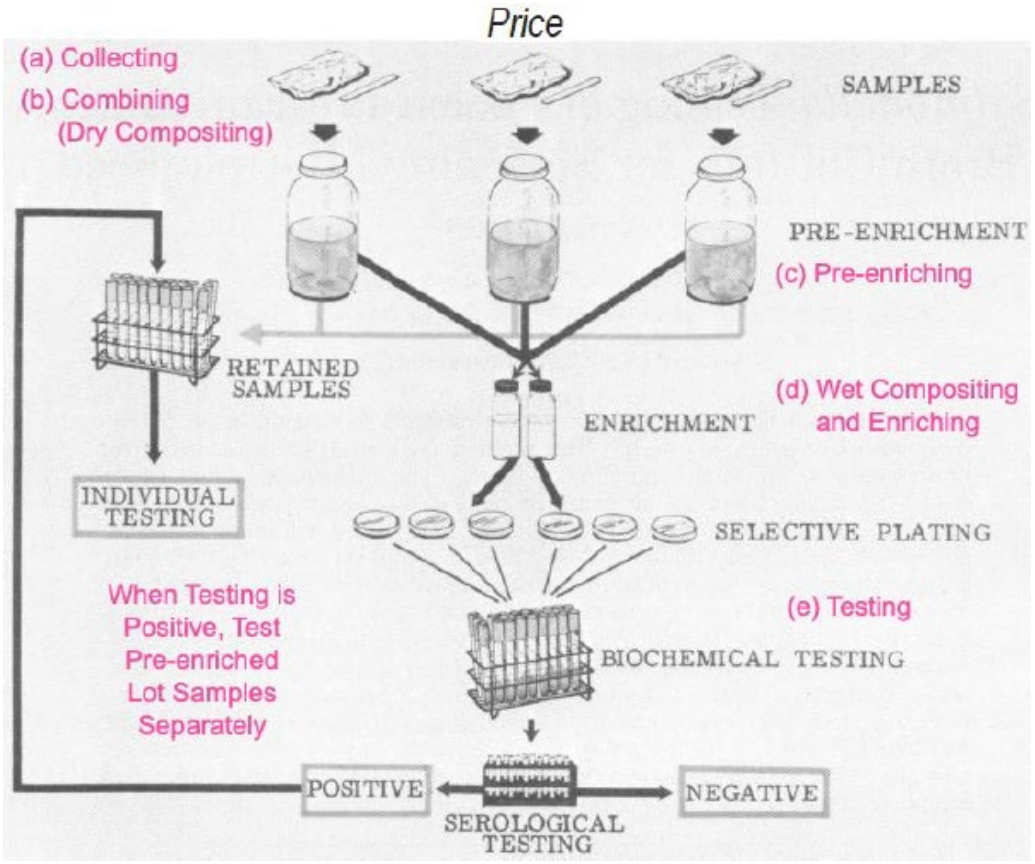
If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment.

A lot should be composed of food produced with as little variation as possible for a given process or commodity. Because of the uncertainties in identifying a lot commercially, however, the use of the word *lot* in this book is usually in its statistical sense, as a collection of units of a product, the acceptability of which is determined by examining a sample drawn from it.

ICMSF, 118-19 (annotated).

	Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Because Hughes explains the steps to be taken when testing is positive (*see* ie.3)*,* a POSITA would also expect negative testing results. Hughes, 638. Determining whether meat is safe for consumption so it may be released into commerce is a well-understood purpose of testing meat. Moreover, the recitation of a lot being validated for "release . . . into commerce" is not limiting. Prosecution History for Reexamination Control No. 95/011,294.
[17e.3] wherein when such testing is positive, individual separate single-unit production lots are validated for release into commerce, according to the statistical-based sampling plan, by further testing of a portion of the respective enriched separate single-unit production lot sample using the same or a more sensitive protocol and obtaining a negative test result for each single-unit production lot,	Price, alone or in combination with ICMSF and Hughes, wherein when such testing is positive, individual separate single-unit production lots are validated for release into commerce, according to the statistical-based sampling plan, by further testing of a portion of the respective enriched separate single-unit production lot sample using the same or a more sensitive protocol and obtaining a negative test result for each single-unit production lot. The combination of Price, ICMSF, and Hughes describes retaining a portion of the enriched lot samples (steps (b)-(d)). Price indicates "a portion of each pre-enrichment is . . . retained . . . for later reference to the individual samples" and "individual tests on the retained samples can be made to determine which sample or samples contributed *Salmonella* to the pool." Price, 679. Price states, "If the *Salmonella* test of the pooled preenrichment broths is positive, individual tests on the retained samples can be made to determine which sample or samples contributed *Salmonella* to the pool." *Id.,* 679. Price discloses "the pooling of broth cultures permits small portions of each individual culture to be retained for subsequent analysis if a positive test is detected

following enrichment, thus maintaining the individuality of each test sample." Price, 681.



Price, 680 (Figure 1 (annotated)).

Price states "[i]f the *Salmonella* test of the pooled pre-enrichment broths is positive, individual tests on the retained samples can be made to determine which sample or samples contributed *Salmonella* to the pool":

MATERIALS AND METHODS

Salmonella analyses were carried out as described in the *Bacteriological Analytical Manual* (BAM) (2) except for the pooling of pre-enrichment broths described in the following paragraph.

The procedure for *Salmonella* testing of pooled pre-enrichment broths is illustrated in Fig. 1. Samples are individually pre-enriched followed by transfer from multiple pre-enrichments to single selenite and tetrathionate broths at the enrichment stage. During pooling, a portion of each pre-enrichment is transferred to a sterile culture tube and retained at 4 C for later reference to the individual samples. If the *Salmonella* test of the pooled pre-enrichment broths is positive, individual tests on the retained samples can be made to determine which sample or samples contributed *Salmonella* to the pool.

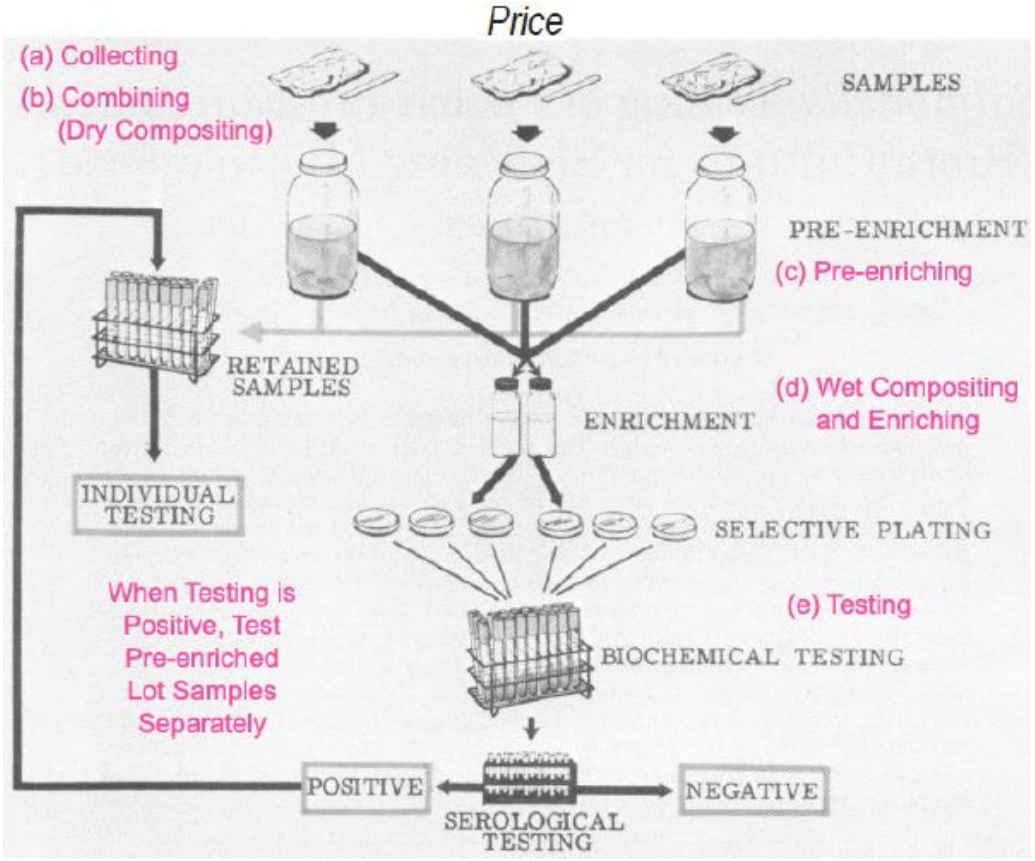
Price, 679 (annotated). POSITA would have considered retention of samples that had been selectively enriched as an option.

A POSITA would have replaced Price's steps of testing a composite sample from the pre-enriched lot samples to obtain a negative result and further testing retained portions of the pre-enriched lot samples with the combined teachings of ICMSF and Hughes. These combined teachings provide for testing a composite made from the selectively enriched lot samples to obtain a negative result and further testing retained portions of the enriched lot samples after a positive test of the composite by a more sensitive protocol.

"Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119.

	"There is often a desire to retest retained samples." ICMSF, 191. If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment. ICMSF, 119 (annotated). ICMSF discloses validating for release into commerce (ICMSF, 113–15 (§§ 6.2–6.6), 118–19 (§§ 6.9–6.10), 184–85 (§ 10.2), 325–27 (§§ 17.5.3–17.5.3.4); *see also id.*, 186–89 (§ 10.4–10.6)) according to a statistically-based sampling plan (*id.*, 113 (§ 6.1); *see also id.*, 115–18 (§§ 6.4–6.8), 184 (§ 10.2), 319–21 (§§ 17.2.3.3–17.2.3.4), 326–30 (§§ 17.5.3.1–17.6), as described regarding steps (a) and (e)(2). Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Hughes explicitly teaches retention of the enriched test lot samples for further testing after a positive result. Hughes provides instructions to refrigerate the "remaining M broth and TT and Rappaport-Vassiliadis R10 tubes [that were enriched] for culture confirmation of any EIA-positive samples." Hughes, 638. Hughes refers to the results as the "confirmed positive samples" (Hughes, 635) and also states that diagnostic reagents are "necessary for culture confirmation of presumptive EIA tests." Hughes, 636. Hughes indicates "[e]nrichment broths and M broths from samples positive by an

	enzyme immunoassay (EIA) method must be streaked on selective media." *Id.*, 635. Culture confirmation using streaking is more sensitive than the EIA method, as was commonly understood and confirmed by the teachings of D'Haese. *See generally*, D'Haese. Each sample was analyzed by the BAM method (7th Ed.) and the TSVIA method using the enrichment protocol described in section F of the method, *Preparation of Sample*. Equal portions (0.5–1 mL) from each of the 2M broths arising from the 2 selective-enrichment broths were combined in a small screw-capped tube and heat treated in boiling water for 15 min prior to performing the TSVIA. Confirmation for the immunoassay was by streaking from the M broth culture onto selective agar, as specified in the BAM reference culture method. Hughes, 635. Moreover, the recitation of a lot being validated for "release . . . into commerce" is not limiting. Prosecution History for Reexamination Control No. 95/011,294.
[17e.4] wherein the enrichment in step c), prior to removing portions in step d), is sufficient to enable said validation of individual separate single-unit production lots with no requirement for further enrichment thereof, and with no requirement for further sampling in a).	Price, alone or in combination with ICMSF and Hughes, discloses wherein the enrichment in step c), prior to removing portions in step d), is sufficient to enable said validation of individual separate single-unit production lots with no requirement for further enrichment thereof, and with no requirement for further sampling in a).



Price, 680 (Figure 1 (annotated)).

ICMSF and Hughes disclose selectively enriching prior to removing portions, thus a POSITA would consider the enrichment sufficient to enable validation of the lots without further enrichment or sampling.

Hughes explicitly indicates portions of the enrichment cultures are retained for further testing; thus Hughes has no need for further sampling.

Hughes' "Preparation of Sample" section identifies four phases including (a) pre-

	enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. ICMSF and Hughes disclose selective enrichment so further enrichment is unnecessary. Also, as discussed regarding step (e)(3), Hughes explicitly indicates portions of the enrichment cultures are retained for further testing; thus Hughes has no need for further sampling. The same would be expected for enrichment cultures in ICMSF because retaining samples at each process stage was considered standard practice for a POSITA. in flowing steam 15 min. Refrigerate (2°–8°C) remaining M broth and TT and Rappaport-Vassiliadis R10 tubes from (c) for culture confirmation of any EIA-positive samples (*see* **967.26B, 967.26C, 967.27** and **967.28**). Cool heated M broths to 25°–37°C prior to analysis. Hughes, 638 (annotated). Thus, the negative limitation of step (e)(4) is disclosed by and is obvious based on the combination of ICSMF and Hughes. *Id.*
Claim 18	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 1, wherein removing portions in step d) is removing equal portions, aseptically, so as to preclude contamination of the enriched separate test lot samples.	Price, in combination with ICMSF and Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. Price, in combination with ICMSF and Hughes, further discloses wherein removing portions in step d) is removing equal portions, aseptically, so as to preclude contamination of the enriched separate test lot samples.

	ICMSF describes aseptically withdrawing and weighing sample units into the appropriate diluent. ICMSF, 231, 232. Hughes instructs that after enrichment "[c]ombine equal volumes (0.5-1 mL) from each M broth tube in a clean screw-cap tube." Hughes, 638. Price further discloses removing portions in step d) is removing equal portions, aseptically, so as to preclude contamination of the enriched separate test lot samples. A POSITA would consider this to be standard practice. A POSITA would have aseptically removed equal volume portions of each enriched separate test lot sample to preclude contamination.
Claim 19	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 1, wherein the target microbial agent or organism comprises at least one selected from the group consisting of *Listeria*, *Salmonella* and *Escherichia coli.*	Price, in combination with ICMSF and Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. Price, in combination with ICMSF and Hughes, further discloses wherein the target microbial agent or organism comprises at least one selected from the group consisting of *Listeria*, *Salmonella* and *Escherichia coli.* ICMSF describes testing for *Listeria* (ICMSF, 285-312), *Salmonella* (*id.*, 273-84), and *Escherichia coli* (*id.*, 313-32). Hughes focuses on *Salmonella*. Hughes, 634. Price further discloses a target microbial agent or organism comprises at least one selected from the group consisting of *Listeria*, *Salmonella* and *Escherichia coli*. Price established that "pre-enrichment broths can be pooled for analysis without loss in sensitivity to *Salmonella* in the individual samples." Price, 679. Price tested dry food materials inoculated (purposefully contaminated) with *Salmonella. Id.*
Claim 20	**Disclosure of Price, ICMSF, and Hughes**

The method of claim 1, wherein the target microbial agent or organism comprises at least one selected from the group consisting of *Listeria* spp., *Listeria monocytogenes*, *Salmonella* spp., *Escherichia coli* O157:H7, coliforms and fecal coliforms.	Price, in combination with ICMSF and Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. Price, in combination with Hughes and ICMSF, further discloses the target microbial agent or organism comprises at least one selected from the group consisting of *Listeria* spp*., Listeria monocytogenes*, *Salmonella* spp., *Escherichia coli* O157:H7, coliforms and fecal coliforms. ICMSF describes testing for *Listeria* (ICMSF, 285-312), *Salmonella* (*id.*, 273-84), and *Escherichia coli* (*id.*, 313-32). Hughes focuses on *Salmonella*. Hughes, 634. Price established that "pre-enrichment broths can be pooled for analysis without loss in sensitivity to *Salmonella* in the individual samples." Price, 679. Price tested dry food materials inoculated (purposefully contaminated) with *Salmonella. Id.*
Claim 21	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 1, wherein the target microbial agent or organism comprises a spoilage organism.	Price, in combination with ICMSF and Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. Price, in combination with Hughes and ICMSF and the teachings of a POSITA, further discloses the target microbial agent or organism comprises a spoilage organism. A POSITA would find it obvious to apply the teachings of ICMSF and Hughes to target spoilage organisms. "Examination of every piece of whole meat is clearly impractical, hence any sampling procedure for estimating shelf-life would involve taking enough samples to give some statistical assurance of the keeping qualities of meat." Gill, 449.

	Borch is titled "Bacterial spoilage of meat and cured meat products." Borch. The reference discusses spoilage organisms in meat: Lactic acid bacteria are the major bacterial group associated with the spoilage of refrigerated vacuum- or MA-packaged cooked, cured meat products (Blickstad and Molin, 1983b; Shaw and Harding, 1989; von Holy et al., 1991). At the time of spoilage some products contain a 'pure' culture of only one species, while in others a mixture of *Lactobacillus* spp. and *Leuconostoc* spp. was found (Kant-Muermans, unpublished results). The great diversity of bacteria isolated from spoiled meat products is confirmed by several studies (Korkeala and Mäkelä, 1989; von Holy et al., 1991; Yang and Ray, 1994). The genus/species of lactic acid bacteria responsible for spoilage, depend on the product composition (product-related flora) as well as the manufacturing site (house-related flora; Korkeala and Mäkelä, 1989). Borch, 113. "Lactic acid bacteria spoil refrigerated meat products by causing defects such as sour off-flavours, discolouration, gas production, slime production and decrease in PH." Borch, 114; *see also, id.*, 115-17 (Section 4 Analysis of Spoilage). It is understood in the art that aerobic plate counts ("APC") can test for spoilage organisms. *See. e.g.*, ICMSF 1986, 122 ("tests to be made (e.g., APC, *Salmonella*, Clostridium perfringens"); *id.* at 132 ("However, aerobic plate counts (APC) can be used to monitor Good Commercial Practice (GCP), and criteria based on such examinations are a valuable aid to in-plant quality control.").

TABLE 19

Sampling plans and recommended microbiological limits for raw meat[a]

Product[b]	Test	Method reference[c]	Case	Plan class	n	c	Limit per cm² or gram m	M
Carcass meat, before chilling	APC	118	1	3	5	3	10^5	10^6
Carcass meat, chilled	APC	118	1	3	5	3	10^6	10^7
Edible offal, chilled	APC	118	1	3	5	3	10^6	10^7
Carcass meat, frozen	APC	118	1	3	5	3	5×10^5	10^7
Boneless meat, frozen (beef, veal, pork, mutton)	APC	118	1	3	5	3	5×10^5	10^7
Comminuted meat, frozen	APC	118	1	3	5	3	10^6	10^7
Edible offal, frozen	APC	118	1	3	5	3	5×10^5	10^7

a **Not for use at port of entry** but for in-plant quality control. Refer to Section B for appropriate applications.
b Unfrozen carcasses and primal cuts, swab counts per cm²; other meats and meat products, per gram.
c Refers to page number in ICMSF, 1978.

ICMSF 1986, 133 (Table 19 (annotated)).

Although for “quality control,” a POSITA would understand that "quality control" means testing for spoilage organisms could also be accomplished by this well-known method. It is disclosed within the prior art during the production lifecycle of beef production. ICMSF 1986, 134 (“The FAOIWHO Working Group (FAQ/WHO, 1979) considered that the only point at which APC could be used for chilled meat, to evaluate the hygienic conditions under which it was produced, was at the slaughterhouse, and because of its perishable nature it would not be possible to set

APC limits for use in guidelines or specifications for chilled meats at port of entry."); *see also, id.*, 153 ("Monitoring for APC, E. coli, and Staph. aureus would give some indication of inadequate processing (heating, curing, smoking), postprocess contamination, or storage at temperatures above those recommended for frozen or refrigerated distribution and storage.")

E. TEST PROCEDURES

(a) *Aerobic plate counts*

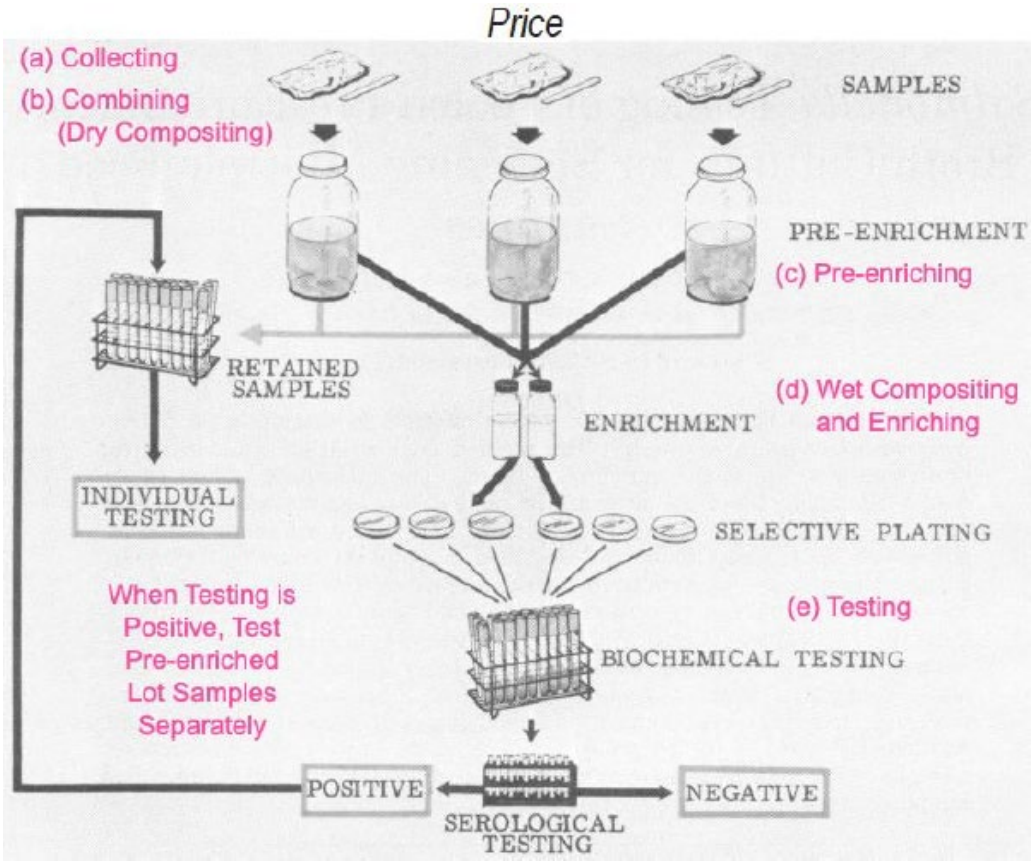
Comminute, blend, or stomach the various portions of meat composing the sample unit and withdraw the analytical unit. Combine the swabs to form an analytical unit, or treat each separately. Prepare dilutions and perform the APC test according to the directions given in ICMSF, 1978, p. 118. Spread plates are preferable to pour plates when examining raw meats.

The temperature used for incubation of plates should be related to the material being examined. To estimate numbers of mesophilic contaminants on freshly dressed carcasses it is advisable to incubate plates at 35 or 37°C. For meat that has been held under chilled conditions, plates should be incubated at 25°C to include psychrotrophs and thus give a better indication of the microbial growth that has occurred. Counts at 20°C give similar results after a longer incubation time.

ICMSF 1986, 136 (annotated).

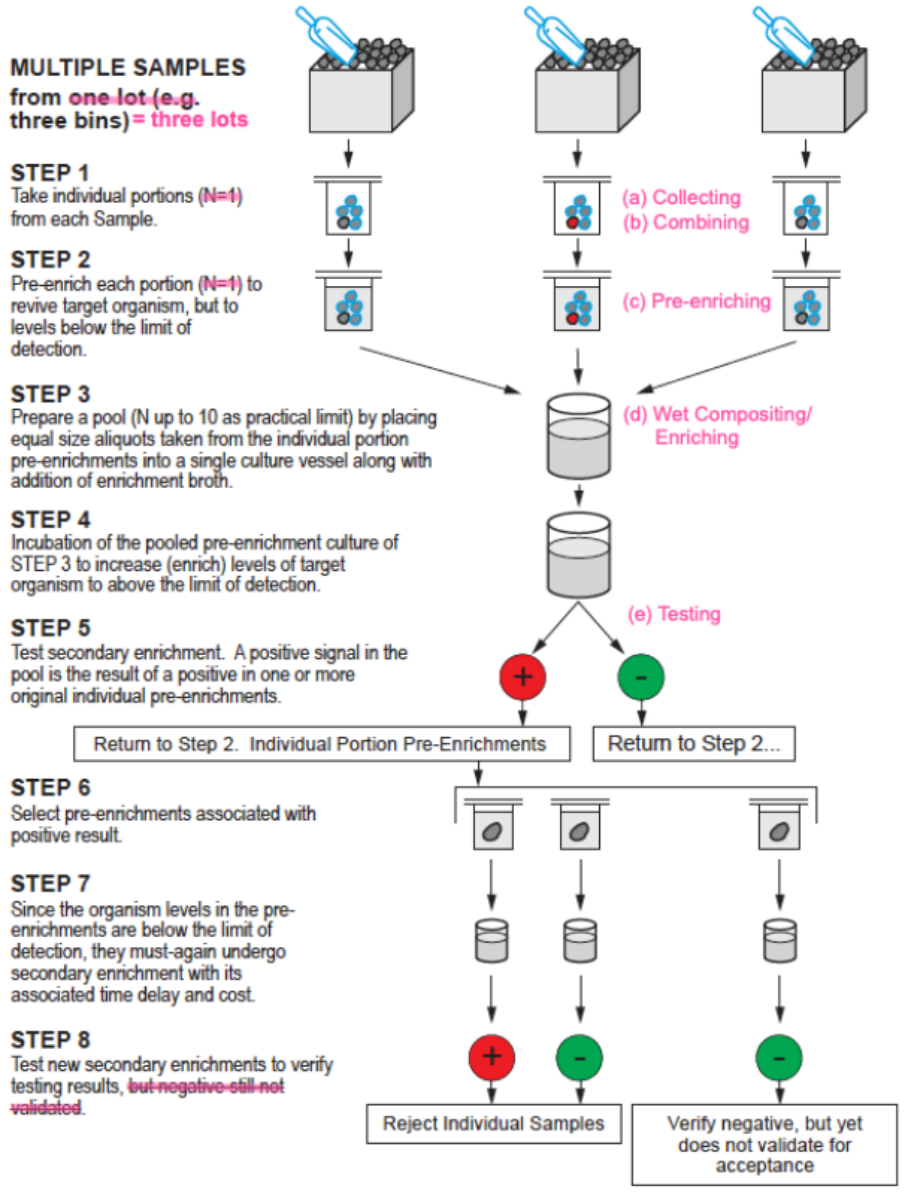
See also, Bhunia 4 ("Bacteria-specific generic rapid methods such ATP-based luminescence and total plate count (APC) are also needed for monitoring cleaning and hygienic practices employed in a processing plant."); Wheeler, 1606 (use of APC in a beef-processing plant); PR/HAACP, 38849 ("Various alternative indicator

	organisms were suggested, including generic E. coli (biotype I), total plate counts, Enterobacteriaceae, Total Viable Counts (TVC), and Aerobic Plate Counts (APC).”).
Claim 22	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 1, wherein removing portions of each enriched separate test lot sample comprises removing equal volume portions of each enriched separate test lot sample.	Price, in combination with ICMSF and Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. Price, in combination with ICMSF and Hughes, further discloses removing portions of each enriched separate test lot sample comprises removing equal volume portions of each enriched separate test lot sample. ICMSF describes aseptically withdrawing and weighing sample units into the appropriate diluent. ICMSF, 231, 232. Hughes instructs that after enrichment “[c]ombine equal volumes (0.5-1 mL) from each M broth tube in a clean screw-cap tube.” Hughes, 638. Moreover, a POSITA would consider this to be standard practice. A POSITA would have removed equal volume portions of each enriched separate test lot sample.
Claim 23	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 1, wherein testing of the modular composite sample, using a suitable detection assay comprises use of at least one of a DNA-based method, an immunochemistry-based method, and a biosensor-based method.	Price, in combination with ICMSF and Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. Price, in combination with Hughes and ICMSF, further discloses testing of the modular composite sample, using a suitable detection assay comprises use of at least one of a DNA-based method, an immunochemistry-based method, and a biosensor-based method. Price discloses biochemical testing of the modular composite sample. A POSITA would consider other suitable detection assays to test for the presence of *Salmonella*,

	which can include DNA-based methods, immunochemistry-based methods, and biosensor-based methods.  Price, 680 (Figure 1 (annotated)). Hughes discloses use of "an enzyme immunoassay (EIA) method," which is an immunochemistry-based method. Hughes, 635.

	Each sample was analyzed by the BAM method (7th Ed.) and the TSVIA method using the enrichment protocol described in section F of the method, *Preparation of Sample*. Equal portions (0.5–1 mL) from each of the 2M broths arising from the 2 selective-enrichment broths were combined in a small screw-capped tube and heat treated in boiling water for 15 min prior to performing the TSVIA. Confirmation for the immunoassay was by streaking from the M broth culture onto selective agar, as specified in the BAM reference culture method. Hughes, 635.
Claim 24	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 1, wherein the separate test lots are production lots of a meat or poultry product, comprising an assemblage of one or more meat or poultry specimens, respectively.	Claim 24 was cancelled during reexamination. IEH cannot assert a cancelled claim against National Beef. As such, National Beef does not substantively address this claim.
Claim 27	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 2, wherein product specimens are synonymous with product portions.	Price, in combination with ICMSF and Hughes, discloses Claim 2, upon which this claim relies. See above re Claim 2. Price, in combination with ICMSF and/or Hughes, further discloses product specimens are synonymous with product portions.

Figure 3: CITED PRIOR ART (PRICE et al, 1972)



	Prosecution History for Reexamination Control No. 95/001,294, 298 (annotated). IEH's diagram indicates multiple samples and portions are removed from the bins/lots, and uses the terminology interchangeably. The specification of the '584 Patent indicates a test lot is a single-unit production lot ('584 Patent, 5:21-32) such as a single combo, bin, pallet, or segment of production ('584 Patent, 7:28-36; *see also* AAPA #3 and #4). ICMSF teaches collecting portions or samples, which were known synonyms for product portions or specimens. ICMSF states that "[a] sample unit is a small portion of the lot." ICMSF, 225. **12.1 INTRODUCTION** Many different types of samples are collected and submitted to the laboratory for analysis. Some are sample units from lots or consignments of foods or ingredients for lot acceptance determination. Others may be for investigational purposes to assess control of the environment, investigate the source of a problem, or validate a process. Some may have legal implications relative to a lawsuit or for regulatory compliance. This chapter briefly discusses some of the major factors that should be considered when collecting sample units, shipping them to a laboratory, preparing them for analysis, and analyzing them. A sample unit is a small portion of the lot in which the distribution of both numbers and types of microorganisms should be representative of the whole lot. This requirement is only completely fulfilled when the food and processing conditions result in a homogeneous distribution of microorganisms. This only occurs in well-agitated fluid products. In general, the distribution of microorganisms is presumed to be random. ICMSF at 225 annotated).
Claim 28	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 4, wherein the medium comprises a selective agent that will inhibit a competing non-target microbial agent or organism.	Price, in combination with ICMSF and Hughes, discloses Claim 4, upon which this claim relies. See above re Claim 4. Price, in combination with ICMSF and Hughes, further discloses a medium comprises a selective agent that will inhibit a competing non-target microbial agent or organism.

	Price discusses organisms facing competition from other organisms during the enrichment stage. A POSITA would conclude that inhibiting competition would be beneficial to the growth of target microbial agents or organisms. **Pooled samples versus pooled pre-enrichment broths.** Silliker (4) reported a loss in sensitivity when several samples were pooled in a large container of pre-enrichment broth as compared to individual sample analysis. This is not surprising since, to be detected, the desiccated and debilitated *Salmonella* cells in a dried food sample must grow out in pre-enrichment broth in competition with other bacteria in the sample. If many food samples are pooled at the pre-enrichment stage, the salmonellae face competition from the flora of all the samples in the pool. If, on the other hand, the samples are individually pre-enriched, the actively growing salmonellae might be expected to compete more favorably with organisms introduced from other samples when pooled at the enrichment stage. In addition, pooling pre-enrichment broths avoids the hazards and inconvenience of handling very large flasks of culture. Price, 681. Additionally, Claim 28 recites what a POSITA would consider the well-known property of selective enrichment, inhibition of a competing non-target microorganism. D'Aoust describes "selective enrichment [that] inhibits growth of

	non-*Salmonella*e and facilitates isolation of *Salmonella* on differential plating media." D'Aoust, 369. A POSITA would have been motivated to use an enrichment medium comprising a selective agent that inhibits a non-target agent or organisms as described by D'Aoust, for the test lot samples described by ICMSF and Hughes, to ensure an accurate test. A POSITA would understand that incubating samples at different temperature ranges for different target microorganisms would, effectively, inhibit the growth of other organisms. "Incubation temperature markedly affects productivity of selective enrichment broths by suppressing growth of competing microorganisms but not *Salmonella*." D'Aoust, 372. "This may be achieved by utilization of enrichment media that through various mechanisms may favor and enhance growth and multiplication of *Salmonella*e while other gram-negative organisms such as coliforms, Proteus, and Pseudomonas are suppressed or inhibited." Fagerberg, 630.
Claim 29	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 5, wherein the concentration step comprises at least one of immunomagnetic bead separation, and affinity chromatography.	Price, in combination with ICMSF and Hughes, discloses Claim 5, upon which this claim relies. See above re Claim 5. Price, in combination with ICMSF and Hughes, further discloses the concentration step comprises at least one of immunomagnetic bead separation, and affinity chromatography. Price discloses an enrichment stage after compositing where "[s]amples are individually pre-enriched followed by transfer from multiple pre-enrichments to single selenite and tetrathionate broths at the enrichment stage." Price, 679. A POSITA would understand that a detection assay that includes enrichment could further comprise a concentration step. Enrichment for the microbial agent or

	organism increases its concentration. A POSITA would use a standard concentration procedure such as immunomagnetic bead separation or affinity chromatography in order to concentrate the sample. ICMSF discloses the use of "immunomagnetic beads to concentrate cells." ICMSF, 318. A POSITA would consider this a well-known technique before the priority date. Fratamico discusses the rapid isolation of target microorganisms using an immunomagnetic separation method. Fratamico, 105. Fratamico discloses that use of immunomagnetic beads resulted in "an increase in the level of E. coli B1409 in the sample," as well as "an increase in the number of organisms recovered." *Id.*, 107. Additionally, Fratamico provides detailed instructions for preparing and using immunomagnetic bead separation to concentrate a target microbial agent or organism. Fratamico developed "[a] method employing immunomagnetic beads" to concentrate and thereby improve the isolation and detection of E. coli. *Id.* For example, Fratamico was able to use immunomagnetic beads to isolate *E. coli* strains from enrichment cultures of raw ground beef "when the initial inoculum level was 1 cfu g-1." *Id.*, 109. Fratamico further teaches that "[t]he percentage of bacteria recovered increased with increasing amounts of beads." *Id.*, 108. Consequently, Fratamico "demonstrates that immunomagnetic separation [using immunomagnetic beads] is a simple and rapid technique" for concentrating a microbe for isolation and detection. *Id.*, 109. These teachings of Fratamico would have motivated a POSITA to modify the combination of ICMSF and Gombas and ICMSF and Hughes to use immunomagnetic beads to concentrate microbes in a modular composite sample to benefit from accurate tests. Use of immunomagnetic beads was already known in the art for concentrating during testing beef samples. "Tu et al. (78) detected pathogenic E. coli O157:H7 in beef hamburger with the use of IMS and ATP measurement. After 6 h of enrichment at 37°C, inoculated beef samples (0.9 ml each) were filtered to remove large particles

	prior to 10 min of incubation with ca. 10^5 magnetic beads coated with anti–*E. coli* O157:H7 (Dynabeads, Dynal Inc., Oslo, Norway). Following incubation, the magnetic beads were collected with a Dynal magnetic concentrator and resuspended in 0.9 ml of Trypticase soy broth for ATP analysis." Benoit, 1941. "Hudson et al. (26) used IMS to directly separate and concentrate L. monocytogenes from ham samples prior to detection by PCR. IMS was used on the basis of the commercial availability of anti-Listeria magnetic beads (Dynal Inc.) to avoid time-consuming cultural enrichment steps and to remove PCR inhibitors." Benoit, 1941-42. Cudjoe investigates the use of immunomagnetic beads in pooling methods. In a study designed to evaluate the performance of Dynabeads® Anti-Salmonella, immunomagnetic separation (IMS) followed by plating (IMS-Plating) proved far superior to the conventional ISO *Salmonella* methodology and the Modified Semi-solid Rappaport-Vassiliadis (MSRV) method. *Salmonella* species were isolated and detected from 135 out of the 180 diverse poultry samples by IMS analysis as against 98 by the conventional method and 33 by the MSRV technique. All results were confirmed biochemically and serologically. It appeared from the data generated that some of the salmonellae isolated using IMS were greatly inhibited in Rappaport-Vassiliadis enrichment broth at 42°C and to a lesser extent in selenite cystine broth at 37°C. A moderately selective plating medium like XLD proved to be better in isolating these possible sensitive wild-type strains of salmonellae than the more selective BGA. Cudjoe, Abstract.

	Samples. One hundred and eighty (180) diverse poultry samples, consisting of 40 pools of cloacal/faecal swabs (5 swabs/pool; 5 pools samples/flock) from eight different flocks, 10 raw chicken breast meat samples, 10 pooled egg shell samples, 60 raw chicken liver samples and 60 chicken neck skins sampled from the slaughter line, were used in this study. All samples were obtained from a poultry slaughter and processing plant in the Netherlands. A pool of five swab samples was pre-enriched in 10 ml of buffered peptone water (BPW; Difco Laboratories, Detroit, Michigan, USA) at 37°C for 16–20 h, whilst 25 g of each of the other samples above, were pre-enriched similarly in 225 ml of BPW and examined as detailed below. Cudjoe, 160.
Claim 30	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 6, wherein the more sensitive protocol further comprises a concentration step to concentrate the microbial agent or organism, in combination with the same test protocol that is used to test the modular composite sample.	Price, in combination with ICMSF and Hughes, discloses Claim 6, upon which this claim relies. See above re Claim 6. Price, in combination with ICMSF and Hughes, further discloses more sensitive protocol that further comprises a concentration step to concentrate the microbial agent or organism, in combination with the same test protocol that is used to test the modular composite sample. Price discloses an enrichment stage after compositing where "[s]amples are individually pre-enriched followed by transfer from multiple pre-enrichments to single selenite and tetrathionate broths at the enrichment stage." Price, 679. A POSITA would understand that a detection assay that includes enrichment could further comprise a concentration step. Enrichment for the microbial agent or organism increases its concentration. A POSITA would use a standard concentration procedure such as immunomagnetic bead separation or affinity chromatography in order to concentrate the sample.

	A POSITA would understand that retesting the non-pooled, singular samples is an inherently more sensitive process because the sample is not diluted with other samples in the testing pool. Therefore, the retesting of a singular sample is more concentrated and therefore, more sensitive. ICMSF discloses the use of "immunomagnetic beads to concentrate cells." ICMSF, 318. A POSITA would consider this a well-known technique before the priority date. Hughes uses an EIA method followed by a more sensitive protocol—streaking on selective media. Hughes, 635. Each sample was analyzed by the BAM method (7th Ed.) and the TSVIA method using the enrichment protocol described in section F of the method, *Preparation of Sample*. Equal portions (0.5–1 mL) from each of the 2M broths arising from the 2 selective-enrichment broths were combined in a small screw-capped tube and heat treated in boiling water for 15 min prior to performing the TSVIA. Confirmation for the immunoassay was by streaking from the M broth culture onto selective agar, as specified in the BAM reference culture method. Hughes, 635. A POSITA would find that modifying Hughes based on D'Haese ensures a more sensitive test is used for further testing. A POSITA would have recognized the concentration step could be employed to provide a more sensitive protocol for further testing. A POSITA would understand that retesting the non-pooled, singular samples is an inherently more sensitive process because the sample is not diluted with other

	samples in the testing pool. Therefore, the retesting of a singular sample is more concentrated and therefore, more sensitive. *See also, generally*, Jarvis. Fratamico discusses the rapid isolation of target microorganisms using an immunomagnetic separation method. Fratamico, 105. Fratamico discloses that use of immunomagnetic beads resulted in "an increase in the level of E. coli B1409 in the sample," as well as "an increase in the number of organisms recovered." *Id.*, 107. Additionally, Fratamico provides detailed instructions for preparing and using immunomagnetic bead separation to concentrate a target microbial agent or organism. Fratamico developed "[a] method employing immunomagnetic beads" to concentrate and thereby improve the isolation and detection of E. coli. *Id.* For example, Fratamico was able to use immunomagnetic beads to isolate *E. coli* strains from enrichment cultures of raw ground beef "when the initial inoculum level was 1 cfu g-1." *Id.*, 109. Fratamico further teaches that "[t]he percentage of bacteria recovered increased with increasing amounts of beads." *Id.*, 108. Consequently, Fratamico "demonstrates that immunomagnetic separation [using immunomagnetic beads] is a simple and rapid technique" for concentrating a microbe for isolation and detection. *Id.*, 109. These teachings of Fratamico would have motivated a POSITA to modify the combination of ICMSF and Gombas and ICMSF and Hughes to use immunomagnetic beads to concentrate microbes in a modular composite sample to benefit from accurate tests. Use of immunomagnetic beads was already known in the art for concentrating during testing beef samples. "Tu et al. (78) detected pathogenic E. coli O157:H7 in beef hamburger with the use of IMS and ATP measurement. After 6 h of enrichment at 37°C, inoculated beef samples (0.9 ml each) were filtered to remove large particles prior to 10 min of incubation with ca. 10^5 magnetic beads coated with anti–*E. coli* O157:H7 (Dynabeads, Dynal Inc., Oslo, Norway). Following incubation, the

	magnetic beads were collected with a Dynal magnetic concentrator and resuspended in 0.9 ml of Trypticase soy broth for ATP analysis." Benoit, 1941. "Hudson et al. (26) used IMS to directly separate and concentrate L. monocytogenes from ham samples prior to detection by PCR. IMS was used on the basis of the commercial availability of anti-Listeria magnetic beads (Dynal Inc.) to avoid time-consuming cultural enrichment steps and to remove PCR inhibitors." Benoit, 1941-42. Cudjoe investigates the use of immunomagnetic beads in pooling methods. In a study designed to evaluate the performance of Dynabeads® Anti-Salmonella, immunomagnetic separation (IMS) followed by plating (IMS-Plating) proved far superior to the conventional ISO *Salmonella* methodology and the Modified Semi-solid Rappaport-Vassiliadis (MSRV) method. *Salmonella* species were isolated and detected from 135 out of the 180 diverse poultry samples by IMS analysis as against 98 by the conventional method and 33 by the MSRV technique. All results were confirmed biochemically and serologically. It appeared from the data generated that some of the salmonellae isolated using IMS were greatly inhibited in Rappaport-Vassiliadis enrichment broth at 42°C and to a lesser extent in selenite cystine broth at 37°C. A moderately selective plating medium like XLD proved to be better in isolating these possible sensitive wild-type strains of salmonellae than the more selective BGA. Cudjoe, Abstract.

	Samples. One hundred and eighty (180) diverse poultry samples, consisting of 40 pools of cloacal/faecal swabs (5 swabs/pool; 5 pools samples/flock) from eight different flocks, 10 raw chicken breast meat samples, 10 pooled egg shell samples, 60 raw chicken liver samples and 60 chicken neck skins sampled from the slaughter line, were used in this study. All samples were obtained from a poultry slaughter and processing plant in the Netherlands. A pool of five swab samples was pre-enriched in 10 ml of buffered peptone water (BPW; Difco Laboratories, Detroit, Michigan, USA) at 37°C for 16–20 h, whilst 25 g of each of the other samples above, were pre-enriched similarly in 225 ml of BPW and examined as detailed below. Cudjoe, 160.
Claim 31	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 17, wherein removing portions in step d) is removing equal portions, aseptically, so as to preclude contamination of the enriched separate single-unit production lot samples.	Price, in combination with ICMSF and Hughes, discloses Claim 17, upon which this claim relies. See above re Claim 17. Price, in combination with Hughes and ICMSF, further discloses removing portions in step d) is removing equal portions, aseptically, so as to preclude contamination of the enriched separate single-unit production lot samples. ICMSF describes aseptically withdrawing and weighing sample units into the appropriate diluent. ICMSF, 231, 232. Hughes instructs that after enrichment "[c]ombine equal volumes (0.5-1 mL) from each M broth tube in a clean screw-cap tube." Hughes, 638. Moreover, a POSITA would consider this to be standard practice. A POSITA would have aseptically removed equal volume portions of each enriched separate test lot sample.
Claim 32	**Disclosure of Price, ICMSF, and Hughes**

The method of claim 1, wherein the beef specimens are meat trim pieces.	Price, in combination with ICMSF and Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. Price, in combination with Hughes and ICMSF, further discloses wherein the beef specimens are meat trim pieces A POSITA would find it obvious to apply the teachings of Price to meat trim pieces. ICMSF indicates "[t]rimmings to be used for ground beef could be pretested for E. coli O157:H7 by various sampling plans." ICMSF, 323. ICMSF indicates "[r]esearch has validated that up to fifteen 25 g analytical units of ground beef or trimmings can be composited without significant loss in E. coli O157:H7 detection sensitivity." *Id.*, 327 *see also id.*, 226, 314, 320, and 324. ICMSF includes many other references to testing lots of beef or poultry, two of the most common types of meat in commerce. *Id.* at 114 (§ 6.3), 184–191 (§§ 10.2–10.6), 225–226 (§ 12.1), 285 (§ 16.1), 313–330 (Chapter 17). ICMSF is relevant to any kind of food product including beef or poultry.
Claim 33	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 17, wherein the meat is that of beef, pork, sheep or bison.	Price, in combination with ICMSF and Hughes, discloses Claim 17, upon which this claim relies. See above re Claim 17. Price, in combination with Hughes and ICMSF, further discloses wherein the meat is that of beef, pork, sheep or bison A POSITA would find it obvious to apply the teachings of Price to meat. ICMSF indicates "[t]rimmings to be used for ground beef could be pretested for E. coli O157:H7 by various sampling plans." ICMSF, 323. ICMSF indicates "[r]esearch has validated that up to fifteen 25 g analytical units of ground beef or trimmings can be composited without significant loss in E. coli O157:H7 detection sensitivity." *Id.*, 327 *see also id.*, 226, 314, 320, and 324.

	ICMSF includes many other references to testing lots of beef or poultry, two of the most common types of meat in commerce. *Id.* at 114 (§ 6.3), 184–91 (§§ 10.2–10.6), 225–26 (§ 12.1), 285 (§ 16.1), 313–30 (Chapter 17). ICMSF is relevant to any kind of food product including beef or poultry.

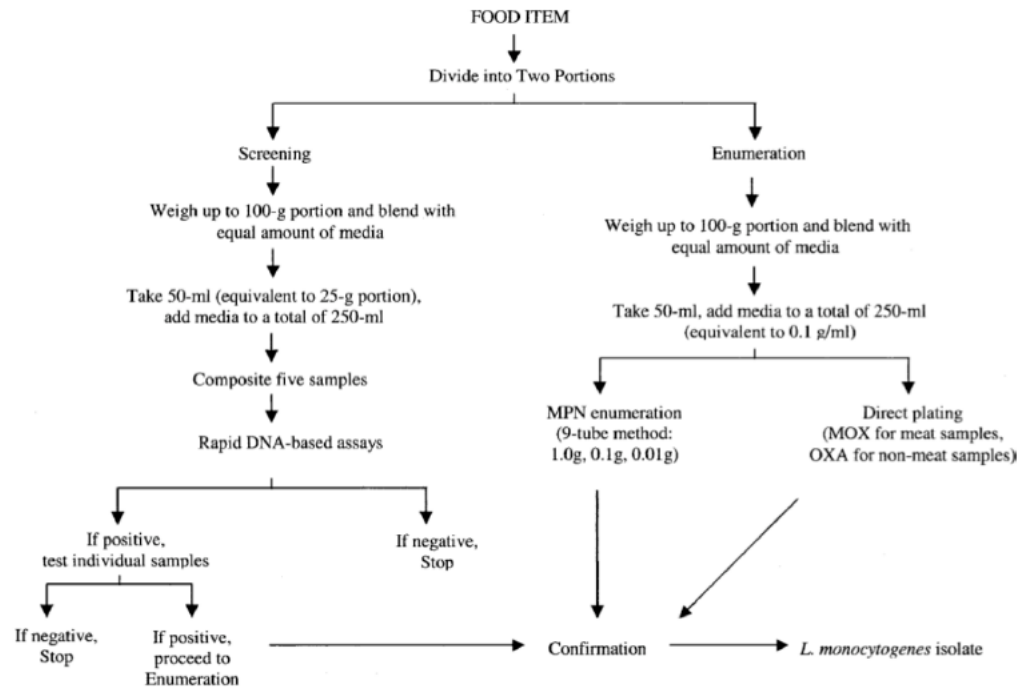
EXHIBIT B1

Invalidity of U.S. Patent No. 9,637,771 in light of ICMSF & Gombas

National Beef denies that any of its microbiological testing methods infringe any claim of U.S. Patent No. U.S. Patent No. 9,637,771 ("the '771 Patent"). If the claims of the '771 Patent were construed so broadly as to cover the Pooled Testing Methods, they would be invalid at least in view of INT'L COMM'N ON MICROBIOLOGICAL SPEC'S FOR FOODS (ICMSF), MICROORGANISMS IN FOODS 7: MICROBIOLOGICAL TESTING IN FOOD SAFETY MANAGEMENT (Kluwer Academic/Plenum Publishers, 2002) ("ICMSF") and David Gombas et al., *Survey of Listeria monocytogenes in Ready-to-Eat Foods*, 66 J. FOOD PROTECTION 559–569 (2003) ("Gombas"), which anticipate and/or disclose the limitations of the '771 Patent under at least pre-AIA 35 U.S.C. § 102(a), pre-AIA 35 U.S.C. § 102(b), pre-AIA 35 U.S.C. §§ 102(f)/(g), and/or pre-AIA 35 U.S.C. § 103. To the extent that ICMSF and/or Gombas do not expressly disclose one or more limitations of the asserted claims, as they would have to be construed to support IEH's claim of infringement, they disclose the limitations alone or in combination with one or more additional prior art references and/or the knowledge of one of ordinary skill in the art.

Claim 1	**Disclosure of ICMSF & Gombas**
[1pre] A method of sampling and testing for microbes in multiple separate test lots, comprising:	To the extent the preamble is limiting, as discussed herein, ICMSF, alone or in combination with Gombas, discloses a method of sampling and testing for microbes in multiple separate test lots. ICMSF and Gombas both disclose a method of sampling. ICMSF provides an example of "[u]sing a sampling plan involving n = 15 (25 g analytical units) and a composite of 375 g." *Id.*, 195 (§ 10.7.2). ICMSF also states "[f]or qualitative analyses, 25 g analytical units are frequently analyzed individually or as composites of 100 g, 200 g, or more." *Id.*, 232 (§ 12.5.2). ICMSF discloses collecting a plurality of samples or sample units (other synonyms include portions, specimens, and sub-samples) from lots of beef or poultry. ICMSF describes collecting from a lot or lots: "If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can

	have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." *Id.*, 119. Additionally, ICMSF indicates a consignment may be "a very large quantity of food" and may be "composed of several lots, representing for example, different days of production from the same plant." *Id.*, 119, 120; *see also* Applicant Admitted Prior Art ("AAPA") #3-#5. ICMSF and Gombas both disclose testing for microbes in multiple separate test lots. ICMSF describes testing for *Listeria* (ICMSF, 285-312), *Salmonella* (*id.*, 273-84), and *Escherichia coli* (*id.*, 313-32). Gombas focuses on *Listeria*. Gombas, 559.

	 FIGURE 1. *Detection and enumeration of* L. monocytogenes *in RTE food samples.* Gombas, Figure 1.
[1a] a) separately collecting multiple independent samples from each of multiple separate lots, wherein each separate lot is separately sampled by taking said multiple independent samples thereof;	ICMSF states: "To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units of the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. . . . This finding suggests that considerable reductions may be made in the cost of laboratory analysis by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, n, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort." ICMSF, 187.

	To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. A laboratory investigation undertaken for ICMSF (Silliker & Gabis, 1973) indicated that different sized analytical units drawn from a single well-mixed sample unit of dried food give very similar results. A subsequent investigation (Gabis & Silliker, 1974) using foods of high moisture content (including eggs, poultry meat, meat, and meat products) showed comparable reliability in the use of composite analytical units; for example, examination of three composites of 20 × 25 g analytical units gave the same result as examination of all 60 analytical units individually (the total amount examined being 1500 g in both instances). This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n*, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort. ICMSF, 187.

10.5.1 Conceivable Alternative Plans

As indicated above, for an infectious agent such as *Salmonella*, there are strong reasons for adopting sampling plans in which $c = 0$:

1. It is philosophically objectionable to some people to accept a lot containing a recognized pathogen, regardless of how the food is to be used in a manufacturing process or by the consumer.
2. There is the very great technical advantage of combining many analytical units for bacteriological examination in systems with $c = 0$, because a single positive decides the outcome.
3. The plan with $c = 1$ (or more) would always require the examination of (many) more analytical units, for equal probability of acceptance, than a plan with $c = 0$.
4. Even if the indicated probabilities of plans with $c = 0$ versus $c = 1$ are the same, their implications are not. For example, when a lot is accepted with the plan $n = 60$, $c = 0$, it is possible (though not certain) that the lot may not be contaminated at all. But using the plan $n = 95$, $c = 1$ and accepting one positive, it is known that the lot certainly is contaminated (though the probability that a lot with 5% of analytical units contaminated would be accepted is in both instances $P_a = 0.05$).

ICMSF, 188 (annotated). ICMSF also provides an example of "[u]sing a sampling plan involving n = 15 (25 g analytical units) and a composite of 375 g." *Id.*, 195 (§ 10.7.2). ICMSF also states "[f]or qualitative analyses, 25 g analytical units are frequently analyzed individually or as composites of 100 g, 200 g, or more." *Id.*, 232 (§ 12.5.2).

The ICMSF incorporates two references, Silliker & Gabis (1973) and Gabis & Silliker (1974). Silliker notes that "[c]ompositing of multiple samples (lumping) and wet compositing of pre-enrichment cultures have been tested as economical approaches to *Salmonella* control." Silliker, 475.

Silliker further notes that "[b]oth approaches are being used widely by the food industry in the United States." *Id.*.

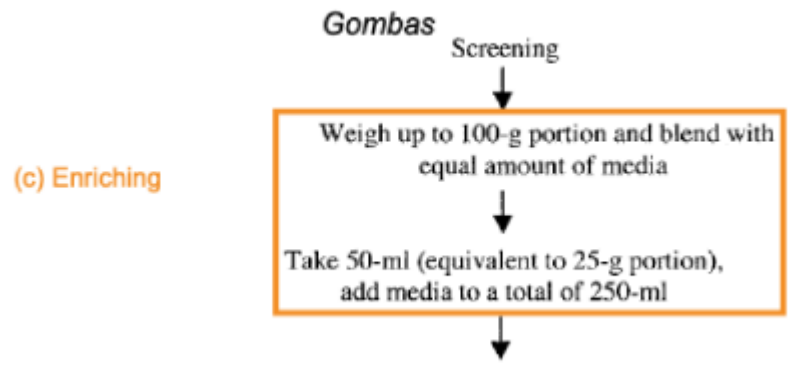
	To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. A laboratory investigation undertaken for ICMSF (Silliker & Gabis, 1973) indicated that different sized analytical units drawn from a single well-mixed sample unit of dried food give very similar results. A subsequent investigation (Gabis & Silliker, 1974) using foods of high moisture content (including eggs, poultry meat, meat, and meat products) showed comparable reliability in the use of composite analytical units; for example, examination of three composites of 20 × 25 g analytical units gave the same result as examination of all 60 analytical units individually (the total amount examined being 1500 g in both instances). This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n*, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort. "There is the very great technical advantage of combining many analytical units for bacteriological examination." ICMSF, 188. ICMSF also describes composites prepared by combining "15 (25 g analytical units)" and "composites of 100 g, 200 g, or more." *Id.,* 195, 232; *see* AAPA #5. ICMSF states, "All steps up to the analysis must be controlled . . . to allow reliable traceability." ICMSF, 227. ICMSF indicates lot samples are traceable to a particular corresponding lot: "It is therefore helpful . . . to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load). . . . Treating the individual production

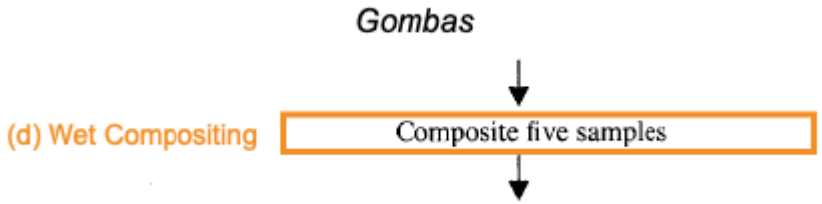
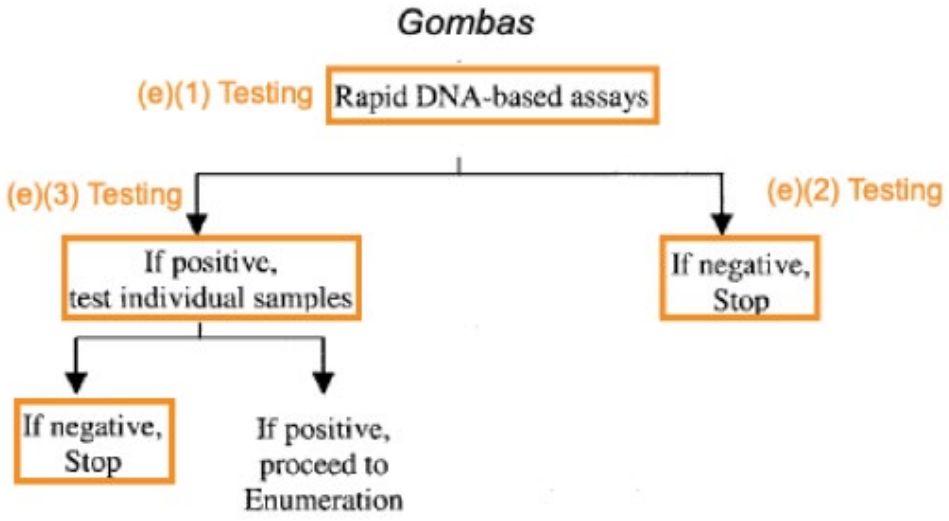
	batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119. Gombas also discloses this element. Gombas states "samples were assigned codes" ad product information was recorded for the luncheon meats including "sampling location." Gombas, 563. A POSITA would find Gombas' system for testing individual samples, which composited samples test positive, inherently requires tracking the individual samples that contributed to the composite. *See, e.g.*, Gombas, 564 ("If a composite tested positive, samples in the composite were tested individually by Gene-Trak assay with the PBS from the second mLCA plate or by the BAX assay with the secondary enrichment broths. Individual samples that tested negative were not tested further If an individual sample tested positive, the retained portion was subjected to an enumeration assay.") Thus, Gombas also discloses that each of the separate composited lot samples is attributed to a particular corresponding separate lot. ICMSF discloses collecting a plurality of samples or sample units (other synonyms include portions, specimens, and sub-samples) from lots of beef or poultry. ICMSF describes collecting from a lot or lots: "If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." *Id.*, 119. Additionally, ICMSF indicates a consignment may be "a very large quantity of food" and may be "composed of several lots, representing for example, different days of production from the same plant." *Id.*, 119, 120; *see also* AAPA #3-#5.

[1b] b) separately compositing the collected multiple independent samples from each of the separate lots to provide a corresponding set of separate composited lot samples, wherein each of the separate composited lot samples is attributed to a particular corresponding separate lot;	ICMSF states: "To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units of the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. . . . This finding suggests that considerable reductions may be made in the cost of laboratory analysis by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, n, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort." ICMSF, 187. To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. A laboratory investigation undertaken for ICMSF (Silliker & Gabis, 1973) indicated that different sized analytical units drawn from a single well-mixed sample unit of dried food give very similar results. A subsequent investigation (Gabis & Silliker, 1974) using foods of high moisture content (including eggs, poultry meat, meat, and meat products) showed comparable reliability in the use of composite analytical units; for example, examination of three composites of 20 × 25 g analytical units gave the same result as examination of all 60 analytical units individually (the total amount examined being 1500 g in both instances). This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n*, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort. ICMSF, 187.

[1c] c) enriching each of the separate composited lot samples to provide a set of separate composited lot samples enriched for the target microbe(s);	ICMSF states: To reduce laboratory effort . . . it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. ICMSF, 187. Also, ICMSF describes preparing composites of analytical units then diluting "for qualitative (enrichment)" analysis. *Id.,* 231-32; *see also* AAPA #5, #7. For practical reasons, however, analytical units of 10 g up to 50 g are typical for quantitative methods and are diluted 1:10. For qualitative analyses, 25 g analytical units are frequently analyzed individually or as composites of 100 g, 200 g, or more. ICMSF, 232 (annotated). Preparation of the first dilution, either for qualitative (enrichment) or quantitative (direct) analyses, can have a marked impact on the microbial population in the sample. This is often due to changes in the physico-chemical characteristics of the suspension as compared to the food matrix. Where the food does not confer protection, the composition of the diluent is important to prevent or minimize shock/injury to the microorganisms that can result from rapid changes in the ionic concentrations. ICMSF, 232 (annotated). ICMSF also describes strategic use of non-selective enrichment to resuscitate microorganisms and selective enrichments for growing microorganisms. *Id.*, 232–33 (§ 12.6); *see e.g.*, *id.,* 233 ("use of nonselective medium . . . to enhance recovery of injured or stressed cells."). ICMSF, § 12.6 also describes optimizing the incubation period. S*ee e.g.*, *id.,* 232. It was well known long before the priority date to use non-selective enrichments after dry compositing and prior to wet compositing as evidenced by Price, Silliker, Gabis, and MLG. A POSITA would also know to use selective enrichments after dry compositing and prior to wet compositing as disclosed by ICMSF and Gombas. Gombas further indicates that: "For each California sample, 0.1 ml of the demi-Fraser enrichment culture was transferred to 10 ml of morpholinepropanesulfonic

	acid-*Listeria* enrichment broth and incubated at 35°C for 20 to 24 h. After the secondary enrichment, 1 ml of the broth culture was used for screening with the BAX assay as described below." *Id.,* 564. **Sample screening.** Each sample was divided into two portions for screening and enumeration. For screening, approximately half (up to 100 g) of a sample was aseptically transferred to a sterile stomacher bag and blended with an equal amount of enrichment broth. For Maryland samples, University of Vermont broth 2 (UVM-2 broth) was used as the enrichment broth for luncheon meats, smoked seafood, and seafood salads; phosphate-buffered *Listeria* enrichment broth was used for the other product types. For northern California samples, demi-Fraser broth was used as the enrichment broth for all products. After blending, 50 g of the homogenate was added to 200 ml of enrichment broth and stomached for 1 min. This procedure resulted in a detection sensitivity equivalent to that of the current regulatory methods (i.e., 1 CFU/25 g). The initial sample-blending step was performed to account for the potential heterogeneous distribution of *L. monocytogenes* in the sample. The 250-ml enrichment was incubated at 35°C for 24 ± 2 h. Gombas, 563 (annotated).

	 Gombas, 563 (Figure 1 (annotated) showing an enriching step).
[1d] d) removing portions of each separate enriched composited lot sample, and combining the removed portions to provide a pooled modular composite sample; and	ICMSF describes compositing the enriched lot samples: "it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. . . . This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n,* with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort." ICMSF, 187. AAPA #5-#7. Additionally, Gombas discloses wet compositing of enriched samples: "Up to five like-product samples were composited and screened. . . . For California samples, five 1-ml samples of secondary enrichment broths were composited." Gombas, 564. Gombas's Figure 1 depicts wet compositing as shown in the excerpt below:

	 Gombas, 563 (Figure 1 (annotated) showing a wet compositing step).
[1e.1] e) testing of the pooled modular composite sample, using a suitable detection assay, for the target microbe(s),	ICMSF describes testing enrichment cultures of composite samples with suitable detection assays (ICMSF, 187) and "presence/absence tests" (ICMSF, 233). Suitable detection assays were established in Silliker and Gabis as discussed in ICMSF. Gombas provides a complete framework for testing, and a POSITA would have been motivated to modify ICMSF based on Gombas's framework:  Gombas, 563 (Figure 1 (annotated) showing the testing step).

	Gombas also discloses a suitable detection assay: "For California samples, . . . the 5-ml composite was tested by the BAX assay according to the manufacturer's procedures." Gombas, 564. These assay types were "art recognized." '771 Patent (AAPA #7).
[1e.2] wherein when such testing is negative all of said samples that were composited to form the separate composited lot samples are deemed negative for the target microbe(s) and each of the multiple separate lots is validated, and	ICMSF describes correlating lots in a consignment with lot samples so a negative or positive test result enables a lot to be validated or rejected. ICMSF, 118-19. ICMSF describes sampling plans in which "c is the maximum allowable number of sample units yielding a positive result." ICMSF, 115. For many scenarios, the requirement is c = 0, which indicates the testing is negative. *Id.*, 118, 183-90, 309, 325, 326. A negative test result (*id.*, 113-15, 325, 326) is the expected and desired outcome to determine meat is not contaminated and is safe for consumption (*id.*, 184-89) such that a lot is validated and it is "commercially possible to market" (*id.*, 185) the meat.

6.10 WHAT IS A LOT?

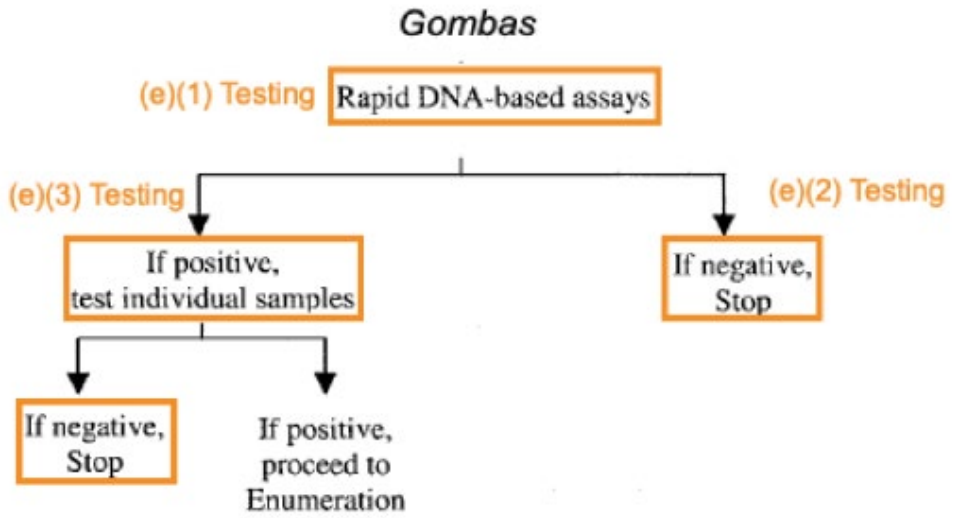
Ideally, a lot is a quantity of food or food units produced and handled under uniform conditions. Under certain circumstances, it has been shown that the logarithms of the counts from a batch of food are likely to be normally distributed (Baird-Parker, 1980; Kilsby & Baird-Parker, 1983 [their Table 2]; Ingram & Roberts, 1976). The implication is that there is homogeneity within a lot, which, when considering microbial levels and distributions, rarely occurs in practice. In most instances, the distribution of microorganisms within lots of food is heterogeneous. Even in food factories, this heterogeneity makes the interpretation of sample results difficult, and it becomes even more so in circumstances where a lot is less well defined, such as in a consignment of a very large quantity of food.

It is therefore helpful if suppliers can be persuaded to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load).

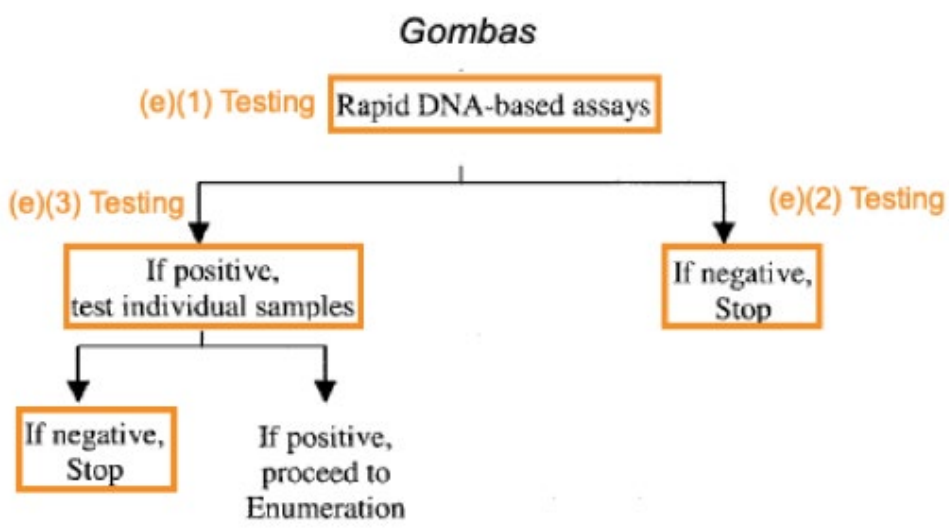
If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment.

A lot should be composed of food produced with as little variation as possible for a given process or commodity. Because of the uncertainties in identifying a lot commercially, however, the use of the word *lot* in this book is usually in its statistical sense, as a collection of units of a product, the acceptability of which is determined by examining a sample drawn from it.

ICMSF, 118-19 (annotated).

	To the extent ICMSF is considered not to disclose some aspect of step (e)(2), Gombas indicates a negative test result is the end of the process: "If a composite tested negative, all samples in the composite were considered negative (in 25 g) and were not tested further." Gombas, 564. A POSITA would find that in the combination, a negative test result equates to being validated.  Gombas, 563 (Figure 1 (annotated) showing "if negative, stop.").
[1e.3] wherein when such testing is positive, each of the individual separate composited lot samples that were used to form the pooled modular composite sample are individually	The combination of ICMSF and Gombas describes retaining a portion of the enriched lot samples (steps (b)-(d)). "Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more

tested to determine which of the separate composited lot samples is positive for the target microbe(s),	analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119. "There is often a desire to retest retained samples." ICMSF, 191. If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment. ICMSF, 119 (annotated). ICMSF discloses validating for release into commerce. ICMSF, 113–15 (§§ 6.2–6.6), 118–19 (§§ 6.9–6.10), 184–85 (§ 10.2), 325–27 (§§ 17.5.3–17.5.3.4); *see also id.*, 186–89 (§ 10.4–10.6)) according to a statistically-based sampling plan (*id.*, 113 (§ 6.1); *see also id.*, 115–18 (§§ 6.4–6.8), 184 (§ 10.2), 319–21 (§§ 17.2.3.3–17.2.3.4), 326–30 (§§ 17.5.3.1–17.6), as described regarding steps (a) and (e)(2). "If a composite tested positive, samples in the composite were tested individually . . . by the BAX assay with the secondary enrichment broths. Individual samples that tested negative were not tested further." Gombas, 564.

	 Gombas, 563 (Figure 1 (annotated) showing testing procedures for further testing). "Individual samples that tested negative were not tested further. If an individual sample tested positive, the retained portion was subjected to an enumeration assay." Gombas, 564. The combination teaches that after the composited samples test positive, then the individual samples used to form the composited sample are individually tested with the same protocol to determine which of the separate composited lot samples is positive for the target microbe(s).
[1e.4] wherein the lots corresponding to any negatively testing composited lot samples are validated.	ICMSF describes correlating lots in a consignment with lot samples so a negative or positive test result enables a lot to be validated or rejected. ICMSF, 118-19. ICMSF describes sampling plans in which "c is the maximum allowable number of sample units yielding a positive result." ICMSF, 115. For many scenarios, the requirement is c = 0, which indicates the testing is negative. *Id.*, 118, 183-90, 309, 325, 326. A negative test result (*id.*, 113-15, 325, 326) is the expected and desired

	outcome to determine meat is not contaminated and is safe for consumption (*id.*, 184-89) such that a lot is validated and it is "commercially possible to market" (*id.*, 185) the meat.

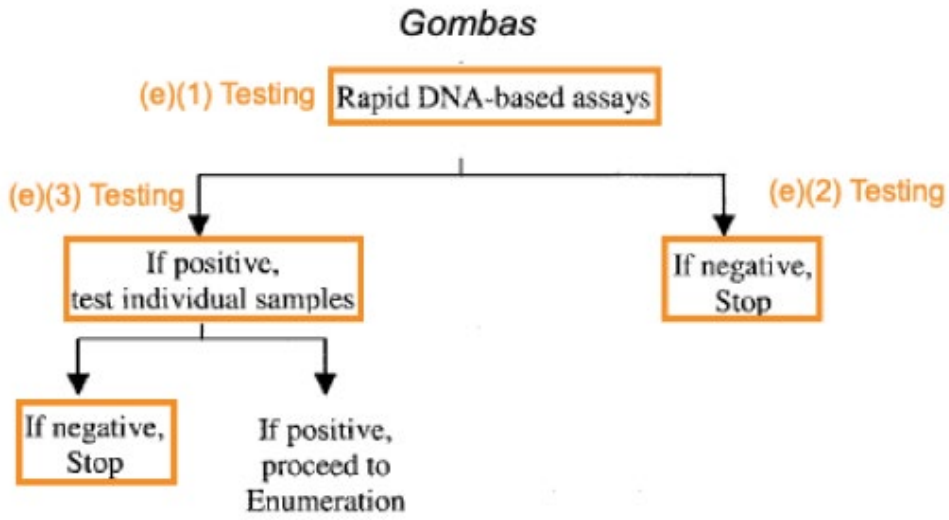
6.10 WHAT IS A LOT?

Ideally, a lot is a quantity of food or food units produced and handled under uniform conditions. Under certain circumstances, it has been shown that the logarithms of the counts from a batch of food are likely to be normally distributed (Baird-Parker, 1980; Kilsby & Baird-Parker, 1983 [their Table 2]; Ingram & Roberts, 1976). The implication is that there is homogeneity within a lot, which, when considering microbial levels and distributions, rarely occurs in practice. In most instances, the distribution of microorganisms within lots of food is heterogeneous. Even in food factories, this heterogeneity makes the interpretation of sample results difficult, and it becomes even more so in circumstances where a lot is less well defined, such as in a consignment of a very large quantity of food.

It is therefore helpful if suppliers can be persuaded to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load).

If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment.

A lot should be composed of food produced with as little variation as possible for a given process or commodity. Because of the uncertainties in identifying a lot commercially, however, the use of the word *lot* in this book is usually in its statistical sense, as a collection of units of a product, the acceptability of which is determined by examining a sample drawn from it.

	ICMSF, 118-19 (annotated). To the extent ICMSF is considered not to disclose some aspect of step (e)(2), Gombas indicates a negative test result is the end of the process: "If a composite tested negative, all samples in the composite were considered negative (in 25 g) and were not tested further." Gombas, 564. A POSITA would find that in the combination, a negative test result equates to being validated.  Gombas, 563 (Figure 1 (annotated) showing "if negative, stop.").
Claim 2	**Disclosure of ICMSF & Gombas**

The method of claim 1, wherein each of the multiple separate lots is selected from the group consisting of an environmental lot comprising multiple operationally-linked environmental specimens, pharmaceutical lot comprising multiple operationally-linked pharmaceutical specimens, single product lot comprising multiple operationally-linked product specimens, and combinations thereof.	ICMSF, alone or in combination with Gombas, discloses Claim 1, upon which this claim relies. See above re Claim 1. The specification of the '771 Patent indicates a test lot is a single-unit production lot ('771 Patent, 7:54-56) such as a single combo, bin. pallet, or segment of production ('771 Patent, 7:54-59; *see also* AAPA #3 and #4). ICMSF teaches collecting from "individual production batches as lots" (ICMSF, 119) and "[a] lot is considered as a collection of units" (ICMSF, 327). "Operationally-linked" is simply grouping like items, which is also disclosed by ICMSF. "Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119. ICMSF states, "All steps up to the analysis must be controlled . . . to allow reliable traceability." ICMSF, 227. ICMSF indicates lot samples are traceable to a particular corresponding lot: "It is therefore helpful . . . to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load). . . . Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119.

	Gombas also discloses this element. Gombas states "samples were assigned codes" ad product information was recorded for the luncheon meats including "sampling location." Gombas, 563. A POSITA would find Gombas' system for testing individual samples, which composited samples test positive, inherently requires tracking the individual samples that contributed to the composite. *See, e.g.*, Gombas, 564 ("If a composite tested positive, samples in the composite were tested individually by Gene-Trak assay with the PBS from the second mLCA plate or by the BAX assay with the secondary enrichment broths. Individual samples that tested negative were not tested further If an individual sample tested positive, the retained portion was subjected to an enumeration assay.") Thus, Gombas also discloses that each of the separate composited lot samples is attributed to a particular corresponding separate lot.
Claim 3	**Disclosure of ICMSF & Gombas**
The method of claim 1, wherein each of the multiple separate lots is a single production lot, comprised of operationally-linked product specimens.	ICMSF, alone or in combination with Gombas, discloses Claim 1, upon which this claim relies. See above re Claim 1. The specification of the '771 Patent indicates a test lot is a single-unit production lot ('771 Patent, 7:54-56) such as a single combo, bin. pallet, or segment of production ('771 Patent, 7:54-59; *see also* AAPA #3 and #4). ICMSF teaches collecting from "individual production batches as lots" (ICMSF, 119) and "[a] lot is considered as a collection of units" (ICMSF, 327). "Operationally-linked" is simply grouping like items, which is also disclosed by ICMSF. "Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119. ICMSF states, "All steps up to the analysis must be controlled . . . to allow reliable traceability." ICMSF, 227.

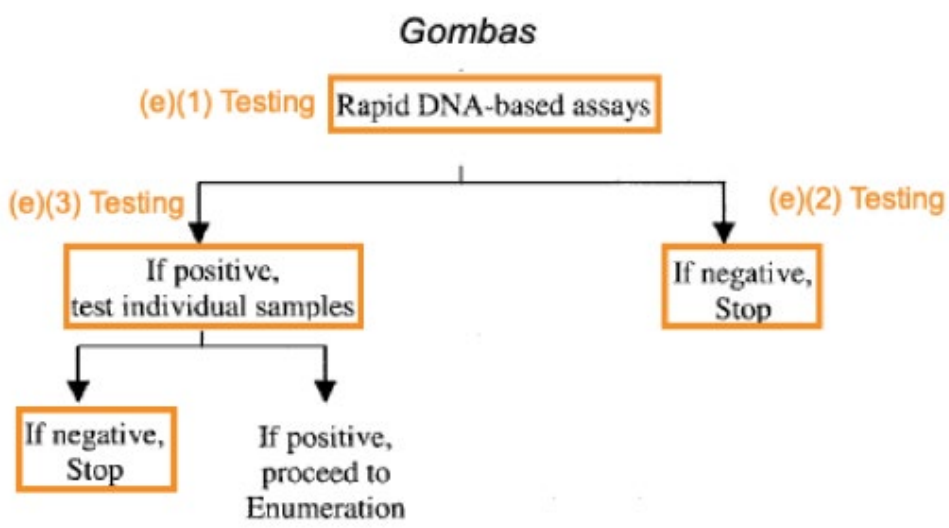
	ICMSF indicates lot samples are traceable to a particular corresponding lot: "It is therefore helpful . . . to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load). . . . Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119. Gombas also discloses this element. Gombas states "samples were assigned codes" ad product information was recorded for the luncheon meats including "sampling location." Gombas, 563. A POSITA would find Gombas' system for testing individual samples, which composited samples test positive, inherently requires tracking the individual samples that contributed to the composite. *See, e.g.*, Gombas, 564 ("If a composite tested positive, samples in the composite were tested individually by Gene-Trak assay with the PBS from the second mLCA plate or by the BAX assay with the secondary enrichment broths. Individual samples that tested negative were not tested further If an individual sample tested positive, the retained portion was subjected to an enumeration assay.") Thus, Gombas also discloses that each of the separate composited lot samples is attributed to a particular corresponding separate lot.
Claim 4	
The method of claim 3, wherein product specimens are synonymous with product portions.	ICMSF, alone or in combination with Gombas, discloses Claim 3, upon which this claim relies. See above re Claim 3.

	ICMSF teaches collecting portions or samples, which were known synonyms for product portions or specimens. ICMSF states that "[a] sample unit is a small portion of the lot." ICMSF, 225. **12.1 INTRODUCTION** Many different types of samples are collected and submitted to the laboratory for analysis. Some are sample units from lots or consignments of foods or ingredients for lot acceptance determination. Others may be for investigational purposes to assess control of the environment, investigate the source of a problem, or validate a process. Some may have legal implications relative to a lawsuit or for regulatory compliance. This chapter briefly discusses some of the major factors that should be considered when collecting sample units, shipping them to a laboratory, preparing them for analysis, and analyzing them. A sample unit is a small portion of the lot in which the distribution of both numbers and types of microorganisms should be representative of the whole lot. This requirement is only completely fulfilled when the food and processing conditions result in a homogeneous distribution of microorganisms. This only occurs in well-agitated fluid products. In general, the distribution of microorganisms is presumed to be random. ICMSF at 225 annotated).
Claim 5	**Disclosure of ICMSF & Gombas**
The method of claim 1, further comprising incubating the pooled modular composite sample of d), prior to testing in e).	ICMSF, alone or in combination with Gombas, discloses Claim 1, upon which this claim relies. See above re Claim 1. ICMSF and Gombas, in combination with Stewart, discloses the method of claim 1, further comprising incubating the pooled modular composite sample of d), prior to testing in e). Claim 1 is disclosed by ICMSF and Gombas. Stewart discloses that after the selective enrichment in Rappaport-Vassiliadis (RV) broth and tetrathionate (TT) broths, the two selective enrichments were combined into a single sample for further incubation and analysis. "[P]ost-enrichment by

	transfer and combination of 0.5 mL RV and 1 mL TT into a single tube containing 10 mL prewarmed trypticase soy broth supplemented with novobiocin (TSB + n) at 10 µg/mL with incubation for 16–20 h at 42°C." Stewart, 398.
Claim 6	**Disclosure of ICMSF & Gombas**
The method of claim 1, wherein each separate composited lot sample in c) comprises incubating each separate composited lot sample with enrichment medium suitable to enrich for the target microbe(s), and wherein removing in d) comprises removing a portion of the enrichment medium from each enriched separate composited lot sample and combining the removed portions to provide a pooled modular composite sample.	ICMSF, alone or in combination with Gombas, discloses Claim 1, upon which this claim relies. See above re Claim 1. ICMSF describes the well-known benefits of combining enrichment cultures from analytical units from lots in a consignment. To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. A laboratory investigation undertaken for ICMSF (Silliker & Gabis, 1973) indicated that different sized analytical units drawn from a single well-mixed sample unit of dried food give very similar results. A subsequent investigation (Gabis & Silliker, 1974) using foods of high moisture content (including eggs, poultry meat, meat, and meat products) showed comparable reliability in the use of composite analytical units; for example, examination of three composites of 20 × 25 g analytical units gave the same result as examination of all 60 analytical units individually (the total amount examined being 1500 g in both instances). This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n*, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort. ICMSF, 187. Gombas describes incubating an enriched test lot sample. "The 250-ml enrichment was incubated at 35°C for 24 ± 2 h." Gombas, 563. "[A]pproximately half (up to 100 g) of a sample was aseptically transferred to a

	sterile stomacher bag and blended with an equal amount of enrichment broth. . . . For northern California samples, demi-Fraser broth was used as the enrichment broth for all products. After blending, 50 g of the homogenate was added to 200 ml of enrichment broth and stomached for 1 min. This procedure resulted in a detection sensitivity equivalent to that of the current regulatory methods (i.e., 1 CFU/25 g). The initial sample-blending step was performed to account for the potential heterogeneous distribution of *L. monocytogenes* in the sample. The 250-ml enrichment was incubated at 35°C for 24 ± 2 h." Gombas, 563; *see also id.,* 564. Gombas further indicates that: "For each California sample, 0.1 ml of the demi-Fraser enrichment culture was transferred to 10 ml of morpholinepropanesulfonic acid-*Listeria* enrichment broth and incubated at 35°C for 20 to 24 h. After the secondary enrichment, 1 ml of the broth culture was used for screening with the BAX assay as described below." *Id.,* 564.
Claim 7	**Disclosure of ICMSF & Gombas**
The method of claim 6, wherein the enrichment medium comprises a selective agent that will inhibit a competing non-target microbe.	ICMSF, alone or in combination with Gombas, discloses Claim 6, upon which this claim relies. See above re Claim 6. Additionally, Claim 7 recites what a POSITA would consider the well-known property of selective enrichment, inhibition of a competing non-target microorganism. D'Aoust describes "selective enrichment [that] inhibits growth of non-salmonellae and facilitates isolation of *Salmonella* on differential plating media." D'Aoust, 369. A POSITA would have been motivated to use an enrichment medium comprising a selective agent that inhibits a non-target agent or organisms as described by D'Aoust, for the test lot samples described by ICMSF and Gombas, to ensure an accurate test. A POSITA would understand that incubating samples at different temperature ranges for different target microorganisms would, effectively, inhibit the growth of other

	organisms. "Incubation temperature markedly affects productivity of selective enrichment broths by suppressing growth of competing microorganisms but not *Salmonella*." D'Aoust, 372. "This may be achieved by utilization of enrichment media that through various mechanisms may favor and enhance growth and multiplication of salmonellae while other gram-negative organisms such as coliforms, Proteus, and Pseudomonas are suppressed or inhibited." Fagerberg, 630.
Claim 8	**Disclosure of ICMSF & Gombas**
The method of claim 1, wherein testing of the pooled modular composite sample, using a suitable detection assay comprises use of a concentration step to concentrate the target microbe(s).	ICMSF, alone or in combination with Gombas, discloses Claim 1, upon which this claim relies. See above re Claim 1. ICMSF describes testing enrichment cultures of composite samples with suitable detection assays (ICMSF, 187) and "presence/absence tests" (ICMSF, 233). Suitable detection assays were established in Silliker and Gabis as discussed in ICMSF. Gombas provides a complete framework for testing, and a POSITA would have been motivated to modify ICMSF based on Gombas's framework:



Gombas, 563 (Figure 1 (annotated) showing the testing step).

Gombas also discloses a suitable detection assay: "For California samples, . . . the 5-ml composite was tested by the BAX assay according to the manufacturer's procedures." Gombas, 564.

These assay types were "art recognized." '771 Patent, (AAPA #7).

Fratamico discusses the rapid isolation of target microorganisms using an immunomagnetic separation method. Fratamico, 105. Fratamico discloses that use of immunomagnetic beads resulted in "an increase in the level of E. coli B1409 in the sample," as well as "an increase in the number of organisms recovered." *Id.*, 107. Additionally, Fratamico provides detailed instructions for preparing and using immunomagnetic bead separation to concentrate a target microbial agent or organism.

Fratamico developed "[a] method employing immunomagnetic beads" to concentrate and thereby improve the isolation and detection of E. coli. *Id.* For example,

	Fratamico was able to use immunomagnetic beads to isolate *E. coli* strains from enrichment cultures of raw ground beef "when the initial inoculum level was 1 cfu g-1." *Id.*, 109. Fratamico further teaches that "[t]he percentage of bacteria recovered increased with increasing amounts of beads." *Id.*, 108. Consequently, Fratamico "demonstrates that immunomagnetic separation [using immunomagnetic beads] is a simple and rapid technique" for concentrating a microbe for isolation and detection. *Id.*, 109. These teachings of Fratamico would have motivated a POSA to modify the combination of ICMSF and Gombas and ICMSF and Hughes to use immunomagnetic beads to concentrate microbes in a modular composite sample to benefit from accurate tests. Use of immunomagnetic beads was already known in the art for concentrating during testing beef samples. "Tu et al. (78) detected pathogenic E. coli O157:H7 in beef hamburger with the use of IMS and ATP measurement. After 6 h of enrichment at 37°C, inoculated beef samples (0.9 ml each) were filtered to remove large particles prior to 10 min of incubation with ca. 10^5 magnetic beads coated with anti–*E. coli* O157:H7 (Dynabeads, Dynal Inc., Oslo, Norway). Following incubation, the magnetic beads were collected with a Dynal magnetic concentrator and resuspended in 0.9 ml of Trypticase soy broth for ATP analysis." Benoit, 1941. "Hudson et al. (26) used IMS to directly separate and concentrate L. monocytogenes from ham samples prior to detection by PCR. IMS was used on the basis of the commercial availability of anti-Listeria magnetic beads (Dynal Inc.) to avoid time-consuming cultural enrichment steps and to remove PCR inhibitors." Benoit, 1941-42. Cudjoe investigates the use of immunomagnetic beads in pooling methods.

	In a study designed to evaluate the performance of Dynabeads® Anti-Salmonella, immunomagnetic separation (IMS) followed by plating (IMS-Plating) proved far superior to the conventional ISO *Salmonella* methodology and the Modified Semi-solid Rappaport-Vassiliadis (MSRV) method. *Salmonella* species were isolated and detected from 135 out of the 180 diverse poultry samples by IMS analysis as against 98 by the conventional method and 33 by the MSRV technique. All results were confirmed biochemically and serologically. It appeared from the data generated that some of the salmonellae isolated using IMS were greatly inhibited in Rappaport-Vassiliadis enrichment broth at 42°C and to a lesser extent in selenite cystine broth at 37°C. A moderately selective plating medium like XLD proved to be better in isolating these possible sensitive wild-type strains of salmonellae than the more selective BGA. Cudjoe, Abstract. *Samples*. One hundred and eighty (180) diverse poultry samples, consisting of 40 pools of cloacal/faecal swabs (5 swabs/pool; 5 pools samples/flock) from eight different flocks, 10 raw chicken breast meat samples, 10 pooled egg shell samples, 60 raw chicken liver samples and 60 chicken neck skins sampled from the slaughter line, were used in this study. All samples were obtained from a poultry slaughter and processing plant in the Netherlands. A pool of five swab samples was pre-enriched in 10 ml of buffered peptone water (BPW; Difco Laboratories, Detroit, Michigan, USA) at 37°C for 16–20 h, whilst 25 g of each of the other samples above, were pre-enriched similarly in 225 ml of BPW and examined as detailed below. Cudjoe, 160.
Claim 9	**Disclosure of ICMSF & Gombas**
The method of claim 8, wherein the concentration step comprises at least one of immunomagnetic bead separation, and affinity chromatography.	ICMSF, alone or in combination with Gombas, discloses Claim 8, upon which this claim relies. See above re Claim 8. ICMSF discloses the use of "immunomagnetic beads to concentrate cells." ICMSF, 318. A POSITA would consider this a well-known technique before the priority date.

	Fratamico discusses the rapid isolation of target microorganisms using an immunomagnetic separation method. Fratamico, 105. Fratamico discloses that use of immunomagnetic beads resulted in "an increase in the level of E. coli B1409 in the sample," as well as "an increase in the number of organisms recovered." *Id.*, 107. Additionally, Fratamico provides detailed instructions for preparing and using immunomagnetic bead separation to concentrate a target microbial agent or organism. Fratamico developed "[a] method employing immunomagnetic beads" to concentrate and thereby improve the isolation and detection of E. coli. *Id.* For example, Fratamico was able to use immunomagnetic beads to isolate *E. coli* strains from enrichment cultures of raw ground beef "when the initial inoculum level was 1 cfu g-1." *Id.*, 109. Fratamico further teaches that "[t]he percentage of bacteria recovered increased with increasing amounts of beads." *Id.*, 108. Consequently, Fratamico "demonstrates that immunomagnetic separation [using immunomagnetic beads] is a simple and rapid technique" for concentrating a microbe for isolation and detection. *Id.*, 109. These teachings of Fratamico would have motivated a POSA to modify the combination of ICMSF and Gombas and ICMSF and Hughes to use immunomagnetic beads to concentrate microbes in a modular composite sample to benefit from accurate tests. Use of immunomagnetic beads was already known in the art for concentrating during testing beef samples. "Tu et al. (78) detected pathogenic E. coli O157:H7 in beef hamburger with the use of IMS and ATP measurement. After 6 h of enrichment at 37°C, inoculated beef samples (0.9 ml each) were filtered to remove large particles prior to 10 min of incubation with ca. 10^5 magnetic beads coated with anti–*E. coli* O157:H7 (Dynabeads, Dynal Inc., Oslo, Norway). Following incubation, the magnetic beads were collected with a Dynal magnetic concentrator and resuspended in 0.9 ml of Trypticase soy broth for ATP analysis." Benoit, 1941.

	"Hudson et al. (26) used IMS to directly separate and concentrate L. monocytogenes from ham samples prior to detection by PCR. IMS was used on the basis of the commercial availability of anti-Listeria magnetic beads (Dynal Inc.) to avoid time-consuming cultural enrichment steps and to remove PCR inhibitors." Benoit, 1941-42. Cudjoe investigates the use of immunomagnetic beads in pooling methods. In a study designed to evaluate the performance of Dynabeads® Anti-Salmonella, immunomagnetic separation (IMS) followed by plating (IMS-Plating) proved far superior to the conventional ISO *Salmonella* methodology and the Modified Semi-solid Rappaport-Vassiliadis (MSRV) method. *Salmonella* species were isolated and detected from 135 out of the 180 diverse poultry samples by IMS analysis as against 98 by the conventional method and 33 by the MSRV technique. All results were confirmed biochemically and serologically. It appeared from the data generated that some of the salmonellae isolated using IMS were greatly inhibited in Rappaport-Vassiliadis enrichment broth at 42°C and to a lesser extent in selenite cystine broth at 37°C. A moderately selective plating medium like XLD proved to be better in isolating these possible sensitive wild-type strains of salmonellae than the more selective BGA. Cudjoe, Abstract. *Samples*. One hundred and eighty (180) diverse poultry samples, consisting of 40 pools of cloacal/faecal swabs (5 swabs/pool; 5 pools samples/flock) from eight different flocks, 10 raw chicken breast meat samples, 10 pooled egg shell samples, 60 raw chicken liver samples and 60 chicken neck skins sampled from the slaughter line, were used in this study. All samples were obtained from a poultry slaughter and processing plant in the Netherlands. A pool of five swab samples was pre-enriched in 10 ml of buffered peptone water (BPW; Difco Laboratories, Detroit, Michigan, USA) at 37°C for 16–20 h, whilst 25 g of each of the other samples above, were pre-enriched similarly in 225 ml of BPW and examined as detailed below. Cudjoe, 160.

Claim 10	Disclosure of ICMSF & Gombas
The method of claim 1, wherein where testing in e) of the pooled modular composite sample is positive, and wherein the separate individual composited lot samples are further tested using a more sensitive protocol.	ICMSF, alone or in combination with Gombas, discloses Claim 1, upon which this claim relies. See above re Claim 1. ICMSF discloses the use of "immunomagnetic beads to concentrate cells." ICMSF, 318. A POSITA would consider this a well-known technique before the priority date. Gombas discloses retesting using the same protocol. Gombas discusses the use of "biochemical confirmation" to confirm testing results. Gombas, 565. A POSITA would have recognized the concentration step could be employed to provide a more sensitive protocol for further testing. A POSITA would understand that retesting the non-pooled, singular samples is an inherently more sensitive process because the sample is not diluted with other samples in the testing pool. Therefore, the retesting of a singular sample is more concentrated and therefore, more sensitive. *See also, generally*, Jarvis. Fratamico discusses the rapid isolation of target microorganisms using an immunomagnetic separation method. Fratamico, 105. Fratamico discloses that use of immunomagnetic beads resulted in "an increase in the level of E. coli B1409 in the sample," as well as "an increase in the number of organisms recovered." *Id.*, 107. Additionally, Fratamico provides detailed instructions for preparing and using immunomagnetic bead separation to concentrate a target microbial agent or organism. Fratamico developed "[a] method employing immunomagnetic beads" to concentrate and thereby improve the isolation and detection of E. coli. *Id.* For example, Fratamico was able to use immunomagnetic beads to isolate *E. coli* strains from enrichment cultures of raw ground beef "when the initial inoculum level was 1 cfu g-1." *Id.*, 109. Fratamico further teaches that "[t]he percentage of bacteria recovered increased with increasing amounts of beads." *Id.*, 108. Consequently, Fratamico "demonstrates that immunomagnetic separation [using immunomagnetic beads] is a

	simple and rapid technique" for concentrating a microbe for isolation and detection. *Id.*, 109. These teachings of Fratamico would have motivated a POSA to modify the combination of ICMSF and Gombas and ICMSF and Hughes to use immunomagnetic beads to concentrate microbes in a modular composite sample to benefit from accurate tests. Use of immunomagnetic beads was already known in the art for concentrating during testing beef samples. "Tu et al. (78) detected pathogenic E. coli O157:H7 in beef hamburger with the use of IMS and ATP measurement. After 6 h of enrichment at 37°C, inoculated beef samples (0.9 ml each) were filtered to remove large particles prior to 10 min of incubation with ca. 10^5 magnetic beads coated with anti–*E. coli* O157:H7 (Dynabeads, Dynal Inc., Oslo, Norway). Following incubation, the magnetic beads were collected with a Dynal magnetic concentrator and resuspended in 0.9 ml of Trypticase soy broth for ATP analysis." Benoit, 1941. "Hudson et al. (26) used IMS to directly separate and concentrate L. monocytogenes from ham samples prior to detection by PCR. IMS was used on the basis of the commercial availability of anti-Listeria magnetic beads (Dynal Inc.) to avoid time-consuming cultural enrichment steps and to remove PCR inhibitors." Benoit, 1941-42. Cudjoe investigates the use of immunomagnetic beads in pooling methods.

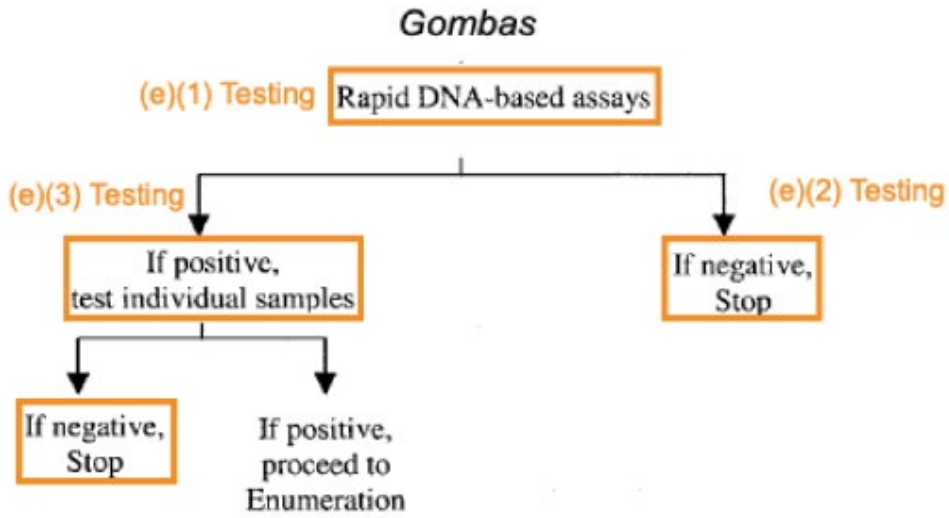
	In a study designed to evaluate the performance of Dynabeads® Anti-Salmonella, immunomagnetic separation (IMS) followed by plating (IMS-Plating) proved far superior to the conventional ISO *Salmonella* methodology and the Modified Semi-solid Rappaport-Vassiliadis (MSRV) method. *Salmonella* species were isolated and detected from 135 out of the 180 diverse poultry samples by IMS analysis as against 98 by the conventional method and 33 by the MSRV technique. All results were confirmed biochemically and serologically. It appeared from the data generated that some of the salmonellae isolated using IMS were greatly inhibited in Rappaport-Vassiliadis enrichment broth at 42°C and to a lesser extent in selenite cystine broth at 37°C. A moderately selective plating medium like XLD proved to be better in isolating these possible sensitive wild-type strains of salmonellae than the more selective BGA. Cudjoe, Abstract. *Samples*. One hundred and eighty (180) diverse poultry samples, consisting of 40 pools of cloacal/faecal swabs (5 swabs/pool; 5 pools samples/flock) from eight different flocks, 10 raw chicken breast meat samples, 10 pooled egg shell samples, 60 raw chicken liver samples and 60 chicken neck skins sampled from the slaughter line, were used in this study. All samples were obtained from a poultry slaughter and processing plant in the Netherlands. A pool of five swab samples was pre-enriched in 10 ml of buffered peptone water (BPW; Difco Laboratories, Detroit, Michigan, USA) at 37°C for 16–20 h, whilst 25 g of each of the other samples above, were pre-enriched similarly in 225 ml of BPW and examined as detailed below. Cudjoe, 160.
Claim 11	**Disclosure of ICMSF & Gombas**
The method of claim 10, wherein the more sensitive protocol comprises use of a concentration step to concentrate the target microbe(s), in combination with the same test	ICMSF, alone or in combination with Gombas, discloses Claim 10, upon which this claim relies. See above re Claim 10. ICMSF discloses the use of "immunomagnetic beads to concentrate cells." ICMSF, 318. A POSITA would consider this a well-known technique before the priority date.

protocol used to test the pooled modular composite sample.	Gombas discloses retesting using the same protocol. Gombas discusses the use of "biochemical confirmation" to confirm testing results. Gombas, 565. A POSITA would have recognized the concentration step could be employed to provide a more sensitive protocol for further testing. A POSITA would understand that retesting the non-pooled, singular samples is an inherently more sensitive process because the sample is not diluted with other samples in the testing pool. Therefore, the retesting of a singular sample is more concentrated and therefore, more sensitive. *See also, generally*, Jarvis. Fratamico discusses the rapid isolation of target microorganisms using an immunomagnetic separation method. Fratamico, 105. Fratamico discloses that use of immunomagnetic beads resulted in "an increase in the level of E. coli B1409 in the sample," as well as "an increase in the number of organisms recovered." *Id.*, 107. Additionally, Fratamico provides detailed instructions for preparing and using immunomagnetic bead separation to concentrate a target microbial agent or organism. Fratamico developed "[a] method employing immunomagnetic beads" to concentrate and thereby improve the isolation and detection of E. coli. *Id.* For example, Fratamico was able to use immunomagnetic beads to isolate *E. coli* strains from enrichment cultures of raw ground beef "when the initial inoculum level was 1 cfu g-1." *Id.*, 109. Fratamico further teaches that "[t]he percentage of bacteria recovered increased with increasing amounts of beads." *Id.*, 108. Consequently, Fratamico "demonstrates that immunomagnetic separation [using immunomagnetic beads] is a simple and rapid technique" for concentrating a microbe for isolation and detection. *Id.*, 109. These teachings of Fratamico would have motivated a POSA to modify the combination of ICMSF and Gombas and ICMSF and Hughes to use immunomagnetic beads to concentrate microbes in a modular composite sample to benefit from accurate tests.

	Use of immunomagnetic beads was already known in the art for concentrating during testing beef samples. "Tu et al. (78) detected pathogenic E. coli O157:H7 in beef hamburger with the use of IMS and ATP measurement. After 6 h of enrichment at 37°C, inoculated beef samples (0.9 ml each) were filtered to remove large particles prior to 10 min of incubation with ca. 10^5 magnetic beads coated with anti–*E. coli* O157:H7 (Dynabeads, Dynal Inc., Oslo, Norway). Following incubation, the magnetic beads were collected with a Dynal magnetic concentrator and resuspended in 0.9 ml of Trypticase soy broth for ATP analysis." Benoit, 1941. "Hudson et al. (26) used IMS to directly separate and concentrate L. monocytogenes from ham samples prior to detection by PCR. IMS was used on the basis of the commercial availability of anti-Listeria magnetic beads (Dynal Inc.) to avoid time-consuming cultural enrichment steps and to remove PCR inhibitors." Benoit, 1941-42. Cudjoe investigates the use of immunomagnetic beads in pooling methods. In a study designed to evaluate the performance of Dynabeads® Anti-Salmonella, immunomagnetic separation (IMS) followed by plating (IMS-Plating) proved far superior to the conventional ISO *Salmonella* methodology and the Modified Semi-solid Rappaport-Vassiliadis (MSRV) method. *Salmonella* species were isolated and detected from 135 out of the 180 diverse poultry samples by IMS analysis as against 98 by the conventional method and 33 by the MSRV technique. All results were confirmed biochemically and serologically. It appeared from the data generated that some of the salmonellae isolated using IMS were greatly inhibited in Rappaport-Vassiliadis enrichment broth at 42°C and to a lesser extent in selenite cystine broth at 37°C. A moderately selective plating medium like XLD proved to be better in isolating these possible sensitive wild-type strains of salmonellae than the more selective BGA. Cudjoe, Abstract.

	Samples. One hundred and eighty (180) diverse poultry samples, consisting of 40 pools of cloacal/faecal swabs (5 swabs/pool; 5 pools samples/flock) from eight different flocks, 10 raw chicken breast meat samples, 10 pooled egg shell samples, 60 raw chicken liver samples and 60 chicken neck skins sampled from the slaughter line, were used in this study. All samples were obtained from a poultry slaughter and processing plant in the Netherlands. A pool of five swab samples was pre-enriched in 10 ml of buffered peptone water (BPW; Difco Laboratories, Detroit, Michigan, USA) at 37°C for 16–20 h, whilst 25 g of each of the other samples above, were pre-enriched similarly in 225 ml of BPW and examined as detailed below. Cudjoe, 160.
Claim 15	**Disclosure of ICMSF & Gombas**
The method of claim 1, wherein each of the lots is selected from the lot group consisting of a single combo, single bin, single pallet, and a production assemblage.	ICMSF, alone or in combination with Gombas, discloses Claim 1, upon which this claim relies. See above re Claim 1. The specification of the '771 Patent indicates a test lot is a single-unit production lot ('771 Patent, 7:54-56) such as a single combo, bin. pallet, or segment of production ('771 Patent, 7:54-59; *see also* AAPA #3 and #4). ICMSF teaches collecting from "individual production batches as lots" (ICMSF, 119) and "[a] lot is considered as a collection of units." ICMSF, 327. "Operationally-linked" is simply grouping like items, which is also disclosed by ICMSF. "Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119. ICMSF states, "All steps up to the analysis must be controlled . . . to allow reliable traceability." ICMSF, 227.

	ICMSF indicates lot samples are traceable to a particular corresponding lot: "It is therefore helpful . . . to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load). . . . Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119. Gombas also discloses this element. Gombas states "samples were assigned codes" ad product information was recorded for the luncheon meats including "sampling location." Gombas, 563. A POSITA would find Gombas' system for testing individual samples, which composited samples test positive, inherently requires tracking the individual samples that contributed to the composite. *See, e.g.*, Gombas, 564 ("If a composite tested positive, samples in the composite were tested individually by Gene-Trak assay with the PBS from the second mLCA plate or by the BAX assay with the secondary enrichment broths. Individual samples that tested negative were not tested further If an individual sample tested positive, the retained portion was subjected to an enumeration assay.") Thus, Gombas also discloses that each of the separate composited lot samples is attributed to a particular corresponding separate lot.
Claim 16	**Disclosure of ICMSF & Gombas**
The method of claim 1, wherein the number of separate lots is from 3 to 10.	ICMSF, alone or in combination with Gombas, discloses Claim 1, upon which this claim relies. See above re Claim 1.

	ICMSF indicates a consignment may be "composed of several lots." ICMSF, 119-20. "Several" lots includes at least three lots. Additionally, Gombas tests five lots. *See, e.g.*, Gombas, Fig. 1 ("composite five samples"), *id.*, 564 ("up to five like-product samples were composited and screened.").
Claim 17	**Disclosure of ICMSF & Gombas**
The method of claim 1, further comprising, where testing of the pooled modular composite sample is positive, determining which individual separate lots are positive, application of remedial measures to said separate positive lots, and validating by further testing of the respective remediated separate lots and obtaining a negative test result.	ICMSF, alone or in combination with Gombas, discloses Claim 1, upon which this claim relies. See above re Claim 1.  Gombas, 563 (Figure 1 (annotated) showing testing procedures for further testing). A POSITA understands the purpose of testing for pathogens and other organisms in food is to determine if a food is safe for entry into commerce to be sold for consumption. One having ordinary skill in the art understands that if a sample tests positive, a remediation step, like cooking, needs to be completed before it can be

	sold into commerce to comply with USDA guidelines.
Claim 18	**Disclosure of ICMSF & Gombas**
The method of claim 1, wherein, in a), separately collecting said multiple independent samples from each of the multiple separate lots comprises separately collecting from each separate lot a number of samples from 5 to 100.	ICMSF, alone or in combination with Gombas, discloses Claim 1, upon which this claim relies. See above re Claim 1. ICMSF teaches collecting 5, 30, 60, 95, or 100 portions. ICMSF, 184-90, 276, 282, 309, 320, 326-30. Gombas also teaches collecting 25, 50, or 100 samples. "After culling unacceptable samples from luncheon meats and deli salads, 100 samples were selected for testing. For the other product categories, 50 samples were selected for the first several weeks of sampling, and 25 samples were selected each week thereafter for testing." Gombas, 563. *See also* MPEP § 2144.05.
Claim 19	**Disclosure of ICMSF & Gombas**
The method of claim 1 wherein, in a), separately collecting said multiple independent samples from each of the multiple separate lots comprises separately collecting 30 to 60 samples from each separate lot.	ICMSF, alone or in combination with Gombas, discloses Claim 1, upon which this claim relies. See above re Claim 1. ICMSF teaches collecting 5, 30, 60, 95, or 100 portions. ICMSF, 184-90, 276, 282, 309, 320, 326-30. Gombas also teaches collecting 25, 50, or 100 samples. "After culling unacceptable samples from luncheon meats and deli salads, 100 samples were selected for testing. For the other product categories, 50 samples were selected for the first several weeks of sampling, and 25 samples were selected each week thereafter for testing." Gombas, 563. *See also* MPEP § 2144.05.

Claim 20	**Disclosure of ICMSF & Gombas**
The method of claim 1, wherein, in a), separately collecting said multiple independent samples from each of the multiple separate lots comprises separately collecting at least 30 samples from each separate lot.	ICMSF, alone or in combination with Gombas, discloses Claim 1, upon which this claim relies. See above re Claim 1. ICMSF teaches collecting 30, 60, 95, or 100 portions. ICMSF, 184-90, 276, 282, 309, 320, 326-30. Gombas also teaches collecting at least 30 samples. "In a week when < 100 or <25 samples were purchased (owing to product shortages in stores), all acceptable samples were tested." Gombas, 563. *See also* MPEP § 2144.05.
Claim 22	**Disclosure of ICMSF & Gombas**
The method of claim 1, wherein removing portions in step d) is removing equal portions, aseptically, so as to preclude contamination of the enriched separate composited lot samples.	ICMSF, alone or in combination with Gombas, discloses Claim 1, upon which this claim relies. See above re Claim 1. ICMSF describes aseptically withdrawing and weighing sample units into the appropriate diluent. ICMSF, 231, 232. Gombas indicates "a sample was aseptically transferred to a sterile stomacher bag and blended with an equal amount of enrichment broth." Gombas, 563. Gombas further indicates "five 1-ml samples of secondary enrichment broths were composited." *Id.*, 564. Moreover, a POSITA would consider this to be standard practice. A POSITA would have aseptically removed equal volume portions of each enriched separate test lot sample.
Claim 23	**Disclosure of ICMSF & Gombas**

The method of claim 1, wherein the target microbe(s) comprises at least one selected from the group consisting of *Listeria*, *Salmonella* and *Escherichia coli*.	ICMSF, alone or in combination with Gombas, discloses Claim 1, upon which this claim relies. See above re Claim 1. ICMSF describes testing for *Listeria* (ICMSF, 285-312), *Salmonella* (*id.*, 273-84), and *Escherichia coli* (*id.*, 313-32). Gombas focuses on *Listeria*. Gombas, 559.
Claim 24	**Disclosure of ICMSF & Gombas**
The method of claim 1, wherein the target microbe(s) comprises at least one selected from the group consisting of *Listeria* spp., *Listeria monocytogenes*, *Salmonella* spp., *Escherichia coli* O157:H7, coliforms and fecal coliforms.	ICMSF, alone or in combination with Gombas, discloses Claim 1, upon which this claim relies. See above re Claim 1. ICMSF describes testing for *Listeria* (ICMSF, 285-312), *Salmonella* (*id.*, 273-84), and *Escherichia coli* (*id.*, 313-32). Gombas focuses on *Listeria*. Gombas, 559.
Claim 25	**Disclosure of ICMSF & Gombas**
The method of claim 1, wherein the target microbe(s) comprises a spoilage organism.	ICMSF, alone or in combination with Gombas, discloses Claim 1, upon which this claim relies. See above re Claim 1. A POSITA would find it obvious to apply the teachings of ICMSF and Gombas to target spoilage organisms. "Examination of every piece of whole meat is clearly impractical, hence any sampling procedure for estimating shelf-life would involve taking enough samples to give some statistical assurance of the keeping qualities of meat." Gill, 449. Borch is titled "Bacterial spoilage of meat and cured meat products." Borch. The reference discusses spoilage organisms in meat:

	Lactic acid bacteria are the major bacterial group associated with the spoilage of refrigerated vacuum- or MA-packaged cooked, cured meat products (Blickstad and Molin, 1983b; Shaw and Harding, 1989; von Holy et al., 1991). At the time of spoilage some products contain a 'pure' culture of only one species, while in others a mixture of *Lactobacillus* spp. and *Leuconostoc* spp. was found (Kant-Muermans, unpublished results). The great diversity of bacteria isolated from spoiled meat products is confirmed by several studies (Korkeala and Mäkelä, 1989; von Holy et al., 1991; Yang and Ray, 1994). The genus/species of lactic acid bacteria responsible for spoilage, depend on the product composition (product-related flora) as well as the manufacturing site (house-related flora; Korkeala and Mäkelä, 1989). Borch, 113. "Lactic acid bacteria spoil refrigerated meat products by causing defects such as sour off-flavours, discolouration, gas production, slime production and decrease in PH." Borch, 114; *see also, id.*, 115-17 (Section 4 Analysis of Spoilage). It is understood in the art that aerobic plate counts ("APC") can test for spoilage organisms. *See. e.g.*, ICMSF 1986, 122 ("tests to be made (e.g., APC, *Salmonella*, Clostridium perfringens"); *id.* at 132 ("However, aerobic plate counts (APC) can be used to monitor Good Commercial Practice (GCP), and criteria based on such examinations are a valuable aid to in-plant quality control.").

TABLE 19

Sampling plans and recommended microbiological limits for raw meat[a]

Product[b]	Test	Method reference[c]	Case	Plan class	n	c	Limit per cm^2 or gram m	M
Carcass meat, before chilling	APC	118	1	3	5	3	10^5	10^6
Carcass meat, chilled	APC	118	1	3	5	3	10^6	10^7
Edible offal, chilled	APC	118	1	3	5	3	10^6	10^7
Carcass meat, frozen	APC	118	1	3	5	3	5×10^5	10^7
Boneless meat, frozen (beef, veal, pork, mutton)	APC	118	1	3	5	3	5×10^5	10^7
Comminuted meat, frozen	APC	118	1	3	5	3	10^6	10^7
Edible offal, frozen	APC	118	1	3	5	3	5×10^5	10^7

a **Not for use at port of entry** but for in-plant quality control. Refer to Section B for appropriate applications.
b Unfrozen carcasses and primal cuts, swab counts per cm^2; other meats and meat products, per gram.
c Refers to page number in ICMSF, 1978.

ICMSF 1986, 133 (Table 19 (annotated)).

Although for “quality control,” a POSITA would understand that "quality control" means testing for spoilage organisms could also be accomplished by this well-known method. It is disclosed within the prior art during the production lifecycle of beef production. ICMSF 1986, 134 (“The FAOIWHO Working Group (FAQ/WHO, 1979) considered that the only point at which APC could be used for chilled meat, to evaluate the hygienic conditions under which it was produced, was at the slaughterhouse, and because of its perishable nature it would not be possible to set

APC limits for use in guidelines or specifications for chilled meats at port of entry."); *see also, id.*, 153 ("Monitoring for APC, E. coli, and Staph. aureus would give some indication of inadequate processing (heating, curing, smoking), postprocess contamination, or storage at temperatures above those recommended for frozen or refrigerated distribution and storage.")

E. TEST PROCEDURES

(a) *Aerobic plate counts*

Comminute, blend, or stomach the various portions of meat composing the sample unit and withdraw the analytical unit. Combine the swabs to form an analytical unit, or treat each separately. Prepare dilutions and perform the APC test according to the directions given in ICMSF, 1978, p. 118. Spread plates are preferable to pour plates when examining raw meats.

The temperature used for incubation of plates should be related to the material being examined. To estimate numbers of mesophilic contaminants on freshly dressed carcasses it is advisable to incubate plates at 35 or 37°C. For meat that has been held under chilled conditions, plates should be incubated at 25°C to include psychrotrophs and thus give a better indication of the microbial growth that has occurred. Counts at 20°C give similar results after a longer incubation time.

ICMSF 1986, 136 (annotated).

See also, Bhunia 4 ("Bacteria-specific generic rapid methods such ATP-based luminescence and total plate count (APC) are also needed for monitoring cleaning and hygienic practices employed in a processing plant."); Wheeler, 1606 (use of APC in a beef-processing plant); PR/HAACP, 38849 ("Various alternative indicator

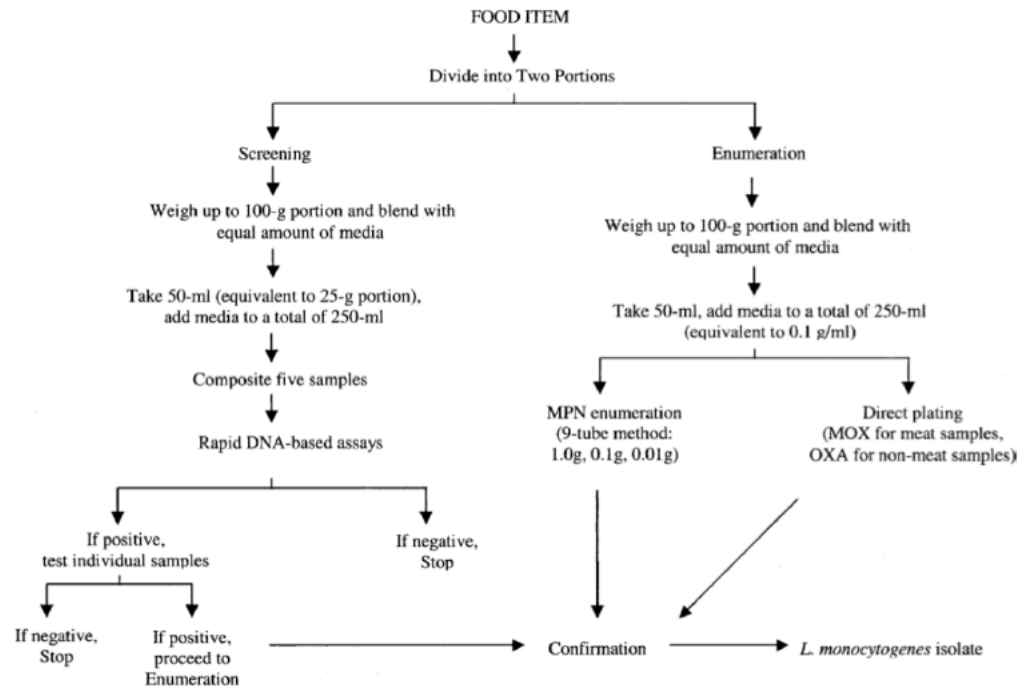
	organisms were suggested, including generic E. coli (biotype I), total plate counts, Enterobacteriaceae, Total Viable Counts (TVC), and Aerobic Plate Counts (APC).”).
Claim 26	**Disclosure of ICMSF & Gombas**
The method of claim 1, wherein removing portions of each separate enriched composited lot sample comprises removing equal volume portions of each separate enriched composited lot sample.	ICMSF, alone or in combination with Gombas, discloses Claim 1, upon which this claim relies. See above re Claim 1. ICMSF describes aseptically withdrawing and weighing sample units into the appropriate diluent. ICMSF, 231, 232. Gombas indicates “a sample was aseptically transferred to a sterile stomacher bag and blended with an equal amount of enrichment broth.” Gombas, 563. Gombas further indicates “five 1-ml samples of secondary enrichment broths were composited.” *Id.*, 564. Moreover, a POSITA would consider this to be standard practice. A POSITA would have aseptically removed equal volume portions of each enriched separate test lot sample.
Claim 27	**Disclosure of ICMSF & Gombas**
The method of claim 1, wherein testing of the pooled modular composite sample, using a suitable detection assay comprises use of at least one of a DNA-based method, an immunochemistry-based method, and a biosensor-based method.	ICMSF, alone or in combination with Gombas, discloses Claim 1, upon which this claim relies. See above re Claim 1. Gombas discloses use of DNA-based assays. Gombas, 559 (abstract).
Claim 28	**Disclosure of ICMSF & Gombas**

The method of claim 1, wherein each of the multiple separate lots are production lots of a meat or poultry product, comprising an assemblage of one or more meat or poultry specimens, respectively.	ICMSF, alone or in combination with Gombas, discloses Claim 1, upon which this claim relies. See above re Claim 1. ICMSF indicates "[t]rimmings to be used for ground beef could be pretested for E. coli O157:H7 by various sampling plans." ICMSF, 323. ICMSF indicates "[r]esearch has validated that up to fifteen 25 g analytical units of ground beef or trimmings can be composited without significant loss in E. coli O157:H7 detection sensitivity." *Id.*, 327 *see also id.*, 226, 314, 320, and 324. ICMSF includes many other references to testing lots of beef or poultry, two of the most common types of meat in commerce. *Id.* at 114 (§ 6.3), 184–191 (§§ 10.2–10.6), 225–226 (§ 12.1), 285 (§ 16.1), 313–330 (Chapter 17). ICMSF is relevant to any kind of food product including beef or poultry. Gombas discloses testing of "luncheon meats." " For example, for the sampling of luncheon meats in California, collectors were instructed to purchase 39 samples of ham, 27 samples of bologna, and 24 samples of poultry from stores on list A and 13 samples of ham, 9 samples of bologna, and 8 samples of poultry from stores on list B. Ham could include products made from pork or poultry (such as turkey) and could include regular low-salt or low-sodium, low-fat, extra-lean, and fat-free varieties. Bologna could include products made from pork, beef, turkey, or mixtures of these meats and could include regular low-salt or low-sodium, low-fat, and fat-free varieties. Turkey or chicken could be smoked or not smoked." Gombas, 562.
Claim 29	**Disclosure of ICMSF & Gombas**
The method of claim 28, wherein the meat or poultry specimens are meat trim pieces or poultry trim pieces, respectively.	ICMSF, alone or in combination with Gombas, discloses Claim 28, upon which this claim relies. See above re Claim 28. ICMSF indicates "[t]rimmings to be used for ground beef could be pretested for E. coli O157:H7 by various sampling plans." ICMSF, 323.

	ICMSF indicates "[r]esearch has validated that up to fifteen 25 g analytical units of ground beef or trimmings can be composited without significant loss in E. coli O157:H7 detection sensitivity." *Id.*, 327 *see also id.*, 226, 314, 320, and 324. ICMSF includes many other references to testing lots of beef or poultry, two of the most common types of meat in commerce. *Id.* at 114 (§ 6.3), 184–191 (§§ 10.2–10.6), 225–226 (§ 12.1), 285 (§ 16.1), 313–330 (Chapter 17). ICMSF is relevant to any kind of food product including beef or poultry.
Claim 30	**Disclosure of ICMSF & Gombas**
The method of claim 28, wherein the meat is that of beef, pork, sheep or bison.	ICMSF, alone or in combination with Gombas, discloses Claim 28, upon which this claim relies. See above re Claim 28. ICMSF indicates "[t]rimmings to be used for ground beef could be pretested for E. coli O157:H7 by various sampling plans." ICMSF, 323. ICMSF indicates "[r]esearch has validated that up to fifteen 25 g analytical units of ground beef or trimmings can be composited without significant loss in E. coli O157:H7 detection sensitivity." *Id.*, 327 *see also id.*, 226, 314, 320, and 324. ICMSF includes many other references to testing lots of beef or poultry, two of the most common types of meat in commerce. *Id.* at 114 (§ 6.3), 184–191 (§§ 10.2–10.6), 225–226 (§ 12.1), 285 (§ 16.1), 313–330 (Chapter 17). ICMSF is relevant to any kind of food product including beef or poultry. Gombas discloses testing of "luncheon meats." " For example, for the sampling of luncheon meats in California, collectors were instructed to purchase 39 samples of ham, 27 samples of bologna, and 24 samples of poultry from stores on list A and 13 samples of ham, 9 samples of bologna, and 8 samples of poultry from stores on list B. Ham could include products made from pork or poultry (such as turkey) and could include regular low-salt or low-sodium, low-fat, extra-lean, and fat-free varieties. Bologna could include products made from pork, beef, turkey, or mixtures

	of these meats and could include regular low-salt or low-sodium, low-fat, and fat-free varieties. Turkey or chicken could be smoked or not smoked." Gombas, 562.
Claim 31	**Disclosure of ICMSF & Gombas**
The method of claim 30, wherein the meat is that of beef.	ICMSF, alone or in combination with Gombas, discloses Claim 30, upon which this claim relies. See above re Claim 30. ICMSF indicates "[t]rimmings to be used for ground beef could be pretested for E. coli O157:H7 by various sampling plans." ICMSF, 323. ICMSF indicates "[r]esearch has validated that up to fifteen 25 g analytical units of ground beef or trimmings can be composited without significant loss in E. coli O157:H7 detection sensitivity." *Id.*, 327 *see also id.*, 226, 314, 320, and 324. ICMSF includes many other references to testing lots of beef or poultry, two of the most common types of meat in commerce. *Id.* at 114 (§ 6.3), 184–191 (§§ 10.2–10.6), 225–226 (§ 12.1), 285 (§ 16.1), 313–330 (Chapter 17). ICMSF is relevant to any kind of food product including beef or poultry.
Claim 37	**Disclosure of ICMSF & Gombas**
[37pre] A method of sampling and testing products for microbe(s) in multiple separate lots, comprising:	To the extent the preamble is limiting, as discussed herein, ICMSF, alone or in combination with Gombas, discloses a method of sampling and testing for microbes in multiple separate test lots. ICMSF and Gombas both disclose a method of sampling. ICMSF provides an example of "[u]sing a sampling plan involving n = 15 (25 g analytical units) and a composite of 375 g." *Id.*, 195 (§ 10.7.2). ICMSF also states "[f]or qualitative analyses, 25 g analytical units are frequently analyzed individually or as composites of 100 g, 200 g, or more." *Id.*, 232 (§ 12.5.2). ICMSF discloses collecting a plurality of samples or sample units (other synonyms

	include portions, specimens, and sub-samples) from lots of beef or poultry. ICMSF describes collecting from a lot or lots: "If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." *Id.*, 119. Additionally, ICMSF indicates a consignment may be "a very large quantity of food" and may be "composed of several lots, representing for example, different days of production from the same plant." *Id.*, 119, 120; *see also* AAPA #3-#5. ICMSF and Gombas both disclose testing for microbes in multiple separate test lots. ICMSF describes testing for *Listeria* (ICMSF, 285-312), *Salmonella* (*id.*, 273-84), and *Escherichia coli* (*id.*, 313-32). Gombas focuses on *Listeria*. Gombas, 559.

	 FIGURE 1. *Detection and enumeration of* L. monocytogenes *in RTE food samples.* Gombas, Figure 1.
[37a] a) separately collecting multiple independent samples from each of multiple separate single production lots, wherein each separate single production lot is separately sampled by taking said multiple independent samples thereof;	"Many different types of samples are collected and submitted to the laboratory for analysis. Some are sample units from lots or consignments of food or ingredients for lot acceptance determination. . . . A sample unit is a small portion of the lot in which the distribution of both numbers and types of microorganisms should be representative of the whole lot." ICMSF, 255. Thus, ICMSF discloses collecting multiple samples or sample units (other synonyms include portions, specimens, and sub-samples). ICMSF describes collecting from a lot or lots:

	"If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." *Id.*, 119. Additionally, ICMSF indicates a consignment may be "a very large quantity of food" and may be "composed of several lots, representing for example, different days of production from the same plant." *Id.*, 119, 120; *see also* Applicant Admitted Prior Art ("AAPA") #3-#5.

6.11 WHAT IS A REPRESENTATIVE SAMPLE?

A *representative sample* reflects, as far as is possible, the composition of the lot from which it is drawn.

How then should a representative sample be drawn? It is important to avoid bias and to draw a sufficient number of sample units to confidently make a judgment about a lot. Sampling at random is the universally recognized way of avoiding bias and is more reliable than consciously trying to draw the sample units from the various parts of a lot. The units (cartons or containers, particular weights of solid, or volumes of liquid) are drawn by using random numbers. There is, of course, no guarantee that a random sample has characteristics identical with those of the lot, but the randomness of sampling is the basis for calculation of the probability that a sample will give a biased result.

It is also possible, and may be desirable, to use a *stratified random sampling* approach, drawing a random sample of a given number of sample units from each *stratum* (e.g., each sublot or carton). The distribution of sample units on strata should correspond to the distribution of lot units on strata. This means that if each stratum contains the same quantity as every other one, the number of sample units per stratum should be the same. Otherwise, they should differ just as the proportions of the lot contained in the strata differ. Stratification is a device for handling known sources of variation and may be used where one has prior knowledge that the consignment is potentially not of uniform quality. A consignment might not be of uniform quality if portions of it are shipped in different vehicles or in different holds of a vessel, or if it is known that it is really composed of several lots representing, for example, different days of production from the same plant or different plants of the same company. The results for different strata should be assessed separately and then pooled if they appear to be homogeneous.

The calculation of acceptance probabilities is based on the assumption that random samples have been drawn and analyzed. Where physical or practical constraints prevent random sampling, such calculated probabilities may not be valid. Their reliability depends on how close reality approaches the ideal situation of random sampling. This fact should be borne in mind when subsequently interpreting results.

ICMSF, 119-20 (annotated).

6.10 WHAT IS A LOT?

Ideally, a lot is a quantity of food or food units produced and handled under uniform conditions. Under certain circumstances, it has been shown that the logarithms of the counts from a batch of food are likely to be normally distributed (Baird-Parker, 1980; Kilsby & Baird-Parker, 1983 [their Table 2]; Ingram & Roberts, 1976). The implication is that there is homogeneity within a lot, which, when considering microbial levels and distributions, rarely occurs in practice. In most instances, the distribution of microorganisms within lots of food is heterogeneous. Even in food factories, this heterogeneity makes the interpretation of sample results difficult, and it becomes even more so in circumstances where a lot is less well defined, such as in a consignment of a very large quantity of food.

It is therefore helpful if suppliers can be persuaded to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load).

If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment.

A lot should be composed of food produced with as little variation as possible for a given process or commodity. Because of the uncertainties in identifying a lot commercially, however, the use of the word *lot* in this book is usually in its statistical sense, as a collection of units of a product, the acceptability of which is determined by examining a sample drawn from it.

ICMSF, 118-19 (annotated).

	The ICMSF has defined a lot as follows: In the commercial sense, a lot is a quantity of food supposedly produced under identical conditions, all packages of which would normally bear a lot number that identifies the production during a particular time interval, and usually from a particular line, retort, or other critical processing unit. Statistically, a lot is considered as a collection of units of a product from which a sample is drawn to determine acceptability of the lot (ICMSF, 1986). In this example, the maximum size of a lot consists of all the ground beef produced on common equipment from cleanup to cleanup. Smaller lots could be established (e.g., hourly). ICMSF, 327 (annotated). This definition also reflects the common practice referred to in the IEH Patents at Example II, wherein "[p]referably, the producer defines and produces these single-unit lots in such a fashion as to maximize the information available." '771 Patent,13:13–15. This corresponds with the specification of IEH's Patents which reflects the common practice of producers defining what constitutes a lot grouped for traceability purposes. '771 Patent, 2:15-67, 5:43-54 (AAPA #3), and 12:46-49. Thus, ICMSF discloses collecting multiple independent samples. ICMSF describes a lot as "a collection of units of a product." IMCSF, 119. ICMSF further indicates a lot has been defined as: "a quantity of food supposedly produced under identical conditions, all packages of which would normally bear a lot number that identifies the production during a particular time interval, and usually from a particular line, retort, or other critical processing unit. Statistically, a lot is considered as a collection of units of a product from which a sample is drawn to determine acceptability of the lot." ICMSF, 327. ICMSF advocates use of a "statistically based sampling plan." *Id.*, 113-15, 119-21, 320, 327, 328. In the sampling plans, "*n* is the number of sample units chosen separately and independently, and . . . *c* is the maximum allowable number of sample

	units yielding a positive result." *Id.*, 115. ICMSF discloses a wide range for the number of sample units (portions) for a particular microbial agent or organism. For example, ICMSF discusses collecting 15, 30, 60 or 100 sample units from a lot. *Id.*, 320, 326. **6.5 THE SAMPLING PLAN** The sample units so drawn will, after examination, yield results that will be compared with certain criteria to reach a decision as to whether the entire lot should be accepted or rejected (see section 6.9 for explanation of rejection). The particular choice of sampling procedure and the decision criteria make up the *sampling plan*. Proper drawing of samples is essential if the decision making criteria of the sampling plan are to give unbiased results. By sampling randomly, we are able to reduce the risk of making biased decisions. A simple example of a sampling plan follows. Take 10 sample units of a food from a lot and subject them to appropriate laboratory procedure to test for the presence or absence of a microorganism. If two or fewer of the 10 sample units show presence of the organism, that is, give a positive result, then the whole lot of food is acceptable (relative to this organism). But if three or more give a positive result, the whole lot is to be rejected. This plan is described by the use of two terms $n = 10$ and $c = 2$, where n is the number of sample units chosen separately and independently, and where c is the maximum allowable number of sample units yielding a positive result. ICMSF, 115 (annotated).

	6.1 INTRODUCTION It is important to recognize that the management of food safety using the approaches outlined in the first five chapters, based on controlling hazards through Good Hygienic Practices (GHP) and Hazard Analysis Critical Control Point (HACCP), is much more effective than trying to ensure safety by lot acceptance through end-product testing. This chapter discusses the concepts of probability and sampling that determine the limitations of end-product testing. Those concepts form the basis of the design of statistically based sampling plans (Chapter 7) and the establishment of cases (Chapter 8). In Chapters 6 and 7, it is assumed that the microbiological test will give an absolutely accurate result, with no false positives or false negatives. This is not a realistic assumption, and problems arising from sampling, sample transport and preparation for analysis, and the imprecision of microbiological methods are addressed in Chapters 10 and 12 and elsewhere in the text. ICMSF, 113 (annotated); *see also id.*, 115–21 (§§ 6.4–6.13), 319–20 (§ 17.2.3.3), and 326–30 (§§ 17.5.3.1–17.6). ICMSF discusses the number of portions to be taken sufficient for validation of a particular microorganism including *Salmonella* (*id.*, 276 (§ 15.3.3); *see also id.*, 282 (§ 15.6.3)); *Listeria* (*id.*, 309 (§ 16.6.3)), and *E. coli* (*id.*, 318–21 (§§ 17.2.3.2–17.2.3.4), 326 (§ 17.5.3.1)). IEH's AAPA discloses statistical-based sampling plans taking 10-60 sample units. '771 Patent, 6:12-29; *see* AAPA #1, #2, #5, and #6.

	It is therefore helpful if suppliers can be persuaded to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load). If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment. ICMSF, 119 (annotated).
[37b] b) separately compositing the collected multiple independent samples from each of the separate lots to provide a corresponding set of separate composited single production lot samples, wherein each of the separate composited single production lot samples is attributed to a particular corresponding separate single production lot;	ICMSF states: "To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units of the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. . . . This finding suggests that considerable reductions may be made in the cost of laboratory analysis by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, n, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort."

	To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. A laboratory investigation undertaken for ICMSF (Silliker & Gabis, 1973) indicated that different sized analytical units drawn from a single well-mixed sample unit of dried food give very similar results. A subsequent investigation (Gabis & Silliker, 1974) using foods of high moisture content (including eggs, poultry meat, meat, and meat products) showed comparable reliability in the use of composite analytical units; for example, examination of three composites of 20 × 25 g analytical units gave the same result as examination of all 60 analytical units individually (the total amount examined being 1500 g in both instances). This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n*, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort. ICMSF, 187.

10.5.1 Conceivable Alternative Plans

As indicated above, for an infectious agent such as *Salmonella*, there are strong reasons for adopting sampling plans in which $c = 0$:

1. It is philosophically objectionable to some people to accept a lot containing a recognized pathogen, regardless of how the food is to be used in a manufacturing process or by the consumer.
2. There is the very great technical advantage of combining many analytical units for bacteriological examination in systems with $c = 0$, because a single positive decides the outcome.
3. The plan with $c = 1$ (or more) would always require the examination of (many) more analytical units, for equal probability of acceptance, than a plan with $c = 0$.
4. Even if the indicated probabilities of plans with $c = 0$ versus $c = 1$ are the same, their implications are not. For example, when a lot is accepted with the plan $n = 60$, $c = 0$, it is possible (though not certain) that the lot may not be contaminated at all. But using the plan $n = 95$, $c = 1$ and accepting one positive, it is known that the lot certainly is contaminated (though the probability that a lot with 5% of analytical units contaminated would be accepted is in both instances $P_a = 0.05$).

ICMSF, 188 (annotated). ICMSF also provides an example of "[u]sing a sampling plan involving n = 15 (25 g analytical units) and a composite of 375 g." *Id.* at 195 (§ 10.7.2). ICMSF also states "[f]or qualitative analyses, 25 g analytical units are frequently analyzed individually or as composites of 100 g, 200 g, or more." *Id.* at 232 (§ 12.5.2).

The ICMSF incorporates two references, Silliker & Gabis (1973) and Gabis & Silliker (1974). Silliker notes that "[c]ompositing of multiple samples (lumping) and wet compositing of pre-enrichment cultures have been tested as economical approaches to *Salmonella* control." Silliker, 475.

Silliker further notes that "[b]oth approaches are being used widely by the food industry in the United States." *Id.*.

	To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. A laboratory investigation undertaken for ICMSF (Silliker & Gabis, 1973) indicated that different sized analytical units drawn from a single well-mixed sample unit of dried food give very similar results. A subsequent investigation (Gabis & Silliker, 1974) using foods of high moisture content (including eggs, poultry meat, meat, and meat products) showed comparable reliability in the use of composite analytical units; for example, examination of three composites of 20 × 25 g analytical units gave the same result as examination of all 60 analytical units individually (the total amount examined being 1500 g in both instances). This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n*, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort. "There is the very great technical advantage of combining many analytical units for bacteriological examination." ICMSF, 188. ICMSF also describes composites prepared by combining "15 (25 g analytical units)" and "composites of 100 g, 200 g, or more." *Id.,* 195, 232; *see* AAPA #5.
[37c] c) enriching of the separate composited single production lot samples to provide a set of separate composited single production lot samples enriched for the target microbe(s);	ICMSF states: To reduce laboratory effort . . . it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom**,** using plans in which the presence of a single positive rejects the consignment. ICMSF, 187. Also, ICMSF describes preparing composites of analytical units then diluting "for qualitative (enrichment)" analysis. *Id.,* 231-32; *see also* AAPA #5, #7. For practical reasons, however, analytical units of 10 g up to 50 g are typical for quantitative methods and are diluted 1:10. For qualitative analyses, 25 g analytical units are frequently analyzed individually or as composites of 100 g, 200 g, or more.

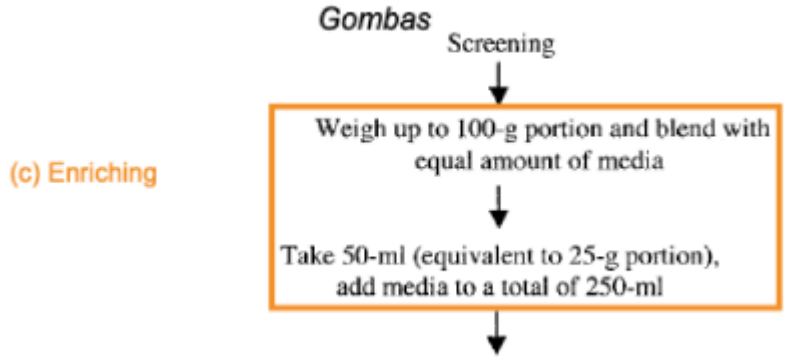
ICMSF, 232 (annotated).

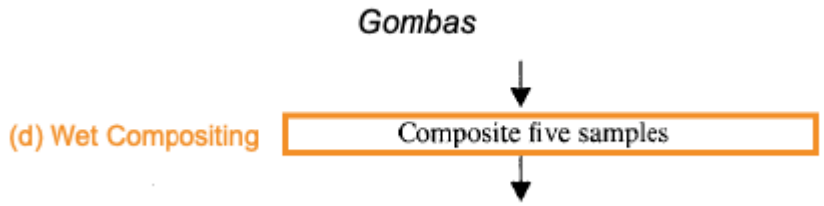
> Preparation of the first dilution, either for qualitative (enrichment) or quantitative (direct) analyses, can have a marked impact on the microbial population in the sample. This is often due to changes in the physico-chemical characteristics of the suspension as compared to the food matrix. Where the food does not confer protection, the composition of the diluent is important to prevent or minimize shock/injury to the microorganisms that can result from rapid changes in the ionic concentrations.

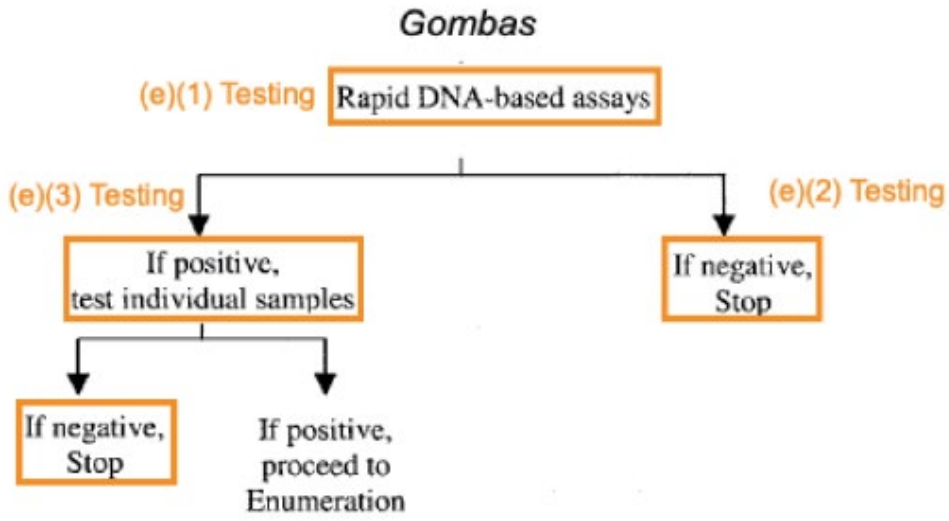
ICMSF, 232 (annotated).

ICMSF also describes strategic use of non-selective enrichment to resuscitate microorganisms and selective enrichments for growing microorganisms. *Id.*, 232–33 (§ 12.6); *see e.g.*, *id.,* 233 ("use of nonselective medium . . . to enhance recovery of injured or stressed cells."). ICMSF, § 12.6 also describes optimizing the incubation period. S*ee e.g.*, *id.,* 232.

It was well known long before the priority date to use non-selective enrichments after dry compositing and prior to wet compositing as evidenced by Price, Silliker, Gabis, and MLG. A POSITA would also know to use selective enrichments after dry compositing and prior to wet compositing as disclosed by ICMSF and Gombas.



	Gombas, 563 (Figure 1 (annotated) showing an enriching step).
[37d] d) removing portions of each separate enriched composited single production lot sample, and combining the removed portions to provide a pooled modular composite sample; and	ICMSF describes compositing the enriched lot samples: "it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. . . . This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n,* with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort." ICMSF, 187. AAPA #5-#7. Additionally, Gombas discloses wet compositing of enriched samples: "Up to five like-product samples were composited and screened. . . . For California samples, five 1-ml samples of secondary enrichment broths were composited." Gombas, 564. Gombas's Figure 1 depicts wet compositing as shown in the excerpt below:  Gombas, 563 (Figure 1 (annotated) showing a wet compositing step).

[37e.1] e) testing of the pooled modular composite sample, using a suitable detection assay, for the target microbe(s),	ICMSF describes testing enrichment cultures of composite samples with suitable detection assays (ICMSF, 187) and "presence/absence tests" (ICMSF, 233). Suitable detection assays were established in Silliker and Gabis as discussed in ICMSF. Gombas provides a complete framework for testing, and a POSITA would have been motivated to modify ICMSF based on Gombas's framework:  Gombas, 563 (Figure 1 (annotated) showing the testing step). Gombas also discloses a suitable detection assay: "For California samples, . . . the 5-ml composite was tested by the BAX assay according to the manufacturer's procedures." Gombas, 564. These assay types were "art recognized." '771 Patent (AAPA #7).
[35e.2] wherein when such testing is negative all of said samples that were composited to form the separate composited single	ICMSF describes correlating lots in a consignment with lot samples so a negative or positive test result enables a lot to be validated or rejected. ICMSF, 118-19. ICMSF describes sampling plans in which "c is the maximum allowable number of

production lot samples are deemed negative for the target microbe(s) and each of the multiple separate single production lots is validated, and	sample units yielding a positive result." ICMSF, 115. For many scenarios, the requirement is c = 0, which indicates the testing is negative. *Id.*, 118, 183-90, 309, 325, 326. A negative test result (*id.*, 113-15, 325, 326) is the expected and desired outcome to determine meat is not contaminated and is safe for consumption (*id.*, 184-89) such that a lot is validated and it is "commercially possible to market" (*id.*, 185) the meat.

6.10 WHAT IS A LOT?

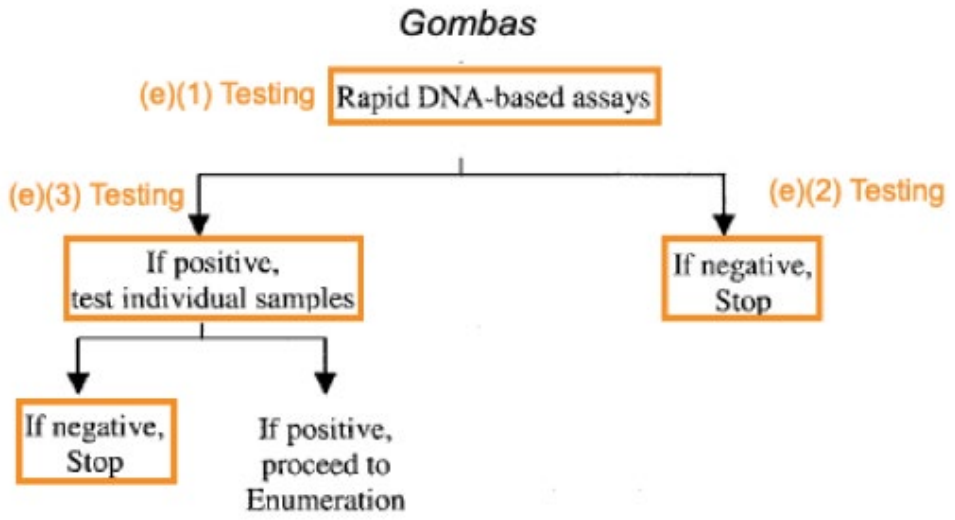
Ideally, a lot is a quantity of food or food units produced and handled under uniform conditions. Under certain circumstances, it has been shown that the logarithms of the counts from a batch of food are likely to be normally distributed (Baird-Parker, 1980; Kilsby & Baird-Parker, 1983 [their Table 2]; Ingram & Roberts, 1976). The implication is that there is homogeneity within a lot, which, when considering microbial levels and distributions, rarely occurs in practice. In most instances, the distribution of microorganisms within lots of food is heterogeneous. Even in food factories, this heterogeneity makes the interpretation of sample results difficult, and it becomes even more so in circumstances where a lot is less well defined, such as in a consignment of a very large quantity of food.

It is therefore helpful if suppliers can be persuaded to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load).

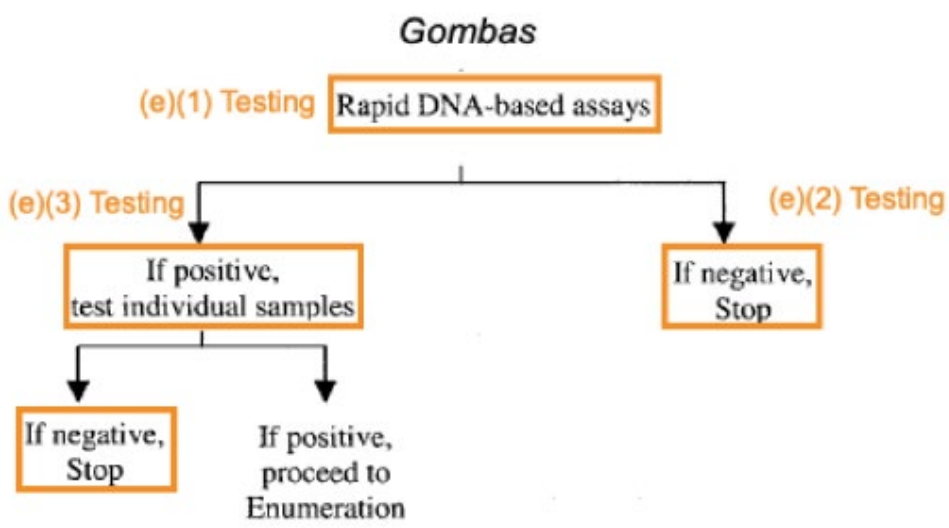
If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment.

A lot should be composed of food produced with as little variation as possible for a given process or commodity. Because of the uncertainties in identifying a lot commercially, however, the use of the word *lot* in this book is usually in its statistical sense, as a collection of units of a product, the acceptability of which is determined by examining a sample drawn from it.

ICMSF, 118-19 (annotated).

	To the extent ICMSF is considered not to disclose some aspect of step (e)(2), Gombas indicates a negative test result is the end of the process: "If a composite tested negative, all samples in the composite were considered negative (in 25 g) and were not tested further." Gombas, 564. A POSITA would find that in the combination, a negative test result equates to being validated.  Gombas, 563 (Figure 1 (annotated) showing "if negative, stop.").
[35e.3] wherein when such testing is positive, each of the individual separate composited single production lot samples that were used to form the pooled modular composite	The combination of ICMSF and Gombas describes retaining a portion of the enriched lot samples (steps (b)-(d)). "Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more

sample are individually tested to determine which of the separate composited single production lot samples is positive for the target microbe(s),	analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119. "There is often a desire to retest retained samples." ICMSF, 191. If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment. ICMSF, 119 (annotated). ICMSF discloses validating for release into commerce. ICMSF, 113–15 (§§ 6.2–6.6), 118–19 (§§ 6.9–6.10), 184–85 (§ 10.2), 325–27 (§§ 17.5.3–17.5.3.4); *see also id.*, 186–89 (§ 10.4–10.6)) according to a statistically-based sampling plan (*id.*, 113 (§ 6.1); *see also id.*, 115–18 (§§ 6.4–6.8), 184 (§ 10.2), 319–21 (§§ 17.2.3.3–17.2.3.4), 326–30 (§§ 17.5.3.1–17.6), as described regarding steps (a) and (e)(2). "If a composite tested positive, samples in the composite were tested individually . . . by the BAX assay with the secondary enrichment broths. Individual samples that tested negative were not tested further." Gombas, 564.

	 Gombas, 563 (Figure 1 (annotated) showing testing procedures for further testing). "Individual samples that tested negative were not tested further. If an individual sample tested positive, the retained portion was subjected to an enumeration assay." Gombas, 564. The combination teaches that after the composited samples test positive, then the individual samples used to form the composited sample are individually tested with the same protocol to determine which of the separate composited lot samples is positive for the target microbe(s).
[35e.4] wherein the single production lots corresponding to any negatively testing composited single production lot samples are validated.	ICMSF describes correlating lots in a consignment with lot samples so a negative or positive test result enables a lot to be validated or rejected. ICMSF, 118-19. ICMSF describes sampling plans in which "c is the maximum allowable number of sample units yielding a positive result." ICMSF, 115. For many scenarios, the requirement is c = 0, which indicates the testing is negative. *Id.*, 118, 183-90, 309, 325, 326. A negative test result (*id.*, 113-15, 325, 326) is the expected and desired

	outcome to determine meat is not contaminated and is safe for consumption (*id.*, 184-89) such that a lot is validated and it is "commercially possible to market" (*id.*, 185) the meat.

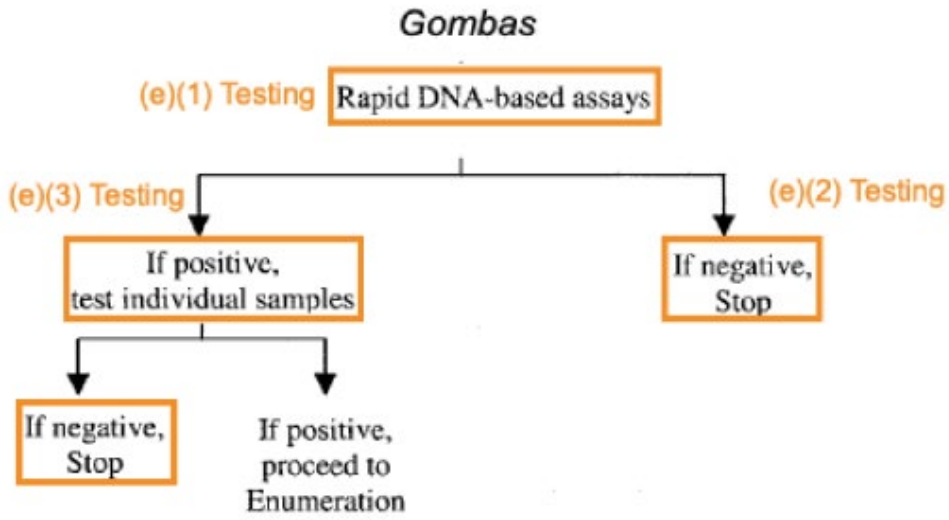
6.10 WHAT IS A LOT?

Ideally, a lot is a quantity of food or food units produced and handled under uniform conditions. Under certain circumstances, it has been shown that the logarithms of the counts from a batch of food are likely to be normally distributed (Baird-Parker, 1980; Kilsby & Baird-Parker, 1983 [their Table 2]; Ingram & Roberts, 1976). The implication is that there is homogeneity within a lot, which, when considering microbial levels and distributions, rarely occurs in practice. In most instances, the distribution of microorganisms within lots of food is heterogeneous. Even in food factories, this heterogeneity makes the interpretation of sample results difficult, and it becomes even more so in circumstances where a lot is less well defined, such as in a consignment of a very large quantity of food.

It is therefore helpful if suppliers can be persuaded to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load).

If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment.

A lot should be composed of food produced with as little variation as possible for a given process or commodity. Because of the uncertainties in identifying a lot commercially, however, the use of the word *lot* in this book is usually in its statistical sense, as a collection of units of a product, the acceptability of which is determined by examining a sample drawn from it.

	ICMSF, 118-19 (annotated). To the extent ICMSF is considered not to disclose some aspect of step (e)(2), Gombas indicates a negative test result is the end of the process: "If a composite tested negative, all samples in the composite were considered negative (in 25 g) and were not tested further." Gombas, 564. A POSITA would find that in the combination, a negative test result equates to being validated.  Gombas, 563 (Figure 1 (annotated) showing "if negative, stop.").
Claim 38	**Disclosure of ICMSF & Gombas**

The method of claim 37, wherein removing portions in step d) is removing equal portions, aseptically, so as to preclude contamination of the enriched separate composited single production lot samples.	ICMSF, alone or in combination with Gombas, discloses Claim 37, upon which this claim relies. See above re Claim 37. ICMSF describes aseptically withdrawing and weighing sample units into the appropriate diluent. ICMSF, 231, 232. Gombas indicates "a sample was aseptically transferred to a sterile stomacher bag and blended with an equal amount of enrichment broth." Gombas, 563. Gombas further indicates "five 1-ml samples of secondary enrichment broths were composited." *Id.*, 564. Moreover, a POSITA would consider this to be standard practice. A POSITA would have aseptically removed equal volume portions of each enriched separate test lot sample.
Claim 41	**Disclosure of ICMSF & Gombas**
The method of claim 37, wherein each of the multiple separate single production lots are separate single production lots of a meat or poultry product, comprising an assemblage of one or more meat or poultry specimens, respectively.	ICMSF, alone or in combination with Gombas, discloses Claim 37, upon which this claim relies. See above re Claim 37. ICMSF indicates "[t]rimmings to be used for ground beef could be pretested for E. coli O157:H7 by various sampling plans." ICMSF, 323. ICMSF indicates "[r]esearch has validated that up to fifteen 25 g analytical units of ground beef or trimmings can be composited without significant loss in E. coli O157:H7 detection sensitivity." *Id.*, 327 *see also id.*, 226, 314, 320, and 324. ICMSF includes many other references to testing lots of beef or poultry, two of the most common types of meat in commerce. *Id.* at 114 (§ 6.3), 184–191 (§§ 10.2–10.6), 225–226 (§ 12.1), 285 (§ 16.1), 313–330 (Chapter 17). ICMSF is relevant to any kind of food product including beef or poultry.

	Gombas discloses testing of "luncheon meats." " For example, for the sampling of luncheon meats in California, collectors were instructed to purchase 39 samples of ham, 27 samples of bologna, and 24 samples of poultry from stores on list A and 13 samples of ham, 9 samples of bologna, and 8 samples of poultry from stores on list B. Ham could include products made from pork or poultry (such as turkey) and could include regular low-salt or low-sodium, low-fat, extra-lean, and fat-free varieties. Bologna could include products made from pork, beef, turkey, or mixtures of these meats and could include regular low-salt or low-sodium, low-fat, and fat-free varieties. Turkey or chicken could be smoked or not smoked." Gombas, 562.
Claim 42	**Disclosure of ICMSF & Gombas**
The method of claim 41, wherein the meat or poultry specimens are meat trim pieces or poultry trim pieces, respectively.	ICMSF, alone or in combination with Gombas, discloses Claim 41, upon which this claim relies. See above re Claim 41. ICMSF indicates "[t]rimmings to be used for ground beef could be pretested for E. coli O157:H7 by various sampling plans." ICMSF, 323. ICMSF indicates "[r]esearch has validated that up to fifteen 25 g analytical units of ground beef or trimmings can be composited without significant loss in E. coli O157:H7 detection sensitivity." *Id.*, 327 *see also id.*, 226, 314, 320, and 324. ICMSF includes many other references to testing lots of beef or poultry, two of the most common types of meat in commerce. *Id.* at 114 (§ 6.3), 184–191 (§§ 10.2–10.6), 225–226 (§ 12.1), 285 (§ 16.1), 313–330 (Chapter 17). ICMSF is relevant to any kind of food product including beef or poultry.
Claim 43	**Disclosure of ICMSF & Gombas**
The method of claim 42, wherein the meat is that of beef, pork, sheep, or bison.	ICMSF, alone or in combination with Gombas, discloses Claim 42, upon which this claim relies. See above re Claim 42.

	ICMSF indicates "[t]rimmings to be used for ground beef could be pretested for E. coli O157:H7 by various sampling plans." ICMSF, 323. ICMSF indicates "[r]esearch has validated that up to fifteen 25 g analytical units of ground beef or trimmings can be composited without significant loss in E. coli O157:H7 detection sensitivity." *Id.*, 327 *see also id.*, 226, 314, 320, and 324. ICMSF includes many other references to testing lots of beef or poultry, two of the most common types of meat in commerce. *Id.* at 114 (§ 6.3), 184–191 (§§ 10.2–10.6), 225–226 (§ 12.1), 285 (§ 16.1), 313–330 (Chapter 17). ICMSF is relevant to any kind of food product including beef or poultry. Gombas discloses testing of "luncheon meats." " For example, for the sampling of luncheon meats in California, collectors were instructed to purchase 39 samples of ham, 27 samples of bologna, and 24 samples of poultry from stores on list A and 13 samples of ham, 9 samples of bologna, and 8 samples of poultry from stores on list B. Ham could include products made from pork or poultry (such as turkey) and could include regular low-salt or low-sodium, low-fat, extra-lean, and fat-free varieties. Bologna could include products made from pork, beef, turkey, or mixtures of these meats and could include regular low-salt or low-sodium, low-fat, and fat-free varieties. Turkey or chicken could be smoked or not smoked." Gombas, 562.
Claim 44	**Disclosure of ICMSF & Gombas**
The method of claim 43, wherein the meat is that of beef.	ICMSF, alone or in combination with Gombas, discloses Claim 43, upon which this claim relies. See above re Claim 43. ICMSF indicates "[t]rimmings to be used for ground beef could be pretested for E. coli O157:H7 by various sampling plans." ICMSF, 323. ICMSF indicates "[r]esearch has validated that up to fifteen 25 g analytical units of ground beef or trimmings can be composited without significant loss in E. coli O157:H7 detection sensitivity." *Id.*, 327 *see also id.*, 226, 314, 320, and 324.

	ICMSF includes many other references to testing lots of beef or poultry, two of the most common types of meat in commerce. *Id.* at 114 (§ 6.3), 184–191 (§§ 10.2–10.6), 225–226 (§ 12.1), 285 (§ 16.1), 313–330 (Chapter 17). ICMSF is relevant to any kind of food product including beef or poultry. Gombas discloses testing of "luncheon meats." " For example, for the sampling of luncheon meats in California, collectors were instructed to purchase 39 samples of ham, 27 samples of bologna, and 24 samples of poultry from stores on list A and 13 samples of ham, 9 samples of bologna, and 8 samples of poultry from stores on list B. Ham could include products made from pork or poultry (such as turkey) and could include regular low-salt or low-sodium, low-fat, extra-lean, and fat-free varieties. Bologna could include products made from pork, beef, turkey, or mixtures of these meats and could include regular low-salt or low-sodium, low-fat, and fat-free varieties. Turkey or chicken could be smoked or not smoked." Gombas, 562.
Claim 48	**Disclosure of ICMSF & Gombas**
The method of claim 37, wherein the target microbe(s) comprises at least one selected from the group consisting of *Listeria*, *Salmonella*, and *Escherichia coli*.	ICMSF, alone or in combination with Gombas, discloses Claim 37, upon which this claim relies. See above re Claim 37. ICMSF describes testing for *Listeria* (ICMSF, 285-312), *Salmonella* (*id.*, 273-84), and *Escherichia coli* (*id.*, 313-32). Gombas focuses on *Listeria*. Gombas, 559.
Claim 49	**Disclosure of ICMSF & Gombas**
The method of claim 48, wherein the target microbe(s) comprises at least one selected from the group consisting of *Listeria* spp., *Listeria monocytogenes*, *Salmonella* spp.,	ICMSF, alone or in combination with Gombas, discloses Claim 48, upon which this claim relies. See above re Claim 48. ICMSF describes testing for *Listeria* (ICMSF, 285-312), *Salmonella* (*id.*, 273-84), and *Escherichia coli* (*id.*, 313-32).

Escherichia coli O157:H7, coliforms, and fecal coliforms.	Gombas focuses on *Listeria*. Gombas, 559.

EXHIBIT B2

Invalidity of U.S. Patent No. 9,637,771 in light of ICMSF and Hughes

National Beef denies that any of its microbiological testing methods infringe any claim of U.S. Patent No. U.S. Patent No. 9,637,771 ("the '771 Patent"). If the claims of the '771 Patent were construed so broadly as to cover the Pooled Testing Methods, they would be invalid at least in view of INT'L COMM'N ON MICROBIOLOGICAL SPEC'S FOR FOODS (ICMSF), MICROORGANISMS IN FOODS 7: MICROBIOLOGICAL TESTING IN FOOD SAFETY MANAGEMENT (Kluwer Academic/Plenum Publishers, 2002) ("ICMSF") and Denise Hughes et al., *Salmonella in Foods—A New Enrichment Procedure for Use with the TECRA Salmonella Visual Immunoassay: Collaborative Study*, 82 J. AOAC INT'L 634–647 (1999) ("Hughes"), which anticipate and/or disclose the limitations of the '771 Patent under at least pre-AIA 35 U.S.C. § 102(a), pre-AIA 35 U.S.C. § 102(b), pre-AIA 35 U.S.C. §§ 102(f)/(g), and/or pre-AIA 35 U.S.C. § 103. To the extent that ICMSF and/or Hughes do not expressly disclose one or more limitations of the asserted claims, as they would have to be construed to support IEH's claim of infringement, they disclose the limitations alone or in combination with one or more additional prior art references and/or the knowledge of one of ordinary skill in the art.

Claim 1	**Disclosure of ICMSF and Hughes**
[1pre] A method of sampling and testing for microbes in multiple separate test lots, comprising:	To the extent the preamble is limiting, as discussed herein, ICMSF, alone or in combination with Hughes, discloses a method of sampling and testing for microbes in multiple separate test lots. ICMSF includes many other references to testing lots of beef or poultry, two of the most common types of meat in commerce. *Id.*, 114 (§ 6.3), 184–191 (§§ 10.2–10.6), 225–226 (§ 12.1), 285 (§ 16.1), 313–330 (Chapter 17). ICMSF is relevant to any kind of food product including beef. Hughes discusses turkey (Hughes, Abstract), and a POSITA would be motivated to combine the teachings of ICMSF and Hughes as they both test meat for release into commerce. Hughes discloses testing turkey for *Salmonella*. "Three food types (. . . and raw turkey) were analyzed in the United States." Hughes, 634, 645, Table 8.

	Table 8 shows results from 12 collaborators for naturally contaminated raw turkey. For the 180 samples tested, using confirmed data, there was one false negative at the low level for the TECRA method. There were 3 false negatives at the low level and 3 false negatives at the high level for the cultural method. The incidence of false-positive results was very high for the cultural method, with a total of 41 false positives, compared with 5 for the TECRA method. It should, however, be taken into account that the comparison was with the method in BAM 7th Ed., not the current 8th Ed. The method for raw flesh food in the BAM 8th Ed. uses elevated temperature with RV and TT broth, which would be more selective. No significant Hughes, 646. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Moreover, the recitation of a lot being validated for "release . . . into commerce" is not limiting. Prosecution History for Reexamination Control No. 95/011,294 .
[1a] a) separately collecting multiple independent samples from each of multiple separate lots, wherein each separate lot is separately sampled by taking said multiple independent samples thereof;	ICMSF, alone or in combination with Hughes, discloses separately collecting multiple independent samples from each of multiple separate lots, wherein each separate lot is separately sampled by taking said multiple independent samples thereof. ICMSF describes collecting from a lot or lots:

	"If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119.

6.10 WHAT IS A LOT?

Ideally, a lot is a quantity of food or food units produced and handled under uniform conditions. Under certain circumstances, it has been shown that the logarithms of the counts from a batch of food are likely to be normally distributed (Baird-Parker, 1980; Kilsby & Baird-Parker, 1983 [their Table 2]; Ingram & Roberts, 1976). The implication is that there is homogeneity within a lot, which, when considering microbial levels and distributions, rarely occurs in practice. In most instances, the distribution of microorganisms within lots of food is heterogeneous. Even in food factories, this heterogeneity makes the interpretation of sample results difficult, and it becomes even more so in circumstances where a lot is less well defined, such as in a consignment of a very large quantity of food.

It is therefore helpful if suppliers can be persuaded to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load).

If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment.

A lot should be composed of food produced with as little variation as possible for a given process or commodity. Because of the uncertainties in identifying a lot commercially, however, the use of the word *lot* in this book is usually in its statistical sense, as a collection of units of a product, the acceptability of which is determined by examining a sample drawn from it.

ICMSF, 118-19 (annotated).

	The ICMSF has defined a lot as follows: In the commercial sense, a lot is a quantity of food supposedly produced under identical conditions, all packages of which would normally bear a lot number that identifies the production during a particular time interval, and usually from a particular line, retort, or other critical processing unit. Statistically, a lot is considered as a collection of units of a product from which a sample is drawn to determine acceptability of the lot (ICMSF, 1986). In this example, the maximum size of a lot consists of all the ground beef produced on common equipment from cleanup to cleanup. Smaller lots could be established (e.g., hourly). ICMSF, 327 (annotated). This definition also reflects the common practice referred to in the IEH Patents at Example II, wherein "[p]referably, the producer defines and produces these single-unit lots in such a fashion as to maximize the information available." '771 Patent,13:13–15. This corresponds with the specification of IEH's Patents which reflects the common practice of producers defining what constitutes a lot grouped for traceability purposes. '771 Patent, 2:15-67, 5:43-54 (AAPA #3), and 12:46-49. Thus, ICMSF discloses collecting a plurality of portions from separate lots. ICMSF describes a lot as "a collection of units of a product." IMCSF, 119. ICMSF further indicates a lot has been defined as: "a quantity of food supposedly produced under identical conditions, all packages of which would normally bear a lot number that identifies the production during a particular time interval, and usually from a particular line, retort, or other critical processing unit. Statistically, a lot is considered as a collection of units of a product from which a sample is drawn to determine acceptability of the lot." *Id.*, 327.
[1b] b) separately compositing the collected multiple independent samples from each of the separate lots to provide a corresponding	ICMSF, alone or in combination with Hughes, separately compositing the collected multiple independent samples from each of the separate lots to provide a

set of separate composited lot samples, wherein each of the separate composited lot samples is attributed to a particular corresponding separate lot;	corresponding set of separate composited lot samples, wherein each of the separate composited lot samples is attributed to a particular corresponding separate lot. ICMSF states: "To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units of the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. . . . This finding suggests that considerable reductions may be made in the cost of laboratory analysis by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, n, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort." ICMSF, 187. To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. A laboratory investigation undertaken for ICMSF (Silliker & Gabis, 1973) indicated that different sized analytical units drawn from a single well-mixed sample unit of dried food give very similar results. A subsequent investigation (Gabis & Silliker, 1974) using foods of high moisture content (including eggs, poultry meat, meat, and meat products) showed comparable reliability in the use of composite analytical units; for example, examination of three composites of 20 × 25 g analytical units gave the same result as examination of all 60 analytical units individually (the total amount examined being 1500 g in both instances). This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n*, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort. *Id.*, 187.

10.5.1 Conceivable Alternative Plans

As indicated above, for an infectious agent such as *Salmonella*, there are strong reasons for adopting sampling plans in which $c = 0$:

1. It is philosophically objectionable to some people to accept a lot containing a recognized pathogen, regardless of how the food is to be used in a manufacturing process or by the consumer.
2. There is the very great technical advantage of combining many analytical units for bacteriological examination in systems with $c = 0$, because a single positive decides the outcome.
3. The plan with $c = 1$ (or more) would always require the examination of (many) more analytical units, for equal probability of acceptance, than a plan with $c = 0$.
4. Even if the indicated probabilities of plans with $c = 0$ versus $c = 1$ are the same, their implications are not. For example, when a lot is accepted with the plan $n = 60$, $c = 0$, it is possible (though not certain) that the lot may not be contaminated at all. But using the plan $n = 95$, $c = 1$ and accepting one positive, it is known that the lot certainly is contaminated (though the probability that a lot with 5% of analytical units contaminated would be accepted is in both instances $P_a = 0.05$).

ICMSF, 188 (annotated). ICMSF also provides an example of "[u]sing a sampling plan involving n = 15 (25 g analytical units) and a composite of 375 g." *Id.*, 195 (§ 10.7.2). ICMSF also states "[f]or qualitative analyses, 25 g analytical units are frequently analyzed individually or as composites of 100 g, 200 g, or more." *Id.*, 232 (§ 12.5.2).

The ICMSF incorporates two references, Silliker & Gabis (1973) and Gabis & Silliker (1974). Silliker notes that "[c]ompositing of multiple samples (lumping) and wet compositing of pre-enrichment cultures have been tested as economical approaches to *Salmonella* control." Silliker, 475.

Silliker further notes that "[b]oth approaches are being used widely by the food industry in the United States." *Id.*.

	To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. A laboratory investigation undertaken for ICMSF (Silliker & Gabis, 1973) indicated that different sized analytical units drawn from a single well-mixed sample unit of dried food give very similar results. A subsequent investigation (Gabis & Silliker, 1974) using foods of high moisture content (including eggs, poultry meat, meat, and meat products) showed comparable reliability in the use of composite analytical units; for example, examination of three composites of 20 × 25 g analytical units gave the same result as examination of all 60 analytical units individually (the total amount examined being 1500 g in both instances). This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n*, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort. "There is the very great technical advantage of combining many analytical units for bacteriological examination." ICMSF, 188. ICMSF also describes composites prepared by combining "15 (25 g analytical units)" and "composites of 100 g, 200 g, or more." *Id.,* 195, 232; *see* AAPA #5. ICMSF states, "All steps up to the analysis must be controlled . . . to allow reliable traceability." ICMSF, 227. ICMSF indicates lot samples are traceable to a particular corresponding lot: "It is therefore helpful . . . to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load). . . . Treating the individual production

	batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119.
[1c] c) enriching each of the separate composited lot samples to provide a set of separate composited lot samples enriched for the target microbe(s);	ICMSF, alone or in combination with Hughes, discloses enriching each of the separate composited lot samples to provide a set of separate composited lot samples enriched for the target microbe(s). ICMSF states: To reduce laboratory effort . . . it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. ICMSF, 187. Also, ICMSF describes preparing composites of analytical units then diluting "for qualitative (enrichment)" analysis. *Id.*, 231-32; *see also* AAPA #5, #7. For practical reasons, however, analytical units of 10 g up to 50 g are typical for quantitative methods and are diluted 1:10. For qualitative analyses, 25 g analytical units are frequently analyzed individually or as composites of 100 g, 200 g, or more. ICMSF, 232 (annotated). Preparation of the first dilution, either for qualitative (enrichment) or quantitative (direct) analyses, can have a marked impact on the microbial population in the sample. This is often due to changes in the physico-chemical characteristics of the suspension as compared to the food matrix. Where the food does not confer protection, the composition of the diluent is important to prevent or minimize shock/injury to the microorganisms that can result from rapid changes in the ionic concentrations. ICMSF, 232 (annotated). ICMSF also describes strategic use of non-selective enrichment to resuscitate microorganisms and selective enrichments for growing microorganisms. *Id.*, 232–33 (§ 12.6); *see e.g.*, *id.,* 233 ("use of nonselective medium . . . to enhance recovery of injured or stressed cells."). ICMSF, § 12.6 also describes optimizing the incubation

	period. S*ee e.g.*, *id.,* 232. It was well known long before the priority date to use non-selective enrichments after dry compositing and prior to wet compositing as evidenced by Price, Silliker, Gabis, and MLG. A POSITA would also know to use selective enrichments after dry compositing and prior to wet compositing as disclosed by ICMSF and Hughes. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. **(a)** *Pre-enrichment.*—Pre-enrich product in noninhibitory broth to initiate growth of *Salmonella* spp. Methods used may vary with product and should be performed as indicated in **967.26A** or BAM, 7th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, chapter 5, section C. Pre-enrichment broths should be incubated at 35°–37°C. **(b)** *Selective enrichment.*—Transfer 0.1 mL incubated pre-enrichment mixtures to Rappaport-Vassiliadis R10 broth (9.9 mL) and 1 mL to TT broth (9 mL). For all foods other than raw foods or foods having a high microbial load, incubate 6–8 h at 41°–43°C. Selective enrichments of raw foods or foods having a high microbial load must be incubated 16–20 h at 41°–43°C. An air incubator is suitable for this purpose. Hughes, 636.

[1d] d) removing portions of each separate enriched composited lot sample, and combining the removed portions to provide a pooled modular composite sample; and	ICMSF, alone or in combination with Hughes, discloses removing portions of each separate enriched composited lot sample, and combining the removed portions to provide a pooled modular composite sample. ICMSF describes compositing the enriched lot samples: "it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. . . . This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n,* with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort." ICMSF, 187. *See also*, AAPA #5-#7. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Hughes also discloses a method of removing portions of each enriched separate test lot sample, and combining the removed portions to provide a modular composite sample. Hughes instructs to "[c]ombine equal volumes (0.5-1 mL) from each M broth tube in a clean screw-cap tube." Hughes, 638.

	that has been warmed to 35°–37°C. Also remove 1 mL from Rappaport-Vassiliadis R10 tube and transfer to a separate tube of prewarmed M broth (10 mL). For all foods other than raw foods or foods having a high microbial load, incubate M broth tubes 16–20 h at 35°–37°C and return selective enrichment broth tubes to 41°–43°C incubator and incubate for additional 16–18 h. For raw foods or foods having a high microbial load, incubate M broth tubes 6 h at 35°–37°C and return selective enrichment broth tubes to 41°–43°C and incubate for an additional 6 h. (**d**) *Preparation of sample for EIA analysis.*—Remove M broth tubes from incubation and mix tubes by hand or Vortex mixer. Combine equal volumes (0.5–1 mL) from each M broth tube in a clean screw-cap tube and heat in boiling H_2O bath or in flowing steam 15 min. Refrigerate (2°–8°C) remaining M broth and TT and Rappaport-Vassiliadis R10 tubes from (**c**) for culture confirmation of any EIA-positive samples (*see* **967.26B, 967.26C, 967.27** and **967.28**). Cool heated M broths to 25°–37°C prior to analysis. Hughes, 638. Hughes 2003 confirms the pooling of enrichments. Hughes 2003, 775-76.
[1e.1] e) testing of the pooled modular composite sample, using a suitable detection assay, for the target microbe(s),	ICMSF, alone or in combination with Hughes, discloses e) testing of the pooled modular composite sample, using a suitable detection assay, for the target microbe(s). ICMSF describes testing enrichment cultures of composite samples with suitable detection assays (ICMSF, 187) and “presence/absence tests” (ICMSF, 233). Suitable

	detection assays were established in Silliker and Gabis as discussed in ICMSF. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Hughes states testing of the composite sample is "by an enzyme immunoassay (EIA) method." Hughes, 635, 638. These assay types were "art recognized." '771 Patent, 3:8-12 (AAPA #7).
[1e.2] wherein when such testing is negative all of said samples that were composited to form the separate composited lot samples are deemed negative for the target microbe(s) and each of the multiple separate lots is validated, and	ICMSF, alone or in combination with Hughes, discloses wherein when such testing is negative all of said samples that were composited to form the separate composited lot samples are deemed negative for the target microbe(s) and each of the multiple separate lots is validated. ICMSF describes correlating lots in a consignment with lot samples so a negative or positive test result enables a lot to be validated or rejected. ICMSF, 118-19. ICMSF describes sampling plans in which "c is the maximum allowable number of sample units yielding a positive result." ICMSF, 115. For many scenarios, the requirement is c = 0, which indicates the testing is negative. *Id.*, 118, 183-90, 309, 325, 326. A negative test result (*id.*, 113-15, 325, 326) is the expected and desired outcome to determine meat is not contaminated and is safe for consumption (*id.*, 184-89) such that a lot is validated and it is "commercially possible to market" (*id.*, 185) the meat.

6.10 WHAT IS A LOT?

Ideally, a lot is a quantity of food or food units produced and handled under uniform conditions. Under certain circumstances, it has been shown that the logarithms of the counts from a batch of food are likely to be normally distributed (Baird-Parker, 1980; Kilsby & Baird-Parker, 1983 [their Table 2]; Ingram & Roberts, 1976). The implication is that there is homogeneity within a lot, which, when considering microbial levels and distributions, rarely occurs in practice. In most instances, the distribution of microorganisms within lots of food is heterogeneous. Even in food factories, this heterogeneity makes the interpretation of sample results difficult, and it becomes even more so in circumstances where a lot is less well defined, such as in a consignment of a very large quantity of food.

It is therefore helpful if suppliers can be persuaded to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load).

If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment.

A lot should be composed of food produced with as little variation as possible for a given process or commodity. Because of the uncertainties in identifying a lot commercially, however, the use of the word *lot* in this book is usually in its statistical sense, as a collection of units of a product, the acceptability of which is determined by examining a sample drawn from it.

ICMSF, 118-19 (annotated).

	Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Because Hughes explains the steps to be taken when testing is positive (*see* ie.3)*,* a POSITA would also expect negative testing results. Hughes, 638. Determining meat is safe for consumption so it may be released into commerce is the well-understood point of testing meat. Moreover, the recitation of a lot being validated for "release . . . into commerce" is not limiting. Prosecution History for Reexamination Control No. 95/011,294 .
[1e.3] wherein when such testing is positive, each of the individual separate composited lot samples that were used to form the pooled modular composite sample are individually tested to determine which of the separate composited lot samples is positive for the target microbe(s),	ICMSF, alone or in combination with Hughes, discloses wherein when such testing is positive, each of the individual separate composited lot samples that were used to form the pooled modular composite sample are individually tested to determine which of the separate composited lot samples is positive for the target microbe(s). The combination of ICMSF and Hughes describes retaining a portion of the enriched lot samples (steps (b)-(d)). "Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119. "There is often a desire to retest retained samples." ICMSF, 191.

	If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment. ICMSF, 119 (annotated). Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Hughes explicitly teaches retention of the enriched test lot samples for further testing after a positive result. Hughes provides instructions to refrigerate the "remaining M broth and TT and Rappaport-Vassiliadis R10 tubes [that were enriched] for culture confirmation of any EIA-positive samples." Hughes, 638. Hughes refers to the results as the "confirmed positive samples" (Hughes, 635) and also states that diagnostic reagents are "necessary for culture confirmation of presumptive EIA tests." Hughes, 636. Hughes indicates "[e]nrichment broths and M broths from samples positive by an enzyme immunoassay (EIA) method must be streaked on selective media." *Id.*, 635
[1e.4] wherein the lots corresponding to any negatively testing composited lot samples are validated.	ICMSF, alone or in combination with Hughes, discloses wherein the lots corresponding to any negatively testing composited lot samples are validated. ICMSF describes correlating lots in a consignment with lot samples so a negative or positive test result enables a lot to be validated or rejected. ICMSF, 118-19.

	ICMSF describes sampling plans in which "c is the maximum allowable number of sample units yielding a positive result." ICMSF, 115. For many scenarios, the requirement is c = 0, which indicates the testing is negative. *Id.*, 118, 183-90, 309, 325, 326. A negative test result (*id.*, 113-15, 325, 326) is the expected and desired outcome to determine meat is not contaminated and is safe for consumption (*id.*, 184-89) such that a lot is validated and it is "commercially possible to market" (*id.*, 185) the meat.

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A lot should be composed of food produced with as little variation as possible for a given process or commodity. Because of the uncertainties in identifying a lot commercially, however, the use of the word *lot* in this book is usually in its statistical sense, as a collection of units of a product, the acceptability of which is determined by examining a sample drawn from it.

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	Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Because Hughes explains the steps to be taken when testing is positive (*see* ie.3)*,* a POSITA would also expect negative testing results. Hughes, 638. Determining meat is safe for consumption so it may be released into commerce is the well-understood point of testing meat. Moreover, the recitation of a lot being validated for "release . . . into commerce" is not limiting. Prosecution History for Reexamination Control No. 95/011,294 .
Claim 2	**Disclosure of ICMSF and Hughes**
The method of claim 1, wherein each of the multiple separate lots is selected from the group consisting of an environmental lot comprising multiple operationally-linked environmental specimens, pharmaceutical lot comprising multiple operationally-linked pharmaceutical specimens, single product lot comprising multiple operationally-linked product specimens, and combinations thereof.	ICMSF, alone or in combination with Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. The specification of the '771 Patent indicates a test lot is a single-unit production lot ('771 Patent, 7:54-56) such as a single combo, bin. pallet, or segment of production ('771 Patent, 7:54-59; *see also* AAPA #3 and #4). ICMSF teaches collecting from "individual production batches as lots" (ICMSF, 119) and "[a] lot is considered as a collection of units" (ICMSF, 327). "Operationally-linked" is simply grouping like items, which is also disclosed by ICMSF. "Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119.
Claim 3	**Disclosure of ICMSF and Hughes**

The method of claim 1, wherein each of the multiple separate lots is a single production lot, comprised of operationally-linked product specimens.	ICMSF, alone or in combination with Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. The specification of the '771 Patent indicates a test lot is a single-unit production lot ('771 Patent, 7:54-56) such as a single combo, bin. pallet, or segment of production ('771 Patent, 7:54-59; *see also* AAPA #3 and #4). ICMSF teaches collecting from "individual production batches as lots" (ICMSF, 119) and "[a] lot is considered as a collection of units" (ICMSF, 327). "Operationally-linked" is simply grouping like items, which is also disclosed by ICMSF. "Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119.
Claim 4	**Disclosure of ICMSF and Hughes**
The method of claim 3, wherein product specimens are synonymous with product portions.	ICMSF, alone or in combination with Hughes, discloses Claim 3, upon which this claim relies. See above re Claim 3. ICMSF teaches collecting portions or samples, which were known synonyms for product portions or specimens. ICMSF states that "[a] sample unit is a small portion of the lot." ICMSF, 225.

	12.1 INTRODUCTION Many different types of samples are collected and submitted to the laboratory for analysis. Some are sample units from lots or consignments of foods or ingredients for lot acceptance determination. Others may be for investigational purposes to assess control of the environment, investigate the source of a problem, or validate a process. Some may have legal implications relative to a lawsuit or for regulatory compliance. This chapter briefly discusses some of the major factors that should be considered when collecting sample units, shipping them to a laboratory, preparing them for analysis, and analyzing them. A sample unit is a small portion of the lot in which the distribution of both numbers and types of microorganisms should be representative of the whole lot. This requirement is only completely fulfilled when the food and processing conditions result in a homogeneous distribution of microorganisms. This only occurs in well-agitated fluid products. In general, the distribution of microorganisms is presumed to be random. ICMSF at 225 annotated).
Claim 5	**Disclosure of ICMSF and Hughes**
The method of claim 1, further comprising incubating the pooled modular composite sample of d), prior to testing in e).	ICMSF, alone or in combination with Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. ICMSF and Hughes, in combination with Stewart, discloses the method of claim 1, further comprising incubating the modular composite sample of d), prior to testing in e). Hughes describes incubating an enriched test lot sample. Hughes, 636, 638. Claim 1 is disclosed by ICMSF and Hughes. Stewart discloses that after the selective enrichment in Rappaport-Vassiliadis (RV) broth and tetrathionate (TT) broths, the two selective enrichments were combined into a single sample for further incubation and analysis. “[P]ost-enrichment by transfer and combination of 0.5 mL RV and 1 mL TT into a single tube containing 10 mL prewarmed trypticase soy broth supplemented with novobiocin (TSB + n) at 10 µg/mL with incubation for 16–20 h at 42°C.” Stewart, 398.

	Hughes also discloses an incubation protocol. "For the previous AOAC studies on TSVIA, a cultural protocol using selective enrichment in selenite cystine (SC) and tetrathionate (Tr) broths at 35°C was used. However, there has been a recent trend to use elevated incubation temperatures and to replace SC broth with the Rappaport-Vassiliadis (RV) medium (2)." Hughes, 634. A POSITA would have been motivated to incubate the pooled modular composite sample of Gombas or Hughes, as taught by Stewart, for reliable testing results.
Claim 6	**Disclosure of ICMSF and Hughes**
The method of claim 1, wherein each separate composited lot sample in c) comprises incubating each separate composited lot sample with enrichment medium suitable to enrich for the target microbe(s), and wherein removing in d) comprises removing a portion of the enrichment medium from each enriched separate composited lot sample and combining the removed portions to provide a pooled modular composite sample.	ICMSF, alone or in combination with Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. ICMSF describes the well-known benefits of combining enrichment cultures from analytical units from lots in a consignment. To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. A laboratory investigation undertaken for ICMSF (Silliker & Gabis, 1973) indicated that different sized analytical units drawn from a single well-mixed sample unit of dried food give very similar results. A subsequent investigation (Gabis & Silliker, 1974) using foods of high moisture content (including eggs, poultry meat, meat, and meat products) showed comparable reliability in the use of composite analytical units; for example, examination of three composites of 20 × 25 g analytical units gave the same result as examination of all 60 analytical units individually (the total amount examined being 1500 g in both instances). This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n*, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort. ICMSF, 187.

	Hughes describes incubating an enriches test lot sample. Hughes 636, 638.
Claim 7	**Disclosure of ICMSF and Hughes**
The method of claim 6, wherein the enrichment medium comprises a selective agent that will inhibit a competing non-target microbe.	ICMSF, alone or in combination with Hughes, discloses Claim 6, upon which this claim relies. See above re Claim 6. Additionally, Claim 7 recites what a POSITA would consider the well-known property of selective enrichment, inhibition of a competing non-target microorganism. D'Aoust describes "selective enrichment [that] inhibits growth of non-*Salmonella*e and facilitates isolation of *Salmonella* on differential plating media." D'Aoust, 369. A POSITA would have been motivated to use an enrichment medium comprising a selective agent that inhibits a non-target agent or organisms as described by D'Aoust, for the test lot samples described by ICMSF and Hughes, to ensure an accurate test. A POSITA would understand that incubating samples at different temperature ranges for different target microorganisms would, effectively, inhibit the growth of other organisms. "Incubation temperature markedly affects productivity of selective enrichment broths by suppressing growth of competing microorganisms but not *Salmonella*." D'Aoust, 372. "This may be achieved by utilization of enrichment media that through various mechanisms may favor and enhance growth and multiplication of salmonellae while other gram-negative organisms such as coliforms, Proteus, and Pseudomonas are suppressed or inhibited." Fagerberg, 630.
Claim 8	**Disclosure of ICMSF and Hughes**
The method of claim 1, wherein testing of the pooled modular composite sample, using a suitable detection assay comprises use of a	ICMSF, alone or in combination with Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. ICMSF describes testing enrichment cultures of composite samples with suitable

concentration step to concentrate the target microbe(s).	detection assays (ICMSF, 187) and "presence/absence tests" (ICMSF, 233). Suitable detection assays were established in Silliker and Gabis as discussed in ICMSF. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Hughes states testing of the composite sample is "by an enzyme immunoassay (EIA) method." Hughes, 635, 638. These assay types were "art recognized." '771 Patent, 3:8-12 (AAPA #7). Fratamico discusses the rapid isolation of target microorganisms using an immunomagnetic separation method. Fratamico, 105. Fratamico discloses that use of immunomagnetic beads resulted in "an increase in the level of E. coli B1409 in the sample," as well as "an increase in the number of organisms recovered." *Id.*, 107. Additionally, Fratamico provides detailed instructions for preparing and using immunomagnetic bead separation to concentrate a target microbial agent or organism. Fratamico developed "[a] method employing immunomagnetic beads" to concentrate and thereby improve the isolation and detection of E. coli. *Id.* For example, Fratamico was able to use immunomagnetic beads to isolate *E. coli* strains from enrichment cultures of raw ground beef "when the initial inoculum level was 1 cfu g-1." *Id.*, 109. Fratamico further teaches that "[t]he percentage of bacteria recovered increased with increasing amounts of beads." *Id.*, 108. Consequently, Fratamico "demonstrates that immunomagnetic separation [using immunomagnetic beads] is a simple and rapid technique" for concentrating a microbe for isolation and detection. *Id.*, 109. These teachings of Fratamico would have motivated a POSA to modify the combination of ICMSF and Gombas and ICMSF and Hughes to use

	immunomagnetic beads to concentrate microbes in a modular composite sample to benefit from accurate tests. Use of immunomagnetic beads was already known in the art for concentrating during testing beef samples. “Tu et al. (78) detected pathogenic E. coli O157:H7 in beef hamburger with the use of IMS and ATP measurement. After 6 h of enrichment at 37°C, inoculated beef samples (0.9 ml each) were filtered to remove large particles prior to 10 min of incubation with ca. 10^5 magnetic beads coated with anti–*E. coli* O157:H7 (Dynabeads, Dynal Inc., Oslo, Norway). Following incubation, the magnetic beads were collected with a Dynal magnetic concentrator and resuspended in 0.9 ml of Trypticase soy broth for ATP analysis.” Benoit, 1941. “Hudson et al. (26) used IMS to directly separate and concentrate L. monocytogenes from ham samples prior to detection by PCR. IMS was used on the basis of the commercial availability of anti-Listeria magnetic beads (Dynal Inc.) to avoid time-consuming cultural enrichment steps and to remove PCR inhibitors.” Benoit, 1941-42. Cudjoe investigates the use of immunomagnetic beads in pooling methods. In a study designed to evaluate the performance of Dynabeads® Anti-Salmonella, immunomagnetic separation (IMS) followed by plating (IMS-Plating) proved far superior to the conventional ISO *Salmonella* methodology and the Modified Semi-solid Rappaport-Vassiliadis (MSRV) method. *Salmonella* species were isolated and detected from 135 out of the 180 diverse poultry samples by IMS analysis as against 98 by the conventional method and 33 by the MSRV technique. All results were confirmed biochemically and serologically. It appeared from the data generated that some of the salmonellae isolated using IMS were greatly inhibited in Rappaport-Vassiliadis enrichment broth at 42°C and to a lesser extent in selenite cystine broth at 37°C. A moderately selective plating medium like XLD proved to be better in isolating these possible sensitive wild-type strains of salmonellae than the more selective BGA. Cudjoe, Abstract.

	Samples. One hundred and eighty (180) diverse poultry samples, consisting of 40 pools of cloacal/faecal swabs (5 swabs/pool; 5 pools samples/flock) from eight different flocks, 10 raw chicken breast meat samples, 10 pooled egg shell samples, 60 raw chicken liver samples and 60 chicken neck skins sampled from the slaughter line, were used in this study. All samples were obtained from a poultry slaughter and processing plant in the Netherlands. A pool of five swab samples was pre-enriched in 10 ml of buffered peptone water (BPW; Difco Laboratories, Detroit, Michigan, USA) at 37°C for 16–20 h, whilst 25 g of each of the other samples above, were pre-enriched similarly in 225 ml of BPW and examined as detailed below. Cudjoe, 160.
Claim 9	**Disclosure of ICMSF and Hughes**
The method of claim 8, wherein the concentration step comprises at least one of immunomagnetic bead separation, and affinity chromatography.	ICMSF, alone or in combination with Hughes, discloses Claim 8, upon which this claim relies. See above re Claim 8. ICMSF discloses the use of "immunomagnetic beads to concentrate cells." ICMSF, 318. A POSITA would consider this a well-known technique before the priority date. Fratamico discusses the rapid isolation of target microorganisms using an immunomagnetic separation method. Fratamico, 105. Fratamico discloses that use of immunomagnetic beads resulted in "an increase in the level of E. coli B1409 in the sample," as well as "an increase in the number of organisms recovered." *Id.*, 107. Additionally, Fratamico provides detailed instructions for preparing and using immunomagnetic bead separation to concentrate a target microbial agent or organism. Fratamico developed "[a] method employing immunomagnetic beads" to concentrate and thereby improve the isolation and detection of E. coli. *Id.* For example, Fratamico was able to use immunomagnetic beads to isolate *E. coli* strains from enrichment cultures of raw ground beef "when the initial inoculum level was 1 cfu g-1." *Id.*, 109. Fratamico further teaches that "[t]he percentage of bacteria recovered

	increased with increasing amounts of beads." *Id.*, 108. Consequently, Fratamico "demonstrates that immunomagnetic separation [using immunomagnetic beads] is a simple and rapid technique" for concentrating a microbe for isolation and detection. *Id.*, 109. These teachings of Fratamico would have motivated a POSA to modify the combination of ICMSF and Gombas and ICMSF and Hughes to use immunomagnetic beads to concentrate microbes in a modular composite sample to benefit from accurate tests. Use of immunomagnetic beads was already known in the art for concentrating during testing beef samples. "Tu et al. (78) detected pathogenic E. coli O157:H7 in beef hamburger with the use of IMS and ATP measurement. After 6 h of enrichment at 37°C, inoculated beef samples (0.9 ml each) were filtered to remove large particles prior to 10 min of incubation with ca. 10^5 magnetic beads coated with anti–*E. coli* O157:H7 (Dynabeads, Dynal Inc., Oslo, Norway). Following incubation, the magnetic beads were collected with a Dynal magnetic concentrator and resuspended in 0.9 ml of Trypticase soy broth for ATP analysis." Benoit, 1941. "Hudson et al. (26) used IMS to directly separate and concentrate L. monocytogenes from ham samples prior to detection by PCR. IMS was used on the basis of the commercial availability of anti-Listeria magnetic beads (Dynal Inc.) to avoid time-consuming cultural enrichment steps and to remove PCR inhibitors." Benoit, 1941-42. Cudjoe investigates the use of immunomagnetic beads in pooling methods.

	In a study designed to evaluate the performance of Dynabeads® Anti-Salmonella, immunomagnetic separation (IMS) followed by plating (IMS-Plating) proved far superior to the conventional ISO *Salmonella* methodology and the Modified Semi-solid Rappaport-Vassiliadis (MSRV) method. *Salmonella* species were isolated and detected from 135 out of the 180 diverse poultry samples by IMS analysis as against 98 by the conventional method and 33 by the MSRV technique. All results were confirmed biochemically and serologically. It appeared from the data generated that some of the salmonellae isolated using IMS were greatly inhibited in Rappaport-Vassiliadis enrichment broth at 42°C and to a lesser extent in selenite cystine broth at 37°C. A moderately selective plating medium like XLD proved to be better in isolating these possible sensitive wild-type strains of salmonellae than the more selective BGA. Cudjoe, Abstract. *Samples*. One hundred and eighty (180) diverse poultry samples, consisting of 40 pools of cloacal/faecal swabs (5 swabs/pool; 5 pools samples/flock) from eight different flocks, 10 raw chicken breast meat samples, 10 pooled egg shell samples, 60 raw chicken liver samples and 60 chicken neck skins sampled from the slaughter line, were used in this study. All samples were obtained from a poultry slaughter and processing plant in the Netherlands. A pool of five swab samples was pre-enriched in 10 ml of buffered peptone water (BPW; Difco Laboratories, Detroit, Michigan, USA) at 37°C for 16–20 h, whilst 25 g of each of the other samples above, were pre-enriched similarly in 225 ml of BPW and examined as detailed below. Cudjoe, 160.
Claim 10	**Disclosure of ICMSF and Hughes**
The method of claim 1, wherein where testing in e) of the pooled modular composite sample is positive, and wherein the separate individual composited lot samples are further tested using a more sensitive protocol.	ICMSF, alone or in combination with Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. ICMSF discloses the use of "immunomagnetic beads to concentrate cells." ICMSF, 318. A POSITA would consider this a well-known technique before the priority date.

	Hughes uses an EIA method followed by a more sensitive protocol—streaking on selective media. Hughes, 635. Each sample was analyzed by the BAM method (7th Ed.) and the TSVIA method using the enrichment protocol described in section F of the method, *Preparation of Sample*. Equal portions (0.5–1 mL) from each of the 2M broths arising from the 2 selective-enrichment broths were combined in a small screw-capped tube and heat treated in boiling water for 15 min prior to performing the TSVIA. Confirmation for the immunoassay was by streaking from the M broth culture onto selective agar, as specified in the BAM reference culture method. Hughes, 635. A POSITA would find that modifying Hughes based on D'Haese ensures a more sensitive test is used for further testing. A POSITA would have recognized the concentration step could be employed to provide a more sensitive protocol for further testing. A POSITA would understand that retesting the non-pooled, singular samples is an inherently more sensitive process because the sample is not diluted with other samples in the testing pool. Therefore, the retesting of a singular sample is more concentrated and therefore, more sensitive. *See also, generally*, Jarvis. Fratamico discusses the rapid isolation of target microorganisms using an immunomagnetic separation method. Fratamico, 105. Fratamico discloses that use of immunomagnetic beads resulted in "an increase in the level of E. coli B1409 in the sample," as well as "an increase in the number of organisms recovered." *Id.*, 107.

	Additionally, Fratamico provides detailed instructions for preparing and using immunomagnetic bead separation to concentrate a target microbial agent or organism. Fratamico developed "[a] method employing immunomagnetic beads" to concentrate and thereby improve the isolation and detection of E. coli. *Id.* For example, Fratamico was able to use immunomagnetic beads to isolate *E. coli* strains from enrichment cultures of raw ground beef "when the initial inoculum level was 1 cfu g-1." *Id.*, 109. Fratamico further teaches that "[t]he percentage of bacteria recovered increased with increasing amounts of beads." *Id.*, 108. Consequently, Fratamico "demonstrates that immunomagnetic separation [using immunomagnetic beads] is a simple and rapid technique" for concentrating a microbe for isolation and detection. *Id.*, 109. These teachings of Fratamico would have motivated a POSA to modify the combination of ICMSF and Gombas and ICMSF and Hughes to use immunomagnetic beads to concentrate microbes in a modular composite sample to benefit from accurate tests. Use of immunomagnetic beads was already known in the art for concentrating during testing beef samples. "Tu et al. (78) detected pathogenic E. coli O157:H7 in beef hamburger with the use of IMS and ATP measurement. After 6 h of enrichment at 37°C, inoculated beef samples (0.9 ml each) were filtered to remove large particles prior to 10 min of incubation with ca. 10^5 magnetic beads coated with anti–*E. coli* O157:H7 (Dynabeads, Dynal Inc., Oslo, Norway). Following incubation, the magnetic beads were collected with a Dynal magnetic concentrator and resuspended in 0.9 ml of Trypticase soy broth for ATP analysis." Benoit, 1941. "Hudson et al. (26) used IMS to directly separate and concentrate L. monocytogenes from ham samples prior to detection by PCR. IMS was used on the basis of the commercial availability of anti-Listeria magnetic beads (Dynal Inc.) to avoid time-

	consuming cultural enrichment steps and to remove PCR inhibitors." Benoit, 1941-42. Cudjoe investigates the use of immunomagnetic beads in pooling methods. In a study designed to evaluate the performance of Dynabeads® Anti-Salmonella, immunomagnetic separation (IMS) followed by plating (IMS-Plating) proved far superior to the conventional ISO *Salmonella* methodology and the Modified Semi-solid Rappaport-Vassiliadis (MSRV) method. *Salmonella* species were isolated and detected from 135 out of the 180 diverse poultry samples by IMS analysis as against 98 by the conventional method and 33 by the MSRV technique. All results were confirmed biochemically and serologically. It appeared from the data generated that some of the salmonellae isolated using IMS were greatly inhibited in Rappaport-Vassiliadis enrichment broth at 42°C and to a lesser extent in selenite cystine broth at 37°C. A moderately selective plating medium like XLD proved to be better in isolating these possible sensitive wild-type strains of salmonellae than the more selective BGA. Cudjoe, Abstract. *Samples*. One hundred and eighty (180) diverse poultry samples, consisting of 40 pools of cloacal/faecal swabs (5 swabs/pool; 5 pools samples/flock) from eight different flocks, 10 raw chicken breast meat samples, 10 pooled egg shell samples, 60 raw chicken liver samples and 60 chicken neck skins sampled from the slaughter line, were used in this study. All samples were obtained from a poultry slaughter and processing plant in the Netherlands. A pool of five swab samples was pre-enriched in 10 ml of buffered peptone water (BPW; Difco Laboratories, Detroit, Michigan, USA) at 37°C for 16–20 h, whilst 25 g of each of the other samples above, were pre-enriched similarly in 225 ml of BPW and examined as detailed below. Cudjoe, 160.
Claim 11	**Disclosure of ICMSF and Hughes**

The method of claim 10, wherein the more sensitive protocol comprises use of a concentration step to concentrate the target microbe(s), in combination with the same test protocol used to test the pooled modular composite sample.	ICMSF, alone or in combination with Hughes, discloses Claim 10, upon which this claim relies. See above re Claim 10. ICMSF discloses the use of "immunomagnetic beads to concentrate cells." ICMSF, 318. A POSITA would consider this a well-known technique before the priority date. Hughes uses an EIA method followed by a more sensitive protocol—streaking on selective media. Hughes, 635. Each sample was analyzed by the BAM method (7th Ed.) and the TSVIA method using the enrichment protocol described in section F of the method, *Preparation of Sample*. Equal portions (0.5–1 mL) from each of the 2M broths arising from the 2 selective-enrichment broths were combined in a small screw-capped tube and heat treated in boiling water for 15 min prior to performing the TSVIA. Confirmation for the immunoassay was by streaking from the M broth culture onto selective agar, as specified in the BAM reference culture method. Hughes, 635. A POSITA would find that modifying Hughes based on D'Haese ensures a more sensitive test is used for further testing. A POSITA would have recognized the concentration step could be employed to provide a more sensitive protocol for further testing. A POSITA would understand that retesting the non-pooled, singular samples is an inherently more sensitive process because the sample is not diluted with other samples in the testing pool. Therefore, the retesting of a singular sample is more concentrated and therefore, more sensitive. *See also, generally*, Jarvis.

	Fratamico discusses the rapid isolation of target microorganisms using an immunomagnetic separation method. Fratamico, 105. Fratamico discloses that use of immunomagnetic beads resulted in "an increase in the level of E. coli B1409 in the sample," as well as "an increase in the number of organisms recovered." *Id.*, 107. Additionally, Fratamico provides detailed instructions for preparing and using immunomagnetic bead separation to concentrate a target microbial agent or organism. Fratamico developed "[a] method employing immunomagnetic beads" to concentrate and thereby improve the isolation and detection of E. coli. *Id.* For example, Fratamico was able to use immunomagnetic beads to isolate *E. coli* strains from enrichment cultures of raw ground beef "when the initial inoculum level was 1 cfu g-1." *Id.*, 109. Fratamico further teaches that "[t]he percentage of bacteria recovered increased with increasing amounts of beads." *Id.*, 108. Consequently, Fratamico "demonstrates that immunomagnetic separation [using immunomagnetic beads] is a simple and rapid technique" for concentrating a microbe for isolation and detection. *Id.*, 109. These teachings of Fratamico would have motivated a POSA to modify the combination of ICMSF and Gombas and ICMSF and Hughes to use immunomagnetic beads to concentrate microbes in a modular composite sample to benefit from accurate tests. Use of immunomagnetic beads was already known in the art for concentrating during testing beef samples. "Tu et al. (78) detected pathogenic E. coli O157:H7 in beef hamburger with the use of IMS and ATP measurement. After 6 h of enrichment at 37°C, inoculated beef samples (0.9 ml each) were filtered to remove large particles prior to 10 min of incubation with ca. 10^5 magnetic beads coated with anti–*E. coli* O157:H7 (Dynabeads, Dynal Inc., Oslo, Norway). Following incubation, the magnetic beads were collected with a Dynal magnetic concentrator and resuspended in 0.9 ml of Trypticase soy broth for ATP analysis." Benoit, 1941.

	"Hudson et al. (26) used IMS to directly separate and concentrate *L. monocytogenes* from ham samples prior to detection by PCR. IMS was used on the basis of the commercial availability of anti-Listeria magnetic beads (Dynal Inc.) to avoid time-consuming cultural enrichment steps and to remove PCR inhibitors." Benoit, 1941-42. Cudjoe investigates the use of immunomagnetic beads in pooling methods. In a study designed to evaluate the performance of Dynabeads® Anti-Salmonella, immunomagnetic separation (IMS) followed by plating (IMS-Plating) proved far superior to the conventional ISO *Salmonella* methodology and the Modified Semi-solid Rappaport-Vassiliadis (MSRV) method. *Salmonella* species were isolated and detected from 135 out of the 180 diverse poultry samples by IMS analysis as against 98 by the conventional method and 33 by the MSRV technique. All results were confirmed biochemically and serologically. It appeared from the data generated that some of the salmonellae isolated using IMS were greatly inhibited in Rappaport-Vassiliadis enrichment broth at 42°C and to a lesser extent in selenite cystine broth at 37°C. A moderately selective plating medium like XLD proved to be better in isolating these possible sensitive wild-type strains of salmonellae than the more selective BGA. Cudjoe, Abstract. *Samples*. One hundred and eighty (180) diverse poultry samples, consisting of 40 pools of cloacal/faecal swabs (5 swabs/pool; 5 pools samples/flock) from eight different flocks, 10 raw chicken breast meat samples, 10 pooled egg shell samples, 60 raw chicken liver samples and 60 chicken neck skins sampled from the slaughter line, were used in this study. All samples were obtained from a poultry slaughter and processing plant in the Netherlands. A pool of five swab samples was pre-enriched in 10 ml of buffered peptone water (BPW; Difco Laboratories, Detroit, Michigan, USA) at 37°C for 16–20 h, whilst 25 g of each of the other samples above, were pre-enriched similarly in 225 ml of BPW and examined as detailed below. Cudjoe, 160.

Claim 15	Disclosure of ICMSF and Hughes
The method of claim 1, wherein each of the lots is selected from the lot group consisting of a single combo, single bin, single pallet, and a production assemblage.	ICMSF, alone or in combination with Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. The specification of the '771 Patent indicates a test lot is a single-unit production lot ('771 Patent, 7:54-56) such as a single combo, bin. pallet, or segment of production ('771 Patent, 7:54-59; *see also* AAPA #3 and #4). ICMSF teaches collecting from "individual production batches as lots" (ICMSF, 119) and "[a] lot is considered as a collection of units." ICMSF, 327. "Operationally-linked" is simply grouping like items, which is also disclosed by ICMSF. "Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119.
Claim 16	**Disclosure of ICMSF and Hughes**
The method of claim 1, wherein the number of separate lots is from 3 to 10.	ICMSF, alone or in combination with Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. ICMSF indicates a consignment may be "composed of several lots." ICMSF, 119-20. "Several" lots includes at least three lots.
Claim 17	**Disclosure of ICMSF and Hughes**
The method of claim 1, further comprising, where testing of the pooled modular composite sample is positive, determining which individual separate lots are positive, application of remedial measures to said separate positive lots, and validating by further testing of the respective remediated	ICMSF, alone or in combination with Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite

separate lots and obtaining a negative test result.	sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Each sample was analyzed by the BAM method (7th Ed.) and the TSVIA method using the enrichment protocol described in section F of the method, *Preparation of Sample.* Equal portions (0.5–1 mL) from each of the 2M broths arising from the 2 selective-enrichment broths were combined in a small screw-capped tube and heat treated in boiling water for 15 min prior to performing the TSVIA. Confirmation for the immunoassay was by streaking from the M broth culture onto selective agar, as specified in the BAM reference culture method. Hughes, 635. A POSITA understands the purpose of testing for pathogens and other organisms in food is to determine if a food is safe for entry into commerce to be sold for consumption. One having ordinary skill in the art understands that if a sample tests positive, a remediation step, like cooking, needs to be completed before it can be sold into commerce to comply with USDA guidelines.
Claim 18	**Disclosure of ICMSF and Hughes**
The method of claim 1, wherein, in a), separately collecting said multiple independent samples from each of the multiple separate lots comprises separately collecting from each separate lot a number of samples from 5 to 100.	ICMSF, alone or in combination with Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. ICMSF teaches collecting 5, 30, 60, 95, or 100 portions. ICMSF, 184-90, 276, 282, 309, 320, 326-30. *See also* MPEP § 2144.05.

Claim 19	Disclosure of ICMSF and Hughes
The method of claim 1 wherein, in a), separately collecting said multiple independent samples from each of the multiple separate lots comprises separately collecting 30 to 60 samples from each separate lot.	ICMSF, alone or in combination with Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. ICMSF teaches collecting 5, 30, 60, 95, or 100 portions. ICMSF, 184-90, 276, 282, 309, 320, 326-30. *See also* MPEP § 2144.05.
Claim 20	**Disclosure of ICMSF and Hughes**
The method of claim 1, wherein, in a), separately collecting said multiple independent samples from each of the multiple separate lots comprises separately collecting at least 30 samples from each separate lot.	ICMSF, alone or in combination with Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. ICMSF teaches collecting 5, 30, 60, 95, or 100 portions. ICMSF, 184-90, 276, 282, 309, 320, 326-30. *See also* MPEP § 2144.05.
Claim 22	**Disclosure of ICMSF and Hughes**
The method of claim 1, wherein removing portions in step d) is removing equal portions, aseptically, so as to preclude contamination of the enriched separate composited lot samples.	ICMSF, alone or in combination with Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. ICMSF describes aseptically withdrawing and weighing sample units into the appropriate diluent. ICMSF, 231, 232. Hughes instructs that after enrichment "[c]ombine equal volumes (0.5-1 mL) from each M broth tube in a clean screw-cap tube." Hughes, 638. Moreover, a POSITA would consider this to be standard practice. A POSITA would have aseptically removed equal volume portions of each enriched separate test lot sample.
Claim 23	**Disclosure of ICMSF and Hughes**

The method of claim 1, wherein the target microbe(s) comprises at least one selected from the group consisting of *Listeria*, *Salmonella* and *Escherichia coli*.	ICMSF, alone or in combination with Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. ICMSF describes testing for *Listeria* (ICMSF, 285-312), *Salmonella* (*id.*, 273-84), and *Escherichia coli* (*id.*, 313-32). Hughes focuses on *Salmonella*. Hughes, 634.
Claim 24	**Disclosure of ICMSF and Hughes**
The method of claim 1, wherein the target microbe(s) comprises at least one selected from the group consisting of *Listeria* spp., *Listeria monocytogenes*, *Salmonella* spp., *Escherichia coli* O157:H7, coliforms and fecal coliforms.	ICMSF, alone or in combination with Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. ICMSF describes testing for *Listeria* (ICMSF, 285-312), *Salmonella* (*id.*, 273-84), and *Escherichia coli* (*id.*, 313-32). Hughes focuses on *Salmonella*. Hughes, 634.
Claim 25	**Disclosure of ICMSF and Hughes**
The method of claim 1, wherein the target microbe(s) comprises a spoilage organism.	ICMSF, alone or in combination with Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. A POSITA would find it obvious to apply the teachings of ICMSF and Hughes to target spoilage organisms. "Examination of every piece of whole meat is clearly impractical, hence any sampling procedure for estimating shelf-life would involve taking enough samples to give some statistical assurance of the keeping qualities of meat." Gill, 449. Borch is titled "Bacterial spoilage of meat and cured meat products." Borch. The reference discusses spoilage organisms in meat:

	Lactic acid bacteria are the major bacterial group associated with the spoilage of refrigerated vacuum- or MA-packaged cooked, cured meat products (Blickstad and Molin, 1983b; Shaw and Harding, 1989; von Holy et al., 1991). At the time of spoilage some products contain a 'pure' culture of only one species, while in others a mixture of *Lactobacillus* spp. and *Leuconostoc* spp. was found (Kant-Muermans, unpublished results). The great diversity of bacteria isolated from spoiled meat products is confirmed by several studies (Korkeala and Mäkelä, 1989; von Holy et al., 1991; Yang and Ray, 1994). The genus/species of lactic acid bacteria responsible for spoilage, depend on the product composition (product-related flora) as well as the manufacturing site (house-related flora; Korkeala and Mäkelä, 1989). Borch, 113. "Lactic acid bacteria spoil refrigerated meat products by causing defects such as sour off-flavours, discolouration, gas production, slime production and decrease in PH." Borch, 114; *see also, id.*, 115-17 (Section 4 Analysis of Spoilage). It is understood in the art that aerobic plate counts ("APC") can test for spoilage organisms. *See. e.g.*, ICMSF 1986, 122 ("tests to be made (e.g., APC, *Salmonella*, Clostridium perfringens"); *id.* at 132 ("However, aerobic plate counts (APC) can be used to monitor Good Commercial Practice (GCP), and criteria based on such examinations are a valuable aid to in-plant quality control.").

TABLE 19

Sampling plans and recommended microbiological limits for raw meat[a]

Product[b]	Test	Method reference[c]	Case	Plan class	n	c	Limit per cm² or gram m	M
Carcass meat, before chilling	APC	118	1	3	5	3	10^5	10^6
Carcass meat, chilled	APC	118	1	3	5	3	10^6	10^7
Edible offal, chilled	APC	118	1	3	5	3	10^6	10^7
Carcass meat, frozen	APC	118	1	3	5	3	5×10^5	10^7
Boneless meat, frozen (beef, veal, pork, mutton)	APC	118	1	3	5	3	5×10^5	10^7
Comminuted meat, frozen	APC	118	1	3	5	3	10^6	10^7
Edible offal, frozen	APC	118	1	3	5	3	5×10^5	10^7

a **Not for use at port of entry** but for in-plant quality control. Refer to Section B for appropriate applications.
b Unfrozen carcasses and primal cuts, swab counts per cm²; other meats and meat products, per gram.
c Refers to page number in ICMSF, 1978.

ICMSF 1986, 133 (Table 19 (annotated)).

Although for "quality control," a POSITA would understand that "quality control" means testing for spoilage organisms could also be accomplished by this well-known method. It is disclosed within the prior art during the production lifecycle of beef production. ICMSF 1986, 134 ("The FAOIWHO Working Group (FAQ/WHO, 1979) considered that the only point at which APC could be used for chilled meat, to evaluate the hygienic conditions under which it was produced, was at the slaughterhouse, and because of its perishable nature it would not be possible to set

APC limits for use in guidelines or specifications for chilled meats at port of entry."); *see also, id.*, 153 ("Monitoring for APC, E. coli, and Staph. aureus would give some indication of inadequate processing (heating, curing, smoking), postprocess contamination, or storage at temperatures above those recommended for frozen or refrigerated distribution and storage.")

E. TEST PROCEDURES

(a) *Aerobic plate counts*

Comminute, blend, or stomach the various portions of meat composing the sample unit and withdraw the analytical unit. Combine the swabs to form an analytical unit, or treat each separately. Prepare dilutions and perform the APC test according to the directions given in ICMSF, 1978, p. 118. Spread plates are preferable to pour plates when examining raw meats.

The temperature used for incubation of plates should be related to the material being examined. To estimate numbers of mesophilic contaminants on freshly dressed carcasses it is advisable to incubate plates at 35 or 37°C. For meat that has been held under chilled conditions, plates should be incubated at 25°C to include psychrotrophs and thus give a better indication of the microbial growth that has occurred. Counts at 20°C give similar results after a longer incubation time.

ICMSF 1986, 136 (annotated).

See also, Bhunia 4 ("Bacteria-specific generic rapid methods such ATP-based luminescence and total plate count (APC) are also needed for monitoring cleaning and hygienic practices employed in a processing plant."); Wheeler, 1606 (use of APC in a beef-processing plant); PR/HAACP, 38849 ("Various alternative indicator

	organisms were suggested, including generic E. coli (biotype I), total plate counts, Enterobacteriaceae, Total Viable Counts (TVC), and Aerobic Plate Counts (APC).”).
Claim 26	**Disclosure of ICMSF and Hughes**
The method of claim 1, wherein removing portions of each separate enriched composited lot sample comprises removing equal volume portions of each separate enriched composited lot sample.	ICMSF, alone or in combination with Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. ICMSF describes aseptically withdrawing and weighing sample units into the appropriate diluent. ICMSF, 231, 232. Hughes instructs that after enrichment “[c]ombine equal volumes (0.5-1 mL) from each M broth tube in a clean screw-cap tube.” Hughes, 638. Moreover, a POSITA would consider this to be standard practice. A POSITA would have aseptically removed equal volume portions of each enriched separate test lot sample.
Claim 27	**Disclosure of ICMSF and Hughes**
The method of claim 1, wherein testing of the pooled modular composite sample, using a suitable detection assay comprises use of at least one of a DNA-based method, an immunochemistry-based method, and a biosensor-based method.	ICMSF, alone or in combination with Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. Hughes discloses use of “an enzyme immunoassay (EIA) method,” which is an immunochemistry-based method. Hughes, 635.

	Each sample was analyzed by the BAM method (7th Ed.) and the TSVIA method using the enrichment protocol described in section F of the method, *Preparation of Sample*. Equal portions (0.5–1 mL) from each of the 2M broths arising from the 2 selective-enrichment broths were combined in a small screw-capped tube and heat treated in boiling water for 15 min prior to performing the TSVIA. Confirmation for the immunoassay was by streaking from the M broth culture onto selective agar, as specified in the BAM reference culture method. Hughes, 635.
Claim 28	**Disclosure of ICMSF and Hughes**
The method of claim 1, wherein each of the multiple separate lots are production lots of a meat or poultry product, comprising an assemblage of one or more meat or poultry specimens, respectively.	ICMSF, alone or in combination with Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. ICMSF indicates "[t]rimmings to be used for ground beef could be pretested for E. coli O157:H7 by various sampling plans." ICMSF, 323. ICMSF indicates "[r]esearch has validated that up to fifteen 25 g analytical units of ground beef or trimmings can be composited without significant loss in E. coli O157:H7 detection sensitivity." *Id.*, 327 *see also id.*, 226, 314, 320, and 324. ICMSF includes many other references to testing lots of beef or poultry, two of the most common types of meat in commerce. *Id.* at 114 (§ 6.3), 184–191 (§§ 10.2–10.6), 225–226 (§ 12.1), 285 (§ 16.1), 313–330 (Chapter 17). ICMSF is relevant to any kind of food product including beef or poultry.

	Hughes discloses testing turkey for *Salmonella*. "Three food types (. . . and raw turkey) were analyzed in the United States." Hughes, 634, 645, Table 8. Table 8 shows results from 12 collaborators for naturally contaminated raw turkey. For the 180 samples tested, using confirmed data, there was one false negative at the low level for the TECRA method. There were 3 false negatives at the low level and 3 false negatives at the high level for the cultural method. The incidence of false-positive results was very high for the cultural method, with a total of 41 false positives, compared with 5 for the TECRA method. It should, however, be taken into account that the comparison was with the method in BAM 7th Ed., not the current 8th Ed. The method for raw flesh food in the BAM 8th Ed. uses elevated temperature with RV and TT broth, which would be more selective. No significant Hughes, 646.
Claim 29	**Disclosure of ICMSF and Hughes**
The method of claim 28, wherein the meat or poultry specimens are meat trim pieces or poultry trim pieces, respectively.	ICMSF, alone or in combination with Hughes, discloses Claim 28, upon which this claim relies. See above re Claim 28. ICMSF indicates "[t]rimmings to be used for ground beef could be pretested for E. coli O157:H7 by various sampling plans." ICMSF, 323. ICMSF indicates "[r]esearch has validated that up to fifteen 25 g analytical units of ground beef or trimmings can be composited without significant loss in E. coli O157:H7 detection sensitivity." *Id.*, 327 *see also id.*, 226, 314, 320, and 324. ICMSF includes many other references to testing lots of beef or poultry, two of the most common types of meat in commerce. *Id.* at 114 (§ 6.3), 184–191 (§§ 10.2–

	10.6), 225–226 (§ 12.1), 285 (§ 16.1), 313–330 (Chapter 17). ICMSF is relevant to any kind of food product including beef or poultry.
Claim 30	**Disclosure of ICMSF and Hughes**
The method of claim 28, wherein the meat is that of beef, pork, sheep or bison.	ICMSF, alone or in combination with Hughes, discloses Claim 28, upon which this claim relies. See above re Claim 28. ICMSF indicates "[t]rimmings to be used for ground beef could be pretested for E. coli O157:H7 by various sampling plans." ICMSF, 323. ICMSF indicates "[r]esearch has validated that up to fifteen 25 g analytical units of ground beef or trimmings can be composited without significant loss in E. coli O157:H7 detection sensitivity." *Id.*, 327 *see also id.*, 226, 314, 320, and 324. ICMSF includes many other references to testing lots of beef or poultry, two of the most common types of meat in commerce. *Id.* at 114 (§ 6.3), 184–191 (§§ 10.2–10.6), 225–226 (§ 12.1), 285 (§ 16.1), 313–330 (Chapter 17). ICMSF is relevant to any kind of food product including beef or poultry.
Claim 31	**Disclosure of ICMSF and Hughes**
The method of claim 30, wherein the meat is that of beef.	ICMSF, alone or in combination with Hughes, discloses Claim 30, upon which this claim relies. See above re Claim 30. ICMSF indicates "[t]rimmings to be used for ground beef could be pretested for E. coli O157:H7 by various sampling plans." ICMSF, 323. ICMSF indicates "[r]esearch has validated that up to fifteen 25 g analytical units of ground beef or trimmings can be composited without significant loss in E. coli O157:H7 detection sensitivity." *Id.*, 327 *see also id.*, 226, 314, 320, and 324. ICMSF includes many other references to testing lots of beef or poultry, two of the most common types of meat in commerce. *Id.* at 114 (§ 6.3), 184–191 (§§ 10.2–10.6), 225–226 (§ 12.1), 285 (§ 16.1), 313–330 (Chapter 17). ICMSF is relevant to any kind of food product including beef or poultry.

Claim 37	Disclosure of ICMSF and Hughes
[37pre] A method of sampling and testing products for microbe(s) in multiple separate lots, comprising:	To the extent the preamble is limiting, as discussed herein, ICMSF, alone or in combination with Hughes, discloses collecting a plurality of portions from each of a plurality of separate operationally-linked test lots of beef, each to be validated for release into commerce, the test lots each comprising an assemblage of one or more specimens from which the portions are collected, wherein each test lot is separately sampled to conform to a statistical-based sampling plan specifying, for a particular microbial agent or organism, a number of portions to be taken sufficient to provide for validation and release of the test lot into commerce. "Many different types of samples are collected and submitted to the laboratory for analysis. Some are sample units from lots or consignments of food or ingredients for lot acceptance determination. . . . A sample unit is a small portion of the lot in which the distribution of both numbers and types of microorganisms should be representative of the whole lot." ICMSF, 255. ICMSF describes collecting from a lot or lots: "If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." *Id.*, 119. Additionally, ICMSF indicates a consignment may be "a very large quantity of food" and may be "composed of several lots, representing for example, different days of production from the same plant." *Id.*, 119, 120; *see also* Applicant Admitted Prior Art ("AAPA") #3-#5.

6.11 WHAT IS A REPRESENTATIVE SAMPLE?

A *representative sample* reflects, as far as is possible, the composition of the lot from which it is drawn.

How then should a representative sample be drawn? It is important to avoid bias and to draw a sufficient number of sample units to confidently make a judgment about a lot. Sampling at random is the universally recognized way of avoiding bias and is more reliable than consciously trying to draw the sample units from the various parts of a lot. The units (cartons or containers, particular weights of solid, or volumes of liquid) are drawn by using random numbers. There is, of course, no guarantee that a random sample has characteristics identical with those of the lot, but the randomness of sampling is the basis for calculation of the probability that a sample will give a biased result.

It is also possible, and may be desirable, to use a *stratified random sampling* approach, drawing a random sample of a given number of sample units from each *stratum* (e.g., each sublot or carton). The distribution of sample units on strata should correspond to the distribution of lot units on strata. This means that if each stratum contains the same quantity as every other one, the number of sample units per stratum should be the same. Otherwise, they should differ just as the proportions of the lot contained in the strata differ. Stratification is a device for handling known sources of variation and may be used where one has prior knowledge that the consignment is potentially not of uniform quality. A consignment might not be of uniform quality if portions of it are shipped in different vehicles or in different holds of a vessel, or if it is known that it is really composed of several lots representing, for example, different days of production from the same plant or different plants of the same company. The results for different strata should be assessed separately and then pooled if they appear to be homogeneous.

The calculation of acceptance probabilities is based on the assumption that random samples have been drawn and analyzed. Where physical or practical constraints prevent random sampling, such calculated probabilities may not be valid. Their reliability depends on how close reality approaches the ideal situation of random sampling. This fact should be borne in mind when subsequently interpreting results.

ICMSF, 119-20 (annotated).

6.10 WHAT IS A LOT?

Ideally, a lot is a quantity of food or food units produced and handled under uniform conditions. Under certain circumstances, it has been shown that the logarithms of the counts from a batch of food are likely to be normally distributed (Baird-Parker, 1980; Kilsby & Baird-Parker, 1983 [their Table 2]; Ingram & Roberts, 1976). The implication is that there is homogeneity within a lot, which, when considering microbial levels and distributions, rarely occurs in practice. In most instances, the distribution of microorganisms within lots of food is heterogeneous. Even in food factories, this heterogeneity makes the interpretation of sample results difficult, and it becomes even more so in circumstances where a lot is less well defined, such as in a consignment of a very large quantity of food.

It is therefore helpful if suppliers can be persuaded to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load).

If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment.

A lot should be composed of food produced with as little variation as possible for a given process or commodity. Because of the uncertainties in identifying a lot commercially, however, the use of the word *lot* in this book is usually in its statistical sense, as a collection of units of a product, the acceptability of which is determined by examining a sample drawn from it.

ICMSF, 118-19 (annotated).

	The ICMSF has defined a lot as follows: In the commercial sense, a lot is a quantity of food supposedly produced under identical conditions, all packages of which would normally bear a lot number that identifies the production during a particular time interval, and usually from a particular line, retort, or other critical processing unit. Statistically, a lot is considered as a collection of units of a product from which a sample is drawn to determine acceptability of the lot (ICMSF, 1986). In this example, the maximum size of a lot consists of all the ground beef produced on common equipment from cleanup to cleanup. Smaller lots could be established (e.g., hourly). ICMSF, 327 (annotated). This definition also reflects the common practice referred to in the IEH Patents at Example II, wherein "[p]referably, the producer defines and produces these single-unit lots in such a fashion as to maximize the information available." '771 Patent, 13:13–15. This corresponds with the specification of IEH's Patents which reflects the common practice of producers defining what constitutes a lot grouped for traceability purposes. '771 Patent, 2:15-67, 5:43-54 (AAPA #3), and 12:46-49. Thus, ICMSF discloses collecting a plurality of portions from separate lots. ICMSF describes a lot as "a collection of units of a product." IMCSF, 119. ICMSF further indicates a lot has been defined as: "a quantity of food supposedly produced under identical conditions, all packages of which would normally bear a lot number that identifies the production during a particular time interval, and usually from a particular line, retort, or other critical processing unit. Statistically, a lot is considered as a collection of units of a product from which a sample is drawn to determine acceptability of the lot." ICMSF, 327. ICMSF advocates use of a "statistically based sampling plan." *Id.*, 113-15, 119-21, 320, 327, 328. In the sampling plans, "*n* is the number of sample units chosen

	separately and independently, and . . . *c* is the maximum allowable number of sample units yielding a positive result." *Id.*, 115. ICMSF discloses a wide range for the number of sample units (portions) for a particular microbial agent or organism. For example, ICMSF discusses collecting 15, 30, 60 or 100 sample units from a lot. *Id.*, 320, 326. **6.5 THE SAMPLING PLAN** The sample units so drawn will, after examination, yield results that will be compared with certain criteria to reach a decision as to whether the entire lot should be accepted or rejected (see section 6.9 for explanation of rejection). The particular choice of sampling procedure and the decision criteria make up the *sampling plan*. Proper drawing of samples is essential if the decision making criteria of the sampling plan are to give unbiased results. By sampling randomly, we are able to reduce the risk of making biased decisions. A simple example of a sampling plan follows. Take 10 sample units of a food from a lot and subject them to appropriate laboratory procedure to test for the presence or absence of a microorganism. If two or fewer of the 10 sample units show presence of the organism, that is, give a positive result, then the whole lot of food is acceptable (relative to this organism). But if three or more give a positive result, the whole lot is to be rejected. This plan is described by the use of two terms $n = 10$ and $c = 2$, where n is the number of sample units chosen separately and independently, and where c is the maximum allowable number of sample units yielding a positive result. ICMSF, 115 (annotated).
[37a] a) separately collecting multiple independent samples from each of multiple separate single production lots, wherein each separate single production lot is separately sampled by taking said multiple independent samples thereof;	ICMSF, alone or in combination with Hughes, discloses collecting a plurality of portions from each of a plurality of separate operationally-linked test lots of beef, each to be validated for release into commerce, the test lots each comprising an assemblage of one or more specimens from which the portions are collected, wherein each test lot is separately sampled to conform to a statistical-based sampling plan specifying, for a particular microbial agent or organism, a number of portions to be taken sufficient to provide for validation and release of the test lot into commerce.

	"Many different types of samples are collected and submitted to the laboratory for analysis. Some are sample units from lots or consignments of food or ingredients for lot acceptance determination. . . . A sample unit is a small portion of the lot in which the distribution of both numbers and types of microorganisms should be representative of the whole lot." ICMSF, 255. ICMSF describes collecting from a lot or lots: "If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." *Id.*, 119. Additionally, ICMSF indicates a consignment may be "a very large quantity of food" and may be "composed of several lots, representing for example, different days of production from the same plant." *Id.*, 119, 120; *see also* Applicant Admitted Prior Art ("AAPA") #3-#5.

6.11 WHAT IS A REPRESENTATIVE SAMPLE?

A *representative sample* reflects, as far as is possible, the composition of the lot from which it is drawn.

How then should a representative sample be drawn? It is important to avoid bias and to draw a sufficient number of sample units to confidently make a judgment about a lot. Sampling at random is the universally recognized way of avoiding bias and is more reliable than consciously trying to draw the sample units from the various parts of a lot. The units (cartons or containers, particular weights of solid, or volumes of liquid) are drawn by using random numbers. There is, of course, no guarantee that a random sample has characteristics identical with those of the lot, but the randomness of sampling is the basis for calculation of the probability that a sample will give a biased result.

It is also possible, and may be desirable, to use a *stratified random sampling* approach, drawing a random sample of a given number of sample units from each *stratum* (e.g., each sublot or carton). The distribution of sample units on strata should correspond to the distribution of lot units on strata. This means that if each stratum contains the same quantity as every other one, the number of sample units per stratum should be the same. Otherwise, they should differ just as the proportions of the lot contained in the strata differ. Stratification is a device for handling known sources of variation and may be used where one has prior knowledge that the consignment is potentially not of uniform quality. A consignment might not be of uniform quality if portions of it are shipped in different vehicles or in different holds of a vessel, or if it is known that it is really composed of several lots representing, for example, different days of production from the same plant or different plants of the same company. The results for different strata should be assessed separately and then pooled if they appear to be homogeneous.

The calculation of acceptance probabilities is based on the assumption that random samples have been drawn and analyzed. Where physical or practical constraints prevent random sampling, such calculated probabilities may not be valid. Their reliability depends on how close reality approaches the ideal situation of random sampling. This fact should be borne in mind when subsequently interpreting results.

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It is therefore helpful if suppliers can be persuaded to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load).

If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment.

A lot should be composed of food produced with as little variation as possible for a given process or commodity. Because of the uncertainties in identifying a lot commercially, however, the use of the word *lot* in this book is usually in its statistical sense, as a collection of units of a product, the acceptability of which is determined by examining a sample drawn from it.

ICMSF, 118-19 (annotated).

	The ICMSF has defined a lot as follows: In the commercial sense, a lot is a quantity of food supposedly produced under identical conditions, all packages of which would normally bear a lot number that identifies the production during a particular time interval, and usually from a particular line, retort, or other critical processing unit. Statistically, a lot is considered as a collection of units of a product from which a sample is drawn to determine acceptability of the lot (ICMSF, 1986). In this example, the maximum size of a lot consists of all the ground beef produced on common equipment from cleanup to cleanup. Smaller lots could be established (e.g., hourly). ICMSF, 327 (annotated). This definition also reflects the common practice referred to in the IEH Patents at Example II, wherein "[p]referably, the producer defines and produces these single-unit lots in such a fashion as to maximize the information available." '771 Patent,13:13–15. This corresponds with the specification of IEH's Patents which reflects the common practice of producers defining what constitutes a lot grouped for traceability purposes. '771 Patent, 2:15-67, 5:43-54 (AAPA #3), and 12:46-49. Thus, ICMSF discloses collecting a plurality of portions from separate lots. ICMSF describes a lot as "a collection of units of a product." IMCSF, 119. ICMSF further indicates a lot has been defined as: "a quantity of food supposedly produced under identical conditions, all packages of which would normally bear a lot number that identifies the production during a particular time interval, and usually from a particular line, retort, or other critical processing unit. Statistically, a lot is considered as a collection of units of a product from which a sample is drawn to determine acceptability of the lot." ICMSF, 327. ICMSF advocates use of a "statistically based sampling plan." *Id.*, 113-15, 119-21, 320, 327, 328. In the sampling plans, "*n* is the number of sample

	units chosen separately and independently, and . . . *c* is the maximum allowable number of sample units yielding a positive result." *Id.*, 115. ICMSF discloses a wide range for the number of sample units (portions) for a particular microbial agent or organism. For example, ICMSF discusses collecting 15, 30, 60 or 100 sample units from a lot. *Id.*, 320, 326. **6.5 THE SAMPLING PLAN** The sample units so drawn will, after examination, yield results that will be compared with certain criteria to reach a decision as to whether the entire lot should be accepted or rejected (see section 6.9 for explanation of rejection). The particular choice of sampling procedure and the decision criteria make up the *sampling plan*. Proper drawing of samples is essential if the decision making criteria of the sampling plan are to give unbiased results. By sampling randomly, we are able to reduce the risk of making biased decisions. A simple example of a sampling plan follows. Take 10 sample units of a food from a lot and subject them to appropriate laboratory procedure to test for the presence or absence of a microorganism. If two or fewer of the 10 sample units show presence of the organism, that is, give a positive result, then the whole lot of food is acceptable (relative to this organism). But if three or more give a positive result, the whole lot is to be rejected. This plan is described by the use of two terms $n = 10$ and $c = 2$, where n is the number of sample units chosen separately and independently, and where c is the maximum allowable number of sample units yielding a positive result. ICMSF, 115 (annotated).
[37b] b) separately compositing the collected multiple independent samples from each of the separate lots to provide a corresponding set of separate composited single production lot samples, wherein each of the separate composited single production lot samples is attributed to a particular corresponding separate single production lot;	ICMSF, alone or in combination with Hughes, discloses combining the collected conforming plurality of portions corresponding to each of the separate test lots to provide a corresponding set of separate test lot samples, wherein each separate test lot sample is attributed to a particular corresponding separate test lot. ICMSF states: "To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units of the enrichment cultures therefrom, using plans in which the presence of a single positive

	rejects the consignment. . . . This finding suggests that considerable reductions may be made in the cost of laboratory analysis by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, n, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort." ICMSF, 187. To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. A laboratory investigation undertaken for ICMSF (Silliker & Gabis, 1973) indicated that different sized analytical units drawn from a single well-mixed sample unit of dried food give very similar results. A subsequent investigation (Gabis & Silliker, 1974) using foods of high moisture content (including eggs, poultry meat, meat, and meat products) showed comparable reliability in the use of composite analytical units; for example, examination of three composites of 20 × 25 g analytical units gave the same result as examination of all 60 analytical units individually (the total amount examined being 1500 g in both instances). This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n*, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort. ICMSF, 187.

10.5.1 Conceivable Alternative Plans

As indicated above, for an infectious agent such as *Salmonella*, there are strong reasons for adopting sampling plans in which $c = 0$:

1. It is philosophically objectionable to some people to accept a lot containing a recognized pathogen, regardless of how the food is to be used in a manufacturing process or by the consumer.
2. There is the very great technical advantage of combining many analytical units for bacteriological examination in systems with $c = 0$, because a single positive decides the outcome.
3. The plan with $c = 1$ (or more) would always require the examination of (many) more analytical units, for equal probability of acceptance, than a plan with $c = 0$.
4. Even if the indicated probabilities of plans with $c = 0$ versus $c = 1$ are the same, their implications are not. For example, when a lot is accepted with the plan $n = 60$, $c = 0$, it is possible (though not certain) that the lot may not be contaminated at all. But using the plan $n = 95$, $c = 1$ and accepting one positive, it is known that the lot certainly is contaminated (though the probability that a lot with 5% of analytical units contaminated would be accepted is in both instances $P_a = 0.05$).

ICMSF, 188 (annotated). ICMSF also provides an example of "[u]sing a sampling plan involving n = 15 (25 g analytical units) and a composite of 375 g." *Id.*, 195 (§ 10.7.2). ICMSF also states "[f]or qualitative analyses, 25 g analytical units are frequently analyzed individually or as composites of 100 g, 200 g, or more." *Id.*, 232 (§ 12.5.2).

The ICMSF incorporates two references, Silliker & Gabis (1973) and Gabis & Silliker (1974). Silliker notes that "[c]ompositing of multiple samples (lumping) and wet compositing of pre-enrichment cultures have been tested as economical approaches to *Salmonella* control." Silliker, 475.

Silliker further notes that "[b]oth approaches are being used widely by the food industry in the United States." *Id.*.

	To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. A laboratory investigation undertaken for ICMSF (Silliker & Gabis, 1973) indicated that different sized analytical units drawn from a single well-mixed sample unit of dried food give very similar results. A subsequent investigation (Gabis & Silliker, 1974) using foods of high moisture content (including eggs, poultry meat, meat, and meat products) showed comparable reliability in the use of composite analytical units; for example, examination of three composites of 20 × 25 g analytical units gave the same result as examination of all 60 analytical units individually (the total amount examined being 1500 g in both instances). This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n*, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort. "There is the very great technical advantage of combining many analytical units for bacteriological examination." ICMSF, 188. ICMSF also describes composites prepared by combining "15 (25 g analytical units)" and "composites of 100 g, 200 g, or more." *Id.,* 195, 232; *see* AAPA #5. ICMSF states, "All steps up to the analysis must be controlled . . . to allow reliable traceability." ICMSF, 227. ICMSF indicates lot samples are traceable to a particular corresponding lot: "It is therefore helpful . . . to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load). . . . Treating the individual production

	batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119.
[37c] c) enriching of the separate composited single production lot samples to provide a set of separate composited single production lot samples enriched for the target microbe(s);	ICMSF, alone or in combination with Hughes, discloses incubating the set of separate test lot samples under conditions suitable to allow levels of a target microbial agent or organism that is present in one or more of the separate test lot samples to reach detectable levels and become uniform, or substantially uniform, throughout the respective one or more separate test lot samples, to provide a set of separate test lot samples enriched for the target microbial agent or organism. ICMSF states: To reduce laboratory effort . . . it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. ICMSF, 187. Also, ICMSF describes preparing composites of analytical units then diluting "for qualitative (enrichment)" analysis. *Id.,* 231-32; *see also* AAPA #5, #7. For practical reasons, however, analytical units of 10 g up to 50 g are typical for quantitative methods and are diluted 1:10. For qualitative analyses, 25 g analytical units are frequently analyzed individually or as composites of 100 g, 200 g, or more. ICMSF, 232 (annotated). Preparation of the first dilution, either for qualitative (enrichment) or quantitative (direct) analyses, can have a marked impact on the microbial population in the sample. This is often due to changes in the physico-chemical characteristics of the suspension as compared to the food matrix. Where the food does not confer protection, the composition of the diluent is important to prevent or minimize shock/injury to the microorganisms that can result from rapid changes in the ionic concentrations. ICMSF, 232 (annotated). ICMSF also describes strategic use of non-selective enrichment to resuscitate microorganisms and selective enrichments for growing microorganisms. *Id.*, 232–33

	(§ 12.6); *see e.g.*, *id.,* 233 ("use of nonselective medium . . . to enhance recovery of injured or stressed cells."). ICMSF, § 12.6 also describes optimizing the incubation period. S*ee e.g.*, *id.,* 232. It was well known long before the priority date to use non-selective enrichments after dry compositing and prior to wet compositing as evidenced by Price, Silliker, Gabis, and MLG. A POSITA would also know to use selective enrichments after dry compositing and prior to wet compositing as disclosed by ICMSF and Hughes. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. **(a)** *Pre-enrichment.*—Pre-enrich product in noninhibitory broth to initiate growth of *Salmonella* spp. Methods used may vary with product and should be performed as indicated in **967.26A** or BAM, 7th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, chapter 5, section C. Pre-enrichment broths should be incubated at 35°–37°C. **(b)** *Selective enrichment.*—Transfer 0.1 mL incubated pre-enrichment mixtures to Rappaport-Vassiliadis R10 broth (9.9 mL) and 1 mL to TT broth (9 mL). For all foods other than raw foods or foods having a high microbial load, incubate 6–8 h at 41°–43°C. Selective enrichments of raw foods or foods having a high microbial load must be incubated 16–20 h at 41°–43°C. An air incubator is suitable for this purpose. Hughes, 636.

[37d] d) removing portions of each separate enriched composited single production lot sample, and combining the removed portions to provide a pooled modular composite sample; and	ICMSF describes compositing the enriched lot samples: "it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. . . . This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n,* with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort." ICMSF, 187. AAPA #5-#7. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Hughes also discloses a method of removing portions of each enriched separate test lot sample, and combining the removed portions to provide a modular composite sample. Hughes instructs to "[c]ombine equal volumes (0.5-1 mL) from each M broth tube in a clean screw-cap tube." Hughes, 638.

	that has been warmed to 35°–37°C. Also remove 1 mL from Rappaport-Vassiliadis R10 tube and transfer to a separate tube of prewarmed M broth (10 mL). For all foods other than raw foods or foods having a high microbial load, incubate M broth tubes 16–20 h at 35°–37°C and return selective enrichment broth tubes to 41°–43°C incubator and incubate for additional 16–18 h. For raw foods or foods having a high microbial load, incubate M broth tubes 6 h at 35°–37°C and return selective enrichment broth tubes to 41°–43°C and incubate for an additional 6 h. (**d**) *Preparation of sample for EIA analysis.*—Remove M broth tubes from incubation and mix tubes by hand or Vortex mixer. Combine equal volumes (0.5–1 mL) from each M broth tube in a clean screw-cap tube and heat in boiling H_2O bath or in flowing steam 15 min. Refrigerate (2°–8°C) remaining M broth and TT and Rappaport-Vassiliadis R10 tubes from (**c**) for culture confirmation of any EIA-positive samples (*see* **967.26B, 967.26C, 967.27** and **967.28**). Cool heated M broths to 25°–37°C prior to analysis. Hughes, 638. Hughes 2003 confirms the pooling of enrichments. Hughes 2003, 775-76.
[37e.1] e) testing of the pooled modular composite sample, using a suitable detection assay, for the target microbe(s),	ICMSF describes testing enrichment cultures of composite samples with suitable detection assays (ICMSF, 187) and "presence/absence tests" (ICMSF, 233). Suitable detection assays were established in Silliker and Gabis as discussed in ICMSF. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section

	further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Hughes states testing of the composite sample is "by an enzyme immunoassay (EIA) method." Hughes, 635, 638. These assay types were "art recognized." '771 Patent, 3:8-12 (AAPA #7).
[35e.2] wherein when such testing is negative all of said samples that were composited to form the separate composited single production lot samples are deemed negative for the target microbe(s) and each of the multiple separate single production lots is validated, and	ICMSF describes correlating lots in a consignment with lot samples so a negative or positive test result enables a lot to be validated or rejected. ICMSF, 118-19. ICMSF describes sampling plans in which "c is the maximum allowable number of sample units yielding a positive result." ICMSF, 115. For many scenarios, the requirement is c = 0, which indicates the testing is negative. *Id.*, 118, 183-90, 309, 325, 326. A negative test result (*id.*, 113-15, 325, 326) is the expected and desired outcome to determine meat is not contaminated and is safe for consumption (*id.*, 184-89) such that a lot is validated and it is "commercially possible to market" (*id.*, 185) the meat.

6.10 WHAT IS A LOT?

Ideally, a lot is a quantity of food or food units produced and handled under uniform conditions. Under certain circumstances, it has been shown that the logarithms of the counts from a batch of food are likely to be normally distributed (Baird-Parker, 1980; Kilsby & Baird-Parker, 1983 [their Table 2]; Ingram & Roberts, 1976). The implication is that there is homogeneity within a lot, which, when considering microbial levels and distributions, rarely occurs in practice. In most instances, the distribution of microorganisms within lots of food is heterogeneous. Even in food factories, this heterogeneity makes the interpretation of sample results difficult, and it becomes even more so in circumstances where a lot is less well defined, such as in a consignment of a very large quantity of food.

It is therefore helpful if suppliers can be persuaded to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load).

If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment.

A lot should be composed of food produced with as little variation as possible for a given process or commodity. Because of the uncertainties in identifying a lot commercially, however, the use of the word *lot* in this book is usually in its statistical sense, as a collection of units of a product, the acceptability of which is determined by examining a sample drawn from it.

ICMSF, 118-19 (annotated).

	Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Because Hughes explains the steps to be taken when testing is positive (*see* ie.3), a POSITA would also expect negative testing results. Hughes, 638.
[35e.3] wherein when such testing is positive, each of the individual separate composited single production lot samples that were used to form the pooled modular composite sample are individually tested to determine which of the separate composited single production lot samples is positive for the target microbe(s),	ICMSF discloses the use of "immunomagnetic beads to concentrate cells." ICMSF, 318. A POSITA would consider this a well-known technique before the priority date. Hughes uses an EIA method followed by a more sensitive protocol—streaking on selective media. Hughes, 635. Each sample was analyzed by the BAM method (7th Ed.) and the TSVIA method using the enrichment protocol described in section F of the method, *Preparation of Sample*. Equal portions (0.5–1 mL) from each of the 2M broths arising from the 2 selective-enrichment broths were combined in a small screw-capped tube and heat treated in boiling water for 15 min prior to performing the TSVIA. Confirmation for the immunoassay was by streaking from the M broth culture onto selective agar, as specified in the BAM reference culture method. Hughes, 635. A POSITA would find that modifying Hughes based on D'Haese ensures a more sensitive test is used for further testing.

	A POSITA would have recognized the concentration step could be employed to provide a more sensitive protocol for further testing. A POSITA would understand that retesting the non-pooled, singular samples is an inherently more sensitive process because the sample is not diluted with other samples in the testing pool. Therefore, the retesting of a singular sample is more concentrated and therefore, more sensitive. *See also, generally*, Jarvis.
[35e.4] wherein the single production lots corresponding to any negatively testing composited single production lot samples are validated.	ICMSF describes correlating lots in a consignment with lot samples so a negative or positive test result enables a lot to be validated or rejected. ICMSF, 118-19. ICMSF describes sampling plans in which "c is the maximum allowable number of sample units yielding a positive result." ICMSF, 115. For many scenarios, the requirement is c = 0, which indicates the testing is negative. *Id.*, 118, 183-90, 309, 325, 326. A negative test result (*id.*, 113-15, 325, 326) is the expected and desired outcome to determine meat is not contaminated and is safe for consumption (*id.*, 184-89) such that a lot is validated and it is "commercially possible to market" (*id.*, 185) the meat.

6.10 WHAT IS A LOT?

Ideally, a lot is a quantity of food or food units produced and handled under uniform conditions. Under certain circumstances, it has been shown that the logarithms of the counts from a batch of food are likely to be normally distributed (Baird-Parker, 1980; Kilsby & Baird-Parker, 1983 [their Table 2]; Ingram & Roberts, 1976). The implication is that there is homogeneity within a lot, which, when considering microbial levels and distributions, rarely occurs in practice. In most instances, the distribution of microorganisms within lots of food is heterogeneous. Even in food factories, this heterogeneity makes the interpretation of sample results difficult, and it becomes even more so in circumstances where a lot is less well defined, such as in a consignment of a very large quantity of food.

It is therefore helpful if suppliers can be persuaded to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load).

If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment.

A lot should be composed of food produced with as little variation as possible for a given process or commodity. Because of the uncertainties in identifying a lot commercially, however, the use of the word *lot* in this book is usually in its statistical sense, as a collection of units of a product, the acceptability of which is determined by examining a sample drawn from it.

ICMSF, 118-19 (annotated).

	Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Because Hughes explains the steps to be taken when testing is positive (*see* ie.3)*,* a POSITA would also expect negative testing results. Hughes, 638. Determining meat is safe for consumption so it may be released into commerce is the well-understood point of testing meat. Moreover, the recitation of a lot being validated for "release . . . into commerce" is not limiting. Prosecution History for Reexamination Control No. 95/011,294 .
Claim 38	**Disclosure of ICMSF and Hughes**
The method of claim 37, wherein removing portions in step d) is removing equal portions, aseptically, so as to preclude contamination of the enriched separate composited single production lot samples.	ICMSF, alone or in combination with Hughes, discloses Claim 37, upon which this claim relies. See above re Claim 37. ICMSF describes aseptically withdrawing and weighing sample units into the appropriate diluent. ICMSF, 231, 232. Hughes instructs that after enrichment "[c]ombine equal volumes (0.5-1 mL) from each M broth tube in a clean screw-cap tube." Hughes, 638. Moreover, a POSITA would consider this to be standard practice. A POSITA would have aseptically removed equal volume portions of each enriched separate test lot sample.
Claim 41	**Disclosure of ICMSF and Hughes**
The method of claim 37, wherein each of the multiple separate single production lots are	ICMSF, alone or in combination with Hughes, discloses Claim 37, upon which this claim relies. See above re Claim 37.

separate single production lots of a meat or poultry product, comprising an assemblage of one or more meat or poultry specimens, respectively.	ICMSF indicates "[t]rimmings to be used for ground beef could be pretested for E. coli O157:H7 by various sampling plans." ICMSF, 323. ICMSF indicates "[r]esearch has validated that up to fifteen 25 g analytical units of ground beef or trimmings can be composited without significant loss in E. coli O157:H7 detection sensitivity." *Id.*, 327 *see also id.*, 226, 314, 320, and 324. ICMSF includes many other references to testing lots of beef or poultry, two of the most common types of meat in commerce. *Id.* at 114 (§ 6.3), 184–191 (§§ 10.2–10.6), 225–226 (§ 12.1), 285 (§ 16.1), 313–330 (Chapter 17). ICMSF is relevant to any kind of food product including beef or poultry. Hughes discloses testing turkey for *Salmonella*. "Three food types (. . . and raw turkey) were analyzed in the United States." Hughes, 634, 645, Table 8. Table 8 shows results from 12 collaborators for naturally contaminated raw turkey. For the 180 samples tested, using confirmed data, there was one false negative at the low level for the TECRA method. There were 3 false negatives at the low level and 3 false negatives at the high level for the cultural method. The incidence of false-positive results was very high for the cultural method, with a total of 41 false positives, compared with 5 for the TECRA method. It should, however, be taken into account that the comparison was with the method in BAM 7th Ed., not the current 8th Ed. The method for raw flesh food in the BAM 8th Ed. uses elevated temperature with RV and TT broth, which would be more selective. No significant Hughes, 646.
Claim 42	**Disclosure of ICMSF and Hughes**

The method of claim 41, wherein the meat or poultry specimens are meat trim pieces or poultry trim pieces, respectively.	ICMSF, alone or in combination with Hughes, discloses Claim 41, upon which this claim relies. See above re Claim 41. ICMSF indicates "[t]rimmings to be used for ground beef could be pretested for E. coli O157:H7 by various sampling plans." ICMSF, 323. ICMSF indicates "[r]esearch has validated that up to fifteen 25 g analytical units of ground beef or trimmings can be composited without significant loss in E. coli O157:H7 detection sensitivity." *Id.*, 327 *see also id.*, 226, 314, 320, and 324. ICMSF includes many other references to testing lots of beef or poultry, two of the most common types of meat in commerce. *Id.* at 114 (§ 6.3), 184–191 (§§ 10.2–10.6), 225–226 (§ 12.1), 285 (§ 16.1), 313–330 (Chapter 17). ICMSF is relevant to any kind of food product including beef or poultry.
Claim 43	**Disclosure of ICMSF and Hughes**
The method of claim 42, wherein the meat is that of beef, pork, sheep, or bison.	ICMSF, alone or in combination with Hughes, discloses Claim 42, upon which this claim relies. See above re Claim 42. ICMSF indicates "[t]rimmings to be used for ground beef could be pretested for E. coli O157:H7 by various sampling plans." ICMSF, 323. ICMSF indicates "[r]esearch has validated that up to fifteen 25 g analytical units of ground beef or trimmings can be composited without significant loss in E. coli O157:H7 detection sensitivity." *Id.*, 327 *see also id.*, 226, 314, 320, and 324. ICMSF includes many other references to testing lots of beef or poultry, two of the most common types of meat in commerce. *Id.* at 114 (§ 6.3), 184–191 (§§ 10.2–10.6), 225–226 (§ 12.1), 285 (§ 16.1), 313–330 (Chapter 17). ICMSF is relevant to any kind of food product including beef or poultry.
Claim 44	**Disclosure of ICMSF and Hughes**
The method of claim 43, wherein the meat is that of beef.	ICMSF, alone or in combination with Hughes, discloses Claim 43, upon which this claim relies. See above re Claim 43.

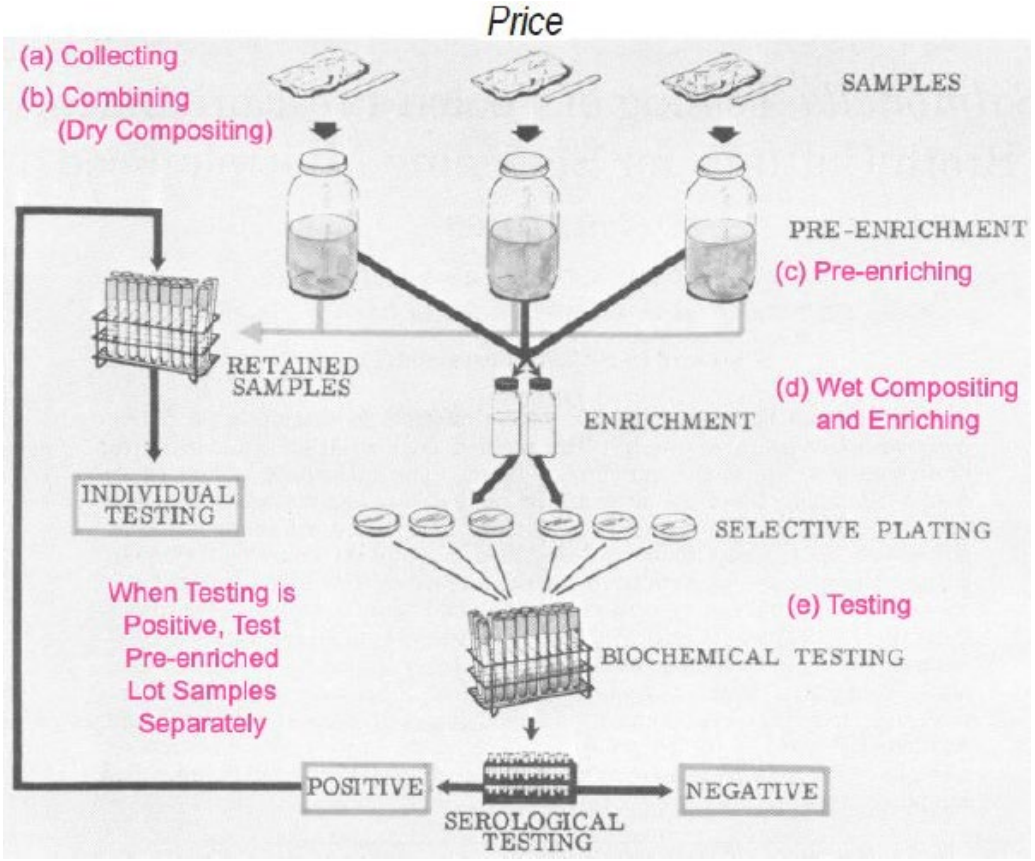
	ICMSF indicates "[t]rimmings to be used for ground beef could be pretested for E. coli O157:H7 by various sampling plans." ICMSF, 323. ICMSF indicates "[r]esearch has validated that up to fifteen 25 g analytical units of ground beef or trimmings can be composited without significant loss in E. coli O157:H7 detection sensitivity." *Id.*, 327 *see also id.*, 226, 314, 320, and 324. ICMSF includes many other references to testing lots of beef or poultry, two of the most common types of meat in commerce. *Id.* at 114 (§ 6.3), 184–191 (§§ 10.2–10.6), 225–226 (§ 12.1), 285 (§ 16.1), 313–330 (Chapter 17). ICMSF is relevant to any kind of food product including beef or poultry.
Claim 48	**Disclosure of ICMSF and Hughes**
The method of claim 37, wherein the target microbe(s) comprises at least one selected from the group consisting of *Listeria*, *Salmonella*, and *Escherichia coli*.	ICMSF, alone or in combination with Hughes, discloses Claim 37, upon which this claim relies. See above re Claim 37. ICMSF describes testing for *Listeria* (ICMSF, 285-312), *Salmonella* (*id.*, 273-84), and *Escherichia coli* (*id.*, 313-32). Hughes focuses on *Salmonella*. Hughes, 634.
Claim 49	**Disclosure of ICMSF and Hughes**
The method of claim 48, wherein the target microbe(s) comprises at least one selected from the group consisting of *Listeria* spp., *Listeria monocytogenes*, *Salmonella* spp., *Escherichia coli* O157:H7, coliforms, and fecal coliforms.	ICMSF, alone or in combination with Hughes, discloses Claim 48, upon which this claim relies. See above re Claim 48. ICMSF describes testing for *Listeria* (ICMSF, 285-312), *Salmonella* (*id.*, 273-84), and *Escherichia coli* (*id.*, 313-32). Hughes focuses on *Salmonella*. Hughes, 634.

EXHIBIT B3

Invalidity of U.S. Patent No. 9,637,771 in light of Price, ICMSF and Hughes

National Beef denies that any of its microbiological testing methods infringe any claim of 9,637,771 ("the '771 Patent"). If the claims of the '771 Patent were construed so broadly as to cover the microbiological testing methods used by National Beef, they would be invalid at least in view of W.R. Price et al., *Salmonella Testing of Pooled PreEnrichment Broth Cultures for Screening Multiple Food Samples*, 23 APPLIED MICROBIOLOGY 679–682 (1972) ("Price"); INT'L COMM'N ON MICROBIOLOGICAL SPEC'S FOR FOODS (ICMSF), MICROORGANISMS IN FOODS 7: MICROBIOLOGICAL TESTING IN FOOD SAFETY MANAGEMENT (Kluwer Academic/Plenum Publishers, 2002) ("ICMSF"); and Denise Hughes et al., *Salmonella in Foods—A New Enrichment Procedure for Use with the TECRA Salmonella Visual Immunoassay: Collaborative Study*, 82 J. AOAC INT'L 634–647 (1999) ("Hughes"), which anticipate and/or disclose the limitations of the '771 Patent under at least pre-AIA 35 U.S.C. § 102(a), pre-AIA 35 U.S.C. § 102(b), pre-AIA 35 U.S.C. §§ 102(f)/(g), and/or pre-AIA 35 U.S.C. § 103. To the extent that Price, ICMSF, and/or Hughes do not expressly disclose one or more limitations of the asserted claims, as they would have to be construed to support IEH's claim of infringement, they disclose the limitations alone or in combination with one or more additional prior art references and/or the knowledge of one of ordinary skill in the art.

Claim 1	**Disclosure of Price, ICMSF, and Hughes**
[1pre] A method of sampling and testing for microbes in multiple separate test lots, comprising:	To the extent the preamble is limiting, as discussed herein, Price, alone or in combination with ICMSF and Hughes, discloses a method of sampling and testing for microbes in multiple separate test lots. Price established that "pre-enrichment broths can be pooled for analysis without loss in sensitivity to *Salmonella* in the individual samples." Price, 679. Price tested dry food materials inoculated (purposefully contaminated) with *Salmonella. Id.*



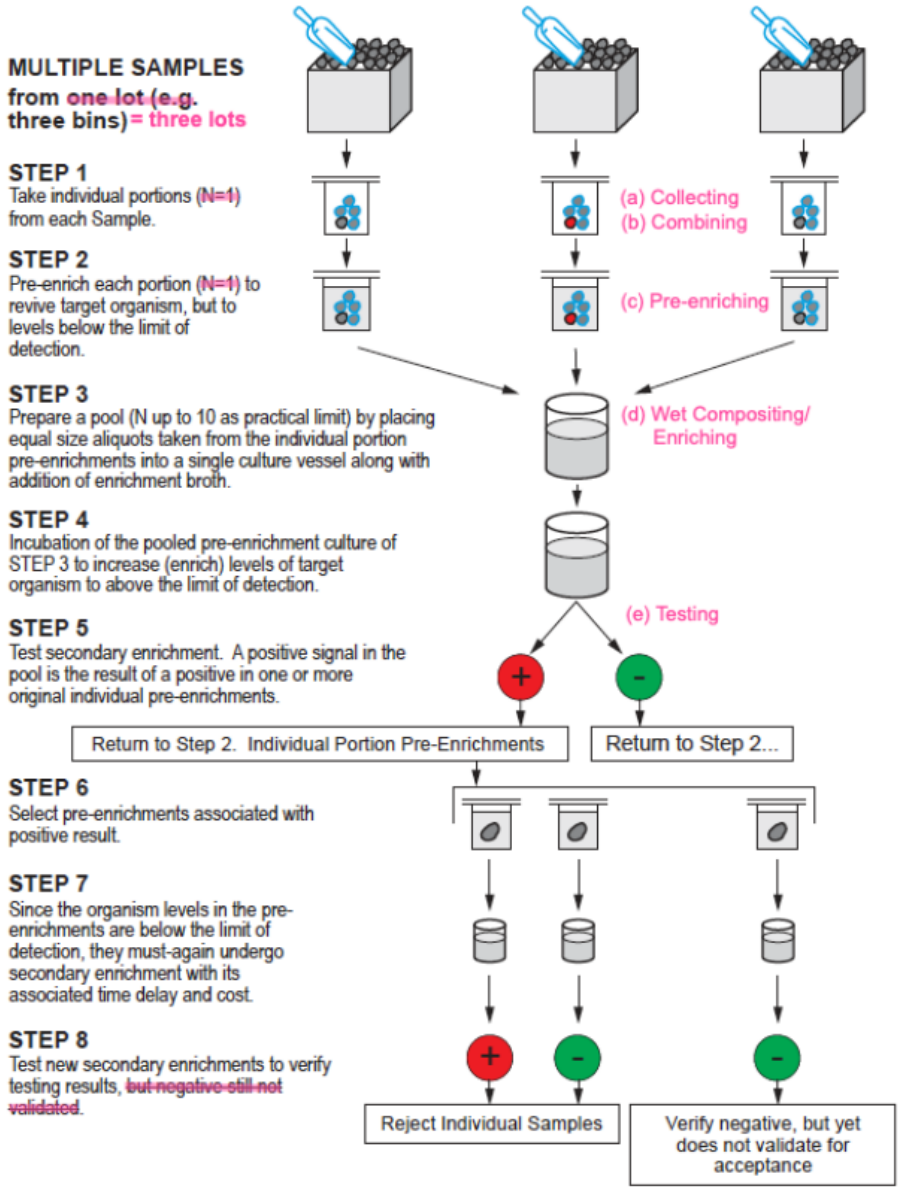
Price, 680 (Figure 1 (annotated)).

ICMSF includes many other references to testing lots of beef or poultry, two of the most common types of meat in commerce. *Id.*, 114 (§ 6.3), 184–191 (§§ 10.2–10.6), 225–226 (§ 12.1), 285 (§ 16.1), 313–330 (Chapter 17). ICMSF is relevant to any kind of food product including beef.

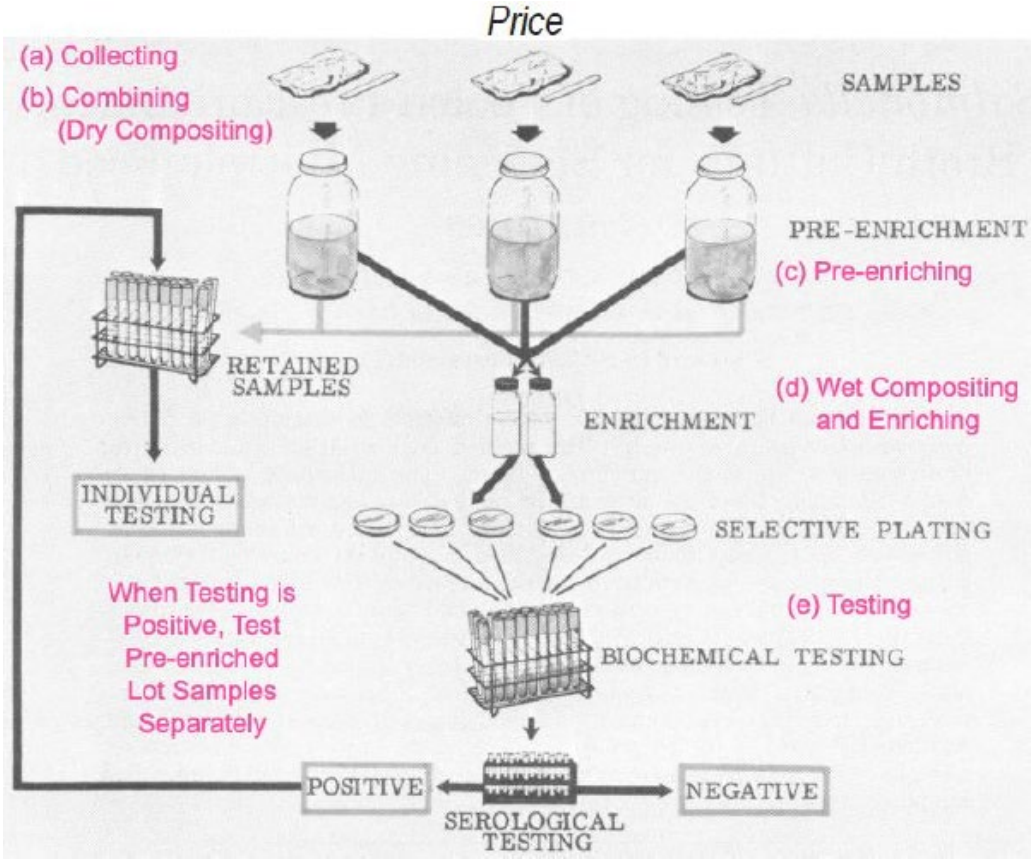
	Hughes discusses turkey (Hughes, Abstract), and a POSITA would be motivated to combine the teachings of ICMSF and Hughes as they both test meat for release into commerce. Hughes discloses testing turkey for *Salmonella*. "Three food types (. . . and raw turkey) were analyzed in the United States." Hughes, 634, 645, Table 8. Table 8 shows results from 12 collaborators for naturally contaminated raw turkey. For the 180 samples tested, using confirmed data, there was one false negative at the low level for the TECRA method. There were 3 false negatives at the low level and 3 false negatives at the high level for the cultural method. The incidence of false-positive results was very high for the cultural method, with a total of 41 false positives, compared with 5 for the TECRA method. It should, however, be taken into account that the comparison was with the method in BAM 7th Ed., not the current 8th Ed. The method for raw flesh food in the BAM 8th Ed. uses elevated temperature with RV and TT broth, which would be more selective. No significant Hughes, 646. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Moreover, the recitation of a lot being validated for "release . . . into commerce" is not limiting. Prosecution History for Reexamination Control No. 95/011,294.

[1a] a) separately collecting multiple independent samples from each of multiple separate lots, wherein each separate lot is separately sampled by taking said multiple independent samples thereof;	Price, alone or in combination with ICMSF and Hughes, discloses separately collecting multiple independent samples from each of multiple separate test lots, wherein each separate test lot is separately sampled by taking said multiple independent samples thereof. Price's teachings for collecting a plurality of portions are based on standard practices as described in BAM 1969 (BAM 1969, 1 § 10.04) which cites to JAOAC 1967 (JAOAC, 234). Price, 679, 682. ***Salmonella* analyses were carried out as described in the *Bacteriological Analytical Manual* (BAM) (2) except for the pooling of pre-enrichment broths described in the following paragraph.** Price, 679. IEH's diagram of Price from Prosecution History for Reexamination Control No. 95/001,294 (reproduced below) shows step 1 corresponds to IEH's step (a), with instructions to "[t]ake individual portions . . . from each Sample."

Figure 3: CITED PRIOR ART (PRICE et al, 1972)



	Prosecution History for Reexamination Control No. 95/001,294, 298 (annotated). IEH's diagram also indicates multiple samples are taken from three bins, which is analogous to three lots rather than a single lot because each bin is an assemblage of specimens. The purpose of Jarvis and Price was to determine whether pooling samples reduced the ability to detect contaminated food lots. It would have been commonsense to combine samples from the same bin and subject them to enrichment, since each bin represents a discrete unit of the production run and results obtained from the enriched sample could be attributed to the bin from which the sample was obtained. Prosecution History for Reexamination Control No. 95/001,294, at 1575 (First Board Decision of January 20, 2015 at 16). Additionally, the specification describes a bin of dry food materials (almonds), like those in Price, as one example of a test lot/single unit of production, similar to a combo of beef. '771 Patent, 5:55-60. Price discloses testing of "Dried egg albumen, cocoa, and nonfat dry milk" (Price, 679) and "wheat flour, coconut, and cottonseed flour" (Price, 681).



Price, 680 (Figure 1 (annotated)).

	A method has been described for testing multiple food samples for *Salmonella* without loss in sensitivity. The method pools multiple pre-enrichment broth cultures into single enrichment broths. The subsequent stages of the *Salmonella* analysis are not altered. The method was found applicable to several dry food materials including nonfat dry milk, dried egg albumin, cocoa, cottonseed flour, wheat flour, and shredded coconut. As many as 25 pre-enrichment broth cultures were pooled without apparent loss in the sensitivity of *Salmonella* detection as compared to individual sample analysis. The procedure offers a simple, yet effective, way to increase sample capacity in the *Salmonella* testing of foods, particularly where a large proportion of samples ordinarily is negative. It also permits small portions of pre-enrichment broth cultures to be retained for subsequent individual analysis if positive tests are found. *Salmonella* testing of pooled pre-enrichment broths provides increased consumer protection for a given amount of analytical effort as compared to individual sample analysis. Price, 679. A POSITA would have replaced Price's collection and combination of dry food with ICMSF's collection and combination of portions from a consignment of meat lots in conformance to a statistical-based sampling plan for release into commerce. "Many different types of samples are collected and submitted to the laboratory for analysis. Some are sample units from lots or consignments of food or ingredients for lot acceptance determination. . . . A sample unit is a small portion of the lot in which the distribution of both numbers and types of microorganisms should be representative of the whole lot." ICMSF, 255. The lots may be beef or poultry. *Id.*, 184-91, 226, 230. Thus, ICMSF discloses collecting a plurality of samples or sample units (other synonyms include portions, specimens, and sub-samples) from lots of beef or poultry. ICMSF describes collecting from a lot or lots:

	"If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." *Id.*, 119. Additionally, ICMSF indicates a consignment may be "a very large quantity of food" and may be "composed of several lots, representing for example, different days of production from the same plant." *Id.*, 119, 120; *see also* Applicant Admitted Prior Art ("AAPA") #3-#5.

6.11 WHAT IS A REPRESENTATIVE SAMPLE?

A *representative sample* reflects, as far as is possible, the composition of the lot from which it is drawn.

How then should a representative sample be drawn? It is important to avoid bias and to draw a sufficient number of sample units to confidently make a judgment about a lot. Sampling at random is the universally recognized way of avoiding bias and is more reliable than consciously trying to draw the sample units from the various parts of a lot. The units (cartons or containers, particular weights of solid, or volumes of liquid) are drawn by using random numbers. There is, of course, no guarantee that a random sample has characteristics identical with those of the lot, but the randomness of sampling is the basis for calculation of the probability that a sample will give a biased result.

It is also possible, and may be desirable, to use a *stratified random sampling* approach, drawing a random sample of a given number of sample units from each *stratum* (e.g., each sublot or carton). The distribution of sample units on strata should correspond to the distribution of lot units on strata. This means that if each stratum contains the same quantity as every other one, the number of sample units per stratum should be the same. Otherwise, they should differ just as the proportions of the lot contained in the strata differ. Stratification is a device for handling known sources of variation and may be used where one has prior knowledge that the consignment is potentially not of uniform quality. A consignment might not be of uniform quality if portions of it are shipped in different vehicles or in different holds of a vessel, or if it is known that it is really composed of several lots representing, for example, different days of production from the same plant or different plants of the same company. The results for different strata should be assessed separately and then pooled if they appear to be homogeneous.

The calculation of acceptance probabilities is based on the assumption that random samples have been drawn and analyzed. Where physical or practical constraints prevent random sampling, such calculated probabilities may not be valid. Their reliability depends on how close reality approaches the ideal situation of random sampling. This fact should be borne in mind when subsequently interpreting results.

ICMSF, 119-20 (annotated).

6.10 WHAT IS A LOT?

Ideally, a lot is a quantity of food or food units produced and handled under uniform conditions. Under certain circumstances, it has been shown that the logarithms of the counts from a batch of food are likely to be normally distributed (Baird-Parker, 1980; Kilsby & Baird-Parker, 1983 [their Table 2]; Ingram & Roberts, 1976). The implication is that there is homogeneity within a lot, which, when considering microbial levels and distributions, rarely occurs in practice. In most instances, the distribution of microorganisms within lots of food is heterogeneous. Even in food factories, this heterogeneity makes the interpretation of sample results difficult, and it becomes even more so in circumstances where a lot is less well defined, such as in a consignment of a very large quantity of food.

It is therefore helpful if suppliers can be persuaded to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load).

If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment.

A lot should be composed of food produced with as little variation as possible for a given process or commodity. Because of the uncertainties in identifying a lot commercially, however, the use of the word *lot* in this book is usually in its statistical sense, as a collection of units of a product, the acceptability of which is determined by examining a sample drawn from it.

ICMSF, 118-19 (annotated).

The ICMSF has defined a lot as follows:

> In the commercial sense, a lot is a quantity of food supposedly produced under identical conditions, all packages of which would normally bear a lot number that identifies the production during a particular time interval, and usually from a particular line, retort, or other critical processing unit. Statistically, a lot is considered as a collection of units of a product from which a sample is drawn to determine acceptability of the lot (ICMSF, 1986).

In this example, the maximum size of a lot consists of all the ground beef produced on common equipment from cleanup to cleanup. Smaller lots could be established (e.g., hourly).

ICMSF, 327 (annotated). This definition also reflects the common practice referred to in the IEH Patents at Example II, wherein "[p]referably, the producer defines and produces these single-unit lots in such a fashion as to maximize the information available." '771 Patent,13:12–16.

This corresponds with the specification of IEH's Patents which reflects the common practice of producers defining what constitutes a lot grouped for traceability purposes. '771 Patent, 3:14-24, 11:37-40 (AAPA #3), and 13:12-15.

Thus, ICMSF discloses collecting a plurality of portions from separate lots.

ICMSF describes a lot as "a collection of units of a product." IMCSF, 119. ICMSF further indicates a lot has been defined as: "a quantity of food supposedly produced under identical conditions, all packages of which would normally bear a lot number that identifies the production during a particular time interval, and usually from a particular line, retort, or other critical processing unit. Statistically, a lot is considered as a collection of units of a product from which a sample is drawn to determine acceptability of the lot." ICMSF, 327.

ICMSF advocates use of a "statistically based sampling plan." *Id.*, 113-15, 119-21, 320, 327, 328. In the sampling plans, "*n* is the number of sample units chosen separately and independently, and . . . *c* is the maximum allowable number of sample

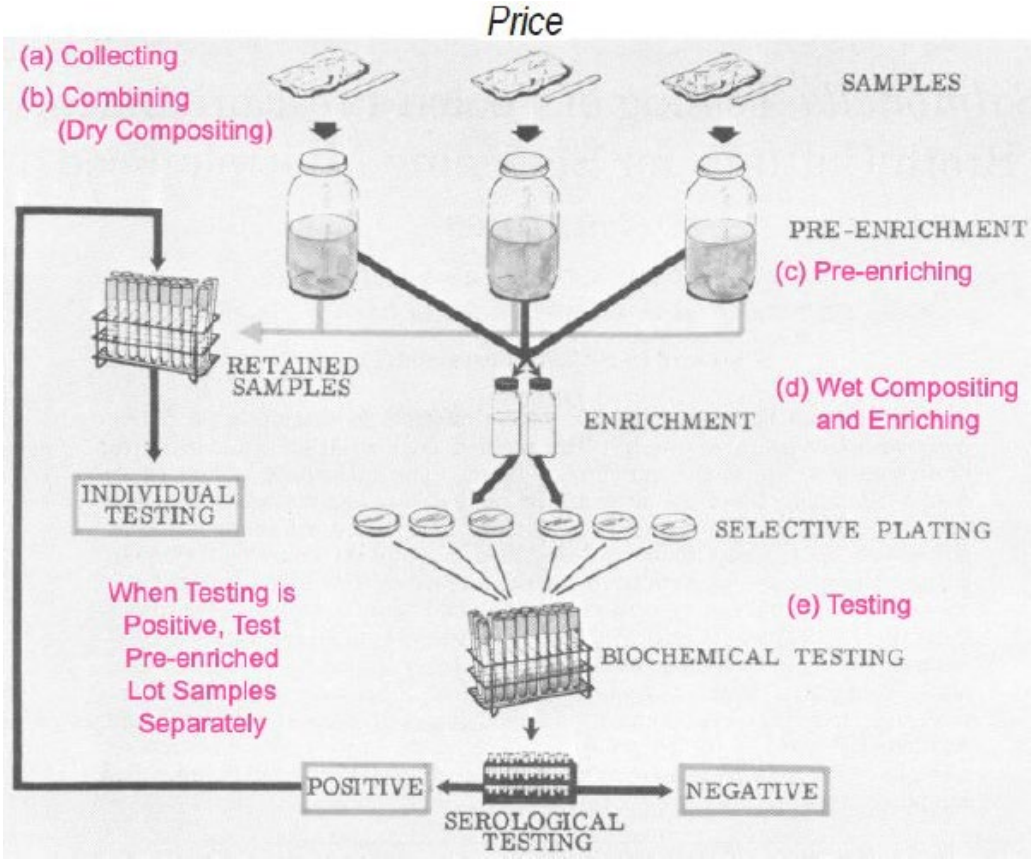
	units yielding a positive result." *Id.*, 115. ICMSF discloses a wide range for the number of sample units (portions) for a particular microbial agent or organism. For example, ICMSF discusses collecting 15, 30, 60 or 100 sample units from a lot. *Id.*, 320, 326. **6.5 THE SAMPLING PLAN** The sample units so drawn will, after examination, yield results that will be compared with certain criteria to reach a decision as to whether the entire lot should be accepted or rejected (see section 6.9 for explanation of rejection). The particular choice of sampling procedure and the decision criteria make up the *sampling plan*. Proper drawing of samples is essential if the decision making criteria of the sampling plan are to give unbiased results. By sampling randomly, we are able to reduce the risk of making biased decisions. A simple example of a sampling plan follows. Take 10 sample units of a food from a lot and subject them to appropriate laboratory procedure to test for the presence or absence of a microorganism. If two or fewer of the 10 sample units show presence of the organism, that is, give a positive result, then the whole lot of food is acceptable (relative to this organism). But if three or more give a positive result, the whole lot is to be rejected. This plan is described by the use of two terms $n = 10$ and $c = 2$, where n is the number of sample units chosen separately and independently, and where c is the maximum allowable number of sample units yielding a positive result. ICMSF, 115 (annotated).

	6.1 INTRODUCTION It is important to recognize that the management of food safety using the approaches outlined in the first five chapters, based on controlling hazards through Good Hygienic Practices (GHP) and Hazard Analysis Critical Control Point (HACCP), is much more effective than trying to ensure safety by lot acceptance through end-product testing. This chapter discusses the concepts of probability and sampling that determine the limitations of end-product testing. Those concepts form the basis of the design of statistically based sampling plans (Chapter 7) and the establishment of cases (Chapter 8). In Chapters 6 and 7, it is assumed that the microbiological test will give an absolutely accurate result, with no false positives or false negatives. This is not a realistic assumption, and problems arising from sampling, sample transport and preparation for analysis, and the imprecision of microbiological methods are addressed in Chapters 10 and 12 and elsewhere in the text. ICMSF, 113 (annotated); *see also id.*, 115–21 (§§ 6.4–6.13), 319–20 (§ 17.2.3.3), and 326–30 (§§ 17.5.3.1–17.6). ICMSF discusses the number of portions to be taken sufficient for validation of a particular microorganism including *Salmonella* (*id.*, 276 (§ 15.3.3); *see also id.*, 282 (§ 15.6.3)); *Listeria* (*id.*, 309 (§ 16.6.3)), and *E. coli* (*id.*, 318–21 (§§ 17.2.3.2–17.2.3.4), 326 (§ 17.5.3.1)). IEH's AAPA discloses statistical-based sampling plans taking 10-60 sample units. '771 Patent, 6:12-29; *see* AAPA #1, #2, #5, and #6. Determining meat is safe for consumption so it is "commercially possible to market" the meat, is the well-understood point of testing meat and is taught by ICMSF. ICMSF, 185-89. Moreover, the recitation of a lot being validated for "release . . . into commerce" is not limiting. Prosecution History for Reexamination Control No. 95/011,294.
[1b] b) separately compositing the collected multiple independent samples from each of the separate lots to provide a corresponding set of separate composited lot samples,	Price, alone or in combination with ICMSF and Hughes, discloses separately compositing the collected multiple independent samples from each of the separate test lots to provide a corresponding set of separate composited test lot samples,

wherein each of the separate composited lot samples is attributed to a particular corresponding separate lot;	wherein each of the separate composited test lot samples is attributed to a particular corresponding separate test lot. Like ICMSF, the test lot samples in Price are attributable to a particular lot. For example, Price's abstract states that the procedure "permits small portions of pre-enrichment broth cultures to be retained for subsequent individual analysis if positive tests are found," as shown below: **A method has been described for testing multiple food samples for *Salmonella* without loss in sensitivity. The method pools multiple pre-enrichment broth cultures into single enrichment broths. The subsequent stages of the *Salmonella* analysis are not altered. The method was found applicable to several dry food materials including nonfat dry milk, dried egg albumin, cocoa, cottonseed flour, wheat flour, and shredded coconut. As many as 25 pre-enrichment broth cultures were pooled without apparent loss in the sensitivity of *Salmonella* detection as compared to individual sample analysis. The procedure offers a simple, yet effective, way to increase sample capacity in the *Salmonella* testing of foods, particularly where a large proportion of samples ordinarily is negative. It also permits small portions of pre-enrichment broth cultures to be retained for subsequent individual analysis if positive tests are found. *Salmonella* testing of pooled pre-enrichment broths provides increased consumer protection for a given amount of analytical effort as compared to individual sample analysis.** Price, 679. Price further describes this retention step as enabling "individual tests on the retained sample" to "be made to determine which sample or samples contributed *Salmonella* to the pool" as shown below:

	MATERIALS AND METHODS *Salmonella* analyses were carried out as described in the *Bacteriological Analytical Manual* (BAM) (2) except for the pooling of pre-enrichment broths described in the following paragraph. The procedure for *Salmonella* testing of pooled pre-enrichment broths is illustrated in Fig. 1. Samples are individually pre-enriched followed by transfer from multiple pre-enrichments to single selenite and tetrathionate broths at the enrichment stage. During pooling, a portion of each pre-enrichment is transferred to a sterile culture tube and retained at 4 C for later reference to the individual samples. If the *Salmonella* test of the pooled pre-enrichment broths is positive, individual tests on the retained samples can be made to determine which sample or samples contributed *Salmonella* to the pool. *Id.* (highlighting added). A POSITA would understand Price to implicitly disclose combining the collected confirming plurality of portions based on the inherent mixing involved with dry food materials.

	Salmonella-positive test samples were prepared by blending the dry test food with one of three dry inocula. Inoculum 1 was a dry enzyme drain cleaner which had been found to contain multiple *Salmonella* serotypes including E_1, z_{10}; G, z_{29} (*S. cubana*); and C_1, g complex. Inoculum 2 was a freeze-dried, skim milk suspension of serotypes *S. anatum*, *S. binza*, *S. tennessee*, *S. worthington*, *S. cubana*, *S. braenderup*, and a B_1, g complex. Inoculum 3 consisted of four laboratory isolates of serotypes G, z_{29} (*S. cubana*); E_1, b; E_1, l complex; and C_1, l complex grown in a sterile aqueous suspension of cottonseed flour and freeze dried. Price, 679.



Price, 680 (Figure 1 (annotated)).

A POSITA would have replaced Price's collection and combination of dry food with ICMSF's collection and combination of portions from a consignment of meat lots in conformance to a statistical-based sampling plan for release into commerce.

ICMSF states: "To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units of the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. . . . This finding suggests that considerable reductions may

	be made in the cost of laboratory analysis by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, n, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort." ICMSF, 187. To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. A laboratory investigation undertaken for ICMSF (Silliker & Gabis, 1973) indicated that different sized analytical units drawn from a single well-mixed sample unit of dried food give very similar results. A subsequent investigation (Gabis & Silliker, 1974) using foods of high moisture content (including eggs, poultry meat, meat, and meat products) showed comparable reliability in the use of composite analytical units; for example, examination of three composites of 20 × 25 g analytical units gave the same result as examination of all 60 analytical units individually (the total amount examined being 1500 g in both instances). This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n*, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort. ICMSF, 187.

10.5.1 Conceivable Alternative Plans

As indicated above, for an infectious agent such as *Salmonella*, there are strong reasons for adopting sampling plans in which $c = 0$:

1. It is philosophically objectionable to some people to accept a lot containing a recognized pathogen, regardless of how the food is to be used in a manufacturing process or by the consumer.
2. There is the very great technical advantage of combining many analytical units for bacteriological examination in systems with $c = 0$, because a single positive decides the outcome.
3. The plan with $c = 1$ (or more) would always require the examination of (many) more analytical units, for equal probability of acceptance, than a plan with $c = 0$.
4. Even if the indicated probabilities of plans with $c = 0$ versus $c = 1$ are the same, their implications are not. For example, when a lot is accepted with the plan $n = 60$, $c = 0$, it is possible (though not certain) that the lot may not be contaminated at all. But using the plan $n = 95$, $c = 1$ and accepting one positive, it is known that the lot certainly is contaminated (though the probability that a lot with 5% of analytical units contaminated would be accepted is in both instances $P_a = 0.05$).

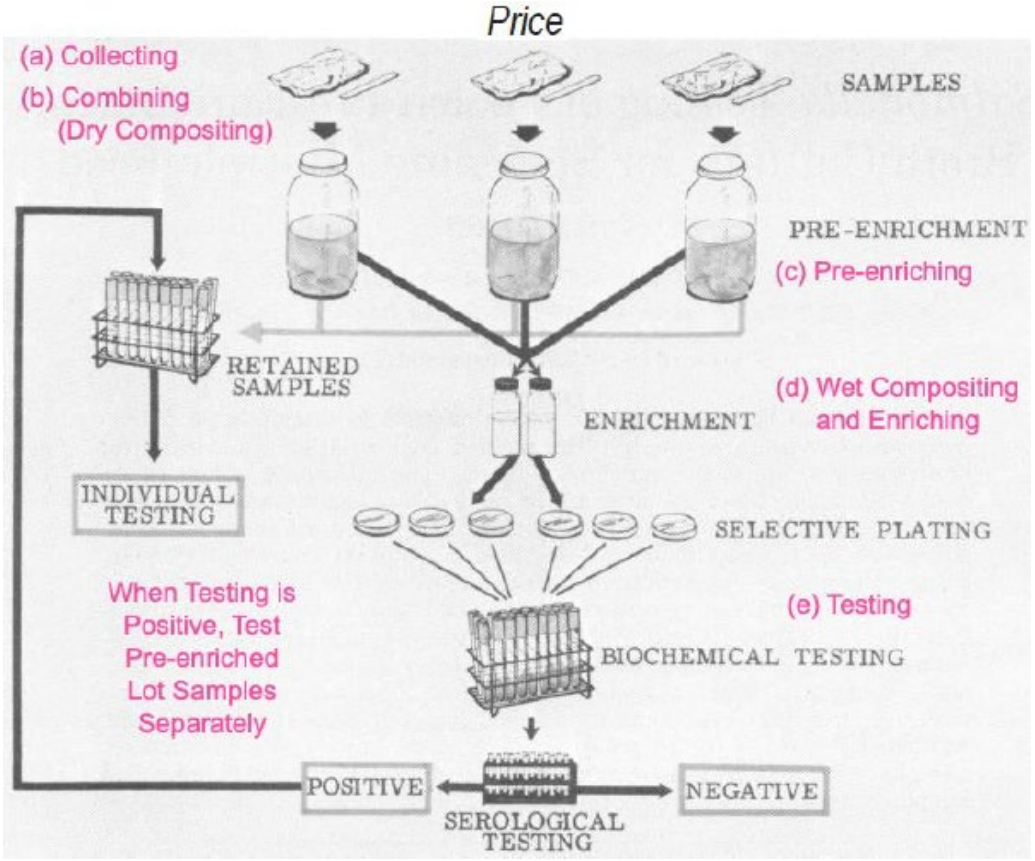
ICMSF, 188 (annotated). ICMSF also provides an example of "[u]sing a sampling plan involving n = 15 (25 g analytical units) and a composite of 375 g." *Id.*, 195 (§ 10.7.2). ICMSF also states "[f]or qualitative analyses, 25 g analytical units are frequently analyzed individually or as composites of 100 g, 200 g, or more." *Id.*, 232 (§ 12.5.2).

The ICMSF incorporates two references, Silliker & Gabis (1973) and Gabis & Silliker (1974). Silliker notes that "[c]ompositing of multiple samples (lumping) and wet compositing of pre-enrichment cultures have been tested as economical approaches to *Salmonella* control." Silliker, 475.

Silliker further notes that "[b]oth approaches are being used widely by the food industry in the United States." *Id.*.

	To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. A laboratory investigation undertaken for ICMSF (Silliker & Gabis, 1973) indicated that different sized analytical units drawn from a single well-mixed sample unit of dried food give very similar results. A subsequent investigation (Gabis & Silliker, 1974) using foods of high moisture content (including eggs, poultry meat, meat, and meat products) showed comparable reliability in the use of composite analytical units; for example, examination of three composites of 20 × 25 g analytical units gave the same result as examination of all 60 analytical units individually (the total amount examined being 1500 g in both instances). This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n*, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort. "There is the very great technical advantage of combining many analytical units for bacteriological examination." ICMSF, 188. ICMSF also describes composites prepared by combining "15 (25 g analytical units)" and "composites of 100 g, 200 g, or more." *Id.,* 195, 232; *see* AAPA #5. ICMSF states, "All steps up to the analysis must be controlled . . . to allow reliable traceability." ICMSF, 227. ICMSF indicates lot samples are traceable to a particular corresponding lot: "It is therefore helpful . . . to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load). . . . Treating the individual production

	batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119.
[1c] c) enriching each of the separate composited lot samples to provide a set of separate composited lot samples enriched for the target microbe(s);	Price, alone or in combination with ICMSF and Hughes, discloses enriching each of the separate composited test lot samples to provide a set of separate composited test lot samples enriched for the target microbe(s). Price discloses "[s]amples are individually pre-enriched followed by transfer from multiple pre-enrichments to single selenite and tetrathionate broths at the enrichment stage." Price, 679. These pre-enrichment and enrichment steps inherently disclose an incubation period in which levels of the target microbial agent, in this case, *Salmonella*, grow to detectable levels for testing. Price states "[i]n the multiple pooling of pre-enrichment broth cultures . . . , 1-ml quantities of cultures were transferred, generally, to a volume of enrichment broth." Price, 682. Price also states "[a]lternatively, problems with interfering organisms may warrant the use of direct selective enrichments." *Id.* Based on this statement, the Board noted "there is a suggestion in Price (at 682) . . . of direct enrichment." Prosecution History for Reexamination Control No. 95/001,294, 1576.



Price, 680 (Figure 1 (annotated)).

Price states "[a]lternatively, problems with interfering organisms may warrant the use of direct selective enrichments" and further mentions "a reduction in the incubation time of the pre-enrichment cultures." Price inherently discloses an incubation period of the pre-enrichment samples.

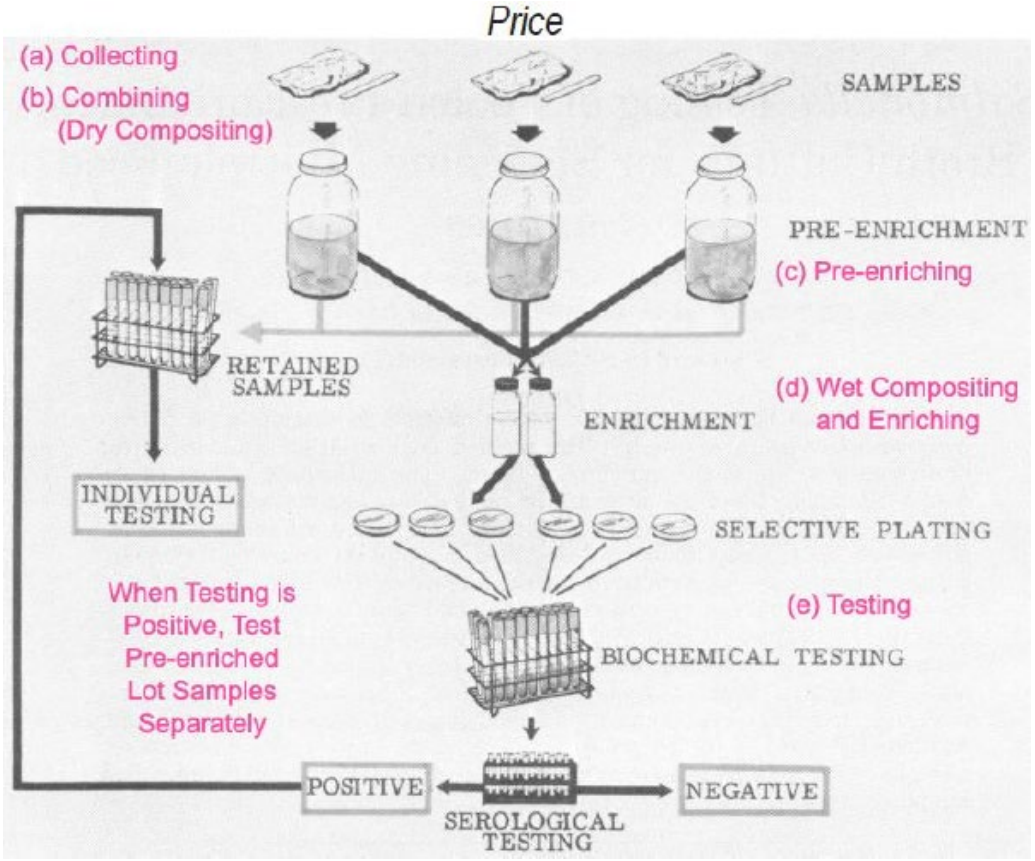
	Whether pooled pre-enrichments are less sensitive than individual tests in the presence of interfering organisms probably is not known. For the present, we would recommend that, if numerous non-*Salmonella* colonies that resemble *Salmonella* on selective agar media are encountered in the pooled test, the retained pre-enrichment cultures should be tested individually. Alternatively, problems with interfering organisms may warrant the use of direct selective enrichments, two selective enrichments, or possibly even a reduction in the incubation time of the pre-enrichment cultures. Price, 682 (highlighted added). A POSITA would have replaced Price's pre-enriching and compositing pre-enrichments with teachings of ICMSF and Hughes for enriching lot samples, removing portions of each enriched lot sample, and combining the portions to provide a composite sample. Also, instead of retaining portions of pre-enrichments like Price, a POSITA would have retained portions of enriched lot samples as taught by ICMSF and Hughes. ICMSF states: To reduce laboratory effort . . . it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. ICMSF, 187. Also, ICMSF describes preparing composites of analytical units then diluting "for qualitative (enrichment)" analysis. *Id.,* 231-32; *see also* AAPA #5, #7.

	For practical reasons, however, analytical units of 10 g up to 50 g are typical for quantitative methods and are diluted 1:10. For qualitative analyses, 25 g analytical units are frequently analyzed individually or as composites of 100 g, 200 g, or more. ICMSF, 232 (annotated). Preparation of the first dilution, either for qualitative (enrichment) or quantitative (direct) analyses, can have a marked impact on the microbial population in the sample. This is often due to changes in the physico-chemical characteristics of the suspension as compared to the food matrix. Where the food does not confer protection, the composition of the diluent is important to prevent or minimize shock/injury to the microorganisms that can result from rapid changes in the ionic concentrations. ICMSF, 232 (annotated). ICMSF also describes strategic use of non-selective enrichment to resuscitate microorganisms and selective enrichments for growing microorganisms. *Id.*, 232–33 (§ 12.6); *see e.g.*, *id.,* 233 ("use of nonselective medium . . . to enhance recovery of injured or stressed cells."). ICMSF, § 12.6 also describes optimizing the incubation period. S*ee e.g.*, *id.,* 232. It was well known long before the priority date to use non-selective enrichments after dry compositing and prior to wet compositing as evidenced by Price, Silliker, Gabis, and MLG. A POSITA would also know to use selective enrichments after dry compositing and prior to wet compositing as disclosed by ICMSF and Hughes. A POSITA would find that incubating so microorganisms are detectable and at least substantially uniform in the selective enrichment broth was considered the standard practice in official methods because the methods otherwise fail. For example, D'Aoust states "standard methodology recommends incubation of selective enrichment broths." D'Aoust, 372. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section

	further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. **(a)** *Pre-enrichment.*—Pre-enrich product in noninhibitory broth to initiate growth of *Salmonella* spp. Methods used may vary with product and should be performed as indicated in **967.26A** or BAM, 7th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, chapter 5, section C. Pre-enrichment broths should be incubated at 35°–37°C. **(b)** *Selective enrichment.*—Transfer 0.1 mL incubated pre-enrichment mixtures to Rappaport-Vassiliadis R10 broth (9.9 mL) and 1 mL to TT broth (9 mL). For all foods other than raw foods or foods having a high microbial load, incubate 6–8 h at 41°–43°C. Selective enrichments of raw foods or foods having a high microbial load must be incubated 16–20 h at 41°–43°C. An air incubator is suitable for this purpose. Hughes, 636. Hughes instructs that, after pre-enrichment, portions of the "incubated pre-enrichment mixtures" should be transferred to two separate selective enrichment broths, Rappaport-Vassiliadis R10 broth and TT broth, and incubated for 16-20 hours at 41°-43°C. Hughes, 636, 638. Additionally, Hughes instructs that these selective broths should be removed from incubation and mixed by hand or by Vortex mixer. Hughes, 638. Hughes further instructs to then remove 1 mL from the TT tube and 1 mL from the Rappaport-Vassiliadis R10 tube and transfer to separate, sterile M broths. *Id.,* 636, 638. The separate M broths are then incubated ("16-20 h at 35°-37°C" for "all foods other than raw foods or foods having a high microbial load" or "6 h at 35°-37°C" for

	"raw foods or foods having a high microbial load"). *Id.*, 638. A POSITA would find that the long incubation at an elevated temperature and mixing ensures the target microbial agent or organism reaches detectable levels and becomes substantially uniform throughout the separate lot samples.
[1d] d) removing portions of each separate enriched composited lot sample, and combining the removed portions to provide a pooled modular composite sample; and	Price, alone or in combination with ICMSF and Hughes, discloses removing portions of each enriched separate test lot sample, and combining the removed portions to provide a modular composite sample. Price discloses wet compositing as "[s]amples are individually pre-enriched followed by transfer from multiple pre-enrichments to single selenite and tetrathionate broths at the enrichment stage." Price, 679. **MATERIALS AND METHODS** ***Salmonella*** **analyses were carried out as described in the *Bacteriological Analytical Manual* (BAM) (2) except for the pooling of pre-enrichment broths described in the following paragraph.** **The procedure for *Salmonella* testing of pooled pre-enrichment broths is illustrated in Fig. 1. Samples are individually pre-enriched followed by transfer from multiple pre-enrichments to single selenite and tetrathionate broths at the enrichment stage. During pooling, a portion of each pre-enrichment is transferred to a sterile culture tube and retained at 4 C for later reference to the individual samples. If the *Salmonella* test of the pooled pre-enrichment broths is positive, individual tests on the retained samples can be made to determine which sample or samples contributed *Salmonella* to the pool.** Price 679.

	Pooled samples versus pooled pre-enrichment broths. Silliker (4) reported a loss in sensitivity when several samples were pooled in a large container of pre-enrichment broth as compared to individual sample analysis. This is not surprising since, to be detected, the desiccated and debilitated *Salmonella* cells in a dried food sample must grow out in pre-enrichment broth in competition with other bacteria in the sample. If many food samples are pooled at the pre-enrichment stage, the salmonellae face competition from the flora of all the samples in the pool. If, on the other hand, the samples are individually pre-enriched, the actively growing salmonellae might be expected to compete more favorably with organisms introduced from other samples when pooled at the enrichment stage. In addition, pooling pre-enrichment broths avoids the hazards and inconvenience of handling very large flasks of culture. Price, 681 (discussing Silliker).

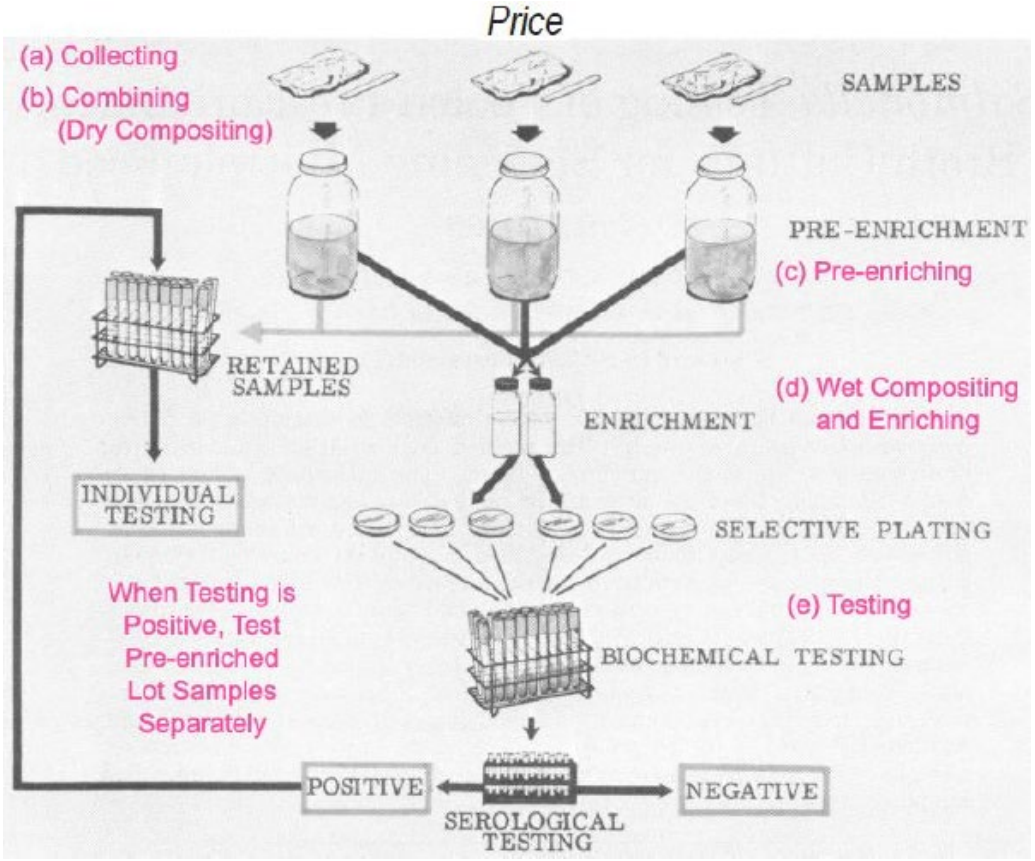


Price, 680 (Figure 1 (annotated)). Price's Figure 1 indicates that selective plating, biochemical testing, and serological testing are used.

A POSITA would have replaced Price's pre-enriching and compositing pre-enrichments with teachings of ICMSF and Hughes for enriching lot samples, removing portions of each enriched lot sample, and combining the portions to provide a composite sample. Also, instead of retaining portions of pre-enrichments like Price, a POSITA would have retained portions of enriched lot samples as taught by ICMSF and Hughes.

	ICMSF describes compositing the enriched lot samples: "it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. . . . This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n,* with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort." ICMSF, 187. AAPA #5-#7. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Hughes also discloses a method of removing portions of each enriched separate test lot sample, and combining the removed portions to provide a modular composite sample. Hughes instructs to "[c]ombine equal volumes (0.5-1 mL) from each M broth tube in a clean screw-cap tube." Hughes, 638.

	that has been warmed to 35°–37°C. Also remove 1 mL from Rappaport-Vassiliadis R10 tube and transfer to a separate tube of prewarmed M broth (10 mL). For all foods other than raw foods or foods having a high microbial load, incubate M broth tubes 16–20 h at 35°–37°C and return selective enrichment broth tubes to 41°–43°C incubator and incubate for additional 16–18 h. For raw foods or foods having a high microbial load, incubate M broth tubes 6 h at 35°–37°C and return selective enrichment broth tubes to 41°–43°C and incubate for an additional 6 h. (**d**) *Preparation of sample for EIA analysis.*—Remove M broth tubes from incubation and mix tubes by hand or Vortex mixer. Combine equal volumes (0.5–1 mL) from each M broth tube in a clean screw-cap tube and heat in boiling H_2O bath or in flowing steam 15 min. Refrigerate (2°–8°C) remaining M broth and TT and Rappaport-Vassiliadis R10 tubes from (**c**) for culture confirmation of any EIA-positive samples (*see* **967.26B, 967.26C, 967.27** and **967.28**). Cool heated M broths to 25°–37°C prior to analysis. Hughes, 638. Hughes 2003 confirms the pooling of enrichments. Hughes 2003, 775-76.
[1e.1] e) testing of the pooled modular composite sample, using a suitable detection assay, for the target microbe(s),	Price, alone or in combination with ICMSF and Hughes, discloses testing of the modular composite sample, using a suitable detection assay, for the target microbe(s).



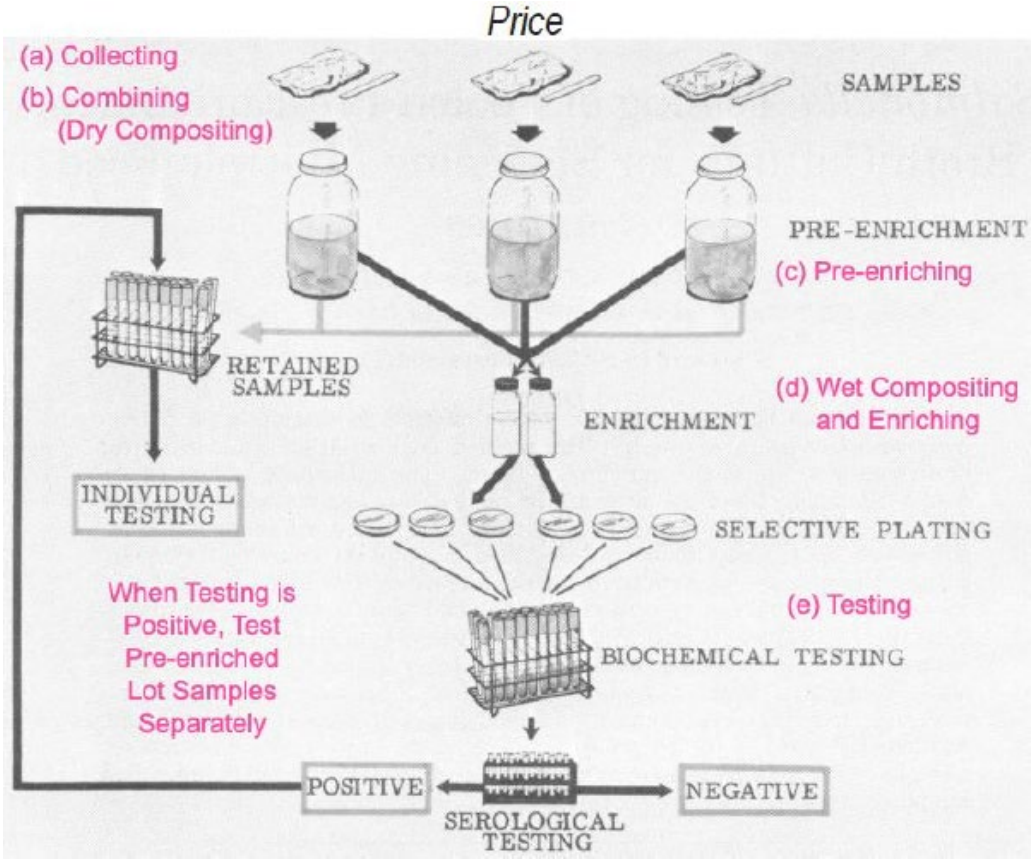
Price, 680 (Figure 1 (annotated)).

Price teaches testing the composite sample using a detection assay for *Salmonella*. Price, 680 (Figure 1).

Price states "a test procedure that gives a single negative answer for many negative samples offers an important saving in the analytical effort required for quality control":

	Positive test results are relatively rare among the many samples of food ingredients and products which are analyzed for *Salmonella*. Therefore, a test procedure that gives a single negative answer for many negative samples offers an important saving in the analytical effort required for quality control. These studies support the conclusion that *Salmonella* pre-enrichment broths can be pooled for analysis without loss in sensitivity to *Salmonella* in the individual samples. Price, 679. Price indicates that "positive test results are relatively rare." *Id.* A POSITA would have replaced Price's steps of testing a composite sample from the pre-enriched lot samples to obtain a negative result and further testing retained portions of the pre-enriched lot samples with the combined teachings of ICMSF and Hughes. These combined teachings provide for testing a composite made from the selectively enriched lot samples to obtain a negative result and further testing retained portions of the enriched lot samples after a positive test of the composite by a more sensitive protocol. ICMSF describes testing enrichment cultures of composite samples with suitable detection assays (ICMSF, 187) and "presence/absence tests" (ICMSF, 233). Suitable detection assays were established in Silliker and Gabis as discussed in ICMSF. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture

	confirmation of any EIA-positive samples." *Id.,* 638. Hughes states testing of the composite sample is "by an enzyme immunoassay (EIA) method." Hughes, 635, 638. These assay types were "art recognized." '771 Patent, 3:9-13 (AAPA #7).
[1e.2] wherein when such testing is negative all of said samples that were composited to form the separate composited lot samples are deemed negative for the target microbe(s) and each of the multiple separate lots is validated, and	Price, alone or in combination with ICMSF and Hughes, discloses wherein when such testing is negative all of said samples that were composited to form the separate composited test lot samples are deemed negative for the target microbe(s) and each of the multiple separate test lots is validated.



Price, 680 (Figure 1 (annotated)).

Price states "a test procedure that gives a single negative answer for many negative samples offers an important saving in the analytical effort required for quality control." Price, 679.

A POSITA would have replaced Price's steps of testing a composite sample from the pre-enriched lot samples to obtain a negative result and further testing retained portions of the pre-enriched lot samples with the combined teachings of ICMSF and Hughes. These combined teachings provide for testing a composite made from the

	selectively enriched lot samples to obtain a negative result and further testing retained portions of the enriched lot samples after a positive test of the composite by a more sensitive protocol. ICMSF describes correlating lots in a consignment with lot samples so a negative or positive test result enables a lot to be validated or rejected. ICMSF, 118-19. ICMSF describes sampling plans in which "c is the maximum allowable number of sample units yielding a positive result." ICMSF, 115. For many scenarios, the requirement is c = 0, which indicates the testing is negative. *Id.*, 118, 183-90, 309, 325, 326. A negative test result (*id.*, 113-15, 325, 326) is the expected and desired outcome to determine meat is not contaminated and is safe for consumption (*id.*, 184-89) such that a lot is validated and it is "commercially possible to market" (*id.*, 185) the meat.

6.10 WHAT IS A LOT?

Ideally, a lot is a quantity of food or food units produced and handled under uniform conditions. Under certain circumstances, it has been shown that the logarithms of the counts from a batch of food are likely to be normally distributed (Baird-Parker, 1980; Kilsby & Baird-Parker, 1983 [their Table 2]; Ingram & Roberts, 1976). The implication is that there is homogeneity within a lot, which, when considering microbial levels and distributions, rarely occurs in practice. In most instances, the distribution of microorganisms within lots of food is heterogeneous. Even in food factories, this heterogeneity makes the interpretation of sample results difficult, and it becomes even more so in circumstances where a lot is less well defined, such as in a consignment of a very large quantity of food.

It is therefore helpful if suppliers can be persuaded to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load).

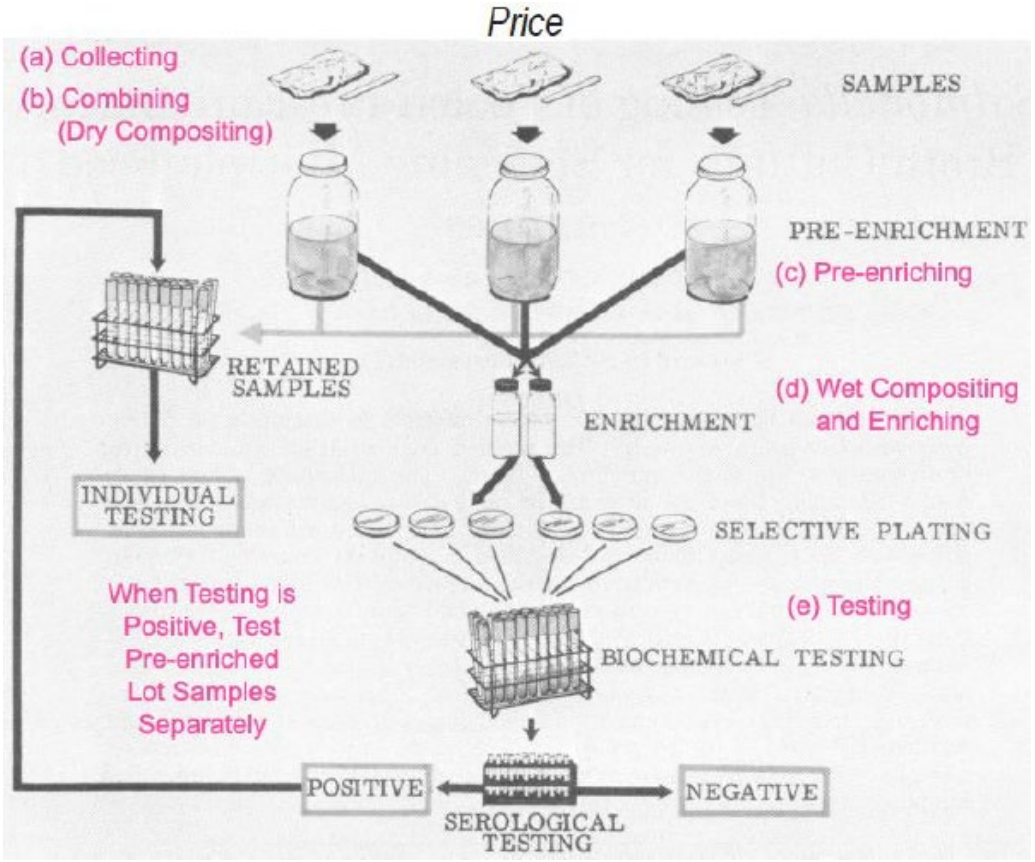
If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment.

A lot should be composed of food produced with as little variation as possible for a given process or commodity. Because of the uncertainties in identifying a lot commercially, however, the use of the word *lot* in this book is usually in its statistical sense, as a collection of units of a product, the acceptability of which is determined by examining a sample drawn from it.

ICMSF, 118-19 (annotated).

	Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Because Hughes explains the steps to be taken when testing is positive (*see* ie.3)*,* a POSITA would also expect negative testing results. Hughes, 638. Determining meat is safe for consumption so it may be released into commerce is the well-understood point of testing meat. Moreover, the recitation of a lot being validated for "release . . . into commerce" is not limiting. Prosecution History for Reexamination Control No. 95/011,294.
[1e.3] wherein when such testing is positive, each of the individual separate composited lot samples that were used to form the pooled modular composite sample are individually tested to determine which of the separate composited lot samples is positive for the target microbe(s),	Price, alone or in combination with ICMSF and Hughes, discloses wherein when such testing is positive, each of the individual separate composited lot samples that were used to form the pooled modular composite sample are individually tested to determine which of the separate composited lot samples is positive for the target microbe(s). The combination of Price, ICMSF, and Hughes describes retaining a portion of the enriched lot samples (steps (b)-(d)). Price indicates "a portion of each pre-enrichment is . . . retained . . . for later reference to the individual samples" and "individual tests on the retained samples can be made to determine which sample or samples contributed *Salmonella* to the pool." Price, 679. Price states, "If the *Salmonella* test of the pooled preenrichment broths is positive, individual tests on the retained samples can be made to determine which sample or samples contributed *Salmonella* to the pool." *Id.,* 679. Price discloses "the pooling of broth cultures permits small portions of each individual culture to be retained for subsequent analysis if a positive test is detected following enrichment, thus maintaining the individuality of each test sample." Price,

681.



Price, 680 (Figure 1 (annotated)).

Price states "[i]f the *Salmonella* test of the pooled pre-enrichment broths is positive, individual tests on the retained samples can be made to determine which sample or samples contributed *Salmonella* to the pool":

MATERIALS AND METHODS

Salmonella analyses were carried out as described in the *Bacteriological Analytical Manual* (BAM) (2) except for the pooling of pre-enrichment broths described in the following paragraph.

The procedure for *Salmonella* testing of pooled pre-enrichment broths is illustrated in Fig. 1. Samples are individually pre-enriched followed by transfer from multiple pre-enrichments to single selenite and tetrathionate broths at the enrichment stage. During pooling, a portion of each pre-enrichment is transferred to a sterile culture tube and retained at 4 C for later reference to the individual samples. If the *Salmonella* test of the pooled pre-enrichment broths is positive, individual tests on the retained samples can be made to determine which sample or samples contributed *Salmonella* to the pool.

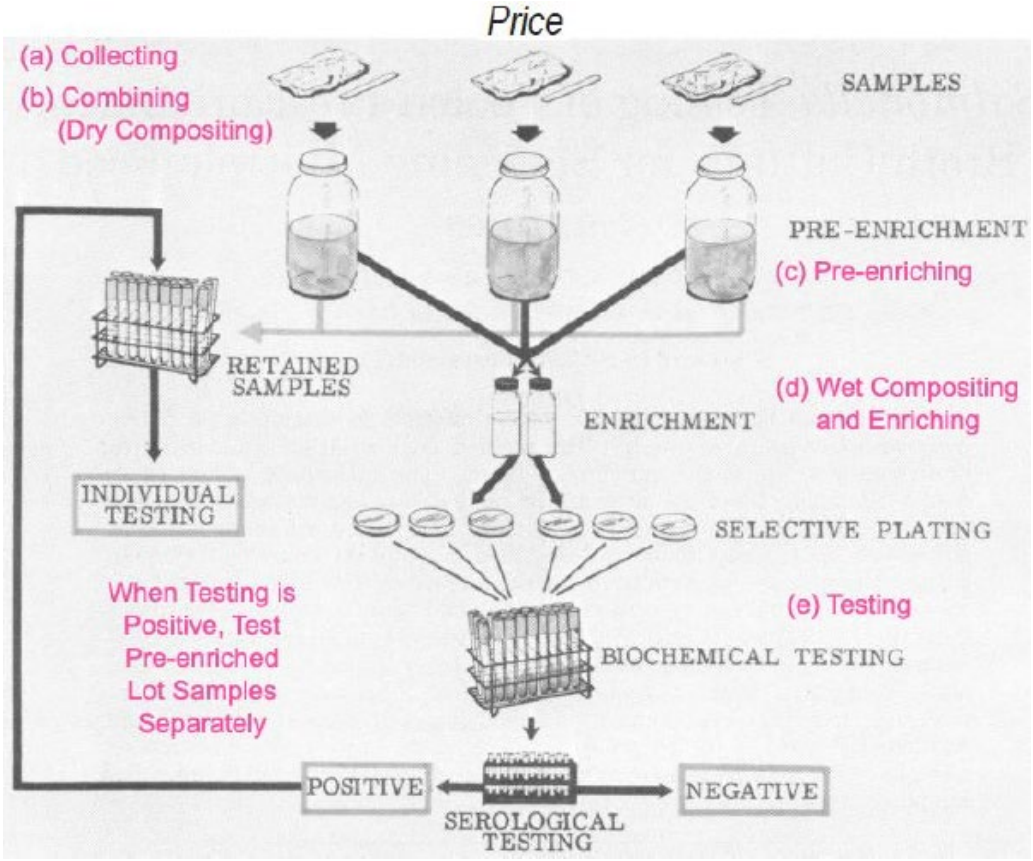
Price, 679. A POSITA would have considered retention of samples that had been selectively enriched as an option.

A POSITA would have replaced Price's steps of testing a composite sample from the pre-enriched lot samples to obtain a negative result and further testing retained portions of the pre-enriched lot samples with the combined teachings of ICMSF and Hughes. These combined teachings provide for testing a composite made from the selectively enriched lot samples to obtain a negative result and further testing retained portions of the enriched lot samples after a positive test of the composite by a more sensitive protocol.

"Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119.

	"There is often a desire to retest retained samples." ICMSF, 191. If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment. ICMSF, 119 (annotated). ICMSF discloses validating for release into commerce. ICMSF, 113–15 (§§ 6.2–6.6), 118–19 (§§ 6.9–6.10), 184–85 (§ 10.2), 325–27 (§§ 17.5.3–17.5.3.4); *see also id.*, 186–89 (§ 10.4–10.6)) according to a statistically-based sampling plan (*id.*, 113 (§ 6.1); *see also id.*, 115–18 (§§ 6.4–6.8), 184 (§ 10.2), 319–21 (§§ 17.2.3.3–17.2.3.4), 326–30 (§§ 17.5.3.1–17.6), as described regarding steps (a) and (e)(2). Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Hughes explicitly teaches retention of the enriched test lot samples for further testing after a positive result. Hughes provides instructions to refrigerate the "remaining M broth and TT and Rappaport-Vassiliadis R10 tubes [that were enriched] for culture confirmation of any EIA-positive samples." Hughes, 638. Hughes refers to the results as the "confirmed positive samples" (Hughes, 635) and also states that diagnostic reagents are "necessary for culture confirmation of presumptive EIA tests." Hughes, 636. Hughes indicates "[e]nrichment broths and M broths from samples positive by an

	enzyme immunoassay (EIA) method must be streaked on selective media." *Id.*, 635. Culture confirmation using streaking is more sensitive than the EIA method, as was commonly understood and confirmed by the teachings of D'Haese. *See generally*, D'Haese. Each sample was analyzed by the BAM method (7th Ed.) and the TSVIA method using the enrichment protocol described in section F of the method, *Preparation of Sample*. Equal portions (0.5–1 mL) from each of the 2M broths arising from the 2 selective-enrichment broths were combined in a small screw-capped tube and heat treated in boiling water for 15 min prior to performing the TSVIA. Confirmation for the immunoassay was by streaking from the M broth culture onto selective agar, as specified in the BAM reference culture method. Hughes, 635. Moreover, the recitation of a lot being validated for "release . . . into commerce" is not limiting. Prosecution History for Reexamination Control No. 95/011,294.
[1e.4] wherein the lots corresponding to any negatively testing composited lot samples are validated.	Price, alone or in combination with ICMSF and Hughes, discloses wherein the lots corresponding to any negatively testing composited lot samples are validated.



Price, 680 (Figure 1 (annotated)).

ICMSF and Hughes disclose selectively enriching prior to removing portions, thus a POSITA would consider the enrichment sufficient to enable validation of the lots without further enrichment or sampling.

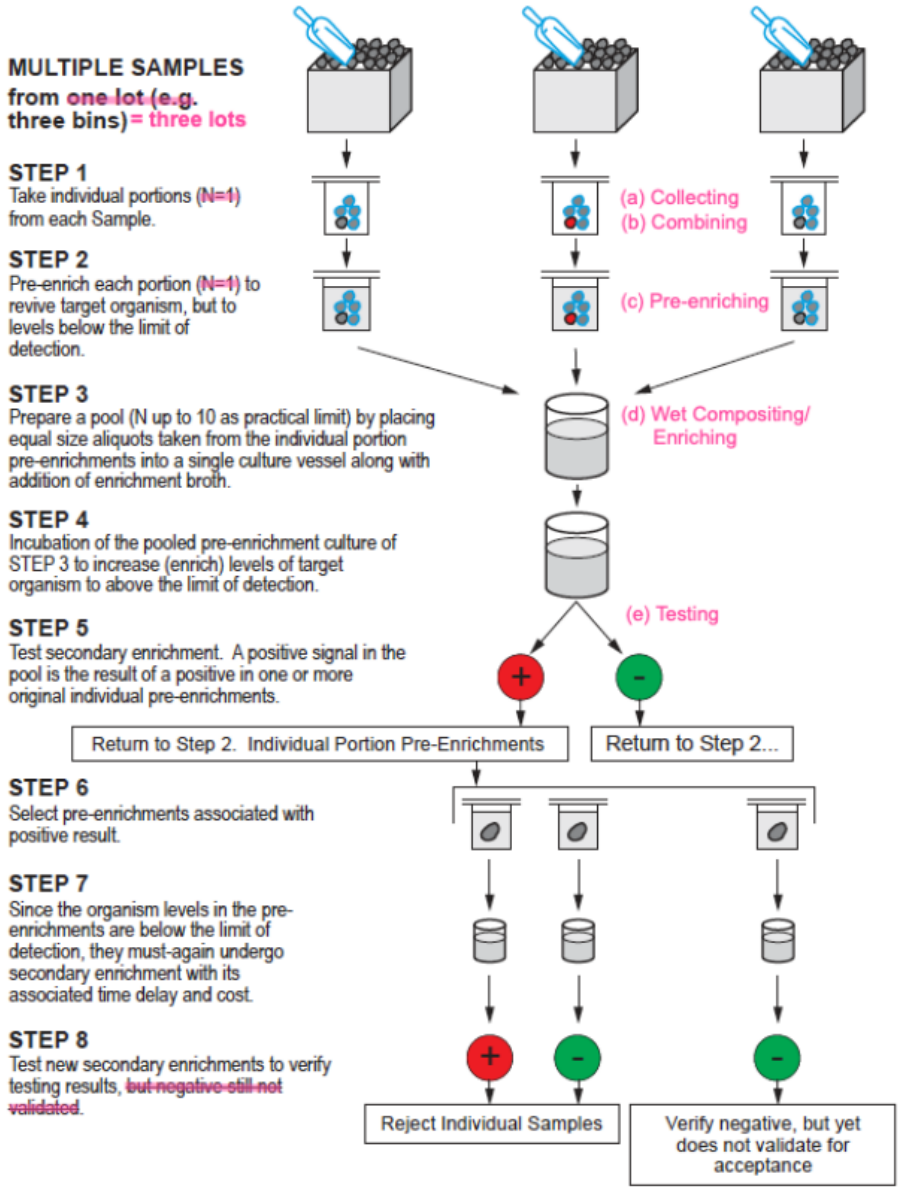
Hughes explicitly indicates portions of the enrichment cultures are retained for further testing; thus Hughes has no need for further sampling.

Hughes' "Preparation of Sample" section identifies four phases including (a) pre-

	enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. ICMSF and Hughes disclose selective enrichment so further enrichment is unnecessary. Also, as discussed regarding step (e)(3), Hughes explicitly indicates portions of the enrichment cultures are retained for further testing; thus Hughes has no need for further sampling. The same would be expected for enrichment cultures in ICMSF because retaining samples at each process stage was considered standard practice for a POSITA. in flowing steam 15 min. Refrigerate (2°–8°C) remaining M broth and TT and Rappaport-Vassiliadis R10 tubes from (c) for culture confirmation of any EIA-positive samples (*see* **967.26B, 967.26C, 967.27** and **967.28**). Cool heated M broths to 25°–37°C prior to analysis. Hughes, 638. Thus, the negative limitation of step (e)(4) is disclosed by and is obvious based on the combination of ICSMF and Hughes. *Id.*
Claim 2	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 1, wherein each of the multiple separate lots is selected from the group consisting of an environmental lot comprising multiple operationally-linked environmental specimens, pharmaceutical lot comprising multiple operationally-linked pharmaceutical specimens, single product lot	Price, in combination with ICMSF and Gombas discloses Claim 1, upon which this claim relies. See above re Claim 1. Price further discloses test lots that are a single-unit production lot, comprised of operationally-linked product specimens.

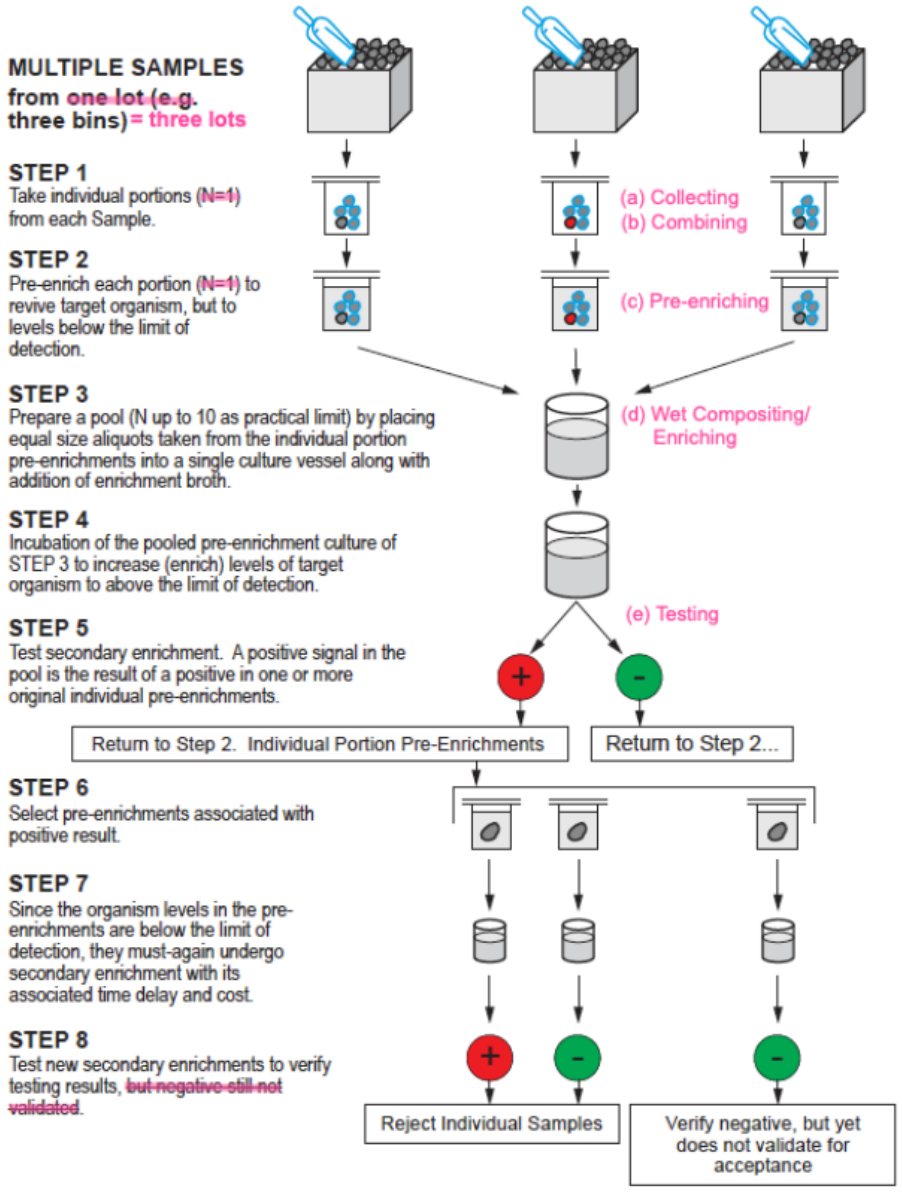
comprising multiple operationally-linked product specimens, and combinations thereof.

Figure 3: CITED PRIOR ART (PRICE et al, 1972)



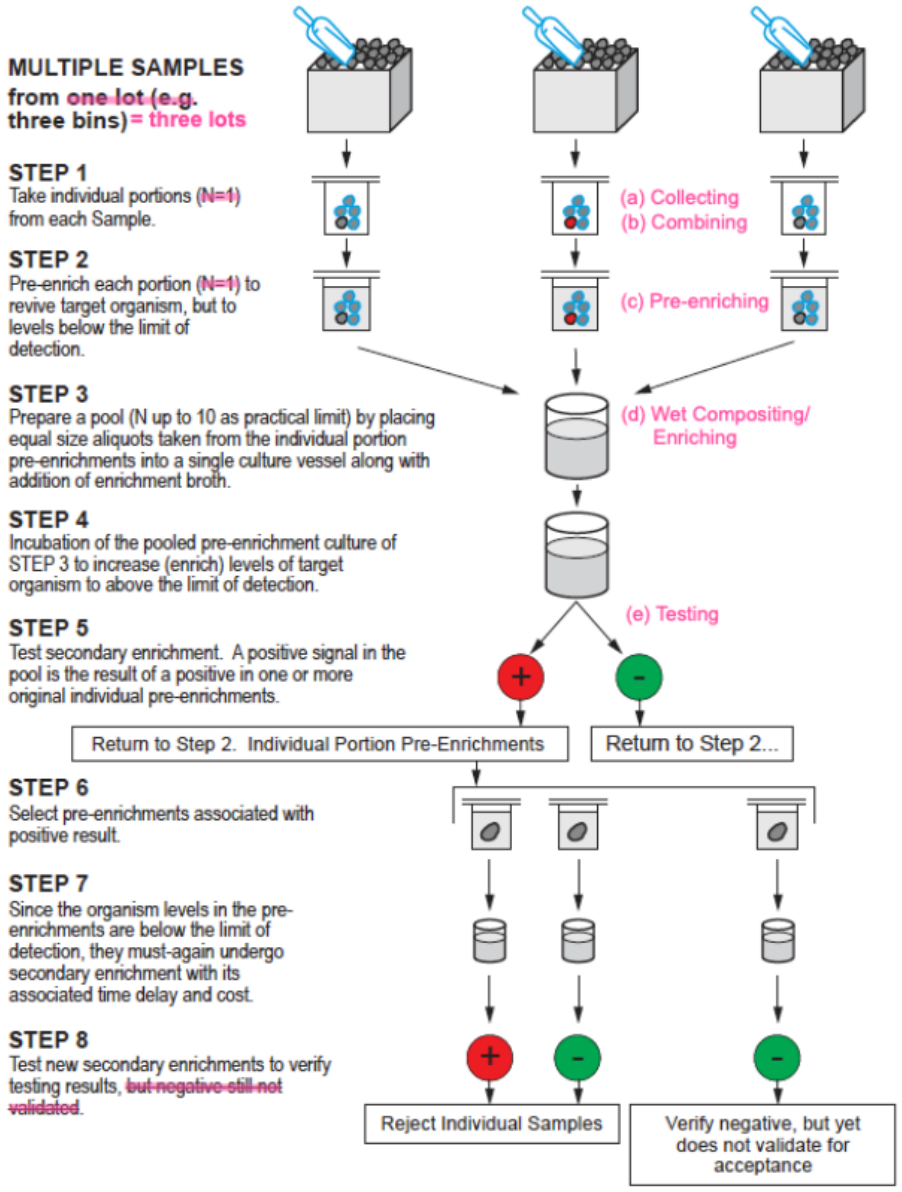
	Prosecution History for Reexamination Control No. 95/001,294, 298 (annotated). IEH's diagram also indicates multiple samples are taken from three bins, which is analogous to three lots rather than a single lot because each bin is an assemblage of specimens. The specification of the '771 Patent indicates a test lot is a single-unit production lot ('771 Patent, 5:43-54) such as a single combo, bin. pallet, or segment of production ('771 Patent, 7:54-63; *see also* AAPA #3 and #4). ICMSF teaches collecting from "individual production batches as lots" (ICMSF, 119) and "[a] lot is considered as a collection of units" (ICMSF, 327). "Operationally-linked" is simply grouping like items, which is also disclosed by ICMSF. "Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119.
Claim 3	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 1, wherein each of the multiple separate lots is a single production lot, comprised of operationally-linked product specimens.	Price, in combination with ICMSF and Gombas discloses Claim 1, upon which this claim relies. See above re Claim 1. Price further discloses test lots that are a single-unit production lot, comprised of operationally-linked product specimens.

Figure 3: CITED PRIOR ART (PRICE et al, 1972)



	Prosecution History for Reexamination Control No. 95/001,294, 298 (annotated). IEH's diagram also indicates multiple samples are taken from three bins, which is analogous to three lots rather than a single lot because each bin is an assemblage of specimens. The specification of the '771 Patent indicates a test lot is a single-unit production lot ('771 Patent, 5:43-54) such as a single combo, bin. pallet, or segment of production ('771 Patent, 7:54-63; *see also* AAPA #3 and #4). ICMSF teaches collecting from "individual production batches as lots" (ICMSF, 119) and "[a] lot is considered as a collection of units" (ICMSF, 327). "Operationally-linked" is simply grouping like items, which is also disclosed by ICMSF. "Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119.
Claim 4	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 3, wherein product specimens are synonymous with product portions.	Price, in combination with ICMSF and Gombas discloses Claim 3, upon which this claim relies. See above re Claim 3. Price further discloses product specimens are synonymous with product portions.

Figure 3: CITED PRIOR ART (PRICE et al, 1972)



	Prosecution History for Reexamination Control No. 95/001,294, 298 (annotated). IEH's diagram indicates multiple samples and portions are removed from the bins/lots, and uses the terminology interchangeably. The specification of the '771 Patent indicates a test lot is a single-unit production lot ('771 Patent, 5:43-54) such as a single combo, bin. pallet, or segment of production ('771 Patent, 7:54-63; *see also* AAPA #3 and #4). ICMSF teaches collecting portions or samples, which were known synonyms for product portions or specimens. ICMSF states that "[a] sample unit is a small portion of the lot." ICMSF, 225. **12.1 INTRODUCTION** Many different types of samples are collected and submitted to the laboratory for analysis. Some are sample units from lots or consignments of foods or ingredients for lot acceptance determination. Others may be for investigational purposes to assess control of the environment, investigate the source of a problem, or validate a process. Some may have legal implications relative to a lawsuit or for regulatory compliance. This chapter briefly discusses some of the major factors that should be considered when collecting sample units, shipping them to a laboratory, preparing them for analysis, and analyzing them. A sample unit is a small portion of the lot in which the distribution of both numbers and types of microorganisms should be representative of the whole lot. This requirement is only completely fulfilled when the food and processing conditions result in a homogeneous distribution of microorganisms. This only occurs in well-agitated fluid products. In general, the distribution of microorganisms is presumed to be random. ICMSF at 225 annotated).
Claim 5	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 1, further comprising incubating the pooled modular composite sample of d), prior to testing in e).	Price, in combination with ICMSF and Gombas discloses Claim 1, upon which this claim relies. See above re Claim 1. Price further discloses incubating the modular composite sample of d), prior to testing in e). Price discloses "[s]amples are individually pre-enriched followed by transfer from multiple pre-enrichments to single selenite and tetrathionate broths at the enrichment

	stage." Price, 679. These pre-enrichment and enrichment steps (after the samples are composited) inherently disclose an incubation period in which levels of the target microbial agent, in this case, *Salmonella*, grow to detectable levels for testing. Price states "[a]lternatively, problems with interfering organisms may warrant the use of direct selective enrichments" and further mentions "a reduction in the incubation time of the pre-enrichment cultures." Price inherently discloses an incubation period of the pre-enrichment samples. **Whether pooled pre-enrichments are less sensitive than individual tests in the presence of interfering organisms probably is not known. For the present, we would recommend that, if numerous non-*Salmonella* colonies that resemble *Salmonella* on selective agar media are encountered in the pooled test, the retained pre-enrichment cultures should be tested individually. Alternatively, problems with interfering organisms may warrant the use of direct selective enrichments, two selective enrichments, or possibly even a reduction in the incubation time of the pre-enrichment cultures.** Price, 682 (highlighted added). ICMSF and Hughes, in combination with Stewart, discloses the method of claim 1, further comprising incubating the modular composite sample of d), prior to testing in e). Hughes describes incubating an enriched test lot sample. Hughes, 636, 638.

	Claim 1 is disclosed by ICMSF and Hughes. Stewart discloses that after the selective enrichment in Rappaport-Vassiliadis (RV) broth and tetrathionate (TT) broths, the two selective enrichments were combined into a single sample for further incubation and analysis. "[P]ost-enrichment by transfer and combination of 0.5 mL RV and 1 mL TT into a single tube containing 10 mL prewarmed trypticase soy broth supplemented with novobiocin (TSB + n) at 10 µg/mL with incubation for 16–20 h at 42°C." Stewart, 398. Hughes also discloses an incubation protocol. "For the previous AOAC studies on TSVIA, a cultural protocol using selective enrichment in selenite cystine (SC) and tetrathionate (Tr) broths at 35°C was used. However, there has been a recent trend to use elevated incubation temperatures and to replace SC broth with the Rappaport-Vassiliadis (RV) medium (2)." Hughes, 634. A POSITA would have been motivated to incubate the pooled modular composite sample of Gombas or Hughes, as taught by Stewart, for reliable testing results.
Claim 6	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 1, wherein each separate composited lot sample in c) comprises incubating each separate composited lot sample with enrichment medium suitable to enrich for the target microbe(s), and wherein removing in d) comprises removing a portion of the enrichment medium from each enriched separate composited lot sample and combining the removed portions to provide a pooled modular composite sample.	Price, in combination with ICMSF and Gombas discloses Claim 1, upon which this claim relies. See above re Claim 1. Price further discloses incubating each separate test lot sample in c) comprises incubating each separate test lot sample with enrichment medium suitable to enrich for the target microbial agent or organism, and wherein removing in d) comprises removing a portion of the enrichment medium from each enriched separate test lot sample and combining the removed portions to provide a modular composite sample. Price discloses "[s]amples are individually pre-enriched followed by transfer from multiple pre-enrichments to single selenite and tetrathionate broths at the enrichment stage." Price, 679. The pre-enrichment step inherently discloses an incubation period for each individual sample in which levels of the target microbial agent, in this case, *Salmonella*, grow to detectable levels for testing. The enriched sample is then

	transferred and combined with other samples. Price states "[a]lternatively, problems with interfering organisms may warrant the use of direct selective enrichments" and further mentions "a reduction in the incubation time of the pre-enrichment cultures." Price inherently discloses an incubation period of the pre-enrichment samples. **Whether pooled pre-enrichments are less sensitive than individual tests in the presence of interfering organisms probably is not known. For the present, we would recommend that, if numerous non-*Salmonella* colonies that resemble *Salmonella* on selective agar media are encountered in the pooled test, the retained pre-enrichment cultures should be tested individually. Alternatively, problems with interfering organisms may warrant the use of direct selective enrichments, two selective enrichments, or possibly even a reduction in the incubation time of the pre-enrichment cultures.** Price, 682 (highlighted added). Price discloses wet compositing as "[s]amples are individually pre-enriched followed by transfer from multiple pre-enrichments to single selenite and tetrathionate broths at the enrichment stage." Price, 679.

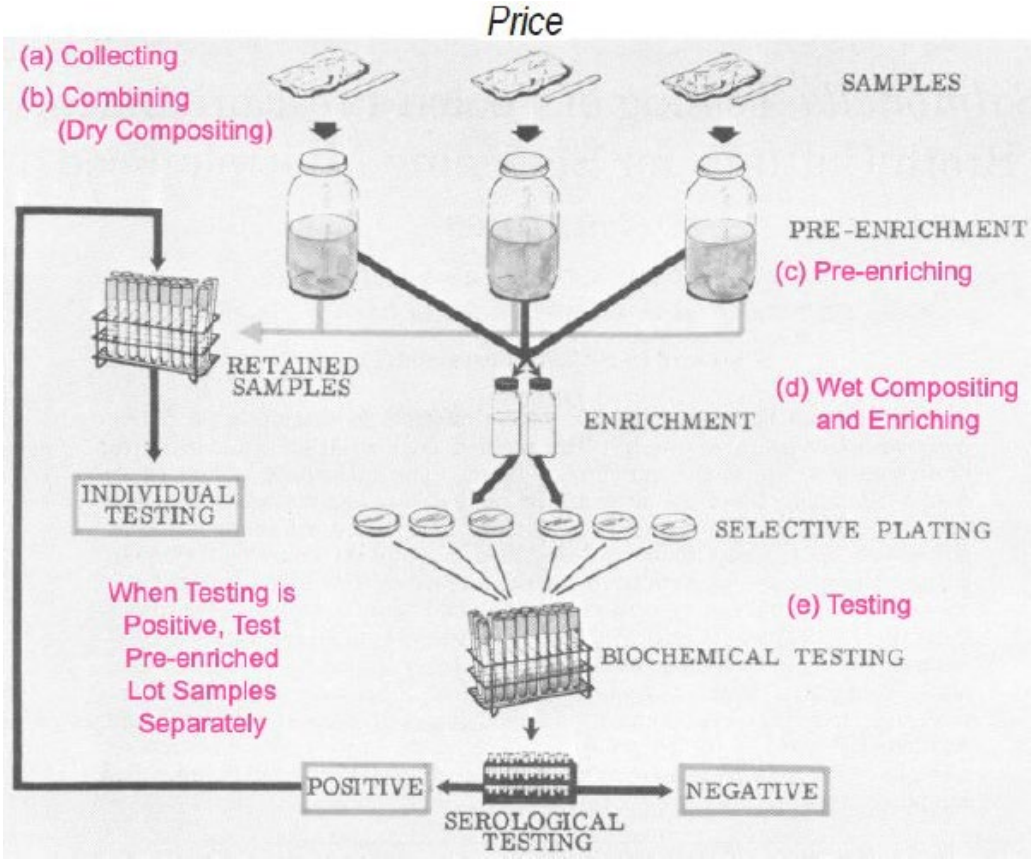
MATERIALS AND METHODS

Salmonella analyses were carried out as described in the *Bacteriological Analytical Manual* (BAM) (2) except for the pooling of pre-enrichment broths described in the following paragraph.

The procedure for *Salmonella* testing of pooled pre-enrichment broths is illustrated in Fig. 1. Samples are individually pre-enriched followed by transfer from multiple pre-enrichments to single selenite and tetrathionate broths at the enrichment stage. During pooling, a portion of each pre-enrichment is transferred to a sterile culture tube and retained at 4 C for later reference to the individual samples. If the *Salmonella* test of the pooled pre-enrichment broths is positive, individual tests on the retained samples can be made to determine which sample or samples contributed *Salmonella* to the pool.

Price 679.

	Pooled samples versus pooled pre-enrichment broths. Silliker (4) reported a loss in sensitivity when several samples were pooled in a large container of pre-enrichment broth as compared to individual sample analysis. This is not surprising since, to be detected, the desiccated and debilitated *Salmonella* cells in a dried food sample must grow out in pre-enrichment broth in competition with other bacteria in the sample. If many food samples are pooled at the pre-enrichment stage, the salmonellae face competition from the flora of all the samples in the pool. If, on the other hand, the samples are individually pre-enriched, the actively growing salmonellae might be expected to compete more favorably with organisms introduced from other samples when pooled at the enrichment stage. In addition, pooling pre-enrichment broths avoids the hazards and inconvenience of handling very large flasks of culture. Price, 681 (discussing Silliker).



Price, 680 (Figure 1 (annotated)). Price's Figure 1 indicates that selective plating, biochemical testing, and serological testing are used.

ICMSF describes the well-known benefits of combining enrichment cultures from analytical units from lots in a consignment.

	To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. A laboratory investigation undertaken for ICMSF (Silliker & Gabis, 1973) indicated that different sized analytical units drawn from a single well-mixed sample unit of dried food give very similar results. A subsequent investigation (Gabis & Silliker, 1974) using foods of high moisture content (including eggs, poultry meat, meat, and meat products) showed comparable reliability in the use of composite analytical units; for example, examination of three composites of 20 × 25 g analytical units gave the same result as examination of all 60 analytical units individually (the total amount examined being 1500 g in both instances). This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n*, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort. ICMSF, 187. Hughes describes incubating an enriches test lot sample. Hughes 636, 638.
Claim 7	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 6, wherein the enrichment medium comprises a selective agent that will inhibit a competing non-target microbe.	Price, alone or in combination with ICMSF and Hughes discloses Claim 6, upon which this claim relies. See above re Claim 6. Price further discloses a medium comprises a selective agent that will inhibit a competing non-target microbial agent or organism. Price discusses organisms facing competition from other organisms during the enrichment stage. A POSITA would conclude that inhibiting competition would be beneficial to the growth of target microbial agents or organisms.

	Pooled samples versus pooled pre-enrichment broths. Silliker (4) reported a loss in sensitivity when several samples were pooled in a large container of pre-enrichment broth as compared to individual sample analysis. This is not surprising since, to be detected, the desiccated and debilitated *Salmonella* cells in a dried food sample must grow out in pre-enrichment broth in competition with other bacteria in the sample. If many food samples are pooled at the pre-enrichment stage, the salmonellae face competition from the flora of all the samples in the pool. If, on the other hand, the samples are individually pre-enriched, the actively growing salmonellae might be expected to compete more favorably with organisms introduced from other samples when pooled at the enrichment stage. In addition, pooling pre-enrichment broths avoids the hazards and inconvenience of handling very large flasks of culture. Price, 681. Additionally, Claim 28 recites what a POSITA would consider the well-known property of selective enrichment, inhibition of a competing non-target microorganism. D'Aoust describes "selective enrichment [that] inhibits growth of non-*Salmonella*e and facilitates isolation of *Salmonella* on differential plating media." D'Aoust, 369. A POSITA would have been motivated to use an enrichment medium comprising a selective agent that inhibits a non-target agent or organisms as described by

	D'Aoust, for the test lot samples described by ICMSF and Hughes, to ensure an accurate test. A POSITA would understand that incubating samples at different temperature ranges for different target microorganisms would, effectively, inhibit the growth of other organisms. "Incubation temperature markedly affects productivity of selective enrichment broths by suppressing growth of competing microorganisms but not *Salmonella*." D'Aoust, 372. "This may be achieved by utilization of enrichment media that through various mechanisms may favor and enhance growth and multiplication of salmonellae while other gram-negative organisms such as coliforms, Proteus, and Pseudomonas are suppressed or inhibited." Fagerberg, 630.
Claim 8	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 1, wherein testing of the pooled modular composite sample, using a suitable detection assay comprises use of a concentration step to concentrate the target microbe(s).	Price, alone or in combination with ICMSF and Hughes discloses Claim 1, upon which this claim relies. See above re Claim 1. Price further discloses testing of the modular composite sample, using a suitable detection assay further comprises a concentration step to concentrate the microbial agent or organism. Price discloses an enrichment stage after compositing where "[s]amples are individually pre-enriched followed by transfer from multiple pre-enrichments to single selenite and tetrathionate broths at the enrichment stage." Price, 679. A POSITA would understand that a detection assay that includes enrichment could further comprise a concentration step. Enrichment for the microbial agent or organism increases its concentration. ICMSF discloses the use of "immunomagnetic beads to concentrate cells." ICMSF, 318. A POSITA would consider this a well-known technique before the priority date.

	Hughes uses an EIA method followed by a more sensitive protocol—streaking on selective media. Hughes, 635. Each sample was analyzed by the BAM method (7th Ed.) and the TSVIA method using the enrichment protocol described in section F of the method, *Preparation of Sample*. Equal portions (0.5–1 mL) from each of the 2M broths arising from the 2 selective-enrichment broths were combined in a small screw-capped tube and heat treated in boiling water for 15 min prior to performing the TSVIA. Confirmation for the immunoassay was by streaking from the M broth culture onto selective agar, as specified in the BAM reference culture method. Hughes, 635. A POSITA would find that modifying Hughes based on D'Haese ensures a more sensitive test is used for further testing. A POSITA would have recognized the concentration step could be employed to provide a more sensitive protocol for further testing. Fratamico discusses the rapid isolation of target microorganisms using an immunomagnetic separation method. Fratamico, 105. Fratamico discloses that use of immunomagnetic beads resulted in "an increase in the level of E. coli B1409 in the sample," as well as "an increase in the number of organisms recovered." *Id.*, 107. Additionally, Fratamico provides detailed instructions for preparing and using immunomagnetic bead separation to concentrate a target microbial agent or organism. Fratamico developed "[a] method employing immunomagnetic beads" to concentrate and thereby improve the isolation and detection of E. coli. *Id.* For example,

	Fratamico was able to use immunomagnetic beads to isolate *E. coli* strains from enrichment cultures of raw ground beef "when the initial inoculum level was 1 cfu g-1." *Id.*, 109. Fratamico further teaches that "[t]he percentage of bacteria recovered increased with increasing amounts of beads." *Id.*, 108. Consequently, Fratamico "demonstrates that immunomagnetic separation [using immunomagnetic beads] is a simple and rapid technique" for concentrating a microbe for isolation and detection. *Id.*, 109. These teachings of Fratamico would have motivated a POSA to modify the combination of ICMSF and Gombas and ICMSF and Hughes to use immunomagnetic beads to concentrate microbes in a modular composite sample to benefit from accurate tests. Use of immunomagnetic beads was already known in the art for concentrating during testing beef samples. "Tu et al. (78) detected pathogenic E. coli O157:H7 in beef hamburger with the use of IMS and ATP measurement. After 6 h of enrichment at 37°C, inoculated beef samples (0.9 ml each) were filtered to remove large particles prior to 10 min of incubation with ca. 10^5 magnetic beads coated with anti–*E. coli* O157:H7 (Dynabeads, Dynal Inc., Oslo, Norway). Following incubation, the magnetic beads were collected with a Dynal magnetic concentrator and resuspended in 0.9 ml of Trypticase soy broth for ATP analysis." Benoit, 1941. "Hudson et al. (26) used IMS to directly separate and concentrate L. monocytogenes from ham samples prior to detection by PCR. IMS was used on the basis of the commercial availability of anti-Listeria magnetic beads (Dynal Inc.) to avoid time-consuming cultural enrichment steps and to remove PCR inhibitors." Benoit, 1941-42. Cudjoe investigates the use of immunomagnetic beads in pooling methods.

	In a study designed to evaluate the performance of Dynabeads® Anti-Salmonella, immunomagnetic separation (IMS) followed by plating (IMS-Plating) proved far superior to the conventional ISO *Salmonella* methodology and the Modified Semi-solid Rappaport-Vassiliadis (MSRV) method. *Salmonella* species were isolated and detected from 135 out of the 180 diverse poultry samples by IMS analysis as against 98 by the conventional method and 33 by the MSRV technique. All results were confirmed biochemically and serologically. It appeared from the data generated that some of the salmonellae isolated using IMS were greatly inhibited in Rappaport-Vassiliadis enrichment broth at 42°C and to a lesser extent in selenite cystine broth at 37°C. A moderately selective plating medium like XLD proved to be better in isolating these possible sensitive wild-type strains of salmonellae than the more selective BGA. Cudjoe, Abstract. *Samples*. One hundred and eighty (180) diverse poultry samples, consisting of 40 pools of cloacal/faecal swabs (5 swabs/pool; 5 pools samples/flock) from eight different flocks, 10 raw chicken breast meat samples, 10 pooled egg shell samples, 60 raw chicken liver samples and 60 chicken neck skins sampled from the slaughter line, were used in this study. All samples were obtained from a poultry slaughter and processing plant in the Netherlands. A pool of five swab samples was pre-enriched in 10 ml of buffered peptone water (BPW; Difco Laboratories, Detroit, Michigan, USA) at 37°C for 16–20 h, whilst 25 g of each of the other samples above, were pre-enriched similarly in 225 ml of BPW and examined as detailed below. Cudjoe, 160.
Claim 9	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 8, wherein the concentration step comprises at least one of immunomagnetic bead separation, and affinity chromatography.	Price, alone or in combination with ICMSF and Hughes discloses Claim 8, upon which this claim relies. See above re Claim 8.

	Price further discloses the concentration step comprises at least one of immunomagnetic bead separation, and affinity chromatography. Price discloses an enrichment stage after compositing where "[s]amples are individually pre-enriched followed by transfer from multiple pre-enrichments to single selenite and tetrathionate broths at the enrichment stage." Price, 679. A POSITA would understand that a detection assay that includes enrichment could further comprise a concentration step. Enrichment for the microbial agent or organism increases its concentration. A POSITA would use a standard concentration procedure such as immunomagnetic bead separation or affinity chromatography in order to concentrate the sample. ICMSF discloses the use of "immunomagnetic beads to concentrate cells." ICMSF, 318. A POSITA would consider this a well-known technique before the priority date. Fratamico discusses the rapid isolation of target microorganisms using an immunomagnetic separation method. Fratamico, 105. Fratamico discloses that use of immunomagnetic beads resulted in "an increase in the level of E. coli B1409 in the sample," as well as "an increase in the number of organisms recovered." *Id.*, 107. Additionally, Fratamico provides detailed instructions for preparing and using immunomagnetic bead separation to concentrate a target microbial agent or organism. Fratamico developed "[a] method employing immunomagnetic beads" to concentrate and thereby improve the isolation and detection of E. coli. *Id.* For example, Fratamico was able to use immunomagnetic beads to isolate *E. coli* strains from enrichment cultures of raw ground beef "when the initial inoculum level was 1 cfu g-1." *Id.*, 109. Fratamico further teaches that "[t]he percentage of bacteria recovered increased with increasing amounts of beads." *Id.*, 108. Consequently, Fratamico "demonstrates that immunomagnetic separation [using immunomagnetic beads] is a simple and rapid technique" for concentrating a microbe for isolation and detection. *Id.*, 109.

	These teachings of Fratamico would have motivated a POSA to modify the combination of ICMSF and Gombas and ICMSF and Hughes to use immunomagnetic beads to concentrate microbes in a modular composite sample to benefit from accurate tests. Use of immunomagnetic beads was already known in the art for concentrating during testing beef samples. "Tu et al. (78) detected pathogenic E. coli O157:H7 in beef hamburger with the use of IMS and ATP measurement. After 6 h of enrichment at 37°C, inoculated beef samples (0.9 ml each) were filtered to remove large particles prior to 10 min of incubation with ca. 10^5 magnetic beads coated with anti–*E. coli* O157:H7 (Dynabeads, Dynal Inc., Oslo, Norway). Following incubation, the magnetic beads were collected with a Dynal magnetic concentrator and resuspended in 0.9 ml of Trypticase soy broth for ATP analysis." Benoit, 1941. "Hudson et al. (26) used IMS to directly separate and concentrate L. monocytogenes from ham samples prior to detection by PCR. IMS was used on the basis of the commercial availability of anti-Listeria magnetic beads (Dynal Inc.) to avoid time-consuming cultural enrichment steps and to remove PCR inhibitors." Benoit, 1941-42. Cudjoe investigates the use of immunomagnetic beads in pooling methods. In a study designed to evaluate the performance of Dynabeads® Anti-Salmonella, immunomagnetic separation (IMS) followed by plating (IMS-Plating) proved far superior to the conventional ISO *Salmonella* methodology and the Modified Semi-solid Rappaport-Vassiliadis (MSRV) method. *Salmonella* species were isolated and detected from 135 out of the 180 diverse poultry samples by IMS analysis as against 98 by the conventional method and 33 by the MSRV technique. All results were confirmed biochemically and serologically. It appeared from the data generated that some of the salmonellae isolated using IMS were greatly inhibited in Rappaport-Vassiliadis enrichment broth at 42°C and to a lesser extent in selenite cystine broth at 37°C. A moderately selective plating medium like XLD proved to be better in isolating these possible sensitive wild-type strains of salmonellae than the more selective BGA.

	Cudjoe, Abstract. *Samples*. One hundred and eighty (180) diverse poultry samples, consisting of 40 pools of cloacal/faecal swabs (5 swabs/pool; 5 pools samples/flock) from eight different flocks, 10 raw chicken breast meat samples, 10 pooled egg shell samples, 60 raw chicken liver samples and 60 chicken neck skins sampled from the slaughter line, were used in this study. All samples were obtained from a poultry slaughter and processing plant in the Netherlands. A pool of five swab samples was pre-enriched in 10 ml of buffered peptone water (BPW; Difco Laboratories, Detroit, Michigan, USA) at 37°C for 16–20 h, whilst 25 g of each of the other samples above, were pre-enriched similarly in 225 ml of BPW and examined as detailed below. Cudjoe, 160.
Claim 10	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 1, wherein where testing in e) of the pooled modular composite sample is positive, and wherein the separate individual composited lot samples are further tested using a more sensitive protocol.	Price, alone or in combination with ICMSF and Hughes discloses Claim 1, upon which this claim relies. See above re Claim 1. A POSITA would understand that retesting the individual test lot samples is an inherently more sensitive process because the sample is not diluted with other samples in the testing pool. Therefore, the retesting of a singular sample is more concentrated and therefore, more sensitive. *See also, generally*, Jarvis. ICMSF discloses the use of "immunomagnetic beads to concentrate cells." ICMSF, 318. A POSITA would consider this a well-known technique before the priority date. Hughes uses an EIA method followed by a more sensitive protocol—streaking on selective media. Hughes, 635.

	Each sample was analyzed by the BAM method (7th Ed.) and the TSVIA method using the enrichment protocol described in section F of the method, *Preparation of Sample*. Equal portions (0.5–1 mL) from each of the 2M broths arising from the 2 selective-enrichment broths were combined in a small screw-capped tube and heat treated in boiling water for 15 min prior to performing the TSVIA. Confirmation for the immunoassay was by streaking from the M broth culture onto selective agar, as specified in the BAM reference culture method. Hughes, 635. A POSITA would find that modifying Hughes based on D'Haese ensures a more sensitive test is used for further testing. A POSITA would have recognized the concentration step could be employed to provide a more sensitive protocol for further testing. A POSITA would understand that retesting the non-pooled, singular samples is an inherently more sensitive process because the sample is not diluted with other samples in the testing pool. Therefore, the retesting of a singular sample is more concentrated and therefore, more sensitive. *See also, generally*, Jarvis. Fratamico discusses the rapid isolation of target microorganisms using an immunomagnetic separation method. Fratamico, 105. Fratamico discloses that use of immunomagnetic beads resulted in "an increase in the level of E. coli B1409 in the sample," as well as "an increase in the number of organisms recovered." *Id.*, 107. Additionally, Fratamico provides detailed instructions for preparing and using immunomagnetic bead separation to concentrate a target microbial agent or organism.

	Fratamico developed "[a] method employing immunomagnetic beads" to concentrate and thereby improve the isolation and detection of E. coli. *Id.* For example, Fratamico was able to use immunomagnetic beads to isolate *E. coli* strains from enrichment cultures of raw ground beef "when the initial inoculum level was 1 cfu g-1." *Id.*, 109. Fratamico further teaches that "[t]he percentage of bacteria recovered increased with increasing amounts of beads." *Id.*, 108. Consequently, Fratamico "demonstrates that immunomagnetic separation [using immunomagnetic beads] is a simple and rapid technique" for concentrating a microbe for isolation and detection. *Id.*, 109. These teachings of Fratamico would have motivated a POSA to modify the combination of ICMSF and Gombas and ICMSF and Hughes to use immunomagnetic beads to concentrate microbes in a modular composite sample to benefit from accurate tests. Use of immunomagnetic beads was already known in the art for concentrating during testing beef samples. "Tu et al. (78) detected pathogenic E. coli O157:H7 in beef hamburger with the use of IMS and ATP measurement. After 6 h of enrichment at 37°C, inoculated beef samples (0.9 ml each) were filtered to remove large particles prior to 10 min of incubation with ca. 10^5 magnetic beads coated with anti–*E. coli* O157:H7 (Dynabeads, Dynal Inc., Oslo, Norway). Following incubation, the magnetic beads were collected with a Dynal magnetic concentrator and resuspended in 0.9 ml of Trypticase soy broth for ATP analysis." Benoit, 1941. "Hudson et al. (26) used IMS to directly separate and concentrate L. monocytogenes from ham samples prior to detection by PCR. IMS was used on the basis of the commercial availability of anti-Listeria magnetic beads (Dynal Inc.) to avoid time-consuming cultural enrichment steps and to remove PCR inhibitors." Benoit, 1941-42. Cudjoe investigates the use of immunomagnetic beads in pooling methods.

	In a study designed to evaluate the performance of Dynabeads® Anti-Salmonella, immunomagnetic separation (IMS) followed by plating (IMS-Plating) proved far superior to the conventional ISO *Salmonella* methodology and the Modified Semi-solid Rappaport-Vassiliadis (MSRV) method. *Salmonella* species were isolated and detected from 135 out of the 180 diverse poultry samples by IMS analysis as against 98 by the conventional method and 33 by the MSRV technique. All results were confirmed biochemically and serologically. It appeared from the data generated that some of the salmonellae isolated using IMS were greatly inhibited in Rappaport-Vassiliadis enrichment broth at 42°C and to a lesser extent in selenite cystine broth at 37°C. A moderately selective plating medium like XLD proved to be better in isolating these possible sensitive wild-type strains of salmonellae than the more selective BGA. Cudjoe, Abstract. *Samples*. One hundred and eighty (180) diverse poultry samples, consisting of 40 pools of cloacal/faecal swabs (5 swabs/pool; 5 pools samples/flock) from eight different flocks, 10 raw chicken breast meat samples, 10 pooled egg shell samples, 60 raw chicken liver samples and 60 chicken neck skins sampled from the slaughter line, were used in this study. All samples were obtained from a poultry slaughter and processing plant in the Netherlands. A pool of five swab samples was pre-enriched in 10 ml of buffered peptone water (BPW; Difco Laboratories, Detroit, Michigan, USA) at 37°C for 16–20 h, whilst 25 g of each of the other samples above, were pre-enriched similarly in 225 ml of BPW and examined as detailed below. Cudjoe, 160.
Claim 11	
The method of claim 10, wherein the more sensitive protocol comprises use of a concentration step to concentrate the target microbe(s), in combination with the same test protocol used to test the pooled modular composite sample.	Price, alone or in combination with ICMSF and Hughes discloses Claim 10, upon which this claim relies. See above re Claim 10. ICMSF discloses the use of "immunomagnetic beads to concentrate cells." ICMSF, 318. A POSITA would consider this a well-known technique before the priority date.

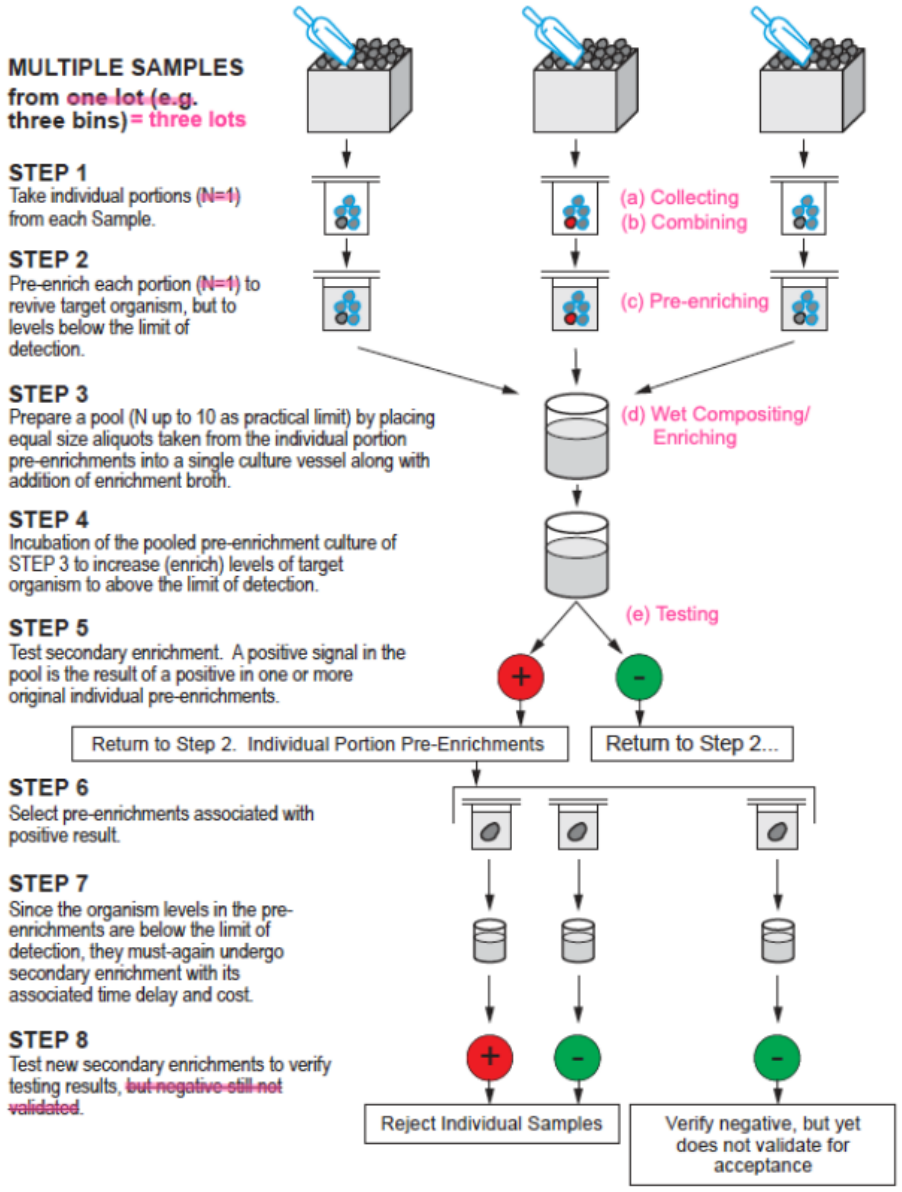
	Hughes uses an EIA method followed by a more sensitive protocol—streaking on selective media. Hughes, 635. Each sample was analyzed by the BAM method (7th Ed.) and the TSVIA method using the enrichment protocol described in section F of the method, *Preparation of Sample*. Equal portions (0.5–1 mL) from each of the 2M broths arising from the 2 selective-enrichment broths were combined in a small screw-capped tube and heat treated in boiling water for 15 min prior to performing the TSVIA. Confirmation for the immunoassay was by streaking from the M broth culture onto selective agar, as specified in the BAM reference culture method. Hughes, 635. A POSITA would find that modifying Hughes based on D'Haese ensures a more sensitive test is used for further testing. A POSITA would have recognized the concentration step could be employed to provide a more sensitive protocol for further testing. A POSITA would understand that retesting the non-pooled, singular samples is an inherently more sensitive process because the sample is not diluted with other samples in the testing pool. Therefore, the retesting of a singular sample is more concentrated and therefore, more sensitive. *See also, generally*, Jarvis. Fratamico discusses the rapid isolation of target microorganisms using an immunomagnetic separation method. Fratamico, 105. Fratamico discloses that use of immunomagnetic beads resulted in "an increase in the level of E. coli B1409 in the sample," as well as "an increase in the number of organisms recovered." *Id.*, 107.

	Additionally, Fratamico provides detailed instructions for preparing and using immunomagnetic bead separation to concentrate a target microbial agent or organism. Fratamico developed "[a] method employing immunomagnetic beads" to concentrate and thereby improve the isolation and detection of E. coli. *Id.* For example, Fratamico was able to use immunomagnetic beads to isolate *E. coli* strains from enrichment cultures of raw ground beef "when the initial inoculum level was 1 cfu g-1." *Id.*, 109. Fratamico further teaches that "[t]he percentage of bacteria recovered increased with increasing amounts of beads." *Id.*, 108. Consequently, Fratamico "demonstrates that immunomagnetic separation [using immunomagnetic beads] is a simple and rapid technique" for concentrating a microbe for isolation and detection. *Id.*, 109. These teachings of Fratamico would have motivated a POSA to modify the combination of ICMSF and Gombas and ICMSF and Hughes to use immunomagnetic beads to concentrate microbes in a modular composite sample to benefit from accurate tests. Use of immunomagnetic beads was already known in the art for concentrating during testing beef samples. "Tu et al. (78) detected pathogenic E. coli O157:H7 in beef hamburger with the use of IMS and ATP measurement. After 6 h of enrichment at 37°C, inoculated beef samples (0.9 ml each) were filtered to remove large particles prior to 10 min of incubation with ca. 10^5 magnetic beads coated with anti–*E. coli* O157:H7 (Dynabeads, Dynal Inc., Oslo, Norway). Following incubation, the magnetic beads were collected with a Dynal magnetic concentrator and resuspended in 0.9 ml of Trypticase soy broth for ATP analysis." Benoit, 1941. "Hudson et al. (26) used IMS to directly separate and concentrate L. monocytogenes from ham samples prior to detection by PCR. IMS was used on the basis of the commercial availability of anti-Listeria magnetic beads (Dynal Inc.) to avoid time-

	consuming cultural enrichment steps and to remove PCR inhibitors." Benoit, 1941-42. Cudjoe investigates the use of immunomagnetic beads in pooling methods. In a study designed to evaluate the performance of Dynabeads® Anti-Salmonella, immunomagnetic separation (IMS) followed by plating (IMS-Plating) proved far superior to the conventional ISO *Salmonella* methodology and the Modified Semi-solid Rappaport-Vassiliadis (MSRV) method. *Salmonella* species were isolated and detected from 135 out of the 180 diverse poultry samples by IMS analysis as against 98 by the conventional method and 33 by the MSRV technique. All results were confirmed biochemically and serologically. It appeared from the data generated that some of the salmonellae isolated using IMS were greatly inhibited in Rappaport-Vassiliadis enrichment broth at 42°C and to a lesser extent in selenite cystine broth at 37°C. A moderately selective plating medium like XLD proved to be better in isolating these possible sensitive wild-type strains of salmonellae than the more selective BGA. Cudjoe, Abstract. *Samples*. One hundred and eighty (180) diverse poultry samples, consisting of 40 pools of cloacal/faecal swabs (5 swabs/pool; 5 pools samples/flock) from eight different flocks, 10 raw chicken breast meat samples, 10 pooled egg shell samples, 60 raw chicken liver samples and 60 chicken neck skins sampled from the slaughter line, were used in this study. All samples were obtained from a poultry slaughter and processing plant in the Netherlands. A pool of five swab samples was pre-enriched in 10 ml of buffered peptone water (BPW; Difco Laboratories, Detroit, Michigan, USA) at 37°C for 16–20 h, whilst 25 g of each of the other samples above, were pre-enriched similarly in 225 ml of BPW and examined as detailed below. Cudjoe, 160.
Claim 15	**Disclosure of Price, ICMSF, and Hughes**

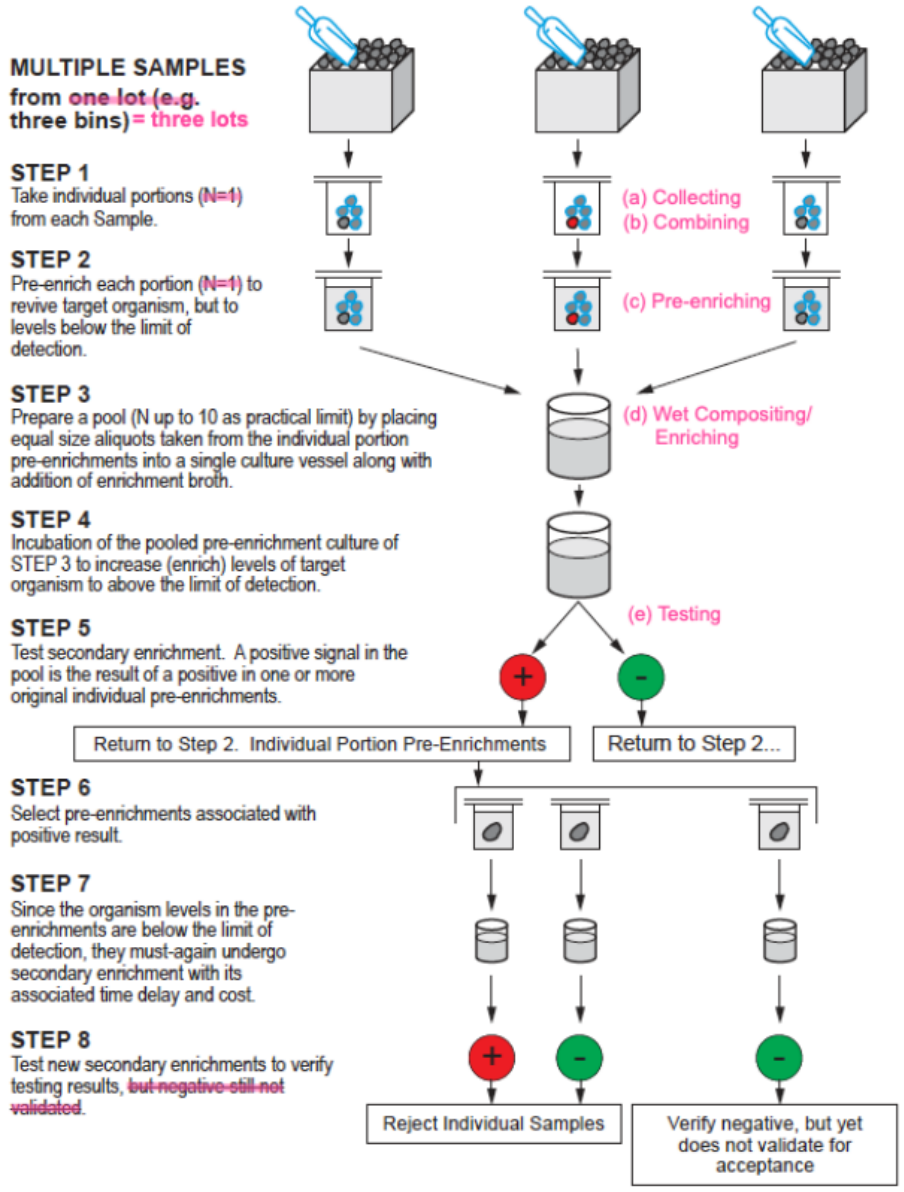
The method of claim 1, wherein each of the lots is selected from the lot group consisting of a single combo, single bin, single pallet, and a production assemblage.	Price, alone or in combination with ICMSF and Hughes discloses Claim 1, upon which this claim relies. See above re Claim 1.

Figure 3: CITED PRIOR ART (PRICE et al, 1972)



	Prosecution History for Reexamination Control No. 95/001,294, 298 (annotated). IEH's diagram also indicates multiple samples are taken from three bins, which is analogous to three lots rather than a single lot because each bin is an assemblage of specimens. The specification of the '771 Patent indicates a test lot is a single-unit production lot ('771 Patent, 5:43-54) such as a single combo, bin. pallet, or segment of production ('771 Patent, 7:54-63; *see also* AAPA #3 and #4). ICMSF teaches collecting from "individual production batches as lots" (ICMSF, 119) and "[a] lot is considered as a collection of units." ICMSF, 327. "Operationally-linked" is simply grouping like items, which is also disclosed by ICMSF. "Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119.
Claim 16	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 1, wherein the number of separate lots is from 3 to 10.	Price, alone or in combination with ICMSF and Hughes discloses Claim 1, upon which this claim relies. See above re Claim 1.

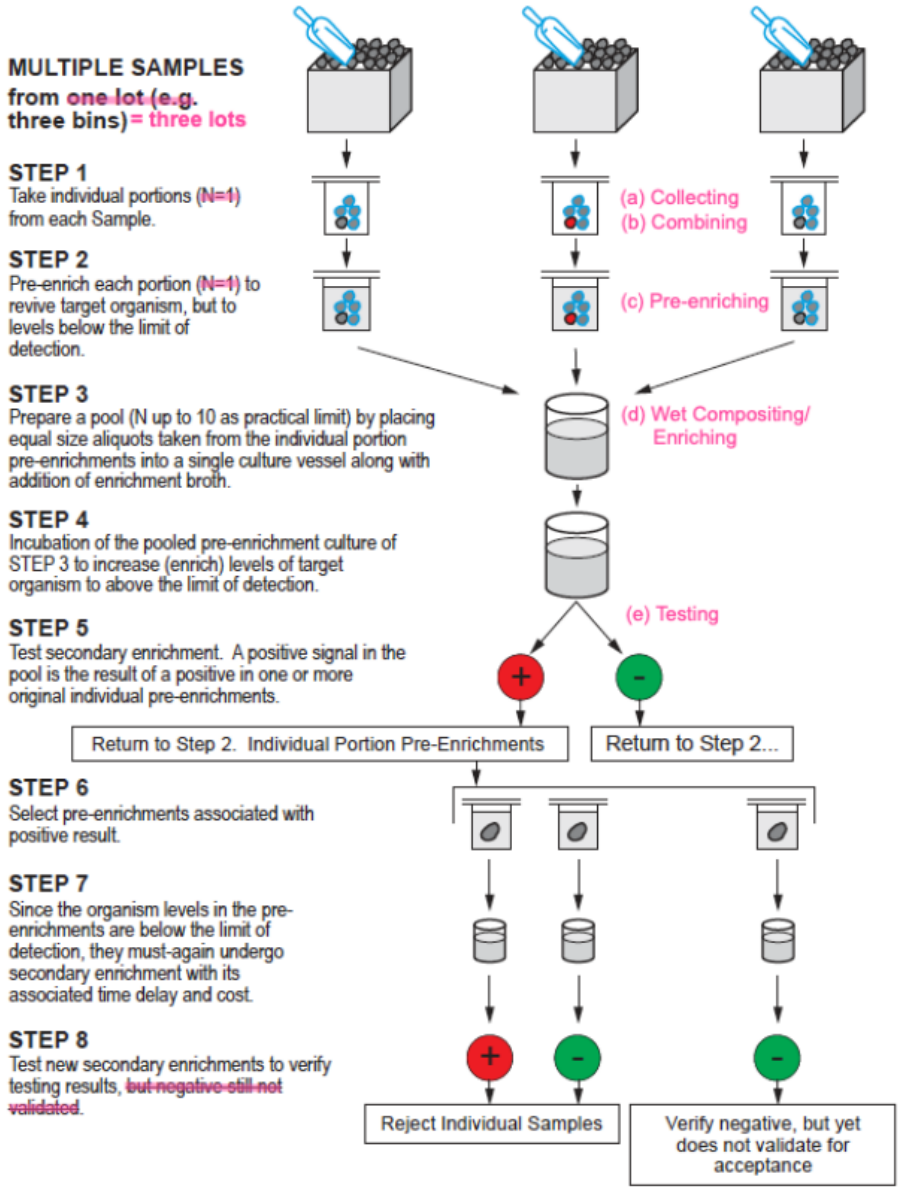
Figure 3: CITED PRIOR ART (PRICE et al, 1972)



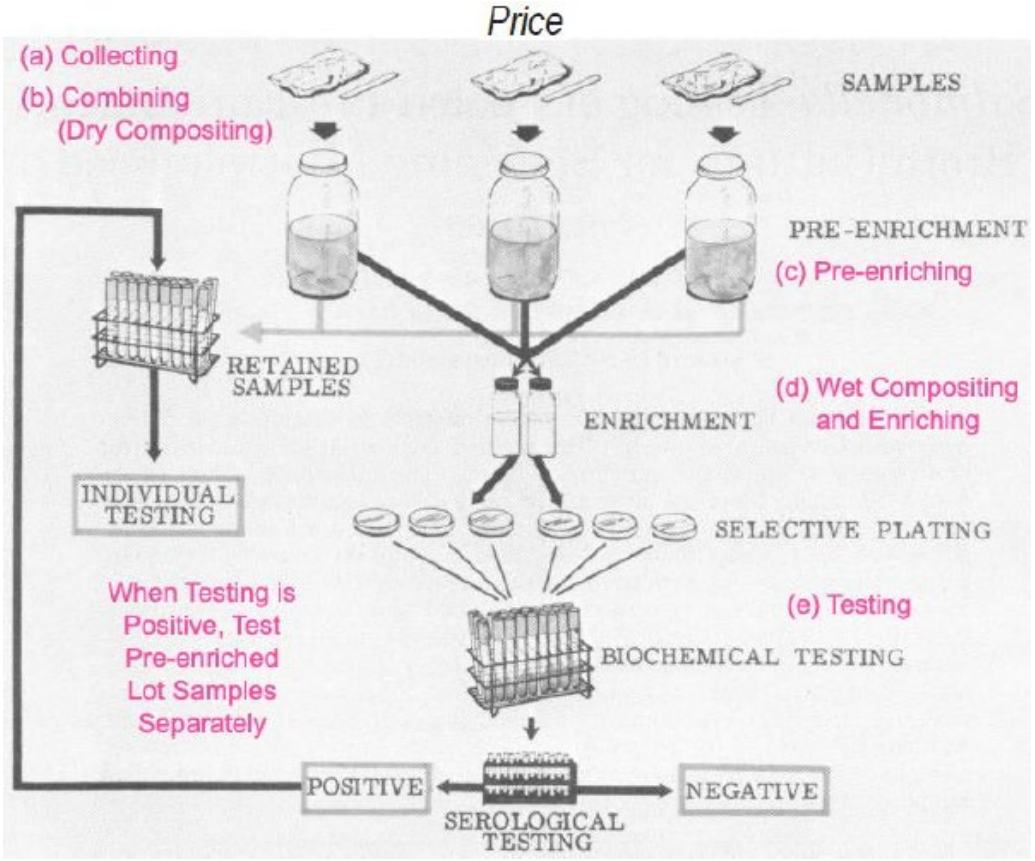
	Prosecution History for Reexamination Control No. 95/001,294, 298 (annotated). IEH's diagram also indicates multiple samples are taken from three bins, which is analogous to three lots rather than a single lot because each bin is an assemblage of specimens. ICMSF indicates a consignment may be "composed of several lots." ICMSF, 119-20. "Several" lots includes at least three lots.
Claim 17	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 1, further comprising, where testing of the pooled modular composite sample is positive, determining which individual separate lots are positive, application of remedial measures to said separate positive lots, and validating by further testing of the respective remediated separate lots and obtaining a negative test result.	Price, alone or in combination with ICMSF and Hughes discloses Claim 1, upon which this claim relies. See above re Claim 1. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Each sample was analyzed by the BAM method (7th Ed.) and the TSVIA method using the enrichment protocol described in section F of the method, *Preparation of Sample*. Equal portions (0.5–1 mL) from each of the 2M broths arising from the 2 selective-enrichment broths were combined in a small screw-capped tube and heat treated in boiling water for 15 min prior to performing the TSVIA. Confirmation for the immunoassay was by streaking from the M broth culture onto selective agar, as specified in the BAM reference culture method.

	Hughes, 635. A POSITA understands the purpose of testing for pathogens and other organisms in food is to determine if a food is safe for entry into commerce to be sold for consumption. One having ordinary skill in the art understands that if a sample tests positive, a remediation step, like cooking, needs to be completed before it can be sold into commerce to comply with USDA guidelines.
Claim 18	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 1, wherein, in a), separately collecting said multiple independent samples from each of the multiple separate lots comprises separately collecting from each separate lot a number of samples from 5 to 100.	Price, alone or in combination with ICMSF and Hughes discloses Claim 1, upon which this claim relies. See above re Claim 1. Price's teachings for collecting a plurality of portions are based on standard practices as described in BAM 1969 (BAM 1969, 1 § 10.04) which cites to JAOAC 1967 (JAOAC, 234). Price, 679, 682. ***Salmonella* analyses were carried out as described in the *Bacteriological Analytical Manual* (BAM) (2) except for the pooling of pre-enrichment broths described in the following paragraph.** Price, 679.

Figure 3: CITED PRIOR ART (PRICE et al, 1972)



	Prosecution History for Reexamination Control No. 95/001,294, 298 (annotated). IEH's diagram also indicates multiple samples are taken from three bins, which is analogous to three lots rather than a single lot because each bin is an assemblage of specimens. The purpose of Jarvis and Price was to determine whether pooling samples reduced the ability to detect contaminated food lots. It would have been commonsense to combine samples from the same bin and subject them to enrichment, since each bin represents a discrete unit of the production run and results obtained from the enriched sample could be attributed to the bin from which the sample was obtained. Prosecution History for Reexamination Control No. 95/001,294, at 1575 (First Board Decision of January 20, 2015 at 16). Additionally, the specification describes a bin of dry food materials (almonds), like those in Price, as one example of a test lot/single unit of production, similar to a combo of beef. '771 Patent, 5:55-60. Price discloses testing of "Dried egg albumen, cocoa, and nonfat dry milk" (Price, 679) and "wheat flour, coconut, and cottonseed flour" Price, 681.

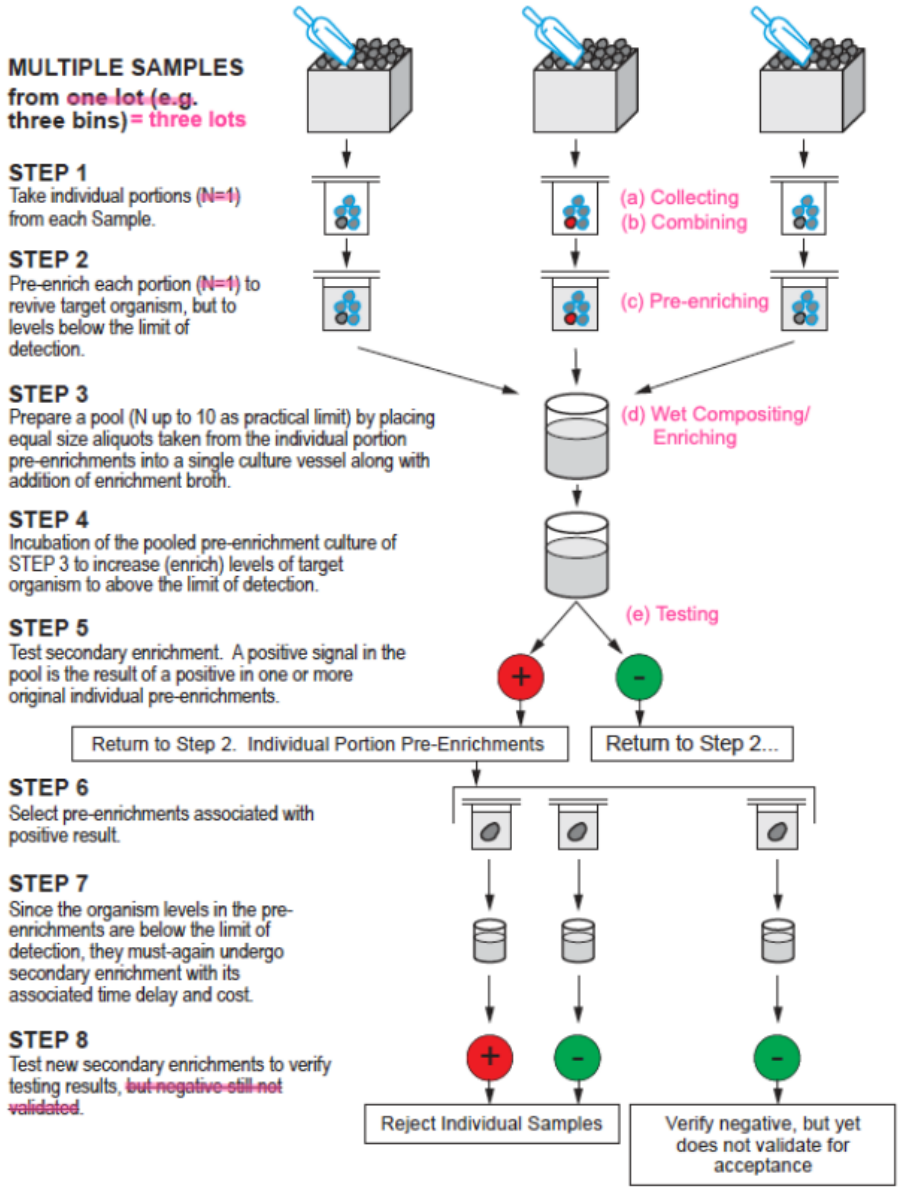


Price, 680 (Figure 1 (annotated)).

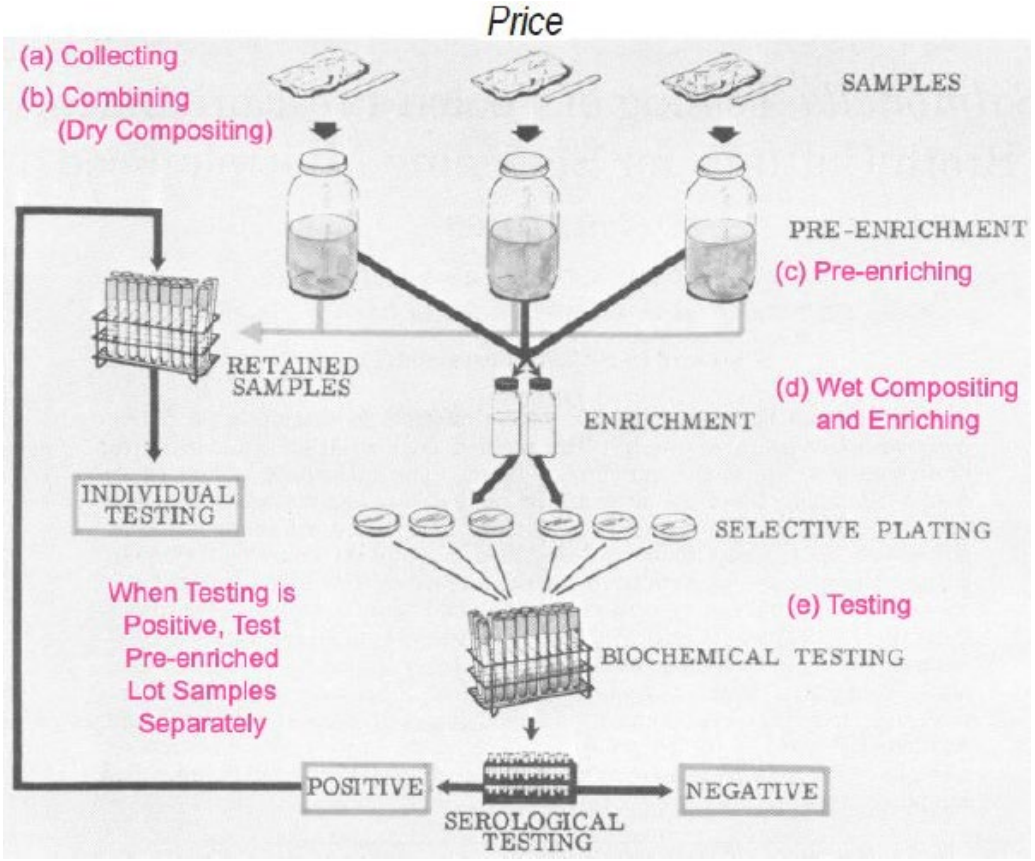
	A method has been described for testing multiple food samples for *Salmonella* without loss in sensitivity. The method pools multiple pre-enrichment broth cultures into single enrichment broths. The subsequent stages of the *Salmonella* analysis are not altered. The method was found applicable to several dry food materials including nonfat dry milk, dried egg albumin, cocoa, cottonseed flour, wheat flour, and shredded coconut. As many as 25 pre-enrichment broth cultures were pooled without apparent loss in the sensitivity of *Salmonella* detection as compared to individual sample analysis. The procedure offers a simple, yet effective, way to increase sample capacity in the *Salmonella* testing of foods, particularly where a large proportion of samples ordinarily is negative. It also permits small portions of pre-enrichment broth cultures to be retained for subsequent individual analysis if positive tests are found. *Salmonella* testing of pooled pre-enrichment broths provides increased consumer protection for a given amount of analytical effort as compared to individual sample analysis. Price, 679. A POSITA would find it obvious to apply the teachings of Price to meat. *See also* MPEP §§ 2144.05, 2131.03. IEH's diagram of Price from Prosecution History for Reexamination Control No. 95/001,294 (reproduced below) shows step 1 corresponds to IEH's step (a), with instructions to "[t]ake individual portions . . . from each Sample." ICMSF teaches collecting 5, 30, 60, 95, or 100 portions. ICMSF, 184-90, 276, 282, 309, 320, 326-30.
Claim 19	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 1 wherein, in a), separately collecting said multiple independent samples from each of the multiple separate lots comprises separately	Price, alone or in combination with ICMSF and Hughes discloses Claim 1, upon which this claim relies. See above re Claim 1. Price's teachings for collecting a plurality of portions are based on standard practices

collecting 30 to 60 samples from each separate lot.	as described in BAM 1969 (BAM 1969, 1 § 10.04) which cites to JAOAC 1967 (JAOAC, 234). Price, 679, 682. ***Salmonella* analyses were carried out as described in the *Bacteriological Analytical Manual* (BAM) (2) except for the pooling of pre-enrichment broths described in the following paragraph.** Price, 679. IEH's diagram of Price from Prosecution History for Reexamination Control No. 95/001,294 (reproduced below) shows step 1 corresponds to IEH's step (a), with instructions to "[t]ake individual portions . . . from each Sample."

Figure 3: CITED PRIOR ART (PRICE et al, 1972)



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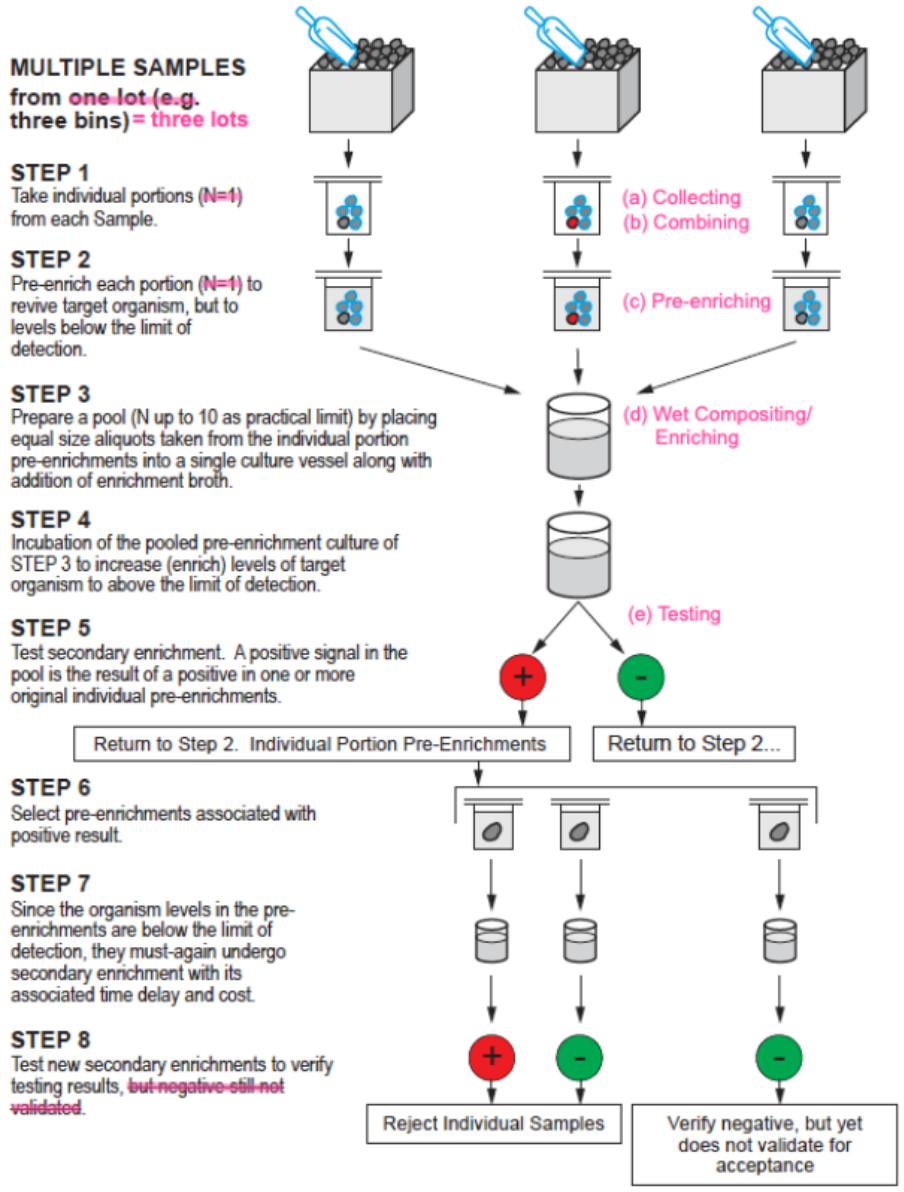


Price, 680 (Figure 1 (annotated)).

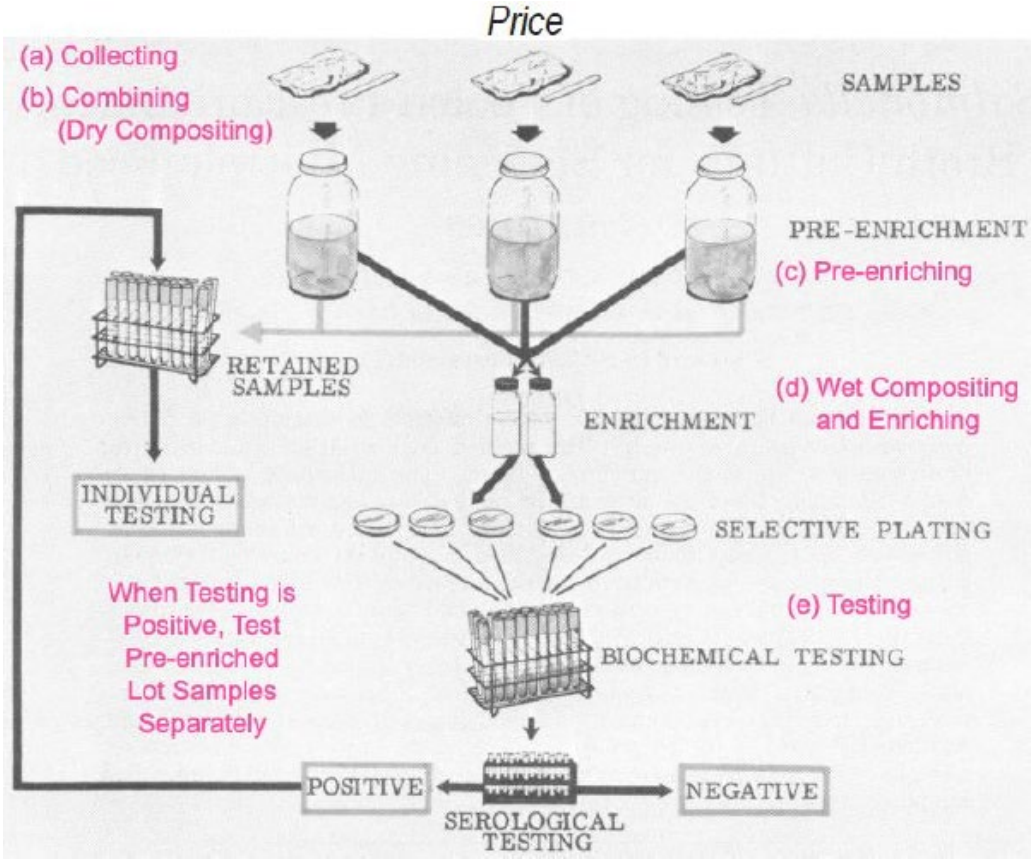
	A method has been described for testing multiple food samples for *Salmonella* without loss in sensitivity. The method pools multiple pre-enrichment broth cultures into single enrichment broths. The subsequent stages of the *Salmonella* analysis are not altered. The method was found applicable to several dry food materials including nonfat dry milk, dried egg albumin, cocoa, cottonseed flour, wheat flour, and shredded coconut. As many as 25 pre-enrichment broth cultures were pooled without apparent loss in the sensitivity of *Salmonella* detection as compared to individual sample analysis. The procedure offers a simple, yet effective, way to increase sample capacity in the *Salmonella* testing of foods, particularly where a large proportion of samples ordinarily is negative. It also permits small portions of pre-enrichment broth cultures to be retained for subsequent individual analysis if positive tests are found. *Salmonella* testing of pooled pre-enrichment broths provides increased consumer protection for a given amount of analytical effort as compared to individual sample analysis. Price, 679. A POSITA would find it obvious to apply the teachings of Price to beef. *See also* MPEP §§ 2144.05, 2131.03. ICMSF teaches collecting 5, 30, 60, 95, or 100 portions. ICMSF, 184-90, 276, 282, 309, 320, 326-30.
Claim 20	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 1, wherein, in a), separately collecting said multiple independent samples from each of the multiple separate lots comprises separately collecting at least 30 samples from each separate lot.	Price, alone or in combination with ICMSF and Hughes discloses Claim 1, upon which this claim relies. See above re Claim 1. Price's teachings for collecting a plurality of portions are based on standard practices as described in BAM 1969 (BAM 1969, 1 § 10.04) which cites to JAOAC 1967 (JAOAC, 234). Price, 679, 682.

	Salmonella **analyses were carried out as described in the *Bacteriological Analytical Manual* (BAM) (2) except for the pooling of pre-enrichment broths described in the following paragraph.** Price, 679. IEH's diagram of Price from Prosecution History for Reexamination Control No. 95/001,294 (reproduced below) shows step 1 corresponds to IEH's step (a), with instructions to "[t]ake individual portions . . . from each Sample."

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	Prosecution History for Reexamination Control No. 95/001,294, 298 (annotated). IEH's diagram also indicates multiple samples are taken from three bins, which is analogous to three lots rather than a single lot because each bin is an assemblage of specimens. The purpose of Jarvis and Price was to determine whether pooling samples reduced the ability to detect contaminated food lots. It would have been commonsense to combine samples from the same bin and subject them to enrichment, since each bin represents a discrete unit of the production run and results obtained from the enriched sample could be attributed to the bin from which the sample was obtained. Prosecution History for Reexamination Control No. 95/001,294, at 1575 (First Board Decision of January 20, 2015 at 16). Additionally, the specification describes a bin of dry food materials (almonds), like those in Price, as one example of a test lot/single unit of production, similar to a combo of beef. '771 Patent, 5:55-60. Price discloses testing of "Dried egg albumen, cocoa, and nonfat dry milk" (Price, 679) and "wheat flour, coconut, and cottonseed flour" Price, 681.



Price, 680 (Figure 1 (annotated)).

	A method has been described for testing multiple food samples for *Salmonella* without loss in sensitivity. The method pools multiple pre-enrichment broth cultures into single enrichment broths. The subsequent stages of the *Salmonella* analysis are not altered. The method was found applicable to several dry food materials including nonfat dry milk, dried egg albumin, cocoa, cottonseed flour, wheat flour, and shredded coconut. As many as 25 pre-enrichment broth cultures were pooled without apparent loss in the sensitivity of *Salmonella* detection as compared to individual sample analysis. The procedure offers a simple, yet effective, way to increase sample capacity in the *Salmonella* testing of foods, particularly where a large proportion of samples ordinarily is negative. It also permits small portions of pre-enrichment broth cultures to be retained for subsequent individual analysis if positive tests are found. *Salmonella* testing of pooled pre-enrichment broths provides increased consumer protection for a given amount of analytical effort as compared to individual sample analysis. Price, 679. A POSITA would find it obvious to apply the teachings of Price to meat. ICMSF teaches collecting 5, 30, 60, 95, or 100 portions. ICMSF, 184-90, 276, 282, 309, 320, 326-30. *See also* MPEP § 2144.05.
Claim 22	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 1, wherein removing portions in step d) is removing equal portions, aseptically, so as to preclude contamination of the enriched separate composited lot samples.	Price, alone or in combination with ICMSF and Hughes discloses Claim 1, upon which this claim relies. See above re Claim 1. Price further discloses removing portions in step d) is removing equal portions, aseptically, so as to preclude contamination of the enriched separate test lot samples. ICMSF describes aseptically withdrawing and weighing sample units into the appropriate diluent. ICMSF, 231, 232.

	Hughes instructs that after enrichment "[c]ombine equal volumes (0.5-1 mL) from each M broth tube in a clean screw-cap tube." Hughes, 638. Moreover, a POSITA would consider this to be standard practice. A POSITA would have aseptically removed equal volume portions of each enriched separate test lot sample to preclude contamination.
Claim 23	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 1, wherein the target microbe(s) comprises at least one selected from the group consisting of *Listeria*, *Salmonella* and *Escherichia coli*.	Price, alone or in combination with ICMSF and Hughes discloses Claim 1, upon which this claim relies. See above re Claim 1. Price further discloses a target microbial agent or organism comprises at least one selected from the group consisting of *Listeria*, *Salmonella* and *Escherichia coli*. ICMSF describes testing for *Listeria* (ICMSF, 285-312), *Salmonella* (*id.*, 273-84), and *Escherichia coli* (*id.*, 313-32). Hughes focuses on *Salmonella*. Hughes, 634. Price established that "pre-enrichment broths can be pooled for analysis without loss in sensitivity to *Salmonella* in the individual samples." Price, 679. Price tested dry food materials inoculated (purposefully contaminated) with *Salmonella. Id.*
Claim 24	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 1, wherein the target microbe(s) comprises at least one selected from the group consisting of *Listeria* spp., *Listeria monocytogenes*, *Salmonella* spp., *Escherichia coli* O157:H7, coliforms and fecal coliforms.	Price, alone or in combination with ICMSF and Hughes discloses Claim 1, upon which this claim relies. See above re Claim 1. Price further discloses the target microbial agent or organism comprises at least one selected from the group consisting of *Listeria* spp*., Listeria monocytogenes*, *Salmonella* spp., *Escherichia coli* O157:H7, coliforms and fecal coliforms.

	ICMSF describes testing for *Listeria* (ICMSF, 285-312), *Salmonella* (*id.*, 273-84), and *Escherichia coli* (*id.*, 313-32). Hughes focuses on *Salmonella*. Hughes, 634. Price established that "pre-enrichment broths can be pooled for analysis without loss in sensitivity to *Salmonella* in the individual samples." Price, 679. Price tested dry food materials inoculated (purposefully contaminated) with *Salmonella. Id.*
Claim 25	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 1, wherein the target microbe(s) comprises a spoilage organism.	Price, alone or in combination with ICMSF and Hughes discloses Claim 1, upon which this claim relies. See above re Claim 1. Price further discloses a target microbial agent or organism comprises a spoilage organism. A POSITA would find it obvious to apply the teachings of Price to target spoilage organisms and would employ a common test like APC, as discussed in regulations such as PR/HACCP. PR/HAACP, 38849 ("Various alternative indicator organisms were suggested, including generic *E. coli* (biotype I), total plate counts, Enterobacteriaceae, Total Viable Counts (TVC), and Aerobic Plate Counts (APC)."). "Examination of every piece of whole meat is clearly impractical, hence any sampling procedure for estimating shelf-life would involve taking enough samples to give some statistical assurance of the keeping qualities of meat." Gill, 449. Borch is titled "Bacterial spoilage of meat and cured meat products." Borch. The reference discusses spoilage organisms in meat:

	Lactic acid bacteria are the major bacterial group associated with the spoilage of refrigerated vacuum- or MA-packaged cooked, cured meat products (Blickstad and Molin, 1983b; Shaw and Harding, 1989; von Holy et al., 1991). At the time of spoilage some products contain a 'pure' culture of only one species, while in others a mixture of *Lactobacillus* spp. and *Leuconostoc* spp. was found (Kant-Muermans, unpublished results). The great diversity of bacteria isolated from spoiled meat products is confirmed by several studies (Korkeala and Mäkelä, 1989; von Holy et al., 1991; Yang and Ray, 1994). The genus/species of lactic acid bacteria responsible for spoilage, depend on the product composition (product-related flora) as well as the manufacturing site (house-related flora; Korkeala and Mäkelä, 1989). Borch, 113. "Lactic acid bacteria spoil refrigerated meat products by causing defects such as sour off-flavours, discolouration, gas production, slime production and decrease in PH." Borch, 114; *see also, id.*, 115-17 (Section 4 Analysis of Spoilage). It is understood in the art that aerobic plate counts ("APC") can test for spoilage organisms. *See. e.g.*, ICMSF 1986, 122 ("tests to be made (e.g., APC, *Salmonella*, Clostridium perfringens"); *id.* at 132 ("However, aerobic plate counts (APC) can be used to monitor Good Commercial Practice (GCP), and criteria based on such examinations are a valuable aid to in-plant quality control.").

TABLE 19

Sampling plans and recommended microbiological limits for raw meat[a]

Product[b]	Test	Method reference[c]	Case	Plan class	n	c	Limit per cm² or gram m	M
Carcass meat, before chilling	APC	118	1	3	5	3	10^5	10^6
Carcass meat, chilled	APC	118	1	3	5	3	10^6	10^7
Edible offal, chilled	APC	118	1	3	5	3	10^6	10^7
Carcass meat, frozen	APC	118	1	3	5	3	5×10^5	10^7
Boneless meat, frozen (beef, veal, pork, mutton)	APC	118	1	3	5	3	5×10^5	10^7
Comminuted meat, frozen	APC	118	1	3	5	3	10^6	10^7
Edible offal, frozen	APC	118	1	3	5	3	5×10^5	10^7

a **Not for use at port of entry** but for in-plant quality control. Refer to Section B for appropriate applications.
b Unfrozen carcasses and primal cuts, swab counts per cm^2; other meats and meat products, per gram.
c Refers to page number in ICMSF, 1978.

ICMSF 1986, 133 (Table 19 (annotated)).

Although for “quality control,” a POSITA would understand that "quality control" means testing for spoilage organisms could also be accomplished by this well-known method. It is disclosed within the prior art during the production lifecycle of beef production. ICMSF 1986, 134 (“The FAOIWHO Working Group (FAQ/WHO, 1979) considered that the only point at which APC could be used for chilled meat, to evaluate the hygienic conditions under which it was produced, was at the slaughterhouse, and because of its perishable nature it would not be possible to set APC limits for use in guidelines or specifications for chilled meats at port of

entry."); *see also, id.*, 153 ("Monitoring for APC, E. coli, and Staph. aureus would give some indication of inadequate processing (heating, curing, smoking), postprocess contamination, or storage at temperatures above those recommended for frozen or refrigerated distribution and storage.")

E. TEST PROCEDURES

(a) *Aerobic plate counts*

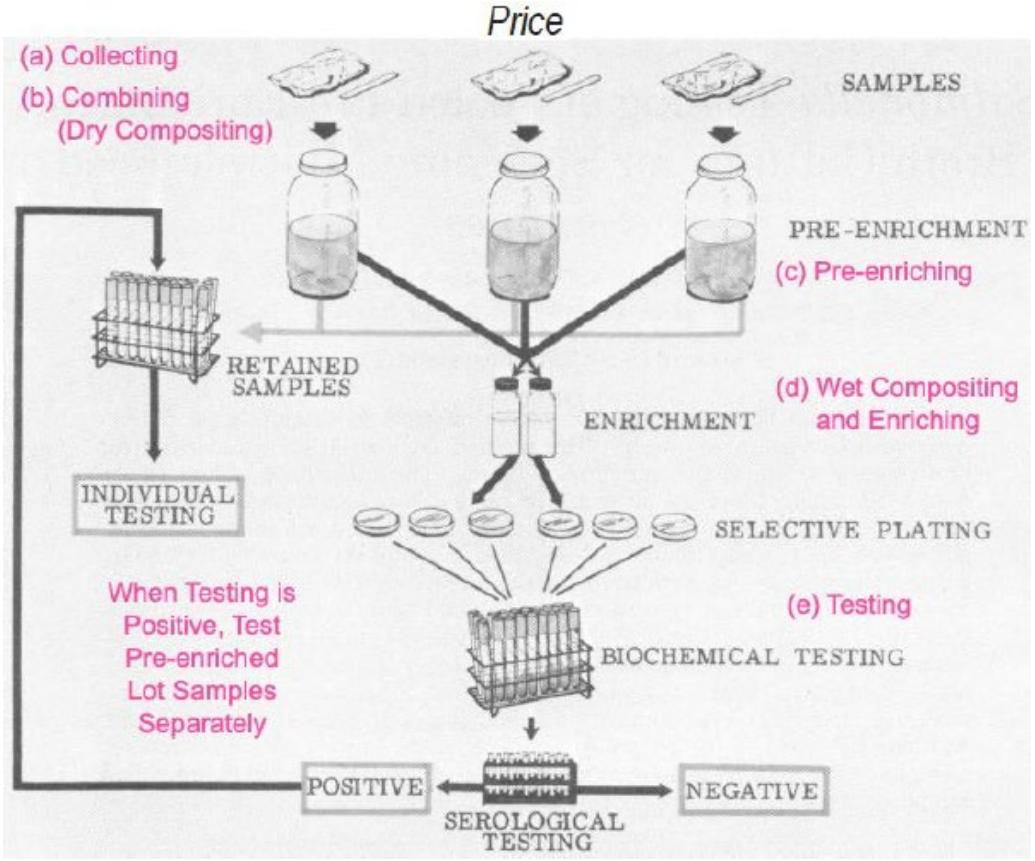
Comminute, blend, or stomach the various portions of meat composing the sample unit and withdraw the analytical unit. Combine the swabs to form an analytical unit, or treat each separately. Prepare dilutions and perform the APC test according to the directions given in ICMSF, 1978, p. 118. Spread plates are preferable to pour plates when examining raw meats.

The temperature used for incubation of plates should be related to the material being examined. To estimate numbers of mesophilic contaminants on freshly dressed carcasses it is advisable to incubate plates at 35 or 37°C. For meat that has been held under chilled conditions, plates should be incubated at 25°C to include psychrotrophs and thus give a better indication of the microbial growth that has occurred. Counts at 20°C give similar results after a longer incubation time.

ICMSF 1986, 136 (annotated).

See also, Bhunia 4 ("Bacteria-specific generic rapid methods such ATP-based luminescence and total plate count (APC) are also needed for monitoring cleaning and hygienic practices employed in a processing plant."); Wheeler, 1606 (use of APC in a beef-processing plant); PR/HAACP, 38849 ("Various alternative indicator organisms were suggested, including generic E. coli (biotype I), total plate counts, Enterobacteriaceae, Total Viable Counts (TVC), and Aerobic Plate Counts (APC).").

Claim 26	
The method of claim 1, wherein removing portions of each separate enriched composited lot sample comprises removing equal volume portions of each separate enriched composited lot sample.	Price, alone or in combination with ICMSF and Hughes discloses Claim 1, upon which this claim relies. See above re Claim 1. Price further discloses removing portions of each enriched separate test lot sample comprises removing equal volume portions of each enriched separate test lot sample. ICMSF describes aseptically withdrawing and weighing sample units into the appropriate diluent. ICMSF, 231, 232. Hughes instructs that after enrichment "[c]ombine equal volumes (0.5-1 mL) from each M broth tube in a clean screw-cap tube." Hughes, 638. Moreover, a POSITA would consider this to be standard practice. A POSITA would have removed equal volume portions of each enriched separate test lot sample.
Claim 27	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 1, wherein testing of the pooled modular composite sample, using a suitable detection assay comprises use of at least one of a DNA-based method, an immunochemistry-based method, and a biosensor-based method.	Price, alone or in combination with ICMSF and Hughes discloses Claim 1, upon which this claim relies. See above re Claim 1. Price further discloses testing of the modular composite sample, using a suitable detection assay comprises use of at least one of a DNA-based method, an immunochemistry-based method, and a biosensor-based method. Price discloses biochemical testing of the modular composite sample. A POSITA would consider other suitable detection assays to test for the presence of *Salmonella*, which can include DNA-based methods, immunochemistry-based methods, and biosensor-based methods.

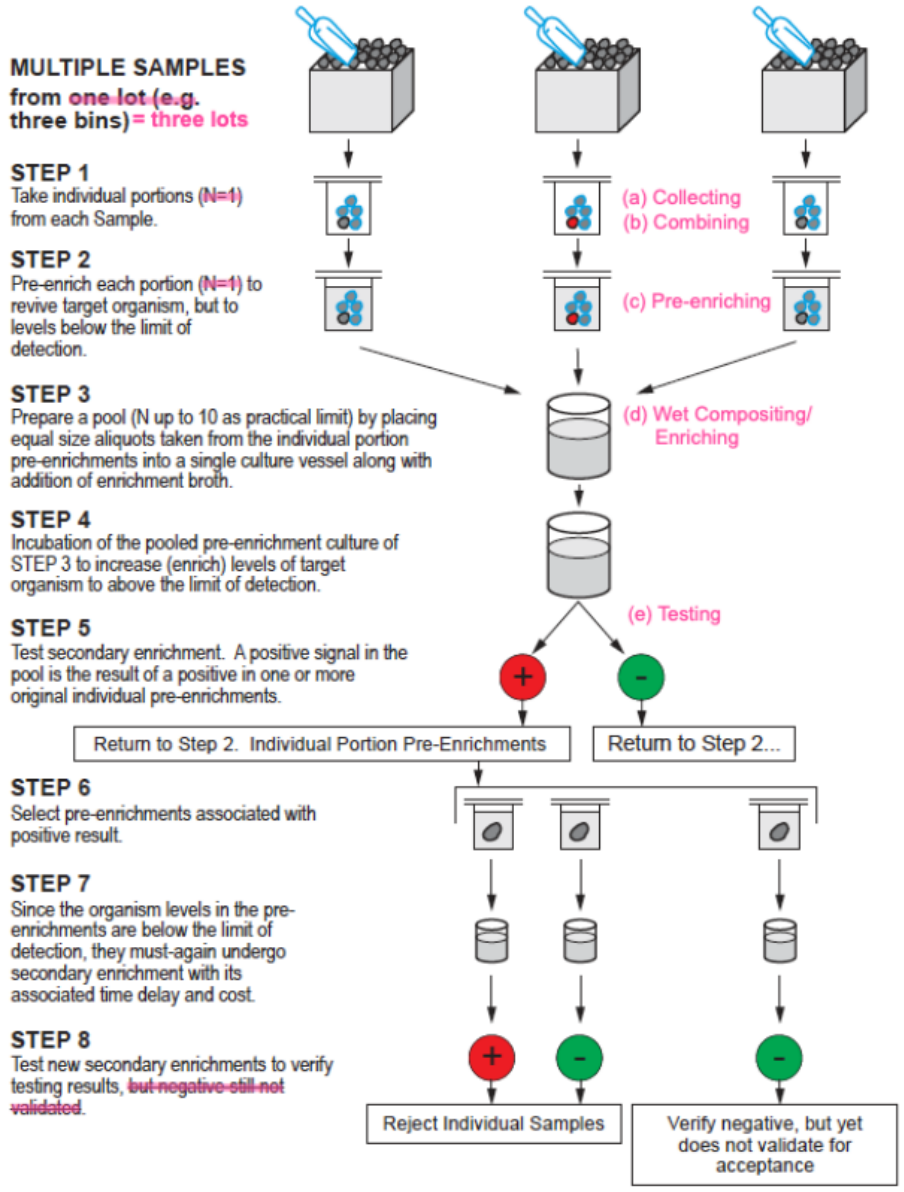


Price, 680 (Figure 1 (annotated)).

Hughes discloses use of "an enzyme immunoassay (EIA) method," which is an immunochemistry-based method. Hughes, 635.

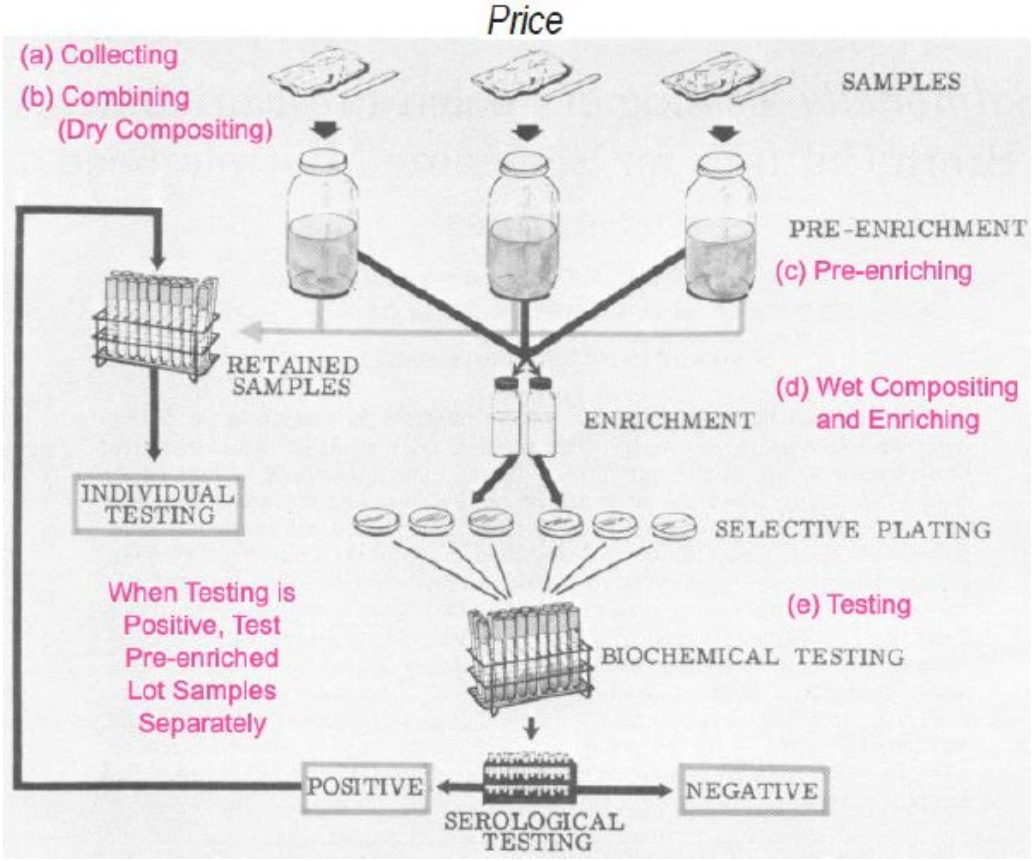
	Each sample was analyzed by the BAM method (7th Ed.) and the TSVIA method using the enrichment protocol described in section F of the method, *Preparation of Sample*. Equal portions (0.5–1 mL) from each of the 2M broths arising from the 2 selective-enrichment broths were combined in a small screw-capped tube and heat treated in boiling water for 15 min prior to performing the TSVIA. Confirmation for the immunoassay was by streaking from the M broth culture onto selective agar, as specified in the BAM reference culture method. Hughes, 635.
Claim 28	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 1, wherein each of the multiple separate lots are production lots of a meat or poultry product, comprising an assemblage of one or more meat or poultry specimens, respectively.	Price, in combination with ICMSF and Hughes, discloses Claim 1 upon which this claim relies. See above re Claim 1.

Figure 3: CITED PRIOR ART (PRICE et al, 1972)



	Prosecution History for Reexamination Control No. 95/001,294, 298 (annotated). IEH's diagram also indicates multiple samples are taken from three bins, analogous to three lots rather than a single lot because each bin is an assemblage of specimens.
Claim 29	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 28, wherein the meat or poultry specimens are meat trim pieces or poultry trim pieces, respectively.	Price, alone or in combination with ICMSF and Hughes discloses Claim 28, upon which this claim relies. See above re Claim 28. A POSITA would find it obvious to apply the teachings of Price to meat or poultry trim pieces. ICMSF indicates "[t]rimmings to be used for ground beef could be pretested for E. coli O157:H7 by various sampling plans." ICMSF, 323. ICMSF indicates "[r]esearch has validated that up to fifteen 25 g analytical units of ground beef or trimmings can be composited without significant loss in E. coli O157:H7 detection sensitivity." *Id.*, 327 *see also id.*, 226, 314, 320, and 324. ICMSF includes many other references to testing lots of beef or poultry, two of the most common types of meat in commerce. *Id.* at 114 (§ 6.3), 184–191 (§§ 10.2–10.6), 225–226 (§ 12.1), 285 (§ 16.1), 313–330 (Chapter 17). ICMSF is relevant to any kind of food product including beef or poultry.
Claim 30	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 28, wherein the meat is that of beef, pork, sheep or bison.	Price, alone or in combination with ICMSF and Hughes discloses Claim 28, upon which this claim relies. See above re Claim 28. A POSITA would find it obvious to apply the teachings of Price to meat. ICMSF indicates "[t]rimmings to be used for ground beef could be pretested for E. coli O157:H7 by various sampling plans." ICMSF, 323.

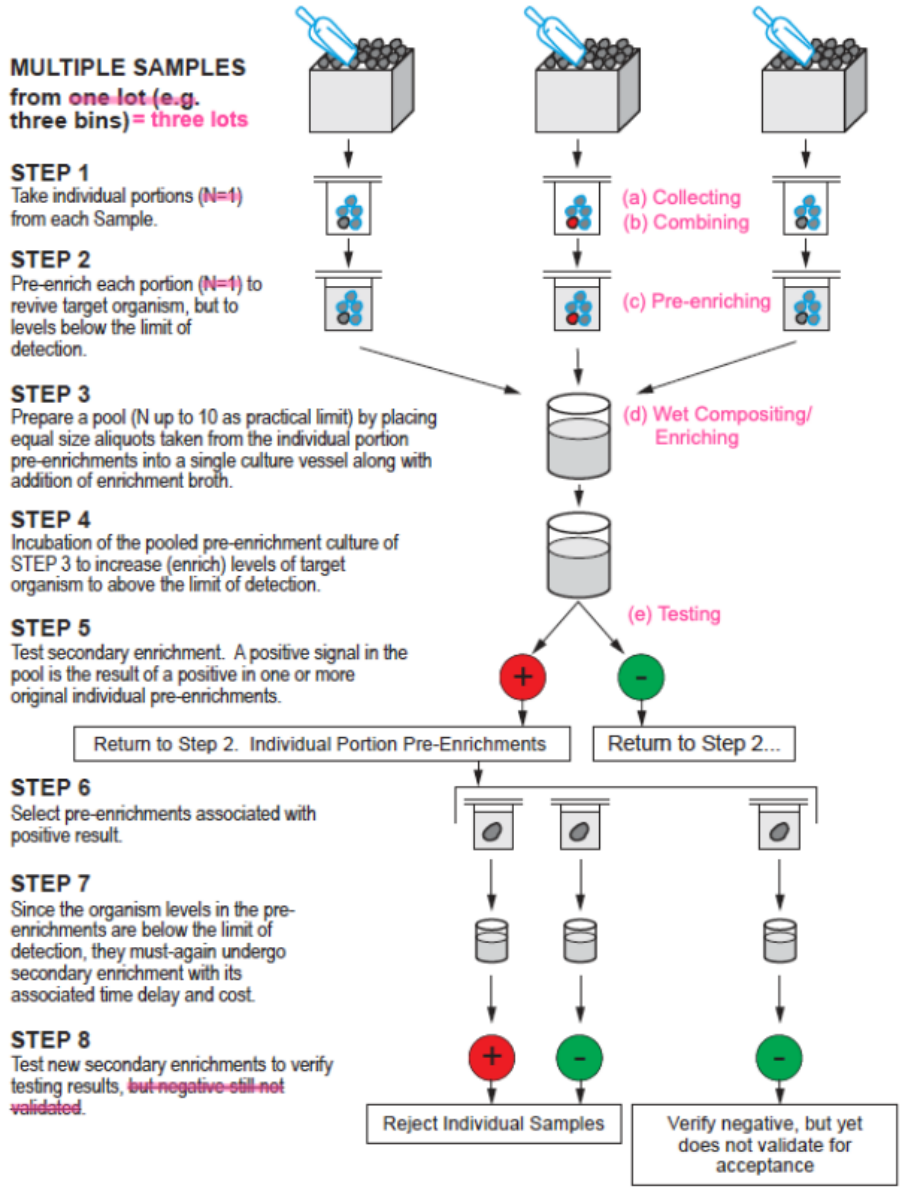
	ICMSF indicates "[r]esearch has validated that up to fifteen 25 g analytical units of ground beef or trimmings can be composited without significant loss in E. coli O157:H7 detection sensitivity." *Id.*, 327 *see also id.*, 226, 314, 320, and 324. ICMSF includes many other references to testing lots of beef or poultry, two of the most common types of meat in commerce. *Id.*, 114 (§ 6.3), 184–191 (§§ 10.2–10.6), 225–226 (§ 12.1), 285 (§ 16.1), 313–330 (Chapter 17). ICMSF is relevant to any kind of food product including beef, pork, sheep or bison.
Claim 31	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 30, wherein the meat is that of beef.	Price, alone or in combination with ICMSF and Hughes discloses Claim 30, upon which this claim relies. See above re Claim 30. A POSITA would find it obvious to apply the teachings of Price to beef. ICMSF indicates "[t]rimmings to be used for ground beef could be pretested for E. coli O157:H7 by various sampling plans." ICMSF, 323. ICMSF indicates "[r]esearch has validated that up to fifteen 25 g analytical units of ground beef or trimmings can be composited without significant loss in E. coli O157:H7 detection sensitivity." *Id.*, 327 *see also id.*, 226, 314, 320, and 324. ICMSF includes many other references to testing lots of beef or poultry, two of the most common types of meat in commerce. *Id.* at 114 (§ 6.3), 184–191 (§§ 10.2–10.6), 225–226 (§ 12.1), 285 (§ 16.1), 313–330 (Chapter 17). ICMSF is relevant to any kind of food product including beef.
Claim 37	**Disclosure of Price, ICMSF, and Hughes**
[37pre] A method of sampling and testing products for microbe(s) in multiple separate lots, comprising:	To the extent the preamble is limiting, as discussed herein, Price, alone or in combination with ICMSF and Hughes, discloses a method of sampling and testing for microbes in multiple separate test lots.

	Price established that "pre-enrichment broths can be pooled for analysis without loss in sensitivity to *Salmonella* in the individual samples." Price, 679. Price tested dry food materials inoculated (purposefully contaminated) with *Salmonella. Id.*  Price, 680 (Figure 1 (annotated)). ICMSF includes many other references to testing lots of beef or poultry, two of the most common types of meat in commerce. *Id.*, 114 (§ 6.3), 184–191 (§§ 10.2–10.6),

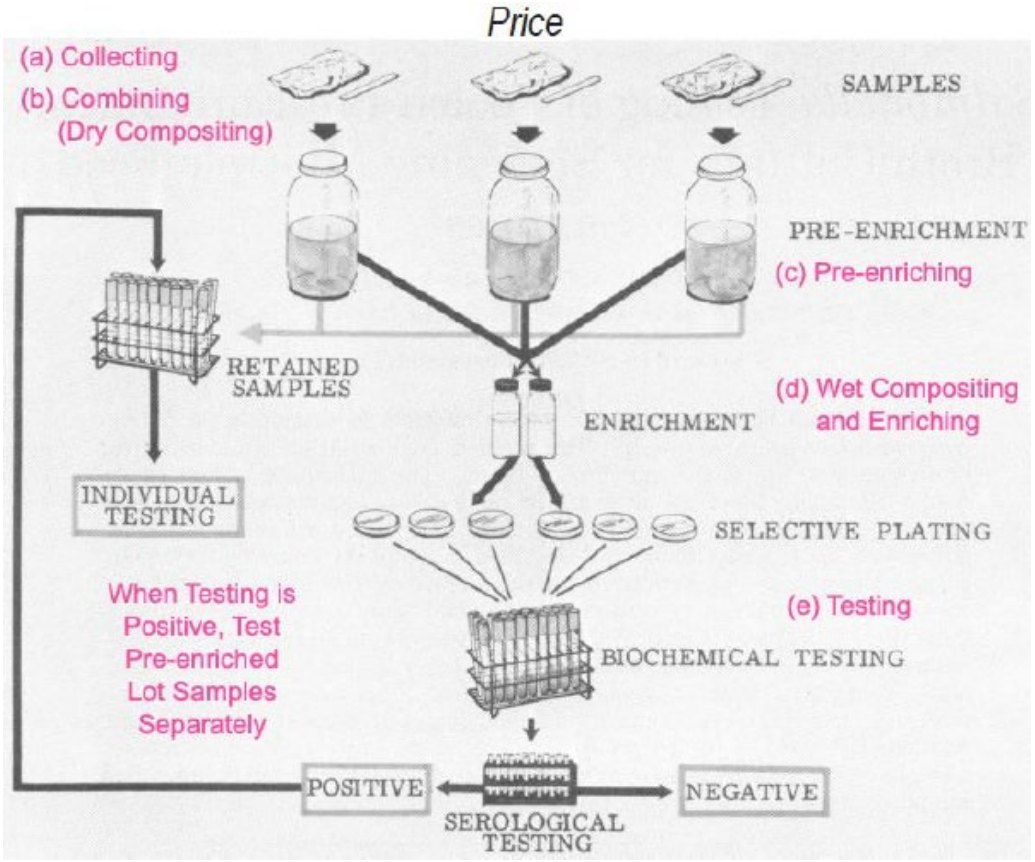
	225–226 (§ 12.1), 285 (§ 16.1), 313–330 (Chapter 17). ICMSF is relevant to any kind of food product including beef. Hughes discusses turkey (Hughes, Abstract), and a POSITA would be motivated to combine the teachings of ICMSF and Hughes as they both test meat for release into commerce. Hughes discloses testing turkey for *Salmonella*. "Three food types (. . . and raw turkey) were analyzed in the United States." Hughes, 634, 645, Table 8. Table 8 shows results from 12 collaborators for naturally contaminated raw turkey. For the 180 samples tested, using confirmed data, there was one false negative at the low level for the TECRA method. There were 3 false negatives at the low level and 3 false negatives at the high level for the cultural method. The incidence of false-positive results was very high for the cultural method, with a total of 41 false positives, compared with 5 for the TECRA method. It should, however, be taken into account that the comparison was with the method in BAM 7th Ed., not the current 8th Ed. The method for raw flesh food in the BAM 8th Ed. uses elevated temperature with RV and TT broth, which would be more selective. No significant Hughes, 646. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638.

	Moreover, the recitation of a lot being validated for "release . . . into commerce" is not limiting. Prosecution History for Reexamination Control No. 95/011,294.
[37a] a) separately collecting multiple independent samples from each of multiple separate single production lots, wherein each separate single production lot is separately sampled by taking said multiple independent samples thereof;	Price, alone or in combination with ICMSF and Hughes, discloses separately collecting multiple independent samples from each of multiple separate single-unit production lots, wherein each separate single-unit production lot is separately sampled by taking said multiple independent samples thereof. Price's teachings for collecting a plurality of portions are based on standard practices as described in BAM 1969 (BAM 1969, 1 § 10.04) which cites to JAOAC 1967 (JAOAC, 234). Price, 679, 682. ***Salmonella* analyses were carried out as described in the *Bacteriological Analytical Manual* (BAM) (2) except for the pooling of pre-enrichment broths described in the following paragraph.** Price, 679. IEH's diagram of Price from Prosecution History for Reexamination Control No. 95/001,294 (reproduced below) shows step 1 corresponds to IEH's step (a), with instructions to "[t]ake individual portions . . . from each Sample."

Figure 3: CITED PRIOR ART (PRICE et al, 1972)



	Prosecution History for Reexamination Control No. 95/001,294, 298 (annotated). IEH's diagram also indicates multiple samples are taken from three bins, which is analogous to three lots rather than a single lot because each bin is an assemblage of specimens. The purpose of Jarvis and Price was to determine whether pooling samples reduced the ability to detect contaminated food lots. It would have been commonsense to combine samples from the same bin and subject them to enrichment, since each bin represents a discrete unit of the production run and results obtained from the enriched sample could be attributed to the bin from which the sample was obtained. Prosecution History for Reexamination Control No. 95/001,294, at 1575 (First Board Decision of January 20, 2015 at 16). Additionally, the specification describes a bin of dry food materials (almonds), like those in Price, as one example of a test lot/single unit of production, similar to a combo of beef. '771 Patent, 5:55-60. Price discloses testing of "Dried egg albumen, cocoa, and nonfat dry milk" (Price, 679) and "wheat flour, coconut, and cottonseed flour" Price, 681.



Price, 680 (Figure 1 (annotated)).

	A method has been described for testing multiple food samples for *Salmonella* without loss in sensitivity. The method pools multiple pre-enrichment broth cultures into single enrichment broths. The subsequent stages of the *Salmonella* analysis are not altered. The method was found applicable to several dry food materials including nonfat dry milk, dried egg albumin, cocoa, cottonseed flour, wheat flour, and shredded coconut. As many as 25 pre-enrichment broth cultures were pooled without apparent loss in the sensitivity of *Salmonella* detection as compared to individual sample analysis. The procedure offers a simple, yet effective, way to increase sample capacity in the *Salmonella* testing of foods, particularly where a large proportion of samples ordinarily is negative. It also permits small portions of pre-enrichment broth cultures to be retained for subsequent individual analysis if positive tests are found. *Salmonella* testing of pooled pre-enrichment broths provides increased consumer protection for a given amount of analytical effort as compared to individual sample analysis. Price, 679. A POSITA would have replaced Price's collection and combination of dry food with ICMSF's collection and combination of portions from a consignment of meat lots in conformance to a statistical-based sampling plan for release into commerce. "Many different types of samples are collected and submitted to the laboratory for analysis. Some are sample units from lots or consignments of food or ingredients for lot acceptance determination. . . . A sample unit is a small portion of the lot in which the distribution of both numbers and types of microorganisms should be representative of the whole lot." ICMSF, 255. The lots may be beef or poultry. *Id.*, 184-91, 226, 230. Thus, ICMSF discloses collecting a plurality of samples or sample units (other synonyms include portions, specimens, and sub-samples) from lots of beef or poultry. ICMSF describes collecting from a lot or lots:

	"If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." *Id.*, 119. Additionally, ICMSF indicates a consignment may be "a very large quantity of food" and may be "composed of several lots, representing for example, different days of production from the same plant." *Id.*, 119, 120; *see also* Applicant Admitted Prior Art ("AAPA") #3-#5.

6.11 WHAT IS A REPRESENTATIVE SAMPLE?

A *representative sample* reflects, as far as is possible, the composition of the lot from which it is drawn.

How then should a representative sample be drawn? It is important to avoid bias and to draw a sufficient number of sample units to confidently make a judgment about a lot. Sampling at random is the universally recognized way of avoiding bias and is more reliable than consciously trying to draw the sample units from the various parts of a lot. The units (cartons or containers, particular weights of solid, or volumes of liquid) are drawn by using random numbers. There is, of course, no guarantee that a random sample has characteristics identical with those of the lot, but the randomness of sampling is the basis for calculation of the probability that a sample will give a biased result.

It is also possible, and may be desirable, to use a *stratified random sampling* approach, drawing a random sample of a given number of sample units from each *stratum* (e.g., each sublot or carton). The distribution of sample units on strata should correspond to the distribution of lot units on strata. This means that if each stratum contains the same quantity as every other one, the number of sample units per stratum should be the same. Otherwise, they should differ just as the proportions of the lot contained in the strata differ. Stratification is a device for handling known sources of variation and may be used where one has prior knowledge that the consignment is potentially not of uniform quality. A consignment might not be of uniform quality if portions of it are shipped in different vehicles or in different holds of a vessel, or if it is known that it is really composed of several lots representing, for example, different days of production from the same plant or different plants of the same company. The results for different strata should be assessed separately and then pooled if they appear to be homogeneous.

The calculation of acceptance probabilities is based on the assumption that random samples have been drawn and analyzed. Where physical or practical constraints prevent random sampling, such calculated probabilities may not be valid. Their reliability depends on how close reality approaches the ideal situation of random sampling. This fact should be borne in mind when subsequently interpreting results.

ICMSF, 119-20 (annotated).

6.10 WHAT IS A LOT?

Ideally, a lot is a quantity of food or food units produced and handled under uniform conditions. Under certain circumstances, it has been shown that the logarithms of the counts from a batch of food are likely to be normally distributed (Baird-Parker, 1980; Kilsby & Baird-Parker, 1983 [their Table 2]; Ingram & Roberts, 1976). The implication is that there is homogeneity within a lot, which, when considering microbial levels and distributions, rarely occurs in practice. In most instances, the distribution of microorganisms within lots of food is heterogeneous. Even in food factories, this heterogeneity makes the interpretation of sample results difficult, and it becomes even more so in circumstances where a lot is less well defined, such as in a consignment of a very large quantity of food.

It is therefore helpful if suppliers can be persuaded to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load).

If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment.

A lot should be composed of food produced with as little variation as possible for a given process or commodity. Because of the uncertainties in identifying a lot commercially, however, the use of the word *lot* in this book is usually in its statistical sense, as a collection of units of a product, the acceptability of which is determined by examining a sample drawn from it.

ICMSF, 118-19 (annotated).

	The ICMSF has defined a lot as follows: In the commercial sense, a lot is a quantity of food supposedly produced under identical conditions, all packages of which would normally bear a lot number that identifies the production during a particular time interval, and usually from a particular line, retort, or other critical processing unit. Statistically, a lot is considered as a collection of units of a product from which a sample is drawn to determine acceptability of the lot (ICMSF, 1986). In this example, the maximum size of a lot consists of all the ground beef produced on common equipment from cleanup to cleanup. Smaller lots could be established (e.g., hourly). ICMSF, 327 (annotated). This definition also reflects the common practice referred to in the IEH Patents at Example II, wherein "[p]referably, the producer defines and produces these single-unit lots in such a fashion as to maximize the information available." '771 Patent,13:12–16. This corresponds with the specification of IEH's Patents which reflects the common practice of producers defining what constitutes a lot grouped for traceability purposes. '771 Patent, 3:14-24, 11:37-40 (AAPA #3), and 13:12-15. Thus, ICMSF discloses collecting a plurality of portions from separate lots. ICMSF describes a lot as "a collection of units of a product." IMCSF, 119. ICMSF further indicates a lot has been defined as: "a quantity of food supposedly produced under identical conditions, all packages of which would normally bear a lot number that identifies the production during a particular time interval, and usually from a particular line, retort, or other critical processing unit. Statistically, a lot is considered as a collection of units of a product from which a sample is drawn to determine acceptability of the lot." ICMSF, 327. ICMSF advocates use of a "statistically based sampling plan." *Id.*, 113-15, 119-21, 320, 327, 328. In the sampling plans, "*n* is the number of sample units chosen separately and independently, and . . . *c* is the maximum allowable number of sample

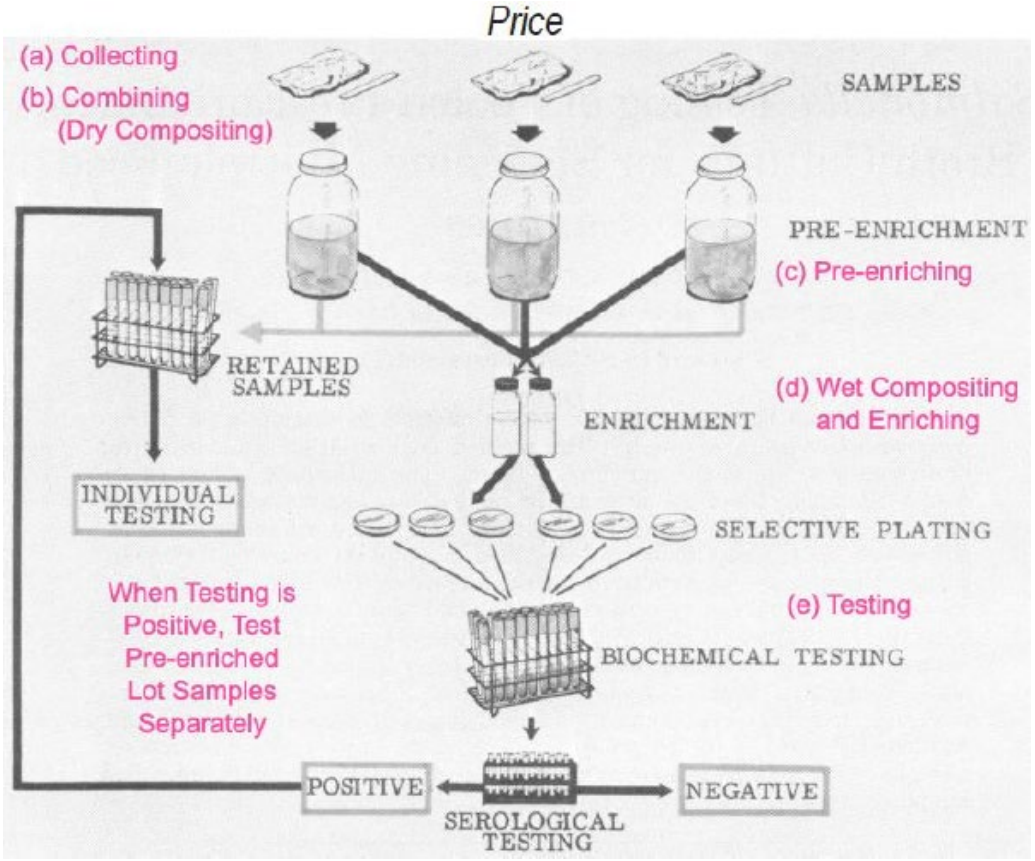
	units yielding a positive result." *Id.*, 115. ICMSF discloses a wide range for the number of sample units (portions) for a particular microbial agent or organism. For example, ICMSF discusses collecting 15, 30, 60 or 100 sample units from a lot. *Id.*, 320, 326. **6.5 THE SAMPLING PLAN** The sample units so drawn will, after examination, yield results that will be compared with certain criteria to reach a decision as to whether the entire lot should be accepted or rejected (see section 6.9 for explanation of rejection). The particular choice of sampling procedure and the decision criteria make up the *sampling plan*. Proper drawing of samples is essential if the decision making criteria of the sampling plan are to give unbiased results. By sampling randomly, we are able to reduce the risk of making biased decisions. A simple example of a sampling plan follows. Take 10 sample units of a food from a lot and subject them to appropriate laboratory procedure to test for the presence or absence of a microorganism. If two or fewer of the 10 sample units show presence of the organism, that is, give a positive result, then the whole lot of food is acceptable (relative to this organism). But if three or more give a positive result, the whole lot is to be rejected. This plan is described by the use of two terms $n = 10$ and $c = 2$, where n is the number of sample units chosen separately and independently, and where c is the maximum allowable number of sample units yielding a positive result. ICMSF, 115 (annotated).

	6.1 INTRODUCTION It is important to recognize that the management of food safety using the approaches outlined in the first five chapters, based on controlling hazards through Good Hygienic Practices (GHP) and Hazard Analysis Critical Control Point (HACCP), is much more effective than trying to ensure safety by lot acceptance through end-product testing. This chapter discusses the concepts of probability and sampling that determine the limitations of end-product testing. Those concepts form the basis of the design of statistically based sampling plans (Chapter 7) and the establishment of cases (Chapter 8). In Chapters 6 and 7, it is assumed that the microbiological test will give an absolutely accurate result, with no false positives or false negatives. This is not a realistic assumption, and problems arising from sampling, sample transport and preparation for analysis, and the imprecision of microbiological methods are addressed in Chapters 10 and 12 and elsewhere in the text. ICMSF, 113 (annotated); *see also id.*, 115–21 (§§ 6.4–6.13), 319–20 (§ 17.2.3.3), and 326–30 (§§ 17.5.3.1–17.6). ICMSF discusses the number of portions to be taken sufficient for validation of a particular microorganism including *Salmonella* (*id.*, 276 (§ 15.3.3); *see also id.*, 282 (§ 15.6.3)); *Listeria* (*id.*, 309 (§ 16.6.3)), and *E. coli* (*id.*, 318–21 (§§ 17.2.3.2–17.2.3.4), 326 (§ 17.5.3.1)). IEH's AAPA discloses statistical-based sampling plans taking 10-60 sample units. '771 Patent, 6:12-29; *see* AAPA #1, #2, #5, and #6. Determining meat is safe for consumption so it is "commercially possible to market" the meat, is the well-understood point of testing meat and is taught by ICMSF. ICMSF, 185-89. Moreover, the recitation of a lot being validated for "release . . . into commerce" is not limiting. Prosecution History for Reexamination Control No. 95/011,294.
[37b] b) separately compositing the collected multiple independent samples from each of the separate lots to provide a corresponding set of separate composited single production	Price, alone or in combination with ICMSF and Hughes, discloses separately compositing the collected multiple independent samples from each of the separate lots to provide a corresponding set of separate composited single production lot

lot samples, wherein each of the separate composited single production lot samples is attributed to a particular corresponding separate single production lot;	samples, wherein each of the separate composited single production lot samples is attributed to a particular corresponding separate single production lot. Like ICMSF, the test lot samples in Price are attributable to a particular lot. For example, Price's abstract states that the procedure "permits small portions of pre-enrichment broth cultures to be retained for subsequent individual analysis if positive tests are found," as shown below: **A method has been described for testing multiple food samples for *Salmonella* without loss in sensitivity. The method pools multiple pre-enrichment broth cultures into single enrichment broths. The subsequent stages of the *Salmonella* analysis are not altered. The method was found applicable to several dry food materials including nonfat dry milk, dried egg albumin, cocoa, cottonseed flour, wheat flour, and shredded coconut. As many as 25 pre-enrichment broth cultures were pooled without apparent loss in the sensitivity of *Salmonella* detection as compared to individual sample analysis. The procedure offers a simple, yet effective, way to increase sample capacity in the *Salmonella* testing of foods, particularly where a large proportion of samples ordinarily is negative. It also permits small portions of pre-enrichment broth cultures to be retained for subsequent individual analysis if positive tests are found. *Salmonella* testing of pooled pre-enrichment broths provides increased consumer protection for a given amount of analytical effort as compared to individual sample analysis.** Price, 679. Price further describes this retention step as enabling "individual tests on the retained sample" to "be made to determine which sample or samples contributed *Salmonella* to the pool" as shown below:

	MATERIALS AND METHODS *Salmonella* analyses were carried out as described in the *Bacteriological Analytical Manual* (BAM) (2) except for the pooling of pre-enrichment broths described in the following paragraph. The procedure for *Salmonella* testing of pooled pre-enrichment broths is illustrated in Fig. 1. Samples are individually pre-enriched followed by transfer from multiple pre-enrichments to single selenite and tetrathionate broths at the enrichment stage. During pooling, a portion of each pre-enrichment is transferred to a sterile culture tube and retained at 4 C for later reference to the individual samples. If the *Salmonella* test of the pooled pre-enrichment broths is positive, individual tests on the retained samples can be made to determine which sample or samples contributed *Salmonella* to the pool. *Id.* (highlighting added). A POSITA would understand Price to implicitly disclose combining the collected confirming plurality of portions based on the inherent mixing involved with dry food materials.

	Salmonella-positive test samples were prepared by blending the dry test food with one of three dry inocula. Inoculum 1 was a dry enzyme drain cleaner which had been found to contain multiple *Salmonella* serotypes including E_1, z_{10}; G, z_{29} (*S. cubana*); and C_1, g complex. Inoculum 2 was a freeze-dried, skim milk suspension of serotypes *S. anatum*, *S. binza*, *S. tennessee*, *S. worthington*, *S. cubana*, *S. braenderup*, and a B_1, g complex. Inoculum 3 consisted of four laboratory isolates of serotypes G, z_{29} (*S. cubana*); E_1, b; E_1, l complex; and C_1, l complex grown in a sterile aqueous suspension of cottonseed flour and freeze dried. Price, 679.



Price, 680 (Figure 1 (annotated)).

A POSITA would have replaced Price's collection and combination of dry food with ICMSF's collection and combination of portions from a consignment of meat lots in conformance to a statistical-based sampling plan for release into commerce.

ICMSF states: "To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units of the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. . . . This finding suggests that considerable reductions may

	be made in the cost of laboratory analysis by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, n, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort." ICMSF, 187. To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. A laboratory investigation undertaken for ICMSF (Silliker & Gabis, 1973) indicated that different sized analytical units drawn from a single well-mixed sample unit of dried food give very similar results. A subsequent investigation (Gabis & Silliker, 1974) using foods of high moisture content (including eggs, poultry meat, meat, and meat products) showed comparable reliability in the use of composite analytical units; for example, examination of three composites of 20 × 25 g analytical units gave the same result as examination of all 60 analytical units individually (the total amount examined being 1500 g in both instances). This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n*, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort. ICMSF, 187.

10.5.1 Conceivable Alternative Plans

As indicated above, for an infectious agent such as *Salmonella*, there are strong reasons for adopting sampling plans in which $c = 0$:

1. It is philosophically objectionable to some people to accept a lot containing a recognized pathogen, regardless of how the food is to be used in a manufacturing process or by the consumer.
2. There is the very great technical advantage of combining many analytical units for bacteriological examination in systems with $c = 0$, because a single positive decides the outcome.
3. The plan with $c = 1$ (or more) would always require the examination of (many) more analytical units, for equal probability of acceptance, than a plan with $c = 0$.
4. Even if the indicated probabilities of plans with $c = 0$ versus $c = 1$ are the same, their implications are not. For example, when a lot is accepted with the plan $n = 60$, $c = 0$, it is possible (though not certain) that the lot may not be contaminated at all. But using the plan $n = 95$, $c = 1$ and accepting one positive, it is known that the lot certainly is contaminated (though the probability that a lot with 5% of analytical units contaminated would be accepted is in both instances $P_a = 0.05$).

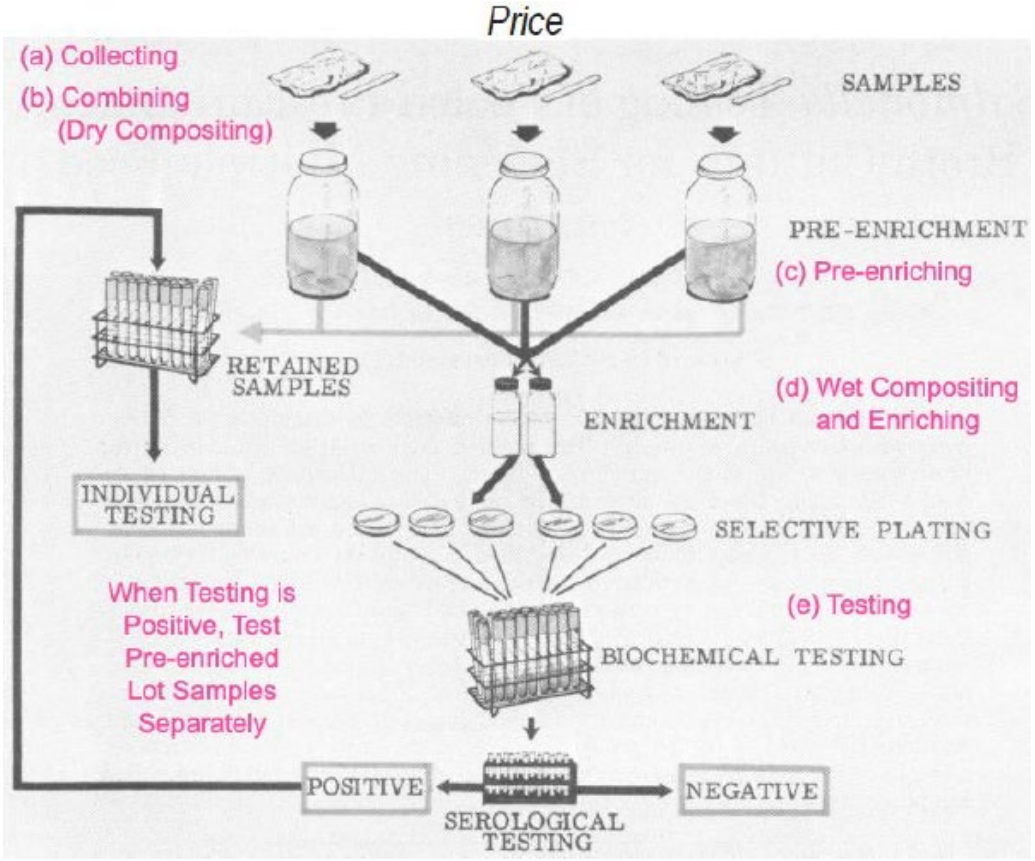
ICMSF, 188 (annotated). ICMSF also provides an example of "[u]sing a sampling plan involving n = 15 (25 g analytical units) and a composite of 375 g." *Id.*, 195 (§ 10.7.2). ICMSF also states "[f]or qualitative analyses, 25 g analytical units are frequently analyzed individually or as composites of 100 g, 200 g, or more." *Id.*, 232 (§ 12.5.2).

The ICMSF incorporates two references, Silliker & Gabis (1973) and Gabis & Silliker (1974). Silliker notes that "[c]ompositing of multiple samples (lumping) and wet compositing of pre-enrichment cultures have been tested as economical approaches to *Salmonella* control." Silliker, 475.

Silliker further notes that "[b]oth approaches are being used widely by the food industry in the United States." *Id.*.

	To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. A laboratory investigation undertaken for ICMSF (Silliker & Gabis, 1973) indicated that different sized analytical units drawn from a single well-mixed sample unit of dried food give very similar results. A subsequent investigation (Gabis & Silliker, 1974) using foods of high moisture content (including eggs, poultry meat, meat, and meat products) showed comparable reliability in the use of composite analytical units; for example, examination of three composites of 20 × 25 g analytical units gave the same result as examination of all 60 analytical units individually (the total amount examined being 1500 g in both instances). This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n*, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort. "There is the very great technical advantage of combining many analytical units for bacteriological examination." ICMSF, 188. ICMSF also describes composites prepared by combining "15 (25 g analytical units)" and "composites of 100 g, 200 g, or more." *Id.,* 195, 232; *see* AAPA #5. ICMSF states, "All steps up to the analysis must be controlled . . . to allow reliable traceability." ICMSF, 227. ICMSF indicates lot samples are traceable to a particular corresponding lot: "It is therefore helpful . . . to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load). . . . Treating the individual production

	batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119.
[37c] c) enriching of the separate composited single production lot samples to provide a set of separate composited single production lot samples enriched for the target microbe(s);	Price, alone or in combination with ICMSF and Hughes, discloses enriching each of the separate composited test lot samples to provide a set of separate composited test lot samples enriched for the target microbe(s). Price discloses "[s]amples are individually pre-enriched followed by transfer from multiple pre-enrichments to single selenite and tetrathionate broths at the enrichment stage." Price, 679. These pre-enrichment and enrichment steps inherently disclose an incubation period in which levels of the target microbial agent, in this case, *Salmonella*, grow to detectable levels for testing. Price states "[i]n the multiple pooling of pre-enrichment broth cultures . . . , 1-ml quantities of cultures were transferred, generally, to a volume of enrichment broth." Price, 682. Price also states "[a]lternatively, problems with interfering organisms may warrant the use of direct selective enrichments." *Id.* Based on this statement, the Board noted "there is a suggestion in Price (at 682) . . . of direct enrichment." Prosecution History for Reexamination Control No. 95/001,294, 1576.



Price, 680 (Figure 1 (annotated)).

Price states "[a]lternatively, problems with interfering organisms may warrant the use of direct selective enrichments" and further mentions "a reduction in the incubation time of the pre-enrichment cultures." Price inherently discloses an incubation period of the pre-enrichment samples.

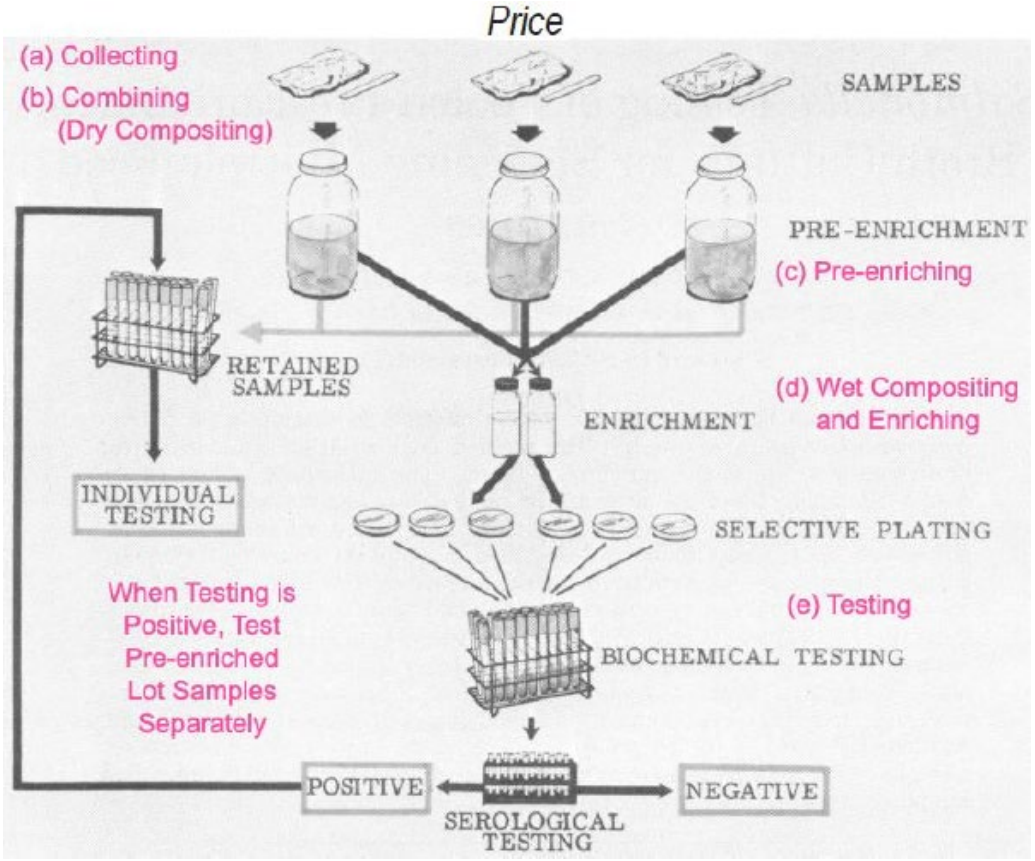
	Whether pooled pre-enrichments are less sensitive than individual tests in the presence of interfering organisms probably is not known. For the present, we would recommend that, if numerous non-*Salmonella* colonies that resemble *Salmonella* on selective agar media are encountered in the pooled test, the retained pre-enrichment cultures should be tested individually. Alternatively, problems with interfering organisms may warrant the use of direct selective enrichments, two selective enrichments, or possibly even a reduction in the incubation time of the pre-enrichment cultures. Price, 682 (highlighted added). A POSITA would have replaced Price's pre-enriching and compositing pre-enrichments with teachings of ICMSF and Hughes for enriching lot samples, removing portions of each enriched lot sample, and combining the portions to provide a composite sample. Also, instead of retaining portions of pre-enrichments like Price, a POSITA would have retained portions of enriched lot samples as taught by ICMSF and Hughes. ICMSF states: To reduce laboratory effort . . . it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. ICMSF, 187. Also, ICMSF describes preparing composites of analytical units then diluting "for qualitative (enrichment)" analysis. *Id.,* 231-32; *see also* AAPA #5, #7.

	For practical reasons, however, analytical units of 10 g up to 50 g are typical for quantitative methods and are diluted 1:10. For qualitative analyses, 25 g analytical units are frequently analyzed individually or as composites of 100 g, 200 g, or more. ICMSF, 232 (annotated). Preparation of the first dilution, either for qualitative (enrichment) or quantitative (direct) analyses, can have a marked impact on the microbial population in the sample. This is often due to changes in the physico-chemical characteristics of the suspension as compared to the food matrix. Where the food does not confer protection, the composition of the diluent is important to prevent or minimize shock/injury to the microorganisms that can result from rapid changes in the ionic concentrations. ICMSF, 232 (annotated). ICMSF also describes strategic use of non-selective enrichment to resuscitate microorganisms and selective enrichments for growing microorganisms. *Id.*, 232–33 (§ 12.6); *see e.g.*, *id.,* 233 ("use of nonselective medium . . . to enhance recovery of injured or stressed cells."). ICMSF, § 12.6 also describes optimizing the incubation period. S*ee e.g.*, *id.,* 232. It was well known long before the priority date to use non-selective enrichments after dry compositing and prior to wet compositing as evidenced by Price, Silliker, Gabis, and MLG. A POSITA would also know to use selective enrichments after dry compositing and prior to wet compositing as disclosed by ICMSF and Hughes. A POSITA would find that incubating so microorganisms are detectable and at least substantially uniform in the selective enrichment broth was considered the standard practice in official methods because the methods otherwise fail. For example, D'Aoust states "standard methodology recommends incubation of selective enrichment broths." D'Aoust, 372. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section

	further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. (a) *Pre-enrichment.*—Pre-enrich product in noninhibitory broth to initiate growth of *Salmonella* spp. Methods used may vary with product and should be performed as indicated in **967.26A** or BAM, 7th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, chapter 5, section C. Pre-enrichment broths should be incubated at 35°–37°C. (b) *Selective enrichment.*—Transfer 0.1 mL incubated pre-enrichment mixtures to Rappaport-Vassiliadis R10 broth (9.9 mL) and 1 mL to TT broth (9 mL). For all foods other than raw foods or foods having a high microbial load, incubate 6–8 h at 41°–43°C. Selective enrichments of raw foods or foods having a high microbial load must be incubated 16–20 h at 41°–43°C. An air incubator is suitable for this purpose. Hughes, 636. Hughes instructs that, after pre-enrichment, portions of the "incubated pre-enrichment mixtures" should be transferred to two separate selective enrichment broths, Rappaport-Vassiliadis R10 broth and TT broth, and incubated for 16-20 hours at 41°-43°C. Hughes, 636, 638. Additionally, Hughes instructs that these selective broths should be removed from incubation and mixed by hand or by Vortex mixer. Hughes, 638. Hughes further instructs to then remove 1 mL from the TT tube and 1 mL from the Rappaport-Vassiliadis R10 tube and transfer to separate, sterile M broths. *Id.,* 636, 638. The separate M broths are then incubated ("16-20 h at 35°-37°C" for "all foods other than raw foods or foods having a high microbial load" or "6 h at 35°-37°C" for

	"raw foods or foods having a high microbial load"). *Id.*, 638. A POSITA would find that the long incubation at an elevated temperature and mixing ensures the target microbial agent or organism reaches detectable levels and becomes substantially uniform throughout the separate lot samples.
[37d] d) removing portions of each separate enriched composited single production lot sample, and combining the removed portions to provide a pooled modular composite sample; and	Price, alone or in combination with ICMSF and Hughes, discloses removing portions of each separate enriched composited single-unit production lot sample, and combining the removed portions to provide a pooled modular composite sample. Price discloses wet compositing as "[s]amples are individually pre-enriched followed by transfer from multiple pre-enrichments to single selenite and tetrathionate broths at the enrichment stage." Price, 679. **MATERIALS AND METHODS** ***Salmonella*** **analyses were carried out as described in the *Bacteriological Analytical Manual* (BAM) (2) except for the pooling of pre-enrichment broths described in the following paragraph.** **The procedure for *Salmonella* testing of pooled pre-enrichment broths is illustrated in Fig. 1. Samples are individually pre-enriched followed by transfer from multiple pre-enrichments to single selenite and tetrathionate broths at the enrichment stage. During pooling, a portion of each pre-enrichment is transferred to a sterile culture tube and retained at 4 C for later reference to the individual samples. If the *Salmonella* test of the pooled pre-enrichment broths is positive, individual tests on the retained samples can be made to determine which sample or samples contributed *Salmonella* to the pool.** Price 679.

	Pooled samples versus pooled pre-enrichment broths. Silliker (4) reported a loss in sensitivity when several samples were pooled in a large container of pre-enrichment broth as compared to individual sample analysis. This is not surprising since, to be detected, the desiccated and debilitated *Salmonella* cells in a dried food sample must grow out in pre-enrichment broth in competition with other bacteria in the sample. If many food samples are pooled at the pre-enrichment stage, the salmonellae face competition from the flora of all the samples in the pool. If, on the other hand, the samples are individually pre-enriched, the actively growing salmonellae might be expected to compete more favorably with organisms introduced from other samples when pooled at the enrichment stage. In addition, pooling pre-enrichment broths avoids the hazards and inconvenience of handling very large flasks of culture. Price, 681 (discussing Silliker).

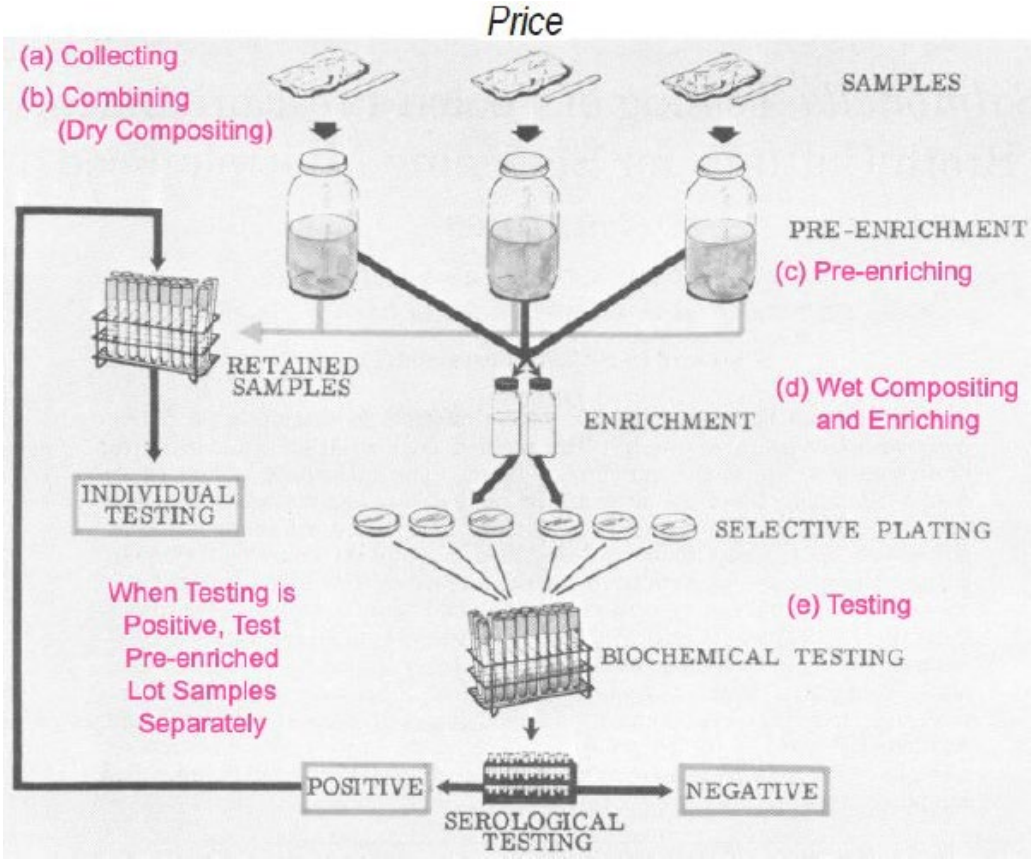


Price, 680 (Figure 1 (annotated)). Price's Figure 1 indicates that selective plating, biochemical testing, and serological testing are used.

A POSITA would have replaced Price's pre-enriching and compositing pre-enrichments with teachings of ICMSF and Hughes for enriching lot samples, removing portions of each enriched lot sample, and combining the portions to provide a composite sample. Also, instead of retaining portions of pre-enrichments like Price, a POSITA would have retained portions of enriched lot samples as taught by ICMSF and Hughes.

	ICMSF describes compositing the enriched lot samples: "it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. . . . This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n,* with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort." ICMSF, 187. AAPA #5-#7. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Hughes also discloses a method of removing portions of each enriched separate test lot sample, and combining the removed portions to provide a modular composite sample. Hughes instructs to "[c]ombine equal volumes (0.5-1 mL) from each M broth tube in a clean screw-cap tube." Hughes, 638.

	that has been warmed to 35°–37°C. Also remove 1 mL from Rappaport-Vassiliadis R10 tube and transfer to a separate tube of prewarmed M broth (10 mL). For all foods other than raw foods or foods having a high microbial load, incubate M broth tubes 16–20 h at 35°–37°C and return selective enrichment broth tubes to 41°–43°C incubator and incubate for additional 16–18 h. For raw foods or foods having a high microbial load, incubate M broth tubes 6 h at 35°–37°C and return selective enrichment broth tubes to 41°–43°C and incubate for an additional 6 h. (**d**) *Preparation of sample for EIA analysis.*—Remove M broth tubes from incubation and mix tubes by hand or Vortex mixer. Combine equal volumes (0.5–1 mL) from each M broth tube in a clean screw-cap tube and heat in boiling H_2O bath or in flowing steam 15 min. Refrigerate (2°–8°C) remaining M broth and TT and Rappaport-Vassiliadis R10 tubes from (**c**) for culture confirmation of any EIA-positive samples (*see* **967.26B, 967.26C, 967.27** and **967.28**). Cool heated M broths to 25°–37°C prior to analysis. Hughes, 638. Hughes 2003 confirms the pooling of enrichments. Hughes 2003, 775-76.
[37e.1] e) testing of the pooled modular composite sample, using a suitable detection assay, for the target microbe(s),	Price, alone or in combination with ICMSF and Hughes, discloses testing of the pooled modular composite sample, using a suitable detection assay, for the target microbe(s).



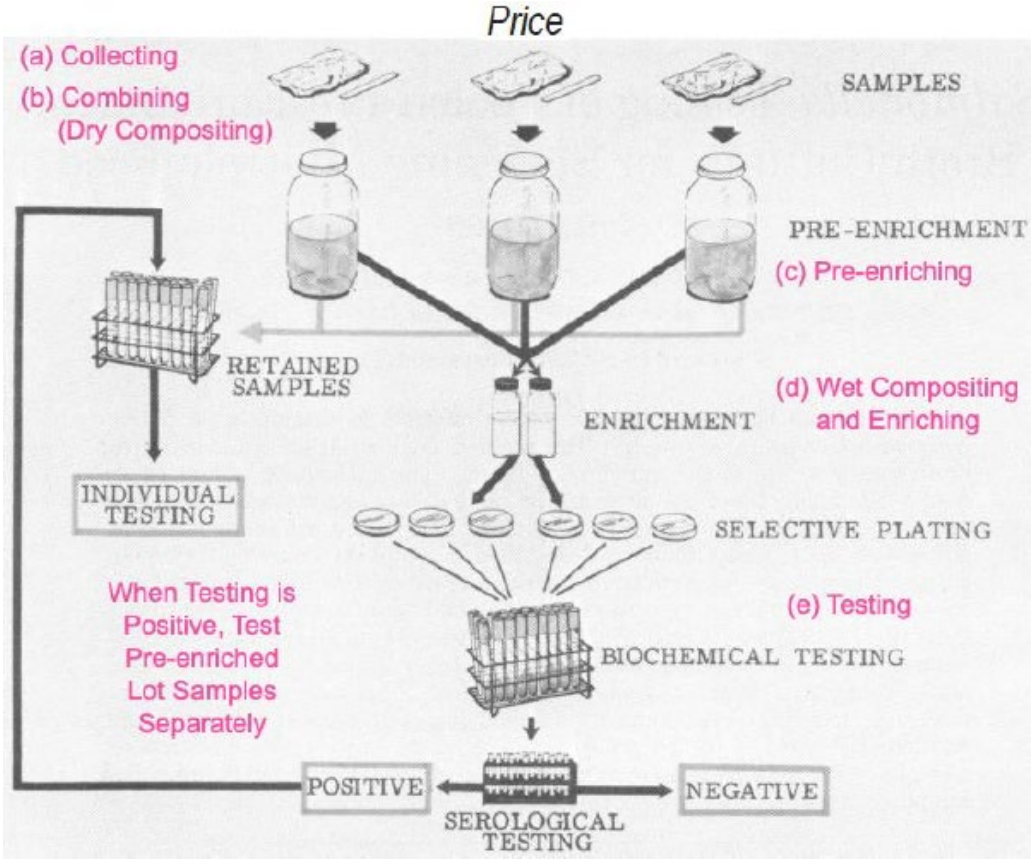
Price, 680 (Figure 1 (annotated)).

Price teaches testing the composite sample using a detection assay for *Salmonella*. Price, 680 (Figure 1).

Price states “a test procedure that gives a single negative answer for many negative samples offers an important saving in the analytical effort required for quality control”:

	Positive test results are relatively rare among the many samples of food ingredients and products which are analyzed for *Salmonella*. Therefore, a test procedure that gives a single negative answer for many negative samples offers an important saving in the analytical effort required for quality control. These studies support the conclusion that *Salmonella* pre-enrichment broths can be pooled for analysis without loss in sensitivity to *Salmonella* in the individual samples. Price, 679. Price indicates that "positive test results are relatively rare." *Id.* A POSITA would have replaced Price's steps of testing a composite sample from the pre-enriched lot samples to obtain a negative result and further testing retained portions of the pre-enriched lot samples with the combined teachings of ICMSF and Hughes. These combined teachings provide for testing a composite made from the selectively enriched lot samples to obtain a negative result and further testing retained portions of the enriched lot samples after a positive test of the composite by a more sensitive protocol. ICMSF describes testing enrichment cultures of composite samples with suitable detection assays (ICMSF, 187) and "presence/absence tests" (ICMSF, 233). Suitable detection assays were established in Silliker and Gabis as discussed in ICMSF. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture

	confirmation of any EIA-positive samples." *Id.,* 638. Hughes states testing of the composite sample is "by an enzyme immunoassay (EIA) method." Hughes, 635, 638. These assay types were "art recognized." '771 Patent, 3:9-13 (AAPA #7).
[35e.2] wherein when such testing is negative all of said samples that were composited to form the separate composited single production lot samples are deemed negative for the target microbe(s) and each of the multiple separate single production lots is validated, and	Price, alone or in combination with ICMSF and Hughes, discloses wherein when such testing is negative all of said samples that were composited to form the separate composited test lot samples are deemed negative for the target microbe(s) and each of the multiple separate test lots is validated.



Price, 680 (Figure 1 (annotated)).

Price states "a test procedure that gives a single negative answer for many negative samples offers an important saving in the analytical effort required for quality control." Price, 679.

A POSITA would have replaced Price's steps of testing a composite sample from the pre-enriched lot samples to obtain a negative result and further testing retained portions of the pre-enriched lot samples with the combined teachings of ICMSF and Hughes. These combined teachings provide for testing a composite made from the

	selectively enriched lot samples to obtain a negative result and further testing retained portions of the enriched lot samples after a positive test of the composite by a more sensitive protocol. ICMSF describes correlating lots in a consignment with lot samples so a negative or positive test result enables a lot to be validated or rejected. ICMSF, 118-19. ICMSF describes sampling plans in which "c is the maximum allowable number of sample units yielding a positive result." ICMSF, 115. For many scenarios, the requirement is c = 0, which indicates the testing is negative. *Id.*, 118, 183-90, 309, 325, 326. A negative test result (*id.*, 113-15, 325, 326) is the expected and desired outcome to determine meat is not contaminated and is safe for consumption (*id.*, 184-89) such that a lot is validated and it is "commercially possible to market" (*id.*, 185) the meat.

6.10 WHAT IS A LOT?

Ideally, a lot is a quantity of food or food units produced and handled under uniform conditions. Under certain circumstances, it has been shown that the logarithms of the counts from a batch of food are likely to be normally distributed (Baird-Parker, 1980; Kilsby & Baird-Parker, 1983 [their Table 2]; Ingram & Roberts, 1976). The implication is that there is homogeneity within a lot, which, when considering microbial levels and distributions, rarely occurs in practice. In most instances, the distribution of microorganisms within lots of food is heterogeneous. Even in food factories, this heterogeneity makes the interpretation of sample results difficult, and it becomes even more so in circumstances where a lot is less well defined, such as in a consignment of a very large quantity of food.

It is therefore helpful if suppliers can be persuaded to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load).

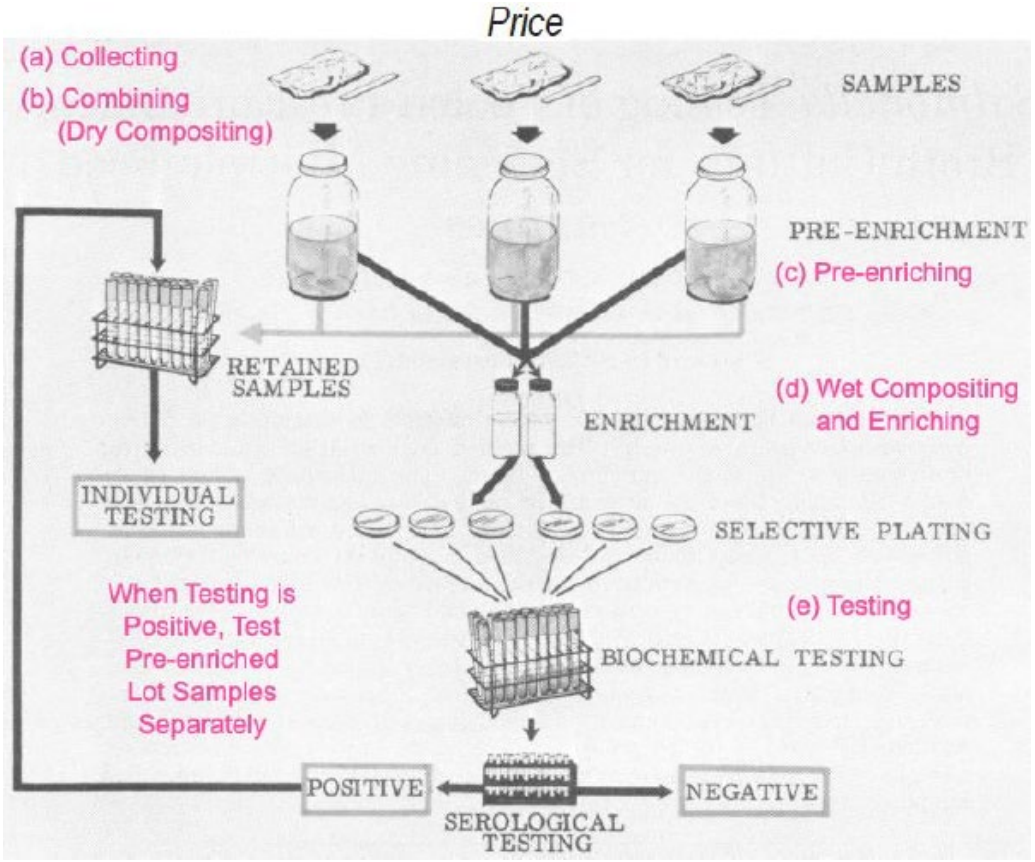
If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment.

A lot should be composed of food produced with as little variation as possible for a given process or commodity. Because of the uncertainties in identifying a lot commercially, however, the use of the word *lot* in this book is usually in its statistical sense, as a collection of units of a product, the acceptability of which is determined by examining a sample drawn from it.

ICMSF, 118-19 (annotated).

	Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Because Hughes explains the steps to be taken when testing is positive (*see* ie.3)*,* a POSITA would also expect negative testing results. Hughes, 638. Determining meat is safe for consumption so it may be released into commerce is the well-understood point of testing meat. Moreover, the recitation of a lot being validated for "release . . . into commerce" is not limiting. Prosecution History for Reexamination Control No. 95/011,294.
[35e.3] wherein when such testing is positive, each of the individual separate composited single production lot samples that were used to form the pooled modular composite sample are individually tested to determine which of the separate composited single production lot samples is positive for the target microbe(s),	Price, alone or in combination with ICMSF and Hughes, discloses wherein when such testing is positive, each of the individual separate composited single production lot samples that were used to form the pooled modular composite sample are individually tested to determine which of the separate composited single production lot samples is positive for the target microbe(s). The combination of Price, ICMSF, and Hughes describes retaining a portion of the enriched lot samples (steps (b)-(d)). Price indicates "a portion of each pre-enrichment is . . . retained . . . for later reference to the individual samples" and "individual tests on the retained samples can be made to determine which sample or samples contributed *Salmonella* to the pool." Price, 679. Price states, "If the *Salmonella* test of the pooled preenrichment broths is positive, individual tests on the retained samples can be made to determine which sample or samples contributed *Salmonella* to the pool." *Id.,* 679. Price discloses "the pooling of broth cultures permits small portions of each individual culture to be retained for subsequent analysis if a positive test is detected following enrichment, thus maintaining the individuality of each test sample." Price,

681.



Price, 680 (Figure 1 (annotated)).

Price states "[i]f the *Salmonella* test of the pooled pre-enrichment broths is positive, individual tests on the retained samples can be made to determine which sample or samples contributed *Salmonella* to the pool":

MATERIALS AND METHODS

Salmonella analyses were carried out as described in the *Bacteriological Analytical Manual* (BAM) (2) except for the pooling of pre-enrichment broths described in the following paragraph.

The procedure for *Salmonella* testing of pooled pre-enrichment broths is illustrated in Fig. 1. Samples are individually pre-enriched followed by transfer from multiple pre-enrichments to single selenite and tetrathionate broths at the enrichment stage. During pooling, a portion of each pre-enrichment is transferred to a sterile culture tube and retained at 4 C for later reference to the individual samples. If the *Salmonella* test of the pooled pre-enrichment broths is positive, individual tests on the retained samples can be made to determine which sample or samples contributed *Salmonella* to the pool.

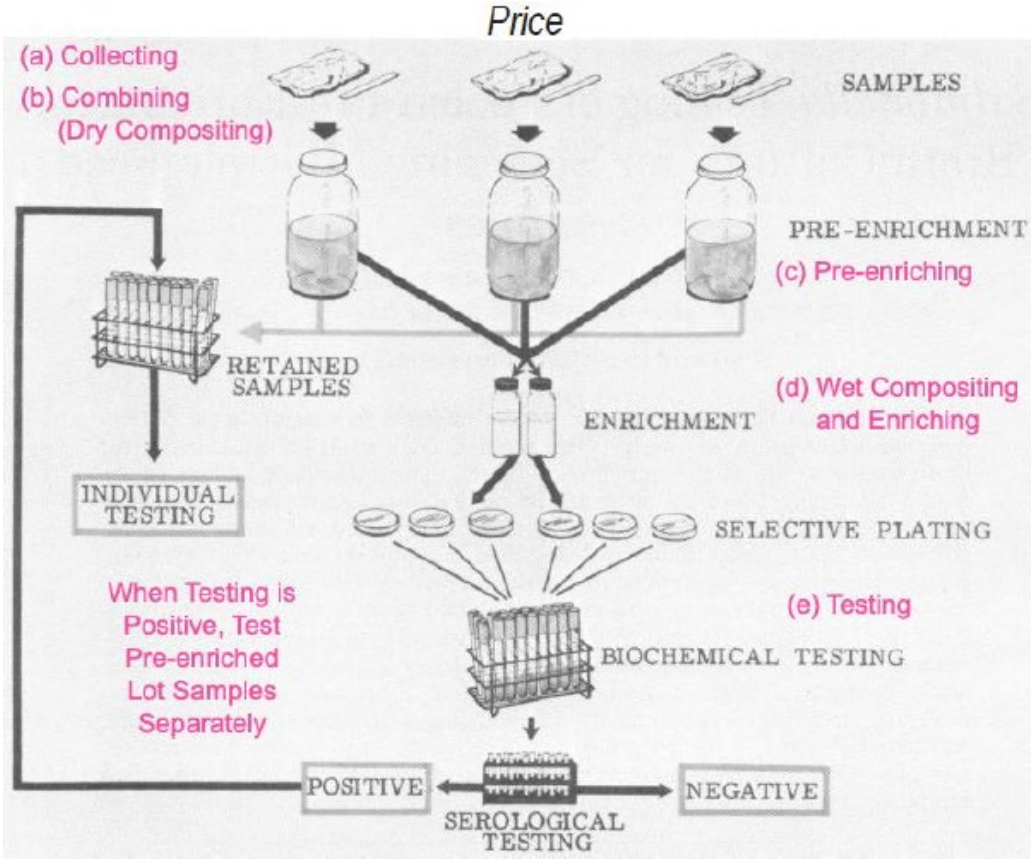
Price, 679. A POSITA would have considered retention of samples that had been selectively enriched as an option.

A POSITA would have replaced Price's steps of testing a composite sample from the pre-enriched lot samples to obtain a negative result and further testing retained portions of the pre-enriched lot samples with the combined teachings of ICMSF and Hughes. These combined teachings provide for testing a composite made from the selectively enriched lot samples to obtain a negative result and further testing retained portions of the enriched lot samples after a positive test of the composite by a more sensitive protocol.

"Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119.

	"There is often a desire to retest retained samples." ICMSF, 191. If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment. ICMSF, 119 (annotated). ICMSF discloses validating for release into commerce. ICMSF, 113–15 (§§ 6.2–6.6), 118–19 (§§ 6.9–6.10), 184–85 (§ 10.2), 325–27 (§§ 17.5.3–17.5.3.4); *see also id.*, 186–89 (§ 10.4–10.6)) according to a statistically-based sampling plan (*id.*, 113 (§ 6.1); *see also id.*, 115–18 (§§ 6.4–6.8), 184 (§ 10.2), 319–21 (§§ 17.2.3.3–17.2.3.4), 326–30 (§§ 17.5.3.1–17.6), as described regarding steps (a) and (e)(2). Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Hughes explicitly teaches retention of the enriched test lot samples for further testing after a positive result. Hughes provides instructions to refrigerate the "remaining M broth and TT and Rappaport-Vassiliadis R10 tubes [that were enriched] for culture confirmation of any EIA-positive samples." Hughes, 638. Hughes refers to the results as the "confirmed positive samples" (Hughes, 635) and also states that diagnostic reagents are "necessary for culture confirmation of presumptive EIA tests." Hughes, 636. Hughes indicates "[e]nrichment broths and M broths from samples positive by an

	enzyme immunoassay (EIA) method must be streaked on selective media." *Id.*, 635. Culture confirmation using streaking is more sensitive than the EIA method, as was commonly understood and confirmed by the teachings of D'Haese. *See generally*, D'Haese. Each sample was analyzed by the BAM method (7th Ed.) and the TSVIA method using the enrichment protocol described in section F of the method, *Preparation of Sample*. Equal portions (0.5–1 mL) from each of the 2M broths arising from the 2 selective-enrichment broths were combined in a small screw-capped tube and heat treated in boiling water for 15 min prior to performing the TSVIA. Confirmation for the immunoassay was by streaking from the M broth culture onto selective agar, as specified in the BAM reference culture method. Hughes, 635. Moreover, the recitation of a lot being validated for "release . . . into commerce" is not limiting. Prosecution History for Reexamination Control No. 95/011,294.
[35e.4] wherein the single production lots corresponding to any negatively testing composited single production lot samples are validated.	Price, alone or in combination with ICMSF and Hughes, discloses wherein the test lots corresponding to any negatively testing composited test lot samples are validated.



Price, 680 (Figure 1 (annotated)).

ICMSF and Hughes disclose selectively enriching prior to removing portions, thus a POSITA would consider the enrichment sufficient to enable validation of the lots without further enrichment or sampling.

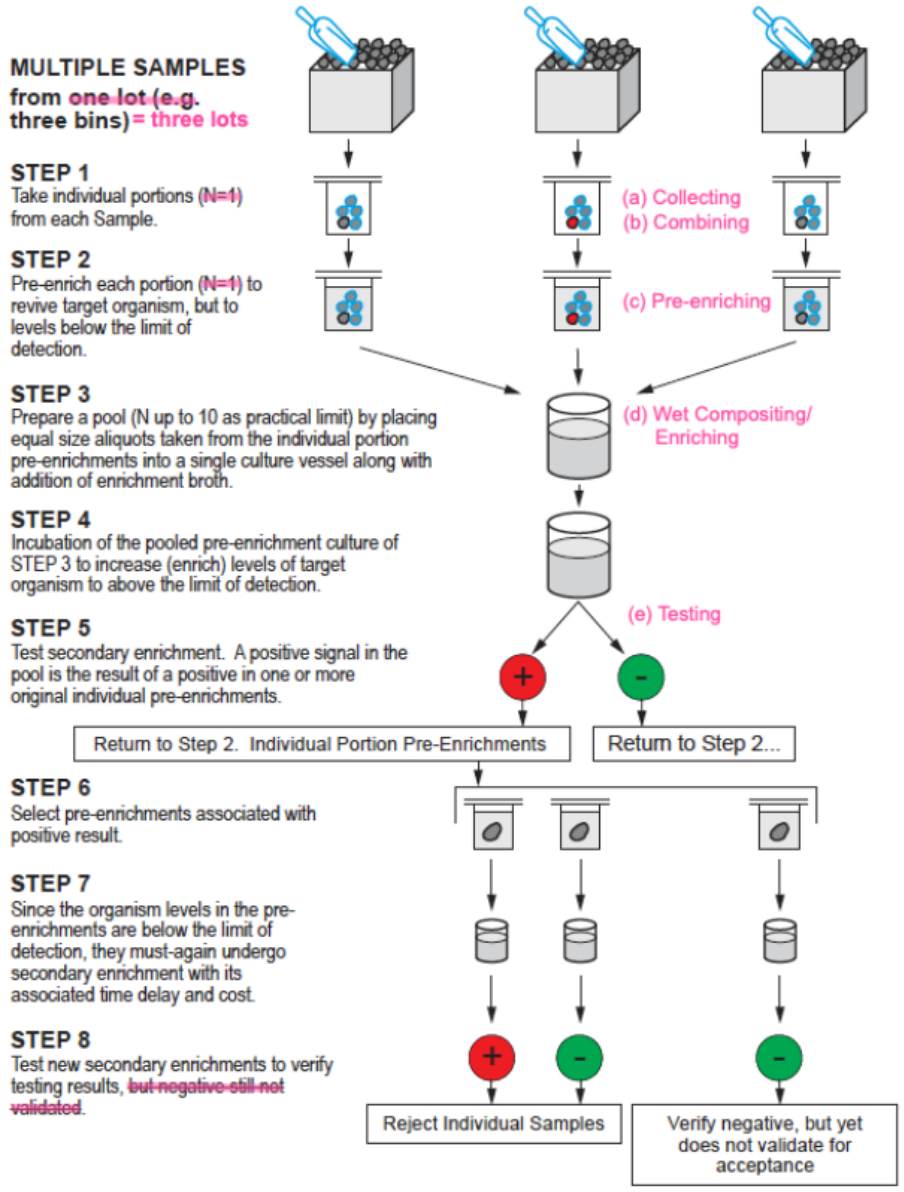
Hughes explicitly indicates portions of the enrichment cultures are retained for further testing; thus Hughes has no need for further sampling.

Hughes' "Preparation of Sample" section identifies four phases including (a) pre-

	enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. ICMSF and Hughes disclose selective enrichment so further enrichment is unnecessary. Also, as discussed regarding step (e)(3), Hughes explicitly indicates portions of the enrichment cultures are retained for further testing; thus Hughes has no need for further sampling. The same would be expected for enrichment cultures in ICMSF because retaining samples at each process stage was considered standard practice for a POSITA. in flowing steam 15 min. Refrigerate (2°–8°C) remaining M broth and TT and Rappaport-Vassiliadis R10 tubes from (c) for culture confirmation of any EIA-positive samples (*see* **967.26B, 967.26C, 967.27** and **967.28**). Cool heated M broths to 25°–37°C prior to analysis. Hughes, 638. Thus, the negative limitation of step (e)(4) is disclosed by and is obvious based on the combination of ICSMF and Hughes. *Id.*
Claim 38	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 37, wherein removing portions in step d) is removing equal portions, aseptically, so as to preclude contamination of the enriched separate composited single production lot samples.	Price, alone or in combination with ICMSF and Hughes discloses Claim 37, upon which this claim relies. See above re Claim 37. Price further discloses removing portions in step d) is removing equal portions, aseptically, so as to preclude contamination of the enriched separate test lot samples.

	ICMSF describes aseptically withdrawing and weighing sample units into the appropriate diluent. ICMSF, 231, 232. Hughes instructs that after enrichment "[c]ombine equal volumes (0.5-1 mL) from each M broth tube in a clean screw-cap tube." Hughes, 638. Moreover, a POSITA would consider this to be standard practice. A POSITA would have aseptically removed equal volume portions of each enriched separate test lot sample to preclude contamination.
Claim 41	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 37, wherein each of the multiple separate single production lots are separate single production lots of a meat or poultry product, comprising an assemblage of one or more meat or poultry specimens, respectively.	Price, in combination with ICMSF and Hughes, discloses Claim 37 upon which this claim relies. See above re Claim 37.

Figure 3: CITED PRIOR ART (PRICE et al, 1972)



	Prosecution History for Reexamination Control No. 95/001,294, 298 (annotated). IEH's diagram also indicates multiple samples are taken from three bins, analogous to three lots rather than a single lot because each bin is an assemblage of specimens.
Claim 42	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 41, wherein the meat or poultry specimens are meat trim pieces or poultry trim pieces, respectively.	Price, alone or in combination with ICMSF and Hughes discloses Claim 41, upon which this claim relies. See above re Claim 41. A POSITA would find it obvious to apply the teachings of Price to meat trim pieces ICMSF indicates "[t]rimmings to be used for ground beef could be pretested for E. coli O157:H7 by various sampling plans." ICMSF, 323. ICMSF indicates "[r]esearch has validated that up to fifteen 25 g analytical units of ground beef or trimmings can be composited without significant loss in E. coli O157:H7 detection sensitivity." *Id.*, 327 *see also id.*, 226, 314, 320, and 324. ICMSF includes many other references to testing lots of beef or poultry, two of the most common types of meat in commerce. *Id.* at 114 (§ 6.3), 184–191 (§§ 10.2–10.6), 225–226 (§ 12.1), 285 (§ 16.1), 313–330 (Chapter 17). ICMSF is relevant to any kind of food product including beef or poultry.
Claim 43	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 42, wherein the meat is that of beef, pork, sheep, or bison.	Price, alone or in combination with ICMSF and Hughes discloses Claim 42, upon which this claim relies. See above re Claim 42. A POSITA would find it obvious to apply the teachings of Price to meat. ICMSF indicates "[t]rimmings to be used for ground beef could be pretested for E. coli O157:H7 by various sampling plans." ICMSF, 323.

	ICMSF indicates "[r]esearch has validated that up to fifteen 25 g analytical units of ground beef or trimmings can be composited without significant loss in E. coli O157:H7 detection sensitivity." *Id.*, 327 *see also id.*, 226, 314, 320, and 324. ICMSF includes many other references to testing lots of beef or poultry, two of the most common types of meat in commerce. *Id.* at 114 (§ 6.3), 184–191 (§§ 10.2–10.6), 225–226 (§ 12.1), 285 (§ 16.1), 313–330 (Chapter 17). ICMSF is relevant to any kind of food product including beef, pork, sheep or bison.
Claim 44	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 43, wherein the meat is that of beef.	Price, alone or in combination with ICMSF and Hughes discloses Claim 43, upon which this claim relies. See above re Claim 43. A POSITA would find it obvious to apply the teachings of Price to meat. ICMSF indicates "[t]rimmings to be used for ground beef could be pretested for E. coli O157:H7 by various sampling plans." ICMSF, 323. ICMSF indicates "[r]esearch has validated that up to fifteen 25 g analytical units of ground beef or trimmings can be composited without significant loss in E. coli O157:H7 detection sensitivity." *Id.*, 327 *see also id.*, 226, 314, 320, and 324. ICMSF includes many other references to testing lots of beef or poultry, two of the most common types of meat in commerce. *Id.* at 114 (§ 6.3), 184–191 (§§ 10.2–10.6), 225–226 (§ 12.1), 285 (§ 16.1), 313–330 (Chapter 17). ICMSF is relevant to any kind of food product including beef.
Claim 48	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 37, wherein the target microbe(s) comprises at least one selected from the group consisting of *Listeria*, *Salmonella*, and *Escherichia coli*.	Price, alone or in combination with ICMSF and Hughes discloses Claim 37, upon which this claim relies. See above re Claim 37. Price further discloses a target microbial agent or organism comprises at least one selected from the group consisting of *Listeria*, *Salmonella* and *Escherichia coli*.

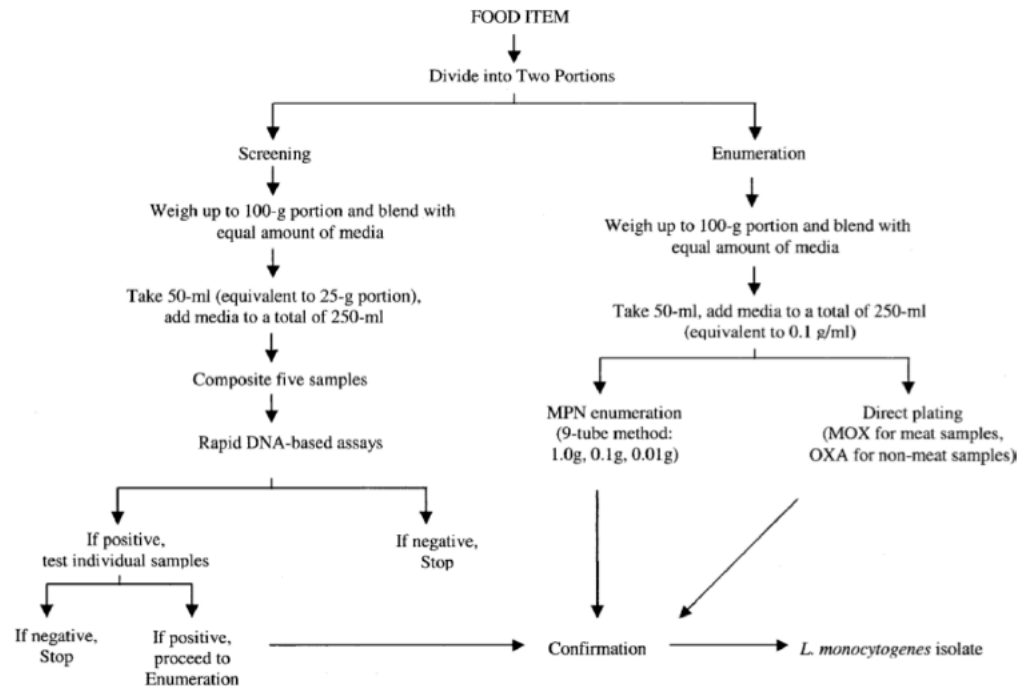
	ICMSF describes testing for *Listeria* (ICMSF, 285-312), *Salmonella* (*id.*, 273-84), and *Escherichia coli* (*id.*, 313-32). Hughes focuses on *Salmonella*. Hughes, 634. Price established that "pre-enrichment broths can be pooled for analysis without loss in sensitivity to *Salmonella* in the individual samples." Price, 679. Price tested dry food materials inoculated (purposefully contaminated) with *Salmonella. Id.*
Claim 49	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 48, wherein the target microbe(s) comprises at least one selected from the group consisting of *Listeria* spp., *Listeria monocytogenes*, *Salmonella* spp., *Escherichia coli* O157:H7, coliforms, and fecal coliforms.	Price, alone or in combination with ICMSF and Hughes discloses Claim 48, upon which this claim relies. See above re Claim 48. Price further discloses the target microbial agent or organism comprises at least one selected from the group consisting of *Listeria* spp*., Listeria monocytogenes*, *Salmonella* spp., *Escherichia coli* O157:H7, coliforms and fecal coliforms. ICMSF describes testing for *Listeria* (ICMSF, 285-312), *Salmonella* (*id.*, 273-84), and *Escherichia coli* (*id.*, 313-32). Hughes focuses on *Salmonella*. Hughes, 634. Price established that "pre-enrichment broths can be pooled for analysis without loss in sensitivity to *Salmonella* in the individual samples." Price, 679. Price tested dry food materials inoculated (purposefully contaminated) with *Salmonella. Id.*

EXHIBIT C1

Invalidity of U.S. Patent No. 8,822,143 in light of ICMSF & Gombas

National Beef denies that any of its microbiological testing methods infringe any claim of U.S. Patent No. 8,822,143 ("the '143 Patent"). If the claims of the '143 Patent were construed so broadly as to cover the microbiological testing methods used by National Beef, they would be invalid at least in view of INT'L COMM'N ON MICROBIOLOGICAL SPEC'S FOR FOODS (ICMSF), MICROORGANISMS IN FOODS 7: MICROBIOLOGICAL TESTING IN FOOD SAFETY MANAGEMENT (Kluwer Academic/Plenum Publishers, 2002) ("ICMSF") and David Gombas et al., *Survey of Listeria monocytogenes in Ready-to-Eat Foods*, 66 J. FOOD PROTECTION 559–569 (2003) ("Gombas"), which anticipate and/or disclose the limitations of the '143 Patent under at least pre-AIA 35 U.S.C. § 102(a), pre-AIA 35 U.S.C. § 102(b), pre-AIA 35 U.S.C. §§ 102(f)/(g) and/or pre-AIA 35 U.S.C. § 103. To the extent that ICMSF and/or Gombas do not expressly disclose one or more limitations of the asserted claims, as they would have to be construed to support IEH's claim of infringement, they disclose the limitations alone or in combination with one or more additional prior art references and/or the knowledge of one of ordinary skill in the art.

Claim 1	**Disclosure of ICMSF and Gombas**
[1pre] A method of sampling and testing for microbes in multiple separate test lots, comprising:	To the extent the preamble is limiting, as discussed herein, ICMSF, alone or in combination with Gombas, discloses a method of sampling and testing for microbes in multiple separate test lots. ICMSF includes many other references to testing lots of beef or poultry, two of the most common types of meat in commerce. *Id.*, 114 (§ 6.3), 184–191 (§§ 10.2–10.6), 225–226 (§ 12.1), 285 (§ 16.1), 313–330 (Chapter 17). ICMSF is relevant to any kind of food product including beef or poultry. Gombas describes sampling from ready to eat ("RTE") products ("[a]ll products were to be sliced (either presliced by manufacturers or sliced by the retail stores")) including beef bologna and "products made from pork or poultry." Gombas, 559, 562.

	 FIGURE 1. *Detection and enumeration of* L. monocytogenes *in RTE food samples.* Gombas, Figure 1.
[1a] a) separately collecting multiple independent samples from each of multiple separate test lots, wherein each separate test lot is separately sampled by taking said multiple independent samples thereof;	ICMSF, alone or in combination with Gombas, discloses separately collecting multiple independent samples from each of multiple separate test lots, wherein each separate test lot is separately sampled by taking said multiple independent samples thereof. Thus, ICMSF discloses collecting a plurality of samples or sample units (other synonyms include portions, specimens, and sub-samples) from lots. ICMSF describes collecting from a lot or lots: "If a consignment consisting of a mixture of production batches is treated as one lot,

	a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." *Id.*, 119. Additionally, ICMSF indicates a consignment may be "a very large quantity of food" and may be "composed of several lots, representing for example, different days of production from the same plant." *Id.*, 119, 120; *see also* Applicant Admitted Prior Art ("AAPA") #3-#5.

6.11 WHAT IS A REPRESENTATIVE SAMPLE?

A *representative sample* reflects, as far as is possible, the composition of the lot from which it is drawn.

How then should a representative sample be drawn? It is important to avoid bias and to draw a sufficient number of sample units to confidently make a judgment about a lot. Sampling at random is the universally recognized way of avoiding bias and is more reliable than consciously trying to draw the sample units from the various parts of a lot. The units (cartons or containers, particular weights of solid, or volumes of liquid) are drawn by using random numbers. There is, of course, no guarantee that a random sample has characteristics identical with those of the lot, but the randomness of sampling is the basis for calculation of the probability that a sample will give a biased result.

It is also possible, and may be desirable, to use a *stratified random sampling* approach, drawing a random sample of a given number of sample units from each *stratum* (e.g., each sublot or carton). The distribution of sample units on strata should correspond to the distribution of lot units on strata. This means that if each stratum contains the same quantity as every other one, the number of sample units per stratum should be the same. Otherwise, they should differ just as the proportions of the lot contained in the strata differ. Stratification is a device for handling known sources of variation and may be used where one has prior knowledge that the consignment is potentially not of uniform quality. A consignment might not be of uniform quality if portions of it are shipped in different vehicles or in different holds of a vessel, or if it is known that it is really composed of several lots representing, for example, different days of production from the same plant or different plants of the same company. The results for different strata should be assessed separately and then pooled if they appear to be homogeneous.

The calculation of acceptance probabilities is based on the assumption that random samples have been drawn and analyzed. Where physical or practical constraints prevent random sampling, such calculated probabilities may not be valid. Their reliability depends on how close reality approaches the ideal situation of random sampling. This fact should be borne in mind when subsequently interpreting results.

ICMSF, 119-20 (annotated).

6.10 WHAT IS A LOT?

Ideally, a lot is a quantity of food or food units produced and handled under uniform conditions. Under certain circumstances, it has been shown that the logarithms of the counts from a batch of food are likely to be normally distributed (Baird-Parker, 1980; Kilsby & Baird-Parker, 1983 [their Table 2]; Ingram & Roberts, 1976). The implication is that there is homogeneity within a lot, which, when considering microbial levels and distributions, rarely occurs in practice. In most instances, the distribution of microorganisms within lots of food is heterogeneous. Even in food factories, this heterogeneity makes the interpretation of sample results difficult, and it becomes even more so in circumstances where a lot is less well defined, such as in a consignment of a very large quantity of food.

It is therefore helpful if suppliers can be persuaded to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load).

If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment.

A lot should be composed of food produced with as little variation as possible for a given process or commodity. Because of the uncertainties in identifying a lot commercially, however, the use of the word *lot* in this book is usually in its statistical sense, as a collection of units of a product, the acceptability of which is determined by examining a sample drawn from it.

ICMSF, 118-19 (annotated).

	The ICMSF has defined a lot as follows: In the commercial sense, a lot is a quantity of food supposedly produced under identical conditions, all packages of which would normally bear a lot number that identifies the production during a particular time interval, and usually from a particular line, retort, or other critical processing unit. Statistically, a lot is considered as a collection of units of a product from which a sample is drawn to determine acceptability of the lot (ICMSF, 1986). In this example, the maximum size of a lot consists of all the ground beef produced on common equipment from cleanup to cleanup. Smaller lots could be established (e.g., hourly). ICMSF, 327 (annotated). This definition also reflects the common practice referred to in the IEH Patents at Example II, wherein "[p]referably, the producer defines and produces these single-unit lots in such a fashion as to maximize the information available." '143 Patent,12:52–54. This corresponds with the specification of IEH's Patents which reflects the common practice of producers defining what constitutes a lot grouped for traceability purposes. '143 Patent, 3:5-15, 11:18-22 (AAPA #3), and 12:52-55. Thus, ICMSF discloses collecting a plurality of portions from separate lots. ICMSF states: "To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units of the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. . . . This finding suggests that considerable reductions may be made in the cost of laboratory analysis by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, n, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort." ICMSF, 187

	To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. A laboratory investigation undertaken for ICMSF (Silliker & Gabis, 1973) indicated that different sized analytical units drawn from a single well-mixed sample unit of dried food give very similar results. A subsequent investigation (Gabis & Silliker, 1974) using foods of high moisture content (including eggs, poultry meat, meat, and meat products) showed comparable reliability in the use of composite analytical units; for example, examination of three composites of 20 × 25 g analytical units gave the same result as examination of all 60 analytical units individually (the total amount examined being 1500 g in both instances). This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n*, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort. ICMSF, 187.

10.5.1 Conceivable Alternative Plans

As indicated above, for an infectious agent such as *Salmonella*, there are strong reasons for adopting sampling plans in which $c = 0$:

1. It is philosophically objectionable to some people to accept a lot containing a recognized pathogen, regardless of how the food is to be used in a manufacturing process or by the consumer.
2. There is the very great technical advantage of combining many analytical units for bacteriological examination in systems with $c = 0$, because a single positive decides the outcome.
3. The plan with $c = 1$ (or more) would always require the examination of (many) more analytical units, for equal probability of acceptance, than a plan with $c = 0$.
4. Even if the indicated probabilities of plans with $c = 0$ versus $c = 1$ are the same, their implications are not. For example, when a lot is accepted with the plan $n = 60$, $c = 0$, it is possible (though not certain) that the lot may not be contaminated at all. But using the plan $n = 95$, $c = 1$ and accepting one positive, it is known that the lot certainly is contaminated (though the probability that a lot with 5% of analytical units contaminated would be accepted is in both instances $P_a = 0.05$).

ICMSF, 188 (annotated). ICMSF also provides an example of "[u]sing a sampling plan involving n = 15 (25 g analytical units) and a composite of 375 g." *Id.*, 195 (§ 10.7.2). ICMSF also states "[f]or qualitative analyses, 25 g analytical units are frequently analyzed individually or as composites of 100 g, 200 g, or more." *Id.*, 232 (§ 12.5.2).

The ICMSF incorporates two references, Silliker & Gabis (1973) and Gabis & Silliker (1974). Silliker notes that "[c]ompositing of multiple samples (lumping) and wet compositing of pre-enrichment cultures have been tested as economical approaches to *Salmonella* control." Silliker, 475.

Silliker further notes that "[b]oth approaches are being used widely by the food industry in the United States." *Id.*. Gabis discloses application of the Silliker teachings to testing meat. Gabis.

	"There is the very great technical advantage of combining many analytical units for bacteriological examination." ICMSF, 188. ICMSF also describes composites prepared by combining "15 (25 g analytical units)" and "composites of 100 g, 200 g, or more." *Id.,* 195, 232; *see* AAPA #5. ICMSF states, "All steps up to the analysis must be controlled . . . to allow reliable traceability." ICMSF, 227.
[1b] b) separately compositing the collected multiple independent samples from each of the separate test lots to provide a corresponding set of separate composited test lot samples, wherein each of the separate composited test lot samples is attributed to a particular corresponding separate test lot;	ICMSF states: "To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units of the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. . . . This finding suggests that considerable reductions may be made in the cost of laboratory analysis by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, n, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort." ICMSF, 187 To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. A laboratory investigation undertaken for ICMSF (Silliker & Gabis, 1973) indicated that different sized analytical units drawn from a single well-mixed sample unit of dried food give very similar results. A subsequent investigation (Gabis & Silliker, 1974) using foods of high moisture content (including eggs, poultry meat, meat, and meat products) showed comparable reliability in the use of composite analytical units; for example, examination of three composites of 20 × 25 g analytical units gave the same result as examination of all 60 analytical units individually (the total amount examined being 1500 g in both instances). This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n*, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort.

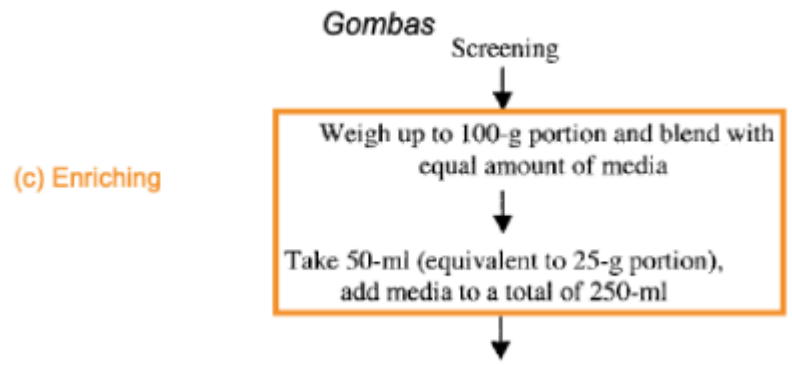
	ICMSF, 187. **10.5.1 Conceivable Alternative Plans** As indicated above, for an infectious agent such as *Salmonella*, there are strong reasons for adopting sampling plans in which $c = 0$: 1. It is philosophically objectionable to some people to accept a lot containing a recognized pathogen, regardless of how the food is to be used in a manufacturing process or by the consumer. 2. There is the very great technical advantage of combining many analytical units for bacteriological examination in systems with $c = 0$, because a single positive decides the outcome. 3. The plan with $c = 1$ (or more) would always require the examination of (many) more analytical units, for equal probability of acceptance, than a plan with $c = 0$. 4. Even if the indicated probabilities of plans with $c = 0$ versus $c = 1$ are the same, their implications are not. For example, when a lot is accepted with the plan $n = 60$, $c = 0$, it is possible (though not certain) that the lot may not be contaminated at all. But using the plan $n = 95$, $c = 1$ and accepting one positive, it is known that the lot certainly is contaminated (though the probability that a lot with 5% of analytical units contaminated would be accepted is in both instances $P_a = 0.05$). ICMSF, 188 (annotated). ICMSF also provides an example of "[u]sing a sampling plan involving n = 15 (25 g analytical units) and a composite of 375 g." *Id.*, 195 (§ 10.7.2). ICMSF also states "[f]or qualitative analyses, 25 g analytical units are frequently analyzed individually or as composites of 100 g, 200 g, or more." *Id.*, 232 (§ 12.5.2). The ICMSF incorporates two references, Silliker & Gabis (1973) and Gabis & Silliker (1974). Silliker notes that "[c]ompositing of multiple samples (lumping) and wet compositing of pre-enrichment cultures have been tested as economical approaches to *Salmonella* control." Silliker, 475. Silliker further notes that "[b]oth approaches are being used widely by the food industry in the United States." *Id.*.

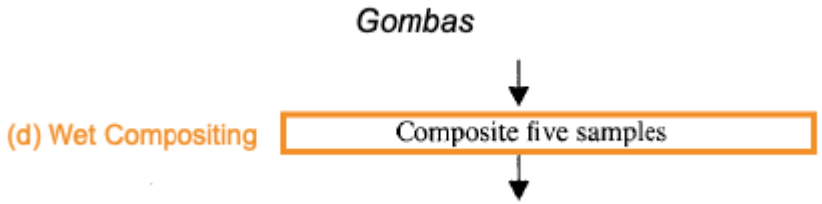
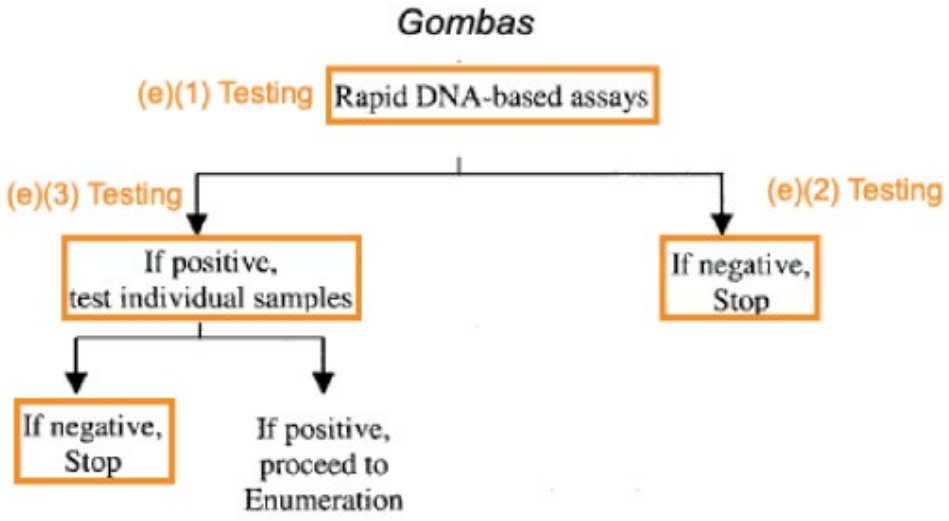
	To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. A laboratory investigation undertaken for ICMSF (Silliker & Gabis, 1973) indicated that different sized analytical units drawn from a single well-mixed sample unit of dried food give very similar results. A subsequent investigation (Gabis & Silliker, 1974) using foods of high moisture content (including eggs, poultry meat, meat, and meat products) showed comparable reliability in the use of composite analytical units; for example, examination of three composites of 20 × 25 g analytical units gave the same result as examination of all 60 analytical units individually (the total amount examined being 1500 g in both instances). This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n*, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort. "There is the very great technical advantage of combining many analytical units for bacteriological examination." ICMSF, 188. ICMSF also describes composites prepared by combining "15 (25 g analytical units)" and "composites of 100 g, 200 g, or more." *Id.,* 195, 232; *see* AAPA #5. ICMSF states, "All steps up to the analysis must be controlled . . . to allow reliable traceability." ICMSF, 227. ICMSF indicates lot samples are traceable to a particular corresponding lot: "It is therefore helpful . . . to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load). . . . Treating the individual production

	batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119. Gombas also discloses this element. Gombas states "samples were assigned codes" ad product information was recorded for the luncheon meats including "sampling location." Gombas, 563. A POSITA would find Gombas' system for testing individual samples, which composited samples test positive, inherently requires tracking the individual samples that contributed to the composite. *See, e.g.*, Gombas, 564 ("If a composite tested positive, samples in the composite were tested individually by Gene-Trak assay with the PBS from the second mLCA plate or by the BAX assay with the secondary enrichment broths. Individual samples that tested negative were not tested further If an individual sample tested positive, the retained portion was subjected to an enumeration assay.") Thus, Gombas also discloses that each of the separate composited lot samples is attributed to a particular corresponding separate lot.
[1c] c) enriching each of the separate composited test lot samples to provide a set of separate composited test lot samples enriched for the target microbe(s);	ICMSF, alone or in combination with Gombas, discloses enriching each of the separate composited test lot samples to provide a set of separate composited test lot samples enriched for the target microbe(s). ICMSF states: To reduce laboratory effort . . . it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. ICMSF, 187. Also, ICMSF describes preparing composites of analytical units then diluting "for qualitative (enrichment)" analysis. *Id.,* 231-32; *see also* AAPA #5, #7. For practical reasons, however, analytical units of 10 g up to 50 g are typical for quantitative methods and are diluted 1:10. For qualitative analyses, 25 g analytical units are frequently analyzed individually or as composites of 100 g, 200 g, or more.

	ICMSF, 232 (annotated). Preparation of the first dilution, either for qualitative (enrichment) or quantitative (direct) analyses, can have a marked impact on the microbial population in the sample. This is often due to changes in the physico-chemical characteristics of the suspension as compared to the food matrix. Where the food does not confer protection, the composition of the diluent is important to prevent or minimize shock/injury to the microorganisms that can result from rapid changes in the ionic concentrations. ICMSF, 232 (annotated). ICMSF also describes strategic use of non-selective enrichment to resuscitate microorganisms and selective enrichments for growing microorganisms. *Id.*, 232–33 (§ 12.6); *see e.g.*, *id.,* 233 ("use of nonselective medium . . . to enhance recovery of injured or stressed cells."). ICMSF, § 12.6 also describes optimizing the incubation period. S*ee e.g.*, *id.,* 232. It was well known long before the priority date to use non-selective enrichments after dry compositing and prior to wet compositing as evidenced by Price, Silliker, Gabis, and MLG. A POSITA would also know to use selective enrichments after dry compositing and prior to wet compositing as disclosed by ICMSF and Gombas. Gombas also discloses incubating: [A]pproximately half (up to 100 g) of a sample was aseptically transferred to a sterile stomacher bag and blended with an equal amount of enrichment broth. . . . For northern California samples, demi-Fraser broth was used as the enrichment broth for all products. After blending, 50 g of the homogenate was added to 200 ml of enrichment broth and stomached for 1 min. This procedure resulted in a detection sensitivity equivalent to that of the current regulatory methods (i.e., 1 CFU/25 g). The initial sample-blending step was performed to account for the potential heterogeneous distribution of *L. monocytogenes* in the sample. The 250-ml enrichment was incubated at 35°C for 24 ± 2 h." Gombas, 563; *see also id.,* 564. Gombas further indicates that: "For each California sample, 0.1 ml of the demi-Fraser enrichment culture was transferred to 10 ml of morpholinepropanesulfonic

	acid-*Listeria* enrichment broth and incubated at 35°C for 20 to 24 h. After the secondary enrichment, 1 ml of the broth culture was used for screening with the BAX assay as described below." *Id.,* 564. Gombas avoids potential heterogenous distribution and ensures the target microorganism is at least substantially uniform in a sample by blending the sample and the enrichment broth in the stomacher bag to yield a homogenate. *Id.,* 563. **Sample screening.** Each sample was divided into two portions for screening and enumeration. For screening, approximately half (up to 100 g) of a sample was aseptically transferred to a sterile stomacher bag and blended with an equal amount of enrichment broth. For Maryland samples, University of Vermont broth 2 (UVM-2 broth) was used as the enrichment broth for luncheon meats, smoked seafood, and seafood salads; phosphate-buffered *Listeria* enrichment broth was used for the other product types. For northern California samples, demi-Fraser broth was used as the enrichment broth for all products. After blending, 50 g of the homogenate was added to 200 ml of enrichment broth and stomached for 1 min. This procedure resulted in a detection sensitivity equivalent to that of the current regulatory methods (i.e., 1 CFU/25 g). The initial sample-blending step was performed to account for the potential heterogeneous distribution of *L. monocytogenes* in the sample. The 250-ml enrichment was incubated at 35°C for 24 ± 2 h. Gombas, 563 (annotated).

	 Gombas, 563 (Figure 1 (annotated) showing an enriching step).
[1d] d) removing portions of each separate enriched composited test lot sample, and combining the removed portions to provide a pooled modular composite sample; and	ICMSF describes compositing the enriched lot samples: "it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. . . . This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n,* with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort." ICMSF, 187. AAPA #5-#7. Additionally, Gombas discloses wet compositing of enriched samples: "Up to five like-product samples were composited and screened. . . . For California samples, five 1-ml samples of secondary enrichment broths were composited." Gombas, 564. Gombas's Figure 1 depicts wet compositing as shown in the excerpt below:

	 Gombas, 563 (Figure 1 (annotated) showing a wet compositing step).
[1e.1] e) testing of the pooled modular composite sample, using a suitable detection assay, for the target microbe(s),	ICMSF describes testing enrichment cultures of composite samples with suitable detection assays (ICMSF, 187) and "presence/absence tests" (ICMSF, 233). Suitable detection assays were established in Silliker and Gabis as discussed in ICMSF. Gombas provides a complete framework for testing, and a POSITA would have been motivated to modify ICMSF based on Gombas's framework:  Gombas, 563 (Figure 1 (annotated) showing the testing step).

	Gombas also discloses a suitable detection assay: "For California samples, . . . the 5-ml composite was tested by the BAX assay according to the manufacturer's procedures." Gombas, 564. These assay types were "art recognized." '143 Patent, AAPA #7.
[1e.2] wherein when such testing is negative all of said samples that were composited to form the separate composited test lot samples are deemed negative for the target microbe(s) and each of the multiple separate test lots is validated, and	ICMSF describes correlating lots in a consignment with lot samples so a negative or positive test result enables a lot to be validated or rejected. ICMSF, 118-19. ICMSF describes sampling plans in which "c is the maximum allowable number of sample units yielding a positive result." ICMSF, 115. For many scenarios, the requirement is c = 0, which indicates the testing is negative. *Id.*, 118, 183-90, 309, 325, 326. A negative test result (*id.*, 113-15, 325, 326) is the expected and desired outcome to determine meat is not contaminated and is safe for consumption (*id.*, 184-89) such that a lot is validated and it is "commercially possible to market" (*id.*, 185) the meat.

6.10 WHAT IS A LOT?

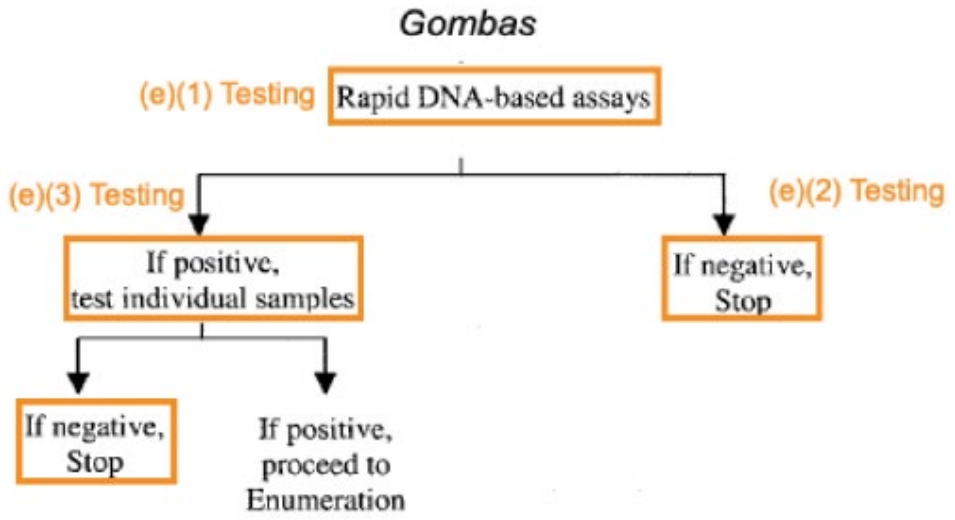
Ideally, a lot is a quantity of food or food units produced and handled under uniform conditions. Under certain circumstances, it has been shown that the logarithms of the counts from a batch of food are likely to be normally distributed (Baird-Parker, 1980; Kilsby & Baird-Parker, 1983 [their Table 2]; Ingram & Roberts, 1976). The implication is that there is homogeneity within a lot, which, when considering microbial levels and distributions, rarely occurs in practice. In most instances, the distribution of microorganisms within lots of food is heterogeneous. Even in food factories, this heterogeneity makes the interpretation of sample results difficult, and it becomes even more so in circumstances where a lot is less well defined, such as in a consignment of a very large quantity of food.

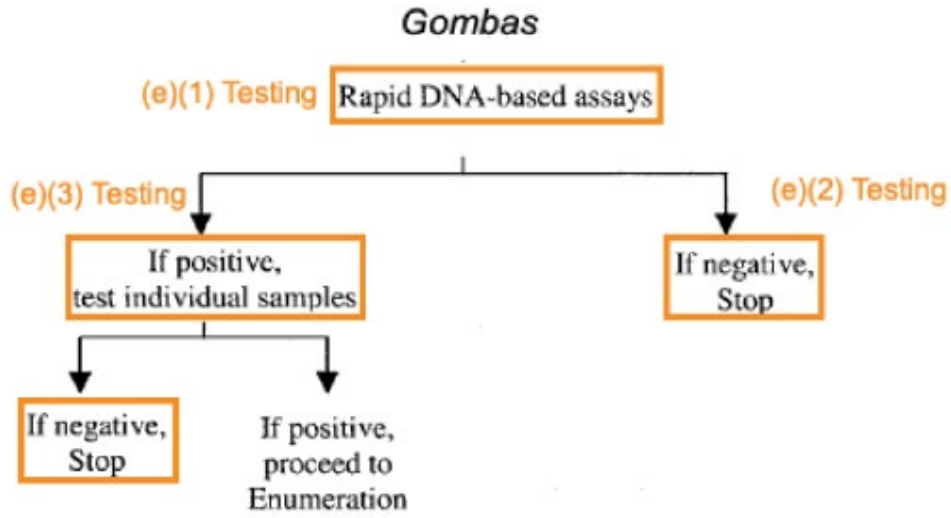
It is therefore helpful if suppliers can be persuaded to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load).

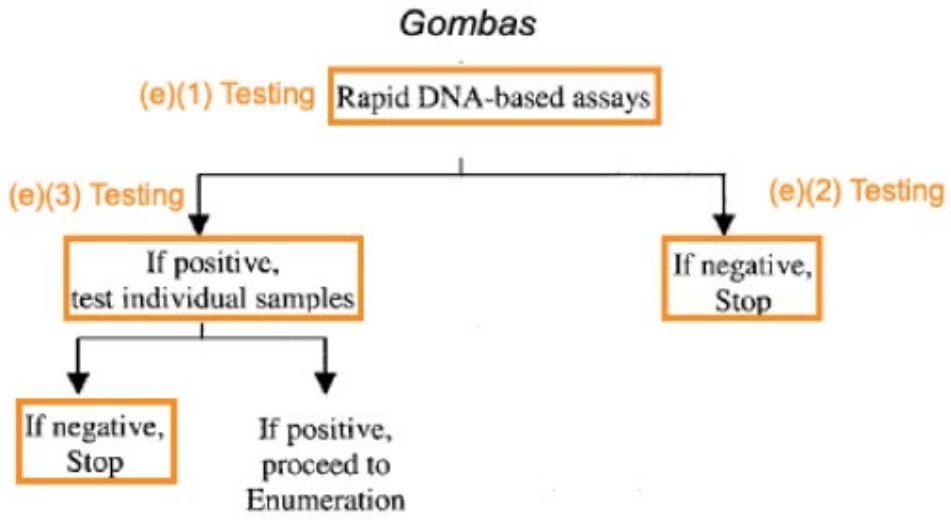
If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment.

A lot should be composed of food produced with as little variation as possible for a given process or commodity. Because of the uncertainties in identifying a lot commercially, however, the use of the word *lot* in this book is usually in its statistical sense, as a collection of units of a product, the acceptability of which is determined by examining a sample drawn from it.

ICMSF, 118-19 (annotated).

	To the extent ICMSF is considered not to disclose some aspect of step (e)(2), Gombas indicates a negative test result is the end of the process: "If a composite tested negative, all samples in the composite were considered negative (in 25 g) and were not tested further." Gombas, 564. In the combination, a POSITA would find that a negative test result equates to being validated.  Gombas, 563 (Figure 1 (annotated) showing "if negative, stop.").
[1e.3] wherein when such testing is positive, each of the individual separate composited test lot samples that were used to form the pooled modular composite sample are individually tested to determine which of the separate composited test lot samples is positive for the target microbe(s),	The combination of ICMSF and Gombas describes retaining a portion of the enriched lot samples (steps (b)-(d)). "Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119.

	"There is often a desire to retest retained samples." ICMSF, 191. If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment. ICMSF, 119 (annotated). "If a composite tested positive, samples in the composite were tested individually . . . by the BAX assay with the secondary enrichment broths. Individual samples that tested negative were not tested further." Gombas, 564.  Gombas, 563 (Figure 1 (annotated) showing testing procedures for further testing). "Individual samples that tested negative were not tested further. If an individual

	sample tested positive, the retained portion was subjected to an enumeration assay." Gombas, 564. The combination of ICMSF and Gombas teaches that after the composited samples test positive, then the individual samples used to form the composited sample are individually tested with the same protocol to determine which of the separate composited lot samples is positive for the target microbe(s).
[1e.4] wherein the test lots corresponding to any negatively testing composited test lot samples are validated.	Gombas teaches that negative tests are not tested again.  Gombas, 563 (Figure 1 (annotated) showing "if negative, stop").
Claim 2	**Disclosure of ICMSF and Gombas**
The method of claim 1, wherein each of the multiple separate test lots is selected from the group consisting of an environmental lot comprising multiple operationally-linked	ICMSF, alone or in combination with Gombas, discloses Claim 1, upon which this claim relies. See above re Claim 1. The specification of the '143 Patent indicates a test lot is a single-unit production lot

environmental specimens, pharmaceutical lot comprising multiple operationally-linked pharmaceutical specimens, single-unit product lot comprising multiple operationally-linked product specimens, and combinations thereof.	('143 Patent, 5:34-45) such as a single combo, bin. pallet, or segment of production ('143 Patent, 7:42-50; *see also* AAPA #3 and #4). ICMSF teaches collecting from "individual production batches as lots" (ICMSF, 119) and "[a] lot is considered as a collection of units" (ICMSF, 327). "Operationally-linked" is simply grouping like items, which is also disclosed by ICMSF. "Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119.
Claim 3	**Disclosure of ICMSF and Gombas**
The method of claim 1, wherein each of the multiple separate test lots is a single-unit production lot, comprised of operationally-linked product specimens.	ICMSF, alone or in combination with Gombas, discloses Claim 1, upon which this claim relies. See above re Claim 1. The specification of the '143 Patent indicates a test lot is a single-unit production lot ('143 Patent, 5:34-45) such as a single combo, bin. pallet, or segment of production ('143 Patent, 7:42-50; *see also* AAPA #3 and #4). ICMSF teaches collecting from "individual production batches as lots" (ICMSF, 119) and "[a] lot is considered as a collection of units" (ICMSF, 327). "Operationally-linked" is simply grouping like items, which is also disclosed by ICMSF. "Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119.
Claim 4	**Disclosure of ICMSF, Gombas, and Stewart**
The method of claim 1, further comprising incubating the pooled modular composite sample of d), prior to testing in e).	ICMSF, alone or in combination with Gombas, discloses Claim 1, upon which this claim relies. See above re Claim 1.

	ICMSF and Gombas, in combination with Stewart, further discloses further comprising incubating the modular composite sample of d), prior to testing in e). Stewart discloses that after the selective enrichment in Rappaport-Vassiliadis (RV) broth and tetrathionate (TT) broths, the two selective enrichments were combined into a single sample for further incubation and analysis. "[P]ost-enrichment by transfer and combination of 0.5 mL RV and 1 mL TT into a single tube containing 10 mL prewarmed trypticase soy broth supplemented with novobiocin (TSB + n) at 10 µg/mL with incubation for 16–20 h at 42°C." Stewart, 398.
Claim 5	**Disclosure of ICMSF and Gombas**
The method of claim 1, wherein enriching each separate composited test lot sample in c) comprises incubating each separate composited test lot sample with enrichment medium suitable to enrich for the target microbe(s), and wherein removing in d) comprises removing a portion of the enrichment medium from each enriched separate composited test lot sample and combining the removed portions to provide a pooled modular composite sample.	ICMSF, alone or in combination with Gombas, discloses Claim 1, upon which this claim relies. See above re Claim 1. ICMSF describes the well-known benefits of combining enrichment cultures from analytical units from lots in a consignment. To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. A laboratory investigation undertaken for ICMSF (Silliker & Gabis, 1973) indicated that different sized analytical units drawn from a single well-mixed sample unit of dried food give very similar results. A subsequent investigation (Gabis & Silliker, 1974) using foods of high moisture content (including eggs, poultry meat, meat, and meat products) showed comparable reliability in the use of composite analytical units; for example, examination of three composites of 20 × 25 g analytical units gave the same result as examination of all 60 analytical units individually (the total amount examined being 1500 g in both instances). This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n*, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort.

	ICMSF, 187. Gombas describes incubating an enriched test lot sample. "The 250-ml enrichment was incubated at 35°C for 24 ± 2 h." Gombas, 563. Gombas also discloses incubating: [A]pproximately half (up to 100 g) of a sample was aseptically transferred to a sterile stomacher bag and blended with an equal amount of enrichment broth. . . . For northern California samples, demi-Fraser broth was used as the enrichment broth for all products. After blending, 50 g of the homogenate was added to 200 ml of enrichment broth and stomached for 1 min. This procedure resulted in a detection sensitivity equivalent to that of the current regulatory methods (i.e., 1 CFU/25 g). The initial sample-blending step was performed to account for the potential heterogeneous distribution of *L. monocytogenes* in the sample. The 250-ml enrichment was incubated at 35°C for 24 ± 2 h." Gombas, 563; *see also id.,* 564. Gombas further indicates that: "For each California sample, 0.1 ml of the demi-Fraser enrichment culture was transferred to 10 ml of morpholinepropanesulfonic acid-*Listeria* enrichment broth and incubated at 35°C for 20 to 24 h. After the secondary enrichment, 1 ml of the broth culture was used for screening with the BAX assay as described below." *Id.,* 564.
Claim 6	**Disclosure of ICMSF and Gombas**
The method of claim 1, wherein testing of the pooled modular composite sample, using a suitable detection assay comprises use of a concentration step to concentrate the target microbe(s).	ICMSF, alone or in combination with Gombas, discloses Claim 1, upon which this claim relies. See above re Claim 1. ICMSF discloses the use of "immunomagnetic beads to concentrate cells." ICMSF, 318. A POSITA would consider this a well-known concentration technique before the priority date. Fratamico discusses the rapid isolation of target microorganisms using an immunomagnetic separation method. Fratamico, 105. Fratamico discloses that use of immunomagnetic beads resulted in "an increase in the level of E. coli B1409 in the sample," as well as "an increase in the number of organisms recovered." *Id.*, 107.

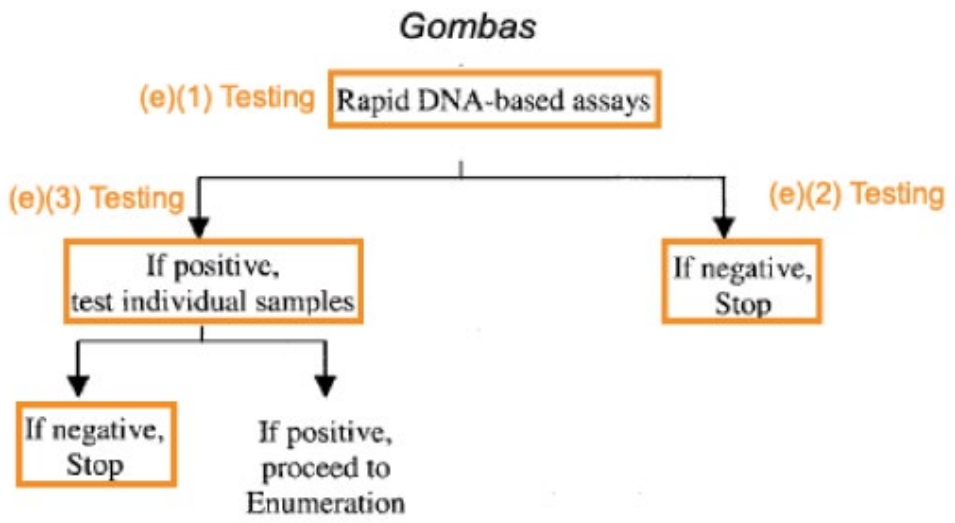
	Additionally, Fratamico provides detailed instructions for preparing and using immunomagnetic bead separation to concentrate a target microbial agent or organism. Fratamico developed "[a] method employing immunomagnetic beads" to concentrate and thereby improve the isolation and detection of E. coli. *Id.* For example, Fratamico was able to use immunomagnetic beads to isolate *E. coli* strains from enrichment cultures of raw ground beef "when the initial inoculum level was 1 cfu g-1." *Id.*, 109. Fratamico further teaches that "[t]he percentage of bacteria recovered increased with increasing amounts of beads." *Id.*, 108. Consequently, Fratamico "demonstrates that immunomagnetic separation [using immunomagnetic beads] is a simple and rapid technique" for concentrating a microbe for isolation and detection. *Id.*, 109. These teachings of Fratamico would have motivated a POSITA to modify the combination of ICMSF and Gombas and ICMSF and Hughes to use immunomagnetic beads to concentrate microbes in a modular composite sample to benefit from accurate tests. Use of immunomagnetic beads was already known in the art for concentrating during testing beef samples. "Tu et al. (78) detected pathogenic E. coli O157:H7 in beef hamburger with the use of IMS and ATP measurement. After 6 h of enrichment at 37°C, inoculated beef samples (0.9 ml each) were filtered to remove large particles prior to 10 min of incubation with ca. 10^5 magnetic beads coated with anti–*E. coli* O157:H7 (Dynabeads, Dynal Inc., Oslo, Norway). Following incubation, the magnetic beads were collected with a Dynal magnetic concentrator and resuspended in 0.9 ml of Trypticase soy broth for ATP analysis." Beniot, 1941. "Hudson et al. (26) used IMS to directly separate and concentrate L. monocytogenes from ham samples prior to detection by PCR. IMS was used on the basis of the commercial availability of anti-Listeria magnetic beads (Dynal Inc.) to avoid time-

	consuming cultural enrichment steps and to remove PCR inhibitors." Beniot, 1941-42. Cudjoe investigates the use of immunomagnetic beads in pooling methods. In a study designed to evaluate the performance of Dynabeads® Anti-Salmonella, immunomagnetic separation (IMS) followed by plating (IMS-Plating) proved far superior to the conventional ISO *Salmonella* methodology and the Modified Semi-solid Rappaport-Vassiliadis (MSRV) method. *Salmonella* species were isolated and detected from 135 out of the 180 diverse poultry samples by IMS analysis as against 98 by the conventional method and 33 by the MSRV technique. All results were confirmed biochemically and serologically. It appeared from the data generated that some of the salmonellae isolated using IMS were greatly inhibited in Rappaport-Vassiliadis enrichment broth at 42°C and to a lesser extent in selenite cystine broth at 37°C. A moderately selective plating medium like XLD proved to be better in isolating these possible sensitive wild-type strains of salmonellae than the more selective BGA. Cudjoe, Abstract. *Samples*. One hundred and eighty (180) diverse poultry samples, consisting of 40 pools of cloacal/faecal swabs (5 swabs/pool; 5 pools samples/flock) from eight different flocks, 10 raw chicken breast meat samples, 10 pooled egg shell samples, 60 raw chicken liver samples and 60 chicken neck skins sampled from the slaughter line, were used in this study. All samples were obtained from a poultry slaughter and processing plant in the Netherlands. A pool of five swab samples was pre-enriched in 10 ml of buffered peptone water (BPW; Difco Laboratories, Detroit, Michigan, USA) at 37°C for 16–20 h, whilst 25 g of each of the other samples above, were pre-enriched similarly in 225 ml of BPW and examined as detailed below. Cudjoe, 160.
Claim 7	**Disclosure of ICMSF and Gombas**

The method of claim 1, wherein where testing in e) of the pooled modular composite sample is positive, and wherein the separate individual composited test lot samples are further tested using a more sensitive protocol.	ICMSF, alone or in combination with Gombas, discloses Claim 1, upon which this claim relies. See above re Claim 1. ICMSF discloses the use of "immunomagnetic beads to concentrate cells." ICMSF, 318. A POSITA would consider this a well-known technique before the priority date. Gombas discloses retesting using the same protocol. Gombas discusses the use of "biochemical confirmation" to confirm testing results. Gombas, 565. A POSITA would have recognized the concentration step could be employed to provide a more sensitive protocol for further testing. A POSITA would understand that retesting the non-pooled, singular samples is an inherently more sensitive process because the sample is not diluted with other samples in the testing pool. Therefore, the retesting of a singular sample is more concentrated and therefore, more sensitive. *See also, generally*, Jarvis. Fratamico discusses the rapid isolation of target microorganisms using an immunomagnetic separation method. Fratamico, 105. Fratamico discloses that use of immunomagnetic beads resulted in "an increase in the level of E. coli B1409 in the sample," as well as "an increase in the number of organisms recovered." *Id.*, 107. Additionally, Fratamico provides detailed instructions for preparing and using immunomagnetic bead separation to concentrate a target microbial agent or organism. Fratamico developed "[a] method employing immunomagnetic beads" to concentrate and thereby improve the isolation and detection of E. coli. *Id.* For example, Fratamico was able to use immunomagnetic beads to isolate *E. coli* strains from enrichment cultures of raw ground beef "when the initial inoculum level was 1 cfu g-1." *Id.*, 109. Fratamico further teaches that "[t]he percentage of bacteria recovered increased with increasing amounts of beads." *Id.*, 108. Consequently, Fratamico "demonstrates that immunomagnetic separation [using immunomagnetic beads] is a simple and rapid technique" for concentrating a microbe for isolation and detection. *Id.*, 109.

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Claim 11	**Disclosure of ICMSF and Gombas**
The method of claim 1, wherein each the test lots is selected from the lot group consisting of a single combo, single bin, single pallet, and a production assemblage.	ICMSF, alone or in combination with Gombas, discloses Claim 1, upon which this claim relies. See above re Claim 1. The specification of the '143 Patent indicates a test lot is a single-unit production lot ('143 Patent, 5:34-45) such as a single combo, bin. pallet, or segment of production ('143 Patent, 7:42-50; *see also* AAPA #3 and #4). ICMSF teaches collecting from "individual production batches as lots" (ICMSF, 119) and "[a] lot is considered as a collection of units." ICMSF, 327. "Operationally-linked" is simply grouping like items, which is also disclosed by ICMSF. "Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119.
Claim 12	**Disclosure of ICMSF and Gombas**

The method of claim 1, wherein the number of separate test lots is from 3 to 10.	ICMSF, alone or in combination with Gombas, discloses Claim 1, upon which this claim relies. See above re Claim 1. ICMSF indicates a consignment may be "composed of several lots." ICMSF, 119-20. "Several" lots includes at least three lots. Additionally, Gombas tests five lots. *See, e.g.*, Gombas, Fig. 1 ("composite five samples"), *id.*, 564 ("up to five like-product samples were composited and screened.").
Claim 13	**Disclosure of ICMSF and Gombas**
The method of claim 1, further comprising, where testing of the pooled modular composite sample is positive, determining which individual separate test lots are positive, application of remedial measures to said separate positive test lots, and validating by further testing of the respective remediated separate test lots and obtaining a negative test result.	ICMSF, alone or in combination with Gombas, discloses Claim 1, upon which this claim relies. See above re Claim 1.  Gombas, 563 (Figure 1 (annotated) showing testing procedures for further testing). A POSITA understands the purpose of testing for pathogens and other organisms in food is to determine if a food is safe for entry into commerce to be sold for

	consumption. One having ordinary skill in the art understands that if a sample tests positive, a remediation step, like cooking, needs to be completed before it can be sold into commerce to comply with USDA guidelines.
Claim 14	**Disclosure of ICMSF and Gombas**
The method of claim 1, wherein, in a), separately collecting said multiple independent samples from each of the multiple separate test lots comprises separately collecting from each separate test lot a number of samples from 5 to 100.	ICMSF, alone or in combination with Gombas, discloses Claim 1, upon which this claim relies. See above re Claim 1. ICMSF teaches collecting 5, 30, 60, 95, or 100 portions. ICMSF, 184-90, 276, 282, 309, 320, 326-30. Gombas also teaches collecting 25, 50, or 100 samples. "After culling unacceptable samples from luncheon meats and deli salads, 100 samples were selected for testing. For the other product categories, 50 samples were selected for the first several weeks of sampling, and 25 samples were selected each week thereafter for testing." Gombas, 563. *See also* MPEP § 2144.05.
Claim 15	**Disclosure of ICMSF and Gombas**
The method of claim 1, wherein, in a), separately collecting said multiple independent samples from each of the multiple separate test lots comprises separately collecting 30 to 60 samples from each separate test lot.	ICMSF, alone or in combination with Gombas, discloses Claim 1, upon which this claim relies. See above re Claim 1. ICMSF teaches collecting 5, 30, 60, 95, or 100 portions. ICMSF, 184-90, 276, 282, 309, 320, 326-30. Gombas also teaches collecting 25, 50, or 100 samples. "After culling unacceptable samples from luncheon meats and deli salads, 100 samples were selected for testing. For the other product categories, 50 samples were selected for the first several weeks of sampling, and 25 samples were selected each week thereafter for testing." Gombas, 563. *See also* MPEP § 2144.05.
Claim 16	**Disclosure of ICMSF and Gombas**

The method of claim 1, wherein, in a), separately collecting said multiple independent samples from each of the multiple separate test lots comprises separately collecting at least 30 samples from each separate test lot.	ICMSF, alone or in combination with Gombas, discloses Claim 1, upon which this claim relies. See above re Claim 1. ICMSF teaches collecting 5, 30, 60, 95, or 100 portions. ICMSF, 184-90, 276, 282, 309, 320, 326-30. Gombas also teaches collecting 30 samples. "In a week when < 100 or <25 samples were purchased (owing to product shortages in stores), all acceptable samples were tested." Gombas, 563. *See also* MPEP § 2144.05.
Claim 18	**Disclosure of ICMSF and Gombas**
The method of claim 1, wherein removing portions in step d) is removing equal portions, aseptically, so as to preclude contamination of the enriched separate composited test lot samples.	ICMSF, alone or in combination with Gombas, discloses Claim 1, upon which this claim relies. See above re Claim 1. ICMSF describes aseptically withdrawing and weighing sample units into the appropriate diluent. ICMSF, 231, 232. Gombas indicates "a sample was aseptically transferred to a sterile stomacher bag and blended with an equal amount of enrichment broth." Gombas, 563. Gombas further indicates "five 1-ml samples of secondary enrichment broths were composited." *Id.*, 564. Moreover, a POSITA would consider this to be standard practice. A POSITA would have aseptically removed equal volume portions of each enriched separate test lot sample.
Claim 19	**Disclosure of ICMSF and Gombas**
The method of claim 1, wherein the target microbe(s) comprises at least one selected from the group consisting of *Listeria*, *Salmonella* and *Escherichia coli*.	ICMSF, alone or in combination with Gombas, discloses Claim 1, upon which this claim relies. See above re Claim 1. ICMSF describes testing for *Listeria* (ICMSF, 285-312), *Salmonella* (*id.*, 273-84), and *Escherichia coli* (*id.*, 313-32).

	Gombas focuses on *Listeria*. Gombas, 559.
Claim 20	**Disclosure of ICMSF and Gombas**
The method of claim 1, wherein the target microbe(s) comprises at least one selected from the group consisting of *Listeria* spp., *Listeria monocytogenes*, *Salmonella* spp., *Escherichia coli* O157:H7, coliforms and fecal coliforms.	ICMSF, alone or in combination with Gombas, discloses Claim 1, upon which this claim relies. See above re Claim 1. ICMSF describes testing for *Listeria* (ICMSF, 285-312), *Salmonella* (*id.*, 273-84), and *Escherichia coli* (*id.*, 313-32). Gombas focuses on *Listeria*. Gombas, 559.
Claim 21	**Disclosure of ICMSF and Gombas**
The method of claim 1, wherein the target microbe(s) comprises a spoilage organism.	ICMSF, alone or in combination with Gombas, discloses Claim 1, upon which this claim relies. See above re Claim 1. A POSITA would find it obvious to apply the teachings of ICMSF and Gombas to target spoilage organisms. "Examination of every piece of whole meat is clearly impractical, hence any sampling procedure for estimating shelf-life would involve taking enough samples to give some statistical assurance of the keeping qualities of meat." Gill, 449. Borch is titled "Bacterial spoilage of meat and cured meat products." Borch. The reference discusses spoilage organisms in meat:

	Lactic acid bacteria are the major bacterial group associated with the spoilage of refrigerated vacuum- or MA-packaged cooked, cured meat products (Blickstad and Molin, 1983b; Shaw and Harding, 1989; von Holy et al., 1991). At the time of spoilage some products contain a 'pure' culture of only one species, while in others a mixture of *Lactobacillus* spp. and *Leuconostoc* spp. was found (Kant-Muermans, unpublished results). The great diversity of bacteria isolated from spoiled meat products is confirmed by several studies (Korkeala and Mäkelä, 1989; von Holy et al., 1991; Yang and Ray, 1994). The genus/species of lactic acid bacteria responsible for spoilage, depend on the product composition (product-related flora) as well as the manufacturing site (house-related flora; Korkeala and Mäkelä, 1989). Borch, 113. "Lactic acid bacteria spoil refrigerated meat products by causing defects such as sour off-flavours, discolouration, gas production, slime production and decrease in PH." Borch, 114; *see also, id.*, 115-17 (Section 4 Analysis of Spoilage). It is understood in the art that aerobic plate counts ("APC") can test for spoilage organisms. *See. e.g.*, ICMSF 1986, 122 ("tests to be made (e.g., APC, *Salmonella*, Clostridium perfringens"); *id.* at 132 ("However, aerobic plate counts (APC) can be used to monitor Good Commercial Practice (GCP), and criteria based on such examinations are a valuable aid to in-plant quality control.").

TABLE 19

Sampling plans and recommended microbiological limits for raw meat[a]

Product[b]	Test	Method reference[c]	Case	Plan class	n	c	Limit per cm² or gram m	M
Carcass meat, before chilling	APC	118	1	3	5	3	10^5	10^6
Carcass meat, chilled	APC	118	1	3	5	3	10^6	10^7
Edible offal, chilled	APC	118	1	3	5	3	10^6	10^7
Carcass meat, frozen	APC	118	1	3	5	3	5×10^5	10^7
Boneless meat, frozen (beef, veal, pork, mutton)	APC	118	1	3	5	3	5×10^5	10^7
Comminuted meat, frozen	APC	118	1	3	5	3	10^6	10^7
Edible offal, frozen	APC	118	1	3	5	3	5×10^5	10^7

a **Not for use at port of entry** but for in-plant quality control. Refer to Section B for appropriate applications.
b Unfrozen carcasses and primal cuts, swab counts per cm²; other meats and meat products, per gram.
c Refers to page number in ICMSF, 1978.

ICMSF 1986, 133 (Table 19 (annotated)).

Although for “quality control,” a POSITA would understand that "quality control" means testing for spoilage organisms could also be accomplished by this well-known method. It is disclosed within the prior art during the production lifecycle of beef production. ICMSF 1986, 134 (“The FAOIWHO Working Group (FAQ/WHO, 1979) considered that the only point at which APC could be used for chilled meat, to evaluate the hygienic conditions under which it was produced, was at the slaughterhouse, and because of its perishable nature it would not be possible to set

	APC limits for use in guidelines or specifications for chilled meats at port of entry."); *see also, id.*, 153 ("Monitoring for APC, E. coli, and Staph. aureus would give some indication of inadequate processing (heating, curing, smoking), postprocess contamination, or storage at temperatures above those recommended for frozen or refrigerated distribution and storage.") E. TEST PROCEDURES (a) *Aerobic plate counts* Comminute, blend, or stomach the various portions of meat composing the sample unit and withdraw the analytical unit. Combine the swabs to form an analytical unit, or treat each separately. Prepare dilutions and perform the APC test according to the directions given in ICMSF, 1978, p. 118. Spread plates are preferable to pour plates when examining raw meats. The temperature used for incubation of plates should be related to the material being examined. To estimate numbers of mesophilic contaminants on freshly dressed carcasses it is advisable to incubate plates at 35 or 37°C. For meat that has been held under chilled conditions, plates should be incubated at 25°C to include psychrotrophs and thus give a better indication of the microbial growth that has occurred. Counts at 20°C give similar results after a longer incubation time. ICMSF 1986, 136 (annotated). *See also*, Bhunia 4 ("Bacteria-specific generic rapid methods such ATP-based luminescence and total plate count (APC) are also needed for monitoring cleaning and hygienic practices employed in a processing plant."); Wheeler, 1606 (use of APC in a beef-processing plant); PR/HAACP, 38849 ("Various alternative indicator

	organisms were suggested, including generic E. coli (biotype I), total plate counts, Enterobacteriaceae, Total Viable Counts (TVC), and Aerobic Plate Counts (APC).”).
Claim 22	**Disclosure of ICMSF and Gombas**
The method of claim 1, wherein removing portions of each separate enriched composited test lot sample comprises removing equal volume portions of each separate enriched composited test lot sample.	ICMSF, alone or in combination with Gombas, discloses Claim 1, upon which this claim relies. See above re Claim 1. ICMSF describes aseptically withdrawing and weighing sample units into the appropriate diluent. ICMSF, 231, 232. Gombas indicates “a sample was aseptically transferred to a sterile stomacher bag and blended with an equal amount of enrichment broth.” Gombas, 563. Gombas further indicates “five 1-ml samples of secondary enrichment broths were composited.” *Id.*, 564. Moreover, a POSITA would consider this to be standard practice. A POSITA would have aseptically removed equal volume portions of each enriched separate test lot sample.
Claim 23	**Disclosure of ICMSF and Gombas**
The method of claim 1, wherein testing of the pooled modular composite sample, using a suitable detection assay comprises use of at least one of a DNA-based method, an immunochemistry-based method, and a biosensor-based method.	ICMSF, alone or in combination with Gombas, discloses Claim 1, upon which this claim relies. See above re Claim 1. Gombas discloses use of DNA-based assays. Gombas, 559 (abstract).
Claim 24	**Disclosure of ICMSF and Gombas**
The method of claim 1, wherein each of the multiple separate test lots are production lots of a meat or poultry product, comprising an	ICMSF, alone or in combination with Gombas, discloses Claim 1, upon which this claim relies. See above re Claim 1.

assemblage of one or more meat or poultry specimens, respectively.	ICMSF indicates "[t]rimmings to be used for ground beef could be pretested for E. coli O157:H7 by various sampling plans." ICMSF, 323. ICMSF indicates "[r]esearch has validated that up to fifteen 25 g analytical units of ground beef or trimmings can be composited without significant loss in E. coli O157:H7 detection sensitivity." *Id.*, 327 *see also id.*, 226, 314, 320, and 324. ICMSF includes many other references to testing lots of beef or poultry, two of the most common types of meat in commerce. *Id.* at 114 (§ 6.3), 184–191 (§§ 10.2–10.6), 225–226 (§ 12.1), 285 (§ 16.1), 313–330 (Chapter 17). ICMSF is relevant to any kind of food product including beef or poultry. Gombas discloses testing of "luncheon meats." " For example, for the sampling of luncheon meats in California, collectors were instructed to purchase 39 samples of ham, 27 samples of bologna, and 24 samples of poultry from stores on list A and 13 samples of ham, 9 samples of bologna, and 8 samples of poultry from stores on list B. Ham could include products made from pork or poultry (such as turkey) and could include regular low-salt or low-sodium, low-fat, extra-lean, and fat-free varieties. Bologna could include products made from pork, beef, turkey, or mixtures of these meats and could include regular low-salt or low-sodium, low-fat, and fat-free varieties. Turkey or chicken could be smoked or not smoked." Gombas, 562.
Claim 29	**Disclosure of ICMSF and Gombas**
The method of claim 3, wherein product specimens are synonymous with product portions.	ICMSF, alone or in combination with Gombas, discloses Claim 3, upon which this claim relies. See above re Claim 3. ICMSF teaches collecting portions or samples, which were known synonyms for product portions or specimens. ICMSF states that "[a] sample unit is a small portion of the lot." ICMSF, 225.

	12.1 INTRODUCTION Many different types of samples are collected and submitted to the laboratory for analysis. Some are sample units from lots or consignments of foods or ingredients for lot acceptance determination. Others may be for investigational purposes to assess control of the environment, investigate the source of a problem, or validate a process. Some may have legal implications relative to a lawsuit or for regulatory compliance. This chapter briefly discusses some of the major factors that should be considered when collecting sample units, shipping them to a laboratory, preparing them for analysis, and analyzing them. A sample unit is a small portion of the lot in which the distribution of both numbers and types of microorganisms should be representative of the whole lot. This requirement is only completely fulfilled when the food and processing conditions result in a homogeneous distribution of microorganisms. This only occurs in well-agitated fluid products. In general, the distribution of microorganisms is presumed to be random. ICMSF at 225 annotated).
Claim 30	**Disclosure of ICMSF and Gombas**
The method of claim 5, wherein the enrichment medium comprises a selective agent that will inhibit a competing non-target microbe.	ICMSF, alone or in combination with Gombas, discloses Claim 5, upon which this claim relies. See above re Claim 5. Additionally, Claim 28 recites what a POSITA would consider the well-known property of selective enrichment, inhibition of a competing non-target microorganism. D'Aoust describes "selective enrichment [that] inhibits growth of non-salmonellae and facilitates isolation of Salmonella on differential plating media." D'Aoust, 369. A POSITA would have been motivated to use an enrichment medium comprising a selective agent that inhibits a non-target agent or organisms as described by D'Aoust, for the test lot samples described by ICMSF and Gombas, to ensure an accurate test. A POSITA would understand that incubating samples at different temperature ranges for different target microorganisms would, effectively, inhibit the growth of other organisms. "Incubation temperature markedly affects productivity of selective

	enrichment broths by suppressing growth of competing microorganisms but not *Salmonella*." D'Aoust, 372. "This may be achieved by utilization of enrichment media that through various mechanisms may favor and enhance growth and multiplication of salmonellae while other gram-negative organisms such as coliforms, Proteus, and Pseudomonas are suppressed or inhibited." Fagerberg, 630.
Claim 31	**Disclosure of ICMSF and Gombas**
The method of claim 6, wherein the concentration step comprises at least one of immunomagnetic bead separation, and affinity chromatography.	ICMSF, alone or in combination with Gombas, discloses Claim 6, upon which this claim relies. See above re Claim 6. ICMSF discloses the use of "immunomagnetic beads to concentrate cells." ICMSF, 318. A POSITA would consider this a well-known technique before the priority date. Fratamico discusses the rapid isolation of target microorganisms using an immunomagnetic separation method. Fratamico, 105. Fratamico discloses that use of immunomagnetic beads resulted in "an increase in the level of E. coli B1409 in the sample," as well as "an increase in the number of organisms recovered." *Id.*, 107. Additionally, Fratamico provides detailed instructions for preparing and using immunomagnetic bead separation to concentrate a target microbial agent or organism. Fratamico developed "[a] method employing immunomagnetic beads" to concentrate and thereby improve the isolation and detection of E. coli. *Id.* For example, Fratamico was able to use immunomagnetic beads to isolate *E. coli* strains from enrichment cultures of raw ground beef "when the initial inoculum level was 1 cfu g-1." *Id.*, 109. Fratamico further teaches that "[t]he percentage of bacteria recovered increased with increasing amounts of beads." *Id.*, 108. Consequently, Fratamico "demonstrates that immunomagnetic separation [using immunomagnetic beads] is a simple and rapid technique" for concentrating a microbe for isolation and detection. *Id.*, 109.

	These teachings of Fratamico would have motivated a POSITA to modify the combination of ICMSF and Gombas and ICMSF and Hughes to use immunomagnetic beads to concentrate microbes in a modular composite sample to benefit from accurate tests. Use of immunomagnetic beads was already known in the art for concentrating during testing beef samples. "Tu et al. (78) detected pathogenic E. coli O157:H7 in beef hamburger with the use of IMS and ATP measurement. After 6 h of enrichment at 37°C, inoculated beef samples (0.9 ml each) were filtered to remove large particles prior to 10 min of incubation with ca. 10^5 magnetic beads coated with anti–*E. coli* O157:H7 (Dynabeads, Dynal Inc., Oslo, Norway). Following incubation, the magnetic beads were collected with a Dynal magnetic concentrator and resuspended in 0.9 ml of Trypticase soy broth for ATP analysis." Beniot, 1941. "Hudson et al. (26) used IMS to directly separate and concentrate L. monocytogenes from ham samples prior to detection by PCR. IMS was used on the basis of the commercial availability of anti-Listeria magnetic beads (Dynal Inc.) to avoid time-consuming cultural enrichment steps and to remove PCR inhibitors." Beniot, 1941-42. Cudjoe investigates the use of immunomagnetic beads in pooling methods. In a study designed to evaluate the performance of Dynabeads® Anti-Salmonella, immunomagnetic separation (IMS) followed by plating (IMS-Plating) proved far superior to the conventional ISO *Salmonella* methodology and the Modified Semi-solid Rappaport-Vassiliadis (MSRV) method. *Salmonella* species were isolated and detected from 135 out of the 180 diverse poultry samples by IMS analysis as against 98 by the conventional method and 33 by the MSRV technique. All results were confirmed biochemically and serologically. It appeared from the data generated that some of the salmonellae isolated using IMS were greatly inhibited in Rappaport-Vassiliadis enrichment broth at 42°C and to a lesser extent in selenite cystine broth at 37°C. A moderately selective plating medium like XLD proved to be better in isolating these possible sensitive wild-type strains of salmonellae than the more selective BGA.

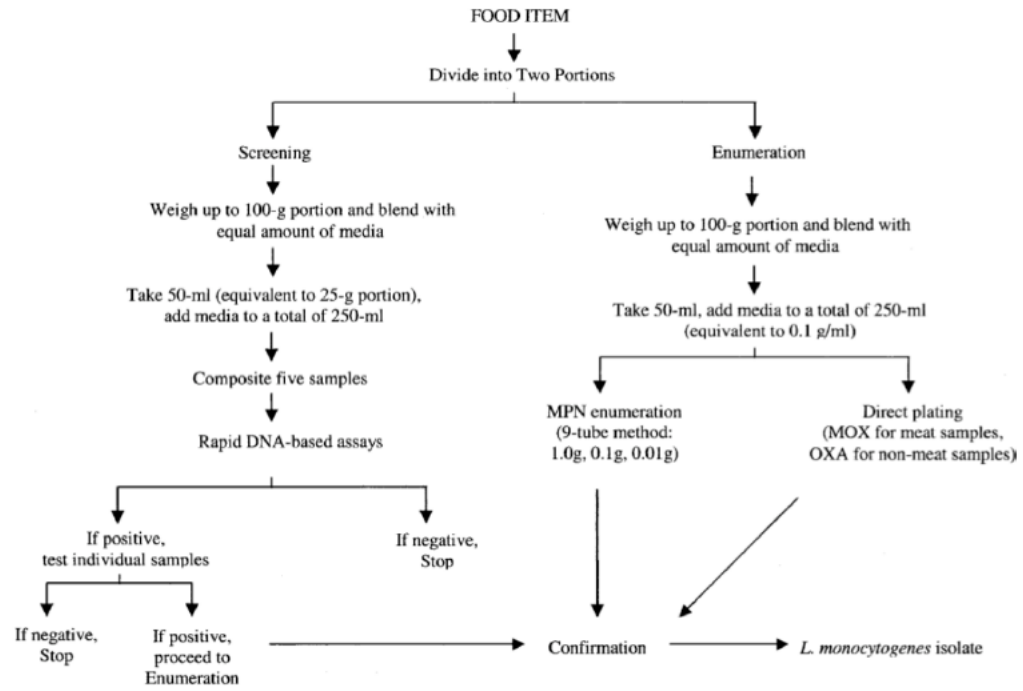
	Cudjoe, Abstract. *Samples*. One hundred and eighty (180) diverse poultry samples, consisting of 40 pools of cloacal/faecal swabs (5 swabs/pool; 5 pools samples/flock) from eight different flocks, 10 raw chicken breast meat samples, 10 pooled egg shell samples, 60 raw chicken liver samples and 60 chicken neck skins sampled from the slaughter line, were used in this study. All samples were obtained from a poultry slaughter and processing plant in the Netherlands. A pool of five swab samples was pre-enriched in 10 ml of buffered peptone water (BPW; Difco Laboratories, Detroit, Michigan, USA) at 37°C for 16–20 h, whilst 25 g of each of the other samples above, were pre-enriched similarly in 225 ml of BPW and examined as detailed below. Cudjoe, 160.
Claim 32	**Disclosure of ICMSF and Gombas**
The method of claim 7, wherein the more sensitive protocol comprises use of a concentration step to concentrate the target microbe(s), in combination with the same test protocol used to test the pooled modular composite sample.	ICMSF, alone or in combination with Gombas, discloses Claim 7, upon which this claim relies. See above re Claim 7. ICMSF discloses the use of "immunomagnetic beads to concentrate cells." ICMSF, 318. A POSITA would consider this a well-known technique before the priority date. Gombas discloses retesting using the same protocol. Gombas discusses the use of "biochemical confirmation" to confirm testing results. Gombas, 565. A POSITA would have recognized the concentration step could be employed to provide a more sensitive protocol for further testing. A POSITA would understand that retesting the non-pooled, singular samples is an inherently more sensitive process because the sample is not diluted with other samples in the testing pool. Therefore, the retesting of a singular sample is more concentrated and therefore, more sensitive. *See also, generally*, Jarvis. Fratamico discusses the rapid isolation of target microorganisms using an immunomagnetic separation method. Fratamico, 105. Fratamico discloses that use of

	immunomagnetic beads resulted in "an increase in the level of E. coli B1409 in the sample," as well as "an increase in the number of organisms recovered." *Id.*, 107. Additionally, Fratamico provides detailed instructions for preparing and using immunomagnetic bead separation to concentrate a target microbial agent or organism. Fratamico developed "[a] method employing immunomagnetic beads" to concentrate and thereby improve the isolation and detection of E. coli. *Id.* For example, Fratamico was able to use immunomagnetic beads to isolate *E. coli* strains from enrichment cultures of raw ground beef "when the initial inoculum level was 1 cfu g-1." *Id.*, 109. Fratamico further teaches that "[t]he percentage of bacteria recovered increased with increasing amounts of beads." *Id.*, 108. Consequently, Fratamico "demonstrates that immunomagnetic separation [using immunomagnetic beads] is a simple and rapid technique" for concentrating a microbe for isolation and detection. *Id.*, 109. These teachings of Fratamico would have motivated a POSITA to modify the combination of ICMSF and Gombas and ICMSF and Hughes to use immunomagnetic beads to concentrate microbes in a modular composite sample to benefit from accurate tests. Use of immunomagnetic beads was already known in the art for concentrating during testing beef samples. "Tu et al. (78) detected pathogenic E. coli O157:H7 in beef hamburger with the use of IMS and ATP measurement. After 6 h of enrichment at 37°C, inoculated beef samples (0.9 ml each) were filtered to remove large particles prior to 10 min of incubation with ca. 10^5 magnetic beads coated with anti–*E. coli* O157:H7 (Dynabeads, Dynal Inc., Oslo, Norway). Following incubation, the magnetic beads were collected with a Dynal magnetic concentrator and resuspended in 0.9 ml of Trypticase soy broth for ATP analysis." Beniot, 1941. "Hudson et al. (26) used IMS to directly separate and concentrate L. monocytogenes from ham samples prior to detection by PCR. IMS was used on the basis of the commercial availability of anti-Listeria magnetic beads (Dynal Inc.) to avoid time-

	consuming cultural enrichment steps and to remove PCR inhibitors." Beniot, 1941-42. Cudjoe investigates the use of immunomagnetic beads in pooling methods. In a study designed to evaluate the performance of Dynabeads® Anti-Salmonella, immunomagnetic separation (IMS) followed by plating (IMS-Plating) proved far superior to the conventional ISO *Salmonella* methodology and the Modified Semi-solid Rappaport-Vassiliadis (MSRV) method. *Salmonella* species were isolated and detected from 135 out of the 180 diverse poultry samples by IMS analysis as against 98 by the conventional method and 33 by the MSRV technique. All results were confirmed biochemically and serologically. It appeared from the data generated that some of the salmonellae isolated using IMS were greatly inhibited in Rappaport-Vassiliadis enrichment broth at 42°C and to a lesser extent in selenite cystine broth at 37°C. A moderately selective plating medium like XLD proved to be better in isolating these possible sensitive wild-type strains of salmonellae than the more selective BGA. Cudjoe, Abstract. *Samples*. One hundred and eighty (180) diverse poultry samples, consisting of 40 pools of cloacal/faecal swabs (5 swabs/pool; 5 pools samples/flock) from eight different flocks, 10 raw chicken breast meat samples, 10 pooled egg shell samples, 60 raw chicken liver samples and 60 chicken neck skins sampled from the slaughter line, were used in this study. All samples were obtained from a poultry slaughter and processing plant in the Netherlands. A pool of five swab samples was pre-enriched in 10 ml of buffered peptone water (BPW; Difco Laboratories, Detroit, Michigan, USA) at 37°C for 16–20 h, whilst 25 g of each of the other samples above, were pre-enriched similarly in 225 ml of BPW and examined as detailed below. Cudjoe, 160.
Claim 33	**Disclosure of ICMSF and Gombas**

The method of claim 24, wherein the meat or poultry specimens are meat trim pieces or poultry trim pieces, respectively.	ICMSF, alone or in combination with Gombas, discloses Claim 24, upon which this claim relies. See above re Claim 24. ICMSF indicates "[t]rimmings to be used for ground beef could be pretested for E. coli O157:H7 by various sampling plans." ICMSF, 323. ICMSF indicates "[r]esearch has validated that up to fifteen 25 g analytical units of ground beef or trimmings can be composited without significant loss in E. coli O157:H7 detection sensitivity." *Id.*, 327 *see also id.*, 226, 314, 320, and 324. ICMSF includes many other references to testing lots of beef or poultry, two of the most common types of meat in commerce. *Id.* at 114 (§ 6.3), 184–191 (§§ 10.2–10.6), 225–226 (§ 12.1), 285 (§ 16.1), 313–330 (Chapter 17). ICMSF is relevant to any kind of food product including beef or poultry.
Claim 34	**Disclosure of ICMSF and Gombas**
The method of claim 24, wherein the meat is that of beef, pork, sheep or bison.	ICMSF, alone or in combination with Gombas, discloses Claim 24, upon which this claim relies. See above re Claim 24. ICMSF indicates "[t]rimmings to be used for ground beef could be pretested for E. coli O157:H7 by various sampling plans." ICMSF, 323. ICMSF indicates "[r]esearch has validated that up to fifteen 25 g analytical units of ground beef or trimmings can be composited without significant loss in E. coli O157:H7 detection sensitivity." *Id.*, 327 *see also id.*, 226, 314, 320, and 324. ICMSF includes many other references to testing lots of beef or poultry, two of the most common types of meat in commerce. *Id.* at 114 (§ 6.3), 184–191 (§§ 10.2–10.6), 225–226 (§ 12.1), 285 (§ 16.1), 313–330 (Chapter 17). ICMSF is relevant to any kind of food product including beef or poultry. Gombas discloses testing of "luncheon meats." " For example, for the sampling of luncheon meats in California, collectors were instructed to purchase 39 samples of ham, 27 samples of bologna, and 24 samples of poultry from stores on list A and 13

	samples of ham, 9 samples of bologna, and 8 samples of poultry from stores on list B. Ham could include products made from pork or poultry (such as turkey) and could include regular low-salt or low-sodium, low-fat, extra-lean, and fat-free varieties. Bologna could include products made from pork, beef, turkey, or mixtures of these meats and could include regular low-salt or low-sodium, low-fat, and fat-free varieties. Turkey or chicken could be smoked or not smoked." Gombas, 562.
Claim 35	
[35pre] A method of sampling, and testing for microbe(s) in multiple separate test lots, comprising:	To the extent the preamble is limiting, as discussed herein ICMSF, alone or in combination with Gombas, discloses A method of sampling, and testing for microbe(s) in multiple separate test lots. "Many different types of samples are collected and submitted to the laboratory for analysis. Some are sample units from lots or consignments of food or ingredients for lot acceptance determination. . . . A sample unit is a small portion of the lot in which the distribution of both numbers and types of microorganisms should be representative of the whole lot." ICMSF, 255.

	 FIGURE 1. *Detection and enumeration of* L. monocytogenes *in RTE food samples.* Gombas, Figure 1.
[35a] a) separately collecting multiple independent samples from each of multiple separate single-unit production lots, wherein each separate single-unit production lot is separately sampled by taking said multiple independent samples thereof;	"Many different types of samples are collected and submitted to the laboratory for analysis. Some are sample units from lots or consignments of food or ingredients for lot acceptance determination. . . . A sample unit is a small portion of the lot in which the distribution of both numbers and types of microorganisms should be representative of the whole lot." ICMSF, 255. The lots may be beef or poultry. *Id.*, 184-91, 226, 230. Thus, ICMSF discloses collecting a plurality of samples or sample units (other

	synonyms include portions, specimens, and sub-samples) from lots of beef or poultry. ICMSF describes collecting from a lot or lots: "If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." *Id.*, 119. Additionally, ICMSF indicates a consignment may be "a very large quantity of food" and may be "composed of several lots, representing for example, different days of production from the same plant." *Id.*, 119, 120; *see also* Applicant Admitted Prior Art ("AAPA") #3-#5.

6.11 WHAT IS A REPRESENTATIVE SAMPLE?

A *representative sample* reflects, as far as is possible, the composition of the lot from which it is drawn.

How then should a representative sample be drawn? It is important to avoid bias and to draw a sufficient number of sample units to confidently make a judgment about a lot. Sampling at random is the universally recognized way of avoiding bias and is more reliable than consciously trying to draw the sample units from the various parts of a lot. The units (cartons or containers, particular weights of solid, or volumes of liquid) are drawn by using random numbers. There is, of course, no guarantee that a random sample has characteristics identical with those of the lot, but the randomness of sampling is the basis for calculation of the probability that a sample will give a biased result.

It is also possible, and may be desirable, to use a *stratified random sampling* approach, drawing a random sample of a given number of sample units from each *stratum* (e.g., each sublot or carton). The distribution of sample units on strata should correspond to the distribution of lot units on strata. This means that if each stratum contains the same quantity as every other one, the number of sample units per stratum should be the same. Otherwise, they should differ just as the proportions of the lot contained in the strata differ. Stratification is a device for handling known sources of variation and may be used where one has prior knowledge that the consignment is potentially not of uniform quality. A consignment might not be of uniform quality if portions of it are shipped in different vehicles or in different holds of a vessel, or if it is known that it is really composed of several lots representing, for example, different days of production from the same plant or different plants of the same company. The results for different strata should be assessed separately and then pooled if they appear to be homogeneous.

The calculation of acceptance probabilities is based on the assumption that random samples have been drawn and analyzed. Where physical or practical constraints prevent random sampling, such calculated probabilities may not be valid. Their reliability depends on how close reality approaches the ideal situation of random sampling. This fact should be borne in mind when subsequently interpreting results.

ICMSF, 119-20 (annotated).

6.10 WHAT IS A LOT?

Ideally, a lot is a quantity of food or food units produced and handled under uniform conditions. Under certain circumstances, it has been shown that the logarithms of the counts from a batch of food are likely to be normally distributed (Baird-Parker, 1980; Kilsby & Baird-Parker, 1983 [their Table 2]; Ingram & Roberts, 1976). The implication is that there is homogeneity within a lot, which, when considering microbial levels and distributions, rarely occurs in practice. In most instances, the distribution of microorganisms within lots of food is heterogeneous. Even in food factories, this heterogeneity makes the interpretation of sample results difficult, and it becomes even more so in circumstances where a lot is less well defined, such as in a consignment of a very large quantity of food.

It is therefore helpful if suppliers can be persuaded to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load).

If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment.

A lot should be composed of food produced with as little variation as possible for a given process or commodity. Because of the uncertainties in identifying a lot commercially, however, the use of the word *lot* in this book is usually in its statistical sense, as a collection of units of a product, the acceptability of which is determined by examining a sample drawn from it.

ICMSF, 118-19 (annotated).

	The ICMSF has defined a lot as follows: In the commercial sense, a lot is a quantity of food supposedly produced under identical conditions, all packages of which would normally bear a lot number that identifies the production during a particular time interval, and usually from a particular line, retort, or other critical processing unit. Statistically, a lot is considered as a collection of units of a product from which a sample is drawn to determine acceptability of the lot (ICMSF, 1986). In this example, the maximum size of a lot consists of all the ground beef produced on common equipment from cleanup to cleanup. Smaller lots could be established (e.g., hourly). ICMSF, 327 (annotated). This definition also reflects the common practice referred to in the IEH Patents at Example II, wherein "[p]referably, the producer defines and produces these single-unit lots in such a fashion as to maximize the information available." '143 Patent,12:52–54. This corresponds with the specification of IEH's Patents which reflects the common practice of producers defining what constitutes a lot grouped for traceability purposes. '143 Patent, 3:5-15, 11:18-22 (AAPA #3), and 12:52-55. Thus, ICMSF discloses collecting a plurality of portions from separate lots. ICMSF describes a lot as "a collection of units of a product." IMCSF, 119. ICMSF further indicates a lot has been defined as: "a quantity of food supposedly produced under identical conditions, all packages of which would normally bear a lot number that identifies the production during a particular time interval, and usually from a particular line, retort, or other critical processing unit. Statistically, a lot is considered as a collection of units of a product from which a sample is drawn to determine acceptability of the lot." ICMSF, 327. ICMSF advocates use of a "statistically based sampling plan." *Id.*, 113-15, 119-21, 320, 327, 328. In the sampling plans, "*n* is the number of sample units chosen separately and independently, and . . . *c* is the maximum allowable number of sample

	units yielding a positive result." *Id.*, 115. ICMSF discloses a wide range for the number of sample units (portions) for a particular microbial agent or organism. For example, ICMSF discusses collecting 15, 30, 60 or 100 sample units from a lot. *Id.*, 320, 326. **6.5 THE SAMPLING PLAN** The sample units so drawn will, after examination, yield results that will be compared with certain criteria to reach a decision as to whether the entire lot should be accepted or rejected (see section 6.9 for explanation of rejection). The particular choice of sampling procedure and the decision criteria make up the *sampling plan*. Proper drawing of samples is essential if the decision making criteria of the sampling plan are to give unbiased results. By sampling randomly, we are able to reduce the risk of making biased decisions. A simple example of a sampling plan follows. Take 10 sample units of a food from a lot and subject them to appropriate laboratory procedure to test for the presence or absence of a microorganism. If two or fewer of the 10 sample units show presence of the organism, that is, give a positive result, then the whole lot of food is acceptable (relative to this organism). But if three or more give a positive result, the whole lot is to be rejected. This plan is described by the use of two terms $n = 10$ and $c = 2$, where n is the number of sample units chosen separately and independently, and where c is the maximum allowable number of sample units yielding a positive result. ICMSF, 115 (annotated).

	6.1 INTRODUCTION It is important to recognize that the management of food safety using the approaches outlined in the first five chapters, based on controlling hazards through Good Hygienic Practices (GHP) and Hazard Analysis Critical Control Point (HACCP), is much more effective than trying to ensure safety by lot acceptance through end-product testing. This chapter discusses the concepts of probability and sampling that determine the limitations of end-product testing. Those concepts form the basis of the design of statistically based sampling plans (Chapter 7) and the establishment of cases (Chapter 8). In Chapters 6 and 7, it is assumed that the microbiological test will give an absolutely accurate result, with no false positives or false negatives. This is not a realistic assumption, and problems arising from sampling, sample transport and preparation for analysis, and the imprecision of microbiological methods are addressed in Chapters 10 and 12 and elsewhere in the text. ICMSF, 113 (annotated); *see also id.* at 115–21 (§§ 6.4–6.13), 319–20 (§ 17.2.3.3), and 326–30 (§§ 17.5.3.1–17.6). ICMSF discusses the number of portions to be taken sufficient for validation of a particular microorganism including *Salmonella* (*id.* at 276 (§ 15.3.3); *see also id.* at 282 (§ 15.6.3)); *Listeria* (*id.* at 309 (§ 16.6.3)), and *E. coli* (*id.* at 318–21 (§§ 17.2.3.2–17.2.3.4), 326 (§ 17.5.3.1)). IEH's AAPA discloses statistical-based sampling plans taking 10-60 sample units. '143 Patent, 6:3-20; *see* AAPA #1, #2, #5, and #6. Determining meat is safe for consumption so it is "commercially possible to market" the meat, is the well-understood point of testing meat and is taught by ICMSF. ICMSF, 185-89. Moreover, the recitation of a lot being validated for "release . . . into commerce" is not limiting. Prosecution History for Reexamination Control No. 95/011,294. "Operationally-linked" is simply grouping like items, which is also disclosed by ICMSF. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole

	consignment." ICMSF, 119. It is therefore helpful if suppliers can be persuaded to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load). If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment. ICMSF, 119 (annotated).
[35b] b) separately compositing the collected multiple independent samples from each of the separate test lots to provide a corresponding set of separate composited single-unit production lot samples, wherein each of the separate composited single-unit production lot samples is attributed to a particular corresponding separate single-unit production lot;	ICMSF states: "To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units of the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. . . . This finding suggests that considerable reductions may be made in the cost of laboratory analysis by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, n, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort." ICMSF, 187.

	To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. A laboratory investigation undertaken for ICMSF (Silliker & Gabis, 1973) indicated that different sized analytical units drawn from a single well-mixed sample unit of dried food give very similar results. A subsequent investigation (Gabis & Silliker, 1974) using foods of high moisture content (including eggs, poultry meat, meat, and meat products) showed comparable reliability in the use of composite analytical units; for example, examination of three composites of 20 × 25 g analytical units gave the same result as examination of all 60 analytical units individually (the total amount examined being 1500 g in both instances). This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n*, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort. ICMSF, 187.

10.5.1 Conceivable Alternative Plans

As indicated above, for an infectious agent such as *Salmonella*, there are strong reasons for adopting sampling plans in which $c = 0$:

1. It is philosophically objectionable to some people to accept a lot containing a recognized pathogen, regardless of how the food is to be used in a manufacturing process or by the consumer.
2. There is the very great technical advantage of combining many analytical units for bacteriological examination in systems with $c = 0$, because a single positive decides the outcome.
3. The plan with $c = 1$ (or more) would always require the examination of (many) more analytical units, for equal probability of acceptance, than a plan with $c = 0$.
4. Even if the indicated probabilities of plans with $c = 0$ versus $c = 1$ are the same, their implications are not. For example, when a lot is accepted with the plan $n = 60$, $c = 0$, it is possible (though not certain) that the lot may not be contaminated at all. But using the plan $n = 95$, $c = 1$ and accepting one positive, it is known that the lot certainly is contaminated (though the probability that a lot with 5% of analytical units contaminated would be accepted is in both instances $P_a = 0.05$).

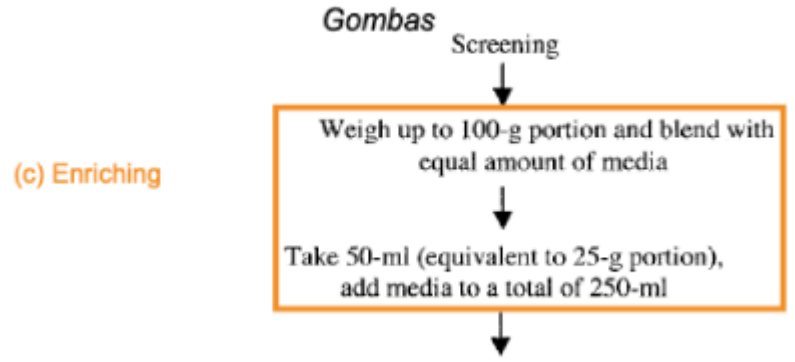
ICMSF, 188 (annotated). ICMSF also provides an example of "[u]sing a sampling plan involving n = 15 (25 g analytical units) and a composite of 375 g." *Id.* at 195 (§ 10.7.2). ICMSF also states "[f]or qualitative analyses, 25 g analytical units are frequently analyzed individually or as composites of 100 g, 200 g, or more." *Id.* at 232 (§ 12.5.2).

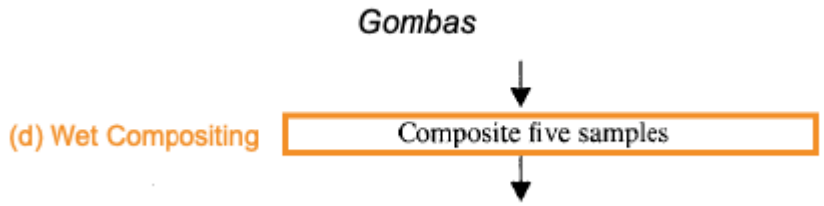
The ICMSF incorporates two references, Silliker & Gabis (1973) and Gabis & Silliker (1974). Silliker notes that "[c]ompositing of multiple samples (lumping) and wet compositing of pre-enrichment cultures have been tested as economical approaches to *Salmonella* control." Silliker, 475.

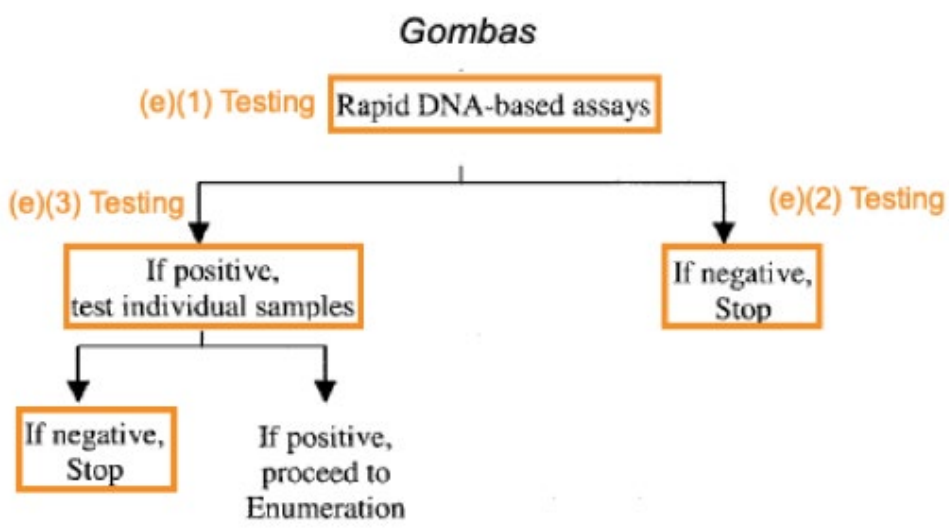
Silliker further notes that "[b]oth approaches are being used widely by the food industry in the United States." *Id.*.

	To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. A laboratory investigation undertaken for ICMSF (Silliker & Gabis, 1973) indicated that different sized analytical units drawn from a single well-mixed sample unit of dried food give very similar results. A subsequent investigation (Gabis & Silliker, 1974) using foods of high moisture content (including eggs, poultry meat, meat, and meat products) showed comparable reliability in the use of composite analytical units; for example, examination of three composites of 20 × 25 g analytical units gave the same result as examination of all 60 analytical units individually (the total amount examined being 1500 g in both instances). This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n*, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort. "There is the very great technical advantage of combining many analytical units for bacteriological examination." ICMSF, 188. ICMSF also describes composites prepared by combining "15 (25 g analytical units)" and "composites of 100 g, 200 g, or more." *Id.,* 195, 232; *see* AAPA #5. ICMSF states, "All steps up to the analysis must be controlled . . . to allow reliable traceability." ICMSF, 227. ICMSF indicates lot samples are traceable to a particular corresponding lot: "It is therefore helpful . . . to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load). . . . Treating the individual production

	batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119. Gombas also discloses this element. Gombas states "samples were assigned codes" ad product information was recorded for the luncheon meats including "sampling location." Gombas, 563. A POSITA would find Gombas' system for testing individual samples, which composited samples test positive, inherently requires tracking the individual samples that contributed to the composite. *See, e.g.*, Gombas, 564 ("If a composite tested positive, samples in the composite were tested individually by Gene-Trak assay with the PBS from the second mLCA plate or by the BAX assay with the secondary enrichment broths. Individual samples that tested negative were not tested further If an individual sample tested positive, the retained portion was subjected to an enumeration assay.") Thus, Gombas also discloses that each of the separate composited lot samples is attributed to a particular corresponding separate lot.
[35c] c) enriching of the separate composited single-unit production lot samples to provide a set of separate composited single-unit production lot samples enriched for the target microbe(s)	ICMSF states: To reduce laboratory effort . . . it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. ICMSF, 187. Also, ICMSF describes preparing composites of analytical units then diluting "for qualitative (enrichment)" analysis. *Id.,* 231-32; *see also* AAPA #5, #7. For practical reasons, however, analytical units of 10 g up to 50 g are typical for quantitative methods and are diluted 1:10. For qualitative analyses, 25 g analytical units are frequently analyzed individually or as composites of 100 g, 200 g, or more. ICMSF at 232 (annotated).

	Preparation of the first dilution, either for qualitative (enrichment) or quantitative (direct) analyses, can have a marked impact on the microbial population in the sample. This is often due to changes in the physico-chemical characteristics of the suspension as compared to the food matrix. Where the food does not confer protection, the composition of the diluent is important to prevent or minimize shock/injury to the microorganisms that can result from rapid changes in the ionic concentrations. ICMSF at 232 (annotated). ICMSF also describes strategic use of non-selective enrichment to resuscitate microorganisms and selective enrichments for growing microorganisms. *Id.* at 232–33 (§ 12.6); *see e.g.*, *id.,* 233 ("use of nonselective medium . . . to enhance recovery of injured or stressed cells."). ICMSF at § 12.6 also describes optimizing the incubation period. S*ee e.g.*, *id.,* 232. It was well known long before the priority date to use non-selective enrichments after dry compositing and prior to wet compositing as evidenced by Price, Silliker, Gabis, and MLG. A POSITA would also know to use selective enrichments after dry compositing and prior to wet compositing as disclosed by ICMSF and Gombas.  Gombas, 563 (Figure 1 (annotated) showing an enriching step).

[35d] d) removing portions of each separate enriched composited single-unit production lot sample, and combining the removed portions to provide a pooled modular composite sample; and	ICMSF describes compositing the enriched lot samples: "it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. . . . This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n,* with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort." ICMSF, 187. AAPA #5-#7. Additionally, Gombas discloses wet compositing of enriched samples: "Up to five like-product samples were composited and screened. . . . For California samples, five 1-ml samples of secondary enrichment broths were composited." Gombas, 564. Gombas's Figure 1 depicts wet compositing as shown in the excerpt below:  Gombas, 563 (Figure 1 (annotated) showing a wet compositing step).
[35e.1] e) testing of the pooled modular composite sample, using a suitable detection assay, for the target microbe(s),	ICMSF describes testing enrichment cultures of composite samples with suitable detection assays (ICMSF, 187) and "presence/absence tests" (ICMSF, 233). Suitable detection assays were established in Silliker and Gabis as discussed in ICMSF. Gombas provides a complete framework for testing, and a POSITA would have been motivated to modify ICMSF based on Gombas's framework:

	 Gombas, 563 (Figure 1 (annotated) showing the testing step). Gombas also discloses a suitable detection assay: "For California samples, . . . the 5-ml composite was tested by the BAX assay according to the manufacturer's procedures." Gombas, 564. These assay types were "art recognized." '143 Patent, AAPA #7.
[35e.2] wherein when such testing is negative all of said samples that were composited to form the separate composited single-unit production lot samples are deemed negative for the target microbe(s) and each of the multiple separate single-unit production lots is validated, and	ICMSF describes correlating lots in a consignment with lot samples so a negative or positive test result enables a lot to be validated or rejected. ICMSF, 118-19. ICMSF describes sampling plans in which "c is the maximum allowable number of sample units yielding a positive result." ICMSF, 115. For many scenarios, the requirement is c = 0, which indicates the testing is negative. *Id.*, 118, 183-90, 309, 325, 326. A negative test result (*id.*, 113-15, 325, 326) is the expected and desired outcome to determine meat is not contaminated and is safe for consumption (*id.*, 184-89) such that a lot is validated and it is "commercially possible to market" (*id.*, 185) the meat.

6.10 WHAT IS A LOT?

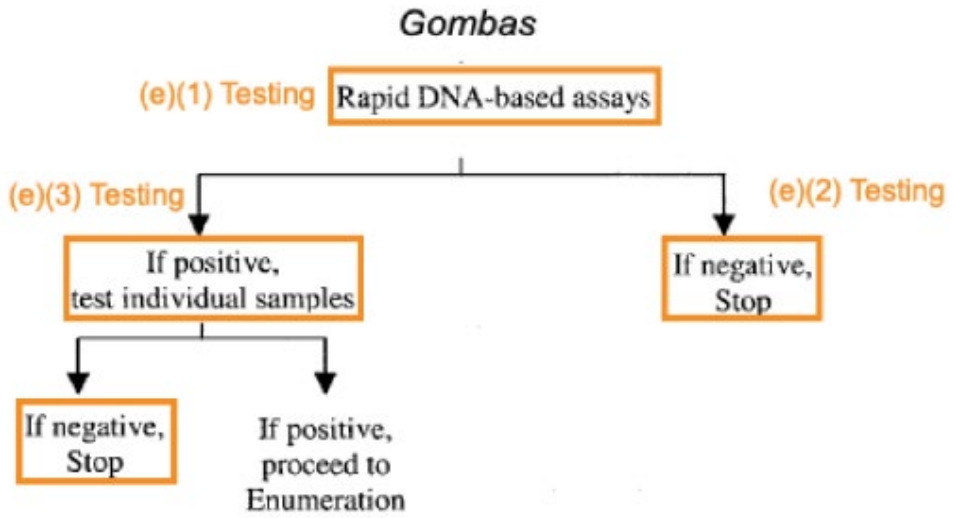
Ideally, a lot is a quantity of food or food units produced and handled under uniform conditions. Under certain circumstances, it has been shown that the logarithms of the counts from a batch of food are likely to be normally distributed (Baird-Parker, 1980; Kilsby & Baird-Parker, 1983 [their Table 2]; Ingram & Roberts, 1976). The implication is that there is homogeneity within a lot, which, when considering microbial levels and distributions, rarely occurs in practice. In most instances, the distribution of microorganisms within lots of food is heterogeneous. Even in food factories, this heterogeneity makes the interpretation of sample results difficult, and it becomes even more so in circumstances where a lot is less well defined, such as in a consignment of a very large quantity of food.

It is therefore helpful if suppliers can be persuaded to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load).

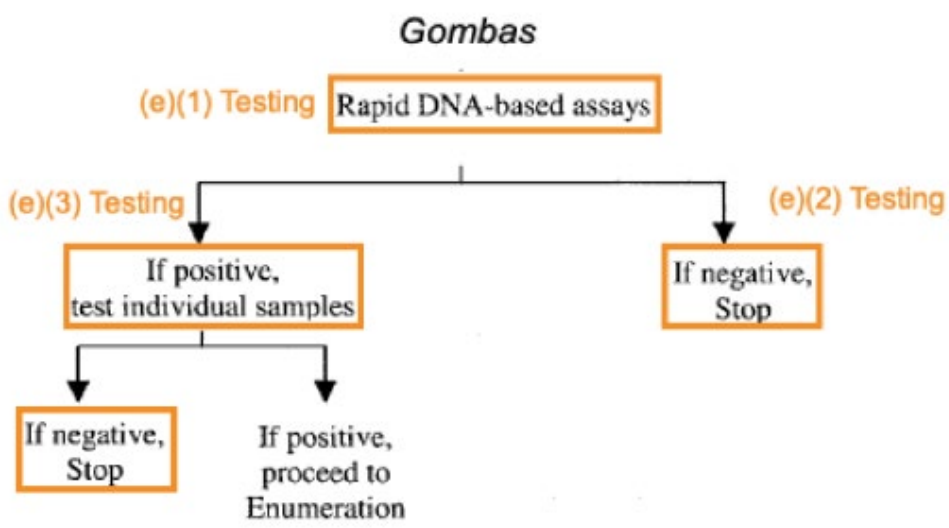
If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment.

A lot should be composed of food produced with as little variation as possible for a given process or commodity. Because of the uncertainties in identifying a lot commercially, however, the use of the word *lot* in this book is usually in its statistical sense, as a collection of units of a product, the acceptability of which is determined by examining a sample drawn from it.

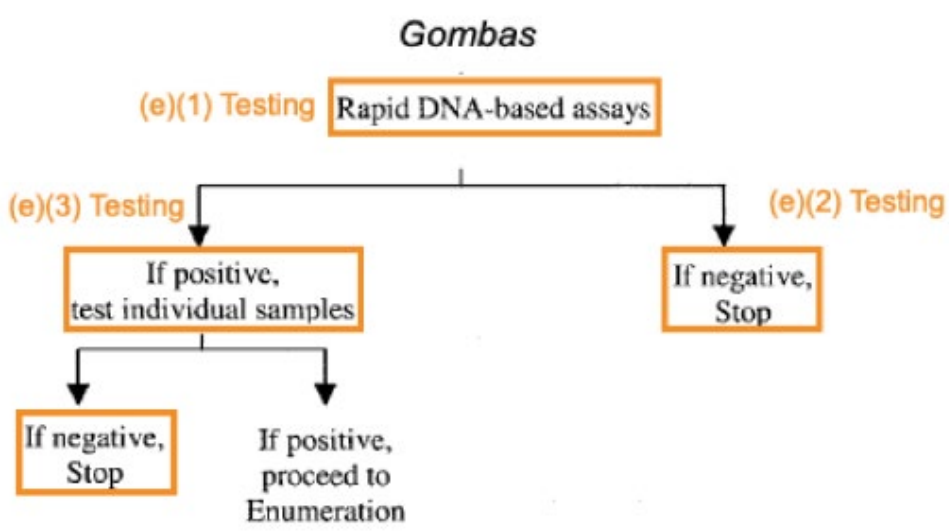
ICMSF, 118-19 (annotated).

	To the extent ICMSF is considered not to disclose some aspect of step (e)(2), Gombas indicates a negative test result is the end of the process: "If a composite tested negative, all samples in the composite were considered negative (in 25 g) and were not tested further." Gombas, 564. A POSITA would find that in the combination, a negative test result equates to being validated.  Gombas, 563 (Figure 1 (annotated) showing "if negative, stop.").
[35e.3] wherein when such testing is positive, each of the individual separate composited single-unit production lot samples that were used to form the pooled modular composite sample are individually tested to determine which of the separate composited single-unit production lot samples is positive for the target microbe(s),	The combination of ICMSF and Gombas describes retaining a portion of the enriched lot samples (steps (b)-(d)). "Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119.

	"There is often a desire to retest retained samples." ICMSF, 191. If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment. ICMSF, 119 (annotated). ICMSF discloses validating for release into commerce. ICMSF, 113–15 (§§ 6.2–6.6), 118–19 (§§ 6.9–6.10), 184–85 (§ 10.2), 325–27 (§§ 17.5.3–17.5.3.4); *see also id.*, 186–89 (§ 10.4–10.6)) according to a statistically-based sampling plan (*id.*, 113 (§ 6.1); *see also id.*, 115–18 (§§ 6.4–6.8), 184 (§ 10.2), 319–21 (§§ 17.2.3.3–17.2.3.4), 326–30 (§§ 17.5.3.1–17.6), as described regarding steps (a) and (e)(2). "If a composite tested positive, samples in the composite were tested individually . . . by the BAX assay with the secondary enrichment broths. Individual samples that tested negative were not tested further." Gombas, 564.

	 Gombas, 563 (Figure 1 (annotated) showing testing procedures for further testing). "Individual samples that tested negative were not tested further. If an individual sample tested positive, the retained portion was subjected to an enumeration assay." Gombas, 564. The combination teaches that after the composited samples test positive, then the individual samples used to form the composited sample are individually tested with the same protocol to determine which of the separate composited lot samples is positive for the target microbe(s).
[35e.4] wherein the single-unit production lots corresponding to any negatively testing composited single-unit production lot samples are validated.	The combination of ICMSF and Gombas describes retaining a portion of the enriched lot samples (steps (b)-(d)). "Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment."

	ICMSF, 119. "There is often a desire to retest retained samples." ICMSF, 191. If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment. ICMSF, 119 (annotated). ICMSF discloses validating for release into commerce. ICMSF, 113–15 (§§ 6.2–6.6), 118–19 (§§ 6.9–6.10), 184–85 (§ 10.2), 325–27 (§§ 17.5.3–17.5.3.4); *see also id.*, 186–89 (§ 10.4–10.6)) according to a statistically-based sampling plan (*id.*, 113 (§ 6.1); *see also id.*, 115–18 (§§ 6.4–6.8), 184 (§ 10.2), 319–21 (§§ 17.2.3.3–17.2.3.4), 326–30 (§§ 17.5.3.1–17.6), as described regarding steps (a) and (e)(2). "If a composite tested positive, samples in the composite were tested individually . . . by the BAX assay with the secondary enrichment broths. Individual samples that tested negative were not tested further." Gombas, 564.

	 Gombas, 563 (Figure 1 (annotated) showing testing procedures for further testing). "Individual samples that tested negative were not tested further. If an individual sample tested positive, the retained portion was subjected to an enumeration assay." Gombas, 564. The combination teaches that after the composited samples test positive, then the individual samples used to form the composited sample are individually tested with the same protocol to determine which of the separate composited lot samples is positive for the target microbe(s).
Claim 36	**Disclosure of ICMSF and Gombas**
The method of claim 35, wherein removing portions in step d) is removing equal portions, aseptically, so as to preclude contamination of the enriched separate composited single-unit production lot samples.	ICMSF, alone or in combination with Gombas, discloses Claim 35, upon which this claim relies. See above re Claim 35. ICMSF describes aseptically withdrawing and weighing sample units into the appropriate diluent. ICMSF, 231, 232.

	Gombas indicates “a sample was aseptically transferred to a sterile stomacher bag and blended with an equal amount of enrichment broth.” Gombas, 563. Gombas further indicates “five 1-ml samples of secondary enrichment broths were composited.” *Id.*, 564. Moreover, a POSITA would consider this to be standard practice. A POSITA would have aseptically removed equal volume portions of each enriched separate test lot sample.

EXHIBIT C2

Invalidity of U.S. Patent No. 8,822,143 in light of ICMSF & Hughes

National Beef denies that any of its microbiological testing methods infringe any claim of U.S. Patent No. 8,822,143 ("the '143 Patent"). If the claims of the '143 Patent were construed so broadly as to cover the microbiological testing methods used by National Beef, they would be invalid at least in view of INT'L COMM'N ON MICROBIOLOGICAL SPEC'S FOR FOODS (ICMSF), Microorganisms in Foods 7: Microbiological Testing in Food Safety Management (Kluwer Academic/Plenum Publishers, 2002) ("ICMSF") and Denise Hughes et al., *Salmonella in Foods—A New Enrichment Procedure for Use with the TECRA Salmonella Visual Immunoassay: Collaborative Study*, 82 J. AOAC Int'l 634–647 (1999) ("Hughes"), which anticipate and/or disclose the limitations of the '143 Patent under at least pre-AIA 35 U.S.C. § 102(a), pre-AIA 35 U.S.C. § 102(b), pre-AIA 35 U.S.C. §§ 102(f)/(g) and/or pre-AIA 35 U.S.C. § 103. To the extent that ICMSF and/or Hughes do not expressly disclose one or more limitations of the asserted claims, as they would have to be construed to support IEH's claim of infringement, they disclose the limitations alone or in combination with one or more additional prior art references and/or the knowledge of one of ordinary skill in the art.

Claim 1	**Disclosure of ICMSF and Hughes**
[1pre] A method of sampling and testing for microbes in multiple separate test lots, comprising:	To the extend the preamble is limiting, as discussed herein, ICMSF, alone or in combination with Hughes, discloses a method of sampling and testing for microbes in multiple separate test lots, comprising: ICMSF includes many other references to testing lots of beef or poultry, two of the most common types of meat in commerce. *Id.*, 114 (§ 6.3), 184–191 (§§ 10.2–10.6), 225–226 (§ 12.1), 285 (§ 16.1), 313–330 (Chapter 17). ICMSF is relevant to any kind of food product including beef. Hughes discusses turkey (Hughes, Abstract), and a POSITA would be motivated to combine the teachings of ICMSF and Hughes as they both test meat for release into commerce. Hughes discloses testing turkey for *Salmonella*. "Three food types (. . . and raw turkey) were analyzed in the United States." Hughes, 634, 645, Table 8.

	Table 8 shows results from 12 collaborators for naturally contaminated raw turkey. For the 180 samples tested, using confirmed data, there was one false negative at the low level for the TECRA method. There were 3 false negatives at the low level and 3 false negatives at the high level for the cultural method. The incidence of false-positive results was very high for the cultural method, with a total of 41 false positives, compared with 5 for the TECRA method. It should, however, be taken into account that the comparison was with the method in BAM 7th Ed., not the current 8th Ed. The method for raw flesh food in the BAM 8th Ed. uses elevated temperature with RV and TT broth, which would be more selective. No significant Hughes, 646. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638.
[1a] a) separately collecting multiple independent samples from each of multiple separate test lots, wherein each separate test lot is separately sampled by taking said multiple independent samples thereof;	ICMSF, alone or in combination with Hughes, discloses separately collecting multiple independent samples from each of multiple separate test lots, wherein each separate test lot is separately sampled by taking said multiple independent samples thereof. "Many different types of samples are collected and submitted to the laboratory for analysis. Some are sample units from lots or consignments of food or ingredients for lot acceptance determination. . . . A sample unit is a small portion of the lot in which the distribution of both numbers and types of microorganisms should be

	representative of the whole lot." ICMSF, 255. The lots may be beef or poultry. *Id.*, 184-91, 226, 230. Thus, ICMSF discloses collecting a plurality of samples or sample units (other synonyms include portions, specimens, and sub-samples) from lots. ICMSF describes collecting from a lot or lots: "If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." *Id.*, 119. Additionally, ICMSF indicates a consignment may be "a very large quantity of food" and may be "composed of several lots, representing for example, different days of production from the same plant." *Id.*, 119, 120; *see also* Applicant Admitted Prior Art ("AAPA") #3-#5.

6.11 WHAT IS A REPRESENTATIVE SAMPLE?

A *representative sample* reflects, as far as is possible, the composition of the lot from which it is drawn.

How then should a representative sample be drawn? It is important to avoid bias and to draw a sufficient number of sample units to confidently make a judgment about a lot. Sampling at random is the universally recognized way of avoiding bias and is more reliable than consciously trying to draw the sample units from the various parts of a lot. The units (cartons or containers, particular weights of solid, or volumes of liquid) are drawn by using random numbers. There is, of course, no guarantee that a random sample has characteristics identical with those of the lot, but the randomness of sampling is the basis for calculation of the probability that a sample will give a biased result.

It is also possible, and may be desirable, to use a *stratified random sampling* approach, drawing a random sample of a given number of sample units from each *stratum* (e.g., each sublot or carton). The distribution of sample units on strata should correspond to the distribution of lot units on strata. This means that if each stratum contains the same quantity as every other one, the number of sample units per stratum should be the same. Otherwise, they should differ just as the proportions of the lot contained in the strata differ. Stratification is a device for handling known sources of variation and may be used where one has prior knowledge that the consignment is potentially not of uniform quality. A consignment might not be of uniform quality if portions of it are shipped in different vehicles or in different holds of a vessel, or if it is known that it is really composed of several lots representing, for example, different days of production from the same plant or different plants of the same company. The results for different strata should be assessed separately and then pooled if they appear to be homogeneous.

The calculation of acceptance probabilities is based on the assumption that random samples have been drawn and analyzed. Where physical or practical constraints prevent random sampling, such calculated probabilities may not be valid. Their reliability depends on how close reality approaches the ideal situation of random sampling. This fact should be borne in mind when subsequently interpreting results.

ICMSF, 119-20 (annotated).

6.10 WHAT IS A LOT?

Ideally, a lot is a quantity of food or food units produced and handled under uniform conditions. Under certain circumstances, it has been shown that the logarithms of the counts from a batch of food are likely to be normally distributed (Baird-Parker, 1980; Kilsby & Baird-Parker, 1983 [their Table 2]; Ingram & Roberts, 1976). The implication is that there is homogeneity within a lot, which, when considering microbial levels and distributions, rarely occurs in practice. In most instances, the distribution of microorganisms within lots of food is heterogeneous. Even in food factories, this heterogeneity makes the interpretation of sample results difficult, and it becomes even more so in circumstances where a lot is less well defined, such as in a consignment of a very large quantity of food.

It is therefore helpful if suppliers can be persuaded to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load).

If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment.

A lot should be composed of food produced with as little variation as possible for a given process or commodity. Because of the uncertainties in identifying a lot commercially, however, the use of the word *lot* in this book is usually in its statistical sense, as a collection of units of a product, the acceptability of which is determined by examining a sample drawn from it.

ICMSF, 118-19 (annotated).

	The ICMSF has defined a lot as follows: In the commercial sense, a lot is a quantity of food supposedly produced under identical conditions, all packages of which would normally bear a lot number that identifies the production during a particular time interval, and usually from a particular line, retort, or other critical processing unit. Statistically, a lot is considered as a collection of units of a product from which a sample is drawn to determine acceptability of the lot (ICMSF, 1986). In this example, the maximum size of a lot consists of all the ground beef produced on common equipment from cleanup to cleanup. Smaller lots could be established (e.g., hourly). ICMSF, 327 (annotated). This definition also reflects the common practice referred to in the IEH Patents at Example II, wherein "[p]referably, the producer defines and produces these single-unit lots in such a fashion as to maximize the information available." '143 Patent,12:52–54. This corresponds with the specification of IEH's Patents which reflects the common practice of producers defining what constitutes a lot grouped for traceability purposes. '143 Patent, 3:5-15, 11:18-22 (AAPA #3), and 12:52-55. Thus, ICMSF discloses collecting a plurality of portions from separate lots. ICMSF describes a lot as "a collection of units of a product." IMCSF, 119. ICMSF further indicates a lot has been defined as: "a quantity of food supposedly produced under identical conditions, all packages of which would normally bear a lot number that identifies the production during a particular time interval, and usually from a particular line, retort, or other critical processing unit. Statistically, a lot is considered as a collection of units of a product from which a sample is drawn to determine acceptability of the lot." ICMSF, 327. ICMSF advocates use of a "statistically based sampling plan." *Id.*, 113-15, 119-21, 320, 327, 328. In the sampling plans, "*n* is the number of sample units chosen separately and independently, and . . . *c* is the maximum allowable number of sample

	units yielding a positive result." *Id.*, 115. ICMSF discloses a wide range for the number of sample units (portions) for a particular microbial agent or organism. For example, ICMSF discusses collecting 15, 30, 60 or 100 sample units from a lot. *Id.*, 320, 326. **6.5 THE SAMPLING PLAN** The sample units so drawn will, after examination, yield results that will be compared with certain criteria to reach a decision as to whether the entire lot should be accepted or rejected (see section 6.9 for explanation of rejection). The particular choice of sampling procedure and the decision criteria make up the *sampling plan*. Proper drawing of samples is essential if the decision making criteria of the sampling plan are to give unbiased results. By sampling randomly, we are able to reduce the risk of making biased decisions. A simple example of a sampling plan follows. Take 10 sample units of a food from a lot and subject them to appropriate laboratory procedure to test for the presence or absence of a microorganism. If two or fewer of the 10 sample units show presence of the organism, that is, give a positive result, then the whole lot of food is acceptable (relative to this organism). But if three or more give a positive result, the whole lot is to be rejected. This plan is described by the use of two terms $n = 10$ and $c = 2$, where n is the number of sample units chosen separately and independently, and where c is the maximum allowable number of sample units yielding a positive result. ICMSF, 115 (annotated).

	6.1 INTRODUCTION It is important to recognize that the management of food safety using the approaches outlined in the first five chapters, based on controlling hazards through Good Hygienic Practices (GHP) and Hazard Analysis Critical Control Point (HACCP), is much more effective than trying to ensure safety by lot acceptance through end-product testing. This chapter discusses the concepts of probability and sampling that determine the limitations of end-product testing. Those concepts form the basis of the design of statistically based sampling plans (Chapter 7) and the establishment of cases (Chapter 8). In Chapters 6 and 7, it is assumed that the microbiological test will give an absolutely accurate result, with no false positives or false negatives. This is not a realistic assumption, and problems arising from sampling, sample transport and preparation for analysis, and the imprecision of microbiological methods are addressed in Chapters 10 and 12 and elsewhere in the text. ICMSF, 113 (annotated); *see also id.*, 115–21 (§§ 6.4–6.13), 319–20 (§ 17.2.3.3), and 326–30 (§§ 17.5.3.1–17.6). ICMSF discusses the number of portions to be taken sufficient for validation of a particular microorganism including *Salmonella* (*id.*, 276 (§ 15.3.3); *see also id.*, 282 (§ 15.6.3)); *Listeria* (*id.*, 309 (§ 16.6.3)), and *E. coli* (*id.*, 318–21 (§§ 17.2.3.2–17.2.3.4), 326 (§ 17.5.3.1)). IEH's AAPA discloses statistical-based sampling plans taking 10-60 sample units. '584 Patent, 6:3-20; *see* AAPA #1, #2, #5, and #6.
[1b] b) separately compositing the collected multiple independent samples from each of the separate test lots to provide a corresponding set of separate composited test lot samples, wherein each of the separate composited test lot samples is attributed to a particular corresponding separate test lot;	ICMSF, alone or in combination with Hughes, discloses separately compositing the collected multiple independent samples from each of the separate test lots to provide a corresponding set of separate composited test lot samples, wherein each of the separate composited test lot samples is attributed to a particular corresponding separate test lot. ICMSF states: "To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units of the enrichment cultures therefrom, using plans in which the presence of a single positive

	rejects the consignment. . . . This finding suggests that considerable reductions may be made in the cost of laboratory analysis by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, n, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort." ICMSF, 187. To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. A laboratory investigation undertaken for ICMSF (Silliker & Gabis, 1973) indicated that different sized analytical units drawn from a single well-mixed sample unit of dried food give very similar results. A subsequent investigation (Gabis & Silliker, 1974) using foods of high moisture content (including eggs, poultry meat, meat, and meat products) showed comparable reliability in the use of composite analytical units; for example, examination of three composites of 20 × 25 g analytical units gave the same result as examination of all 60 analytical units individually (the total amount examined being 1500 g in both instances). This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n*, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort. ICMSF, 187.

10.5.1 Conceivable Alternative Plans

As indicated above, for an infectious agent such as *Salmonella*, there are strong reasons for adopting sampling plans in which $c = 0$:

1. It is philosophically objectionable to some people to accept a lot containing a recognized pathogen, regardless of how the food is to be used in a manufacturing process or by the consumer.
2. There is the very great technical advantage of combining many analytical units for bacteriological examination in systems with $c = 0$, because a single positive decides the outcome.
3. The plan with $c = 1$ (or more) would always require the examination of (many) more analytical units, for equal probability of acceptance, than a plan with $c = 0$.
4. Even if the indicated probabilities of plans with $c = 0$ versus $c = 1$ are the same, their implications are not. For example, when a lot is accepted with the plan $n = 60$, $c = 0$, it is possible (though not certain) that the lot may not be contaminated at all. But using the plan $n = 95$, $c = 1$ and accepting one positive, it is known that the lot certainly is contaminated (though the probability that a lot with 5% of analytical units contaminated would be accepted is in both instances $P_a = 0.05$).

ICMSF, 188 (annotated). ICMSF also provides an example of "[u]sing a sampling plan involving n = 15 (25 g analytical units) and a composite of 375 g." *Id.*, 195 (§ 10.7.2). ICMSF also states "[f]or qualitative analyses, 25 g analytical units are frequently analyzed individually or as composites of 100 g, 200 g, or more." *Id.*, 232 (§ 12.5.2).

The ICMSF incorporates two references, Silliker & Gabis (1973) and Gabis & Silliker (1974). Silliker notes that "[c]ompositing of multiple samples (lumping) and wet compositing of pre-enrichment cultures have been tested as economical approaches to *Salmonella* control." Silliker, 475.

Silliker further notes that "[b]oth approaches are being used widely by the food industry in the United States." *Id.*.

	To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. A laboratory investigation undertaken for ICMSF (Silliker & Gabis, 1973) indicated that different sized analytical units drawn from a single well-mixed sample unit of dried food give very similar results. A subsequent investigation (Gabis & Silliker, 1974) using foods of high moisture content (including eggs, poultry meat, meat, and meat products) showed comparable reliability in the use of composite analytical units; for example, examination of three composites of 20 × 25 g analytical units gave the same result as examination of all 60 analytical units individually (the total amount examined being 1500 g in both instances). This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n*, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort. "There is the very great technical advantage of combining many analytical units for bacteriological examination." ICMSF, 188. ICMSF also describes composites prepared by combining "15 (25 g analytical units)" and "composites of 100 g, 200 g, or more." *Id.,* 195, 232; *see* AAPA #5. ICMSF states, "All steps up to the analysis must be controlled . . . to allow reliable traceability." ICMSF, 227. ICMSF indicates lot samples are traceable to a particular corresponding lot: "It is therefore helpful . . . to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load). . . . Treating the individual production

	batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119.
[1c] c) enriching each of the separate composited test lot samples to provide a set of separate composited test lot samples enriched for the target microbe(s);	ICMSF, alone or in combination with Hughes, discloses enriching each of the separate composited test lot samples to provide a set of separate composited test lot samples enriched for the target microbe(s). ICMSF states: To reduce laboratory effort . . . it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. ICMSF, 187. Also, ICMSF describes preparing composites of analytical units then diluting "for qualitative (enrichment)" analysis. *Id.,* 231-32; *see also* AAPA #5, #7. For practical reasons, however, analytical units of 10 g up to 50 g are typical for quantitative methods and are diluted 1:10. For qualitative analyses, 25 g analytical units are frequently analyzed individually or as composites of 100 g, 200 g, or more. ICMSF, 232 (annotated). Preparation of the first dilution, either for qualitative (enrichment) or quantitative (direct) analyses, can have a marked impact on the microbial population in the sample. This is often due to changes in the physico-chemical characteristics of the suspension as compared to the food matrix. Where the food does not confer protection, the composition of the diluent is important to prevent or minimize shock/injury to the microorganisms that can result from rapid changes in the ionic concentrations. ICMSF, 232 (annotated). ICMSF also describes strategic use of non-selective enrichment to resuscitate microorganisms and selective enrichments for growing microorganisms. *Id.*, 232–33 (§ 12.6); *see e.g.*, *id.,* 233 ("use of nonselective medium . . . to enhance recovery of injured or stressed cells."). ICMSF, § 12.6 also describes optimizing the incubation period. S*ee e.g.*, *id.,* 232.

	It was well known long before the priority date to use non-selective enrichments after dry compositing and prior to wet compositing as evidenced by Price, Silliker, Gabis, and MLG. A POSITA would also know to use selective enrichments after dry compositing and prior to wet compositing as disclosed by ICMSF and Hughes. A POSITA would find that incubating so microorganisms are detectable and at least substantially uniform in the selective enrichment broth was considered the standard practice in official methods because the methods otherwise fail. For example, D'Aoust states "standard methodology recommends incubation of selective enrichment broths." D'Aoust, 372. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638.

	(a) *Pre-enrichment.*—Pre-enrich product in noninhibitory broth to initiate growth of *Salmonella* spp. Methods used may vary with product and should be performed as indicated in **967.26A** or BAM, 7th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, chapter 5, section C. Pre-enrichment broths should be incubated at 35°–37°C. (b) *Selective enrichment.*—Transfer 0.1 mL incubated pre-enrichment mixtures to Rappaport-Vassiliadis R10 broth (9.9 mL) and 1 mL to TT broth (9 mL). For all foods other than raw foods or foods having a high microbial load, incubate 6–8 h at 41°–43°C. Selective enrichments of raw foods or foods having a high microbial load must be incubated 16–20 h at 41°–43°C. An air incubator is suitable for this purpose. Hughes, 636. Hughes instructs that, after pre-enrichment, portions of the "incubated pre-enrichment mixtures" should be transferred to two separate selective enrichment broths, Rappaport-Vassiliadis R10 broth and TT broth, and incubated for 16-20 hours at 41°-43°C. Hughes, 636, 638. Additionally, Hughes instructs that these selective broths should be removed from incubation and mixed by hand or by Vortex mixer. Hughes, 638. Hughes further instructs to then remove 1 mL from the TT tube and 1 mL from the Rappaport-Vassiliadis R10 tube and transfer to separate, sterile M broths. *Id.,* 636, 638. The separate M broths are then incubated ("16-20 h at 35°-37°C" for "all foods other than raw foods or foods having a high microbial load" or "6 h at 35°-37°C" for "raw foods or foods having a high microbial load"). *Id.*, 638. A POSITA would find that the long incubation at an elevated temperature and mixing ensures the target microbial agent or organism reaches detectable levels and

	becomes substantially uniform throughout the separate lot samples.
[1d] d) removing portions of each separate enriched composited test lot sample, and combining the removed portions to provide a pooled modular composite sample; and	ICMSF describes compositing the enriched lot samples: "it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. . . . This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n,* with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort." ICMSF, 187. AAPA #5-#7. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Hughes also discloses a method of removing portions of each enriched separate test lot sample, and combining the removed portions to provide a modular composite sample. Hughes instructs to "[c]ombine equal volumes (0.5-1 mL) from each M broth tube in a clean screw-cap tube." Hughes, 638.

	that has been warmed to 35°–37°C. Also remove 1 mL from Rappaport-Vassiliadis R10 tube and transfer to a separate tube of prewarmed M broth (10 mL). For all foods other than raw foods or foods having a high microbial load, incubate M broth tubes 16–20 h at 35°–37°C and return selective enrichment broth tubes to 41°–43°C incubator and incubate for additional 16–18 h. For raw foods or foods having a high microbial load, incubate M broth tubes 6 h at 35°–37°C and return selective enrichment broth tubes to 41°–43°C and incubate for an additional 6 h. (**d**) *Preparation of sample for EIA analysis.*—Remove M broth tubes from incubation and mix tubes by hand or Vortex mixer. Combine equal volumes (0.5–1 mL) from each M broth tube in a clean screw-cap tube and heat in boiling H_2O bath or in flowing steam 15 min. Refrigerate (2°–8°C) remaining M broth and TT and Rappaport-Vassiliadis R10 tubes from (**c**) for culture confirmation of any EIA-positive samples (*see* **967.26B, 967.26C, 967.27** and **967.28**). Cool heated M broths to 25°–37°C prior to analysis. Hughes, 638. Hughes 2003 confirms the pooling of enrichments. Hughes 2003, 775-76.
[1e.1] e) testing of the pooled modular composite sample, using a suitable detection assay, for the target microbe(s),	ICMSF describes testing enrichment cultures of pooled modular composite samples with suitable detection assays (ICMSF, 187) and "presence/absence tests" (ICMSF, 233). Suitable detection assays were established in Silliker and Gabis as discussed in ICMSF. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-

	enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Hughes states testing of the composite sample is "by an enzyme immunoassay (EIA) method." Hughes, 635, 638. These assay types were "art recognized." '143 Patent, 2:66-3:3 (AAPA #7).
[1e.2] wherein when such testing is negative all of said samples that were composited to form the separate composited test lot samples are deemed negative for the target microbe(s) and each of the multiple separate test lots is validated, and	ICMSF describes correlating lots in a consignment with lot samples so a negative or positive test result enables a lot to be validated or rejected. ICMSF, 118-19. ICMSF describes sampling plans in which "c is the maximum allowable number of sample units yielding a positive result." ICMSF, 115. For many scenarios, the requirement is c = 0, which indicates the testing is negative. *Id.*, 118, 183-90, 309, 325, 326. A negative test result (*id.*, 113-15, 325, 326) is the expected and desired outcome to determine meat is not contaminated and is safe for consumption (*id.*, 184-89) such that a lot is validated and it is "commercially possible to market" (*id.*, 185) the meat.

6.10 WHAT IS A LOT?

Ideally, a lot is a quantity of food or food units produced and handled under uniform conditions. Under certain circumstances, it has been shown that the logarithms of the counts from a batch of food are likely to be normally distributed (Baird-Parker, 1980; Kilsby & Baird-Parker, 1983 [their Table 2]; Ingram & Roberts, 1976). The implication is that there is homogeneity within a lot, which, when considering microbial levels and distributions, rarely occurs in practice. In most instances, the distribution of microorganisms within lots of food is heterogeneous. Even in food factories, this heterogeneity makes the interpretation of sample results difficult, and it becomes even more so in circumstances where a lot is less well defined, such as in a consignment of a very large quantity of food.

It is therefore helpful if suppliers can be persuaded to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load).

If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment.

A lot should be composed of food produced with as little variation as possible for a given process or commodity. Because of the uncertainties in identifying a lot commercially, however, the use of the word *lot* in this book is usually in its statistical sense, as a collection of units of a product, the acceptability of which is determined by examining a sample drawn from it.

ICMSF, 118-19 (annotated).

	Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Because Hughes explains the steps to be taken when testing is positive (*see* ie.3), a POSITA would also expect negative testing results. Hughes, 638. Determining meat is safe for consumption so it may be released into commerce is the well-understood point of testing meat. Moreover, the recitation of a lot being validated for "release . . . into commerce" is not limiting. Prosecution History for Reexamination Control No. 95/011,294).
[1e.3] wherein when such testing is positive, each of the individual separate composited test lot samples that were used to form the pooled modular composite sample are individually tested to determine which of the separate composited test lot samples is positive for the target microbe(s),	The combination of ICMSF and Hughes describes retaining a portion of the enriched lot samples (steps (b)-(d)). "Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119. "There is often a desire to retest retained samples." ICMSF, 191. If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment. ICMSF, 119 (annotated).

	ICMSF discloses validating for release into commerce. ICMSF, 113–15 (§§ 6.2–6.6), 118–19 (§§ 6.9–6.10), 184–85 (§ 10.2), 325–27 (§§ 17.5.3–17.5.3.4); *see also id.*, 186–89 (§ 10.4–10.6)) according to a statistically-based sampling plan (*id.*, 113 (§ 6.1); *see also id.*, 115–18 (§§ 6.4–6.8), 184 (§ 10.2), 319–21 (§§ 17.2.3.3–17.2.3.4), 326–30 (§§ 17.5.3.1–17.6), as described regarding steps (a) and (e)(2). Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Hughes explicitly teaches retention of the enriched test lot samples for further testing after a positive result. Hughes provides instructions to refrigerate the "remaining M broth and TT and Rappaport-Vassiliadis R10 tubes [that were enriched] for culture confirmation of any EIA-positive samples." Hughes, 638. Hughes refers to the results as the "confirmed positive samples" (Hughes, 635) and also states that diagnostic reagents are "necessary for culture confirmation of presumptive EIA tests." Hughes, 636. Hughes indicates "[e]nrichment broths and M broths from samples positive by an enzyme immunoassay (EIA) method must be streaked on selective media." *Id.*, 635. Culture confirmation using streaking is more sensitive than the EIA method, as was commonly understood and confirmed by the teachings of D'Haese. *See generally*, D'Haese.

	Each sample was analyzed by the BAM method (7th Ed.) and the TSVIA method using the enrichment protocol described in section **F** of the method, *Preparation of Sample*. Equal portions (0.5–1 mL) from each of the 2M broths arising from the 2 selective-enrichment broths were combined in a small screw-capped tube and heat treated in boiling water for 15 min prior to performing the TSVIA. Confirmation for the immunoassay was by streaking from the M broth culture onto selective agar, as specified in the BAM reference culture method. Hughes, 635. Moreover, the recitation of a lot being validated for "release . . . into commerce" is not limiting. Prosecution History for Reexamination Control No. 95/011,294).
[1e.4] wherein the test lots corresponding to any negatively testing composited test lot samples are validated.	Hughes explicitly indicates portions of the enrichment cultures are retained for further testing; thus Hughes has no need for further sampling. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. ICMSF and Hughes disclose selective enrichment so further enrichment is unnecessary. Also, as discussed regarding step (e)(3), Hughes explicitly indicates portions of the enrichment cultures are retained for further testing; thus Hughes has no need for further sampling. The same would be expected for enrichment cultures in ICMSF because retaining samples at each process stage was considered standard

	practice for a POSITA. in flowing steam 15 min. Refrigerate (2°–8°C) remaining M broth and TT and Rappaport-Vassiliadis R10 tubes from (c) for culture confirmation of any EIA-positive samples (*see* **967.26B, 967.26C, 967.27** and **967.28**). Cool heated M broths to 25°–37°C prior to analysis. Hughes, 638. Thus, the negative limitation of step (e)(4) is disclosed by and is obvious based on the combination of ICSMF and Hughes. *Id.*
Claim 2	**Disclosure of ICMSF and Hughes**
The method of claim 1, wherein each of the multiple separate test lots is selected from the group consisting of an environmental lot comprising multiple operationally-linked environmental specimens, pharmaceutical lot comprising multiple operationally-linked pharmaceutical specimens, single-unit product lot comprising multiple operationally-linked product specimens, and combinations thereof.	ICMSF, alone or in combination with Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. The specification of the '143 Patent indicates a test lot is a single-unit production lot ('143 Patent, 5:34-45) such as a single combo, bin. pallet, or segment of production ('143 Patent, 7:42-50; *see also* AAPA #3 and #4). ICMSF teaches collecting from "individual production batches as lots" (ICMSF, 119) and "[a] lot is considered as a collection of units" (ICMSF, 327). "Operationally-linked" is simply grouping like items, which is also disclosed by ICMSF. "Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119.
Claim 3	**Disclosure of ICMSF and Hughes**

The method of claim 1, wherein each of the multiple separate test lots is a single-unit production lot, comprised of operationally-linked product specimens.	ICMSF, alone or in combination with Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. The specification of the '143 Patent indicates a test lot is a single-unit production lot ('143 Patent, 5:34-45) such as a single combo, bin. pallet, or segment of production ('143 Patent, 7:42-50; *see also* AAPA #3 and #4). ICMSF teaches collecting from "individual production batches as lots" (ICMSF, 119) and "[a] lot is considered as a collection of units" (ICMSF, 327). "Operationally-linked" is simply grouping like items, which is also disclosed by ICMSF. "Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119.
Claim 4	**Disclosure of ICMSF, Hughes, and Stewart**
The method of claim 1, further comprising incubating the pooled modular composite sample of d), prior to testing in e).	ICMSF, alone or in combination with Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. ICMSF and Hughes, in combination with Stewart, discloses the method of claim 1, further comprising incubating the modular composite sample of d), prior to testing in e). Hughes describes incubating an enriched test lot sample. Hughes, 636, 638. Claim 1 is disclosed by ICMSF and Hughes. Stewart discloses that after the selective enrichment in Rappaport-Vassiliadis (RV) broth and tetrathionate (TT) broths, the two selective enrichments were combined into a single sample for further incubation and analysis. "[P]ost-enrichment by transfer and combination of 0.5 mL RV and 1 mL TT into a single tube containing

	10 mL prewarmed trypticase soy broth supplemented with novobiocin (TSB + n) at 10 µg/mL with incubation for 16–20 h at 42°C." Stewart, 398. Hughes also discloses an incubation protocol. "For the previous AOAC studies on TSVIA, a cultural protocol using selective enrichment in selenite cystine (SC) and tetrathionate (Tr) broths at 35°C was used. However, there has been a recent trend to use elevated incubation temperatures and to replace SC broth with the Rappaport-Vassiliadis (RV) medium (2)." Hughes, 634. A POSITA would have been motivated to incubate the pooled modular composite sample of Gombas or Hughes, as taught by Stewart, for reliable testing results.
Claim 5	**Disclosure of ICMSF and Hughes**
The method of claim 1, wherein enriching each separate composited test lot sample in c) comprises incubating each separate composited test lot sample with enrichment medium suitable to enrich for the target microbe(s), and wherein removing in d) comprises removing a portion of the enrichment medium from each enriched separate composited test lot sample and combining the removed portions to provide a pooled modular composite sample.	ICMSF, alone or in combination with Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. ICMSF describes the well-known benefits of combining enrichment cultures from analytical units from lots in a consignment.

	To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. A laboratory investigation undertaken for ICMSF (Silliker & Gabis, 1973) indicated that different sized analytical units drawn from a single well-mixed sample unit of dried food give very similar results. A subsequent investigation (Gabis & Silliker, 1974) using foods of high moisture content (including eggs, poultry meat, meat, and meat products) showed comparable reliability in the use of composite analytical units; for example, examination of three composites of 20 × 25 g analytical units gave the same result as examination of all 60 analytical units individually (the total amount examined being 1500 g in both instances). This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n*, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort. ICMSF, 187. Hughes describes incubating an enriches test lot sample. Hughes 636, 638.
Claim 6	**Disclosure of ICMSF and Hughes**
The method of claim 1, wherein testing of the pooled modular composite sample, using a suitable detection assay comprises use of a concentration step to concentrate the target microbe(s).	ICMSF, alone or in combination with Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. ICMSF discloses the use of "immunomagnetic beads to concentrate cells." ICMSF, 318. A POSITA would consider this a well-known technique before the priority date. Fratamico discusses the rapid isolation of target microorganisms using an immunomagnetic separation method. Fratamico, 105. Fratamico discloses that use of immunomagnetic beads resulted in "an increase in the level of E. coli B1409 in the sample," as well as "an increase in the number of organisms recovered." *Id.*, 107. Additionally, Fratamico provides detailed instructions for preparing and using

	immunomagnetic bead separation to concentrate a target microbial agent or organism. Fratamico developed "[a] method employing immunomagnetic beads" to concentrate and thereby improve the isolation and detection of E. coli. *Id.* For example, Fratamico was able to use immunomagnetic beads to isolate *E. coli* strains from enrichment cultures of raw ground beef "when the initial inoculum level was 1 cfu g-1." *Id.*, 109. Fratamico further teaches that "[t]he percentage of bacteria recovered increased with increasing amounts of beads." *Id.*, 108. Consequently, Fratamico "demonstrates that immunomagnetic separation [using immunomagnetic beads] is a simple and rapid technique" for concentrating a microbe for isolation and detection. *Id.*, 109. These teachings of Fratamico would have motivated a POSA to modify the combination of ICMSF and Gombas and ICMSF and Hughes to use immunomagnetic beads to concentrate microbes in a modular composite sample to benefit from accurate tests. Use of immunomagnetic beads was already known in the art for concentrating during testing beef samples. "Tu et al. (78) detected pathogenic E. coli O157:H7 in beef hamburger with the use of IMS and ATP measurement. After 6 h of enrichment at 37°C, inoculated beef samples (0.9 ml each) were filtered to remove large particles prior to 10 min of incubation with ca. 10^5 magnetic beads coated with anti–*E. coli* O157:H7 (Dynabeads, Dynal Inc., Oslo, Norway). Following incubation, the magnetic beads were collected with a Dynal magnetic concentrator and resuspended in 0.9 ml of Trypticase soy broth for ATP analysis." Beniot, 1941. "Hudson et al. (26) used IMS to directly separate and concentrate L. monocytogenes from ham samples prior to detection by PCR. IMS was used on the basis of the commercial availability of anti-Listeria magnetic beads (Dynal Inc.) to avoid time-consuming cultural enrichment steps and to remove PCR inhibitors." Beniot, 1941-42.

	Cudjoe investigates the use of immunomagnetic beads in pooling methods. In a study designed to evaluate the performance of Dynabeads® Anti-Salmonella, immunomagnetic separation (IMS) followed by plating (IMS-Plating) proved far superior to the conventional ISO *Salmonella* methodology and the Modified Semi-solid Rappaport-Vassiliadis (MSRV) method. *Salmonella* species were isolated and detected from 135 out of the 180 diverse poultry samples by IMS analysis as against 98 by the conventional method and 33 by the MSRV technique. All results were confirmed biochemically and serologically. It appeared from the data generated that some of the salmonellae isolated using IMS were greatly inhibited in Rappaport-Vassiliadis enrichment broth at 42°C and to a lesser extent in selenite cystine broth at 37°C. A moderately selective plating medium like XLD proved to be better in isolating these possible sensitive wild-type strains of salmonellae than the more selective BGA. Cudjoe, Abstract. *Samples*. One hundred and eighty (180) diverse poultry samples, consisting of 40 pools of cloacal/faecal swabs (5 swabs/pool; 5 pools samples/flock) from eight different flocks, 10 raw chicken breast meat samples, 10 pooled egg shell samples, 60 raw chicken liver samples and 60 chicken neck skins sampled from the slaughter line, were used in this study. All samples were obtained from a poultry slaughter and processing plant in the Netherlands. A pool of five swab samples was pre-enriched in 10 ml of buffered peptone water (BPW; Difco Laboratories, Detroit, Michigan, USA) at 37°C for 16–20 h, whilst 25 g of each of the other samples above, were pre-enriched similarly in 225 ml of BPW and examined as detailed below. Cudjoe, 160.
Claim 7	**Disclosure of ICMSF and Hughes**
The method of claim 1, wherein where testing in e) of the pooled modular composite sample is positive, and wherein the separate	ICMSF, alone or in combination with Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1.

individual composited test lot samples are further tested using a more sensitive protocol.	ICMSF discloses the use of "immunomagnetic beads to concentrate cells." ICMSF, 318. A POSITA would consider this a well-known technique before the priority date. Hughes uses an EIA method followed by a more sensitive protocol—streaking on selective media. Hughes, 635. Each sample was analyzed by the BAM method (7th Ed.) and the TSVIA method using the enrichment protocol described in section F of the method, *Preparation of Sample*. Equal portions (0.5–1 mL) from each of the 2M broths arising from the 2 selective-enrichment broths were combined in a small screw-capped tube and heat treated in boiling water for 15 min prior to performing the TSVIA. Confirmation for the immunoassay was by streaking from the M broth culture onto selective agar, as specified in the BAM reference culture method. Hughes, 635. A POSITA would find that modifying Hughes based on D'Haese ensures a more sensitive test is used for further testing. A POSITA would have recognized the concentration step could be employed to provide a more sensitive protocol for further testing. A POSITA would understand that retesting the non-pooled, singular samples is an inherently more sensitive process because the sample is not diluted with other samples in the testing pool. Therefore, the retesting of a singular sample is more concentrated and therefore, more sensitive. *See also, generally*, Jarvis.

	Fratamico discusses the rapid isolation of target microorganisms using an immunomagnetic separation method. Fratamico, 105. Fratamico discloses that use of immunomagnetic beads resulted in "an increase in the level of E. coli B1409 in the sample," as well as "an increase in the number of organisms recovered." *Id.*, 107. Additionally, Fratamico provides detailed instructions for preparing and using immunomagnetic bead separation to concentrate a target microbial agent or organism. Fratamico developed "[a] method employing immunomagnetic beads" to concentrate and thereby improve the isolation and detection of E. coli. *Id.* For example, Fratamico was able to use immunomagnetic beads to isolate *E. coli* strains from enrichment cultures of raw ground beef "when the initial inoculum level was 1 cfu g-1." *Id.*, 109. Fratamico further teaches that "[t]he percentage of bacteria recovered increased with increasing amounts of beads." *Id.*, 108. Consequently, Fratamico "demonstrates that immunomagnetic separation [using immunomagnetic beads] is a simple and rapid technique" for concentrating a microbe for isolation and detection. *Id.*, 109. These teachings of Fratamico would have motivated a POSA to modify the combination of ICMSF and Gombas and ICMSF and Hughes to use immunomagnetic beads to concentrate microbes in a modular composite sample to benefit from accurate tests. Use of immunomagnetic beads was already known in the art for concentrating during testing beef samples. "Tu et al. (78) detected pathogenic E. coli O157:H7 in beef hamburger with the use of IMS and ATP measurement. After 6 h of enrichment at 37°C, inoculated beef samples (0.9 ml each) were filtered to remove large particles prior to 10 min of incubation with ca. 10^5 magnetic beads coated with anti–*E. coli* O157:H7 (Dynabeads, Dynal Inc., Oslo, Norway). Following incubation, the magnetic beads were collected with a Dynal magnetic concentrator and resuspended in 0.9 ml of Trypticase soy broth for ATP analysis." Beniot, 1941.

	"Hudson et al. (26) used IMS to directly separate and concentrate L. monocytogenes from ham samples prior to detection by PCR. IMS was used on the basis of the commercial availability of anti-Listeria magnetic beads (Dynal Inc.) to avoid time-consuming cultural enrichment steps and to remove PCR inhibitors." Beniot, 1941-42. Cudjoe investigates the use of immunomagnetic beads in pooling methods. In a study designed to evaluate the performance of Dynabeads® Anti-Salmonella, immunomagnetic separation (IMS) followed by plating (IMS-Plating) proved far superior to the conventional ISO *Salmonella* methodology and the Modified Semi-solid Rappaport-Vassiliadis (MSRV) method. *Salmonella* species were isolated and detected from 135 out of the 180 diverse poultry samples by IMS analysis as against 98 by the conventional method and 33 by the MSRV technique. All results were confirmed biochemically and serologically. It appeared from the data generated that some of the salmonellae isolated using IMS were greatly inhibited in Rappaport-Vassiliadis enrichment broth at 42°C and to a lesser extent in selenite cystine broth at 37°C. A moderately selective plating medium like XLD proved to be better in isolating these possible sensitive wild-type strains of salmonellae than the more selective BGA. Cudjoe, Abstract. *Samples*. One hundred and eighty (180) diverse poultry samples, consisting of 40 pools of cloacal/faecal swabs (5 swabs/pool; 5 pools samples/flock) from eight different flocks, 10 raw chicken breast meat samples, 10 pooled egg shell samples, 60 raw chicken liver samples and 60 chicken neck skins sampled from the slaughter line, were used in this study. All samples were obtained from a poultry slaughter and processing plant in the Netherlands. A pool of five swab samples was pre-enriched in 10 ml of buffered peptone water (BPW; Difco Laboratories, Detroit, Michigan, USA) at 37°C for 16–20 h, whilst 25 g of each of the other samples above, were pre-enriched similarly in 225 ml of BPW and examined as detailed below. Cudjoe, 160.

Claim 11	Disclosure of ICMSF and Hughes
The method of claim 1, wherein each the test lots is selected from the lot group consisting of a single combo, single bin, single pallet, and a production assemblage.	ICMSF, alone or in combination with Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. The specification of the '143 Patent indicates a test lot is a single-unit production lot ('143 Patent, 5:34-45) such as a single combo, bin. pallet, or segment of production ('143 Patent, 7:42-50; *see also* AAPA #3 and #4). ICMSF teaches collecting from "individual production batches as lots" (ICMSF, 119) and "[a] lot is considered as a collection of units." ICMSF, 327. "Operationally-linked" is simply grouping like items, which is also disclosed by ICMSF. "Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119.
Claim 12	**Disclosure of ICMSF and Hughes**
The method of claim 1, wherein the number of separate test lots is from 3 to 10.	ICMSF, alone or in combination with Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. ICMSF indicates a consignment may be "composed of several lots." ICMSF, 119-20. "Several" lots includes at least three lots.
Claim 13	**Disclosure of ICMSF and Hughes**
The method of claim 1, further comprising, where testing of the pooled modular composite sample is positive, determining which individual separate test lots are positive, application of remedial measures to said separate positive test lots, and validating by further testing of the respective	ICMSF, alone or in combination with Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite

remediated separate test lots and obtaining a negative test result.	sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Each sample was analyzed by the BAM method (7th Ed.) and the TSVIA method using the enrichment protocol described in section F of the method, *Preparation of Sample.* Equal portions (0.5–1 mL) from each of the 2M broths arising from the 2 selective-enrichment broths were combined in a small screw-capped tube and heat treated in boiling water for 15 min prior to performing the TSVIA. Confirmation for the immunoassay was by streaking from the M broth culture onto selective agar, as specified in the BAM reference culture method. Hughes, 635. A POSITA understands the purpose of testing for pathogens and other organisms in food is to determine if a food is safe for entry into commerce to be sold for consumption. One having ordinary skill in the art understands that if a sample tests positive, a remediation step, like cooking, needs to be completed before it can be sold into commerce to comply with USDA guidelines.
Claim 14	**Disclosure of ICMSF and Hughes**
The method of claim 1, wherein, in a), separately collecting said multiple independent samples from each of the multiple separate test lots comprises separately collecting from each separate test lot a number of samples from 5 to 100.	ICMSF, alone or in combination with Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. ICMSF teaches collecting 5, 30, 60, 95, or 100 portions. ICMSF, 184-90, 276, 282, 309, 320, 326-30. *See also* MPEP § 2144.05.

Claim 15	**Disclosure of ICMSF and Hughes**
The method of claim 1, wherein, in a), separately collecting said multiple independent samples from each of the multiple separate test lots comprises separately collecting 30 to 60 samples from each separate test lot.	ICMSF, alone or in combination with Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. ICMSF teaches collecting 5, 30, 60, 95, or 100 portions. ICMSF, 184-90, 276, 282, 309, 320, 326-30. *See also* MPEP § 2144.05.
Claim 16	**Disclosure of ICMSF and Hughes**
The method of claim 1, wherein, in a), separately collecting said multiple independent samples from each of the multiple separate test lots comprises separately collecting at least 30 samples from each separate test lot.	ICMSF, alone or in combination with Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. ICMSF teaches collecting 5, 30, 60, 95, or 100 portions. ICMSF, 184-90, 276, 282, 309, 320, 326-30. *See also* MPEP § 2144.05.
Claim 18	**Disclosure of ICMSF and Hughes**
The method of claim 1, wherein removing portions in step d) is removing equal portions, aseptically, so as to preclude contamination of the enriched separate composited test lot samples.	ICMSF, alone or in combination with Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. ICMSF describes aseptically withdrawing and weighing sample units into the appropriate diluent. ICMSF, 231, 232. Hughes instructs that after enrichment "[c]ombine equal volumes (0.5-1 mL) from each M broth tube in a clean screw-cap tube." Hughes, 638. Moreover, a POSITA would consider this to be standard practice. A POSITA would have aseptically removed equal volume portions of each enriched separate test lot sample.
Claim 19	**Disclosure of ICMSF and Hughes**

The method of claim 1, wherein the target microbe(s) comprises at least one selected from the group consisting of *Listeria*, *Salmonella* and *Escherichia coli*.	ICMSF, alone or in combination with Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. ICMSF describes testing for *Listeria* (ICMSF, 285-312), *Salmonella* (*id.*, 273-84), and *Escherichia coli* (*id.*, 313-32). Hughes focuses on *Salmonella*. Hughes, 634.
Claim 20	**Disclosure of ICMSF and Hughes**
The method of claim 1, wherein the target microbe(s) comprises at least one selected from the group consisting of *Listeria* spp., *Listeria monocytogenes*, *Salmonella* spp., *Escherichia coli* O157:H7, coliforms and fecal coliforms.	ICMSF, alone or in combination with Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. ICMSF describes testing for *Listeria* (ICMSF, 285-312), *Salmonella* (*id.*, 273-84), and *Escherichia coli* (*id.*, 313-32). Hughes focuses on *Salmonella*. Hughes, 634.
Claim 21	**Disclosure of ICMSF and Hughes**
The method of claim 1, wherein the target microbe(s) comprises a spoilage organism.	ICMSF, alone or in combination with Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. A POSITA would find it obvious to apply the teachings of ICMSF and Hughes to target spoilage organisms. "Examination of every piece of whole meat is clearly impractical, hence any sampling procedure for estimating shelf-life would involve taking enough samples to give some statistical assurance of the keeping qualities of meat." Gill, 449. Borch is titled "Bacterial spoilage of meat and cured meat products." Borch. The reference discusses spoilage organisms in meat:

	Lactic acid bacteria are the major bacterial group associated with the spoilage of refrigerated vacuum- or MA-packaged cooked, cured meat products (Blickstad and Molin, 1983b; Shaw and Harding, 1989; von Holy et al., 1991). At the time of spoilage some products contain a 'pure' culture of only one species, while in others a mixture of *Lactobacillus* spp. and *Leuconostoc* spp. was found (Kant-Muermans, unpublished results). The great diversity of bacteria isolated from spoiled meat products is confirmed by several studies (Korkeala and Mäkelä, 1989; von Holy et al., 1991; Yang and Ray, 1994). The genus/species of lactic acid bacteria responsible for spoilage, depend on the product composition (product-related flora) as well as the manufacturing site (house-related flora; Korkeala and Mäkelä, 1989). Borch, 113. "Lactic acid bacteria spoil refrigerated meat products by causing defects such as sour off-flavours, discolouration, gas production, slime production and decrease in PH." Borch, 114; *see also, id.*, 115-17 (Section 4 Analysis of Spoilage). It is understood in the art that aerobic plate counts ("APC") can test for spoilage organisms. *See. e.g.*, ICMSF 1986, 122 ("tests to be made (e.g., APC, *Salmonella*, Clostridium perfringens"); *id.* at 132 ("However, aerobic plate counts (APC) can be used to monitor Good Commercial Practice (GCP), and criteria based on such examinations are a valuable aid to in-plant quality control.").

TABLE 19

Sampling plans and recommended microbiological limits for raw meat[a]

Product[b]	Test	Method reference[c]	Case	Plan class	n	c	Limit per cm² or gram m	M
Carcass meat, before chilling	APC	118	1	3	5	3	10^5	10^6
Carcass meat, chilled	APC	118	1	3	5	3	10^6	10^7
Edible offal, chilled	APC	118	1	3	5	3	10^6	10^7
Carcass meat, frozen	APC	118	1	3	5	3	5×10^5	10^7
Boneless meat, frozen (beef, veal, pork, mutton)	APC	118	1	3	5	3	5×10^5	10^7
Comminuted meat, frozen	APC	118	1	3	5	3	10^6	10^7
Edible offal, frozen	APC	118	1	3	5	3	5×10^5	10^7

a **Not for use at port of entry** but for in-plant quality control. Refer to Section B for appropriate applications.
b Unfrozen carcasses and primal cuts, swab counts per cm²; other meats and meat products, per gram.
c Refers to page number in ICMSF, 1978.

ICMSF 1986, 133 (Table 19 (annotated)).

Although for “quality control,” a POSITA would understand that "quality control" means testing for spoilage organisms could also be accomplished by this well-known method. It is disclosed within the prior art during the production lifecycle of beef production. ICMSF 1986, 134 (“The FAOIWHO Working Group (FAQ/WHO, 1979) considered that the only point at which APC could be used for chilled meat, to evaluate the hygienic conditions under which it was produced, was at the slaughterhouse, and because of its perishable nature it would not be possible to set

APC limits for use in guidelines or specifications for chilled meats at port of entry."); *see also, id.*, 153 ("Monitoring for APC, E. coli, and Staph. aureus would give some indication of inadequate processing (heating, curing, smoking), postprocess contamination, or storage at temperatures above those recommended for frozen or refrigerated distribution and storage.")

E. TEST PROCEDURES

(a) *Aerobic plate counts*

Comminute, blend, or stomach the various portions of meat composing the sample unit and withdraw the analytical unit. Combine the swabs to form an analytical unit, or treat each separately. Prepare dilutions and perform the APC test according to the directions given in ICMSF, 1978, p. 118. Spread plates are preferable to pour plates when examining raw meats.

The temperature used for incubation of plates should be related to the material being examined. To estimate numbers of mesophilic contaminants on freshly dressed carcasses it is advisable to incubate plates at 35 or 37°C. For meat that has been held under chilled conditions, plates should be incubated at 25°C to include psychrotrophs and thus give a better indication of the microbial growth that has occurred. Counts at 20°C give similar results after a longer incubation time.

ICMSF 1986, 136 (annotated).

See also, Bhunia 4 ("Bacteria-specific generic rapid methods such ATP-based luminescence and total plate count (APC) are also needed for monitoring cleaning and hygienic practices employed in a processing plant."); Wheeler, 1606 (use of APC in a beef-processing plant); PR/HAACP, 38849 ("Various alternative indicator

	organisms were suggested, including generic E. coli (biotype I), total plate counts, Enterobacteriaceae, Total Viable Counts (TVC), and Aerobic Plate Counts (APC).”).
Claim 22	**Disclosure of ICMSF and Hughes**
The method of claim 1, wherein removing portions of each separate enriched composited test lot sample comprises removing equal volume portions of each separate enriched composited test lot sample.	ICMSF, alone or in combination with Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. ICMSF describes aseptically withdrawing and weighing sample units into the appropriate diluent. ICMSF, 231, 232. Hughes instructs that after enrichment “[c]ombine equal volumes (0.5-1 mL) from each M broth tube in a clean screw-cap tube.” Hughes, 638. Moreover, a POSITA would consider this to be standard practice. A POSITA would have aseptically removed equal volume portions of each enriched separate test lot sample.
Claim 23	**Disclosure of ICMSF and Hughes**
The method of claim 1, wherein testing of the pooled modular composite sample, using a suitable detection assay comprises use of at least one of a DNA-based method, an immunochemistry-based method, and a biosensor-based method.	ICMSF, alone or in combination with Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. Hughes discloses use of “an enzyme immunoassay (EIA) method,” which is an immunochemistry-based method. Hughes, 635.

	Each sample was analyzed by the BAM method (7th Ed.) and the TSVIA method using the enrichment protocol described in section F of the method, *Preparation of Sample*. Equal portions (0.5–1 mL) from each of the 2M broths arising from the 2 selective-enrichment broths were combined in a small screw-capped tube and heat treated in boiling water for 15 min prior to performing the TSVIA. Confirmation for the immunoassay was by streaking from the M broth culture onto selective agar, as specified in the BAM reference culture method. Hughes, 635.
Claim 24	**Disclosure of ICMSF and Hughes**
The method of claim 1, wherein each of the multiple separate test lots are production lots of a meat or poultry product, comprising an assemblage of one or more meat or poultry specimens, respectively.	ICMSF, alone or in combination with Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. ICMSF indicates "[t]rimmings to be used for ground beef could be pretested for E. coli O157:H7 by various sampling plans." ICMSF, 323. ICMSF indicates "[r]esearch has validated that up to fifteen 25 g analytical units of ground beef or trimmings can be composited without significant loss in E. coli O157:H7 detection sensitivity." *Id.*, 327 *see also id.*, 226, 314, 320, and 324. ICMSF includes many other references to testing lots of beef or poultry, two of the most common types of meat in commerce. *Id.* at 114 (§ 6.3), 184–191 (§§ 10.2–10.6), 225–226 (§ 12.1), 285 (§ 16.1), 313–330 (Chapter 17). ICMSF is relevant to any kind of food product including beef or poultry.

	Hughes discloses testing turkey for *Salmonella*. "Three food types (. . . and raw turkey) were analyzed in the United States." Hughes, 634, 645, Table 8. Table 8 shows results from 12 collaborators for naturally contaminated raw turkey. For the 180 samples tested, using confirmed data, there was one false negative at the low level for the TECRA method. There were 3 false negatives at the low level and 3 false negatives at the high level for the cultural method. The incidence of false-positive results was very high for the cultural method, with a total of 41 false positives, compared with 5 for the TECRA method. It should, however, be taken into account that the comparison was with the method in BAM 7th Ed., not the current 8th Ed. The method for raw flesh food in the BAM 8th Ed. uses elevated temperature with RV and TT broth, which would be more selective. No significant Hughes, 646.
Claim 29	**Disclosure of ICMSF and Hughes**
The method of claim 3, wherein product specimens are synonymous with product portions.	ICMSF, alone or in combination with Hughes, discloses Claim 3, upon which this claim relies. See above re Claim 3. ICMSF teaches collecting portions or samples, which were known synonyms for product portions or specimens. ICMSF states that "[a] sample unit is a small portion of the lot." ICMSF, 225.

	12.1 INTRODUCTION Many different types of samples are collected and submitted to the laboratory for analysis. Some are sample units from lots or consignments of foods or ingredients for lot acceptance determination. Others may be for investigational purposes to assess control of the environment, investigate the source of a problem, or validate a process. Some may have legal implications relative to a lawsuit or for regulatory compliance. This chapter briefly discusses some of the major factors that should be considered when collecting sample units, shipping them to a laboratory, preparing them for analysis, and analyzing them. A sample unit is a small portion of the lot in which the distribution of both numbers and types of microorganisms should be representative of the whole lot. This requirement is only completely fulfilled when the food and processing conditions result in a homogeneous distribution of microorganisms. This only occurs in well-agitated fluid products. In general, the distribution of microorganisms is presumed to be random. ICMSF at 225 annotated).
Claim 30	**Disclosure of ICMSF and Hughes**
The method of claim 5, wherein the enrichment medium comprises a selective agent that will inhibit a competing non-target microbe.	ICMSF, alone or in combination with Hughes, discloses Claim 5, upon which this claim relies. See above re Claim 5. Additionally, Claim 30 recites what a POSITA would consider the well-known property of selective enrichment, inhibition of a competing non-target microorganism. D'Aoust describes "selective enrichment [that] inhibits growth of non-*Salmonella*e and facilitates isolation of *Salmonella* on differential plating media." D'Aoust, 369. A POSITA would have been motivated to use an enrichment medium comprising a selective agent that inhibits a non-target agent or organisms as described by D'Aoust, for the test lot samples described by ICMSF and Hughes, to ensure an accurate test. A POSITA would understand that incubating samples at different temperature ranges for different target microorganisms would, effectively, inhibit the growth of other organisms. "Incubation temperature markedly affects productivity of selective

	enrichment broths by suppressing growth of competing microorganisms but not *Salmonella*." D'Aoust, 372. "This may be achieved by utilization of enrichment media that through various mechanisms may favor and enhance growth and multiplication of salmonellae while other gram-negative organisms such as coliforms, Proteus, and Pseudomonas are suppressed or inhibited." Fagerberg, 630.
Claim 31	**Disclosure of ICMSF and Hughes**
The method of claim 6, wherein the concentration step comprises at least one of immunomagnetic bead separation, and affinity chromatography.	ICMSF, alone or in combination with Hughes, discloses Claim 6, upon which this claim relies. See above re Claim 6. ICMSF discloses the use of "immunomagnetic beads to concentrate cells." ICMSF, 318. A POSITA would consider this a well-known technique before the priority date. Fratamico discusses the rapid isolation of target microorganisms using an immunomagnetic separation method. Fratamico, 105. Fratamico discloses that use of immunomagnetic beads resulted in "an increase in the level of E. coli B1409 in the sample," as well as "an increase in the number of organisms recovered." *Id.*, 107. Additionally, Fratamico provides detailed instructions for preparing and using immunomagnetic bead separation to concentrate a target microbial agent or organism. Fratamico developed "[a] method employing immunomagnetic beads" to concentrate and thereby improve the isolation and detection of E. coli. *Id.* For example, Fratamico was able to use immunomagnetic beads to isolate *E. coli* strains from enrichment cultures of raw ground beef "when the initial inoculum level was 1 cfu g-1." *Id.*, 109. Fratamico further teaches that "[t]he percentage of bacteria recovered increased with increasing amounts of beads." *Id.*, 108. Consequently, Fratamico "demonstrates that immunomagnetic separation [using immunomagnetic beads] is a simple and rapid technique" for concentrating a microbe for isolation and detection. *Id.*, 109.

	These teachings of Fratamico would have motivated a POSA to modify the combination of ICMSF and Gombas and ICMSF and Hughes to use immunomagnetic beads to concentrate microbes in a modular composite sample to benefit from accurate tests. Use of immunomagnetic beads was already known in the art for concentrating during testing beef samples. "Tu et al. (78) detected pathogenic E. coli O157:H7 in beef hamburger with the use of IMS and ATP measurement. After 6 h of enrichment at 37°C, inoculated beef samples (0.9 ml each) were filtered to remove large particles prior to 10 min of incubation with ca. 10^5 magnetic beads coated with anti–*E. coli* O157:H7 (Dynabeads, Dynal Inc., Oslo, Norway). Following incubation, the magnetic beads were collected with a Dynal magnetic concentrator and resuspended in 0.9 ml of Trypticase soy broth for ATP analysis." Beniot, 1941. "Hudson et al. (26) used IMS to directly separate and concentrate L. monocytogenes from ham samples prior to detection by PCR. IMS was used on the basis of the commercial availability of anti-Listeria magnetic beads (Dynal Inc.) to avoid time-consuming cultural enrichment steps and to remove PCR inhibitors." Beniot, 1941-42. Cudjoe investigates the use of immunomagnetic beads in pooling methods. In a study designed to evaluate the performance of Dynabeads® Anti-Salmonella, immunomagnetic separation (IMS) followed by plating (IMS-Plating) proved far superior to the conventional ISO *Salmonella* methodology and the Modified Semi-solid Rappaport-Vassiliadis (MSRV) method. *Salmonella* species were isolated and detected from 135 out of the 180 diverse poultry samples by IMS analysis as against 98 by the conventional method and 33 by the MSRV technique. All results were confirmed biochemically and serologically. It appeared from the data generated that some of the salmonellae isolated using IMS were greatly inhibited in Rappaport-Vassiliadis enrichment broth at 42°C and to a lesser extent in selenite cystine broth at 37°C. A moderately selective plating medium like XLD proved to be better in isolating these possible sensitive wild-type strains of salmonellae than the more selective BGA.

	Cudjoe, Abstract. *Samples*. One hundred and eighty (180) diverse poultry samples, consisting of 40 pools of cloacal/faecal swabs (5 swabs/pool; 5 pools samples/flock) from eight different flocks, 10 raw chicken breast meat samples, 10 pooled egg shell samples, 60 raw chicken liver samples and 60 chicken neck skins sampled from the slaughter line, were used in this study. All samples were obtained from a poultry slaughter and processing plant in the Netherlands. A pool of five swab samples was pre-enriched in 10 ml of buffered peptone water (BPW; Difco Laboratories, Detroit, Michigan, USA) at 37°C for 16–20 h, whilst 25 g of each of the other samples above, were pre-enriched similarly in 225 ml of BPW and examined as detailed below. Cudjoe, 160.
Claim 32	**Disclosure of ICMSF and Hughes**
The method of claim 7, wherein the more sensitive protocol comprises use of a concentration step to concentrate the target microbe(s), in combination with the same test protocol used to test the pooled modular composite sample.	ICMSF, alone or in combination with Hughes, discloses Claim 7, upon which this claim relies. See above re Claim 7. ICMSF discloses the use of "immunomagnetic beads to concentrate cells." ICMSF, 318. A POSITA would consider this a well-known technique before the priority date. Hughes uses an EIA method followed by a more sensitive protocol—streaking on selective media. Hughes, 635.

	Each sample was analyzed by the BAM method (7th Ed.) and the TSVIA method using the enrichment protocol described in section **F** of the method, *Preparation of Sample*. Equal portions (0.5–1 mL) from each of the 2M broths arising from the 2 selective-enrichment broths were combined in a small screw-capped tube and heat treated in boiling water for 15 min prior to performing the TSVIA. Confirmation for the immunoassay was by streaking from the M broth culture onto selective agar, as specified in the BAM reference culture method. Hughes, 635. A POSITA would find that modifying Hughes based on D'Haese ensures a more sensitive test is used for further testing. A POSITA would have recognized the concentration step could be employed to provide a more sensitive protocol for further testing. A POSITA would understand that retesting the non-pooled, singular samples is an inherently more sensitive process because the sample is not diluted with other samples in the testing pool. Therefore, the retesting of a singular sample is more concentrated and therefore, more sensitive. *See also, generally*, Jarvis. Fratamico discusses the rapid isolation of target microorganisms using an immunomagnetic separation method. Fratamico, 105. Fratamico discloses that use of immunomagnetic beads resulted in "an increase in the level of E. coli B1409 in the sample," as well as "an increase in the number of organisms recovered." *Id.*, 107. Additionally, Fratamico provides detailed instructions for preparing and using

	immunomagnetic bead separation to concentrate a target microbial agent or organism. Fratamico developed "[a] method employing immunomagnetic beads" to concentrate and thereby improve the isolation and detection of E. coli. *Id.* For example, Fratamico was able to use immunomagnetic beads to isolate *E. coli* strains from enrichment cultures of raw ground beef "when the initial inoculum level was 1 cfu g-1." *Id.*, 109. Fratamico further teaches that "[t]he percentage of bacteria recovered increased with increasing amounts of beads." *Id.*, 108. Consequently, Fratamico "demonstrates that immunomagnetic separation [using immunomagnetic beads] is a simple and rapid technique" for concentrating a microbe for isolation and detection. *Id.*, 109. These teachings of Fratamico would have motivated a POSA to modify the combination of ICMSF and Gombas and ICMSF and Hughes to use immunomagnetic beads to concentrate microbes in a modular composite sample to benefit from accurate tests. Use of immunomagnetic beads was already known in the art for concentrating during testing beef samples. "Tu et al. (78) detected pathogenic E. coli O157:H7 in beef hamburger with the use of IMS and ATP measurement. After 6 h of enrichment at 37°C, inoculated beef samples (0.9 ml each) were filtered to remove large particles prior to 10 min of incubation with ca. 10^5 magnetic beads coated with anti–*E. coli* O157:H7 (Dynabeads, Dynal Inc., Oslo, Norway). Following incubation, the magnetic beads were collected with a Dynal magnetic concentrator and resuspended in 0.9 ml of Trypticase soy broth for ATP analysis." Beniot, 1941. "Hudson et al. (26) used IMS to directly separate and concentrate L. monocytogenes from ham samples prior to detection by PCR. IMS was used on the basis of the commercial availability of anti-Listeria magnetic beads (Dynal Inc.) to avoid time-consuming cultural enrichment steps and to remove PCR inhibitors." Beniot, 1941-42.

	Cudjoe investigates the use of immunomagnetic beads in pooling methods. In a study designed to evaluate the performance of Dynabeads® Anti-Salmonella, immunomagnetic separation (IMS) followed by plating (IMS-Plating) proved far superior to the conventional ISO *Salmonella* methodology and the Modified Semi-solid Rappaport-Vassiliadis (MSRV) method. *Salmonella* species were isolated and detected from 135 out of the 180 diverse poultry samples by IMS analysis as against 98 by the conventional method and 33 by the MSRV technique. All results were confirmed biochemically and serologically. It appeared from the data generated that some of the salmonellae isolated using IMS were greatly inhibited in Rappaport-Vassiliadis enrichment broth at 42°C and to a lesser extent in selenite cystine broth at 37°C. A moderately selective plating medium like XLD proved to be better in isolating these possible sensitive wild-type strains of salmonellae than the more selective BGA. Cudjoe, Abstract. *Samples*. One hundred and eighty (180) diverse poultry samples, consisting of 40 pools of cloacal/faecal swabs (5 swabs/pool; 5 pools samples/flock) from eight different flocks, 10 raw chicken breast meat samples, 10 pooled egg shell samples, 60 raw chicken liver samples and 60 chicken neck skins sampled from the slaughter line, were used in this study. All samples were obtained from a poultry slaughter and processing plant in the Netherlands. A pool of five swab samples was pre-enriched in 10 ml of buffered peptone water (BPW; Difco Laboratories, Detroit, Michigan, USA) at 37°C for 16–20 h, whilst 25 g of each of the other samples above, were pre-enriched similarly in 225 ml of BPW and examined as detailed below. Cudjoe, 160.
Claim 33	**Disclosure of ICMSF and Hughes**
The method of claim 24, wherein the meat or poultry specimens are meat trim pieces or poultry trim pieces, respectively.	ICMSF, alone or in combination with Hughes, discloses Claim 24, upon which this claim relies. See above re Claim 24.

	ICMSF indicates "[t]rimmings to be used for ground beef could be pretested for E. coli O157:H7 by various sampling plans." ICMSF, 323. ICMSF indicates "[r]esearch has validated that up to fifteen 25 g analytical units of ground beef or trimmings can be composited without significant loss in E. coli O157:H7 detection sensitivity." *Id.*, 327 *see also id.*, 226, 314, 320, and 324. ICMSF includes many other references to testing lots of beef or poultry, two of the most common types of meat in commerce. *Id.* at 114 (§ 6.3), 184–191 (§§ 10.2–10.6), 225–226 (§ 12.1), 285 (§ 16.1), 313–330 (Chapter 17). ICMSF is relevant to any kind of food product including beef or poultry.
Claim 34	**Disclosure of ICMSF and Hughes**
The method of claim 24, wherein the meat is that of beef, pork, sheep or bison.	ICMSF, alone or in combination with Hughes, discloses Claim 24, upon which this claim relies. See above re Claim 24. ICMSF indicates "[t]rimmings to be used for ground beef could be pretested for E. coli O157:H7 by various sampling plans." ICMSF, 323. ICMSF indicates "[r]esearch has validated that up to fifteen 25 g analytical units of ground beef or trimmings can be composited without significant loss in E. coli O157:H7 detection sensitivity." *Id.*, 327 *see also id.*, 226, 314, 320, and 324. ICMSF includes many other references to testing lots of beef or poultry, two of the most common types of meat in commerce. *Id.* at 114 (§ 6.3), 184–191 (§§ 10.2–10.6), 225–226 (§ 12.1), 285 (§ 16.1), 313–330 (Chapter 17). ICMSF is relevant to any kind of food product including beef or poultry.
Claim 35	**Disclosure of ICMSF and Hughes**
[35pre] A method of sampling, and testing for microbe(s) in multiple separate test lots, comprising:	To the extent the preamble is limiting, as discussed herein, ICMSF, alone or in combination with Hughes, discloses a method of sampling, and testing for microbe(s) in multiple separate test lots.

	Hughes, 646. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638.
[35a] a) separately collecting multiple independent samples from each of multiple separate single-unit production lots, wherein each separate single-unit production lot is separately sampled by taking said multiple independent samples thereof;	ICMSF, alone or in combination with Hughes, discloses separately collecting multiple independent samples from each of multiple separate single-unit production lots, wherein each separate single-unit production lot is separately sampled by taking said multiple independent samples thereof. "Many different types of samples are collected and submitted to the laboratory for analysis. Some are sample units from lots or consignments of food or ingredients for lot acceptance determination. . . . A sample unit is a small portion of the lot in which the distribution of both numbers and types of microorganisms should be representative of the whole lot." ICMSF, 255. The lots may be beef or poultry. *Id.*, 184-91, 226, 230. Thus, ICMSF discloses collecting a plurality of samples or sample units (other synonyms include portions, specimens, and sub-samples) from lots. ICMSF describes collecting from a lot or lots: "If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." *Id.*, 119.

	Additionally, ICMSF indicates a consignment may be "a very large quantity of food" and may be "composed of several lots, representing for example, different days of production from the same plant." *Id.*, 119, 120; *see also* Applicant Admitted Prior Art ("AAPA") #3-#5.

6.11 WHAT IS A REPRESENTATIVE SAMPLE?

A *representative sample* reflects, as far as is possible, the composition of the lot from which it is drawn.

How then should a representative sample be drawn? It is important to avoid bias and to draw a sufficient number of sample units to confidently make a judgment about a lot. Sampling at random is the universally recognized way of avoiding bias and is more reliable than consciously trying to draw the sample units from the various parts of a lot. The units (cartons or containers, particular weights of solid, or volumes of liquid) are drawn by using random numbers. There is, of course, no guarantee that a random sample has characteristics identical with those of the lot, but the randomness of sampling is the basis for calculation of the probability that a sample will give a biased result.

It is also possible, and may be desirable, to use a *stratified random sampling* approach, drawing a random sample of a given number of sample units from each *stratum* (e.g., each sublot or carton). The distribution of sample units on strata should correspond to the distribution of lot units on strata. This means that if each stratum contains the same quantity as every other one, the number of sample units per stratum should be the same. Otherwise, they should differ just as the proportions of the lot contained in the strata differ. Stratification is a device for handling known sources of variation and may be used where one has prior knowledge that the consignment is potentially not of uniform quality. A consignment might not be of uniform quality if portions of it are shipped in different vehicles or in different holds of a vessel, or if it is known that it is really composed of several lots representing, for example, different days of production from the same plant or different plants of the same company. The results for different strata should be assessed separately and then pooled if they appear to be homogeneous.

The calculation of acceptance probabilities is based on the assumption that random samples have been drawn and analyzed. Where physical or practical constraints prevent random sampling, such calculated probabilities may not be valid. Their reliability depends on how close reality approaches the ideal situation of random sampling. This fact should be borne in mind when subsequently interpreting results.

ICMSF, 119-20 (annotated).

6.10 WHAT IS A LOT?

Ideally, a lot is a quantity of food or food units produced and handled under uniform conditions. Under certain circumstances, it has been shown that the logarithms of the counts from a batch of food are likely to be normally distributed (Baird-Parker, 1980; Kilsby & Baird-Parker, 1983 [their Table 2]; Ingram & Roberts, 1976). The implication is that there is homogeneity within a lot, which, when considering microbial levels and distributions, rarely occurs in practice. In most instances, the distribution of microorganisms within lots of food is heterogeneous. Even in food factories, this heterogeneity makes the interpretation of sample results difficult, and it becomes even more so in circumstances where a lot is less well defined, such as in a consignment of a very large quantity of food.

It is therefore helpful if suppliers can be persuaded to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load).

If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment.

A lot should be composed of food produced with as little variation as possible for a given process or commodity. Because of the uncertainties in identifying a lot commercially, however, the use of the word *lot* in this book is usually in its statistical sense, as a collection of units of a product, the acceptability of which is determined by examining a sample drawn from it.

	ICMSF, 118-19 (annotated). The ICMSF has defined a lot as follows: In the commercial sense, a lot is a quantity of food supposedly produced under identical conditions, all packages of which would normally bear a lot number that identifies the production during a particular time interval, and usually from a particular line, retort, or other critical processing unit. Statistically, a lot is considered as a collection of units of a product from which a sample is drawn to determine acceptability of the lot (ICMSF, 1986). In this example, the maximum size of a lot consists of all the ground beef produced on common equipment from cleanup to cleanup. Smaller lots could be established (e.g., hourly). ICMSF, 327 (annotated). This definition also reflects the common practice referred to in the IEH Patents at Example II, wherein "[p]referably, the producer defines and produces these single-unit lots in such a fashion as to maximize the information available." '143 Patent,12:52–54. This corresponds with the specification of IEH's Patents which reflects the common practice of producers defining what constitutes a lot grouped for traceability purposes. '143 Patent, 3:5-15, 11:18-22 (AAPA #3), and 12:52-55. Thus, ICMSF discloses collecting a plurality of portions from separate lots. ICMSF describes a lot as "a collection of units of a product." IMCSF, 119. ICMSF further indicates a lot has been defined as: "a quantity of food supposedly produced under identical conditions, all packages of which would normally bear a lot number that identifies the production during a particular time interval, and usually from a particular line, retort, or other critical processing unit. Statistically, a lot is considered as a collection of units of a product from which a sample is drawn to determine acceptability of the lot." ICMSF, 327. ICMSF advocates use of a "statistically based sampling plan." *Id.*, 113-15, 119-21,

	320, 327, 328. In the sampling plans, "*n* is the number of sample units chosen separately and independently, and . . . *c* is the maximum allowable number of sample units yielding a positive result." *Id.*, 115. ICMSF discloses a wide range for the number of sample units (portions) for a particular microbial agent or organism. For example, ICMSF discusses collecting 15, 30, 60 or 100 sample units from a lot. *Id.*, 320, 326. **6.5 THE SAMPLING PLAN** The sample units so drawn will, after examination, yield results that will be compared with certain criteria to reach a decision as to whether the entire lot should be accepted or rejected (see section 6.9 for explanation of rejection). The particular choice of sampling procedure and the decision criteria make up the *sampling plan*. Proper drawing of samples is essential if the decision making criteria of the sampling plan are to give unbiased results. By sampling randomly, we are able to reduce the risk of making biased decisions. A simple example of a sampling plan follows. Take 10 sample units of a food from a lot and subject them to appropriate laboratory procedure to test for the presence or absence of a microorganism. If two or fewer of the 10 sample units show presence of the organism, that is, give a positive result, then the whole lot of food is acceptable (relative to this organism). But if three or more give a positive result, the whole lot is to be rejected. This plan is described by the use of two terms $n = 10$ and $c = 2$, where n is the number of sample units chosen separately and independently, and where c is the maximum allowable number of sample units yielding a positive result. ICMSF, 115 (annotated).

	6.1 INTRODUCTION It is important to recognize that the management of food safety using the approaches outlined in the first five chapters, based on controlling hazards through Good Hygienic Practices (GHP) and Hazard Analysis Critical Control Point (HACCP), is much more effective than trying to ensure safety by lot acceptance through end-product testing. This chapter discusses the concepts of probability and sampling that determine the limitations of end-product testing. Those concepts form the basis of the design of statistically based sampling plans (Chapter 7) and the establishment of cases (Chapter 8). In Chapters 6 and 7, it is assumed that the microbiological test will give an absolutely accurate result, with no false positives or false negatives. This is not a realistic assumption, and problems arising from sampling, sample transport and preparation for analysis, and the imprecision of microbiological methods are addressed in Chapters 10 and 12 and elsewhere in the text. ICMSF, 113 (annotated); *see also id.*, 115–21 (§§ 6.4–6.13), 319–20 (§ 17.2.3.3), and 326–30 (§§ 17.5.3.1–17.6). ICMSF discusses the number of portions to be taken sufficient for validation of a particular microorganism including *Salmonella* (*id.*, 276 (§ 15.3.3); *see also id.*, 282 (§ 15.6.3)); *Listeria* (*id.*, 309 (§ 16.6.3)), and *E. coli* (*id.*, 318–21 (§§ 17.2.3.2–17.2.3.4), 326 (§ 17.5.3.1)). IEH's AAPA discloses statistical-based sampling plans taking 10-60 sample units. '143 Patent, 6:3-20; *see* AAPA #1, #2, #5, and #6.
[35b] b) separately compositing the collected multiple independent samples from each of the separate test lots to provide a corresponding set of separate composited single-unit production lot samples, wherein each of the separate composited single-unit production lot samples is attributed to a particular corresponding separate single-unit production lot;	ICMSF, alone or in combination with Hughes, discloses separately compositing the collected multiple independent samples from each of the separate test lots to provide a corresponding set of separate composited test lot samples, wherein each of the separate composited single-unit production lot samples is attributed to a particular corresponding separate single-unit production lot. ICMSF states: "To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units of the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. . . . This finding suggests that considerable reductions may

	be made in the cost of laboratory analysis by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, n, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort." ICMSF, 187. To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. A laboratory investigation undertaken for ICMSF (Silliker & Gabis, 1973) indicated that different sized analytical units drawn from a single well-mixed sample unit of dried food give very similar results. A subsequent investigation (Gabis & Silliker, 1974) using foods of high moisture content (including eggs, poultry meat, meat, and meat products) showed comparable reliability in the use of composite analytical units; for example, examination of three composites of 20 × 25 g analytical units gave the same result as examination of all 60 analytical units individually (the total amount examined being 1500 g in both instances). This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n*, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort. ICMSF, 187.

10.5.1 Conceivable Alternative Plans

As indicated above, for an infectious agent such as *Salmonella*, there are strong reasons for adopting sampling plans in which $c = 0$:

1. It is philosophically objectionable to some people to accept a lot containing a recognized pathogen, regardless of how the food is to be used in a manufacturing process or by the consumer.
2. There is the very great technical advantage of combining many analytical units for bacteriological examination in systems with $c = 0$, because a single positive decides the outcome.
3. The plan with $c = 1$ (or more) would always require the examination of (many) more analytical units, for equal probability of acceptance, than a plan with $c = 0$.
4. Even if the indicated probabilities of plans with $c = 0$ versus $c = 1$ are the same, their implications are not. For example, when a lot is accepted with the plan $n = 60$, $c = 0$, it is possible (though not certain) that the lot may not be contaminated at all. But using the plan $n = 95$, $c = 1$ and accepting one positive, it is known that the lot certainly is contaminated (though the probability that a lot with 5% of analytical units contaminated would be accepted is in both instances $P_a = 0.05$).

ICMSF, 188 (annotated). ICMSF also provides an example of "[u]sing a sampling plan involving n = 15 (25 g analytical units) and a composite of 375 g." *Id.*, 195 (§ 10.7.2). ICMSF also states "[f]or qualitative analyses, 25 g analytical units are frequently analyzed individually or as composites of 100 g, 200 g, or more." *Id.*, 232 (§ 12.5.2).

The ICMSF incorporates two references, Silliker & Gabis (1973) and Gabis & Silliker (1974). Silliker notes that "[c]ompositing of multiple samples (lumping) and wet compositing of pre-enrichment cultures have been tested as economical approaches to *Salmonella* control." Silliker, 475.

Silliker further notes that "[b]oth approaches are being used widely by the food industry in the United States." *Id.*.

	To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. A laboratory investigation undertaken for ICMSF (Silliker & Gabis, 1973) indicated that different sized analytical units drawn from a single well-mixed sample unit of dried food give very similar results. A subsequent investigation (Gabis & Silliker, 1974) using foods of high moisture content (including eggs, poultry meat, meat, and meat products) showed comparable reliability in the use of composite analytical units; for example, examination of three composites of 20 × 25 g analytical units gave the same result as examination of all 60 analytical units individually (the total amount examined being 1500 g in both instances). This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n*, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort. "There is the very great technical advantage of combining many analytical units for bacteriological examination." ICMSF, 188. ICMSF also describes composites prepared by combining "15 (25 g analytical units)" and "composites of 100 g, 200 g, or more." *Id.,* 195, 232; *see* AAPA #5. ICMSF states, "All steps up to the analysis must be controlled . . . to allow reliable traceability." ICMSF, 227. ICMSF indicates lot samples are traceable to a particular corresponding lot: "It is therefore helpful . . . to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load). . . . Treating the individual production

	batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119.
[35c] c) enriching of the separate composited single-unit production lot samples to provide a set of separate composited single-unit production lot samples enriched for the target microbe(s)	ICMSF, alone or in combination with Hughes, discloses enriching each of the separate composited test lot samples to provide a set of separate composited test lot samples enriched for the target microbe(s). ICMSF states: To reduce laboratory effort . . . it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. ICMSF, 187. Also, ICMSF describes preparing composites of analytical units then diluting "for qualitative (enrichment)" analysis. *Id.,* 231-32; *see also* AAPA #5, #7. For practical reasons, however, analytical units of 10 g up to 50 g are typical for quantitative methods and are diluted 1:10. For qualitative analyses, 25 g analytical units are frequently analyzed individually or as composites of 100 g, 200 g, or more. ICMSF, 232 (annotated). Preparation of the first dilution, either for qualitative (enrichment) or quantitative (direct) analyses, can have a marked impact on the microbial population in the sample. This is often due to changes in the physico-chemical characteristics of the suspension as compared to the food matrix. Where the food does not confer protection, the composition of the diluent is important to prevent or minimize shock/injury to the microorganisms that can result from rapid changes in the ionic concentrations. ICMSF, 232 (annotated). ICMSF also describes strategic use of non-selective enrichment to resuscitate microorganisms and selective enrichments for growing microorganisms. *Id.*, 232–33 (§ 12.6); *see e.g.*, *id.,* 233 ("use of nonselective medium . . . to enhance recovery of injured or stressed cells."). ICMSF, § 12.6 also describes optimizing the incubation period. S*ee e.g.*, *id.,* 232.

	It was well known long before the priority date to use non-selective enrichments after dry compositing and prior to wet compositing as evidenced by Price, Silliker, Gabis, and MLG. A POSITA would also know to use selective enrichments after dry compositing and prior to wet compositing as disclosed by ICMSF and Hughes. A POSITA would find that incubating so microorganisms are detectable and at least substantially uniform in the selective enrichment broth was considered the standard practice in official methods because the methods otherwise fail. For example, D'Aoust states "standard methodology recommends incubation of selective enrichment broths." D'Aoust, 372. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638.

	(a) *Pre-enrichment.*—Pre-enrich product in noninhibitory broth to initiate growth of *Salmonella* spp. Methods used may vary with product and should be performed as indicated in **967.26A** or BAM, 7th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, chapter 5, section C. Pre-enrichment broths should be incubated at 35°–37°C. (b) *Selective enrichment.*—Transfer 0.1 mL incubated pre-enrichment mixtures to Rappaport-Vassiliadis R10 broth (9.9 mL) and 1 mL to TT broth (9 mL). For all foods other than raw foods or foods having a high microbial load, incubate 6–8 h at 41°–43°C. Selective enrichments of raw foods or foods having a high microbial load must be incubated 16–20 h at 41°–43°C. An air incubator is suitable for this purpose. Hughes, 636. Hughes instructs that, after pre-enrichment, portions of the "incubated pre-enrichment mixtures" should be transferred to two separate selective enrichment broths, Rappaport-Vassiliadis R10 broth and TT broth, and incubated for 16-20 hours at 41°-43°C. Hughes, 636, 638. Additionally, Hughes instructs that these selective broths should be removed from incubation and mixed by hand or by Vortex mixer. Hughes, 638. Hughes further instructs to then remove 1 mL from the TT tube and 1 mL from the Rappaport-Vassiliadis R10 tube and transfer to separate, sterile M broths. *Id.,* 636, 638. The separate M broths are then incubated ("16-20 h at 35°-37°C" for "all foods other than raw foods or foods having a high microbial load" or "6 h at 35°-37°C" for "raw foods or foods having a high microbial load"). *Id.*, 638.

	A POSITA would find that the long incubation at an elevated temperature and mixing ensures the target microbial agent or organism reaches detectable levels and becomes substantially uniform throughout the separate lot samples.
[35d] d) removing portions of each separate enriched composited single-unit production lot sample, and combining the removed portions to provide a pooled modular composite sample; and	ICMSF describes compositing the enriched lot samples: "it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. . . . This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n,* with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort." ICMSF, 187. AAPA #5-#7. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Hughes also discloses a method of removing portions of each enriched separate test lot sample, and combining the removed portions to provide a modular composite sample. Hughes instructs to "[c]ombine equal volumes (0.5-1 mL) from each M broth tube in a clean screw-cap tube." Hughes, 638.

	that has been warmed to 35°–37°C. Also remove 1 mL from Rappaport-Vassiliadis R10 tube and transfer to a separate tube of prewarmed M broth (10 mL). For all foods other than raw foods or foods having a high microbial load, incubate M broth tubes 16–20 h at 35°–37°C and return selective enrichment broth tubes to 41°–43°C incubator and incubate for additional 16–18 h. For raw foods or foods having a high microbial load, incubate M broth tubes 6 h at 35°–37°C and return selective enrichment broth tubes to 41°–43°C and incubate for an additional 6 h. (**d**) *Preparation of sample for EIA analysis.*—Remove M broth tubes from incubation and mix tubes by hand or Vortex mixer. Combine equal volumes (0.5–1 mL) from each M broth tube in a clean screw-cap tube and heat in boiling H_2O bath or in flowing steam 15 min. Refrigerate (2°–8°C) remaining M broth and TT and Rappaport-Vassiliadis R10 tubes from (**c**) for culture confirmation of any EIA-positive samples (*see* **967.26B, 967.26C, 967.27** and **967.28**). Cool heated M broths to 25°–37°C prior to analysis. Hughes, 638. Hughes 2003 confirms the pooling of enrichments. Hughes 2003, 775-76.
[35e.1] e) testing of the pooled modular composite sample, using a suitable detection assay, for the target microbe(s),	ICMSF describes testing enrichment cultures of pooled modular composite samples with suitable detection assays (ICMSF, 187) and "presence/absence tests" (ICMSF, 233). Suitable detection assays were established in Silliker and Gabis as discussed in ICMSF. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of

	sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Hughes states testing of the composite sample is "by an enzyme immunoassay (EIA) method." Hughes, 635, 638. These assay types were "art recognized." '143 Patent, 2:66-3:3 (AAPA #7).
[35e.2] wherein when such testing is negative all of said samples that were composited to form the separate composited single-unit production lot samples are deemed negative for the target microbe(s) and each of the multiple separate single-unit production lots is validated, and	ICMSF describes correlating lots in a consignment with lot samples so a negative or positive test result enables a lot to be validated or rejected. ICMSF, 118-19. ICMSF describes sampling plans in which "c is the maximum allowable number of sample units yielding a positive result." ICMSF, 115. For many scenarios, the requirement is c = 0, which indicates the testing is negative. *Id.*, 118, 183-90, 309, 325, 326. A negative test result (*id.*, 113-15, 325, 326) is the expected and desired outcome to determine meat is not contaminated and is safe for consumption (*id.*, 184-89) such that a lot is validated and it is "commercially possible to market" (*id.*, 185) the meat.

6.10 WHAT IS A LOT?

Ideally, a lot is a quantity of food or food units produced and handled under uniform conditions. Under certain circumstances, it has been shown that the logarithms of the counts from a batch of food are likely to be normally distributed (Baird-Parker, 1980; Kilsby & Baird-Parker, 1983 [their Table 2]; Ingram & Roberts, 1976). The implication is that there is homogeneity within a lot, which, when considering microbial levels and distributions, rarely occurs in practice. In most instances, the distribution of microorganisms within lots of food is heterogeneous. Even in food factories, this heterogeneity makes the interpretation of sample results difficult, and it becomes even more so in circumstances where a lot is less well defined, such as in a consignment of a very large quantity of food.

It is therefore helpful if suppliers can be persuaded to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load).

If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment.

A lot should be composed of food produced with as little variation as possible for a given process or commodity. Because of the uncertainties in identifying a lot commercially, however, the use of the word *lot* in this book is usually in its statistical sense, as a collection of units of a product, the acceptability of which is determined by examining a sample drawn from it.

ICMSF, 118-19 (annotated).

	Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Because Hughes explains the steps to be taken when testing is positive (*see* ie.3), a POSITA would also expect negative testing results. Hughes, 638. Determining meat is safe for consumption so it may be released into commerce is the well-understood point of testing meat. Moreover, the recitation of a lot being validated for "release . . . into commerce" is not limiting. Prosecution History for Reexamination Control No. 95/011,294).
[35e.3] wherein when such testing is positive, each of the individual separate composited single-unit production lot samples that were used to form the pooled modular composite sample are individually tested to determine which of the separate composited single-unit production lot samples is positive for the target microbe(s),	The combination of ICMSF and Hughes describes retaining a portion of the enriched lot samples (steps (b)-(d)). "Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119. "There is often a desire to retest retained samples." ICMSF, 191. If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment. ICMSF, 119 (annotated).

	ICMSF discloses validating for release into commerce. ICMSF, 113–15 (§§ 6.2–6.6), 118–19 (§§ 6.9–6.10), 184–85 (§ 10.2), 325–27 (§§ 17.5.3–17.5.3.4); *see also id.*, 186–89 (§ 10.4–10.6)) according to a statistically-based sampling plan (*id.*, 113 (§ 6.1); *see also id.*, 115–18 (§§ 6.4–6.8), 184 (§ 10.2), 319–21 (§§ 17.2.3.3–17.2.3.4), 326–30 (§§ 17.5.3.1–17.6), as described regarding steps (a) and (e)(2). Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Hughes explicitly teaches retention of the enriched test lot samples for further testing after a positive result. Hughes provides instructions to refrigerate the "remaining M broth and TT and Rappaport-Vassiliadis R10 tubes [that were enriched] for culture confirmation of any EIA-positive samples." Hughes, 638. Hughes refers to the results as the "confirmed positive samples" (Hughes, 635) and also states that diagnostic reagents are "necessary for culture confirmation of presumptive EIA tests." Hughes, 636. Hughes indicates "[e]nrichment broths and M broths from samples positive by an enzyme immunoassay (EIA) method must be streaked on selective media." *Id.*, 635. Culture confirmation using streaking is more sensitive than the EIA method, as was commonly understood and confirmed by the teachings of D'Haese. *See generally*, D'Haese.

	Each sample was analyzed by the BAM method (7th Ed.) and the TSVIA method using the enrichment protocol described in section **F** of the method, *Preparation of Sample*. Equal portions (0.5–1 mL) from each of the 2M broths arising from the 2 selective-enrichment broths were combined in a small screw-capped tube and heat treated in boiling water for 15 min prior to performing the TSVIA. Confirmation for the immunoassay was by streaking from the M broth culture onto selective agar, as specified in the BAM reference culture method. Hughes, 635. Moreover, the recitation of a lot being validated for "release . . . into commerce" is not limiting. Prosecution History for Reexamination Control No. 95/011,294).
[35e.4] wherein the single-unit production lots corresponding to any negatively testing composited single-unit production lot samples are validated.	Hughes explicitly indicates portions of the enrichment cultures are retained for further testing; thus Hughes has no need for further sampling. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. ICMSF and Hughes disclose selective enrichment so further enrichment is unnecessary. Also, as discussed regarding step (e)(3), Hughes explicitly indicates portions of the enrichment cultures are retained for further testing; thus Hughes has no need for further sampling. The same would be expected for enrichment cultures in ICMSF because retaining samples at each process stage was considered standard

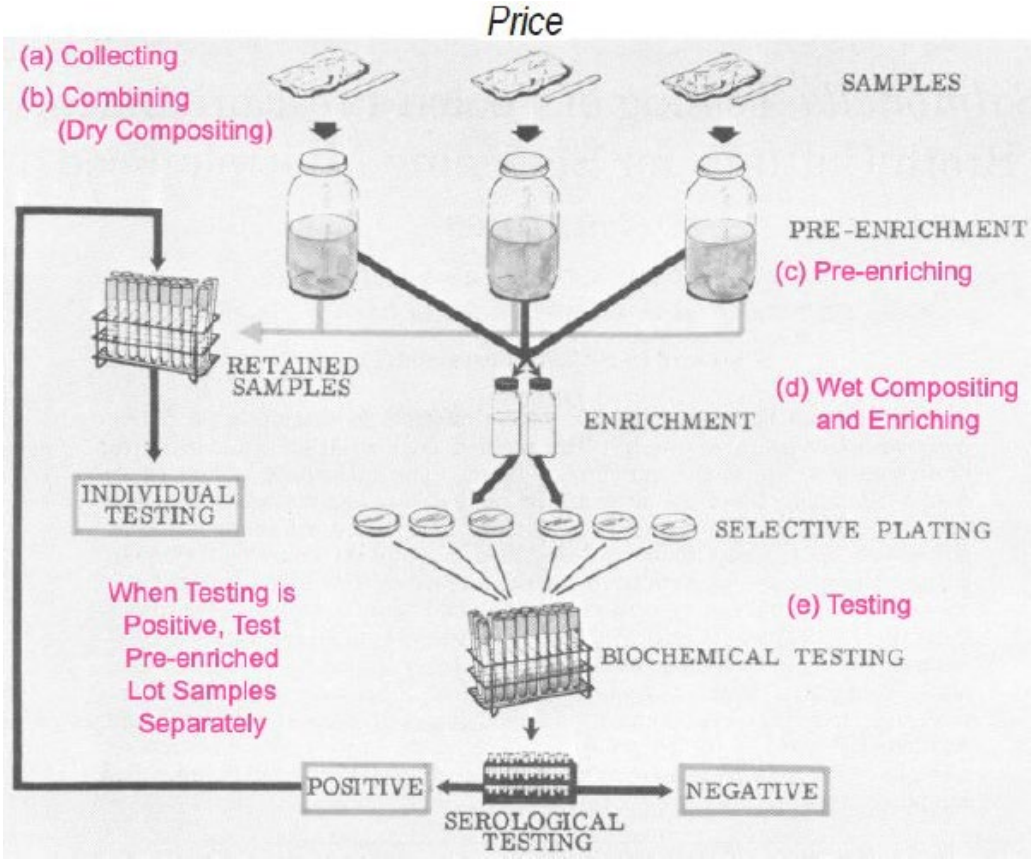
	practice for a POSITA. in flowing steam 15 min. Refrigerate (2°–8°C) remaining M broth and TT and Rappaport-Vassiliadis R10 tubes from (c) for culture confirmation of any EIA-positive samples (*see* **967.26B, 967.26C, 967.27** and **967.28**). Cool heated M broths to 25°–37°C prior to analysis. Hughes, 638. Thus, the negative limitation of step (e)(4) is disclosed by and is obvious based on the combination of ICSMF and Hughes. *Id.*
Claim 36	**Disclosure of ICMSF and Hughes**
The method of claim 35, wherein removing portions in step d) is removing equal portions, aseptically, so as to preclude contamination of the enriched separate composited single-unit production lot samples.	ICMSF, alone or in combination with Hughes, discloses Claim 35, upon which this claim relies. See above re Claim 35. ICMSF describes aseptically withdrawing and weighing sample units into the appropriate diluent. ICMSF, 231, 232. Hughes instructs that after enrichment "[c]ombine equal volumes (0.5-1 mL) from each M broth tube in a clean screw-cap tube." Hughes, 638. Moreover, a POSITA would consider this to be standard practice. A POSITA would have aseptically removed equal volume portions of each enriched separate test lot sample.

EXHIBIT C3

Invalidity of U.S. Patent No. 8,822,143 in light of Price, ICMSF & Hughes

National Beef denies that any of its microbiological testing methods infringe any claim of U.S. Patent No. 8,822,143 ("the '143 Patent"). If the claims of the '143 Patent were construed so broadly as to cover the microbiological testing methods used by National Beef, they would be invalid at least in view of W.R. Price et al., "*Salmonella* Testing of Pooled Pre-Enrichment Broth Cultures for Screening Multiple Food Samples," Appl. Microbiol., vol. 23, no. 4, pages 679-682 (Apr. 1972) ("Price"); INT'L COMM'N ON MICROBIOLOGICAL SPEC'S FOR FOODS (ICMSF), Microorganisms in Foods 7: Microbiological Testing in Food Safety Management (Kluwer Academic/Plenum Publishers, 2002) ("ICMSF"); and Denise Hughes et al., *Salmonella in Foods—A New Enrichment Procedure for Use with the TECRA Salmonella Visual Immunoassay: Collaborative Study*, 82 J. AOAC Int'l 634–647 (1999) ("Hughes"), which anticipate and/or disclose the limitations of the '143 Patent under at least pre-AIA 35 U.S.C. § 102(a), pre-AIA 35 U.S.C. § 102(b), pre-AIA 35 U.S.C. §§ 102(f)/(g) and/or pre-AIA 35 U.S.C. § 103. To the extent that Price, ICMSF, and/or Hughes do not expressly disclose one or more limitations of the asserted claims, as they would have to be construed to support IEH's claim of infringement, they disclose the limitations alone or in combination with one or more additional prior art references and/or the knowledge of one of ordinary skill in the art.

Claim 1	**Disclosure of Price, ICMSF, and Hughes**
[1pre] A method of sampling and testing for microbes in multiple separate test lots, comprising:	To the extent the preamble is limiting, as discussed herein, Price, alone or in combination with ICMSF and Hughes, discloses a method of sampling and testing for microbes in multiple separate test lots. Price established that "pre-enrichment broths can be pooled for analysis without loss in sensitivity to *Salmonella* in the individual samples." Price, 679. Price tested dry food materials inoculated (purposefully contaminated) with *Salmonella. Id.*



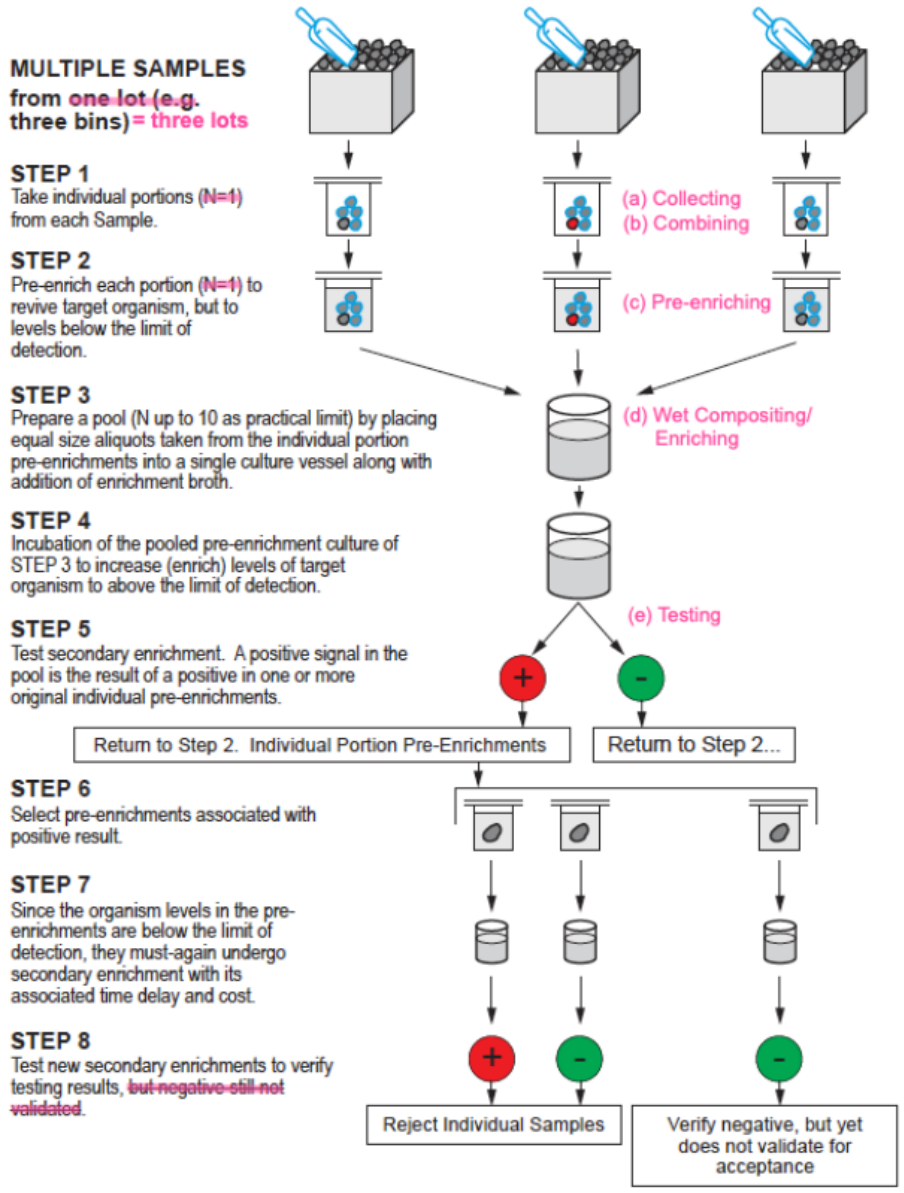
Price, 680 (Figure 1 (annotated)).

ICMSF includes many other references to testing lots of beef or poultry, two of the most common types of meat in commerce. *Id.*, 114 (§ 6.3), 184–91 (§§ 10.2–10.6), 225–226 (§ 12.1), 285 (§ 16.1), 313–30 (Chapter 17). ICMSF is relevant to any kind of food product including beef.

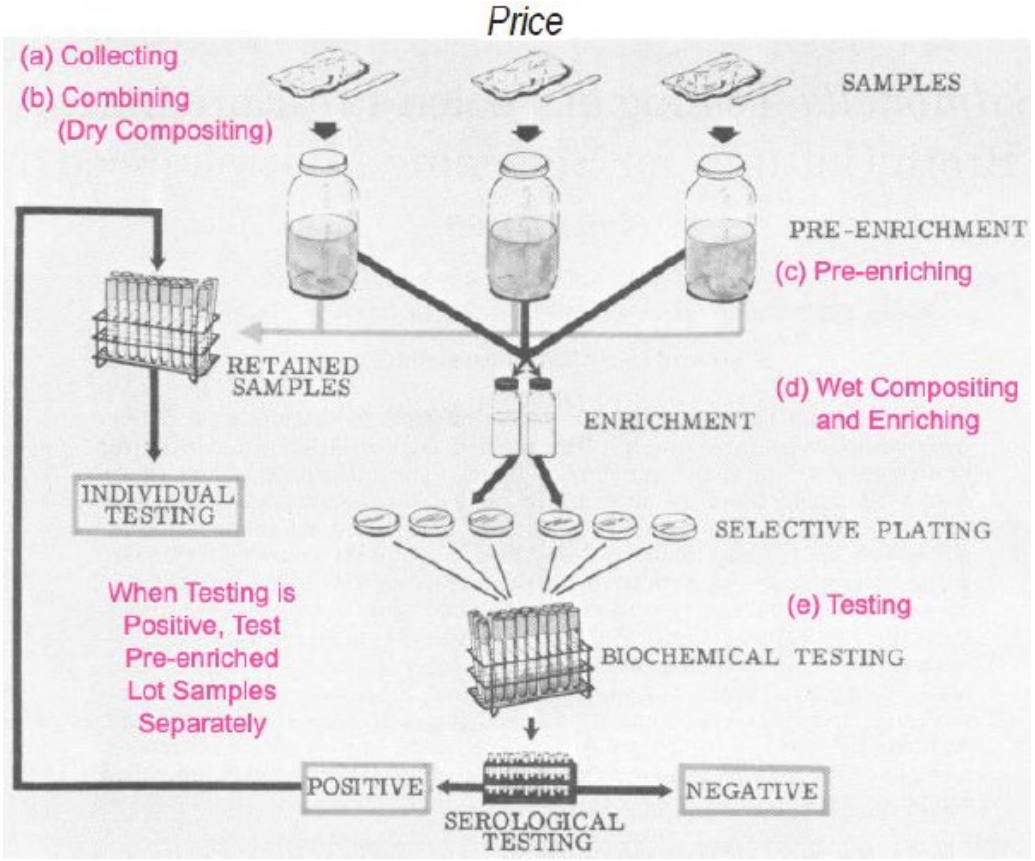
	Hughes discusses turkey (Hughes, Abstract), and a POSITA would be motivated to combine the teachings of ICMSF and Hughes as they both test meat for release into commerce. Hughes discloses testing turkey for *Salmonella*. "Three food types (. . . and raw turkey) were analyzed in the United States." Hughes, 634, 645, Table 8. Table 8 shows results from 12 collaborators for naturally contaminated raw turkey. For the 180 samples tested, using confirmed data, there was one false negative at the low level for the TECRA method. There were 3 false negatives at the low level and 3 false negatives at the high level for the cultural method. The incidence of false-positive results was very high for the cultural method, with a total of 41 false positives, compared with 5 for the TECRA method. It should, however, be taken into account that the comparison was with the method in BAM 7th Ed., not the current 8th Ed. The method for raw flesh food in the BAM 8th Ed. uses elevated temperature with RV and TT broth, which would be more selective. No significant Hughes, 646. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Moreover, the recitation of a lot being validated for "release . . . into commerce" is not limiting. Prosecution History for Reexamination Control No. 95/011,294.

[1a] a) separately collecting multiple independent samples from each of multiple separate test lots, wherein each separate test lot is separately sampled by taking said multiple independent samples thereof;	Price, alone or in combination with ICMSF and Hughes, discloses separately collecting multiple independent samples from each of multiple separate test lots, wherein each separate test lot is separately sampled by taking said multiple independent samples thereof. Price's teachings for collecting a plurality of portions are based on standard practices as described in BAM 1969 (BAM 1969, 1 § 10.04) which cites to JAOAC 1967 (JAOAC, 234). Price, 679, 682. ***Salmonella* analyses were carried out as described in the *Bacteriological Analytical Manual* (BAM) (2) except for the pooling of pre-enrichment broths described in the following paragraph.** Price, 679. IEH's diagram of Price from Prosecution History for Reexamination Control No. 95/001,294 (reproduced below) shows step 1 corresponds to IEH's step (a), with instructions to "[t]ake individual portions . . . from each Sample."

Figure 3: CITED PRIOR ART (PRICE et al, 1972)



	Prosecution History for Reexamination Control No. 95/001,294, 298 (annotated). IEH's diagram also indicates multiple samples are taken from three bins, which is analogous to three lots rather than a single lot because each bin is an assemblage of specimens. The purpose of Jarvis and Price was to determine whether pooling samples reduced the ability to detect contaminated food lots. It would have been commonsense to combine samples from the same bin and subject them to enrichment, since each bin represents a discrete unit of the production run and results obtained from the enriched sample could be attributed to the bin from which the sample was obtained. Prosecution History for Reexamination Control No. 95/001,294, at 1575 (First Board Decision of January 20, 2015 at 16). Additionally, the specification describes a bin of dry food materials (almonds), like those in Price, as one example of a test lot/single unit of production, similar to a combo of beef. '143 Patent, 5:46-51. Price discloses testing of "Dried egg albumen, cocoa, and nonfat dry milk" (Price, 679) and "wheat flour, coconut, and cottonseed flour" (Price, 681).



Price, 680 (Figure 1 (annotated)).

	A method has been described for testing multiple food samples for *Salmonella* without loss in sensitivity. The method pools multiple pre-enrichment broth cultures into single enrichment broths. The subsequent stages of the *Salmonella* analysis are not altered. The method was found applicable to several dry food materials including nonfat dry milk, dried egg albumin, cocoa, cottonseed flour, wheat flour, and shredded coconut. As many as 25 pre-enrichment broth cultures were pooled without apparent loss in the sensitivity of *Salmonella* detection as compared to individual sample analysis. The procedure offers a simple, yet effective, way to increase sample capacity in the *Salmonella* testing of foods, particularly where a large proportion of samples ordinarily is negative. It also permits small portions of pre-enrichment broth cultures to be retained for subsequent individual analysis if positive tests are found. *Salmonella* testing of pooled pre-enrichment broths provides increased consumer protection for a given amount of analytical effort as compared to individual sample analysis. Price, 679. A POSITA would have replaced Price's collection and combination of dry food with ICMSF's collection and combination of portions from a consignment of meat lots in conformance to a statistical-based sampling plan for release into commerce. "Many different types of samples are collected and submitted to the laboratory for analysis. Some are sample units from lots or consignments of food or ingredients for lot acceptance determination. . . . A sample unit is a small portion of the lot in which the distribution of both numbers and types of microorganisms should be representative of the whole lot." ICMSF, 255. The lots may be beef or poultry. *Id.*, 184-91, 226, 230. Thus, ICMSF discloses collecting a plurality of samples or sample units (other synonyms include portions, specimens, and sub-samples) from lots of beef or poultry. ICMSF describes collecting from a lot or lots:

	"If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." *Id.*, 119. Additionally, ICMSF indicates a consignment may be "a very large quantity of food" and may be "composed of several lots, representing for example, different days of production from the same plant." *Id.*, 119, 120; *see also* Applicant Admitted Prior Art ("AAPA") #3-#5.

6.11 WHAT IS A REPRESENTATIVE SAMPLE?

A *representative sample* reflects, as far as is possible, the composition of the lot from which it is drawn.

How then should a representative sample be drawn? It is important to avoid bias and to draw a sufficient number of sample units to confidently make a judgment about a lot. Sampling at random is the universally recognized way of avoiding bias and is more reliable than consciously trying to draw the sample units from the various parts of a lot. The units (cartons or containers, particular weights of solid, or volumes of liquid) are drawn by using random numbers. There is, of course, no guarantee that a random sample has characteristics identical with those of the lot, but the randomness of sampling is the basis for calculation of the probability that a sample will give a biased result.

It is also possible, and may be desirable, to use a *stratified random sampling* approach, drawing a random sample of a given number of sample units from each *stratum* (e.g., each sublot or carton). The distribution of sample units on strata should correspond to the distribution of lot units on strata. This means that if each stratum contains the same quantity as every other one, the number of sample units per stratum should be the same. Otherwise, they should differ just as the proportions of the lot contained in the strata differ. Stratification is a device for handling known sources of variation and may be used where one has prior knowledge that the consignment is potentially not of uniform quality. A consignment might not be of uniform quality if portions of it are shipped in different vehicles or in different holds of a vessel, or if it is known that it is really composed of several lots representing, for example, different days of production from the same plant or different plants of the same company. The results for different strata should be assessed separately and then pooled if they appear to be homogeneous.

The calculation of acceptance probabilities is based on the assumption that random samples have been drawn and analyzed. Where physical or practical constraints prevent random sampling, such calculated probabilities may not be valid. Their reliability depends on how close reality approaches the ideal situation of random sampling. This fact should be borne in mind when subsequently interpreting results.

ICMSF, 119-20 (annotated).

6.10 WHAT IS A LOT?

Ideally, a lot is a quantity of food or food units produced and handled under uniform conditions. Under certain circumstances, it has been shown that the logarithms of the counts from a batch of food are likely to be normally distributed (Baird-Parker, 1980; Kilsby & Baird-Parker, 1983 [their Table 2]; Ingram & Roberts, 1976). The implication is that there is homogeneity within a lot, which, when considering microbial levels and distributions, rarely occurs in practice. In most instances, the distribution of microorganisms within lots of food is heterogeneous. Even in food factories, this heterogeneity makes the interpretation of sample results difficult, and it becomes even more so in circumstances where a lot is less well defined, such as in a consignment of a very large quantity of food.

It is therefore helpful if suppliers can be persuaded to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load).

If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment.

A lot should be composed of food produced with as little variation as possible for a given process or commodity. Because of the uncertainties in identifying a lot commercially, however, the use of the word *lot* in this book is usually in its statistical sense, as a collection of units of a product, the acceptability of which is determined by examining a sample drawn from it.

ICMSF, 118-19 (annotated).

	The ICMSF has defined a lot as follows: In the commercial sense, a lot is a quantity of food supposedly produced under identical conditions, all packages of which would normally bear a lot number that identifies the production during a particular time interval, and usually from a particular line, retort, or other critical processing unit. Statistically, a lot is considered as a collection of units of a product from which a sample is drawn to determine acceptability of the lot (ICMSF, 1986). In this example, the maximum size of a lot consists of all the ground beef produced on common equipment from cleanup to cleanup. Smaller lots could be established (e.g., hourly). ICMSF, 327 (annotated). This definition also reflects the common practice referred to in the IEH Patents at Example II, wherein "[p]referably, the producer defines and produces these single-unit lots in such a fashion as to maximize the information available." '143 Patent,12:52–54. This corresponds with the specification of IEH's Patents which reflects the common practice of producers defining what constitutes a lot grouped for traceability purposes. '143 Patent, 3:5-15, 11:18-22 (AAPA #3), and 12:52-55. Thus, ICMSF discloses collecting a plurality of portions from separate lots. ICMSF describes a lot as "a collection of units of a product." IMCSF, 119. ICMSF further indicates a lot has been defined as: "a quantity of food supposedly produced under identical conditions, all packages of which would normally bear a lot number that identifies the production during a particular time interval, and usually from a particular line, retort, or other critical processing unit. Statistically, a lot is considered as a collection of units of a product from which a sample is drawn to determine acceptability of the lot." ICMSF, 327. ICMSF advocates use of a "statistically based sampling plan." *Id.*, 113-15, 119-21, 320, 327, 328. In the sampling plans, "*n* is the number of sample units chosen separately and independently, and . . . *c* is the maximum allowable number of sample

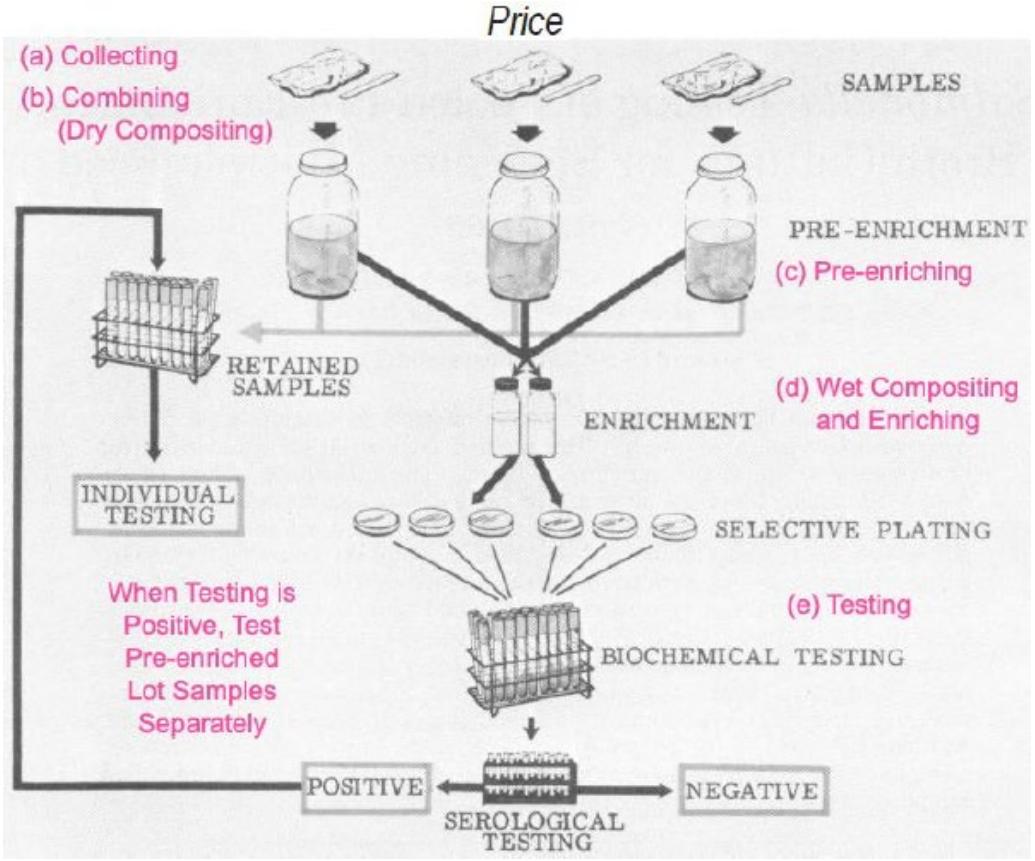
	units yielding a positive result." *Id.*, 115. ICMSF discloses a wide range for the number of sample units (portions) for a particular microbial agent or organism. For example, ICMSF discusses collecting 15, 30, 60 or 100 sample units from a lot. *Id.*, 320, 326. **6.5 THE SAMPLING PLAN** The sample units so drawn will, after examination, yield results that will be compared with certain criteria to reach a decision as to whether the entire lot should be accepted or rejected (see section 6.9 for explanation of rejection). The particular choice of sampling procedure and the decision criteria make up the *sampling plan*. Proper drawing of samples is essential if the decision making criteria of the sampling plan are to give unbiased results. By sampling randomly, we are able to reduce the risk of making biased decisions. A simple example of a sampling plan follows. Take 10 sample units of a food from a lot and subject them to appropriate laboratory procedure to test for the presence or absence of a microorganism. If two or fewer of the 10 sample units show presence of the organism, that is, give a positive result, then the whole lot of food is acceptable (relative to this organism). But if three or more give a positive result, the whole lot is to be rejected. This plan is described by the use of two terms $n = 10$ and $c = 2$, where n is the number of sample units chosen separately and independently, and where c is the maximum allowable number of sample units yielding a positive result. ICMSF, 115 (annotated).

	6.1 INTRODUCTION It is important to recognize that the management of food safety using the approaches outlined in the first five chapters, based on controlling hazards through Good Hygienic Practices (GHP) and Hazard Analysis Critical Control Point (HACCP), is much more effective than trying to ensure safety by lot acceptance through end-product testing. This chapter discusses the concepts of probability and sampling that determine the limitations of end-product testing. Those concepts form the basis of the design of statistically based sampling plans (Chapter 7) and the establishment of cases (Chapter 8). In Chapters 6 and 7, it is assumed that the microbiological test will give an absolutely accurate result, with no false positives or false negatives. This is not a realistic assumption, and problems arising from sampling, sample transport and preparation for analysis, and the imprecision of microbiological methods are addressed in Chapters 10 and 12 and elsewhere in the text. ICMSF, 113 (annotated); *see also id.*, 115–21 (§§ 6.4–6.13), 319–20 (§ 17.2.3.3), and 326–30 (§§ 17.5.3.1–17.6). ICMSF discusses the number of portions to be taken sufficient for validation of a particular microorganism including *Salmonella* (*id.*, 276 (§ 15.3.3); *see also id.*, 282 (§ 15.6.3)); *Listeria* (*id.*, 309 (§ 16.6.3)), and *E. coli* (*id.*, 318–21 (§§ 17.2.3.2–17.2.3.4), 326 (§ 17.5.3.1)). IEH's AAPA discloses statistical-based sampling plans taking 10-60 sample units. '143 Patent, 6:3-20; *see* AAPA #1, #2, #5, and #6. Determining meat is safe for consumption so it is "commercially possible to market" the meat, is the well-understood point of testing meat and is taught by ICMSF. ICMSF, 185-89. Moreover, the recitation of a lot being validated for "release . . . into commerce" is not limiting. Prosecution History for Reexamination Control No. 95/011,294.
[1b] b) separately compositing the collected multiple independent samples from each of the separate test lots to provide a corresponding set of separate composited test	Price, alone or in combination with ICMSF and Hughes, discloses separately compositing the collected multiple independent samples from each of the separate test lots to provide a corresponding set of separate composited test lot samples,

lot samples, wherein each of the separate composited test lot samples is attributed to a particular corresponding separate test lot;	wherein each of the separate composited test lot samples is attributed to a particular corresponding separate test lot. Like ICMSF, the test lot samples in Price are attributable to a particular lot. For example, Price's abstract states that the procedure "permits small portions of pre-enrichment broth cultures to be retained for subsequent individual analysis if positive tests are found," as shown below: A method has been described for testing multiple food samples for *Salmonella* without loss in sensitivity. The method pools multiple pre-enrichment broth cultures into single enrichment broths. The subsequent stages of the *Salmonella* analysis are not altered. The method was found applicable to several dry food materials including nonfat dry milk, dried egg albumin, cocoa, cottonseed flour, wheat flour, and shredded coconut. As many as 25 pre-enrichment broth cultures were pooled without apparent loss in the sensitivity of *Salmonella* detection as compared to individual sample analysis. The procedure offers a simple, yet effective, way to increase sample capacity in the *Salmonella* testing of foods, particularly where a large proportion of samples ordinarily is negative. It also permits small portions of pre-enrichment broth cultures to be retained for subsequent individual analysis if positive tests are found. *Salmonella* testing of pooled pre-enrichment broths provides increased consumer protection for a given amount of analytical effort as compared to individual sample analysis. Price, 679. Price further describes this retention step as enabling "individual tests on the retained sample" to "be made to determine which sample or samples contributed *Salmonella* to the pool" as shown below:

	MATERIALS AND METHODS *Salmonella* analyses were carried out as described in the *Bacteriological Analytical Manual* (BAM) (2) except for the pooling of pre-enrichment broths described in the following paragraph. The procedure for *Salmonella* testing of pooled pre-enrichment broths is illustrated in Fig. 1. Samples are individually pre-enriched followed by transfer from multiple pre-enrichments to single selenite and tetrathionate broths at the enrichment stage. During pooling, a portion of each pre-enrichment is transferred to a sterile culture tube and retained at 4 C for later reference to the individual samples. If the *Salmonella* test of the pooled pre-enrichment broths is positive, individual tests on the retained samples can be made to determine which sample or samples contributed *Salmonella* to the pool. *Id.* (highlighting added). A POSITA would understand Price to implicitly disclose combining the collected confirming plurality of portions based on the inherent mixing involved with dry food materials.

	Salmonella-positive test samples were prepared by blending the dry test food with one of three dry inocula. Inoculum 1 was a dry enzyme drain cleaner which had been found to contain multiple *Salmonella* serotypes including E_1, z_{10}; G, z_{29} (*S. cubana*); and C_1, g complex. Inoculum 2 was a freeze-dried, skim milk suspension of serotypes *S. anatum*, *S. binza*, *S. tennessee*, *S. worthington*, *S. cubana*, *S. braenderup*, and a B_1, g complex. Inoculum 3 consisted of four laboratory isolates of serotypes G, z_{29} (*S. cubana*); E_1, b; E_1, l complex; and C_1, l complex grown in a sterile aqueous suspension of cottonseed flour and freeze dried. Price, 679.



Price, 680 (Figure 1 (annotated)).

A POSITA would have replaced Price's collection and combination of dry food with ICMSF's collection and combination of portions from a consignment of meat lots in conformance to a statistical-based sampling plan for release into commerce.

ICMSF states: "To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units of the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. . . . This finding suggests that considerable reductions may

	be made in the cost of laboratory analysis by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, n, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort." ICMSF, 187. To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. A laboratory investigation undertaken for ICMSF (Silliker & Gabis, 1973) indicated that different sized analytical units drawn from a single well-mixed sample unit of dried food give very similar results. A subsequent investigation (Gabis & Silliker, 1974) using foods of high moisture content (including eggs, poultry meat, meat, and meat products) showed comparable reliability in the use of composite analytical units; for example, examination of three composites of 20 × 25 g analytical units gave the same result as examination of all 60 analytical units individually (the total amount examined being 1500 g in both instances). This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n*, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort. ICMSF, 187.

10.5.1 Conceivable Alternative Plans

As indicated above, for an infectious agent such as *Salmonella*, there are strong reasons for adopting sampling plans in which $c = 0$:

1. It is philosophically objectionable to some people to accept a lot containing a recognized pathogen, regardless of how the food is to be used in a manufacturing process or by the consumer.
2. There is the very great technical advantage of combining many analytical units for bacteriological examination in systems with $c = 0$, because a single positive decides the outcome.
3. The plan with $c = 1$ (or more) would always require the examination of (many) more analytical units, for equal probability of acceptance, than a plan with $c = 0$.
4. Even if the indicated probabilities of plans with $c = 0$ versus $c = 1$ are the same, their implications are not. For example, when a lot is accepted with the plan $n = 60$, $c = 0$, it is possible (though not certain) that the lot may not be contaminated at all. But using the plan $n = 95$, $c = 1$ and accepting one positive, it is known that the lot certainly is contaminated (though the probability that a lot with 5% of analytical units contaminated would be accepted is in both instances $P_a = 0.05$).

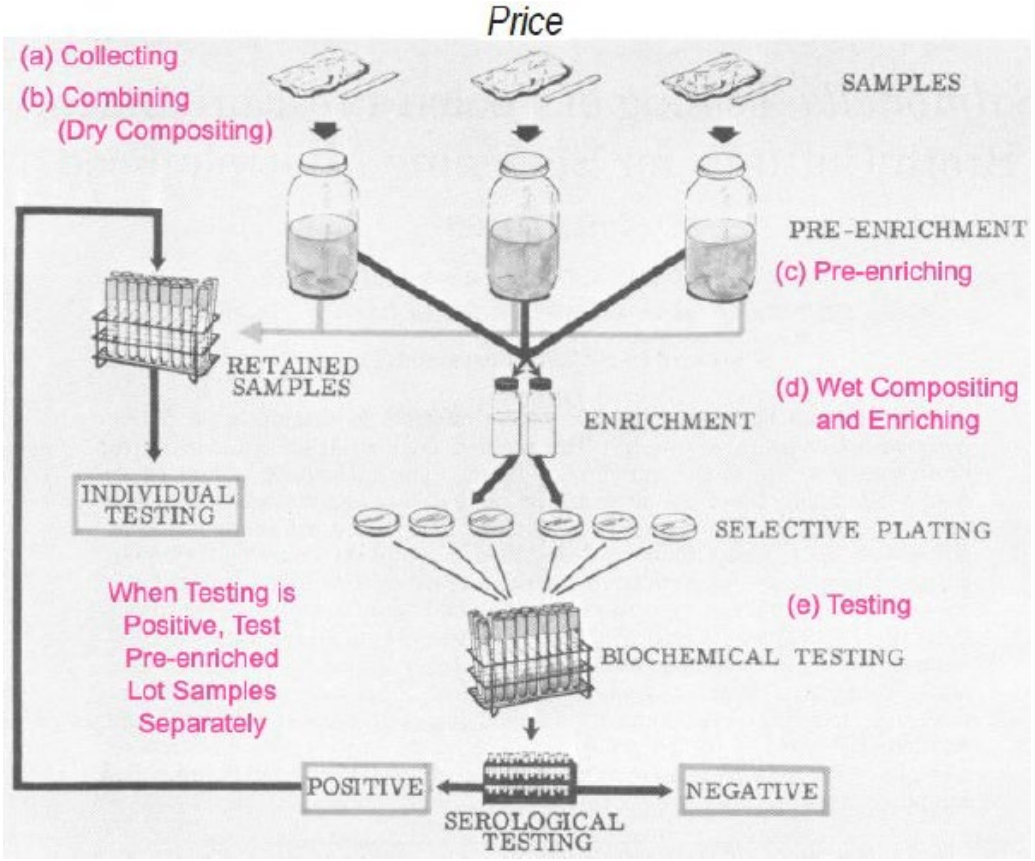
ICMSF, 188 (annotated). ICMSF also provides an example of "[u]sing a sampling plan involving n = 15 (25 g analytical units) and a composite of 375 g." *Id.*, 195 (§ 10.7.2). ICMSF also states "[f]or qualitative analyses, 25 g analytical units are frequently analyzed individually or as composites of 100 g, 200 g, or more." *Id.*, 232 (§ 12.5.2).

The ICMSF incorporates two references, Silliker & Gabis (1973) and Gabis & Silliker (1974). Silliker notes that "[c]ompositing of multiple samples (lumping) and wet compositing of pre-enrichment cultures have been tested as economical approaches to *Salmonella* control." Silliker, 475.

Silliker further notes that "[b]oth approaches are being used widely by the food industry in the United States." *Id.*.

	To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. A laboratory investigation undertaken for ICMSF (Silliker & Gabis, 1973) indicated that different sized analytical units drawn from a single well-mixed sample unit of dried food give very similar results. A subsequent investigation (Gabis & Silliker, 1974) using foods of high moisture content (including eggs, poultry meat, meat, and meat products) showed comparable reliability in the use of composite analytical units; for example, examination of three composites of 20 × 25 g analytical units gave the same result as examination of all 60 analytical units individually (the total amount examined being 1500 g in both instances). This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n*, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort. "There is the very great technical advantage of combining many analytical units for bacteriological examination." ICMSF, 188. ICMSF also describes composites prepared by combining "15 (25 g analytical units)" and "composites of 100 g, 200 g, or more." *Id.,* 195, 232; *see* AAPA #5. ICMSF states, "All steps up to the analysis must be controlled . . . to allow reliable traceability." ICMSF, 227. ICMSF indicates lot samples are traceable to a particular corresponding lot: "It is therefore helpful . . . to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load). . . . Treating the individual production

	batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119.
[1c] c) enriching each of the separate composited test lot samples to provide a set of separate composited test lot samples enriched for the target microbe(s);	Price, alone or in combination with ICMSF and Hughes, discloses enriching each of the separate composited test lot samples to provide a set of separate composited test lot samples enriched for the target microbe(s). Price discloses "[s]amples are individually pre-enriched followed by transfer from multiple pre-enrichments to single selenite and tetrathionate broths at the enrichment stage." Price, 679. These pre-enrichment and enrichment steps inherently disclose an incubation period in which levels of the target microbial agent, in this case, *Salmonella*, grow to detectable levels for testing. Price states "[i]n the multiple pooling of pre-enrichment broth cultures . . . , 1-ml quantities of cultures were transferred, generally, to a volume of enrichment broth." Price, 682. Price also states "[a]lternatively, problems with interfering organisms may warrant the use of direct selective enrichments." *Id.* Based on this statement, the Board noted "there is a suggestion in Price (at 682) . . . of direct enrichment." Prosecution History for Reexamination Control No. 95/001,294, 1576.



Price, 680 (Figure 1 (annotated)).

Price states "[a]lternatively, problems with interfering organisms may warrant the use of direct selective enrichments" and further mentions "a reduction in the incubation time of the pre-enrichment cultures." Price inherently discloses an incubation period of the pre-enrichment samples.

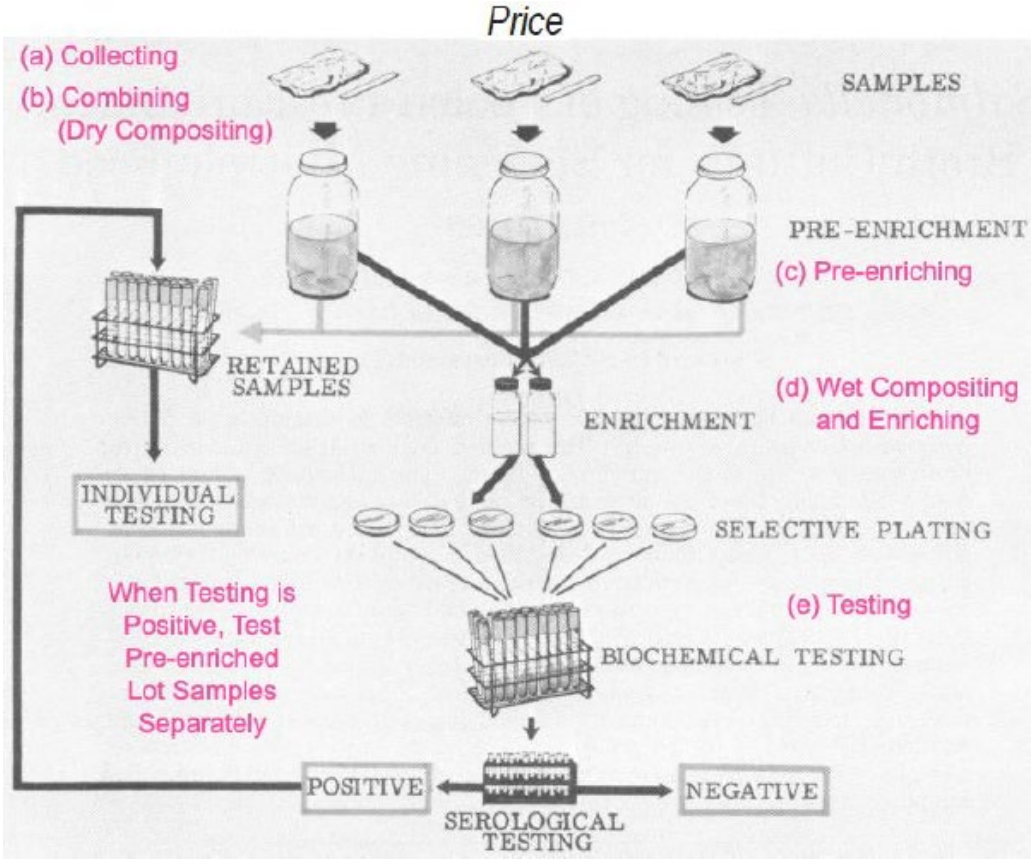
	Whether pooled pre-enrichments are less sensitive than individual tests in the presence of interfering organisms probably is not known. For the present, we would recommend that, if numerous non-*Salmonella* colonies that resemble *Salmonella* on selective agar media are encountered in the pooled test, the retained pre-enrichment cultures should be tested individually. Alternatively, problems with interfering organisms may warrant the use of direct selective enrichments, two selective enrichments, or possibly even a reduction in the incubation time of the pre-enrichment cultures. Price, 682 (highlighted added). A POSITA would have replaced Price's pre-enriching and compositing pre-enrichments with teachings of ICMSF and Hughes for enriching lot samples, removing portions of each enriched lot sample, and combining the portions to provide a composite sample. Also, instead of retaining portions of pre-enrichments like Price, a POSITA would have retained portions of enriched lot samples as taught by ICMSF and Hughes. ICMSF states: To reduce laboratory effort . . . it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. ICMSF, 187. Also, ICMSF describes preparing composites of analytical units then diluting "for qualitative (enrichment)" analysis. *Id.,* 231-32; *see also* AAPA #5, #7.

	For practical reasons, however, analytical units of 10 g up to 50 g are typical for quantitative methods and are diluted 1:10. For qualitative analyses, 25 g analytical units are frequently analyzed individually or as composites of 100 g, 200 g, or more. ICMSF, 232 (annotated). Preparation of the first dilution, either for qualitative (enrichment) or quantitative (direct) analyses, can have a marked impact on the microbial population in the sample. This is often due to changes in the physico-chemical characteristics of the suspension as compared to the food matrix. Where the food does not confer protection, the composition of the diluent is important to prevent or minimize shock/injury to the microorganisms that can result from rapid changes in the ionic concentrations. ICMSF, 232 (annotated). ICMSF also describes strategic use of non-selective enrichment to resuscitate microorganisms and selective enrichments for growing microorganisms. *Id.*, 232–33 (§ 12.6); *see e.g.*, *id.,* 233 ("use of nonselective medium . . . to enhance recovery of injured or stressed cells."). ICMSF, § 12.6 also describes optimizing the incubation period. S*ee e.g.*, *id.,* 232. It was well known long before the priority date to use non-selective enrichments after dry compositing and prior to wet compositing as evidenced by Price, Silliker, Gabis, and MLG. A POSITA would also know to use selective enrichments after dry compositing and prior to wet compositing as disclosed by ICMSF and Hughes. A POSITA would find that incubating so microorganisms are detectable and at least substantially uniform in the selective enrichment broth was considered the standard practice in official methods because the methods otherwise fail. For example, D'Aoust states "standard methodology recommends incubation of selective enrichment broths." D'Aoust, 372. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section

	further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. (a) *Pre-enrichment.*—Pre-enrich product in noninhibitory broth to initiate growth of *Salmonella* spp. Methods used may vary with product and should be performed as indicated in **967.26A** or BAM, 7th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, chapter 5, section C. Pre-enrichment broths should be incubated at 35°–37°C. (b) *Selective enrichment.*—Transfer 0.1 mL incubated pre-enrichment mixtures to Rappaport-Vassiliadis R10 broth (9.9 mL) and 1 mL to TT broth (9 mL). For all foods other than raw foods or foods having a high microbial load, incubate 6–8 h at 41°–43°C. Selective enrichments of raw foods or foods having a high microbial load must be incubated 16–20 h at 41°–43°C. An air incubator is suitable for this purpose. Hughes, 636. Hughes instructs that, after pre-enrichment, portions of the "incubated pre-enrichment mixtures" should be transferred to two separate selective enrichment broths, Rappaport-Vassiliadis R10 broth and TT broth, and incubated for 16-20 hours at 41°-43°C. Hughes, 636, 638. Additionally, Hughes instructs that these selective broths should be removed from incubation and mixed by hand or by Vortex mixer. Hughes, 638. Hughes further instructs to then remove 1 mL from the TT tube and 1 mL from the Rappaport-Vassiliadis R10 tube and transfer to separate, sterile M broths. *Id.,* 636, 638. The separate M broths are then incubated ("16-20 h at 35°-37°C" for "all foods other than raw foods or foods having a high microbial load" or "6 h at 35°-37°C" for

	"raw foods or foods having a high microbial load"). *Id.*, 638. A POSITA would find that the long incubation at an elevated temperature and mixing ensures the target microbial agent or organism reaches detectable levels and becomes substantially uniform throughout the separate lot samples.
[1d] d) removing portions of each separate enriched composited test lot sample, and combining the removed portions to provide a pooled modular composite sample; and	Price, alone or in combination with ICMSF and Hughes, discloses removing portions of each enriched separate test lot sample, and combining the removed portions to provide a modular composite sample. Price discloses wet compositing as "[s]amples are individually pre-enriched followed by transfer from multiple pre-enrichments to single selenite and tetrathionate broths at the enrichment stage." Price, 679. **MATERIALS AND METHODS** ***Salmonella*** **analyses were carried out as described in the *Bacteriological Analytical Manual* (BAM) (2) except for the pooling of pre-enrichment broths described in the following paragraph.** **The procedure for *Salmonella* testing of pooled pre-enrichment broths is illustrated in Fig. 1. Samples are individually pre-enriched followed by transfer from multiple pre-enrichments to single selenite and tetrathionate broths at the enrichment stage. During pooling, a portion of each pre-enrichment is transferred to a sterile culture tube and retained at 4 C for later reference to the individual samples. If the *Salmonella* test of the pooled pre-enrichment broths is positive, individual tests on the retained samples can be made to determine which sample or samples contributed *Salmonella* to the pool.** Price 679.

	Pooled samples versus pooled pre-enrichment broths. Silliker (4) reported a loss in sensitivity when several samples were pooled in a large container of pre-enrichment broth as compared to individual sample analysis. This is not surprising since, to be detected, the desiccated and debilitated *Salmonella* cells in a dried food sample must grow out in pre-enrichment broth in competition with other bacteria in the sample. If many food samples are pooled at the pre-enrichment stage, the salmonellae face competition from the flora of all the samples in the pool. If, on the other hand, the samples are individually pre-enriched, the actively growing salmonellae might be expected to compete more favorably with organisms introduced from other samples when pooled at the enrichment stage. In addition, pooling pre-enrichment broths avoids the hazards and inconvenience of handling very large flasks of culture. Price, 681 (discussing Silliker).

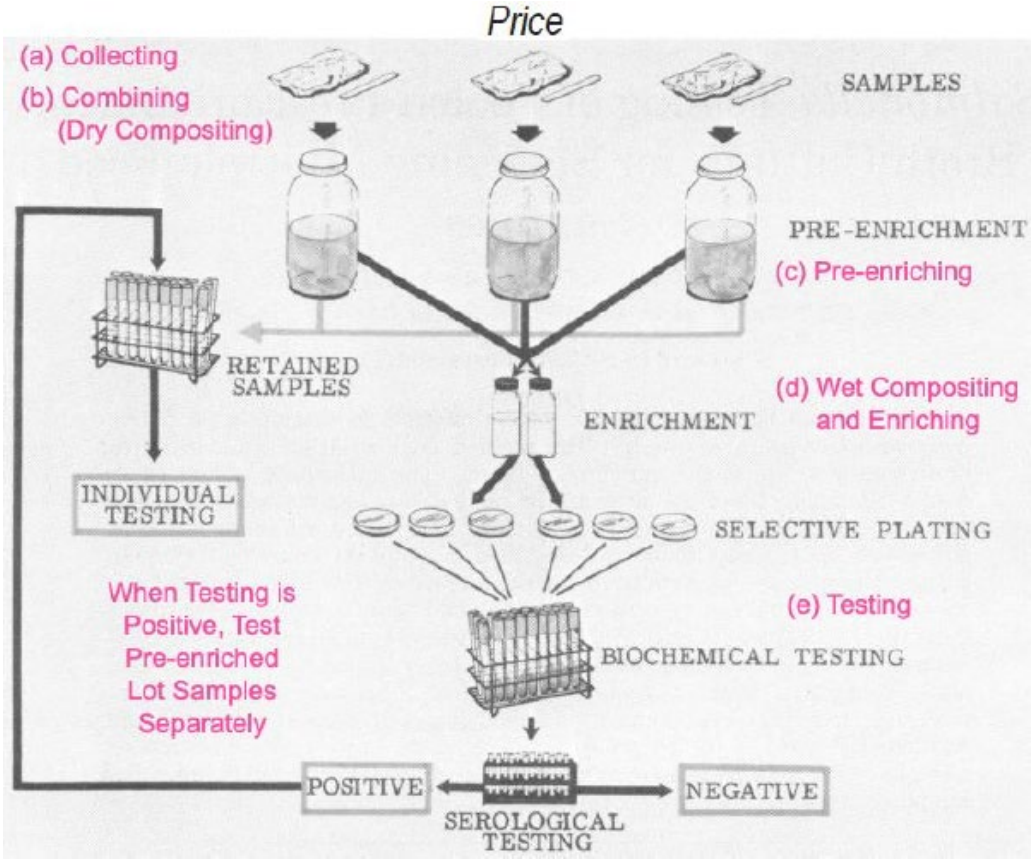


Price, 680 (Figure 1 (annotated)). Price's Figure 1 indicates that selective plating, biochemical testing, and serological testing are used.

A POSITA would have replaced Price's pre-enriching and compositing pre-enrichments with teachings of ICMSF and Hughes for enriching lot samples, removing portions of each enriched lot sample, and combining the portions to provide a composite sample. Also, instead of retaining portions of pre-enrichments like Price, a POSITA would have retained portions of enriched lot samples as taught by ICMSF and Hughes.

	ICMSF describes compositing the enriched lot samples: "it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. . . . This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n,* with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort." ICMSF, 187. AAPA #5-#7. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Hughes also discloses a method of removing portions of each enriched separate test lot sample, and combining the removed portions to provide a modular composite sample. Hughes instructs to "[c]ombine equal volumes (0.5-1 mL) from each M broth tube in a clean screw-cap tube." Hughes, 638.

	that has been warmed to 35°–37°C. Also remove 1 mL from Rappaport-Vassiliadis R10 tube and transfer to a separate tube of prewarmed M broth (10 mL). For all foods other than raw foods or foods having a high microbial load, incubate M broth tubes 16–20 h at 35°–37°C and return selective enrichment broth tubes to 41°–43°C incubator and incubate for additional 16–18 h. For raw foods or foods having a high microbial load, incubate M broth tubes 6 h at 35°–37°C and return selective enrichment broth tubes to 41°–43°C and incubate for an additional 6 h. (**d**) *Preparation of sample for EIA analysis.*—Remove M broth tubes from incubation and mix tubes by hand or Vortex mixer. Combine equal volumes (0.5–1 mL) from each M broth tube in a clean screw-cap tube and heat in boiling H_2O bath or in flowing steam 15 min. Refrigerate (2°–8°C) remaining M broth and TT and Rappaport-Vassiliadis R10 tubes from (**c**) for culture confirmation of any EIA-positive samples (*see* **967.26B, 967.26C, 967.27** and **967.28**). Cool heated M broths to 25°–37°C prior to analysis. Hughes, 638. Hughes 2003 confirms the pooling of enrichments. Hughes 2003, 775-76.
[1e.1] e) testing of the pooled modular composite sample, using a suitable detection assay, for the target microbe(s),	Price, alone or in combination with ICMSF and Hughes, discloses testing of the modular composite sample, using a suitable detection assay, for the target microbe(s).



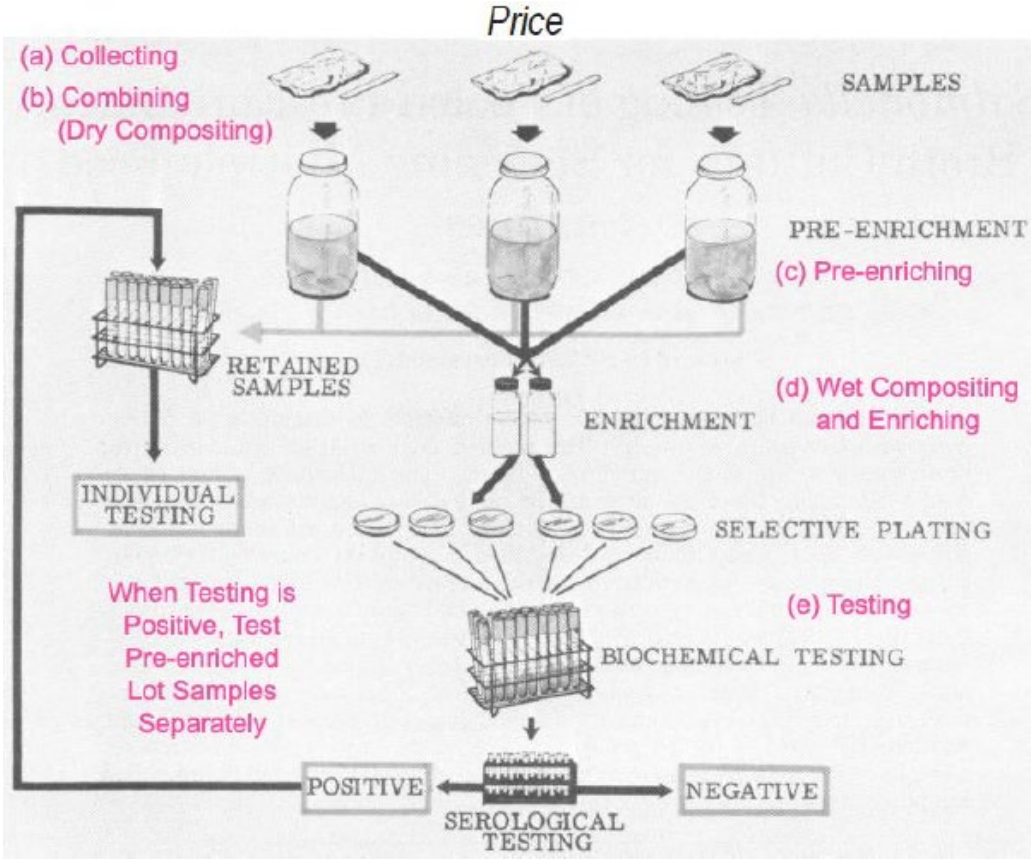
Price, 680 (Figure 1 (annotated)).

Price teaches testing the composite sample using a detection assay for *Salmonella*. Price, 680 (Figure 1).

Price states "a test procedure that gives a single negative answer for many negative samples offers an important saving in the analytical effort required for quality control":

	Positive test results are relatively rare among the many samples of food ingredients and products which are analyzed for *Salmonella*. Therefore, a test procedure that gives a single negative answer for many negative samples offers an important saving in the analytical effort required for quality control. These studies support the conclusion that *Salmonella* pre-enrichment broths can be pooled for analysis without loss in sensitivity to *Salmonella* in the individual samples. Price, 679. Price indicates that "positive test results are relatively rare." *Id.* A POSITA would have replaced Price's steps of testing a composite sample from the pre-enriched lot samples to obtain a negative result and further testing retained portions of the pre-enriched lot samples with the combined teachings of ICMSF and Hughes. These combined teachings provide for testing a composite made from the selectively enriched lot samples to obtain a negative result and further testing retained portions of the enriched lot samples after a positive test of the composite by a more sensitive protocol. ICMSF describes testing enrichment cultures of composite samples with suitable detection assays (ICMSF, 187) and "presence/absence tests" (ICMSF, 233). Suitable detection assays were established in Silliker and Gabis as discussed in ICMSF. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture

	confirmation of any EIA-positive samples." *Id.,* 638. Hughes states testing of the composite sample is "by an enzyme immunoassay (EIA) method." Hughes, 635, 638. These assay types were "art recognized." '143 Patent, 2:66-3:3 (AAPA #7).
[1e.2] wherein when such testing is negative all of said samples that were composited to form the separate composited test lot samples are deemed negative for the target microbe(s) and each of the multiple separate test lots is validated, and	Price, alone or in combination with ICMSF and Hughes, discloses wherein when such testing is negative all of said samples that were composited to form the separate composited test lot samples are deemed negative for the target microbe(s) and each of the multiple separate test lots is validated.



Price, 680 (Figure 1 (annotated)).

Price states "a test procedure that gives a single negative answer for many negative samples offers an important saving in the analytical effort required for quality control." Price, 679.

A POSITA would have replaced Price's steps of testing a composite sample from the pre-enriched lot samples to obtain a negative result and further testing retained portions of the pre-enriched lot samples with the combined teachings of ICMSF and Hughes. These combined teachings provide for testing a composite made from the

	selectively enriched lot samples to obtain a negative result and further testing retained portions of the enriched lot samples after a positive test of the composite by a more sensitive protocol. ICMSF describes correlating lots in a consignment with lot samples so a negative or positive test result enables a lot to be validated or rejected. ICMSF, 118-19. ICMSF describes sampling plans in which "c is the maximum allowable number of sample units yielding a positive result." ICMSF, 115. For many scenarios, the requirement is c = 0, which indicates the testing is negative. *Id.*, 118, 183-90, 309, 325, 326. A negative test result (*id.*, 113-15, 325, 326) is the expected and desired outcome to determine meat is not contaminated and is safe for consumption (*id.*, 184-89) such that a lot is validated and it is "commercially possible to market" (*id.*, 185) the meat.

6.10 WHAT IS A LOT?

Ideally, a lot is a quantity of food or food units produced and handled under uniform conditions. Under certain circumstances, it has been shown that the logarithms of the counts from a batch of food are likely to be normally distributed (Baird-Parker, 1980; Kilsby & Baird-Parker, 1983 [their Table 2]; Ingram & Roberts, 1976). The implication is that there is homogeneity within a lot, which, when considering microbial levels and distributions, rarely occurs in practice. In most instances, the distribution of microorganisms within lots of food is heterogeneous. Even in food factories, this heterogeneity makes the interpretation of sample results difficult, and it becomes even more so in circumstances where a lot is less well defined, such as in a consignment of a very large quantity of food.

It is therefore helpful if suppliers can be persuaded to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load).

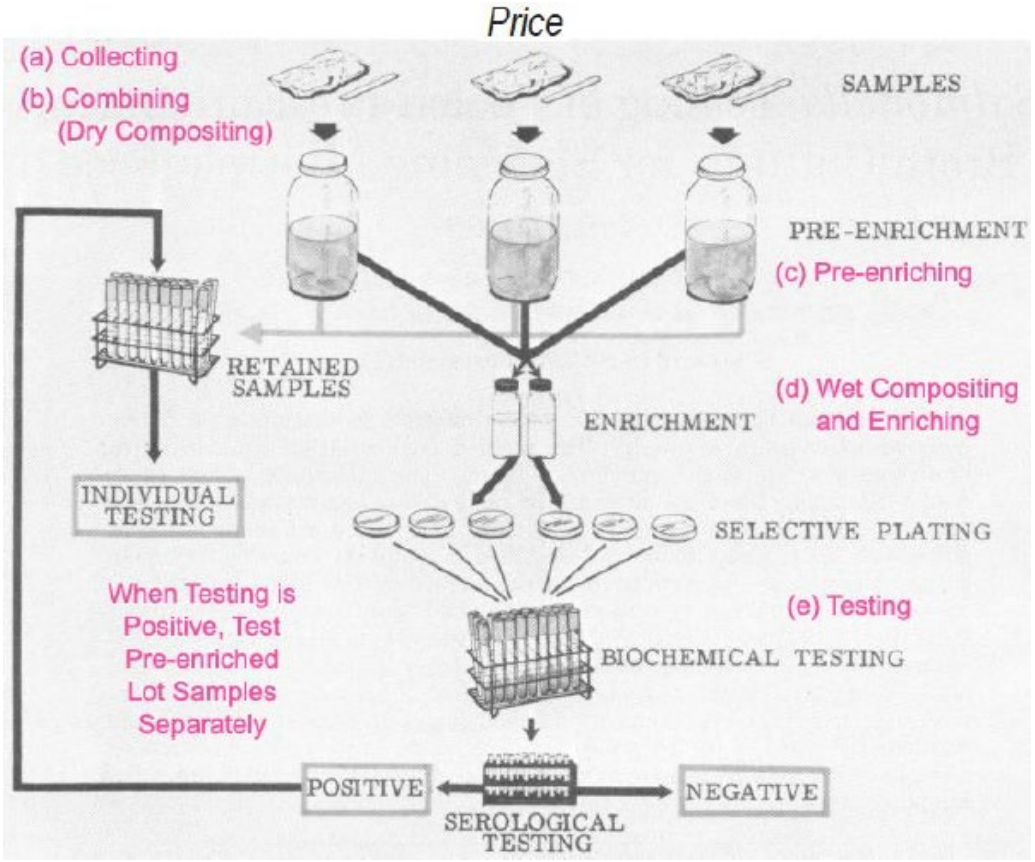
If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment.

A lot should be composed of food produced with as little variation as possible for a given process or commodity. Because of the uncertainties in identifying a lot commercially, however, the use of the word *lot* in this book is usually in its statistical sense, as a collection of units of a product, the acceptability of which is determined by examining a sample drawn from it.

ICMSF, 118-19 (annotated).

	Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Because Hughes explains the steps to be taken when testing is positive (*see* ie.3)*,* a POSITA would also expect negative testing results. Hughes, 638. Determining meat is safe for consumption so it may be released into commerce is the well-understood point of testing meat. Moreover, the recitation of a lot being validated for "release . . . into commerce" is not limiting. Prosecution History for Reexamination Control No. 95/011,294.
[1e.3] wherein when such testing is positive, each of the individual separate composited test lot samples that were used to form the pooled modular composite sample are individually tested to determine which of the separate composited test lot samples is positive for the target microbe(s),	Price, alone or in combination with ICMSF and Hughes, discloses wherein when such testing is positive, each of the individual separate composited test lot samples that were used to form the pooled modular composite sample are individually tested to determine which of the separate composited test lot samples is positive for the target microbe(s). The combination of Price, ICMSF, and Hughes describes retaining a portion of the enriched lot samples (steps (b)-(d)). Price indicates "a portion of each pre-enrichment is . . . retained . . . for later reference to the individual samples" and "individual tests on the retained samples can be made to determine which sample or samples contributed *Salmonella* to the pool." Price, 679. Price states, "If the *Salmonella* test of the pooled preenrichment broths is positive, individual tests on the retained samples can be made to determine which sample or samples contributed *Salmonella* to the pool." *Id.,* 679. Price discloses "the pooling of broth cultures permits small portions of each individual culture to be retained for subsequent analysis if a positive test is detected following enrichment, thus maintaining the individuality of each test sample." Price,

681.



Price, 680 (Figure 1 (annotated)).

Price states "[i]f the *Salmonella* test of the pooled pre-enrichment broths is positive, individual tests on the retained samples can be made to determine which sample or samples contributed *Salmonella* to the pool":

MATERIALS AND METHODS

Salmonella analyses were carried out as described in the *Bacteriological Analytical Manual* (BAM) (2) except for the pooling of pre-enrichment broths described in the following paragraph.

The procedure for *Salmonella* testing of pooled pre-enrichment broths is illustrated in Fig. 1. Samples are individually pre-enriched followed by transfer from multiple pre-enrichments to single selenite and tetrathionate broths at the enrichment stage. During pooling, a portion of each pre-enrichment is transferred to a sterile culture tube and retained at 4 C for later reference to the individual samples. If the *Salmonella* test of the pooled pre-enrichment broths is positive, individual tests on the retained samples can be made to determine which sample or samples contributed *Salmonella* to the pool.

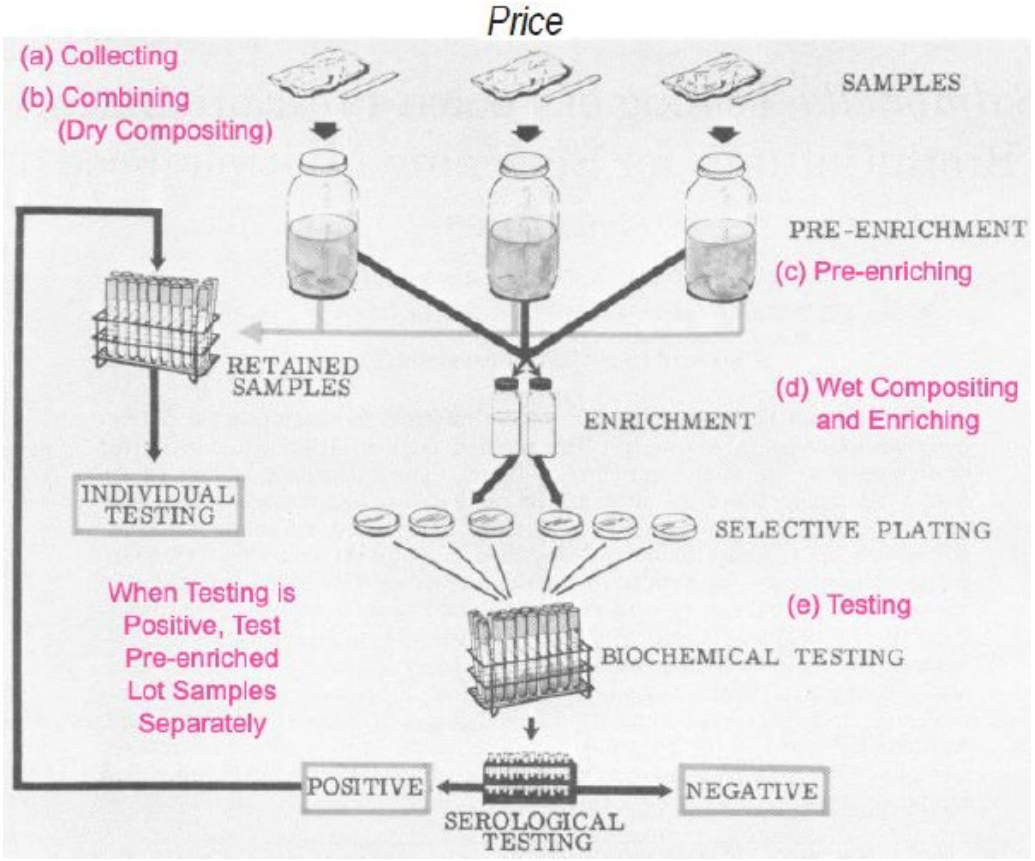
Price, 679. A POSITA would have considered retention of samples that had been selectively enriched as an option.

A POSITA would have replaced Price's steps of testing a composite sample from the pre-enriched lot samples to obtain a negative result and further testing retained portions of the pre-enriched lot samples with the combined teachings of ICMSF and Hughes. These combined teachings provide for testing a composite made from the selectively enriched lot samples to obtain a negative result and further testing retained portions of the enriched lot samples after a positive test of the composite by a more sensitive protocol.

"Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119.

	"There is often a desire to retest retained samples." ICMSF, 191. If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment. ICMSF, 119 (annotated). ICMSF discloses validating for release into commerce. ICMSF, 113–15 (§§ 6.2–6.6), 118–19 (§§ 6.9–6.10), 184–85 (§ 10.2), 325–27 (§§ 17.5.3–17.5.3.4); *see also id.*, 186–89 (§ 10.4–10.6)) according to a statistically-based sampling plan (*id.*, 113 (§ 6.1); *see also id.*, 115–18 (§§ 6.4–6.8), 184 (§ 10.2), 319–21 (§§ 17.2.3.3–17.2.3.4), 326–30 (§§ 17.5.3.1–17.6), as described regarding steps (a) and (e)(2). Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Hughes explicitly teaches retention of the enriched test lot samples for further testing after a positive result. Hughes provides instructions to refrigerate the "remaining M broth and TT and Rappaport-Vassiliadis R10 tubes [that were enriched] for culture confirmation of any EIA-positive samples." Hughes, 638. Hughes refers to the results as the "confirmed positive samples" (Hughes, 635) and also states that diagnostic reagents are "necessary for culture confirmation of presumptive EIA tests." Hughes, 636. Hughes indicates "[e]nrichment broths and M broths from samples positive by an

	enzyme immunoassay (EIA) method must be streaked on selective media." *Id.*, 635. Culture confirmation using streaking is more sensitive than the EIA method, as was commonly understood and confirmed by the teachings of D'Haese. *See generally*, D'Haese. Each sample was analyzed by the BAM method (7th Ed.) and the TSVIA method using the enrichment protocol described in section F of the method, *Preparation of Sample*. Equal portions (0.5–1 mL) from each of the 2M broths arising from the 2 selective-enrichment broths were combined in a small screw-capped tube and heat treated in boiling water for 15 min prior to performing the TSVIA. Confirmation for the immunoassay was by streaking from the M broth culture onto selective agar, as specified in the BAM reference culture method. Hughes, 635. Moreover, the recitation of a lot being validated for "release . . . into commerce" is not limiting. Prosecution History for Reexamination Control No. 95/011,294.
[1e.4] wherein the test lots corresponding to any negatively testing composited test lot samples are validated.	Price, alone or in combination with ICMSF and Hughes, discloses wherein the test lots corresponding to any negatively testing composited test lot samples are validated.



Price, 680 (Figure 1 (annotated)).

ICMSF and Hughes disclose selectively enriching prior to removing portions, thus a POSITA would consider the enrichment sufficient to enable validation of the lots without further enrichment or sampling.

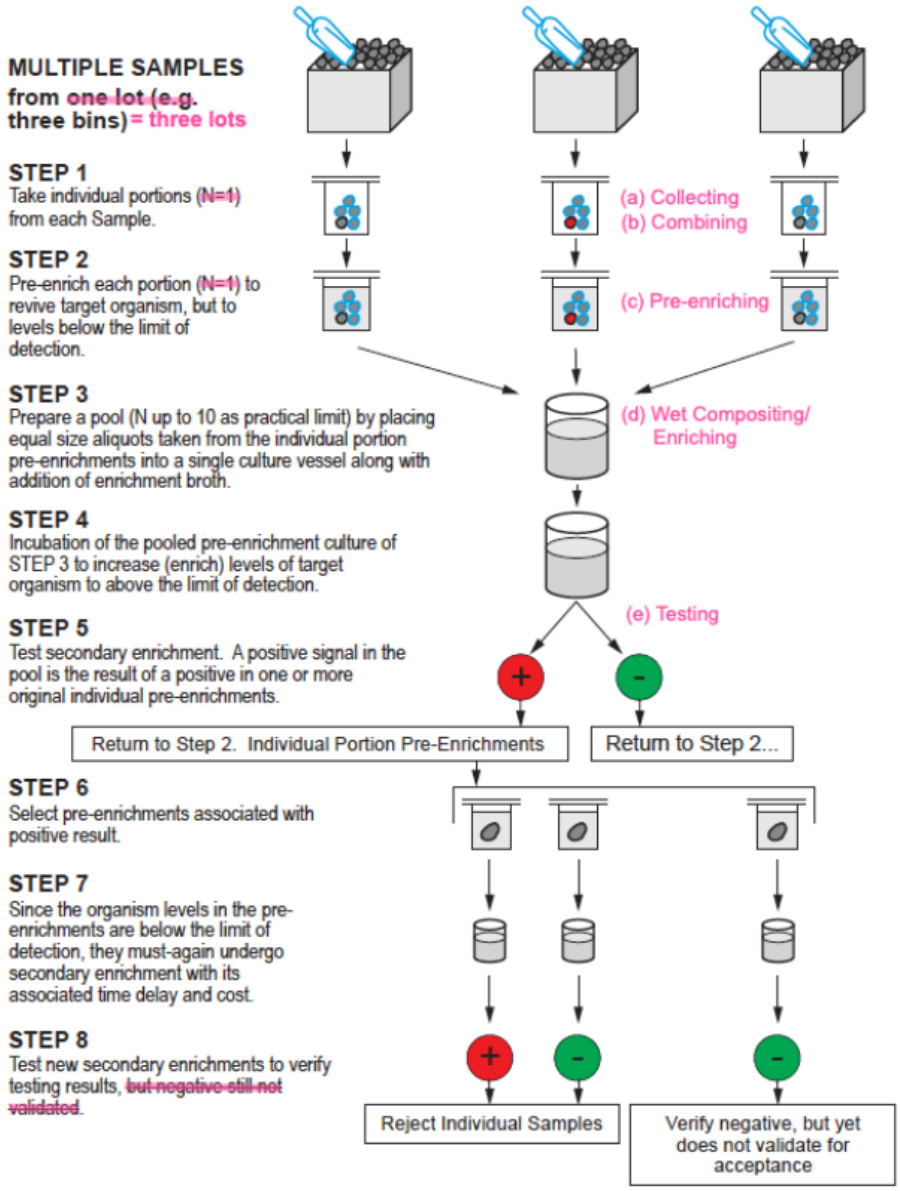
Hughes explicitly indicates portions of the enrichment cultures are retained for further testing; thus Hughes has no need for further sampling.

Hughes' "Preparation of Sample" section identifies four phases including (a) pre-

	enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. ICMSF and Hughes disclose selective enrichment so further enrichment is unnecessary. Also, as discussed regarding step (e)(3), Hughes explicitly indicates portions of the enrichment cultures are retained for further testing; thus Hughes has no need for further sampling. The same would be expected for enrichment cultures in ICMSF because retaining samples at each process stage was considered standard practice for a POSITA. in flowing steam 15 min. Refrigerate (2°–8°C) remaining M broth and TT and Rappaport-Vassiliadis R10 tubes from (c) for culture confirmation of any EIA-positive samples (*see* **967.26B, 967.26C, 967.27** and **967.28**). Cool heated M broths to 25°–37°C prior to analysis. Hughes, 638. Thus, the negative limitation of step (e)(4) is disclosed by and is obvious based on the combination of ICSMF and Hughes. *Id.*
Claim 2	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 1, wherein each of the multiple separate test lots is selected from the group consisting of an environmental lot comprising multiple operationally-linked environmental specimens, pharmaceutical lot comprising multiple operationally-linked pharmaceutical specimens, single-unit	Price, in combination with ICMSF and Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. Price further discloses test lots that are a single-unit production lot, comprise of operationally-linked product specimens.

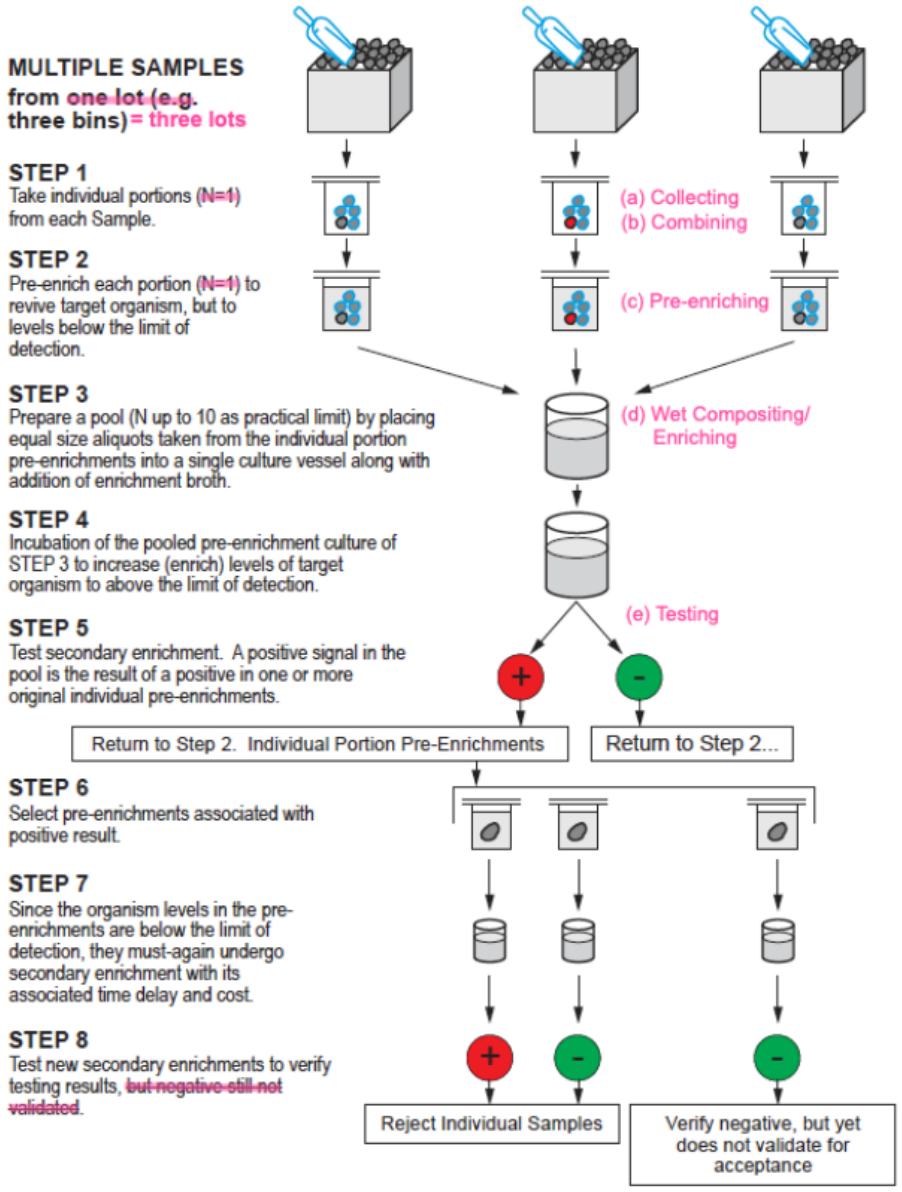
product lot comprising multiple operationally-linked product specimens, and combinations thereof.

Figure 3: CITED PRIOR ART (PRICE et al, 1972)



	Prosecution History for Reexamination Control No. 95/001,294, 298 (annotated). IEH's diagram also indicates multiple samples are taken from three bins, which is analogous to three lots rather than a single lot because each bin is an assemblage of specimens. The specification of the '143 Patent indicates a test lot is a single-unit production lot ('143 Patent, 5:34-45) such as a single combo, bin. pallet, or segment of production ('143 Patent, 7:42-50; *see also* AAPA #3 and #4). ICMSF teaches collecting from "individual production batches as lots" (ICMSF, 119) and "[a] lot is considered as a collection of units" (ICMSF, 327). "Operationally-linked" is simply grouping like items, which is also disclosed by ICMSF. "Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119.
Claim 3	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 1, wherein each of the multiple separate test lots is a single-unit production lot, comprised of operationally-linked product specimens.	Price, in combination with ICMSF and Hughes discloses Claim 1, upon which this claim relies. See above re Claim 1. Price further discloses test lots that are a single-unit production lot, comprise of operationally-linked product specimens.

Figure 3: CITED PRIOR ART (PRICE et al, 1972)



	Prosecution History for Reexamination Control No. 95/001,294, 298 (annotated). IEH's diagram also indicates multiple samples are taken from three bins, which is analogous to three lots rather than a single lot because each bin is an assemblage of specimens. The specification of the '143 Patent indicates a test lot is a single-unit production lot ('143 Patent, 5:34-45) such as a single combo, bin. pallet, or segment of production ('143 Patent, 7:42-50; *see also* AAPA #3 and #4). ICMSF teaches collecting from "individual production batches as lots" (ICMSF, 119) and "[a] lot is considered as a collection of units" (ICMSF, 327). "Operationally-linked" is simply grouping like items, which is also disclosed by ICMSF. "Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119.
Claim 4	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 1, further comprising incubating the pooled modular composite sample of d), prior to testing in e).	Price, alone or in combination with ICMSF and Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. Price further discloses incubating the modular composite sample of d), prior to testing in e). Price discloses "[s]amples are individually pre-enriched followed by transfer from multiple pre-enrichments to single selenite and tetrathionate broths at the enrichment stage." Price, 679. These pre-enrichment and enrichment steps (after the samples are composited) inherently disclose an incubation period in which levels of the target microbial agent, in this case, *Salmonella*, grow to detectable levels for testing. Price states "[a]lternatively, problems with interfering organisms may warrant the use of direct selective enrichments" and further mentions "a reduction in the

	incubation time of the pre-enrichment cultures." Price inherently discloses an incubation period of the pre-enrichment samples. **Whether pooled pre-enrichments are less sensitive than individual tests in the presence of interfering organisms probably is not known. For the present, we would recommend that, if numerous non-*Salmonella* colonies that resemble *Salmonella* on selective agar media are encountered in the pooled test, the retained pre-enrichment cultures should be tested individually. Alternatively, problems with interfering organisms may warrant the use of direct selective enrichments, two selective enrichments, or possibly even a reduction in the incubation time of the pre-enrichment cultures.** Price, 682 (highlighted added). ICMSF and Hughes, in combination with Stewart, discloses the method of claim 1, further comprising incubating the modular composite sample of d), prior to testing in e). Hughes describes incubating an enriched test lot sample. Hughes, 636, 638. Claim 1 is disclosed by ICMSF and Hughes. Stewart discloses that after the selective enrichment in Rappaport-Vassiliadis (RV) broth and tetrathionate (TT) broths, the two selective enrichments were combined into a single sample for further incubation and analysis. "[P]ost-enrichment by transfer and combination of 0.5 mL RV and 1 mL TT into a single tube containing

	10 mL prewarmed trypticase soy broth supplemented with novobiocin (TSB + n) at 10 µg/mL with incubation for 16–20 h at 42°C." Stewart, 398. Hughes also discloses an incubation protocol. "For the previous AOAC studies on TSVIA, a cultural protocol using selective enrichment in selenite cystine (SC) and tetrathionate (Tr) broths at 35°C was used. However, there has been a recent trend to use elevated incubation temperatures and to replace SC broth with the Rappaport-Vassiliadis (RV) medium (2)." Hughes, 634. A POSITA would have been motivated to incubate the pooled modular composite sample of Gombas or Hughes, as taught by Stewart, for reliable testing results.
Claim 5	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 1, wherein enriching each separate composited test lot sample in c) comprises incubating each separate composited test lot sample with enrichment medium suitable to enrich for the target microbe(s), and wherein removing in d) comprises removing a portion of the enrichment medium from each enriched separate composited test lot sample and combining the removed portions to provide a pooled modular composite sample.	Price, alone or in combination with ICMSF and Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. Price further discloses incubating each separate test lot sample in c) comprises incubating each separate test lot sample with enrichment medium suitable to enrich for the target microbial agent or organism, and wherein removing in d) comprises removing a portion of the enrichment medium from each enriched separate test lot sample and combining the removed portions to provide a modular composite sample. Price discloses "[s]amples are individually pre-enriched followed by transfer from multiple pre-enrichments to single selenite and tetrathionate broths at the enrichment stage." Price, 679. The pre-enrichment step inherently discloses an incubation period for each individual sample in which levels of the target microbial agent, in this case, *Salmonella*, grow to detectable levels for testing. The enriched sample is then transferred and combined with other samples. Price states "[a]lternatively, problems with interfering organisms may warrant the use of direct selective enrichments" and further mentions "a reduction in the incubation time of the pre-enrichment cultures." Price inherently discloses an incubation period of the pre-enrichment samples.

	Whether pooled pre-enrichments are less sensitive than individual tests in the presence of interfering organisms probably is not known. For the present, we would recommend that, if numerous non-*Salmonella* colonies that resemble *Salmonella* on selective agar media are encountered in the pooled test, the retained pre-enrichment cultures should be tested individually. Alternatively, problems with interfering organisms may warrant the use of direct selective enrichments, two selective enrichments, or possibly even a reduction in the incubation time of the pre-enrichment cultures. Price, 682 (highlighted added). Price discloses wet compositing as "[s]amples are individually pre-enriched followed by transfer from multiple pre-enrichments to single selenite and tetrathionate broths at the enrichment stage." Price, 679.

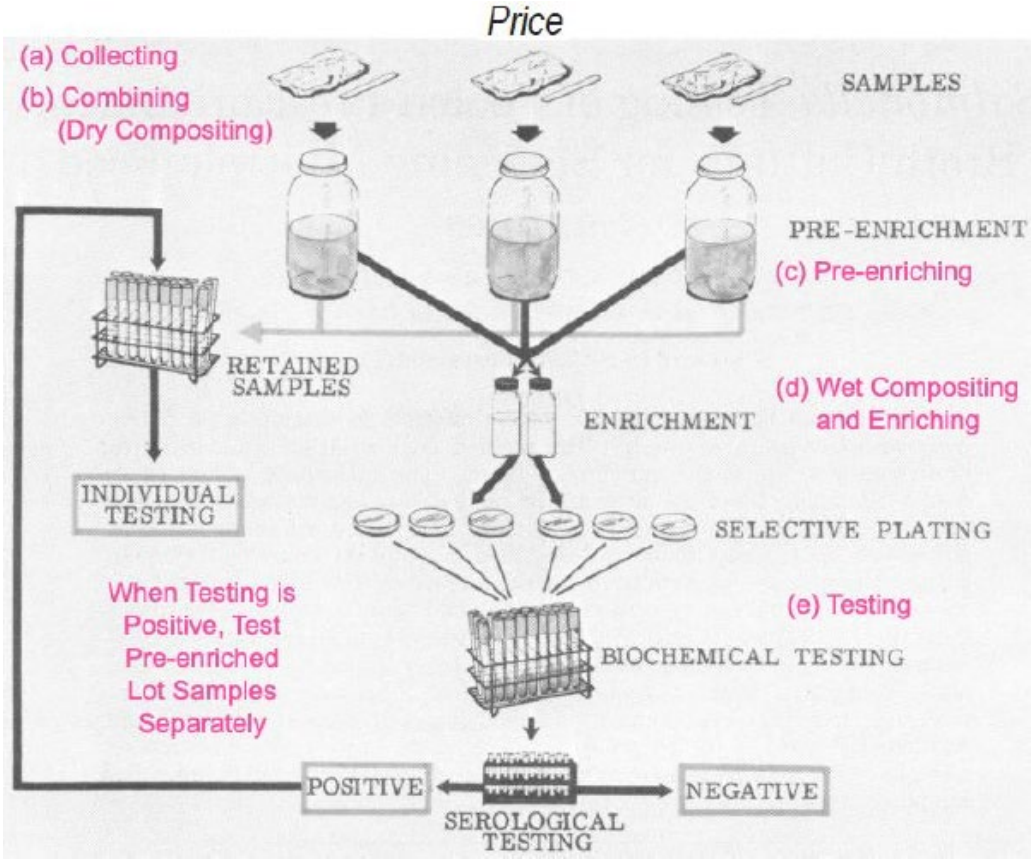
MATERIALS AND METHODS

Salmonella analyses were carried out as described in the *Bacteriological Analytical Manual* (BAM) (2) except for the pooling of pre-enrichment broths described in the following paragraph.

The procedure for *Salmonella* testing of pooled pre-enrichment broths is illustrated in Fig. 1. Samples are individually pre-enriched followed by transfer from multiple pre-enrichments to single selenite and tetrathionate broths at the enrichment stage. During pooling, a portion of each pre-enrichment is transferred to a sterile culture tube and retained at 4 C for later reference to the individual samples. If the *Salmonella* test of the pooled pre-enrichment broths is positive, individual tests on the retained samples can be made to determine which sample or samples contributed *Salmonella* to the pool.

Price 679.

	Pooled samples versus pooled pre-enrichment broths. Silliker (4) reported a loss in sensitivity when several samples were pooled in a large container of pre-enrichment broth as compared to individual sample analysis. This is not surprising since, to be detected, the desiccated and debilitated *Salmonella* cells in a dried food sample must grow out in pre-enrichment broth in competition with other bacteria in the sample. If many food samples are pooled at the pre-enrichment stage, the salmonellae face competition from the flora of all the samples in the pool. If, on the other hand, the samples are individually pre-enriched, the actively growing salmonellae might be expected to compete more favorably with organisms introduced from other samples when pooled at the enrichment stage. In addition, pooling pre-enrichment broths avoids the hazards and inconvenience of handling very large flasks of culture. Price, 681 (discussing Silliker).



Price, 680 (Figure 1 (annotated)). Price's Figure 1 indicates that selective plating, biochemical testing, and serological testing are used.

ICMSF describes the well-known benefits of combining enrichment cultures from analytical units from lots in a consignment.

	To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. A laboratory investigation undertaken for ICMSF (Silliker & Gabis, 1973) indicated that different sized analytical units drawn from a single well-mixed sample unit of dried food give very similar results. A subsequent investigation (Gabis & Silliker, 1974) using foods of high moisture content (including eggs, poultry meat, meat, and meat products) showed comparable reliability in the use of composite analytical units; for example, examination of three composites of 20 × 25 g analytical units gave the same result as examination of all 60 analytical units individually (the total amount examined being 1500 g in both instances). This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n*, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort. ICMSF, 187. Hughes describes incubating an enriches test lot sample. Hughes 636, 638.
Claim 6	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 1, wherein testing of the pooled modular composite sample, using a suitable detection assay comprises use of a concentration step to concentrate the target microbe(s).	Price, alone or in combination with ICMSF and Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. Price further discloses testing of the modular composite sample, using a suitable detection assay further comprises a concentration step to concentrate the microbial agent or organism. Price discloses an enrichment stage after compositing where "[s]amples are individually pre-enriched followed by transfer from multiple pre-enrichments to single selenite and tetrathionate broths at the enrichment stage." Price, 679.

	A POSITA would understand that a detection assay that includes enrichment could further comprise a concentration step. Enrichment for the microbial agent or organism increases its concentration. ICMSF discloses the use of "immunomagnetic beads to concentrate cells." ICMSF, 318. A POSITA would consider this a well-known technique before the priority date. Fratamico discusses the rapid isolation of target microorganisms using an immunomagnetic separation method. Fratamico, 105. Fratamico discloses that use of immunomagnetic beads resulted in "an increase in the level of E. coli B1409 in the sample," as well as "an increase in the number of organisms recovered." *Id.*, 107. Additionally, Fratamico provides detailed instructions for preparing and using immunomagnetic bead separation to concentrate a target microbial agent or organism. Fratamico developed "[a] method employing immunomagnetic beads" to concentrate and thereby improve the isolation and detection of E. coli. *Id.* For example, Fratamico was able to use immunomagnetic beads to isolate *E. coli* strains from enrichment cultures of raw ground beef "when the initial inoculum level was 1 cfu g-1." *Id.*, 109. Fratamico further teaches that "[t]he percentage of bacteria recovered increased with increasing amounts of beads." *Id.*, 108. Consequently, Fratamico "demonstrates that immunomagnetic separation [using immunomagnetic beads] is a simple and rapid technique" for concentrating a microbe for isolation and detection. *Id.*, 109. These teachings of Fratamico would have motivated a POSITA to modify the combination of ICMSF and Gombas and ICMSF and Hughes to use immunomagnetic beads to concentrate microbes in a modular composite sample to benefit from accurate tests. Use of immunomagnetic beads was already known in the art for concentrating during testing beef samples. "Tu et al. (78) detected pathogenic E. coli O157:H7 in beef hamburger with the use of IMS and ATP measurement. After 6 h of enrichment at

	37°C, inoculated beef samples (0.9 ml each) were filtered to remove large particles prior to 10 min of incubation with ca. 10^5 magnetic beads coated with anti–*E. coli* O157:H7 (Dynabeads, Dynal Inc., Oslo, Norway). Following incubation, the magnetic beads were collected with a Dynal magnetic concentrator and resuspended in 0.9 ml of Trypticase soy broth for ATP analysis." Benoit, 1941. "Hudson et al. (26) used IMS to directly separate and concentrate L. monocytogenes from ham samples prior to detection by PCR. IMS was used on the basis of the commercial availability of anti-Listeria magnetic beads (Dynal Inc.) to avoid time-consuming cultural enrichment steps and to remove PCR inhibitors." Benoit, 1941-42. Cudjoe investigates the use of immunomagnetic beads in pooling methods. In a study designed to evaluate the performance of Dynabeads® Anti-Salmonella, immunomagnetic separation (IMS) followed by plating (IMS-Plating) proved far superior to the conventional ISO *Salmonella* methodology and the Modified Semi-solid Rappaport-Vassiliadis (MSRV) method. *Salmonella* species were isolated and detected from 135 out of the 180 diverse poultry samples by IMS analysis as against 98 by the conventional method and 33 by the MSRV technique. All results were confirmed biochemically and serologically. It appeared from the data generated that some of the salmonellae isolated using IMS were greatly inhibited in Rappaport-Vassiliadis enrichment broth at 42°C and to a lesser extent in selenite cystine broth at 37°C. A moderately selective plating medium like XLD proved to be better in isolating these possible sensitive wild-type strains of salmonellae than the more selective BGA. Cudjoe, Abstract.

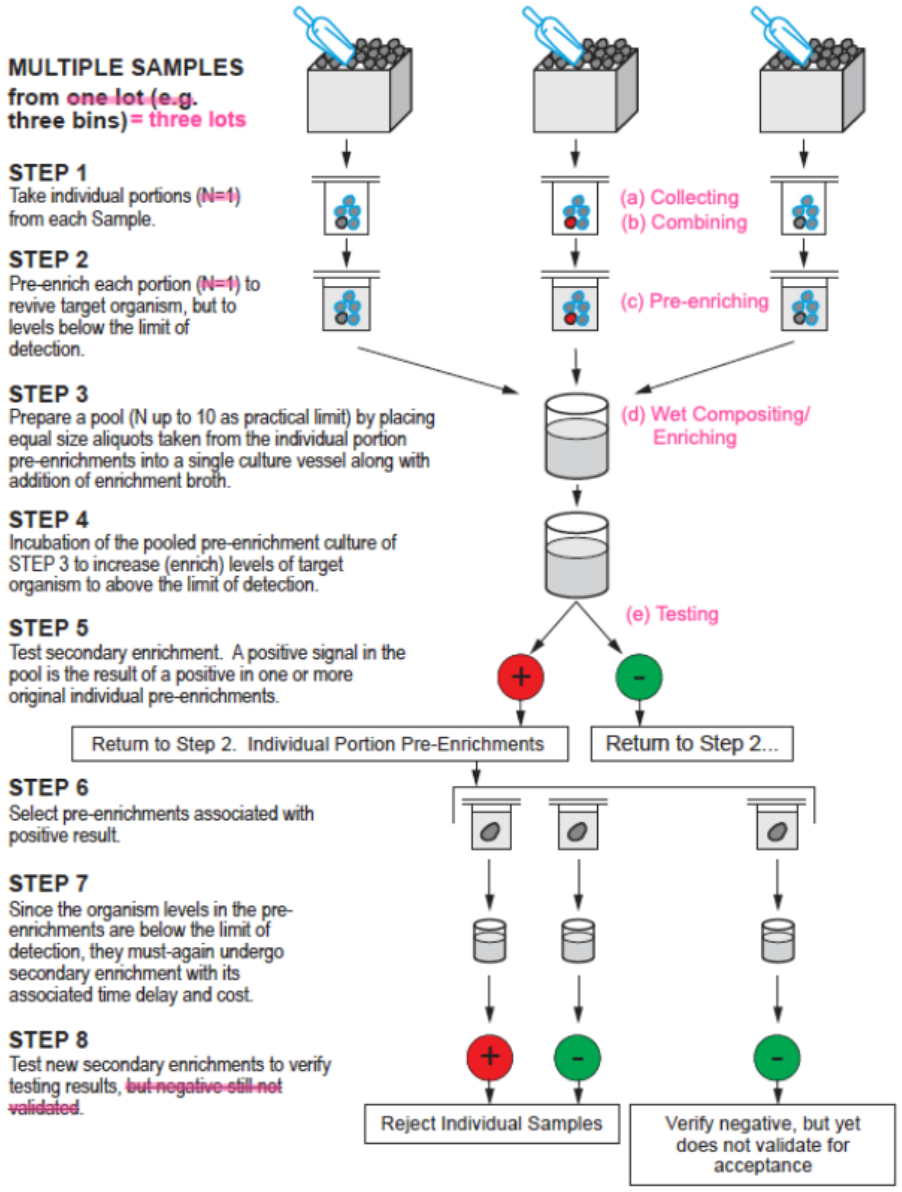
	Samples. One hundred and eighty (180) diverse poultry samples, consisting of 40 pools of cloacal/faecal swabs (5 swabs/pool; 5 pools samples/flock) from eight different flocks, 10 raw chicken breast meat samples, 10 pooled egg shell samples, 60 raw chicken liver samples and 60 chicken neck skins sampled from the slaughter line, were used in this study. All samples were obtained from a poultry slaughter and processing plant in the Netherlands. A pool of five swab samples was pre-enriched in 10 ml of buffered peptone water (BPW; Difco Laboratories, Detroit, Michigan, USA) at 37°C for 16–20 h, whilst 25 g of each of the other samples above, were pre-enriched similarly in 225 ml of BPW and examined as detailed below. Cudjoe, 160.
Claim 7	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 1, wherein where testing in e) of the pooled modular composite sample is positive, and wherein the separate individual composited test lot samples are further tested using a more sensitive protocol.	Price, alone or in combination with ICMSF and Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. A POSITA would understand that retesting the individual test lot samples is an inherently more sensitive process because the sample is not diluted with other samples in the testing pool. Therefore, the retesting of a singular sample is more concentrated and therefore, more sensitive. *See also, generally*, Jarvis. ICMSF discloses the use of "immunomagnetic beads to concentrate cells." ICMSF, 318. A POSITA would consider this a well-known technique before the priority date. Hughes uses an EIA method followed by a more sensitive protocol—streaking on selective media. Hughes, 635.

	Each sample was analyzed by the BAM method (7th Ed.) and the TSVIA method using the enrichment protocol described in section **F** of the method, *Preparation of Sample*. Equal portions (0.5–1 mL) from each of the 2M broths arising from the 2 selective-enrichment broths were combined in a small screw-capped tube and heat treated in boiling water for 15 min prior to performing the TSVIA. Confirmation for the immunoassay was by streaking from the M broth culture onto selective agar, as specified in the BAM reference culture method. Hughes, 635. A POSITA would find that modifying Hughes based on D'Haese ensures a more sensitive test is used for further testing. A POSITA would have recognized the concentration step could be employed to provide a more sensitive protocol for further testing. A POSITA would understand that retesting the non-pooled, singular samples is an inherently more sensitive process because the sample is not diluted with other samples in the testing pool. Therefore, the retesting of a singular sample is more concentrated and therefore, more sensitive. *See also, generally*, Jarvis. Fratamico discusses the rapid isolation of target microorganisms using an immunomagnetic separation method. Fratamico, 105. Fratamico discloses that use of immunomagnetic beads resulted in "an increase in the level of E. coli B1409 in the sample," as well as "an increase in the number of organisms recovered." *Id.*, 107. Additionally, Fratamico provides detailed instructions for preparing and using

	immunomagnetic bead separation to concentrate a target microbial agent or organism. Fratamico developed "[a] method employing immunomagnetic beads" to concentrate and thereby improve the isolation and detection of E. coli. *Id.* For example, Fratamico was able to use immunomagnetic beads to isolate *E. coli* strains from enrichment cultures of raw ground beef "when the initial inoculum level was 1 cfu g-1." *Id.*, 109. Fratamico further teaches that "[t]he percentage of bacteria recovered increased with increasing amounts of beads." *Id.*, 108. Consequently, Fratamico "demonstrates that immunomagnetic separation [using immunomagnetic beads] is a simple and rapid technique" for concentrating a microbe for isolation and detection. *Id.*, 109. These teachings of Fratamico would have motivated a POSITA to modify the combination of ICMSF and Gombas and ICMSF and Hughes to use immunomagnetic beads to concentrate microbes in a modular composite sample to benefit from accurate tests. Use of immunomagnetic beads was already known in the art for concentrating during testing beef samples. "Tu et al. (78) detected pathogenic E. coli O157:H7 in beef hamburger with the use of IMS and ATP measurement. After 6 h of enrichment at 37°C, inoculated beef samples (0.9 ml each) were filtered to remove large particles prior to 10 min of incubation with ca. 10^5 magnetic beads coated with anti–*E. coli* O157:H7 (Dynabeads, Dynal Inc., Oslo, Norway). Following incubation, the magnetic beads were collected with a Dynal magnetic concentrator and resuspended in 0.9 ml of Trypticase soy broth for ATP analysis." Benoit, 1941. "Hudson et al. (26) used IMS to directly separate and concentrate L. monocytogenes from ham samples prior to detection by PCR. IMS was used on the basis of the commercial availability of anti-Listeria magnetic beads (Dynal Inc.) to avoid time-consuming cultural enrichment steps and to remove PCR inhibitors." Benoit, 1941-42.

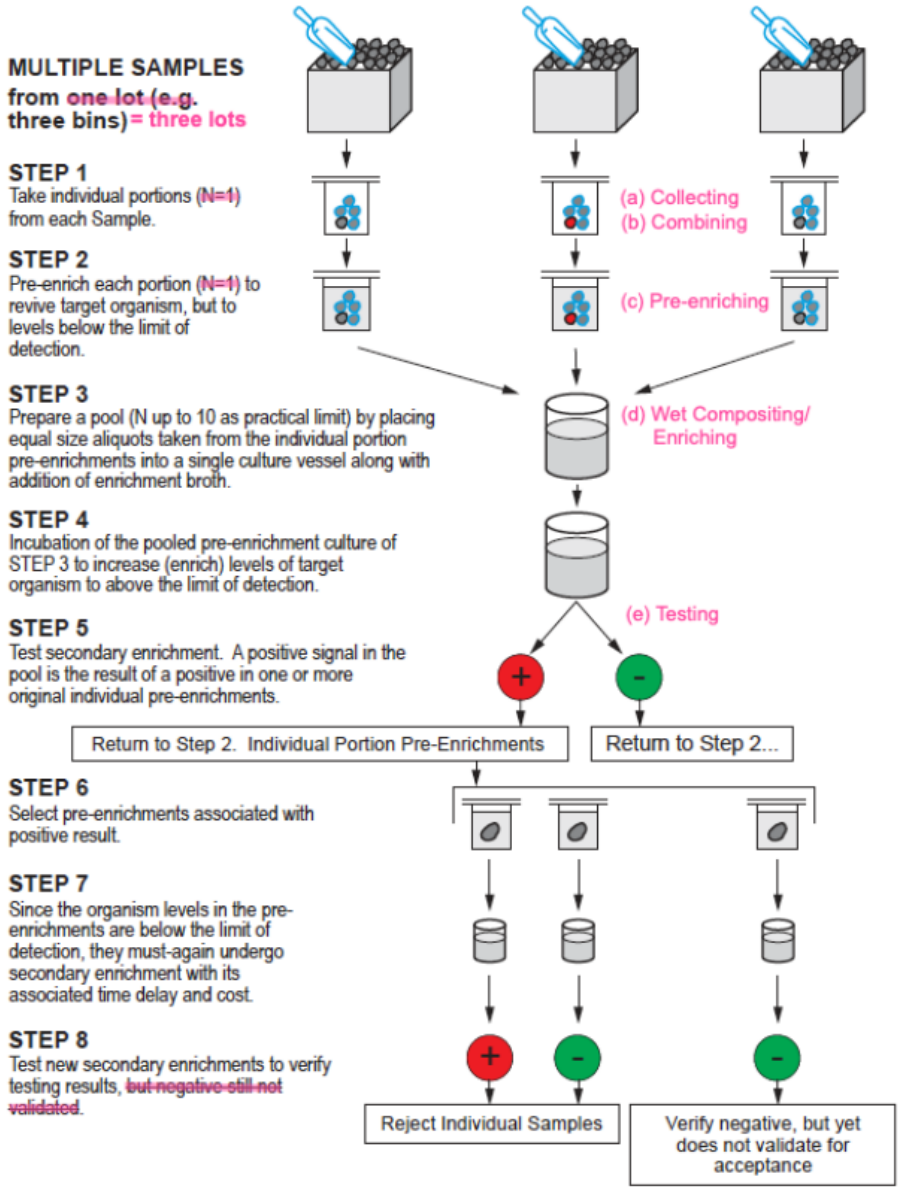
	Cudjoe investigates the use of immunomagnetic beads in pooling methods. In a study designed to evaluate the performance of Dynabeads® Anti-Salmonella, immunomagnetic separation (IMS) followed by plating (IMS-Plating) proved far superior to the conventional ISO *Salmonella* methodology and the Modified Semi-solid Rappaport-Vassiliadis (MSRV) method. *Salmonella* species were isolated and detected from 135 out of the 180 diverse poultry samples by IMS analysis as against 98 by the conventional method and 33 by the MSRV technique. All results were confirmed biochemically and serologically. It appeared from the data generated that some of the salmonellae isolated using IMS were greatly inhibited in Rappaport-Vassiliadis enrichment broth at 42°C and to a lesser extent in selenite cystine broth at 37°C. A moderately selective plating medium like XLD proved to be better in isolating these possible sensitive wild-type strains of salmonellae than the more selective BGA. Cudjoe, Abstract. *Samples*. One hundred and eighty (180) diverse poultry samples, consisting of 40 pools of cloacal/faecal swabs (5 swabs/pool; 5 pools samples/flock) from eight different flocks, 10 raw chicken breast meat samples, 10 pooled egg shell samples, 60 raw chicken liver samples and 60 chicken neck skins sampled from the slaughter line, were used in this study. All samples were obtained from a poultry slaughter and processing plant in the Netherlands. A pool of five swab samples was pre-enriched in 10 ml of buffered peptone water (BPW; Difco Laboratories, Detroit, Michigan, USA) at 37°C for 16–20 h, whilst 25 g of each of the other samples above, were pre-enriched similarly in 225 ml of BPW and examined as detailed below. Cudjoe, 160.
Claim 11	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 1, wherein each the test lots is selected from the lot group consisting of a single combo, single bin, single pallet, and a production assemblage.	Price, alone or in combination with ICMSF and Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1.

Figure 3: CITED PRIOR ART (PRICE et al, 1972)



	Prosecution History for Reexamination Control No. 95/001,294, 298 (annotated). IEH's diagram also indicates multiple samples are taken from three bins, which is analogous to three lots rather than a single lot because each bin is an assemblage of specimens. The specification of the '143 Patent indicates a test lot is a single-unit production lot ('143 Patent, 5:34-45) such as a single combo, bin. pallet, or segment of production ('143 Patent, 7:42-50; *see also* AAPA #3 and #4). ICMSF teaches collecting from "individual production batches as lots" (ICMSF, 119) and "[a] lot is considered as a collection of units." ICMSF, 327. "Operationally-linked" is simply grouping like items, which is also disclosed by ICMSF. "Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119.
Claim 12	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 1, wherein the number of separate test lots is from 3 to 10.	Price, alone or in combination with ICMSF and Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1.

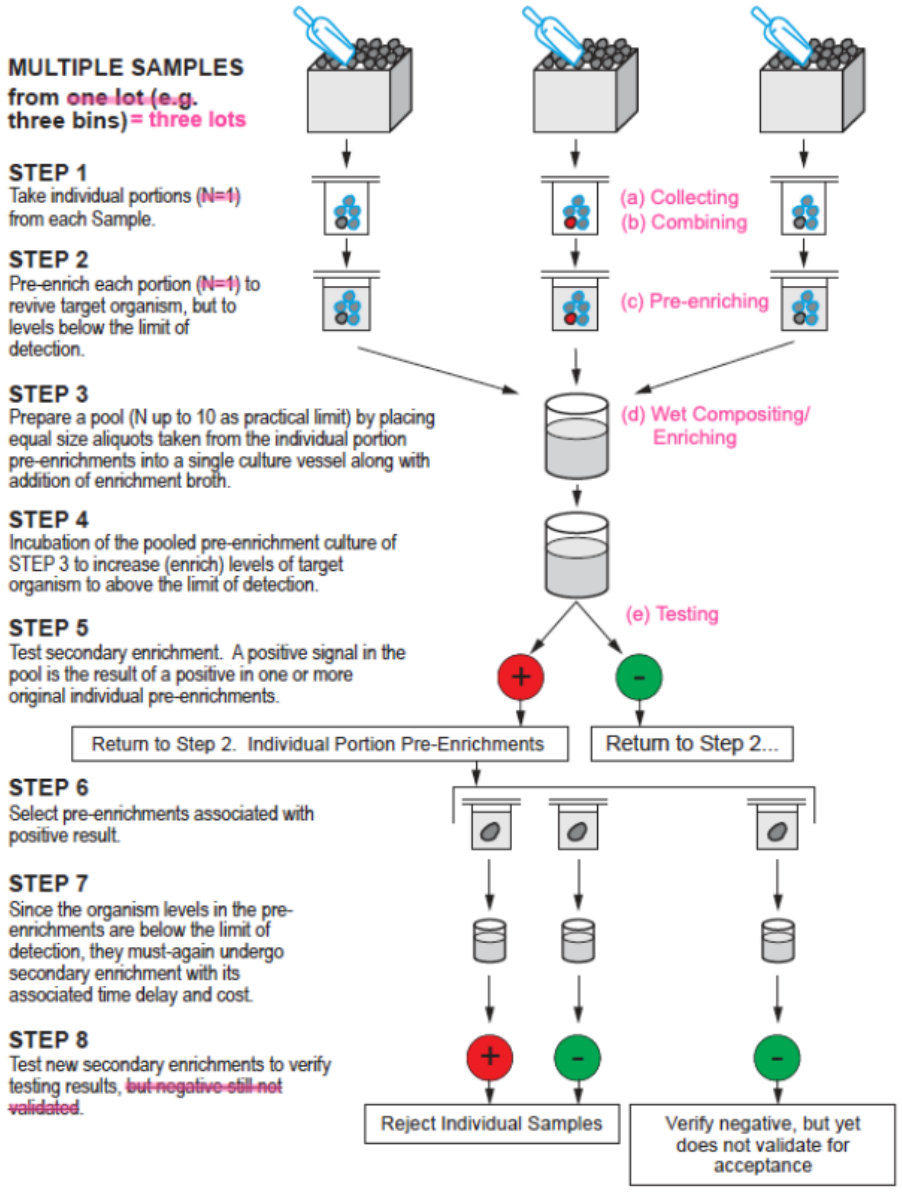
Figure 3: CITED PRIOR ART (PRICE et al, 1972)



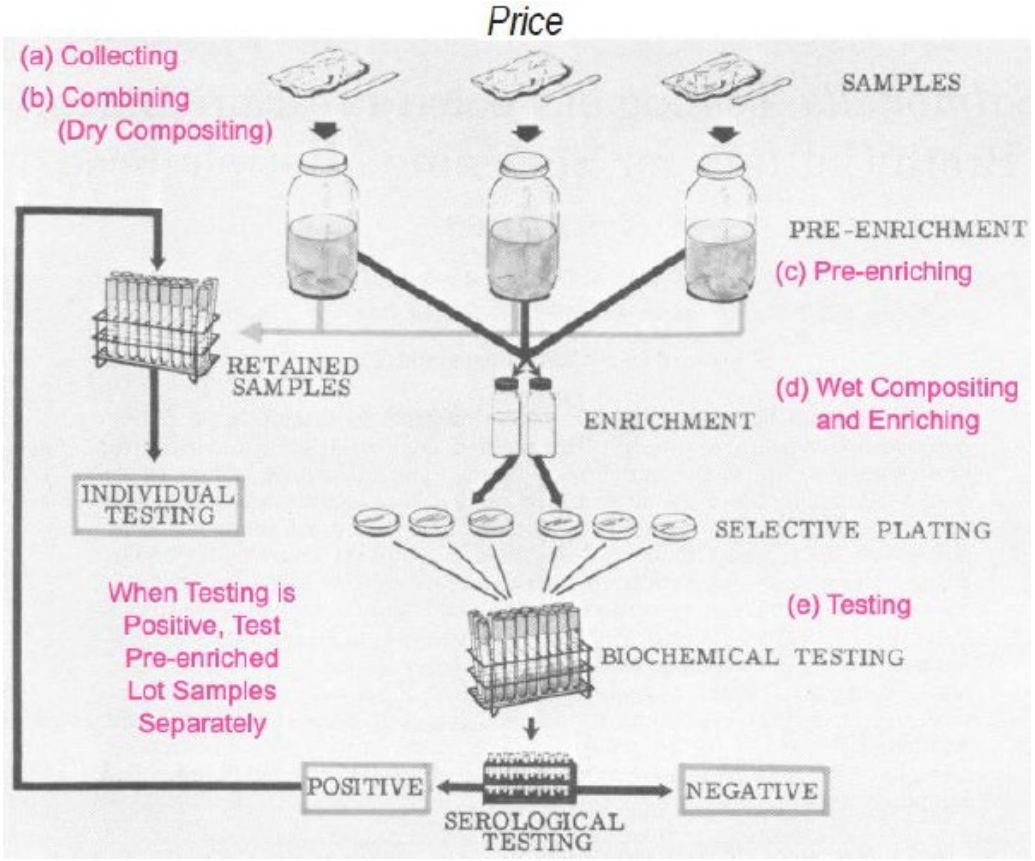
	Prosecution History for Reexamination Control No. 95/001,294, 298 (annotated). IEH's diagram also indicates multiple samples are taken from three bins, which is analogous to three lots rather than a single lot because each bin is an assemblage of specimens. ICMSF indicates a consignment may be "composed of several lots." ICMSF, 119-20. "Several" lots includes at least three lots.
Claim 13	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 1, further comprising, where testing of the pooled modular composite sample is positive, determining which individual separate test lots are positive, application of remedial measures to said separate positive test lots, and validating by further testing of the respective remediated separate test lots and obtaining a negative test result.	Price, alone or in combination with ICMSF and Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Each sample was analyzed by the BAM method (7th Ed.) and the TSVIA method using the enrichment protocol described in section F of the method, *Preparation of Sample*. Equal portions (0.5–1 mL) from each of the 2M broths arising from the 2 selective-enrichment broths were combined in a small screw-capped tube and heat treated in boiling water for 15 min prior to performing the TSVIA. Confirmation for the immunoassay was by streaking from the M broth culture onto selective agar, as specified in the BAM reference culture method.

	Hughes, 635. A POSITA understands the purpose of testing for pathogens and other organisms in food is to determine if a food is safe for entry into commerce to be sold for consumption. One having ordinary skill in the art understands that if a sample tests positive, a remediation step, like cooking, needs to be completed before it can be sold into commerce to comply with USDA guidelines.
Claim 14	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 1, wherein, in a), separately collecting said multiple independent samples from each of the multiple separate test lots comprises separately collecting from each separate test lot a number of samples from 5 to 100.	Price, alone or in combination with ICMSF and Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. Price's teachings for collecting a plurality of portions are based on standard practices as described in BAM 1969 (BAM 1969, 1 § 10.04) which cites to JAOAC 1967 (JAOAC, 234). Price, 679, 682. ***Salmonella* analyses were carried out as described in the *Bacteriological Analytical Manual* (BAM) (2) except for the pooling of pre-enrichment broths described in the following paragraph.** Price, 679. IEH's diagram of Price from Prosecution History for Reexamination Control No. 95/001,294 (reproduced below) shows step 1 corresponds to IEH's step (a), with instructions to "[t]ake individual portions . . . from each Sample."

Figure 3: CITED PRIOR ART (PRICE et al, 1972)



	Prosecution History for Reexamination Control No. 95/001,294, 298 (annotated). IEH's diagram also indicates multiple samples are taken from three bins, which is analogous to three lots rather than a single lot because each bin is an assemblage of specimens. The purpose of Jarvis and Price was to determine whether pooling samples reduced the ability to detect contaminated food lots. It would have been commonsense to combine samples from the same bin and subject them to enrichment, since each bin represents a discrete unit of the production run and results obtained from the enriched sample could be attributed to the bin from which the sample was obtained. Prosecution History for Reexamination Control No. 95/001,294, at 1575 (First Board Decision of January 20, 2015 at 16). Additionally, the specification describes a bin of dry food materials (almonds), like those in Price, as one example of a test lot/single unit of production, similar to a combo of beef. '143 Patent, 5:46-51. Price discloses testing of "Dried egg albumen, cocoa, and nonfat dry milk" (Price, 679) and "wheat flour, coconut, and cottonseed flour" (Price, 681).

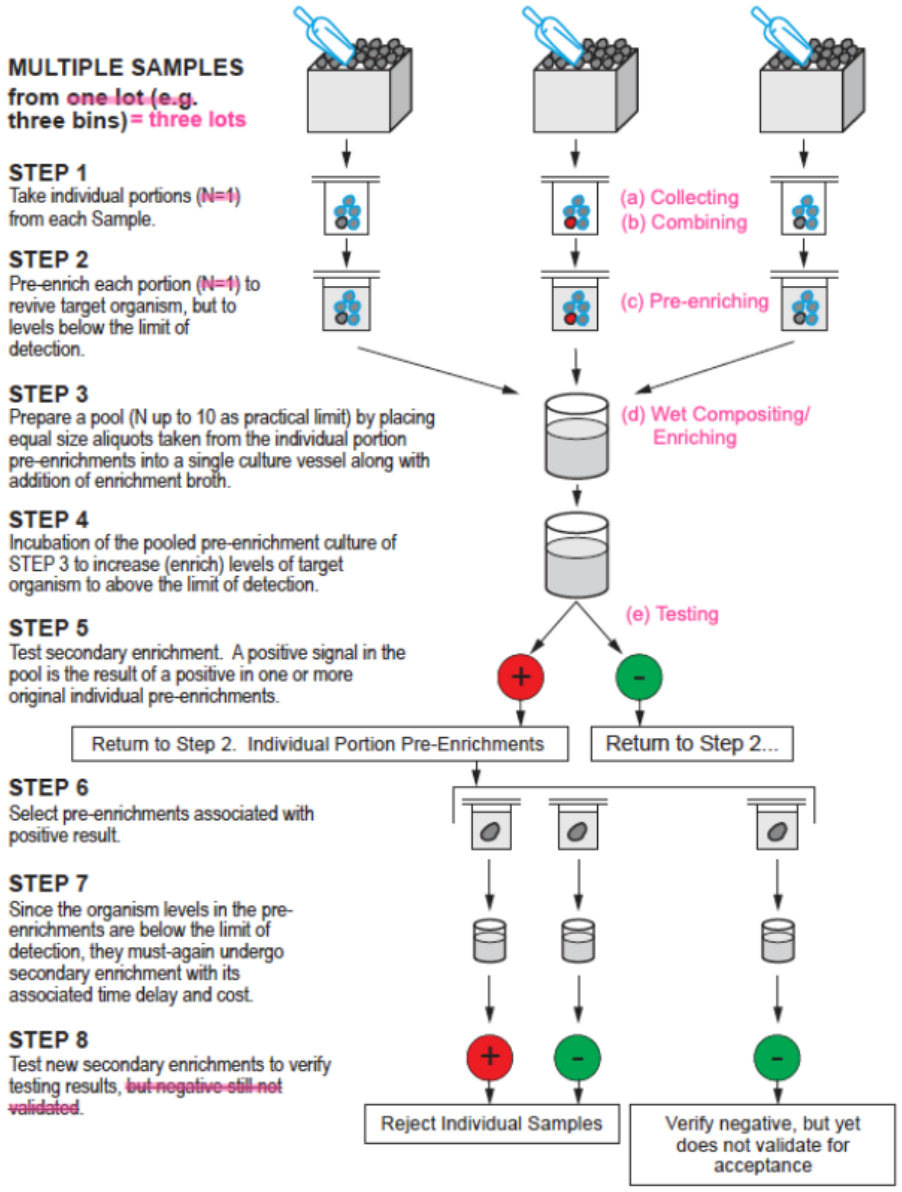


Price, 680 (Figure 1 (annotated)).

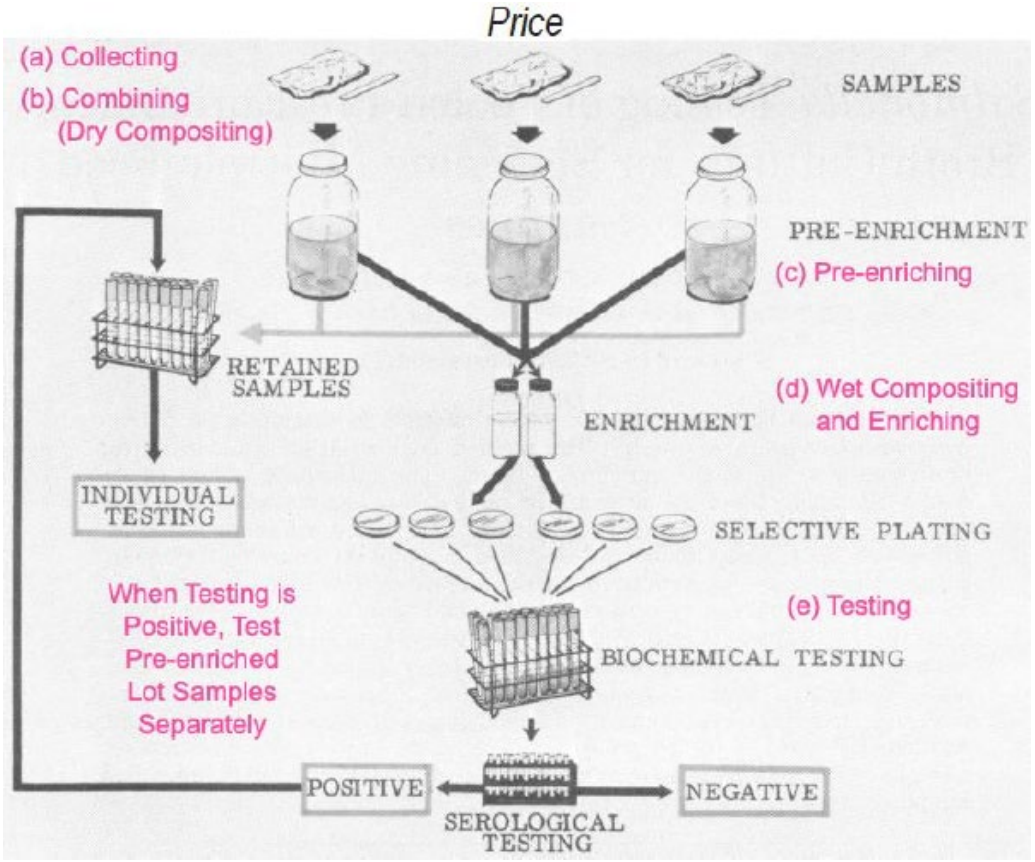
	A method has been described for testing multiple food samples for *Salmonella* without loss in sensitivity. The method pools multiple pre-enrichment broth cultures into single enrichment broths. The subsequent stages of the *Salmonella* analysis are not altered. The method was found applicable to several dry food materials including nonfat dry milk, dried egg albumin, cocoa, cottonseed flour, wheat flour, and shredded coconut. As many as 25 pre-enrichment broth cultures were pooled without apparent loss in the sensitivity of *Salmonella* detection as compared to individual sample analysis. The procedure offers a simple, yet effective, way to increase sample capacity in the *Salmonella* testing of foods, particularly where a large proportion of samples ordinarily is negative. It also permits small portions of pre-enrichment broth cultures to be retained for subsequent individual analysis if positive tests are found. *Salmonella* testing of pooled pre-enrichment broths provides increased consumer protection for a given amount of analytical effort as compared to individual sample analysis. Price, 679. A POSITA would find it obvious to apply the teachings of Price to meat. *See also* MPEP §§ 2144.05, 2131.03. ICMSF teaches collecting 5, 30, 60, 95, or 100 portions. ICMSF, 184-90, 276, 282, 309, 320, 326-30.
Claim 15	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 1, wherein, in a), separately collecting said multiple independent samples from each of the multiple separate test lots comprises separately collecting 30 to 60 samples from each separate test lot.	Price, alone or in combination with ICMSF and Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. Price's teachings for collecting a plurality of portions are based on standard practices as described in BAM 1969 (BAM 1969, 1 § 10.04) which cites to JAOAC 1967 (JAOAC, 234). Price, 679, 682.

	Salmonella **analyses were carried out as described in the *Bacteriological Analytical Manual* (BAM) (2) except for the pooling of pre-enrichment broths described in the following paragraph.** Price, 679. IEH's diagram of Price from Prosecution History for Reexamination Control No. 95/001,294 (reproduced below) shows step 1 corresponds to IEH's step (a), with instructions to "[t]ake individual portions . . . from each Sample."

Figure 3: CITED PRIOR ART (PRICE et al, 1972)



	Prosecution History for Reexamination Control No. 95/001,294, 298 (annotated). IEH's diagram also indicates multiple samples are taken from three bins, which is analogous to three lots rather than a single lot because each bin is an assemblage of specimens. The purpose of Jarvis and Price was to determine whether pooling samples reduced the ability to detect contaminated food lots. It would have been commonsense to combine samples from the same bin and subject them to enrichment, since each bin represents a discrete unit of the production run and results obtained from the enriched sample could be attributed to the bin from which the sample was obtained. Prosecution History for Reexamination Control No. 95/001,294, at 1575 (First Board Decision of January 20, 2015 at 16). Additionally, the specification describes a bin of dry food materials (almonds), like those in Price, as one example of a test lot/single unit of production, similar to a combo of beef. '143 Patent, 5:46-51. Price discloses testing of "Dried egg albumen, cocoa, and nonfat dry milk" (Price, 679) and "wheat flour, coconut, and cottonseed flour" (Price, 681).

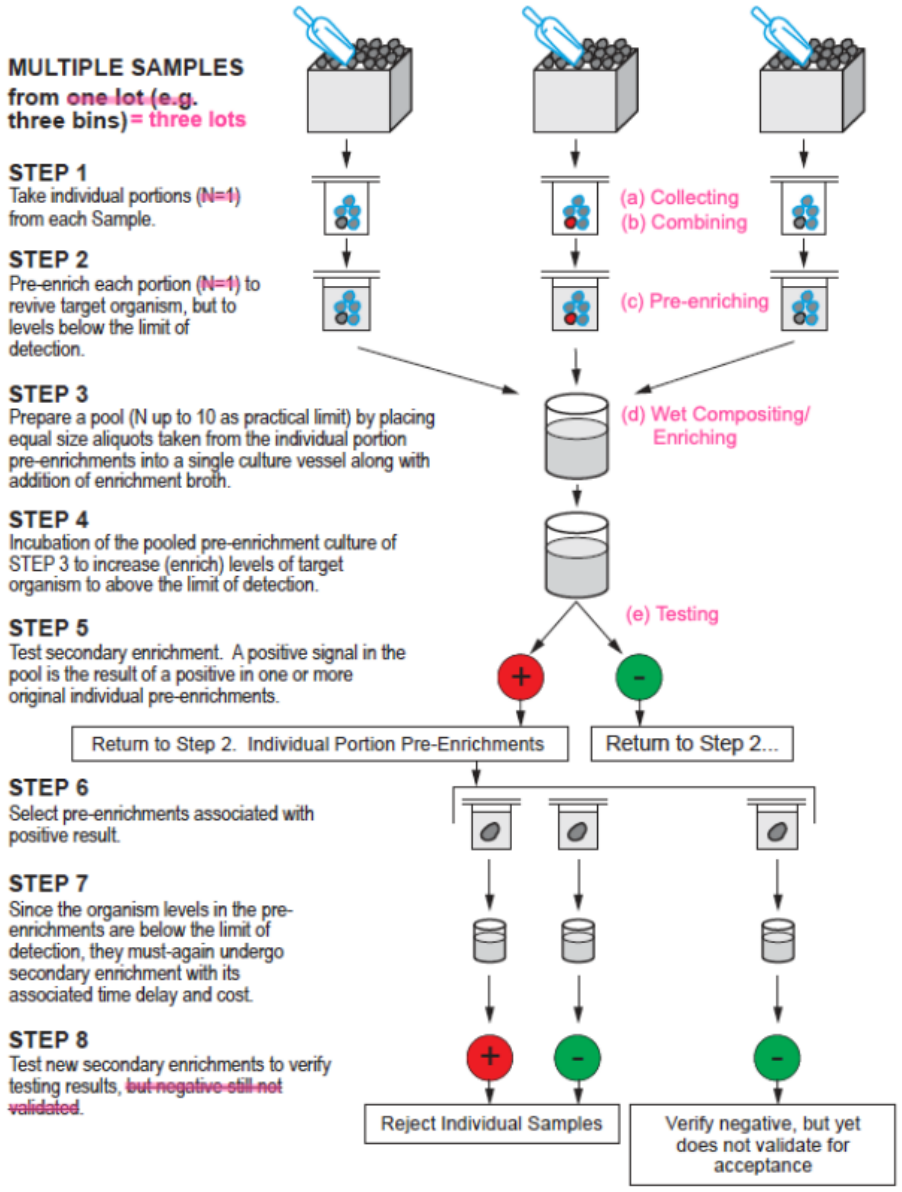


Price, 680 (Figure 1 (annotated)).

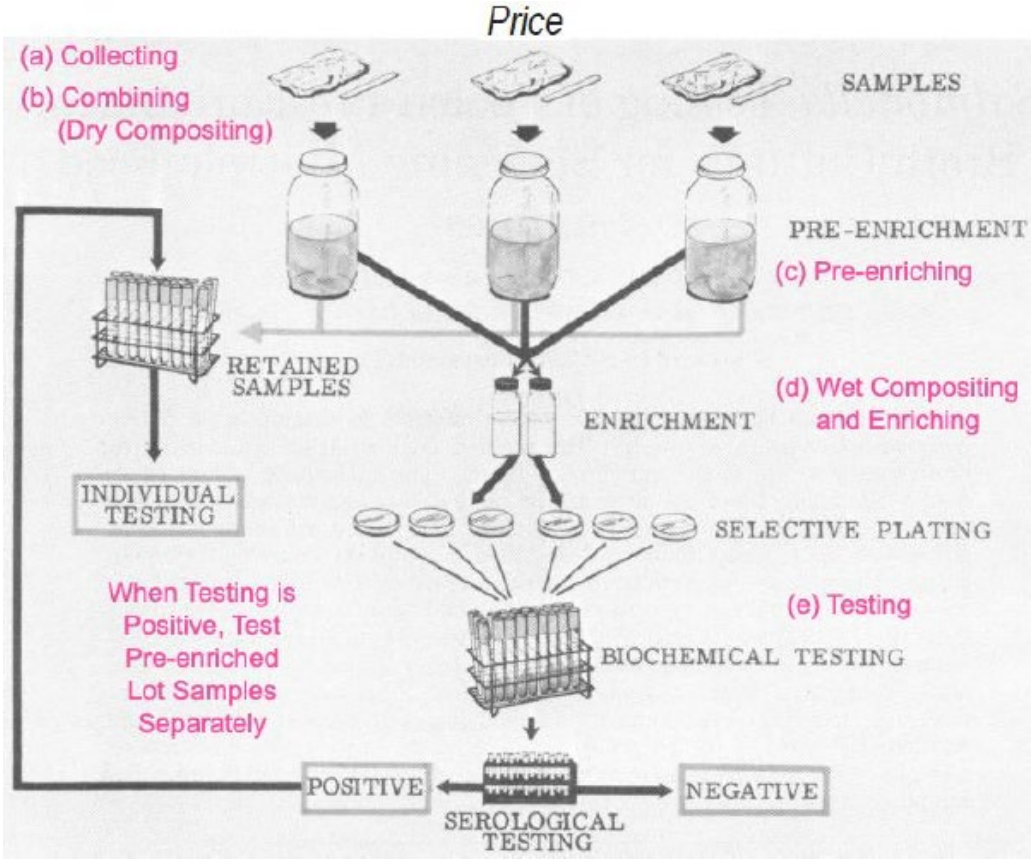
	A method has been described for testing multiple food samples for *Salmonella* without loss in sensitivity. The method pools multiple pre-enrichment broth cultures into single enrichment broths. The subsequent stages of the *Salmonella* analysis are not altered. The method was found applicable to several dry food materials including nonfat dry milk, dried egg albumin, cocoa, cottonseed flour, wheat flour, and shredded coconut. As many as 25 pre-enrichment broth cultures were pooled without apparent loss in the sensitivity of *Salmonella* detection as compared to individual sample analysis. The procedure offers a simple, yet effective, way to increase sample capacity in the *Salmonella* testing of foods, particularly where a large proportion of samples ordinarily is negative. It also permits small portions of pre-enrichment broth cultures to be retained for subsequent individual analysis if positive tests are found. *Salmonella* testing of pooled pre-enrichment broths provides increased consumer protection for a given amount of analytical effort as compared to individual sample analysis. Price, 679. A POSITA would find it obvious to apply the teachings of Price to meat. *See also* MPEP §§ 2144.05, 2131.03. ICMSF teaches collecting 5, 30, 60, 95, or 100 portions. ICMSF, 184-90, 276, 282, 309, 320, 326-30.
Claim 16	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 1, wherein, in a), separately collecting said multiple independent samples from each of the multiple separate test lots comprises separately collecting at least 30 samples from each separate test lot.	Price, alone or in combination with ICMSF and Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. Price's teachings for collecting a plurality of portions are based on standard practices as described in BAM 1969 (BAM 1969, 1 § 10.04) which cites to JAOAC 1967 (JAOAC, 234). Price, 679, 682.

	Salmonella **analyses were carried out as described in the *Bacteriological Analytical Manual* (BAM) (2) except for the pooling of pre-enrichment broths described in the following paragraph.** Price, 679. IEH's diagram of Price from Prosecution History for Reexamination Control No. 95/001,294 (reproduced below) shows step 1 corresponds to IEH's step (a), with instructions to "[t]ake individual portions . . . from each Sample."

Figure 3: CITED PRIOR ART (PRICE et al, 1972)



	Prosecution History for Reexamination Control No. 95/001,294, 298 (annotated). IEH's diagram also indicates multiple samples are taken from three bins, which is analogous to three lots rather than a single lot because each bin is an assemblage of specimens. The purpose of Jarvis and Price was to determine whether pooling samples reduced the ability to detect contaminated food lots. It would have been commonsense to combine samples from the same bin and subject them to enrichment, since each bin represents a discrete unit of the production run and results obtained from the enriched sample could be attributed to the bin from which the sample was obtained. Prosecution History for Reexamination Control No. 95/001,294, at 1575 (First Board Decision of January 20, 2015 at 16). Additionally, the specification describes a bin of dry food materials (almonds), like those in Price, as one example of a test lot/single unit of production, similar to a combo of beef. '143 Patent, 5:46-51. Price discloses testing of "Dried egg albumen, cocoa, and nonfat dry milk" (Price, 679) and "wheat flour, coconut, and cottonseed flour" (Price, 681).



Price, 680 (Figure 1 (annotated)).

	A method has been described for testing multiple food samples for *Salmonella* without loss in sensitivity. The method pools multiple pre-enrichment broth cultures into single enrichment broths. The subsequent stages of the *Salmonella* analysis are not altered. The method was found applicable to several dry food materials including nonfat dry milk, dried egg albumin, cocoa, cottonseed flour, wheat flour, and shredded coconut. As many as 25 pre-enrichment broth cultures were pooled without apparent loss in the sensitivity of *Salmonella* detection as compared to individual sample analysis. The procedure offers a simple, yet effective, way to increase sample capacity in the *Salmonella* testing of foods, particularly where a large proportion of samples ordinarily is negative. It also permits small portions of pre-enrichment broth cultures to be retained for subsequent individual analysis if positive tests are found. *Salmonella* testing of pooled pre-enrichment broths provides increased consumer protection for a given amount of analytical effort as compared to individual sample analysis. Price, 679. A POSITA would find it obvious to apply the teachings of Price to meat. ICMSF teaches collecting 5, 30, 60, 95, or 100 portions. ICMSF, 184-90, 276, 282, 309, 320, 326-30. *See also* MPEP § 2144.05.
Claim 18	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 1, wherein removing portions in step d) is removing equal portions, aseptically, so as to preclude contamination of the enriched separate composited test lot samples.	Price, alone or in combination with ICMSF and Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. Price further discloses removing portions in step d) is removing equal portions, aseptically, so as to preclude contamination of the enriched separate test lot samples. ICMSF describes aseptically withdrawing and weighing sample units into the appropriate diluent. ICMSF, 231, 232.

	Hughes instructs that after enrichment "[c]ombine equal volumes (0.5-1 mL) from each M broth tube in a clean screw-cap tube." Hughes, 638. Moreover, a POSITA would consider this to be standard practice. A POSITA would have aseptically removed equal volume portions of each enriched separate test lot sample to preclude contamination.
Claim 19	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 1, wherein the target microbe(s) comprises at least one selected from the group consisting of *Listeria*, *Salmonella* and *Escherichia coli*.	Price, alone or in combination with ICMSF and Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. Price further discloses a target microbial agent or organism comprises at least one selected from the group consisting of *Listeria*, *Salmonella* and *Escherichia coli*. Price established that "pre-enrichment broths can be pooled for analysis without loss in sensitivity to *Salmonella* in the individual samples." Price, 679. Price tested dry food materials inoculated (purposefully contaminated) with *Salmonella. Id.* ICMSF describes testing for *Listeria* (ICMSF, 285-312), *Salmonella* (*id.*, 273-84), and *Escherichia coli* (*id.*, 313-32). Hughes focuses on *Salmonella*. Hughes, 634. Price established that "pre-enrichment broths can be pooled for analysis without loss in sensitivity to *Salmonella* in the individual samples." Price, 679. Price tested dry food materials inoculated (purposefully contaminated) with *Salmonella. Id.*
Claim 20	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 1, wherein the target microbe(s) comprises at least one selected from the group consisting of *Listeria* spp., *Listeria monocytogenes*, *Salmonella* spp.,	Price, alone or in combination with ICMSF and Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. Price further discloses a target microbial agent or organism comprises at least one selected from the group consisting of *Listeria*, *Salmonella* and *Escherichia coli*.

Escherichia coli O157:H7, coliforms and fecal coliforms.	Price established that "pre-enrichment broths can be pooled for analysis without loss in sensitivity to *Salmonella* in the individual samples." Price, 679. Price tested dry food materials inoculated (purposefully contaminated) with *Salmonella. Id.* ICMSF describes testing for *Listeria* (ICMSF, 285-312), *Salmonella* (*id.*, 273-84), and *Escherichia coli* (*id.*, 313-32). Hughes focuses on *Salmonella*. Hughes, 634. Price established that "pre-enrichment broths can be pooled for analysis without loss in sensitivity to *Salmonella* in the individual samples." Price, 679. Price tested dry food materials inoculated (purposefully contaminated) with *Salmonella. Id.*
Claim 21	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 1, wherein the target microbe(s) comprises a spoilage organism.	Price, alone or in combination with ICMSF and Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. Price further discloses a target microbial agent or organism comprises a spoilage organism. A POSITA would find it obvious to apply the teachings of Price to target spoilage organisms and would employ a common test like APC, as discussed in regulations such as PR/HACCP. PR/HAACP, 38849 ("Various alternative indicator organisms were suggested, including generic *E. coli* (biotype I), total plate counts, Enterobacteriaceae, Total Viable Counts (TVC), and Aerobic Plate Counts (APC)."). A POSITA would find it obvious to apply the teachings of ICMSF and Hughes to target spoilage organisms. "Examination of every piece of whole meat is clearly impractical, hence any sampling procedure for estimating shelf-life would involve taking enough samples to give some statistical assurance of the keeping qualities of meat." Gill, 449.

	Borch is titled "Bacterial spoilage of meat and cured meat products." Borch. The reference discusses spoilage organisms in meat: Lactic acid bacteria are the major bacterial group associated with the spoilage of refrigerated vacuum- or MA-packaged cooked, cured meat products (Blickstad and Molin, 1983b; Shaw and Harding, 1989; von Holy et al., 1991). At the time of spoilage some products contain a 'pure' culture of only one species, while in others a mixture of *Lactobacillus* spp. and *Leuconostoc* spp. was found (Kant-Muermans, unpublished results). The great diversity of bacteria isolated from spoiled meat products is confirmed by several studies (Korkeala and Mäkelä, 1989; von Holy et al., 1991; Yang and Ray, 1994). The genus/species of lactic acid bacteria responsible for spoilage, depend on the product composition (product-related flora) as well as the manufacturing site (house-related flora; Korkeala and Mäkelä, 1989). Borch, 113. "Lactic acid bacteria spoil refrigerated meat products by causing defects such as sour off-flavours, discolouration, gas production, slime production and decrease in PH." Borch, 114; *see also, id.*, 115-17 (Section 4 Analysis of Spoilage). It is understood in the art that aerobic plate counts ("APC") can test for spoilage organisms. *See. e.g.*, ICMSF 1986, 122 ("tests to be made (e.g., APC, *Salmonella*, Clostridium perfringens"); *id.* at 132 ("However, aerobic plate counts (APC) can be used to monitor Good Commercial Practice (GCP), and criteria based on such examinations are a valuable aid to in-plant quality control.").

TABLE 19

Sampling plans and recommended microbiological limits for raw meat[a]

Product[b]	Test	Method reference[c]	Case	Plan class	n	c	Limit per cm² or gram m	M
Carcass meat, before chilling	APC	118	1	3	5	3	10^5	10^6
Carcass meat, chilled	APC	118	1	3	5	3	10^6	10^7
Edible offal, chilled	APC	118	1	3	5	3	10^6	10^7
Carcass meat, frozen	APC	118	1	3	5	3	5×10^5	10^7
Boneless meat, frozen (beef, veal, pork, mutton)	APC	118	1	3	5	3	5×10^5	10^7
Comminuted meat, frozen	APC	118	1	3	5	3	10^6	10^7
Edible offal, frozen	APC	118	1	3	5	3	5×10^5	10^7

a **Not for use at port of entry** but for in-plant quality control. Refer to Section B for appropriate applications.
b Unfrozen carcasses and primal cuts, swab counts per cm²; other meats and meat products, per gram.
c Refers to page number in ICMSF, 1978.

ICMSF 1986, 133 (Table 19 (annotated)).

Although for “quality control,” a POSITA would understand that "quality control" means testing for spoilage organisms could also be accomplished by this well-known method. It is disclosed within the prior art during the production lifecycle of beef production. ICMSF 1986, 134 (“The FAOIWHO Working Group (FAQ/WHO, 1979) considered that the only point at which APC could be used for chilled meat, to evaluate the hygienic conditions under which it was produced, was at the slaughterhouse, and because of its perishable nature it would not be possible to set

APC limits for use in guidelines or specifications for chilled meats at port of entry."); *see also, id.*, 153 ("Monitoring for APC, E. coli, and Staph. aureus would give some indication of inadequate processing (heating, curing, smoking), postprocess contamination, or storage at temperatures above those recommended for frozen or refrigerated distribution and storage.")

E. TEST PROCEDURES

(a) *Aerobic plate counts*

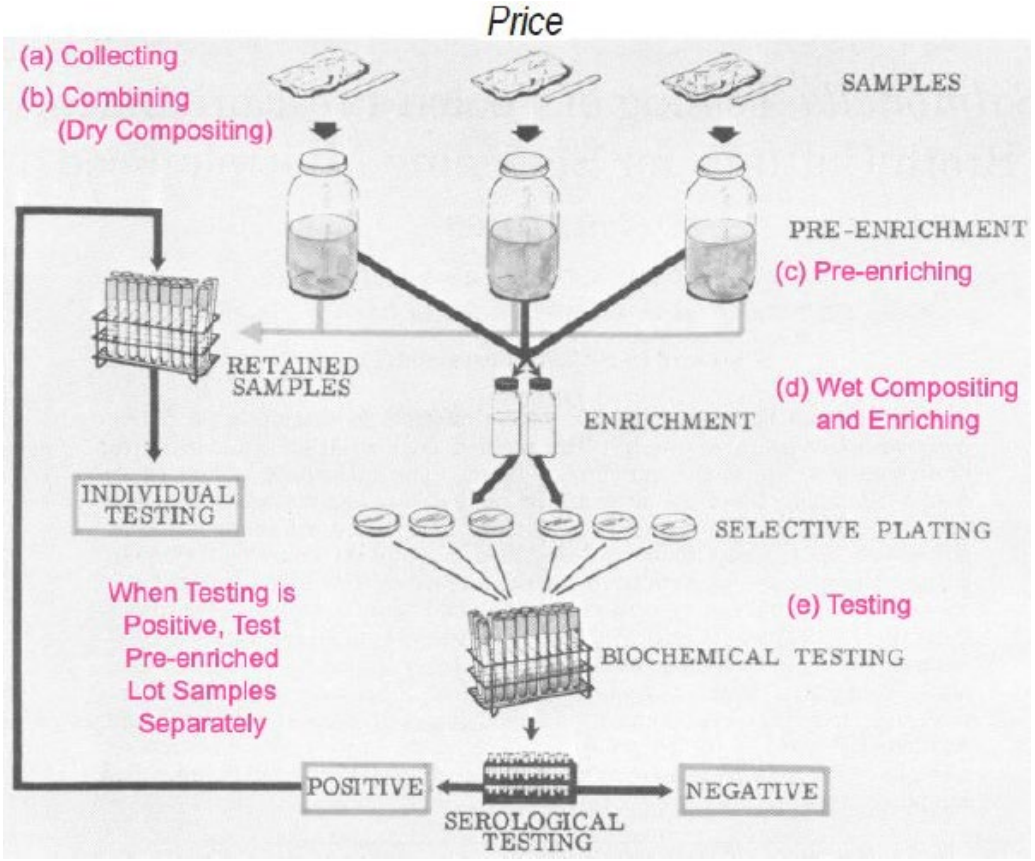
Comminute, blend, or stomach the various portions of meat composing the sample unit and withdraw the analytical unit. Combine the swabs to form an analytical unit, or treat each separately. Prepare dilutions and perform the APC test according to the directions given in ICMSF, 1978, p. 118. Spread plates are preferable to pour plates when examining raw meats.

The temperature used for incubation of plates should be related to the material being examined. To estimate numbers of mesophilic contaminants on freshly dressed carcasses it is advisable to incubate plates at 35 or 37°C. For meat that has been held under chilled conditions, plates should be incubated at 25°C to include psychrotrophs and thus give a better indication of the microbial growth that has occurred. Counts at 20°C give similar results after a longer incubation time.

ICMSF 1986, 136 (annotated).

See also, Bhunia 4 ("Bacteria-specific generic rapid methods such ATP-based luminescence and total plate count (APC) are also needed for monitoring cleaning and hygienic practices employed in a processing plant."); Wheeler, 1606 (use of APC in a beef-processing plant); PR/HAACP, 38849 ("Various alternative indicator

	organisms were suggested, including generic E. coli (biotype I), total plate counts, Enterobacteriaceae, Total Viable Counts (TVC), and Aerobic Plate Counts (APC).”).
Claim 22	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 1, wherein removing portions of each separate enriched composited test lot sample comprises removing equal volume portions of each separate enriched composited test lot sample.	Price, alone or in combination with ICMSF and Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. Price further discloses removing portions of each enriched separate test lot sample comprises removing equal volume portions of each enriched separate test lot sample. ICMSF describes aseptically withdrawing and weighing sample units into the appropriate diluent. ICMSF, 231, 232. Hughes instructs that after enrichment “[c]ombine equal volumes (0.5-1 mL) from each M broth tube in a clean screw-cap tube.” Hughes, 638. Moreover, a POSITA would consider this to be standard practice. A POSITA would have removed equal volume portions of each enriched separate test lot sample.
Claim 23	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 1, wherein testing of the pooled modular composite sample, using a suitable detection assay comprises use of at least one of a DNA-based method, an immunochemistry-based method, and a biosensor-based method.	Price, alone or in combination with ICMSF and Hughes, discloses Claim 1, upon which this claim relies. See above re Claim 1. Price further discloses testing of the modular composite sample, using a suitable detection assay comprises use of at least one of a DNA-based method, an immunochemistry-based method, and a biosensor-based method. Price discloses biochemical testing of the modular composite sample. A POSITA would consider other suitable detection assays to test for the presence of *Salmonella*, which can include DNA-based methods, immunochemistry-based methods, and biosensor-based methods.

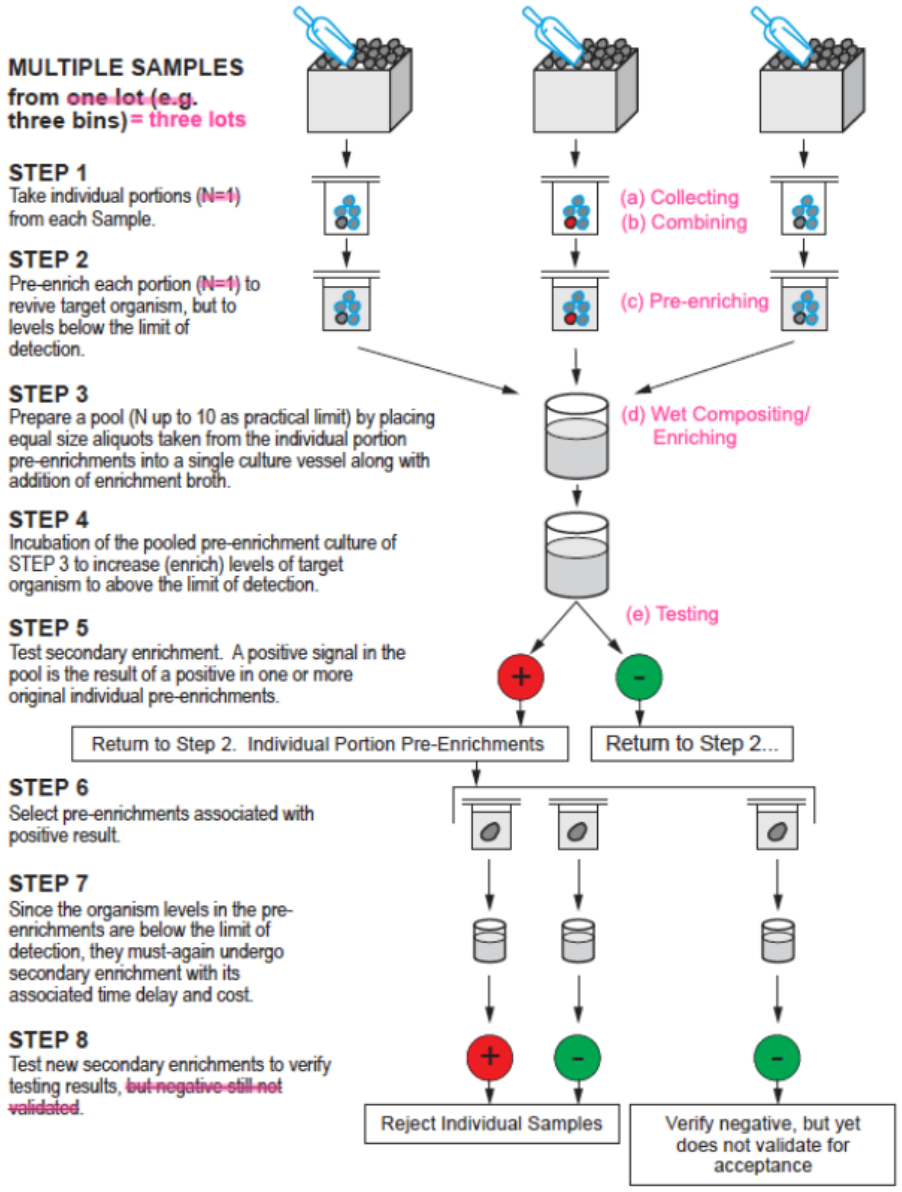


Price, 680 (Figure 1 (annotated)).

Hughes discloses use of "an enzyme immunoassay (EIA) method," which is an immunochemistry-based method. Hughes, 635.

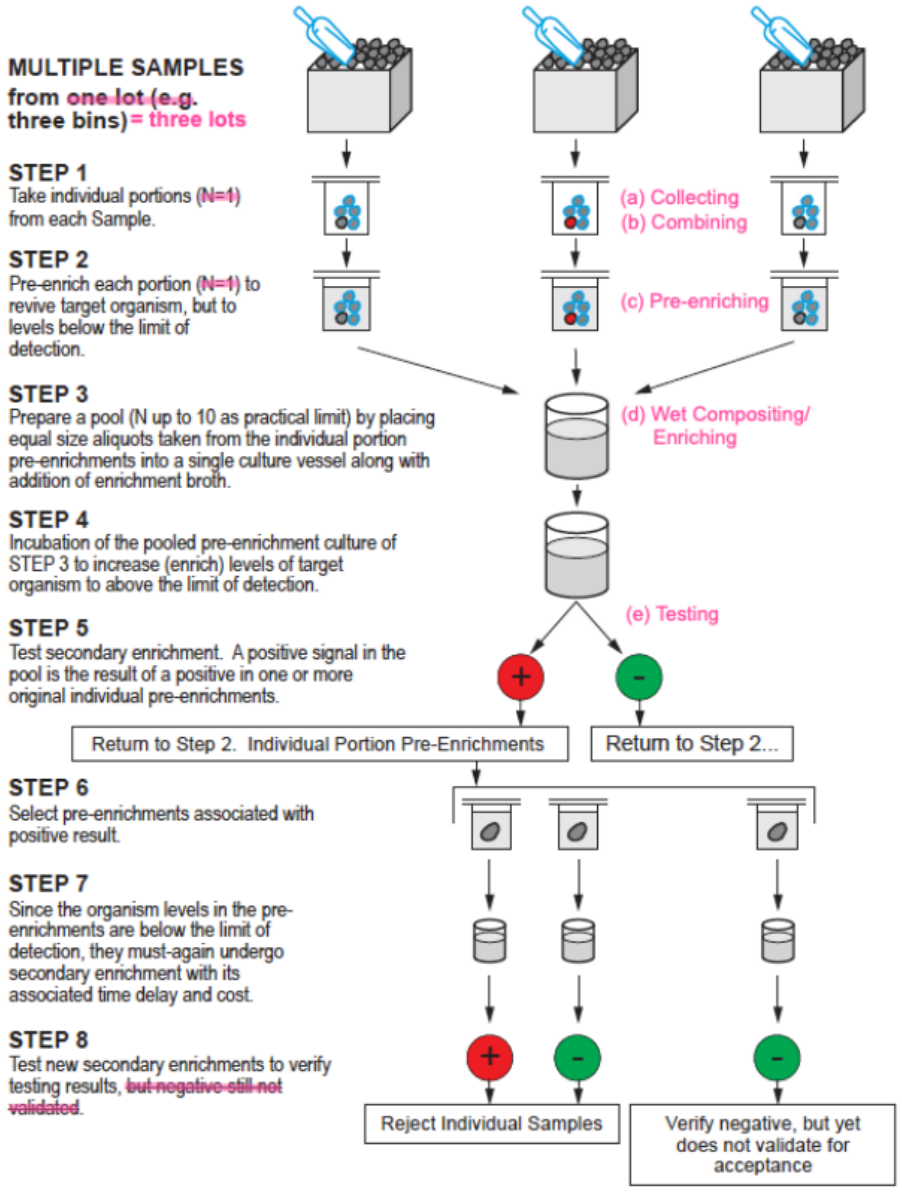
	Each sample was analyzed by the BAM method (7th Ed.) and the TSVIA method using the enrichment protocol described in section F of the method, *Preparation of Sample*. Equal portions (0.5–1 mL) from each of the 2M broths arising from the 2 selective-enrichment broths were combined in a small screw-capped tube and heat treated in boiling water for 15 min prior to performing the TSVIA. Confirmation for the immunoassay was by streaking from the M broth culture onto selective agar, as specified in the BAM reference culture method. Hughes, 635.
Claim 24	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 1, wherein each of the multiple separate test lots are production lots of a meat or poultry product, comprising an assemblage of one or more meat or poultry specimens, respectively.	Price, in combination with ICMSF and Hughes, discloses Claim 1 upon which this claim relies. See above re Claim 1.

Figure 3: CITED PRIOR ART (PRICE et al, 1972)



	Prosecution History for Reexamination Control No. 95/001,294, 298 (annotated). IEH's diagram also indicates multiple samples are taken from three bins, analogous to three lots rather than a single lot because each bin is an assemblage of specimens.
Claim 29	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 3, wherein product specimens are synonymous with product portions.	Price, alone or in combination with ICMSF and Hughes, discloses Claim 3, upon which this claim relies. See above re Claim 3. Price, alone or in combination with ICMSF and Hughes, further discloses product specimens are synonymous with product portions.

Figure 3: CITED PRIOR ART (PRICE et al, 1972)



	Prosecution History for Reexamination Control No. 95/001,294, 298 (annotated). IEH's diagram indicates multiple samples and portions are removed from the bins/lots, and uses the terminology interchangeably. The specification of the '143 Patent indicates a test lot is a single-unit production lot ('143 Patent, 5:34-45) such as a single combo, bin. pallet, or segment of production ('143 Patent, 7:42-50; *see also* AAPA #3 and #4). ICMSF teaches collecting portions or samples, which were known synonyms for product portions or specimens. ICMSF states that "[a] sample unit is a small portion of the lot." ICMSF, 225. **12.1 INTRODUCTION** Many different types of samples are collected and submitted to the laboratory for analysis. Some are sample units from lots or consignments of foods or ingredients for lot acceptance determination. Others may be for investigational purposes to assess control of the environment, investigate the source of a problem, or validate a process. Some may have legal implications relative to a lawsuit or for regulatory compliance. This chapter briefly discusses some of the major factors that should be considered when collecting sample units, shipping them to a laboratory, preparing them for analysis, and analyzing them. A sample unit is a small portion of the lot in which the distribution of both numbers and types of microorganisms should be representative of the whole lot. This requirement is only completely fulfilled when the food and processing conditions result in a homogeneous distribution of microorganisms. This only occurs in well-agitated fluid products. In general, the distribution of microorganisms is presumed to be random. ICMSF at 225 annotated).
Claim 30	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 5, wherein the enrichment medium comprises a selective agent that will inhibit a competing non-target microbe.	Price, alone or in combination with ICMSF and Hughes, discloses Claim 5, upon which this claim relies. See above re Claim 5. Price, alone or in combination with ICMSF and Hughes, further discloses a medium comprises a selective agent that will inhibit a competing non-target microbial agent or organism.

	Price discusses organisms facing competition from other organisms during the enrichment stage. A POSITA would conclude that inhibiting competition would be beneficial to the growth of target microbial agents or organisms. Claim 30 depends on Claims 1 and 5, which are disclosed by ICMSF and Hughes. Price discusses organisms facing competition from other organisms during the enrichment stage. A POSITA would conclude that inhibiting competition would be beneficial to the growth of target microbial agents or organisms. **Pooled samples versus pooled pre-enrichment broths.** Silliker (4) reported a loss in sensitivity when several samples were pooled in a large container of pre-enrichment broth as compared to individual sample analysis. This is not surprising since, to be detected, the desiccated and debilitated *Salmonella* cells in a dried food sample must grow out in pre-enrichment broth in competition with other bacteria in the sample. If many food samples are pooled at the pre-enrichment stage, the salmonellae face competition from the flora of all the samples in the pool. If, on the other hand, the samples are individually pre-enriched, the actively growing salmonellae might be expected to compete more favorably with organisms introduced from other samples when pooled at the enrichment stage. In addition, pooling pre-enrichment broths avoids the hazards and inconvenience of handling very large flasks of culture.

	Price, 681. Additionally, Claim 28 recites what a POSITA would consider the well-known property of selective enrichment, inhibition of a competing non-target microorganism. D'Aoust describes "selective enrichment [that] inhibits growth of non-*Salmonella*e and facilitates isolation of *Salmonella* on differential plating media." D'Aoust, 369. A POSITA would have been motivated to use an enrichment medium comprising a selective agent that inhibits a non-target agent or organisms as described by D'Aoust, for the test lot samples described by ICMSF and Hughes, to ensure an accurate test. A POSITA would understand that incubating samples at different temperature ranges for different target microorganisms would, effectively, inhibit the growth of other organisms. "Incubation temperature markedly affects productivity of selective enrichment broths by suppressing growth of competing microorganisms but not *Salmonella*." D'Aoust, 372. "This may be achieved by utilization of enrichment media that through various mechanisms may favor and enhance growth and multiplication of salmonellae while other gram-negative organisms such as coliforms, Proteus, and Pseudomonas are suppressed or inhibited." Fagerberg, 630.
Claim 31	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 6, wherein the concentration step comprises at least one of immunomagnetic bead separation, and affinity chromatography.	Price, alone or in combination with ICMSF and Hughes, discloses Claim 6, upon which this claim relies. See above re Claim 6. Price, alone or in combination with ICMSF and Hughes, further discloses the concentration step comprises at least one of immunomagnetic bead separation, and affinity chromatography.

	Price discloses an enrichment stage after compositing where "[s]amples are individually pre-enriched followed by transfer from multiple pre-enrichments to single selenite and tetrathionate broths at the enrichment stage." Price, 679. A POSITA would understand that a detection assay that includes enrichment could further comprise a concentration step. Enrichment for the microbial agent or organism increases its concentration. A POSITA would use a standard concentration procedure such as immunomagnetic bead separation or affinity chromatography in order to concentrate the sample. ICMSF discloses the use of "immunomagnetic beads to concentrate cells." ICMSF, 318. A POSITA would consider this a well-known technique before the priority date. Fratamico discusses the rapid isolation of target microorganisms using an immunomagnetic separation method. Fratamico, 105. Fratamico discloses that use of immunomagnetic beads resulted in "an increase in the level of E. coli B1409 in the sample," as well as "an increase in the number of organisms recovered." *Id.*, 107. Additionally, Fratamico provides detailed instructions for preparing and using immunomagnetic bead separation to concentrate a target microbial agent or organism. Fratamico developed "[a] method employing immunomagnetic beads" to concentrate and thereby improve the isolation and detection of E. coli. *Id.* For example, Fratamico was able to use immunomagnetic beads to isolate *E. coli* strains from enrichment cultures of raw ground beef "when the initial inoculum level was 1 cfu g-1." *Id.*, 109. Fratamico further teaches that "[t]he percentage of bacteria recovered increased with increasing amounts of beads." *Id.*, 108. Consequently, Fratamico "demonstrates that immunomagnetic separation [using immunomagnetic beads] is a simple and rapid technique" for concentrating a microbe for isolation and detection. *Id.*, 109. These teachings of Fratamico would have motivated a POSITA to modify the combination of ICMSF and Gombas and ICMSF and Hughes to use

	immunomagnetic beads to concentrate microbes in a modular composite sample to benefit from accurate tests. Use of immunomagnetic beads was already known in the art for concentrating during testing beef samples. "Tu et al. (78) detected pathogenic E. coli O157:H7 in beef hamburger with the use of IMS and ATP measurement. After 6 h of enrichment at 37°C, inoculated beef samples (0.9 ml each) were filtered to remove large particles prior to 10 min of incubation with ca. 10^5 magnetic beads coated with anti–*E. coli* O157:H7 (Dynabeads, Dynal Inc., Oslo, Norway). Following incubation, the magnetic beads were collected with a Dynal magnetic concentrator and resuspended in 0.9 ml of Trypticase soy broth for ATP analysis." Benoit, 1941. "Hudson et al. (26) used IMS to directly separate and concentrate L. monocytogenes from ham samples prior to detection by PCR. IMS was used on the basis of the commercial availability of anti-Listeria magnetic beads (Dynal Inc.) to avoid time-consuming cultural enrichment steps and to remove PCR inhibitors." Benoit, 1941-42. Cudjoe investigates the use of immunomagnetic beads in pooling methods. In a study designed to evaluate the performance of Dynabeads® Anti-Salmonella, immunomagnetic separation (IMS) followed by plating (IMS-Plating) proved far superior to the conventional ISO *Salmonella* methodology and the Modified Semi-solid Rappaport-Vassiliadis (MSRV) method. *Salmonella* species were isolated and detected from 135 out of the 180 diverse poultry samples by IMS analysis as against 98 by the conventional method and 33 by the MSRV technique. All results were confirmed biochemically and serologically. It appeared from the data generated that some of the salmonellae isolated using IMS were greatly inhibited in Rappaport-Vassiliadis enrichment broth at 42°C and to a lesser extent in selenite cystine broth at 37°C. A moderately selective plating medium like XLD proved to be better in isolating these possible sensitive wild-type strains of salmonellae than the more selective BGA. Cudjoe, Abstract.

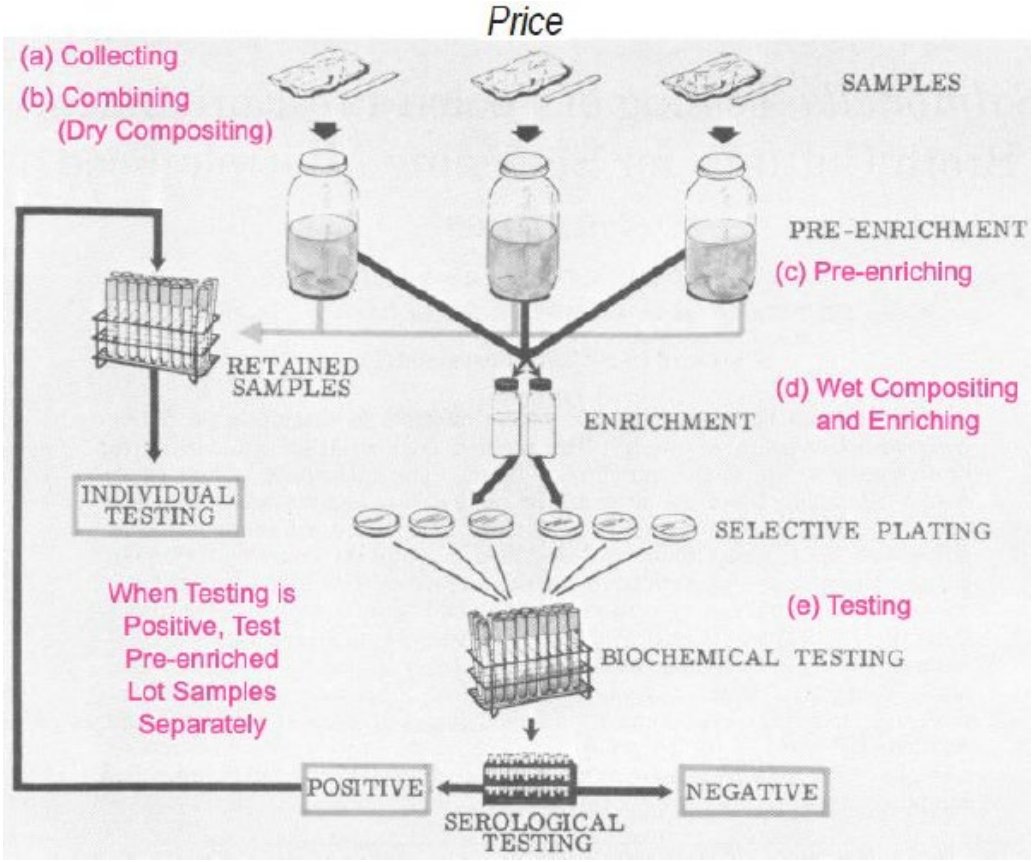
	Samples. One hundred and eighty (180) diverse poultry samples, consisting of 40 pools of cloacal/faecal swabs (5 swabs/pool; 5 pools samples/flock) from eight different flocks, 10 raw chicken breast meat samples, 10 pooled egg shell samples, 60 raw chicken liver samples and 60 chicken neck skins sampled from the slaughter line, were used in this study. All samples were obtained from a poultry slaughter and processing plant in the Netherlands. A pool of five swab samples was pre-enriched in 10 ml of buffered peptone water (BPW; Difco Laboratories, Detroit, Michigan, USA) at 37°C for 16–20 h, whilst 25 g of each of the other samples above, were pre-enriched similarly in 225 ml of BPW and examined as detailed below. Cudjoe, 160.
Claim 32	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 7, wherein the more sensitive protocol comprises use of a concentration step to concentrate the target microbe(s), in combination with the same test protocol used to test the pooled modular composite sample.	Price, alone or in combination with ICMSF and Hughes, discloses Claim 7, upon which this claim relies. See above re Claim 7. Price, alone or in combination with ICMSF and Hughes, further discloses more sensitive protocol that further comprises a concentration step to concentrate the microbial agent or organism, in combination with the same test protocol that is used to test the modular composite sample. Price discloses an enrichment stage after compositing where "[s]amples are individually pre-enriched followed by transfer from multiple pre-enrichments to single selenite and tetrathionate broths at the enrichment stage." Price, 679. A POSITA would understand that a detection assay that includes enrichment could further comprise a concentration step. Enrichment for the microbial agent or organism increases its concentration. A POSITA would use a standard concentration procedure such as immunomagnetic bead separation or affinity chromatography in order to concentrate the sample.

	ICMSF discloses the use of "immunomagnetic beads to concentrate cells." ICMSF, 318. A POSITA would consider this a well-known technique before the priority date. Hughes uses an EIA method followed by a more sensitive protocol—streaking on selective media. Hughes, 635. Each sample was analyzed by the BAM method (7th Ed.) and the TSVIA method using the enrichment protocol described in section F of the method, *Preparation of Sample*. Equal portions (0.5–1 mL) from each of the 2M broths arising from the 2 selective-enrichment broths were combined in a small screw-capped tube and heat treated in boiling water for 15 min prior to performing the TSVIA. Confirmation for the immunoassay was by streaking from the M broth culture onto selective agar, as specified in the BAM reference culture method. Hughes, 635. A POSITA would find that modifying Hughes based on D'Haese ensures a more sensitive test is used for further testing. A POSITA would have recognized the concentration step could be employed to provide a more sensitive protocol for further testing. A POSITA would understand that retesting the non-pooled, singular samples is an inherently more sensitive process because the sample is not diluted with other samples in the testing pool. Therefore, the retesting of a singular sample is more concentrated and therefore, more sensitive. *See also, generally*, Jarvis. Fratamico discusses the rapid isolation of target microorganisms using an immunomagnetic separation method. Fratamico, 105. Fratamico discloses that use of

	immunomagnetic beads resulted in "an increase in the level of E. coli B1409 in the sample," as well as "an increase in the number of organisms recovered." *Id.*, 107. Additionally, Fratamico provides detailed instructions for preparing and using immunomagnetic bead separation to concentrate a target microbial agent or organism. Fratamico developed "[a] method employing immunomagnetic beads" to concentrate and thereby improve the isolation and detection of E. coli. *Id.* For example, Fratamico was able to use immunomagnetic beads to isolate *E. coli* strains from enrichment cultures of raw ground beef "when the initial inoculum level was 1 cfu g-1." *Id.*, 109. Fratamico further teaches that "[t]he percentage of bacteria recovered increased with increasing amounts of beads." *Id.*, 108. Consequently, Fratamico "demonstrates that immunomagnetic separation [using immunomagnetic beads] is a simple and rapid technique" for concentrating a microbe for isolation and detection. *Id.*, 109. These teachings of Fratamico would have motivated a POSITA to modify the combination of ICMSF and Gombas and ICMSF and Hughes to use immunomagnetic beads to concentrate microbes in a modular composite sample to benefit from accurate tests. Use of immunomagnetic beads was already known in the art for concentrating during testing beef samples. "Tu et al. (78) detected pathogenic E. coli O157:H7 in beef hamburger with the use of IMS and ATP measurement. After 6 h of enrichment at 37°C, inoculated beef samples (0.9 ml each) were filtered to remove large particles prior to 10 min of incubation with ca. 10^5 magnetic beads coated with anti–*E. coli* O157:H7 (Dynabeads, Dynal Inc., Oslo, Norway). Following incubation, the magnetic beads were collected with a Dynal magnetic concentrator and resuspended in 0.9 ml of Trypticase soy broth for ATP analysis." Benoit, 1941. "Hudson et al. (26) used IMS to directly separate and concentrate L. monocytogenes from ham samples prior to detection by PCR. IMS was used on the basis of the commercial availability of anti-Listeria magnetic beads (Dynal Inc.) to avoid time-

	consuming cultural enrichment steps and to remove PCR inhibitors." Benoit, 1941-42. Cudjoe investigates the use of immunomagnetic beads in pooling methods. In a study designed to evaluate the performance of Dynabeads® Anti-Salmonella, immunomagnetic separation (IMS) followed by plating (IMS-Plating) proved far superior to the conventional ISO *Salmonella* methodology and the Modified Semi-solid Rappaport-Vassiliadis (MSRV) method. *Salmonella* species were isolated and detected from 135 out of the 180 diverse poultry samples by IMS analysis as against 98 by the conventional method and 33 by the MSRV technique. All results were confirmed biochemically and serologically. It appeared from the data generated that some of the salmonellae isolated using IMS were greatly inhibited in Rappaport-Vassiliadis enrichment broth at 42°C and to a lesser extent in selenite cystine broth at 37°C. A moderately selective plating medium like XLD proved to be better in isolating these possible sensitive wild-type strains of salmonellae than the more selective BGA. Cudjoe, Abstract. *Samples*. One hundred and eighty (180) diverse poultry samples, consisting of 40 pools of cloacal/faecal swabs (5 swabs/pool; 5 pools samples/flock) from eight different flocks, 10 raw chicken breast meat samples, 10 pooled egg shell samples, 60 raw chicken liver samples and 60 chicken neck skins sampled from the slaughter line, were used in this study. All samples were obtained from a poultry slaughter and processing plant in the Netherlands. A pool of five swab samples was pre-enriched in 10 ml of buffered peptone water (BPW; Difco Laboratories, Detroit, Michigan, USA) at 37°C for 16–20 h, whilst 25 g of each of the other samples above, were pre-enriched similarly in 225 ml of BPW and examined as detailed below. Cudjoe, 160.
Claim 33	**Disclosure of Price, ICMSF, and Hughes**

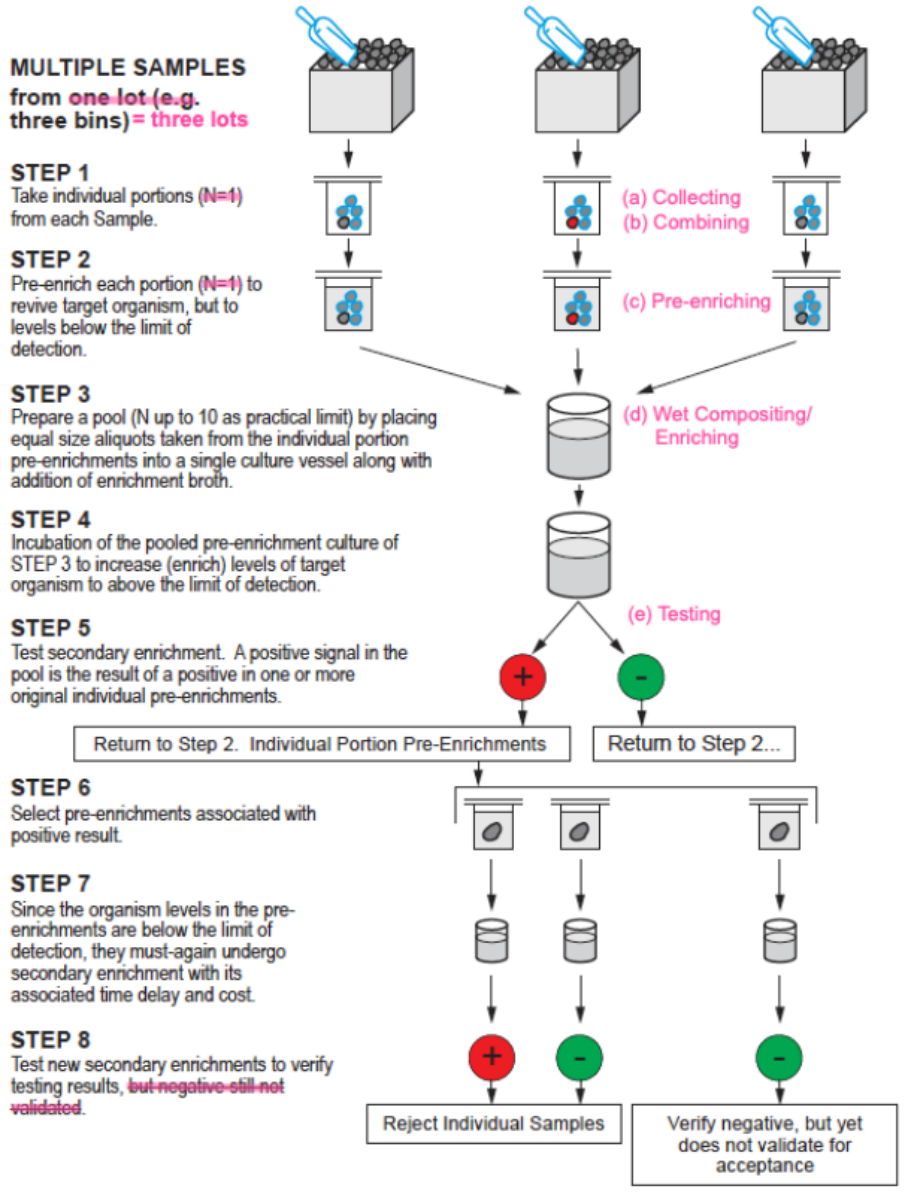
The method of claim 24, wherein the meat or poultry specimens are meat trim pieces or poultry trim pieces, respectively.	Price, alone or in combination with ICMSF and Hughes, discloses Claim 24, upon which this claim relies. See above re Claim 24. A POSITA would find it obvious to apply the teachings of Price to meat or poultry trim pieces. ICMSF indicates "[t]rimmings to be used for ground beef could be pretested for E. coli O157:H7 by various sampling plans." ICMSF, 323. ICMSF indicates "[r]esearch has validated that up to fifteen 25 g analytical units of ground beef or trimmings can be composited without significant loss in E. coli O157:H7 detection sensitivity." *Id.*, 327 *see also id.*, 226, 314, 320, and 324. ICMSF includes many other references to testing lots of beef or poultry, two of the most common types of meat in commerce. *Id.* at 114 (§ 6.3), 184–191 (§§ 10.2–10.6), 225–226 (§ 12.1), 285 (§ 16.1), 313–330 (Chapter 17). ICMSF is relevant to any kind of food product including beef or poultry.
Claim 34	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 24, wherein the meat is that of beef, pork, sheep or bison.	Price, alone or in combination with ICMSF and Hughes, discloses Claim 24, upon which this claim relies. See above re Claim 24. A POSITA would find it obvious to apply the teachings of Price to meat. ICMSF indicates "[t]rimmings to be used for ground beef could be pretested for E. coli O157:H7 by various sampling plans." ICMSF, 323. ICMSF indicates "[r]esearch has validated that up to fifteen 25 g analytical units of ground beef or trimmings can be composited without significant loss in E. coli O157:H7 detection sensitivity." *Id.*, 327 *see also id.*, 226, 314, 320, and 324. ICMSF includes many other references to testing lots of beef or poultry, two of the most common types of meat in commerce. *Id.* at 114 (§ 6.3), 184–191 (§§ 10.2–10.6), 225–226 (§ 12.1), 285 (§ 16.1), 313–330 (Chapter 17). ICMSF is relevant to any kind of food product including beef, pork, sheep or bison.

Claim 35	Disclosure of Price, ICMSF, and Hughes
[35pre] A method of sampling, and testing for microbe(s) in multiple separate test lots, comprising:	To the extent the preamble is limiting, as discussed herein, Price, alone or in combination with ICMSF and Hughes, discloses a method of sampling and testing for microbes in multiple separate test lots. Price established that "pre-enrichment broths can be pooled for analysis without loss in sensitivity to *Salmonella* in the individual samples." Price, 679. Price tested dry food materials inoculated (purposefully contaminated) with *Salmonella. Id.* 

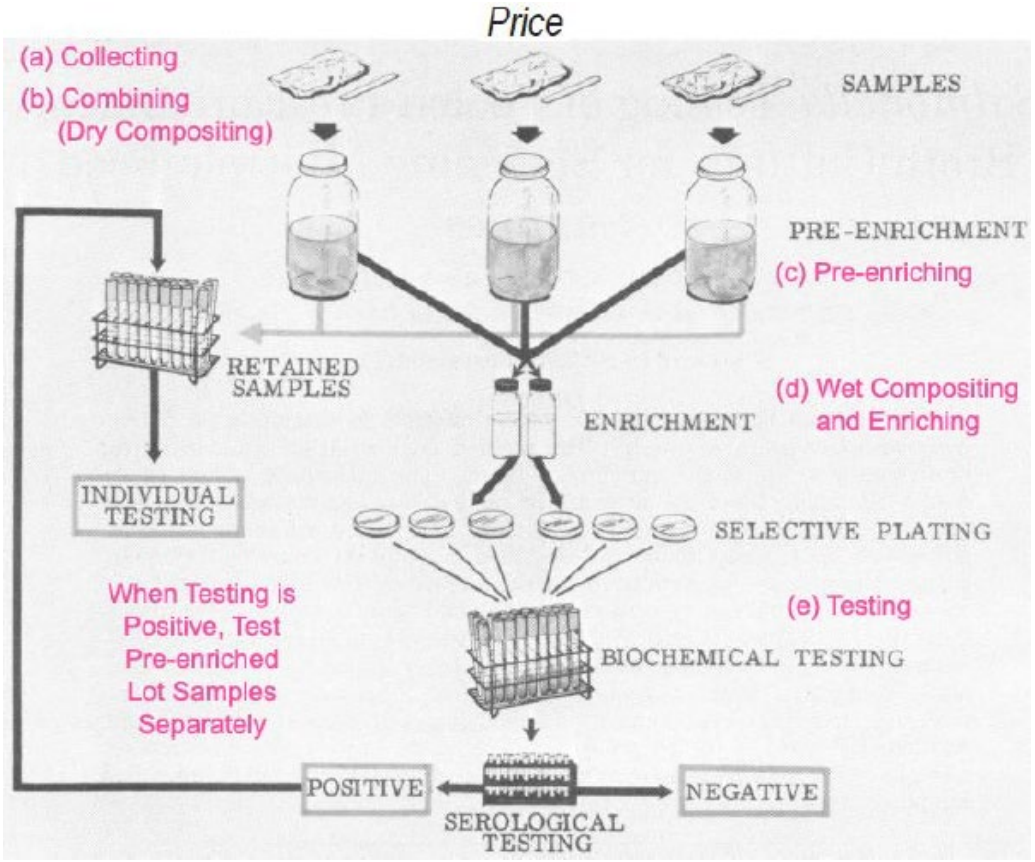
	Price, 680 (Figure 1 (annotated)). ICMSF includes many other references to testing lots of beef or poultry, two of the most common types of meat in commerce. *Id.*, 114 (§ 6.3), 184–191 (§§ 10.2–10.6), 225–226 (§ 12.1), 285 (§ 16.1), 313–330 (Chapter 17). ICMSF is relevant to any kind of food product including beef. Hughes discusses turkey (Hughes, Abstract), and a POSITA would be motivated to combine the teachings of ICMSF and Hughes as they both test meat for release into commerce. Hughes discloses testing turkey for *Salmonella*. "Three food types (. . . and raw turkey) were analyzed in the United States." Hughes, 634, 645, Table 8. Table 8 shows results from 12 collaborators for naturally contaminated raw turkey. For the 180 samples tested, using confirmed data, there was one false negative at the low level for the TECRA method. There were 3 false negatives at the low level and 3 false negatives at the high level for the cultural method. The incidence of false-positive results was very high for the cultural method, with a total of 41 false positives, compared with 5 for the TECRA method. It should, however, be taken into account that the comparison was with the method in BAM 7th Ed., not the current 8th Ed. The method for raw flesh food in the BAM 8th Ed. uses elevated temperature with RV and TT broth, which would be more selective. No significant Hughes, 646. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite

	sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Moreover, the recitation of a lot being validated for "release . . . into commerce" is not limiting. Prosecution History for Reexamination Control No. 95/011,294.
[35a] a) separately collecting multiple independent samples from each of multiple separate single-unit production lots, wherein each separate single-unit production lot is separately sampled by taking said multiple independent samples thereof;	Price, alone or in combination with ICMSF and Hughes, discloses separately collecting multiple independent samples from each of multiple separate single-unit production lots, wherein each separate single-unit production lot is separately sampled by taking said multiple independent samples thereof. Price's teachings for collecting a plurality of portions are based on standard practices as described in BAM 1969 (BAM 1969, 1 § 10.04) which cites to JAOAC 1967 (JAOAC, 234). Price, 679, 682. ***Salmonella* analyses were carried out as described in the *Bacteriological Analytical Manual* (BAM) (2) except for the pooling of pre-enrichment broths described in the following paragraph.** Price, 679. IEH's diagram of Price from Prosecution History for Reexamination Control No. 95/001,294 (reproduced below) shows step 1 corresponds to IEH's step (a), with instructions to "[t]ake individual portions . . . from each Sample."

Figure 3: CITED PRIOR ART (PRICE et al, 1972)



	Prosecution History for Reexamination Control No. 95/001,294, 298 (annotated). IEH's diagram also indicates multiple samples are taken from three bins, which is analogous to three lots rather than a single lot because each bin is an assemblage of specimens. The purpose of Jarvis and Price was to determine whether pooling samples reduced the ability to detect contaminated food lots. It would have been commonsense to combine samples from the same bin and subject them to enrichment, since each bin represents a discrete unit of the production run and results obtained from the enriched sample could be attributed to the bin from which the sample was obtained. Prosecution History for Reexamination Control No. 95/001,294, at 1575 (First Board Decision of January 20, 2015 at 16). Additionally, the specification describes a bin of dry food materials (almonds), like those in Price, as one example of a test lot/single unit of production, like a combo of beef. '143 Patent, 5:46-51. Price discloses testing of "Dried egg albumen, cocoa, and nonfat dry milk" (Price, 679) and "wheat flour, coconut, and cottonseed flour" (Price, 681).



Price, 680 (Figure 1 (annotated)).

	A method has been described for testing multiple food samples for *Salmonella* without loss in sensitivity. The method pools multiple pre-enrichment broth cultures into single enrichment broths. The subsequent stages of the *Salmonella* analysis are not altered. The method was found applicable to several dry food materials including nonfat dry milk, dried egg albumin, cocoa, cottonseed flour, wheat flour, and shredded coconut. As many as 25 pre-enrichment broth cultures were pooled without apparent loss in the sensitivity of *Salmonella* detection as compared to individual sample analysis. The procedure offers a simple, yet effective, way to increase sample capacity in the *Salmonella* testing of foods, particularly where a large proportion of samples ordinarily is negative. It also permits small portions of pre-enrichment broth cultures to be retained for subsequent individual analysis if positive tests are found. *Salmonella* testing of pooled pre-enrichment broths provides increased consumer protection for a given amount of analytical effort as compared to individual sample analysis. Price, 679. A POSITA would have replaced Price's collection and combination of dry food with ICMSF's collection and combination of portions from a consignment of meat lots in conformance to a statistical-based sampling plan for release into commerce. "Many different types of samples are collected and submitted to the laboratory for analysis. Some are sample units from lots or consignments of food or ingredients for lot acceptance determination. . . . A sample unit is a small portion of the lot in which the distribution of both numbers and types of microorganisms should be representative of the whole lot." ICMSF, 255. The lots may be beef or poultry. *Id.*, 184-91, 226, 230. Thus, ICMSF discloses collecting a plurality of samples or sample units (other synonyms include portions, specimens, and sub-samples) from lots of beef or poultry. ICMSF describes collecting from a lot or lots:

	"If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." *Id.*, 119. Additionally, ICMSF indicates a consignment may be "a very large quantity of food" and may be "composed of several lots, representing for example, different days of production from the same plant." *Id.*, 119, 120; *see also* Applicant Admitted Prior Art ("AAPA") #3-#5.

6.11 WHAT IS A REPRESENTATIVE SAMPLE?

A *representative sample* reflects, as far as is possible, the composition of the lot from which it is drawn.

How then should a representative sample be drawn? It is important to avoid bias and to draw a sufficient number of sample units to confidently make a judgment about a lot. Sampling at random is the universally recognized way of avoiding bias and is more reliable than consciously trying to draw the sample units from the various parts of a lot. The units (cartons or containers, particular weights of solid, or volumes of liquid) are drawn by using random numbers. There is, of course, no guarantee that a random sample has characteristics identical with those of the lot, but the randomness of sampling is the basis for calculation of the probability that a sample will give a biased result.

It is also possible, and may be desirable, to use a *stratified random sampling* approach, drawing a random sample of a given number of sample units from each *stratum* (e.g., each sublot or carton). The distribution of sample units on strata should correspond to the distribution of lot units on strata. This means that if each stratum contains the same quantity as every other one, the number of sample units per stratum should be the same. Otherwise, they should differ just as the proportions of the lot contained in the strata differ. Stratification is a device for handling known sources of variation and may be used where one has prior knowledge that the consignment is potentially not of uniform quality. A consignment might not be of uniform quality if portions of it are shipped in different vehicles or in different holds of a vessel, or if it is known that it is really composed of several lots representing, for example, different days of production from the same plant or different plants of the same company. The results for different strata should be assessed separately and then pooled if they appear to be homogeneous.

The calculation of acceptance probabilities is based on the assumption that random samples have been drawn and analyzed. Where physical or practical constraints prevent random sampling, such calculated probabilities may not be valid. Their reliability depends on how close reality approaches the ideal situation of random sampling. This fact should be borne in mind when subsequently interpreting results.

ICMSF, 119-20 (annotated).

6.10 WHAT IS A LOT?

Ideally, a lot is a quantity of food or food units produced and handled under uniform conditions. Under certain circumstances, it has been shown that the logarithms of the counts from a batch of food are likely to be normally distributed (Baird-Parker, 1980; Kilsby & Baird-Parker, 1983 [their Table 2]; Ingram & Roberts, 1976). The implication is that there is homogeneity within a lot, which, when considering microbial levels and distributions, rarely occurs in practice. In most instances, the distribution of microorganisms within lots of food is heterogeneous. Even in food factories, this heterogeneity makes the interpretation of sample results difficult, and it becomes even more so in circumstances where a lot is less well defined, such as in a consignment of a very large quantity of food.

It is therefore helpful if suppliers can be persuaded to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load).

If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment.

A lot should be composed of food produced with as little variation as possible for a given process or commodity. Because of the uncertainties in identifying a lot commercially, however, the use of the word *lot* in this book is usually in its statistical sense, as a collection of units of a product, the acceptability of which is determined by examining a sample drawn from it.

ICMSF, 118-19 (annotated).

	The ICMSF has defined a lot as follows: In the commercial sense, a lot is a quantity of food supposedly produced under identical conditions, all packages of which would normally bear a lot number that identifies the production during a particular time interval, and usually from a particular line, retort, or other critical processing unit. Statistically, a lot is considered as a collection of units of a product from which a sample is drawn to determine acceptability of the lot (ICMSF, 1986). In this example, the maximum size of a lot consists of all the ground beef produced on common equipment from cleanup to cleanup. Smaller lots could be established (e.g., hourly). ICMSF, 327 (annotated). This definition also reflects the common practice referred to in the IEH Patents at Example II, wherein "[p]referably, the producer defines and produces these single-unit lots in such a fashion as to maximize the information available." '143 Patent,12:52–54. This corresponds with the specification of IEH's Patents which reflects the common practice of producers defining what constitutes a lot grouped for traceability purposes. '143 Patent, 3:5-15, 11:18-22 (AAPA #3), and 12:52-55. Thus, ICMSF discloses collecting a plurality of portions from separate lots. ICMSF describes a lot as "a collection of units of a product." IMCSF, 119. ICMSF further indicates a lot has been defined as: "a quantity of food supposedly produced under identical conditions, all packages of which would normally bear a lot number that identifies the production during a particular time interval, and usually from a particular line, retort, or other critical processing unit. Statistically, a lot is considered as a collection of units of a product from which a sample is drawn to determine acceptability of the lot." ICMSF, 327. ICMSF advocates use of a "statistically based sampling plan." *Id.*, 113-15, 119-21, 320, 327, 328. In the sampling plans, "*n* is the number of sample units chosen separately and independently, and . . . *c* is the maximum allowable number of sample

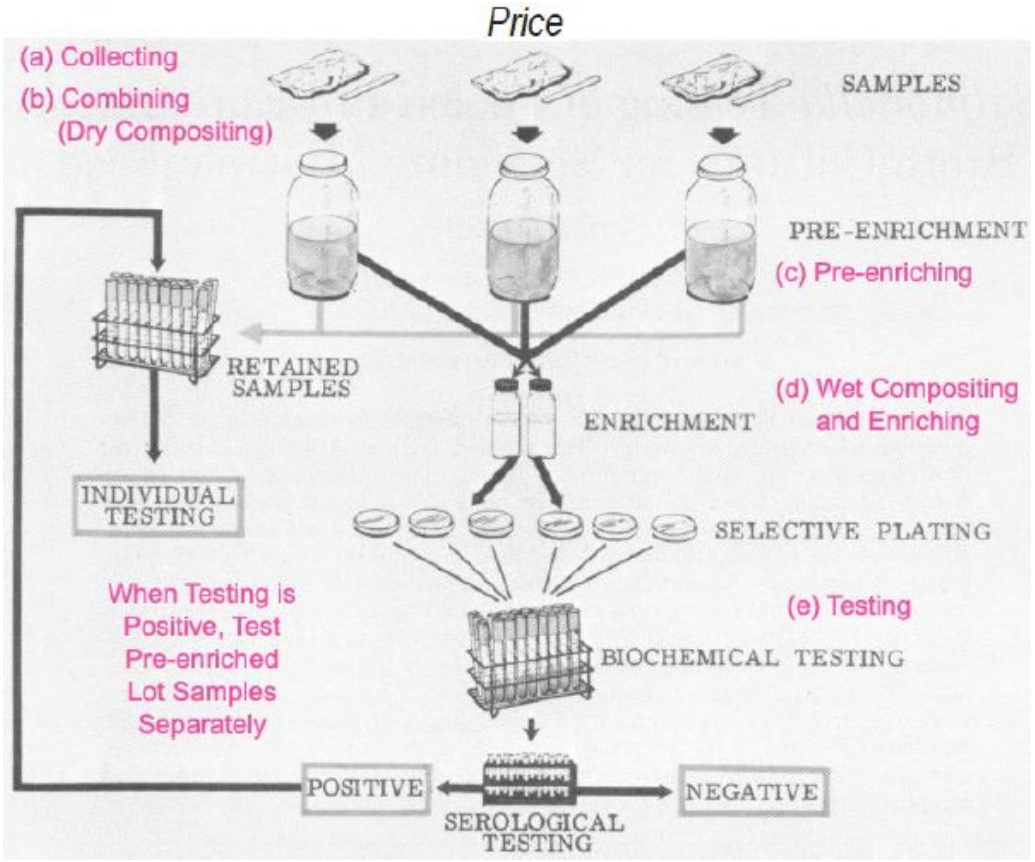
	units yielding a positive result." *Id.*, 115. ICMSF discloses a wide range for the number of sample units (portions) for a particular microbial agent or organism. For example, ICMSF discusses collecting 15, 30, 60 or 100 sample units from a lot. *Id.*, 320, 326. **6.5 THE SAMPLING PLAN** The sample units so drawn will, after examination, yield results that will be compared with certain criteria to reach a decision as to whether the entire lot should be accepted or rejected (see section 6.9 for explanation of rejection). The particular choice of sampling procedure and the decision criteria make up the *sampling plan*. Proper drawing of samples is essential if the decision making criteria of the sampling plan are to give unbiased results. By sampling randomly, we are able to reduce the risk of making biased decisions. A simple example of a sampling plan follows. Take 10 sample units of a food from a lot and subject them to appropriate laboratory procedure to test for the presence or absence of a microorganism. If two or fewer of the 10 sample units show presence of the organism, that is, give a positive result, then the whole lot of food is acceptable (relative to this organism). But if three or more give a positive result, the whole lot is to be rejected. This plan is described by the use of two terms $n = 10$ and $c = 2$, where n is the number of sample units chosen separately and independently, and where c is the maximum allowable number of sample units yielding a positive result. ICMSF, 115 (annotated).

	6.1 INTRODUCTION It is important to recognize that the management of food safety using the approaches outlined in the first five chapters, based on controlling hazards through Good Hygienic Practices (GHP) and Hazard Analysis Critical Control Point (HACCP), is much more effective than trying to ensure safety by lot acceptance through end-product testing. This chapter discusses the concepts of probability and sampling that determine the limitations of end-product testing. Those concepts form the basis of the design of statistically based sampling plans (Chapter 7) and the establishment of cases (Chapter 8). In Chapters 6 and 7, it is assumed that the microbiological test will give an absolutely accurate result, with no false positives or false negatives. This is not a realistic assumption, and problems arising from sampling, sample transport and preparation for analysis, and the imprecision of microbiological methods are addressed in Chapters 10 and 12 and elsewhere in the text. ICMSF, 113 (annotated); *see also id.*, 115–21 (§§ 6.4–6.13), 319–20 (§ 17.2.3.3), and 326–30 (§§ 17.5.3.1–17.6). ICMSF discusses the number of portions to be taken sufficient for validation of a particular microorganism including *Salmonella* (*id.*, 276 (§ 15.3.3); *see also id.*, 282 (§ 15.6.3)); *Listeria* (*id.*, 309 (§ 16.6.3)), and *E. coli* (*id.*, 318–21 (§§ 17.2.3.2–17.2.3.4), 326 (§ 17.5.3.1)). IEH's AAPA discloses statistical-based sampling plans taking 10-60 sample units. '143 Patent, 6:3-20; *see* AAPA #1, #2, #5, and #6. Determining meat is safe for consumption so it is "commercially possible to market" the meat, is the well-understood point of testing meat and is taught by ICMSF. ICMSF, 185-89. Moreover, the recitation of a lot being validated for "release . . . into commerce" is not limiting. Prosecution History for Reexamination Control No. 95/011,294.
[35b] b) separately compositing the collected multiple independent samples from each of the separate test lots to provide a corresponding set of separate composited	Price, alone or in combination with ICMSF and Hughes, discloses separately compositing the collected multiple independent samples from each of the separate test lots to provide a corresponding set of separate composited single-unit production lot samples, wherein each of the separate composited single-unit production lot

single-unit production lot samples, wherein each of the separate composited single-unit production lot samples is attributed to a particular corresponding separate single-unit production lot;	samples is attributed to a particular corresponding separate single-unit production lot. Like ICMSF, the test lot samples in Price are attributable to a particular lot. For example, Price's abstract states that the procedure "permits small portions of pre-enrichment broth cultures to be retained for subsequent individual analysis if positive tests are found," as shown below: A method has been described for testing multiple food samples for *Salmonella* without loss in sensitivity. The method pools multiple pre-enrichment broth cultures into single enrichment broths. The subsequent stages of the *Salmonella* analysis are not altered. The method was found applicable to several dry food materials including nonfat dry milk, dried egg albumin, cocoa, cottonseed flour, wheat flour, and shredded coconut. As many as 25 pre-enrichment broth cultures were pooled without apparent loss in the sensitivity of *Salmonella* detection as compared to individual sample analysis. The procedure offers a simple, yet effective, way to increase sample capacity in the *Salmonella* testing of foods, particularly where a large proportion of samples ordinarily is negative. It also permits small portions of pre-enrichment broth cultures to be retained for subsequent individual analysis if positive tests are found. *Salmonella* testing of pooled pre-enrichment broths provides increased consumer protection for a given amount of analytical effort as compared to individual sample analysis. Price, 679. Price further describes this retention step as enabling "individual tests on the retained sample" to "be made to determine which sample or samples contributed *Salmonella* to the pool" as shown below:

	MATERIALS AND METHODS *Salmonella* analyses were carried out as described in the *Bacteriological Analytical Manual* (BAM) (2) except for the pooling of pre-enrichment broths described in the following paragraph. The procedure for *Salmonella* testing of pooled pre-enrichment broths is illustrated in Fig. 1. Samples are individually pre-enriched followed by transfer from multiple pre-enrichments to single selenite and tetrathionate broths at the enrichment stage. During pooling, a portion of each pre-enrichment is transferred to a sterile culture tube and retained at 4 C for later reference to the individual samples. If the *Salmonella* test of the pooled pre-enrichment broths is positive, individual tests on the retained samples can be made to determine which sample or samples contributed *Salmonella* to the pool. *Id.* (highlighting added). A POSITA would understand Price to implicitly disclose combining the collected confirming plurality of portions based on the inherent mixing involved with dry food materials.

	Salmonella-positive test samples were prepared by blending the dry test food with one of three dry inocula. Inoculum 1 was a dry enzyme drain cleaner which had been found to contain multiple *Salmonella* serotypes including E_1, z_{10}; G, z_{29} (*S. cubana*); and C_1, g complex. Inoculum 2 was a freeze-dried, skim milk suspension of serotypes *S. anatum*, *S. binza*, *S. tennessee*, *S. worthington*, *S. cubana*, *S. braenderup*, and a B_1, g complex. Inoculum 3 consisted of four laboratory isolates of serotypes G, z_{29} (*S. cubana*); E_1, b; E_1, l complex; and C_1, l complex grown in a sterile aqueous suspension of cottonseed flour and freeze dried. Price, 679.



Price, 680 (Figure 1 (annotated)).

A POSITA would have replaced Price's collection and combination of dry food with ICMSF's collection and combination of portions from a consignment of meat lots in conformance to a statistical-based sampling plan for release into commerce.

ICMSF states: "To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units of the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. . . . This finding suggests that considerable reductions may

	be made in the cost of laboratory analysis by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, n, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort." ICMSF, 187. To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. A laboratory investigation undertaken for ICMSF (Silliker & Gabis, 1973) indicated that different sized analytical units drawn from a single well-mixed sample unit of dried food give very similar results. A subsequent investigation (Gabis & Silliker, 1974) using foods of high moisture content (including eggs, poultry meat, meat, and meat products) showed comparable reliability in the use of composite analytical units; for example, examination of three composites of 20 × 25 g analytical units gave the same result as examination of all 60 analytical units individually (the total amount examined being 1500 g in both instances). This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n*, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort. ICMSF, 187.

10.5.1 Conceivable Alternative Plans

As indicated above, for an infectious agent such as *Salmonella*, there are strong reasons for adopting sampling plans in which $c = 0$:

1. It is philosophically objectionable to some people to accept a lot containing a recognized pathogen, regardless of how the food is to be used in a manufacturing process or by the consumer.
2. There is the very great technical advantage of combining many analytical units for bacteriological examination in systems with $c = 0$, because a single positive decides the outcome.
3. The plan with $c = 1$ (or more) would always require the examination of (many) more analytical units, for equal probability of acceptance, than a plan with $c = 0$.
4. Even if the indicated probabilities of plans with $c = 0$ versus $c = 1$ are the same, their implications are not. For example, when a lot is accepted with the plan $n = 60$, $c = 0$, it is possible (though not certain) that the lot may not be contaminated at all. But using the plan $n = 95$, $c = 1$ and accepting one positive, it is known that the lot certainly is contaminated (though the probability that a lot with 5% of analytical units contaminated would be accepted is in both instances $P_a = 0.05$).

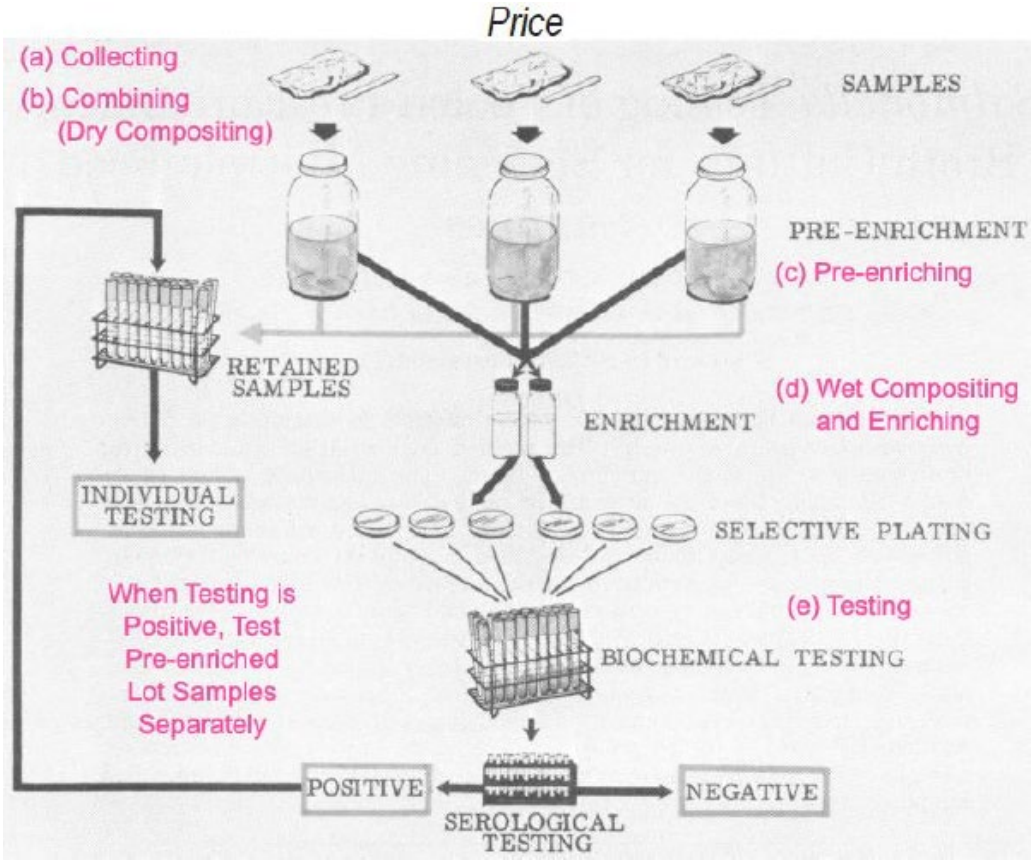
ICMSF, 188 (annotated). ICMSF also provides an example of "[u]sing a sampling plan involving n = 15 (25 g analytical units) and a composite of 375 g." *Id.*, 195 (§ 10.7.2). ICMSF also states "[f]or qualitative analyses, 25 g analytical units are frequently analyzed individually or as composites of 100 g, 200 g, or more." *Id.*, 232 (§ 12.5.2).

The ICMSF incorporates two references, Silliker & Gabis (1973) and Gabis & Silliker (1974). Silliker notes that "[c]ompositing of multiple samples (lumping) and wet compositing of pre-enrichment cultures have been tested as economical approaches to *Salmonella* control." Silliker, 475.

Silliker further notes that "[b]oth approaches are being used widely by the food industry in the United States." *Id.*.

	To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. A laboratory investigation undertaken for ICMSF (Silliker & Gabis, 1973) indicated that different sized analytical units drawn from a single well-mixed sample unit of dried food give very similar results. A subsequent investigation (Gabis & Silliker, 1974) using foods of high moisture content (including eggs, poultry meat, meat, and meat products) showed comparable reliability in the use of composite analytical units; for example, examination of three composites of 20 × 25 g analytical units gave the same result as examination of all 60 analytical units individually (the total amount examined being 1500 g in both instances). This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n*, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort. "There is the very great technical advantage of combining many analytical units for bacteriological examination." ICMSF, 188. ICMSF also describes composites prepared by combining "15 (25 g analytical units)" and "composites of 100 g, 200 g, or more." *Id.,* 195, 232; *see* AAPA #5. ICMSF states, "All steps up to the analysis must be controlled . . . to allow reliable traceability." ICMSF, 227. ICMSF indicates lot samples are traceable to a particular corresponding lot: "It is therefore helpful . . . to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load). . . . Treating the individual production

	batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119.
[35c] c) enriching of the separate composited single-unit production lot samples to provide a set of separate composited single-unit production lot samples enriched for the target microbe(s)	Price, alone or in combination with ICMSF and Hughes, discloses enriching each of the separate composited test lot samples to provide a set of separate composited test lot samples enriched for the target microbe(s). Price discloses "[s]amples are individually pre-enriched followed by transfer from multiple pre-enrichments to single selenite and tetrathionate broths at the enrichment stage." Price, 679. These pre-enrichment and enrichment steps inherently disclose an incubation period in which levels of the target microbial agent, in this case, *Salmonella*, grow to detectable levels for testing. Price states "[i]n the multiple pooling of pre-enrichment broth cultures . . . , 1-ml quantities of cultures were transferred, generally, to a volume of enrichment broth." Price, 682. Price also states "[a]lternatively, problems with interfering organisms may warrant the use of direct selective enrichments." *Id.* Based on this statement, the Board noted "there is a suggestion in Price (at 682) . . . of direct enrichment." Prosecution History for Reexamination Control No. 95/001,294, 1576.



Price, 680 (Figure 1 (annotated)).

Price states "[a]lternatively, problems with interfering organisms may warrant the use of direct selective enrichments":

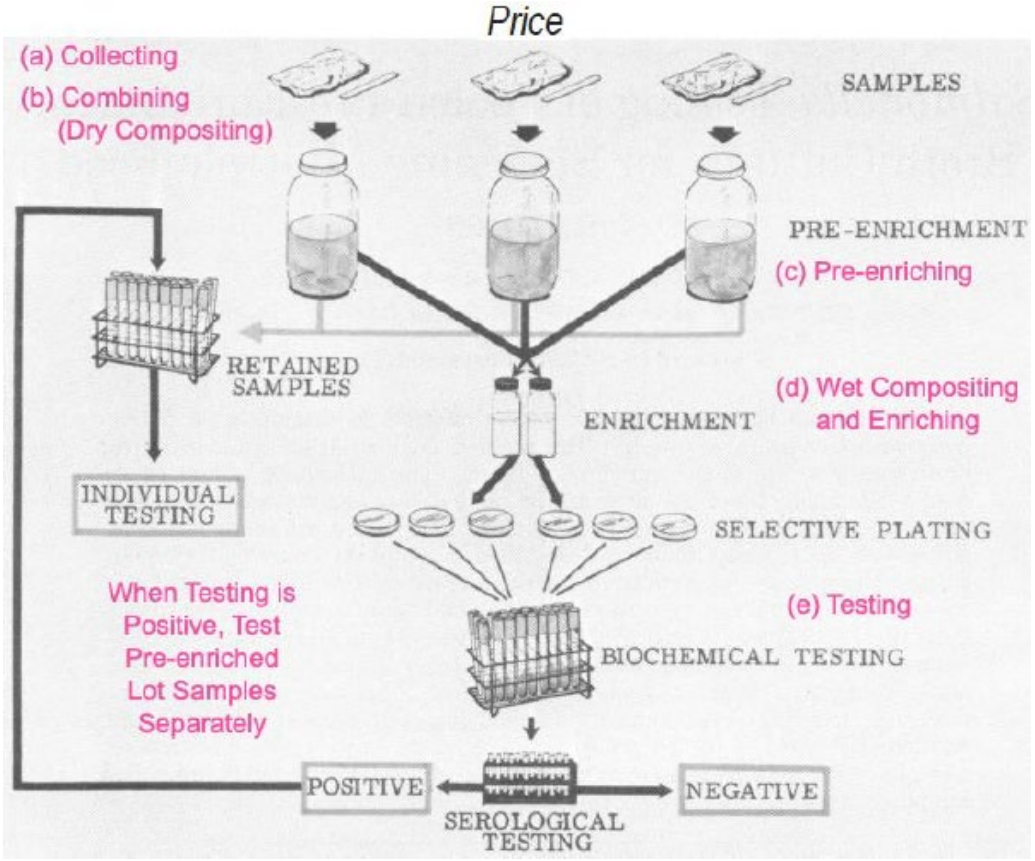
	Whether pooled pre-enrichments are less sensitive than individual tests in the presence of interfering organisms probably is not known. For the present, we would recommend that, if numerous non-*Salmonella* colonies that resemble *Salmonella* on selective agar media are encountered in the pooled test, the retained pre-enrichment cultures should be tested individually. Alternatively, problems with interfering organisms may warrant the use of direct selective enrichments, two selective enrichments, or possibly even a reduction in the incubation time of the pre-enrichment cultures. Price, 682 (highlighted added). A POSITA would have replaced Price's pre-enriching and compositing pre-enrichments with teachings of ICMSF and Hughes for enriching lot samples, removing portions of each enriched lot sample, and combining the portions to provide a composite sample. Also, instead of retaining portions of pre-enrichments like Price, a POSITA would have retained portions of enriched lot samples as taught by ICMSF and Hughes. ICMSF states: To reduce laboratory effort . . . it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. ICMSF, 187. Also, ICMSF describes preparing composites of analytical units then diluting "for qualitative (enrichment)" analysis. *Id.,* 231-32; *see also* AAPA #5, #7.

	For practical reasons, however, analytical units of 10 g up to 50 g are typical for quantitative methods and are diluted 1:10. For qualitative analyses, 25 g analytical units are frequently analyzed individually or as composites of 100 g, 200 g, or more. ICMSF, 232 (annotated). Preparation of the first dilution, either for qualitative (enrichment) or quantitative (direct) analyses, can have a marked impact on the microbial population in the sample. This is often due to changes in the physico-chemical characteristics of the suspension as compared to the food matrix. Where the food does not confer protection, the composition of the diluent is important to prevent or minimize shock/injury to the microorganisms that can result from rapid changes in the ionic concentrations. ICMSF, 232 (annotated). ICMSF also describes strategic use of non-selective enrichment to resuscitate microorganisms and selective enrichments for growing microorganisms. *Id.*, 232–33 (§ 12.6); *see e.g.*, *id.,* 233 ("use of nonselective medium . . . to enhance recovery of injured or stressed cells."). ICMSF, § 12.6 also describes optimizing the incubation period. S*ee e.g.*, *id.,* 232. It was well known long before the priority date to use non-selective enrichments after dry compositing and prior to wet compositing as evidenced by Price, Silliker, Gabis, and MLG. A POSITA would also know to use selective enrichments after dry compositing and prior to wet compositing as disclosed by ICMSF and Hughes. A POSITA would find that incubating so microorganisms are detectable and at least substantially uniform in the selective enrichment broth was considered the standard practice in official methods because the methods otherwise fail. For example, D'Aoust states "standard methodology recommends incubation of selective enrichment broths." D'Aoust, 372. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section

	further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. (a) *Pre-enrichment.*—Pre-enrich product in noninhibitory broth to initiate growth of *Salmonella* spp. Methods used may vary with product and should be performed as indicated in **967.26A** or BAM, 7th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, chapter 5, section C. Pre-enrichment broths should be incubated at 35°–37°C. (b) *Selective enrichment.*—Transfer 0.1 mL incubated pre-enrichment mixtures to Rappaport-Vassiliadis R10 broth (9.9 mL) and 1 mL to TT broth (9 mL). For all foods other than raw foods or foods having a high microbial load, incubate 6–8 h at 41°–43°C. Selective enrichments of raw foods or foods having a high microbial load must be incubated 16–20 h at 41°–43°C. An air incubator is suitable for this purpose. Hughes, 636. Hughes instructs that, after pre-enrichment, portions of the "incubated pre-enrichment mixtures" should be transferred to two separate selective enrichment broths, Rappaport-Vassiliadis R10 broth and TT broth, and incubated for 16-20 hours at 41°-43°C. Hughes, 636, 638. Additionally, Hughes instructs that these selective broths should be removed from incubation and mixed by hand or by Vortex mixer. Hughes, 638. Hughes further instructs to then remove 1 mL from the TT tube and 1 mL from the Rappaport-Vassiliadis R10 tube and transfer to separate, sterile M broths. *Id.,* 636, 638. The separate M broths are then incubated ("16-20 h at 35°-37°C" for "all foods other than raw foods or foods having a high microbial load" or "6 h at 35°-37°C" for

	"raw foods or foods having a high microbial load"). *Id.*, 638. A POSITA would find that the long incubation at an elevated temperature and mixing ensures the target microbial agent or organism reaches detectable levels and becomes substantially uniform throughout the separate lot samples.
[35d] d) removing portions of each separate enriched composited single-unit production lot sample, and combining the removed portions to provide a pooled modular composite sample; and	Price, alone or in combination with ICMSF and Hughes, discloses removing portions of each separate enriched composited single-unit production lot sample, and combining the removed portions to provide a pooled modular composite sample. Price discloses wet compositing as "[s]amples are individually pre-enriched followed by transfer from multiple pre-enrichments to single selenite and tetrathionate broths at the enrichment stage." Price, 679. **MATERIALS AND METHODS** ***Salmonella*** **analyses were carried out as described in the *Bacteriological Analytical Manual* (BAM) (2) except for the pooling of pre-enrichment broths described in the following paragraph.** **The procedure for *Salmonella* testing of pooled pre-enrichment broths is illustrated in Fig. 1. Samples are individually pre-enriched followed by transfer from multiple pre-enrichments to single selenite and tetrathionate broths at the enrichment stage. During pooling, a portion of each pre-enrichment is transferred to a sterile culture tube and retained at 4 C for later reference to the individual samples. If the *Salmonella* test of the pooled pre-enrichment broths is positive, individual tests on the retained samples can be made to determine which sample or samples contributed *Salmonella* to the pool.** Price 679.

	Pooled samples versus pooled pre-enrichment broths. Silliker (4) reported a loss in sensitivity when several samples were pooled in a large container of pre-enrichment broth as compared to individual sample analysis. This is not surprising since, to be detected, the desiccated and debilitated *Salmonella* cells in a dried food sample must grow out in pre-enrichment broth in competition with other bacteria in the sample. If many food samples are pooled at the pre-enrichment stage, the salmonellae face competition from the flora of all the samples in the pool. If, on the other hand, the samples are individually pre-enriched, the actively growing salmonellae might be expected to compete more favorably with organisms introduced from other samples when pooled at the enrichment stage. In addition, pooling pre-enrichment broths avoids the hazards and inconvenience of handling very large flasks of culture. Price, 681 (discussing Silliker).

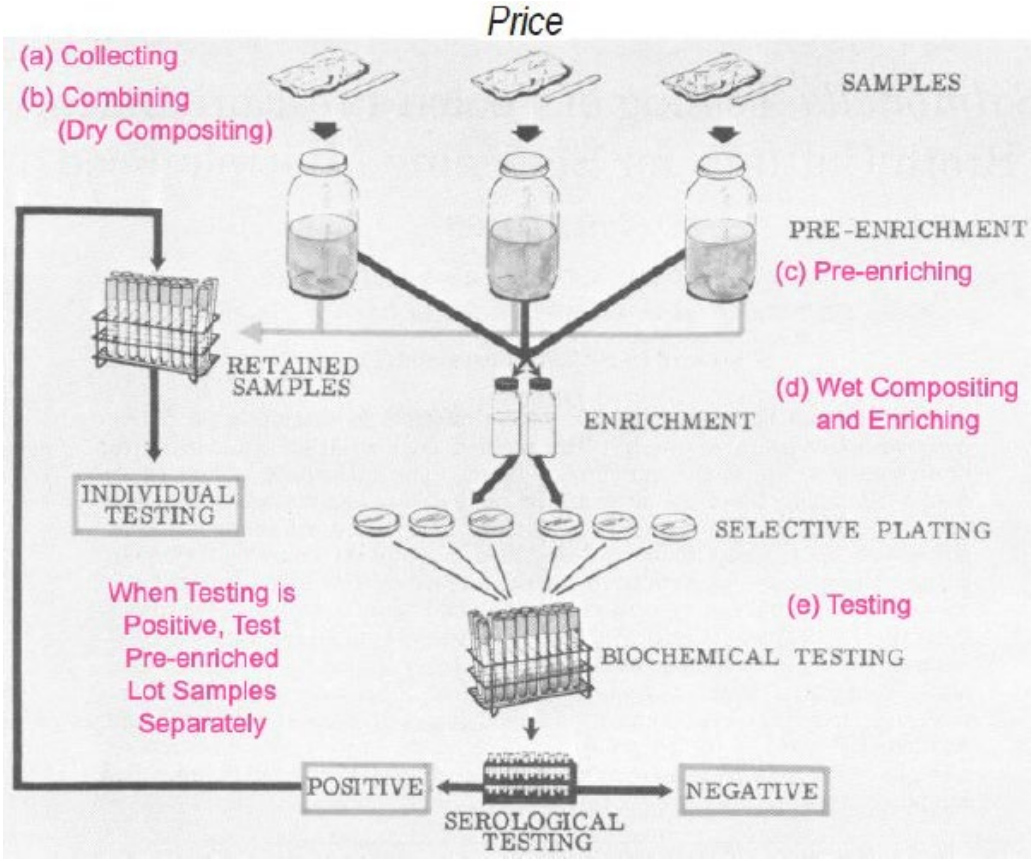


Price, 680 (Figure 1 (annotated)). Price's Figure 1 indicates that selective plating, biochemical testing, and serological testing are used.

A POSITA would have replaced Price's pre-enriching and compositing pre-enrichments with teachings of ICMSF and Hughes for enriching lot samples, removing portions of each enriched lot sample, and combining the portions to provide a composite sample. Also, instead of retaining portions of pre-enrichments like Price, a POSITA would have retained portions of enriched lot samples as taught by ICMSF and Hughes.

	ICMSF describes compositing the enriched lot samples: "it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. . . . This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, *n,* with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort." ICMSF, 187. AAPA #5-#7. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Hughes also discloses a method of removing portions of each enriched separate test lot sample, and combining the removed portions to provide a modular composite sample. Hughes instructs to "[c]ombine equal volumes (0.5-1 mL) from each M broth tube in a clean screw-cap tube." Hughes, 638.

	that has been warmed to 35°–37°C. Also remove 1 mL from Rappaport-Vassiliadis R10 tube and transfer to a separate tube of prewarmed M broth (10 mL). For all foods other than raw foods or foods having a high microbial load, incubate M broth tubes 16–20 h at 35°–37°C and return selective enrichment broth tubes to 41°–43°C incubator and incubate for additional 16–18 h. For raw foods or foods having a high microbial load, incubate M broth tubes 6 h at 35°–37°C and return selective enrichment broth tubes to 41°–43°C and incubate for an additional 6 h. (**d**) *Preparation of sample for EIA analysis.*—Remove M broth tubes from incubation and mix tubes by hand or Vortex mixer. Combine equal volumes (0.5–1 mL) from each M broth tube in a clean screw-cap tube and heat in boiling H_2O bath or in flowing steam 15 min. Refrigerate (2°–8°C) remaining M broth and TT and Rappaport-Vassiliadis R10 tubes from (**c**) for culture confirmation of any EIA-positive samples (*see* **967.26B, 967.26C, 967.27** and **967.28**). Cool heated M broths to 25°–37°C prior to analysis. Hughes, 638. Hughes 2003 confirms the pooling of enrichments. Hughes 2003, 775-76.
[35e.1] e) testing of the pooled modular composite sample, using a suitable detection assay, for the target microbe(s),	Price, alone or in combination with ICMSF and Hughes, discloses testing of the modular composite sample, using a suitable detection assay, for the target microbe(s).



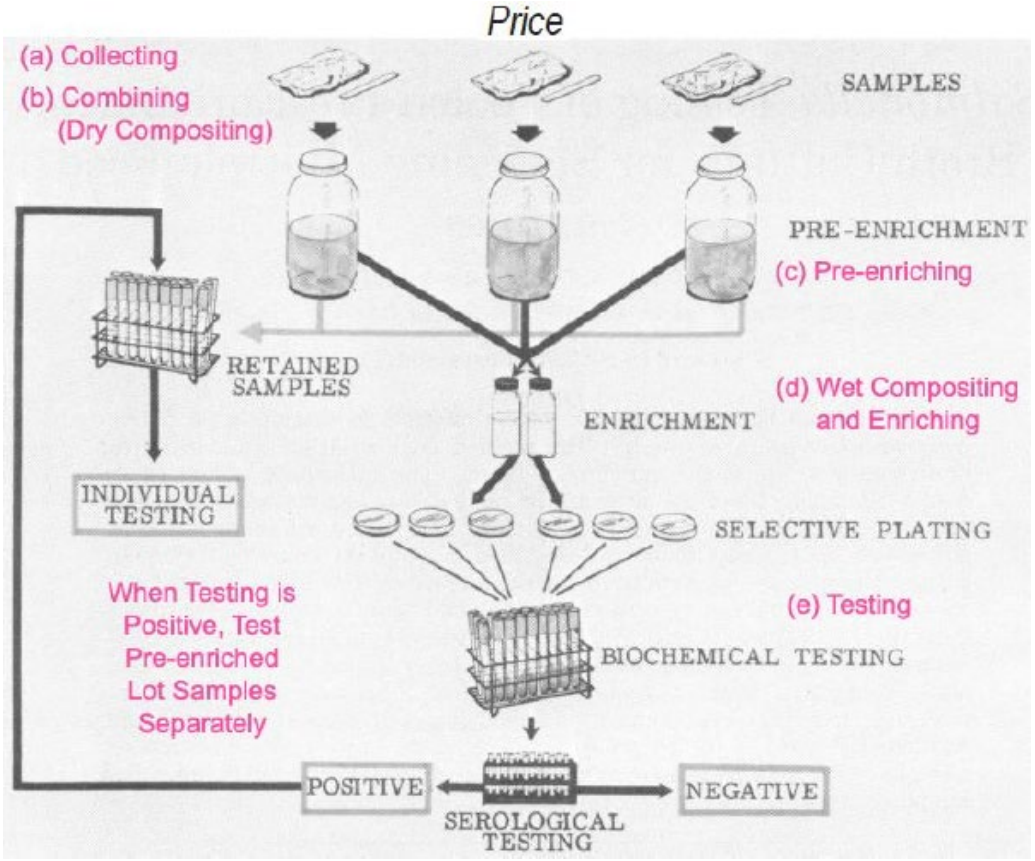
Price, 680 (Figure 1 (annotated)).

Price teaches testing the composite sample using a detection assay for *Salmonella*. Price, 680 (Figure 1).

Price states "a test procedure that gives a single negative answer for many negative samples offers an important saving in the analytical effort required for quality control":

	Positive test results are relatively rare among the many samples of food ingredients and products which are analyzed for *Salmonella*. Therefore, a test procedure that gives a single negative answer for many negative samples offers an important saving in the analytical effort required for quality control. These studies support the conclusion that *Salmonella* pre-enrichment broths can be pooled for analysis without loss in sensitivity to *Salmonella* in the individual samples. Price, 679. Price indicates that "positive test results are relatively rare." *Id.* A POSITA would have replaced Price's steps of testing a composite sample from the pre-enriched lot samples to obtain a negative result and further testing retained portions of the pre-enriched lot samples with the combined teachings of ICMSF and Hughes. These combined teachings provide for testing a composite made from the selectively enriched lot samples to obtain a negative result and further testing retained portions of the enriched lot samples after a positive test of the composite by a more sensitive protocol. ICMSF describes testing enrichment cultures of composite samples with suitable detection assays (ICMSF, 187) and "presence/absence tests" (ICMSF, 233). Suitable detection assays were established in Silliker and Gabis as discussed in ICMSF. Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture

	confirmation of any EIA-positive samples." *Id.,* 638. Hughes states testing of the composite sample is "by an enzyme immunoassay (EIA) method." Hughes, 635, 638. These assay types were "art recognized." '143 Patent, 2:66-3:3 (AAPA #7).
[35e.2] wherein when such testing is negative all of said samples that were composited to form the separate composited single-unit production lot samples are deemed negative for the target microbe(s) and each of the multiple separate single-unit production lots is validated, and	Price, alone or in combination with ICMSF and Hughes, discloses wherein when such testing is negative all of said samples that were composited to form the separate composited test lot samples are deemed negative for the target microbe(s) and each of the multiple separate test lots is validated.



Price, 680 (Figure 1 (annotated)).

Price states "a test procedure that gives a single negative answer for many negative samples offers an important saving in the analytical effort required for quality control." Price, 679.

A POSITA would have replaced Price's steps of testing a composite sample from the pre-enriched lot samples to obtain a negative result and further testing retained portions of the pre-enriched lot samples with the combined teachings of ICMSF and Hughes. These combined teachings provide for testing a composite made from the

	selectively enriched lot samples to obtain a negative result and further testing retained portions of the enriched lot samples after a positive test of the composite by a more sensitive protocol. ICMSF describes correlating lots in a consignment with lot samples so a negative or positive test result enables a lot to be validated or rejected. ICMSF, 118-19. ICMSF describes sampling plans in which "c is the maximum allowable number of sample units yielding a positive result." ICMSF, 115. For many scenarios, the requirement is c = 0, which indicates the testing is negative. *Id.*, 118, 183-90, 309, 325, 326. A negative test result (*id.*, 113-15, 325, 326) is the expected and desired outcome to determine meat is not contaminated and is safe for consumption (*id.*, 184-89) such that a lot is validated and it is "commercially possible to market" (*id.*, 185) the meat.

6.10 WHAT IS A LOT?

Ideally, a lot is a quantity of food or food units produced and handled under uniform conditions. Under certain circumstances, it has been shown that the logarithms of the counts from a batch of food are likely to be normally distributed (Baird-Parker, 1980; Kilsby & Baird-Parker, 1983 [their Table 2]; Ingram & Roberts, 1976). The implication is that there is homogeneity within a lot, which, when considering microbial levels and distributions, rarely occurs in practice. In most instances, the distribution of microorganisms within lots of food is heterogeneous. Even in food factories, this heterogeneity makes the interpretation of sample results difficult, and it becomes even more so in circumstances where a lot is less well defined, such as in a consignment of a very large quantity of food.

It is therefore helpful if suppliers can be persuaded to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load).

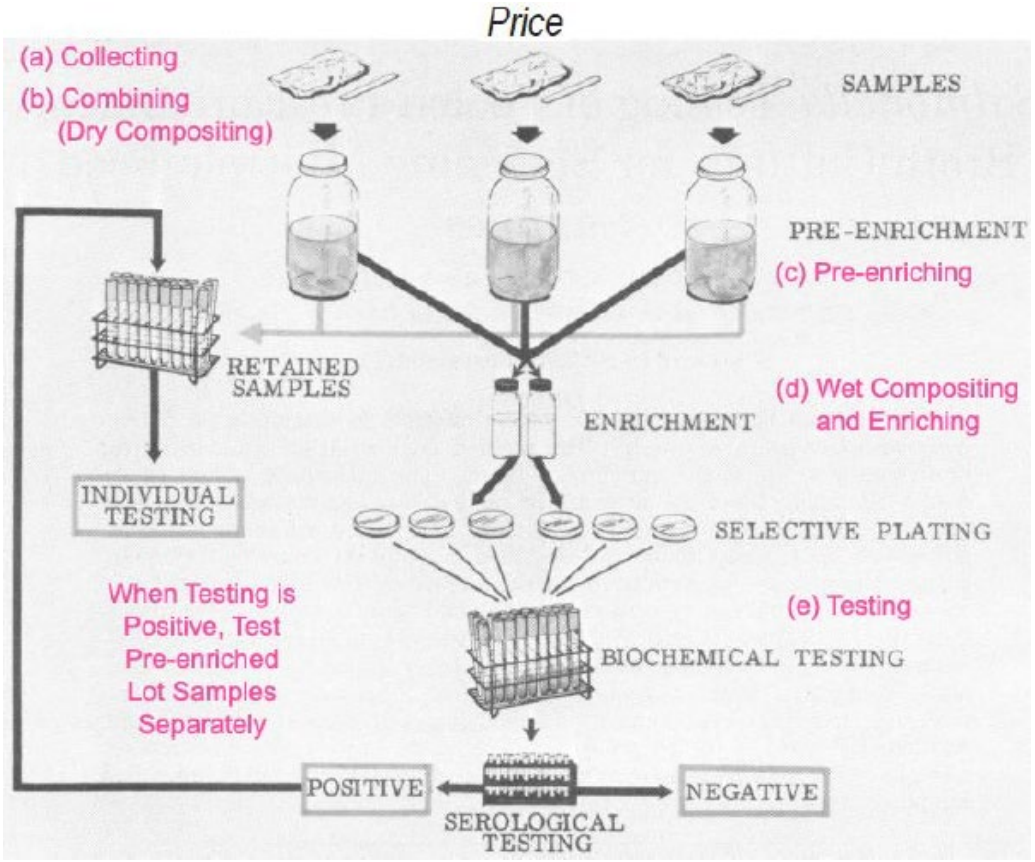
If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment.

A lot should be composed of food produced with as little variation as possible for a given process or commodity. Because of the uncertainties in identifying a lot commercially, however, the use of the word *lot* in this book is usually in its statistical sense, as a collection of units of a product, the acceptability of which is determined by examining a sample drawn from it.

ICMSF, 118-19 (annotated).

	Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Because Hughes explains the steps to be taken when testing is positive (*see* ie.3)*,* a POSITA would also expect negative testing results. Hughes, 638. Determining meat is safe for consumption so it may be released into commerce is the well-understood point of testing meat. Moreover, the recitation of a lot being validated for "release . . . into commerce" is not limiting. Prosecution History for Reexamination Control No. 95/011,294.
[35e.3] wherein when such testing is positive, each of the individual separate composited single-unit production lot samples that were used to form the pooled modular composite sample are individually tested to determine which of the separate composited single-unit production lot samples is positive for the target microbe(s),	Price, alone or in combination with ICMSF and Hughes, discloses wherein when such testing is positive, each of the individual separate composited test lot samples that were used to form the pooled modular composite sample are individually tested to determine which of the separate composited test lot samples is positive for the target microbe(s). The combination of Price, ICMSF, and Hughes describes retaining a portion of the enriched lot samples (steps (b)-(d)). Price indicates "a portion of each pre-enrichment is . . . retained . . . for later reference to the individual samples" and "individual tests on the retained samples can be made to determine which sample or samples contributed *Salmonella* to the pool." Price, 679. Price states, "If the *Salmonella* test of the pooled preenrichment broths is positive, individual tests on the retained samples can be made to determine which sample or samples contributed *Salmonella* to the pool." *Id.,* 679. Price discloses "the pooling of broth cultures permits small portions of each individual culture to be retained for subsequent analysis if a positive test is detected following enrichment, thus maintaining the individuality of each test sample." Price,

681.



Price, 680 (Figure 1 (annotated)).

Price states "[i]f the *Salmonella* test of the pooled pre-enrichment broths is positive, individual tests on the retained samples can be made to determine which sample or samples contributed *Salmonella* to the pool":

MATERIALS AND METHODS

Salmonella analyses were carried out as described in the *Bacteriological Analytical Manual* (BAM) (2) except for the pooling of pre-enrichment broths described in the following paragraph.

The procedure for *Salmonella* testing of pooled pre-enrichment broths is illustrated in Fig. 1. Samples are individually pre-enriched followed by transfer from multiple pre-enrichments to single selenite and tetrathionate broths at the enrichment stage. During pooling, a portion of each pre-enrichment is transferred to a sterile culture tube and retained at 4 C for later reference to the individual samples. If the *Salmonella* test of the pooled pre-enrichment broths is positive, individual tests on the retained samples can be made to determine which sample or samples contributed *Salmonella* to the pool.

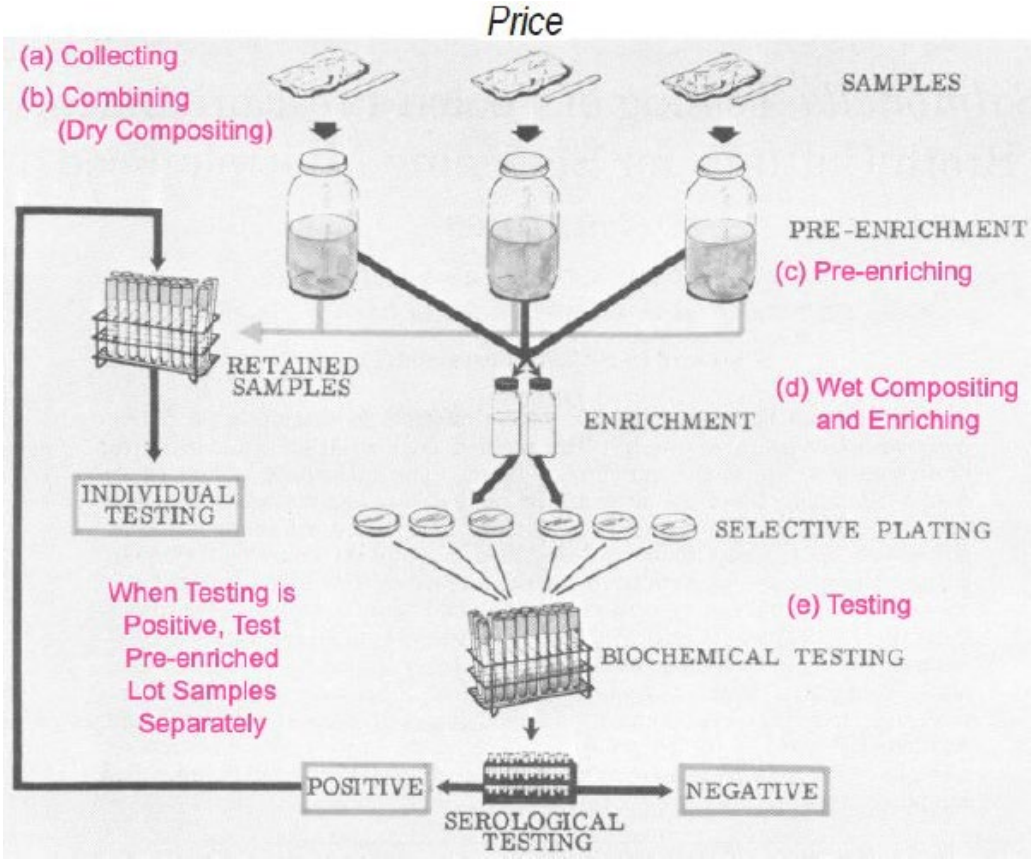
Price, 679. POSITA would have considered retention of samples that had been selectively enriched as an option.

A POSITA would have replaced Price's steps of testing a composite sample from the pre-enriched lot samples to obtain a negative result and further testing retained portions of the pre-enriched lot samples with the combined teachings of ICMSF and Hughes. These combined teachings provide for testing a composite made from the selectively enriched lot samples to obtain a negative result and further testing retained portions of the enriched lot samples after a positive test of the composite by a more sensitive protocol.

"Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment." ICMSF, 119.

	"There is often a desire to retest retained samples." ICMSF, 191. If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment. ICMSF, 119 (annotated). ICMSF discloses validating for release into commerce. ICMSF, 113–15 (§§ 6.2–6.6), 118–19 (§§ 6.9–6.10), 184–85 (§ 10.2), 325–27 (§§ 17.5.3–17.5.3.4); *see also id.*, 186–89 (§ 10.4–10.6)) according to a statistically-based sampling plan (*id.*, 113 (§ 6.1); *see also id.*, 115–18 (§§ 6.4–6.8), 184 (§ 10.2), 319–21 (§§ 17.2.3.3–17.2.3.4), 326–30 (§§ 17.5.3.1–17.6), as described regarding steps (a) and (e)(2). Hughes' "Preparation of Sample" section identifies four phases including (a) pre-enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. Hughes explicitly teaches retention of the enriched test lot samples for further testing after a positive result. Hughes provides instructions to refrigerate the "remaining M broth and TT and Rappaport-Vassiliadis R10 tubes [that were enriched] for culture confirmation of any EIA-positive samples." Hughes, 638. Hughes refers to the results as the "confirmed positive samples" (Hughes, 635) and also states that diagnostic reagents are "necessary for culture confirmation of presumptive EIA tests." Hughes, 636. Hughes indicates "[e]nrichment broths and M broths from samples positive by an

	enzyme immunoassay (EIA) method must be streaked on selective media." *Id.*, 635. Culture confirmation using streaking is more sensitive than the EIA method, as was commonly understood and confirmed by the teachings of D'Haese. *See generally*, D'Haese. Each sample was analyzed by the BAM method (7th Ed.) and the TSVIA method using the enrichment protocol described in section F of the method, *Preparation of Sample*. Equal portions (0.5–1 mL) from each of the 2M broths arising from the 2 selective-enrichment broths were combined in a small screw-capped tube and heat treated in boiling water for 15 min prior to performing the TSVIA. Confirmation for the immunoassay was by streaking from the M broth culture onto selective agar, as specified in the BAM reference culture method. Hughes, 635. Moreover, the recitation of a lot being validated for "release . . . into commerce" is not limiting. Prosecution History for Reexamination Control No. 95/011,294.
[35e.4] wherein the single-unit production lots corresponding to any negatively testing composited single-unit production lot samples are validated.	Price, alone or in combination with ICMSF and Hughes, discloses wherein the test lots corresponding to any negatively testing composited test lot samples are validated.



Price, 680 (Figure 1 (annotated)).

ICMSF and Hughes disclose selectively enriching prior to removing portions, thus a POSITA would consider the enrichment sufficient to enable validation of the lots without further enrichment or sampling.

Hughes explicitly indicates portions of the enrichment cultures are retained for further testing; thus Hughes has no need for further sampling.

Hughes' "Preparation of Sample" section identifies four phases including (a) pre-

	enrichment, (b) selective enrichment, (c) post-enrichment, and (d) preparation of sample for enzyme immunoassay analysis ("EIA"). Hughes, 636, 638. This section further discloses compositing portions of enriched samples to form a composite sample. *Id.* It also discloses retention of the enriched samples "for culture confirmation of any EIA-positive samples." *Id.,* 638. ICMSF and Hughes disclose selective enrichment so further enrichment is unnecessary. Also, as discussed regarding step (e)(3), Hughes explicitly indicates portions of the enrichment cultures are retained for further testing; thus Hughes has no need for further sampling. The same would be expected for enrichment cultures in ICMSF because retaining samples at each process stage was considered standard practice for a POSITA. in flowing steam 15 min. Refrigerate (2°–8°C) remaining M broth and TT and Rappaport-Vassiliadis R10 tubes from (c) for culture confirmation of any EIA-positive samples (*see* **967.26B, 967.26C, 967.27** and **967.28**). Cool heated M broths to 25°–37°C prior to analysis. Hughes, 638. Thus, the negative limitation of step (e)(4) is disclosed by and is obvious based on the combination of ICSMF and Hughes. *Id.*
Claim 36	**Disclosure of Price, ICMSF, and Hughes**
The method of claim 35, wherein removing portions in step d) is removing equal portions, aseptically, so as to preclude contamination of the enriched separate composited single-unit production lot samples.	Price, alone or in combination with ICMSF and Hughes, discloses Claim 35, upon which this claim relies. See above re Claim 35. Price, alone or in combination with ICMSF and Hughes, further discloses removing portions in step d) is removing equal portions, aseptically, so as to preclude contamination of the enriched separate test lot samples.

	ICMSF describes aseptically withdrawing and weighing sample units into the appropriate diluent. ICMSF, 231, 232. Hughes instructs that after enrichment "[c]ombine equal volumes (0.5-1 mL) from each M broth tube in a clean screw-cap tube." Hughes, 638. Moreover, a POSITA would consider this to be standard practice. A POSITA would have aseptically removed equal volume portions of each enriched separate test lot sample to preclude contamination.