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EXAMINER	
TURNER, SHARON L	
ART UNIT	**PAPER NUMBER**
3991	

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

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CENTRAL REEXAMINATION UNIT

Transmittal of Communication to Third Party Requester
Inter Partes Reexamination

REEXAMINATION CONTROL NO. : 95001294
PATENT NO. : 7534584
TECHNOLOGY CENTER : 3999
ART UNIT : 3991

Enclosed is a copy of the latest communication from the United States Patent and Trademark Office in the above identified Reexamination proceeding. 37 CFR 1.903.

Prior to the filing of a Notice of Appeal, each time the patent owner responds to this communication, the third party requester of the inter partes reexamination may once file written comments within a period of 30 days from the date of service of the patent owner's response. This 30-day time period is statutory (35 U.S.C. 314(b)(2)), and, as such, it cannot be extended. See also 37 CFR 1.947.

If an ex parte reexamination has been merged with the inter partes reexamination, no responsive submission by any ex parte third party requester is permitted.

All correspondence relating to this inter partes reexamination proceeding should be directed to the Central Reexamination Unit at the mail, FAX, or hand-carry addresses given at the end of the communication enclosed with this transmittal.

PTOL-2070(Rev.07-04)

Transmittal of Communication to Third Party Requester Inter Partes Reexamination	Control No.	Patent Under Reexamination	
	95/001,294	7534584	
	Examiner	Art Unit	
	SHARON TURNER	3991	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address. --

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All correspondence relating to this inter partes reexamination proceeding should be directed to the **Central Reexamination Unit** at the mail, FAX, or hand-carry addresses given at the end of the communication enclosed with this transmittal.

Right of Appeal Notice (37 CFR 1.953)	Control No.	Patent Under Reexamination
	95/001,294	7534584
	Examiner	Art Unit
	SHARON TURNER	3991

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address. --

Responsive to the communication(s) filed by:
Patent Owner on 05 May, 2011
Third Party(ies) on 02 March, 2011

Patent owner and/or third party requester(s) may file a notice of appeal with respect to any adverse decision with payment of the fee set forth in 37 CFR 41.20(b)(1) within **one-month or thirty-days (whichever is longer)**. See MPEP 2671. In addition, a party may file a notice of **cross** appeal and pay the 37 CFR 41.20(b)(1) fee **within fourteen days of service** of an opposing party's timely filed notice of appeal. See MPEP 2672.

All correspondence relating to this inter partes reexamination proceeding should be directed to the **Central Reexamination Unit** at the mail, FAX, or hand-carry addresses given at the end of this Office action.

If no party timely files a notice of appeal, prosecution on the merits of this reexamination proceeding will be concluded, and the Director of the USPTO will proceed to issue and publish a certificate under 37 CFR 1.997 in accordance with this Office action.

The proposed amendment filed 05 May, 2011 ☒ will be entered ☐ will not be entered*

*Reasons for non-entry are given in the body of this notice.

1a. ☒ Claims 1-33 are subject to reexamination.
1b. ☐ Claims _____ are not subject to reexamination.
2. ☐ Claims _____ have been cancelled.
3. ☐ Claims _____ are confirmed. [Unamended patent claims].
4. ☐ Claims _____ are patentable. [Amended or new claims].
5. ☒ Claims 1-33 are rejected.
6. ☐ Claims _____ are objected to.
7. ☐ The drawings filed on _____ ☐ are acceptable. ☐ are not acceptable.
8. ☐ The drawing correction request filed on _____ is ☐ approved. ☐ disapproved.
9. ☐ Acknowledgment is made of the claim for priority under 35 U.S.C. 119 (a)-(d) or (f). The certified copy has:
   ☐ been received. ☐ not been received. ☐ been filed in Application/Control No. _____.
10. ☐ Other _____

Attachments

1. ☐ Notice of References Cited by Examiner, PTO-892
2. ☐ Information Disclosure Citation, PTO/SB/08
3. ☐ _____

DETAILED ACTION: Inter Partes Reexamination

Right of Appeal Notice

This is the Right of Appeal Notice (RAN) in *inter partes* reexamination proceeding 95/001,294 of U.S. Patent 7,534,584 to Samadpour. The RAN is based upon patent owner's amendment filed after ACP, which has been entered.

Procedural Posture

A corrected Request pursuant to 37 CFR 1.913 for *inter partes* reexamination of claims 1-33 of U.S. Patent 7,534,584 was filed 04/05/10 with a Declaration by J. Stan Bailey, Ph.D.

An Order granting *inter partes* reexamination of claims 1-33 of U.S. Patent 7,534,584 and a Non-final Office Action rejecting claims 1-33 was mailed 05/26/10.

Patent Owner's Response was filed 07/26/10. Included in the response were declarations by Mansour Samadpour, Ph.D. and Mohammad Koohmaraie, Ph.D. Claims 1 and 17 were amended and new claims 34-36 were added.

Comments by Third Party Requester were filed 08/25/10.

An ACP was mailed 12/17/10.

Patent Owner's Response after ACP was filed on 2/3/11 and 5/5/11 (correcting informalities). Included in the response were two declarations by Mansour Samadpour, and declarations by Warren Mirtsching and James Byron. Claims 1, 2 and 17 are proposed to be amended. New claims 34-36 are proposed to be canceled.

Comments by Third Party Requester were filed 3/2/11.

Status of Amendments after ACP

Patent Owner's amendment filed 2/3/11 and 5/5/11 (correcting informalities) has been entered. All submitted declarations have been entered.

Third Party Requester's Comments filed 3/2/11 have been entered.

Status of Claims

Claims 1, 2 and 17 are Amended. Claims 2-16 and 18-33 are Original. New claims 34-36 are Canceled. Claims 1-33 are subject to inter partes reexamination.

As above, the amendment filed 2/3/11 and 5/5/11 (correcting informalities) has been entered. The amendment obviates the rejections necessitated by amendment as set forth in the 12/17/10 ACP at pages 60-63. In particular, the rejections of claims 1, 17 and 34-35 under 35 USC 112, first and second paragraphs and rejection of claims 1-36 under 35 USC 305 for broadening are obviated and withdrawn. The amendment introduces new language within claims 1, 2 and 17. However, these changes merely clarify and do not significantly change the scope of the claims from the form presented prior to ACP. Consistent with Requester's position, these claim amendments fail to obviate any other ground for rejection as set forth in the 12/17/10 ACP. The instant RAN thus merely reiterates the previous grounds for rejection (as set forth in the 12/17/10 ACP) to the claims as instantly amended after-ACP. No new grounds for rejection are set forth. Accordingly, the instant office action is a right of appeal notice (RAN).

Documents Cited

1. DAVIES *et al.*, *Evaluation of the use of pooled serum, pooled muscle tissue fluid (meat juice) and pooled faeces for monitoring pig herds for Salmonella*, J. Appl. Microbiol., 95:1016-25 (2003) (Exhibit 2, hereinafter, "**Davies**").

2, MIDURA, *et al.*, *Sampling Plans, Sample Collection, Shipment and Preparation for Analysis,* Chapter 2, pp.13-23, in Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., *Eds.* Downes and Ito, American Public Health Association (2001) (Exhibit 3, referenced in the Request as "Compendium Chapter 2" and hereinafter as "**Midura**").

3. MENG, *et al.*, *Pathogenic Escherichia coli*, Chapter 35, pp.331-341, in Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., *Eds.* Downes and Ito, American Public Health Association (2001) (Exhibit 4, referenced in the Request as "Compendium Chapter 35" and hereinafter as "**Meng**").

4. ANDREWS, *et al.*, *Salmonella*, Chapter 37, pp. 357-380, in Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., *Eds.* Downes and Ito, American Public Health Association (2001) (Exhibit 5, referenced in the Request as "Compendium Chapter 37" and hereinafter as "**Andrews**").

5. US Dept of Agriculture, FSIS Directive, *Microbial Testing Program and Other Verification Activities for Echerichia coli0157:H7 In Raw Ground Beef Products and Raw Ground Beef Components and Beef Patty Components*, 10.010.1, Revision 1, (3/13/2004), pp. 1-28 (Exhibit 6, hereinafter, "**FSIS**").

6. SILLIKER, *et al.*, "ICMSF Methods Studies. I. Comparison of analytical schemes for detection of Salmonella in dried foods," Can. J. Microbiol. 19:475-479 (1973) (Exhibit 7, hereinafter, "**Silliker**").

7. PRICE, *et al.*, *Salmonella Testing of Pooled Pre-Enrichment Broth Cultures for Screening Multiple Food Samples*, Appl. Microbiol. 23:679 (1972) (Exhibit 8, hereinafter, "**Price**").

8. GABIS, *et al.*, *ICMSF Methods Studies. II. Comparison of analytical schemes for detection of Salmonella in high-moisture foods*," Can. J. Microbiol. 20:663-669 (1974) (Exhibit 9, hereinafter "**Gabis**").

9. ICMSF (The International Committee on Microbiological Specifications for Foods), *Indicator microorganisms* , pp. 1-11; *Important considerations for the analyst*, pp. 91-102; *Preparation and dilution of the food homogenate*, pp. 107-111; *Enumeration of mesophilic aerobes: the agar plate methods*, pp. 113-123; *Coliform bacteria*, pp. 125-139; *Enterobacteriaceae*, pp. 141-143; *The enterococci*, pp. 144-147; *Hemolytic streptococci,* pp. 149-155; *The enumeration of yeasts and molds*, pp. 157-159; and *Salmonellae*, pp. 160-172, in Microorganisms in Foods, Their Significance and Methods of Enumeration, Second Edition, University of Toronto Press (1978) (Exhibit 10, hereinafter "**ICMSF**").

10. BAM (Bacteriological Analytical Manual), *Food Sampling/Preparation of Sample Homogenate*, Chapter 1, pp. 1-11, US FDA, (April 2003) (Exhibit 11, hereinafter, "**BAM**").

11. JARVIS, B., *Errors Associated with Dilution Count and Presence/Absence Methods*, Chapter 8, pp. 117-142, in Statistical Aspects of the Microbiological Analysis of Foods. Progress in Industrial Microbiology, Vol. 21, Elsevier (1989) (Exhibit 13, hereinafter, "**Jarvis**").

12. ENTIS, *et al.*, *Rapid Methods for Detection, Identification, and Enumeration*, Chapter 10, pp. 89-126 in Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., *Eds.* Downes and Ito, American Public Health Association (2001) (Exhibit 15, referenced in the Request as "Compendium, Chapter 10" and hereinafter as "**Entis**").

13. COUSIN, *et al.*, *Psychrotrophic Microorganisms*, , Chapter 13, pp. 159-166, in Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., *Eds.* Downes and Ito, American Public Health Association (2001) (Exhibit 16, referenced in the Request as "Compendium Chapter 13", and hereinafter as "**Cousin**").

14. U.S. Patent 6,080,437, issued 06/27/2000 to Mauer (Exhibit 14, hereinafter, "**Mauer**").

Scope of claims

In reexamination, patent claims are construed broadly. In re Yamamoto, 740 F.2d 1569, 1571,222 USPQ 934, 936 (Fed. Cir. 1984) (claims given "their broadest reasonable interpretation consistent with the specification").

The 7,534,584 patent consists of claims 1-33, drawn to methods of sampling, testing and validating test lots for release into commerce.

Claims 1 and 17, as amended, are representative.

1. A method of sampling, testing and validating a plurality of test lots for release into commerce [microbial contamination], comprising:

a) collecting a plurality of portions from each of a plurality of separate test lots 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 [by taking said plurality of portions thereof];

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;

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;

d) removing portions of each enriched separate test lot sample, and combining the removed portions to provide a modular composite sample; and

e) testing of the modular composite sample, using a suitable detection assay, for the target microbial agent or organism, wherein when such testing is negative all of said separate test lots [samples] are validated for release into commerce, and 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, 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).

17. A method of sampling, testing and validating a plurality of test lots for release into commerce [microbial contamination], comprising:

a) collecting a plurality of portions from each of a plurality of single-unit production lots, 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 samling plan specifying, for a particular microbial agent or organism, a number of portions to be taken sufficient to provide validation and release of the single-unit production lot into commerce [by taking said plurality of portions thereof];

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;

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;

d) removing portions of each enriched separate single-unit production lot sample, and combining the removed portions to provide a modular composite sample; and

e) testing of the modular composite sample, using a suitable detection assay, for the target microbial agent or organism, wherein when such testing is negative all of said separate single-unit production lot samples are validated for release into commerce, and 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, 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).

The following selected claim terms are defined as follows in the '584 patent disclosure:

Test lot or reportable lot: an assemblage of one or more specimens of an operationally linked medium or process (column 5, lines 21-32).

Product portion: piece, aliquot or weight of a product (column 5, lines 38-49).

Test lot sample (or single-unit production lot sample or single production unit lot sample): a sample formed by combining multiple product portions from a test lot (column 5, line 57, through column 6, line 6).

Enrichment: incubating a test lot sample under conditions suitable to allow levels of a target agent or organism to reach detectable levels (see column 6, lines 7-16).

Enrichment medium: mixture which contains nutrients and which has properties (*e.g.*, pH and/or oxygen content) which favor the growth of the target agent/organism (column 6, lines 17-35).

Modular composite sample: a larger sample formed from combining equal portions of a plurality of enriched single-unit production lot samples (column 6, lines 57-61).

Validation: determining that a particular sample tests negative using a detection assay (column 7, lines 1-10). When a modular composite sample tests negative, all single unit production lot samples are validated.

Summary of Proposed Grounds of Rejection Under 35 U.S.C. 103(a)

Grnd #	Reference(s)	Adopted/not adopted/withdrawn
1	Jarvis/FSIS, Midura	Adopted in part/withdrawn in part
2	FSIS/Price, Midura	Adopted
3	Jarvis/P.O. Admissions	Adopted

4	P.O.Admissions/Price	Not adopted
5	Andrews/Price, Midura	Adopted
6	Silliker/Price, Midura	Adopted
7	Gabis/Price, Midura	Adopted
8	Jarvis/BAM	Adopted
9	Price/BAM, Davies	Adopted
10	FSIS/Midura, P.O. Admissions	Not adopted
11	Jarvis/FSIS, Midura, BAM	Not adopted
12	FSIS/Price, Midura, P.O. Admissions	Not adopted
13	FSIS/Price, Midura, BAM	Not adopted
14	Jarvis/FSIS, Midura, Entis	Adopted
15	FSIS/Price, Midura, Entis	Adopted
16	Jarvis/FSIS, Midura, Mauer	Not adopted
17	FSIS/Midura, Mauer, BAM	Not adopted
18	Jarvis/FSIS, Midura, Silliker	Not adopted
19	FSIS/Price, Midura, Silliker	Not adopted
20	FSIS/Price, Midura, Jarvis, Silliker	Not adopted
21	Jarvis/FSIS, Midura, Cousin	Adopted
22	FSIS/Price, Midura, Cousin	Adopted
23	Jarvis/FSIS, Midura, Price	Adopted
24	FSIS/Price, Midura, Gabis	Adopted
25	Jarvis/FSIS, Midura, Andrews	Withdrawn
26	Jarvis/FSIS, Midura, BAM, ICMSF	Not adopted

Rejections, 35 U.S.C 103

Ground #1:

The rejection of claims 1-4, 8, 10, 11, 13, 17-19, 22, 24, 26, 27, and 31 as unpatentable over Jarvis in view of FSIS and Midura (see SNQ #s 1, 10, 16, 18, 24, 27, 29, 33, 47, 49, 56, 61, 66, 68, and 75 at pages 28-34, 54-55, 57-59, 64-65, 67-69, 70-71, 74, 82, 83, 87-88, 91, 94-95, and 100 respectively of the Request) is maintained for claims 1-3, 8, 10, 11, 13, 17-19, 22, 24, 26, 27, and 31. The rejection remains withdrawn for claim 4 as set forth in the 12/17/10 ACP.

This rejection was proposed by the third party requester and **adopted** for the reasons set forth in the 5/26/10 office action and 12/17/10 ACP. The proposed rejections is set forth at pages 28-34, 54-55, 57-59, 64-65, 67-69, 70-71, 74, 82, 83, 87-88, 91, 94-95, 100, 106-114, 117,

119-121, 123, 125, and 126 of the request. Claims 12, 14, 15, 16, 20, 25, 32, and 34-36 were added to the proposed ground of rejection in the 5/26/10 office action. The rejection is reiterated to the claims as amended after-ACP.

Claims 1-3, 8, 10-20, 22, 24-27, 31 and 32 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Jarvis in view of FSIS and Midura.

Regarding ***claims 1 and, 17,*** Jarvis discloses methods of sampling and testing food samples for low numbers of pathogenic organisms using dilution count and presence/ absence methods (sections 8.3 and 8.4). Both methods may be used to produce mean probable number counts for the lots tested (pages 120-121 Example 8.1 A and B and 135-136 Example 8.4 lots A, B and C). Jarvis makes clear that the methods are applied to three different lots of food for decisions regarding whether to accept or reject any particular lot of food.

"For control purposes it may be required to make a decision to accept or reject a consignment of food on the basis of a MPN test (e.g. for *Enterobacteriaceae*). For such purposes it is possible to construct an operating characteristics (OC) curve (§ 5.2.3) for acceptance or rejection at specific confidence levels of tube dilution counts. The best control system would be based on the use of a limit permitting "acceptance of the '**lot**' if less than *c* tubes out of *n* at dilution *i* show evidence of growth" (i.e. if the number of sterile tubes at dilution *i* is > c_i)." (Page 135, 8.3.4, emphasis added).

Jarvis also makes clear that the methods may be based on either, "single or multiple samples." (Page 135-137, Section 8.4)

"In the analysis of foods for low numbers of pathogenic organisms (e.g. salmonellae), it is normal to inoculate one or more samples into a suitable selective or enrichment medium prior to streaking on to a diagnostic medium to determine whether or not the specific organism sought is present."

As above, Jarvis exemplifies the methods with a plurality of separate lots of food, explicitly **lots A, B and C** in which the decisions are alternatively to "Reject lot," Retest lot," or "Accept lot." (Page 135-136, Section 8.4 and Example 8.4, emphasis added). Accordingly, it is clear that Jarvis samples, tests and validates a plurality of test lots for release into commerce.

The method is designed in a statistical-based sampling plan which directs decisions regarding whether the lots should be accepted or rejected based upon levels of microorganism contamination, particularly salmonella (page 135-138, section 8.4, 8.4.1, Example 8.4 and Table 8.9) In particular, Table 8.9 specifies percentage defectives and maximum contamination levels assuming random distribution of organsims, per number of samples (*n*) tested. This is consistent

with the definition of a statistical-based sampling plan and definition of a lot as described in the '584 patent.

The term "test lot or reportable lot as used herein to refer to an assemblage of one or more specimens of a medium or process, (e.g., assemblage of specimens of air, water, solids, or of products of a production process, etc.), where such assemblage can be sampled by taking portions of the one or more specimens thereof, and where the one or more specimens of the assemblage are operationally linked in a manner (e.g., proximity, time, process step, etc.) whereby information derived about sampled portions is operationally applicable to all specimens of the assemblage, and thus to the test lot. In particular instances, portions are synonymous with specimens." ('584 patent, column 5, lines 20-32)

While a statistical-based sampling plan is not specifically defined, the term is discussed in the following passages.

> The term "compositing" as used herein refers to combining a number of single product portions to form one larger sample. In preferred embodiments, the number and selection of single product portions to be combined conform to **statistical-based sampling plans** which seek to form a typical or average sample that is representative of the test lot (e.g., single production unit) being sampled. ('584 patent column 5, lines 50-56).
>
> The term "test lot sample" or "single-unit production lot sample" or "single production unit lot sample," as used herein refers to a sample formed by combining multiple product portions from a test lot (e.g., single production unit), such that each test sample (e.g., each single-unit production lot sample) is attributed to a particular corresponding test lot (e.g., production unit). For production lot samples, the number of potions/pieces combined many be *essentially any number*, but preferably is selected so as to conform to **statistical-based sampling plans**. Preferably the number of pieces combined is selected from the range group consisting of from about 5 to about 100, from about 10 to about 90, from about 20 to about 80, from about 30 to about 70, from about 30 to about 60, and from about 40 to about 50 product portions. Preferably, the number of independent product portions is selected so as to yield a sample size that is equal to or greater than that required by the subsequent analysis to be applied. ('584 patent column 5, lines 57-column 6, line 6).

While the basic method of Jarvis includes inoculating one or more samples in pre-enrichment or enrichment media (pages 135 and 138-139, sections 8.4 and 8.4.2), Jarvis also teaches further compositing of a number of samples via at least 3 different methods (pages 141-142, section 8.4.21.) The methods include generating a series of pre-enriched samples and taking a portion, such as a milliliter (ml.) quantity of each, for composite testing (page 141, section 8.4.2.1, methods (1)-(3). Jarvis notes that testing such composite samples reduces the workload on the laboratory (page 141, section 8.4.2.1). The methods described by Jarvis are as follows.

(1) Combine all samples at pre-enrichment stage; i.e. for 10 x 25 g samples inoculate all 250 g directly into 2250 ml of pre-enrichment medium. After incubation, carry out tests on the same basis as for a single sample (i.e. inoculate 10 ml into 100 ml enrichment medium).

(2) Prepare 10 pre-enrichment series (i.e. one for each 25 g sample) and after incubation composite equal volumes of each pre-enrichment medium (e.g. 10 x I ml) to inoculate the 100 ml enrichment broth.

(3) A combination of (1) and (2) above where, say, 5 samples are composited at pre-enrichment stage and aliquots of the duplicate pre-enrichment media are composited at the stage of inoculating the enrichment medium.

Jarvis teaches that methods 1, 2 and 3 provide the same degree of statistical confidence as would be attained by separately testing each of the 10 samples (see page 141 in its entirety). Jarvis also teaches that composite testing method 2 further provides the advantage of allowing for verification of positive samples by retesting of the stored individual pre-enrichment cultures to provide an indication of the proportion of contaminated samples (pages 141-142). Method (3) is a combination of 1 and 2 and thus also provides the advantages of method 2 in regard to repeat testing of under-lying samples.

As methods 1-3 provide the same degree of statistical confidence, samples which test negative would be indicated as accepted and those that test positive would be indicated as rejected.

Claim 1 differs from claim 17 as claim 1 is directed to separate "test lots" and separate "test lot samples" whereas claim 17 is directed to "single-unit production lots" and "single-unit production lot samples." As in the '584 patent, a "single-unit production lot" is another term referring to a "test lot sample" which is formed by combining multiple product portions from a test lot such that each test sample is attributed to a particular corresponding test lot. As envisioned the number of portions/pieces combined may be essentially any number but preferably is selected so as to conform to statistical-based sampling plans, see '584 patent column 5, lines 57-column 6, line 6. Thus, while the term appears to refer to single portions, one or more portion is permitted.

Regarding ***claim 2***, since Jarvis teaches analysis of foods for low numbers of pathogenic organisms by collecting samples from the food, each of the test lots would necessarily be composed of the operationally-linked product samples or specimens (page 135, section 8.4, first paragraph). With respect to ***claim 3***, Jarvis teaches incubating a modular composite sample in enrichment broth prior to testing for pathogenic organisms, *e.g.*, salmonellae (page 135, section 8.4, first paragraph, and pages 141-142, section 8.4.2.1). In reference to ***claims 11 and 13***, Jarvis teaches a test lot of 10 samples, which falls within the claimed range of 3 to 10. Regarding ***claim 19***, Jarvis teaches testing for *Salmonella* (page 135, section 8.4, first paragraph). With respect to ***claims 18, 22 and 31***, Jarvis teaches aseptically removing and combining equal

portions of each of the pre-enriched separate test lot cultures (10 x 1 ml, see page 141, section 8.4.2.1, method 2). As for ***claims 27 and 36***, the food samples described by Jarvis would be understood by one of skill in the art to be synonymous with product portions or product specimens.

In the compositing method 8.4.2.1, Jarvis defines the medium used for incubation of the individual test lot samples as a "pre-enrichment" medium, while the instant claims do not specify the incubation medium. The instant claims recite incubating the test lot samples under conditions suitable to allow levels of a target microbial agent to reach detectable levels and become uniform, or substantially uniform throughout, to provide a set of separate test lot samples enriched for the target microbial agent or organism (***claims 1 and 17***). As was set forth *supra*, the '584 patent specification defines "enrichment" as incubating a test lot sample under conditions suitable to allow levels of a target agent or organism to reach detectable levels and become uniform or substantially uniform (see column 6, lines 7-12). Jarvis teaches pre-enrichment growth to at least 3 viable salmonellae/ml medium (p. 141 Example 8.6) to ensure the probability of transferring at least one viable organism per transferable inoculum of 1 ml. (pages 138-141, 8.4.2). Also, since the pre-enrichment medium disclosed by Jarvis is designed to recover target organisms which have been compromised by less than optimal growth conditions, and render them "actively growing" (see Price, of record, page 681, second full paragraph), the method disclosed by Jarvis provides a set of separate test lot samples wherein the levels of target organisms are increased to detectable levels, and become uniform or substantially uniform throughout the culture, thereby meeting the limitations of the instant claims.

The method described by Jarvis differs from the claimed method in that Jarvis does not teach repeat testing of negative samples, as in patented ***claim 8***; does not specify that the test lot samples are collected from a single combo, bin, pallet or production assemblage, as in patented ***claim 10***; does not specify testing for *E. coli* 0157:H7 (among others), as in ***claims 19 and 20***, and does not specify the food to be tested, as in patented ***claims 24-26***.

FSIS teaches procedures for verification testing of E. coli 0157:H7 in raw meat. The directives also outline actions to be taken when a sample tests positive. Regarding ***claim 20***, FSIS discloses methods of sampling ground beef or poultry for *E. coli* O157:H7 contamination, comprising aseptically collecting a plurality of pieces or samples and combining the pieces for a

representative sample of a production lot from the current day's production (a production assemblage, see pages 4 and 27-28 in their entirety), thus meeting the limitations of patented ***claims 10, 19, 20, 24, and 26***. Regarding ***claims 8 and 12***, FSIS teaches follow-up sampling upon identification of contaminated lots and corrective or remedial measures (see the paragraph bridging pages 8-9). With respect to ***claims 32 and 36***, FSIS teaches that manufacturing trimmings (e.g., pieces of meat remaining after steaks, roasts, and other intact cuts are removed) may be processed for a food product (page 2, second paragraph).

Midura specifies statistical-based sampling plans used for testing foods for microbial contamination (2.51-2.517). Midura sets forth the standard terminology utilized in the art (pages 14-16).

> Lot-The number of sample units produced in one batch or in some specified period of time such that the units will be about the same quality. Each lot or batch should consist of units of product of a single type, grade, class, size, and composition. The lot should be manufactured under the same conditions and at essentially the same time.
>
> Sampling plan-A design that indicates the number of units to be collected from each lot and the criteria to be applied in accepting or rejecting the lot. A single sampling plan requires the lot to be judged on the basis of one set of sample units (2.512). A double sampling plan (2.513) is a sampling inspection in which the inspection of the first sample leads to a decision to accept, to reject, or to take a second sample. The inspection of a second sample, when required, then leads to a decision to accept or reject. Two or more such collections would mean that a multiple sampling plan was being used. Another procedure where units are drawn one by one (or in groups) and drawing at any stage determines whether to accept, reject, or continue sampling is called a sequential sampling plan.

As above, Midura teaches collecting a plurality of samples from a food lot produced in one batch or in some specified period of time, as in patented ***claim 10*** (2.2), with fish, meats, and shell fish exemplified, as in patented ***claims 24 and 25*** (2.514). Regarding ***claim 8***, Midura teaches subsequent testing of a second sample depending on the results of the first sample as multiple sampling attribute plans (two or three class) (pages 17-20). Midura also teaches that when products with a low incidence of the target pathogen are tested, additional samples need to be collected and tested to ensure a high probability of detecting the pathogen if present (pages 19-20, section 2.516 bridging columns 1 and 2). With respect to ***claims 14-16***, Midura exemplifies collecting 30 or 60 samples or portions per lot (see page 16, Fig. 1, page 17, column 1, first full paragraph; page 19, column 2, last full paragraph; and page 20, column 1, first full

paragraph). Midura serves to clarify the art accepted terms utilized in Jarvis, which dictate testing of a plurality of separate test lot samples, derived from a plurality of separate test lots, where a lot is comprised of a statistically-based number of samples.

It would have been *prima facie* obvious for one of ordinary skill in the art at the time of the invention to have sampled, tested, and validated any number of separate food lots as exemplified in Example 8.4 of Jarvis for three separate lots A, B and C. Regarding the number of portions to be collected as in a) for each test lot, and to be combined in b) so as to assemble a corresponding set of separate test lot samples, each corresponding to the separate lots for incubation testing, one of skill in the art would be motivated to collect a number of portions from each lot consistent with a statistical-based sampling plan specifying for the type of food and particular agent or organism, the number of portions to be taken sufficient to provide for validation and release of the particular lots into commerce as taught particularly in Jarvis and Midura. For example, Midura specifically teaches statistical based single, multiple, three-class and variable sampling plans (pages 16-20, sections 2.512-2.516) with procedures for low contamination level microorganisms such as *Salmonella*, *E. coli* and *Listeria*. As above, Jarvis teaches testing for single or multiple test lot samples and further teaches methods for composite testing (pages 141-142, 8.4.2.1). Thus one of skill in the art would be further motivated to conduct testing for any number of separate test lot samples as directed in Jarvis via composite testing. Such steps include incubating the separate test lot samples under conditions suitable for detection as in c), removing a portion of each separate test lot sample and combining the removed portions for composite testing as in d) to determining the presence or absence of the microorganism, and to decide whether to accept or reject the lots as in e). Jarvis motivates to composite testing of samples as the method has the same degree of sensitivity as individual sample testing, while reducing workload, and also teaches that initial incubation of the food portions or samples in separate broth cultures allows for follow-up testing of individual test lots if the composite enriched sample tests positive for the target pathogen. The person of ordinary skill in the art at the time of the invention would also have found it *prima facie* obvious to have taken the samples from a test lot operationally linked, *e.g.*, by time of production, as is taught by FSIS, in order to pinpoint the contamination to a particular lot of food. Lastly, the person of ordinary skill in the art at the time of the invention would have found it *prima facie* obvious to

have subsequently followed up with additional testing when composites test either positive or negative because the prior art teaches additional testing of positive test lots to isolate, verify and confirm contaminated samples and further teaches that testing production lots with low numbers of target pathogena requires collecting and testing additional samples to verify, confirm and provide for increased confidence levels for any result.

Response to Patent Owner's arguments:

Patent Owner's arguments regarding Ground 1 have been fully considered as they pertain to claims 1-3, 8, 10-20, 22, 24-27, 31 and 32, but are not persuasive for the following reasons:

Patent Owner argues (pages 11-19, 36-40 and 50-60) that Jarvis is limited to testing a single sample or lot as opposed to separate test lot samples or separate test lots. However, Example 8.4 of Jarvis specifically exemplifies testing 3 separate test lots of food, designated **lots A, B and C** (p. 136). Jarvis also teaches testing "single or multiple samples" as specified in section 8.4 at p. 135 and "a number of samples" section 8.4.2.1 at p. 141. Further Jarvis teaches compositing any number of the different test lot samples as follows. "(2) Prepare 10 pre-enrichment series (i.e. one for each 25 g sample) and after incubation composite equal volumes of each pre-enrichment medium (e.g. 10 x 1 ml) to inoculate the 100 ml enrichment broth." Alternatively, "(3) A combination of (1) and (2) above where, say, 5 samples are composited at pre-enrichment stage and aliquots of the duplicate pre-enrichment media are composited at the stage of inoculating the enrichment medium." The use of the terms "pre-enrichment series" and "duplicate pre-enrichment" in this passage clearly indicates tiered testing, where the tiered testing preserves the information for the underlying test lot samples. Thus the testing of composite samples is intended for single or multiple underlying lots. This interpretation is still further supported via Jarvis's direct suggestion to use composite testing method (2) for the advantages provided by composite series which permits the tests to be repeated on stored pre-enrichment cultures so that some indication of the proportion of contaminated samples can be obtained. While Jarvis teaches additional sampling, whereby confidence limits may be increased for any single lot by testing multiple samples from a single lot (pages 121-133, section 8.3.1-8.3.2) such does not teach away from the broad aim in food science testing; which is to validate or reject any number of separate test food lots.

Patent owner's repeated suggestions that the prior art is limited to analysis of single samples and single lots directly opposes the above art standard terminology, which is essentially the same terminology as recognized within the '584 specification and Midura (pages 14-16). As further reiterated in the rejection above, the artisan well recognized statistical-based sampling procedures regarding the number or amount of samples (sampling plans) to be tested for any individual lot (Midura). The prior art is intended for multiple lots (explicitly Jarvis p. 136 Example 8.4), which details experimentation based upon analysis of single or multiple test lot samples, such samples being representative of the under-lying test lot. Jarvis also teaches the use of composite testing. To suggest that the prior art disclosures are only limited to analysis of single samples or single lots is contrary to the above long established principles in food science testing.

Moreover, the instant rejection is one based upon obviousness and cannot be disparaged by discussions of Jarvis alone. The cumulative art motivates to scaling the disclosed testing procedures to any number of different food lots, (Jarvis p. 136, Example 8.4; Midura p. 16, Sampling plan-A design that indicates the number of units to be collected from each lot; FSIS p. 10, for the production lots sent, p. 23, sample every "lot" of ground beef produced). One of skill in the art would be motivated to scale the disclosed testing procedures to sample, test and validate any number of separate food lots. Thus, the cumulative reference teachings render the claimed invention obvious to one of skill in the art.

Patent Owner argues (pages 11-19, 24 and 50-60) that Jarvis does not teach a suitable number of samples to be a "statistical-based sampling plan." Guidance to such plan is provided at columns 5-6 of the '584 patent. As specified, "essentially any number" applies. In fact, at column 5, line 63, through column 6, line 1, the '584 specification states,

> For production lot samples, the number of potions [sic]/pieces combined many [sic] be **essentially any number**, but preferably is selected so as to conform to statistical-based sampling plans. Preferably, the number of pieces combined is selected from the range group consisting of from about 5 to about 100... (emphasis added).

Thus, single samples are applicable (particularly to claim 17), although Jarvis teaches a number of sampling plans as recongnized in art standard terminology, for validation and release of separate lots. This terminology is reiterated in Midura pages 14-16, and the teachings of Midura

are relied upon to show that the sampling in Jarvis, on point at least three different test lots would be as discussed above based upon a statistical-based sampling plan. Moreover, Midura exemplifies a number of different statistically-based sampling plans (with testing for example of n= 3, 5, 10, 15, 20, 30, 60 or 3-60 as disclosed in Midura Figure 1), for testing any lot of food. Jarvis tests least 3 separate lots and further teaching compositing any number of samples via combining for example 5 samples (from 10) (8.4.2.1, Methods 2-3). Midura's numbers fall within the numbers set forth in the '584 specification as exemplary of the number of pieces to be sampled in a statistical-based sampling plan, for any particular lot of food. Jarvis also directs to statistical-based sampling plans (p. 138 Table 8.9) for any particular number of samples tested for different lots of food. The composite testing protocols disclosed by Jarvis at pages 141-142 in no way teach away from such larger sampling numbers. In fact, the entire Jarvis reference is directed to improving accuracy (confidence levels) including by increasing the number of samples or cultures tested (pp. 135-142, Example 8.4, Table 8.9) for any particular lot of food, and includes the use of composite testing for any number of such samples (including from different food lots). Moreover, Midura is specifically on point to the number of portions to be sampled for any food lot.

Regarding claim 2, patent owner argues Jarvis does not teach test lots which are comprised of "operationally-linked" specimens. This language is introduced within the '584 specification with respect to the art standard terms "test lot" or "reportable lot," referring to an assemblage of one or more specimens of a medium or process (e.g., assemblage of specimens of air, water, solids, or of products of a production process, etc.), where such assemblage can be sampled by taking portions of the one or more specimens thereof, and where the one or more specimens of the assemblage are operationally linked in a manner (e.g., proximity, time, process step, etc.) whereby information derived about sampled portions is operationally applicable to all specimens of the assemblage, and thus to the test lot. In particular instances, portions are synonymous with specimens. Jarvis's exemplified samples are food homogenate (or culture), with investigation of samples for contamination with salmonella (page 117-121), and thus are within the scope of the claims. Still further FSIS exemplifies testing lots of meat via sampling multiple pieces or portions from a single day's production (pp. 27-28).

Regarding claims 3, 11 and 13 patent owner argues that Jarvis's composites are not modular composite samples from separate test lots, and are not directed to a statistically sufficient number of different samples. This argument is not persuasive as above. Jarvis teaches sampling from multiple test lots A, B and C, and further teaches compositing at least 5 different test samples. Jarvis teaches compositing any number of samples. The fair reading applies to any number of samples from any number of different test lots. Jarvis exemplifies testing for at least three different test lots, p. 136 Example 8.4, and Jarvis does not teach limiting the composited samples to single lots of food.

Patent Owner argues (pages 11-19, 24, 31-33 and 40-41) that Jarvis does not motivate to increasing work load and cost of testing via compositing, does not teach validation for release or follow-up testing when positive and/or negative samples are tested. Patent owner suggests that increased testing would increase the number of positive lots and would not be practiced due to a fear of decreased sensitivity. However, this position goes against the general teachings of the cited art. Statistics-based sampling, while requiring rigorous testing, allows quantitative assessment of contamination in various bulk food samples. Compositing (Jarvis 8.4.2.1) is a statistically-based method which reduces workload by requiring fewer tests to assess contamination in a larger number of separate underlying samples. In the absence of compositing, a greater number of samples would require testing. Compositing reduces the number of samples which require testing, while providing the same level of statistical significance, as if each of the underlying samples was tested individually. The compositing procedure described by Jarvis also provides the art recognized advantage of preserving the individual underlying samples for subsequent testing if so required or desired; whether the testing is to identify the under-lying test samples/lots that are positive, or to verify test findings (greater confidence level by increasing *n*, the number of samples tested). Clear motivation to composite testing is set forth in Jarvis, for at least these art recognized advantages.

Patent Owner argues (pages 19-24 and 41-49) that Jarvis's "pre-enrichment" cultures do not apply to the claims as the goal is to allow resuscitation and growth, so the aliquot contains at least one viable cell for transfer, and that unlike the prior art, step c) is sufficient for detection; whereas in the prior art it is not. Patent Owner attempts to distinguish such culture procedures as providing dividability vs. detectability. However as discussed, no specific medium is recited in

base claims 1 and 17, therefore the medium is not distinguishing. Jarvis teaches for *Salmonella*, which is the organism exemplified, ICMSF(1978) protocols recommend, "the initial sample is dispersed in 9 volumes of pre-enrichment medium, and after a suitable incubation period 1 ml is transferred to 10 ml of each of two selective enrichment media (p. 139, lines 1-7)." Growth as exemplified at p. 141 provides assurances to propagation and passage of at least a single organism, which as previously discussed, is sufficient for detection with art recognized procedures such as plating as exemplified, or via other art sensitive procedures that may detect even a single organism. The growth in Jarvis also allows uniform or substantially uniform growth throughout the culture, and thus applies to the claims. Further while patent owner suggests that the prior art differs by teaching a first pre-enrichment culture followed by a second enrichment culture, the scope of the broad claims does not preclude such additional culturing steps, which are standard as recognized in the art. Particularly as in Jarvis (page 138, Section 8.4.2), "when samples are tested for presence/absence of specific microbes, the analytical procedure may involve the use of pre-enrichment and selective enrichment stages."

In regard to claim 3, the recitation is, "further comprising incubating the modular composite sample of d) prior to testing in e). Jarvis is on point as it teaches such transfer of aliquots containing at least one organism to enrichment media for further growth. Moreover, while such compositing is recognized as in method (2) attention is also drawn to method (3) which directs parallel sampling of individual lots that are separately maintained and then may be composited as in (2). Method (3) is a combination of (1) and (2) and shows that compositing may proceed at essentially any point amongst a parallel or duplicate series of samples. As above, this sampling is tiered so as to preserve the under-lying test/lot series.

As previously discussed, the broad claims do not recite incubation in any particular medium, particularly "enrichment medium" and does not require that the medium favor growth of or be selective for the target agent. Rather, the claims recite incubating the test lot samples under conditions suitable for the target agent to increase and/or reach detectable levels, to become uniform or substantially uniform, thereby being enriched. As disclosed in Jarvis, at 24 hours of pre-enrichment, there are 6.9×10^8 number of organisms in any 1 ml pre-enrichment medium to be transferred (p. 141). This number is over the 10^4 cells/ml discussed in the first 1/31/11 Samadpour declaration at ¶29, evidencing dividability and detectability. Thus, Jarvis's

pre-enrichment applies; the media allows for sufficient growth, and uniform or substantially uniform growth in culture, such that any standard 1 ml aliquot for transfer would contain *at least* one viable organism. As is reiterated in Price (of record, see page 681, column 1, second full paragraph), the pre-enrichment medium in the individual test lot sample cultures disclosed by Jarvis provides conditions which facilitate recovery of the target agent (e.g., *Salmonella*) from a debilitated condition and which enable the organism to actively grow, *i.e.,* to increase in numbers (see also Andrews, of record, page 360, section 37.123). Such recovery and growth subsequently renders the organisms detectable, *e.g.*, by a DNA-based method such as PCR. Those of ordinary skill in the art at the time of the invention would have readily recognized that sensitive diagnostic methods, such as PCR, are capable of detecting the target agent (see for example, Entis, of record, at page 93 and 94-95). Thus, the organisms in the pre-enrichment cultures taught by Jarvis are increased in numbers over those found in the specimens and have reached detectable levels.. The pre-enrichment broth cultures of Jarvis, therefore, fully meet the limitations set forth in the instant claims.

Regarding claims 8, 10, 19, 20, 24, 26, 32 and 36, Jarvis teaches compositing, and further teaches repeat testing or testing of additional samples (regardless of whether the samples are positive or negative). While the reference does not specifically exemplify retesting negative composite samples, these teachings nonetheless flow from the reference as the method is directed to accepting, retesting or rejecting lots (Example 8.4) including via testing single or multiple samples. The prior art motivates to repeat testing of any test sample to verify findings and or/to improve confidence limits for any particular test by increasing *n* (the number of samples tested) (Jarvis, Midura). In addition, the prior art of FSIS exemplifies production unit lots formed from multiples specimens, pieces or trim as from separate pieces of a day's production, and is on point to investigation of *E. coli* 0157:H7 in meat. Midura also clearly exemplifies statistical-based sampling plans for assessing contamination in a plurality of sample lots, and motivates to repeat testing. FSIS does teach away; the government standard for assessing contamination with *E. coli* 0157:H7, a particularly lethal strain, is not the basis for evaluating the broad testing procedures of the prior art. The prior art is applicable for all it teaches to one of skill in the art. The above references are applied in combination.

Regarding claims 14-16, Midura also does not teach away because it teaches three or more different statistical-based sampling plans; single, multiple, three class and variable sampling. As in Midura, repeat testing may be practiced. Midura also teaches the requisite number of samples (30, 60) to be collected (Figure 1), and such numbers are suggested for collection as in step a). These numbers (including 30 and 60) are also suitable numbers as embodied in the '584 patent. Morever, step a) describes the number of portion for separate test lots. As above, Midura is not relied upon for teaching the principle of compositing, but is relied upon to further describe statistical-based sampling plans for assessing the number of pieces, portions or volumes to sample for any lot that is to be tested for acceptance or rejection.

Patent owner suggests (pages 33-40) that the prior art is not considered as a whole. However, the detailed analysis above is representative of the cumulative prior art taken as a whole, and for what it would suggest to one skilled in the art of food science testing.

The rejection is maintained for claims 1-3, 8, 10-20, 22, 24-27, 31 and 32.

The rejection of claim 4 as being unpatentable over Jarvis in view of FSIS and Midura is withdrawn pursuant to Patent Owner's arguments, which were persuasive with regard to claim 4.

Claim 4, which depends from claim 1, further recites , "...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..." As was set forth *supra*, the '584 patent specification defines "enrichment medium" as a "mixture which contains nutrients and which has properties (*e.g.*, pH and/or oxygen content) which favor the growth of the target agent/organism. The '584 patent specification further describes "enrichment medium" as "...formulated to mimic the environment of the target agent/organism or take advantage of a known metabolic property of the target agent/organism" and as including "selective agents that will inhibit competing undesired agents/organisms" (column 6, lines 17-35). Therefore, the enrichment medium of patent claim 4 is understood to be a selective enrichment medium, which preferentially favors the growth of the target organism over other organisms in the sample. While Jarvis teaches that "In the analysis of foods for low numbers of pathogenic organisms (*e.g.*, salmonellae), it is normal to inoculate one or more samples into a

suitable selective or enrichment medium prior to streaking on to a diagnostic medium to determine whether or not the specific organism sought is present" (page 135, second from last full paragraph), Jarvis does not teach or fairly suggest incubating test lot samples individually in selective enrichment broth and pooling the sample broth cultures for subsequent testing. While the individual pre-enrichment broth cultures of Jarvis do allow levels of the target agent or organism to increase and to reach detectable levels by rendering them actively growing, they do not selectively favor the growth of the target organism over other competing organisms in the sample. Thus, the decision to withdraw claim 4 from rejection as set forth in the 12/17/10 ACP is maintained.

Response to Third Party Requester's arguments:

Requester's position is essentially consistent with the Examiner's position as set forth above. In particular, requester asserts that the claim amendments after-ACP do not distinguish over the prior art rejections set forth in the ACP. Thus requester's arguments are only addressed to the extent they are applicable to withdrawal of the rejection of claim 4 as unpatentable over Jarvis in view of FSIS and Midura. Requester's arguments have been fully considered but are not persuasive for the following reasons.

Requester argues (pages 21-22 of the 3/2/11 response) that Jarvis teaches presence/absence tests which may involve pre-enrichment and selective enrichment stages (Jarvis, p. 138). Requester also argues that FSIS provides motivation for incubation in enrichment media citing the Bailey declaration at ¶18. However, neither the Bailey declaration nor Jarvis teach the limitation of claim 4 requiring steps c) and d) be *in enrichment medium* and *suitable to enrich* for target agent or organism growth (as compared to other species). Accordingly, rejection of claim 4 remains withdrawn as set forth in the 12/17/10 ACP.

Ground #2:

The rejection of claims 1-4, 6-11, 13, 17-18, 22, and 31 as obvious over FSIS in view of Price and Midura (see SNQ #s 2, 13, 15, 17, 19, 22, 23, 25, 26, 28, 30, 34, 48, 57, and 76 at pages 34-36, 58, 59-60, 62-64, 66-67, 69-70, 71, 74-75, 82-83, 88, and 101-102 respectively of the Request) is maintained.

This rejection was proposed by the third party requester and **adopted** for the reasons stated in the 5/26/10 office action and 12/17/10 ACP. The proposed rejections is set forth at pages 34-36, 58, 59-60, 62-64, 66-67, 69-70, 71, 74-75, 82-83, 88, 101-102, 106-116, 118-120, 123, 125, and 126 of the request. Claims 12, 14-16, 20, and 24-26 were added to the proposed ground of rejection in the 5/26/10 office action. The rejection is reiterated to the claims as amended after-ACP.

Claims 1-4, 6-18, 20, 22, 24-26, and 31 are rejected under 35 U.S.C. 103(a) as being unpatentable over FSIS in view of Price and Midura.

Regarding ***claims 1, 17, 18, 20, and 24***, FSIS discloses methods of sampling ground beef for *E. coli* O157:H7 contamination, comprising collecting a plurality of pieces or samples per lot and combining the pieces for a representative sample of a product lot (see pages 4 and 27-28 in their entirety). Regarding ***claims 2 and 10,*** FSIS teaches that the samples are to be aseptically collected from the "current day's production", at specific times, or by specific boxes or combo bins, which is equivalent to a teaching of a test lot consisting of a single combo, single bin, or production assemblage or a teaching of a single unit product lot comprising operationally-linked product specimens (page 4, last paragraph, and page 27 in its entirety). With respect to ***claims 8 and 12,*** FSIS teaches collecting additional samples (follow-up verification) after remedial or corrective action following a positive test result (paragraph bridging pages 8-9 and page 23, last paragraph).

FSIS differs from the claimed invention in that it is silent as to the testing method to be used for detection of microbial contamination and does not specify testing of foods other than ground beef products.

Regarding ***claims 1 and 17***, Price teaches a method for testing multiple food samples for *Salmonella* comprising collecting a plurality of samples (or portions) from the food, inoculating the samples individually into pre-enrichment broth cultures, incubating the cultures to allow for growth of the *Salmonella*, pooling the pre-enrichment broth cultures into wet composite test lots, incubating the pooled test lots into a single enrichment broth (the "modular composite sample" of the patented claims), incubating the composite enrichment broth, and subsequently identifying any *Salmonella* organisms present in the culture. Price teaches that the procedure offers a

simple, effective way to increase sample capacity in the *Salmonella* testing of foods and also permits the pre-enrichment broth cultures to be retained for subsequent individual analysis if a positive result is obtained from the pooled enrichment culture (see, page 679, abstract; page 680, Fig. 1; page 681, column 1, second full paragraph and column 2, first full paragraph). Price specifically teaches test samples prepared by blending dry food with one of three dry inocula, enzyme drain cleaner, freeze-dried skim milk and a aqueous suspension of four lab isolates, (page 679, column 2). The dried food samples were dried egg albumen, cocoa and nonfat dry milk which were either uninoculated or inoculated at different levels of microorganisms ranging from 6 salmonellae/110g-3,000 salmonellae/g. Pre enrichment broths were analyzed both individually and after pooling with 5, 9 and 14 pre-enrichments of uninoculated sample, (page 679-80 and Tables 1-2). Accordingly, Price collects and combines a plurality of portions from separate test lots as in steps a), b) and c) and subsequently pools enriched cultures for composite testing as in steps c), d) and e).

Price defines the medium used for incubation of the individual test lot samples as "pre-enrichment" medium, while the instant claims either do not specify the incubation medium or define the incubation medium as "enrichment medium". As was set forth above, the instant claims recite incubating the test lot samples under conditions suitable to allow levels of a target microbial agent to reach detectable levels, and become uniform or substantially uniform throughout; in order to provide a set of separate test lot samples enriched for the target microbial agent or organism (***claims 1 and 17***). The '584 patent specification defines "enrichment" as incubating a test lot sample under conditions suitable to allow levels of a target agent or organism to reach detectable levels (see column 6, lines 7-16). Since the pre-enrichment medium disclosed by Price is designed to recover target organisms which have been compromised by less than optimal growth conditions and render them "actively growing" (see page 681, second full paragraph), the method disclosed by Price provides a set of separate test lot samples wherein the levels of target organism is increased to detectable levels, and is uniform or substantially uniform, thus meeting the limitations of the instant claims. Price tests for and identifies microorganisms in the individual and composited samples. Regarding ***claim 3,*** Price teaches incubating the composite broth culture *(i.e.*, the modular composite sample) prior to testing. With respect to ***claim 4***, the pre-enrichment medium disclosed by Price (0.5% lactose

broth; see page 679, column 2, first full paragraph) would be equivalent to the enrichment medium of the patented claims as follows. As has been set forth previously herein, the '584 patent specification defines an enrichment medium as "medium which contains nutrients and which has properties (*e.g.*, pH and/or oxygen content) which favor the growth of the target agent/organism." (See the '584 patent specification at column 6, lines 17-35.) Price teaches that the pre-enrichment lactose broth enables the actively growing salmonellae to compete more favorably with organisms introduced from the other samples when pooled at the enrichment stage (see page 681, column 1, second full paragraph). Therefore, absent evidence to the contrary, the pre-enrichment broth disclosed by Price meets the definition of enrichment medium in patented ***claim 4***. Regarding ***claim 6***, Price teaches testing individual pre-enrichment broth cultures if the composite enrichment broth tests positive (see the abstract and page 681, column 2, first full paragraph). Price teaches testing via both biochemical and serological tests, in order to increase the chances of detecting *Salmonella*. With respect to ***claims 22 and 31***, Price teaches aseptically transferring 1 ml each of the pre-enrichment broth cultures to the enrichment broth, which is a teaching of equal volume portions (see page 682, first column, last full paragraph).

Midura, as set forth above, teaches statistical-based sampling plans used for testing lots of food for microbial contamination (2.51-2.517). Midura sets forth the standard terminology utilized in the art (pages 14-16).

Lot-The number of sample units produced in one batch or in some specified period of time such that the units will be about the same quality. Each lot or batch should consist of units of product of a single type, grade, class, size, and composition. The lot should be manufactured under the same conditions and at essentially the same time.

Sampling plan-A design that indicates the number of units to be collected from each lot and the criteria to be applied in accepting or rejecting the lot. A single sampling plan requires the lot to be judged on the basis of one set of sample units (2.512). A double sampling plan (2.513) is a sampling inspection in which the inspection of the first sample leads to a decision to accept, to reject, or to take a second sample. The inspection of a second sample, when required, then leads to a decision to accept or reject. Two or more such collections would mean that a multiple sampling plan was being used. Another procedure where units are drawn one by one (or in groups) and drawing at any stage determines whether to accept, reject, or continue sampling is called a sequential sampling plan.

Regarding ***claims 7 and 9***, Midura teaches that when products with a low incidence of the pathogen are tested, additional samples need to be collected and tested to ensure a high

probability of detecting the pathogen (see page 19, the paragraph bridging columns 1 and 2). Regarding ***claim 8***, Midura teaches subsequent testing of a second sample depending on the results of the first sample in the form of multiple sampling (two or three class) attribute plans (see pages 17-20). Midura teaches collecting a plurality of samples from a food lot produced in one batch or in some specified period of time, as in patented ***claim 10*** (with fish, meats, shell fish, and canned ready-to-eat food products exemplified, as in patented ***claims 24-26***; see page 18, column 1, last full sentence); pooling the samples to obtain a pooled or composite sample; and testing the composite sample for the presence of microbial contaminants, with coliforms and *Salmonella* exemplified. Midura provides recommendations for the number of samples or portions necessary to detect a positive pathogenic organism at varying confidence levels (see section 2.516 beginning on page 19 and Table 1). Midura exemplifies taking 4, 5, 6, 8, 10, 30, 34, 38, 43, 50, and 60 samples, which read on the claimed ranges of 3 to 10 and 30 to 60 in patented ***claims 11 and 14*** respectively and which meets the limitations of ***claims 15 and 16***.

It would have been *prima facie* obvious for one of ordinary skill in the art at the time of the invention to have sampled, tested, and validated any number of separate test food lots as exemplified in Price. Regarding the number of portions to be collected as in a) for each test lot, and to be combined in b) so as to assemble a corresponding set of separate test lot samples, each corresponding to the separate test lots for incubation testing, Price uses an experimental paradigm with known positive (inoculated at different levels) and known negative (uninoculated) test lot samples for each food type. One of skill in the art would be motivated to collect a number of portions from each lot consistent with a statistical-based sampling plan specifying for the type of food and particular agent or organism, the number of portions to be taken sufficient to provide for validation and release of the particular lots into commerce (Midura). For example, Midura specifically teaches statistical-based single, multiple, three-class and variable sampling plans (pages 16-20, sections 2.512-2.516) with procedures for low contamination level microorganisms such as *Salmonella*, *E. coli* and *Listeria*. It would have also been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have utilized the composite testing method disclosed by Price as the testing means for testing contamination in meat production lots according to the FSIS Directive because Price teaches that the method is as

sensitive as individual sample analysis and provides an important savings in the analytical effort required for quality control (see page 679, abstract and first paragraph).

Response to Patent owner's arguments:

Patent owner's arguments have been fully considered but they are not persuasive for the following reasons.

Patent owner argues (pages 60-61, and 64-67) that Price is limited to testing multiple samples from a single lot of food and thus does not apply to the claims. Patent owner suggests the Examiner may be relying on "common knowledge" or "taking official notice" with regard to the prior art samples being tested. However, the separate inoculums of Price represent different sample lots for testing. Note that the lots include different food samples which are separately inoculated with different quantities of test organisms, or are not inoculated to represent negative lots for testing purposes (uninoculated). Also note that the reference reports the test results separately; Nonfat dry milk vs. Cocoa vs. Dried egg albumin; based upon inoculation levels (Series I and II inoculated at different gram quantities) tested individually, as pooled samples and as pooled pre-enrichment broth (composite samples) with one underlying sample being positively inoculated and the rest of the underlying samples being uninoculated (Tables 1-2). A higher number of positives were recovered by pooling pre-enrichments rather than by pooling samples (p. 681, last paragraph column 1-column 2 and Table 2). In addition, pre-enrichment pooling yielded positives at lower levels of inoculation (page 681, last paragraph column 1-column 2 and Table 2). The composite pooling of pre-enrichment broths also retains individual sample (lot) identity and permits portions of the individual (underlying) cultures to be retained for subsequent analysis if a positive test is detected (p. 681, column 2).

Patent owner argues (pages 61-64) that FSIS's teachings are directed to guidelines for the formulation of a lot. Such is not in dispute, however it is also noted that FSIS exemplifies a statistical-based sampling plan with collection of multiple pieces or portions from a single day's production to form individual lots. Further, FSIS exemplifies an art recognized purpose for repeat testing. As patent owner acknowledges, the primary purpose for sampling in FSIS is to generate one or more negative results which verify corrective action (page 63). Patent owner's

arguments suggest FSIS is relied upon for composite testing. As set forth above however, Price is relied upon for composite testing (also referred to as pooling pre-enrichment broths).

As above, patent owner argues (pages 67-72) that there is a distinction between the level of detection attained in Price and the instant claims, and that the claims allow validation without requiring further enrichment. However, further enrichment is not excluded and no specific level, but mere detection is required. As taught in Price, the positive inoculated samples were detected in pooled cultures as effectively as when analyzed individually (p. 680 columns 1-2 and Table 1) and at extremely low levels of inoculation. Moreover, Price teaches that, "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. Thus, the artisan would modify the culture procedures to direct enrichment broths if so required for detection as recognized in the art (p. 682, column 1, second paragraph). Further regarding claims 3 and 6, patent owner argues the intent of the claim is different, if the lab is not prepared to conduct the test, the claim allows for further incubation. However, like claim 3, Price teaches further incubation and specifically exemplifies further incubation in enrichment broth. In regard to claim 6, Price teaches pooled pre-enrichments were more sensitive, and that pooling broth cultures permits small portions of each individual underlying culture to be retained for subsequent analysis (p. 681, column 2). Price also teaches biochemical and serological analysis, for example, "Two or more colonies are picked from each positive selective plate for biochemical and serological testing by the BAM Sallmonella method (2) to increase the chanced of detecting Salmonella." Thus, no distinction between Price and the claims is provided. Further, the claims fail to state the purpose, and in general a statement of purpose would not otherwise distinguish where, as in Price, there is motivation to the same method step.

Regarding claim 4, patent owner argues the pre-enrichment broths of Price do not apply as "enrichment media" as claimed. In the discussion, patentee states that, "the reason that inoculation of pre-enrichment into enrichment media allows for Salmonella to compete better is that the low pH of the spent broth retards growth of all other organisms and it damages some of the background flora, but does not impact Salmonella as much, therefore when a portion containing a few viable Salmonella cells are inoculated into the enrichment media, the cells will compete favorably." However, this argument suggests the pre-enrichment broths of Price do

provide for enrichment. In addition, Price provides motivation to substitute selective enrichment broth for pre-enrichment broth in the individual broth cultures at page 682, first full paragraph, wherein Price teaches,

> 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's testing also allows for detection of positive samples as exemplified in Tables 1-2. Thus, for these reasons Price clearly meets the limitations of claim 4.

Regarding claims 22 and 31, patentee again asserts Price is limited to pooling a single lot. However, Price teaches pooling samples from positive and negative test lots and pooling pre-enrichments from positive and negative test lots, which samples are derived from differently prepared lots, see particularly Materials and Methods pp. 679, Figure 1 and Tables 1-3.

In regard to Midura, patent owner argues (pp. 72-73) that the reference contributes nothing to the claims, and is limited to repeat testing of single lots. However, Midura teaches statistical-based sampling plans for microbiological food testing, particularly with respect to assembling individual lots for validation testing. Consistent with the basis for rejection, Midura confirms that statistical-based sampling plans for individual lots may be represented by single, or multiple samples (2.5-2.517). Price, and not Midura, is relied upon for composite testing (pooling pre-enrichments).

Patent owner also traverses the rejection (pages 73-83) by arguing each of the cited references separately, with reference to exhibits A-D. With respect to FSIS, the position is that the sampling is to a single day production lot. With respect to Price, the position is that the testing is of individual samples, which are not comprised of a statistical -based sampling plan and not enriched to detection. Patent owner concludes Price teaches away from compositing in general. With respect to Midura, the reference is asserted to teach statistical-based sampling plans, but to be limited to a single lot or to multiple samples of a single lot as contrasted to compositing. Patent owner also asserts the invention is more than a predictable use of prior art elements according to their established functions.

In response, exhibits A-D are noted, but the exhibits do not appear to reflect a fair assessment of the cited prior art, or the breadth of the instant claims, and thus the Examiner

merely refers to the cited references for their full teachings as discussed. FSIS is on point to meat sampling plans, Midura to statistical-based sampling plans and Price to Salmonella testing, including for validation testing for the presence or absence of microorganisms in various food lots, and for comparison of individual, pooled samples and pooled pre-enrichment broths which meets the limitation for the claimed composite testing procedures. The references of record are cited in combination and provide all of the requisite elements. The elements are provided according to their art established functions, which are the same as in the instant claims; for sampling, testing and validating any number of multiple test lots. Thus, the cumulative art renders the claimed invention obvious.

For these reasons, Patent owner's arguments are not persuasive. The rejection of claims 1-4, 6-18, 20, 22, 24-26, 31, and 34-36 is maintained.

Response to Third Party Requester's arguments:

Requester's position is essentially consistent with the Examiner's position as set forth above. In particular, requester asserts that the claim amendments after-ACP do not distinguish over the prior art rejections set forth in the ACP. Thus, requester's arguments are not further addressed, and stand on their own.

Ground #3:

Claims 1 and 17 are unpatentable as obvious over Jarvis in view of Patent owner's admissions regarding the state of the prior art (see SNQ # 3 at pages 36-38 of the Request).

This rejection was proposed by the third party requester and **adopted** for the reasons stated in the 5/26/10 office action and 12/17/10 ACP. The proposed rejection is set forth at pages 3-23, 36-38, 106-111 and 119-120 of the request. The rejection is reiterated to the claims as amended after-ACP

Claims 1 and 17 are rejected under 35 U.S.C. 103(a) as being unpatentable over Jarvis in view of Patent owner's admissions regarding the state of the prior art.

As was set forth *supra* (Ground 1), with respect to ***claims 1 and 17***, Jarvis discloses methods of sampling and testing foods for low numbers of pathogenic organisms using

composite samples for testing (pages 135-142, section 8.4.-8.4.2.1). Jarvis teaches that testing composite samples reduces the workload on the laboratory (page 141, section 8.4.2.1). The methods described by Jarvis (methods 2 and 3) comprise collecting a plurality (10) of product samples or portions (25g size); either inoculating the samples separately into one each of a pre-enrichment medium or broth to give test lot samples, each comprising a single specimen (method 2), or alternatively, pooling a plurality (about 5) of specimens and inoculating the pooled specimens into a pre-enrichment culture medium or broth (method 3); incubating the pre-enrichment broth cultures to allow for growth of the target organisms; compositing aliquots of the pre-enrichment cultures and inoculating the composite into a second enrichment culture, incubating the composite enrichment culture, and subsequently testing for the pathogenic organism. Jarvis teaches that methods 1 and 2 both provide the same degree of statistical confidence as would be attained by separately testing each of the 10 samples (see page 141 in its entirety). Jarvis teaches that composite testing method 2 has the advantage of allowing for verification of positive samples by retesting of the stored individual pre-enrichment cultures to provide an indication of the proportion of contaminated samples (see the paragraph bridging pages 141 and 142).

Jarvis defines the medium used for incubation of the individual test lot samples as a "pre-enrichment" medium, while the instant claims do not specify the incubation medium. The instant claims recite incubating the test lot samples under conditions suitable to allow levels of a target microbial agent to reach detectable levels and become uniform or substantially uniform throughout the test lot samples in order to provide a set of separate test lot samples enriched for the target microbial agent or organism (***claims 1 and 17***). As was set forth *supra*, the '584 patent specification defines "enrichment" as incubating a test lot sample under conditions suitable to allow levels of a target agent or organism to reach detectable levels (see column 6, lines 7-16). Since the pre-enrichment medium disclosed by Jarvis is designed to recover target organisms which have been compromised by less than optimal growth conditions and render them "actively growing" (see also Price, of record, page 681, second full paragraph), the method disclosed by Jarvis provides a set of separate test lot samples wherein the levels of target organisms are increased to detectable levels, thus meeting the limitations of the instant claims (p. 140-141, growth chart).

While Jarvis teaches collecting multiple samples (10) and either incubating them individually in pre-enrichment broth cultures or pooling a portion (about 5) of the samples for incubation in the pre-enrichment broth cultures, Jarvis does not specify collecting plural portions from each of a plurality of test lots, each test lot comprising one or more operationally-linked specimens, and combining the portions to provide a corresponding set of separate test lot samples for testing. However, Patent owner admits in the Background section of the '584 patent specification that in the testing of foods, pharmaceuticals, neutraceuticals, water and the environment, collecting plural portions from each of a plurality of test lots, each test lot comprising one or more specimens, and combining the portions for subsequent testing was well known in the art (see column 1, line 37, through column 2, line 26).

One of ordinary skill in the art at the time of the invention would have found it *prima facie* obvious to have applied the testing method disclosed by Jarvis to a collection of a plurality of portions of a food, pharmaceutical, neutraceutical, water, or environmental samples designated as a test lot, as was known in the prior art. The person of ordinary skill in the art at the time of the invention would have been motivated to test by the Jarvis method because Jarvis teaches that enrichment of samples in separate pre-enrichment broth cultures allows for subsequent identification of individual positive samples if the composite sample tests positive for a target agent or organism.

Response to Patent owner's arguments:

Patent owner argues (pages 84-90) essentially as set forth *supra*. In particular, the position is that Jarvis is limited to investigation of a single lot, and that the lumped (composited) pre-enrichment cultures do not correspond to separate underlying lots, with reference to p. 137, 8.4.1., p. 141, 8.4.2.1 and the Samadpour declaration at paragraphs 13-16. However, as above, Jarvis teaches at p. 136, Example 8.4, testing for three different test lots, and thus contrary to patent owner's position, the reference is not limited to testing or compositing single lots. Patentee asserts Jarvis does not validate lots for release to commerce and merely retests after enrichment. Again, Example 8.4, accepts, retests or rejects lots. Patentee also asserts a distinction in the claimed media. As discussed *supra* however, claims 1 and 17 do not recite any particular media but instead recite incubation under conditions suitable to allow detection and to

become uniform, or substantially uniform. Further, the growth exemplified at p. 141 is an amount noted to be within detectable range (Samadpour declaration particularly ¶29. It is also argued that the number of portions in step a) are not of a statistical based sampling plan. However, the admitted prior art teaches art recognition of statistical based sampling plans for any particular lot. Further with respect to steps c) and the new limitations in step e) patentee argues step c) is sufficient to allow for validation, without requiring further enrichment as recited for example in claim 3. In response, step e) does not require further enrichment, this is different from excluding a further enrichment step, and as noted above, growth is within a detectable range as indicated at p. 141 and the positive test results. As pointed out by patentee with respect to claim 3, the scope of claim 1 clearly includes the possibility for any number of further incubation steps. Patentee argues that the prior art does not teach compositing separate underlying test lots and that this admission is not made in the passage at column 1, line 37, through column 2, line 26. However, a fair reading of Jarvis including Example 8.4 makes clear that Jarvis is not limited to testing of single lot units. As also acknowledged in the '584 patent, testing multiple lots was art standard in the art. Patent owner presents separate arguments as to claims 3, 8, 11, 13, 18, 19, 22, 27, and 31. However these claims are not rejected in Ground 3 above, and thus such separate remarks are not further addressed here. A plurality of test lots is specifically exemplified in Jarvis Example 8.4; lots A, B and C being exemplified. Regarding the asserted distinction of the media, Jarvis teaches incubation of individual cultures of pre-enrichment broth, rather than individual cultures of enrichment broth prior to pooling and testing. As was previously set forth, patent owner is arguing limitations which are not found in the claims. The base claims are not limited to any specific media, or to "enrichment medium" and do not require that the medium favor the growth of, or be selective for the target agent. Rather, the claims recite incubating the test lot samples under conditions suitable for the target agent to increase and/or to reach detectable levels, to become uniform or substantially uniform. The pre-enrichment broth cultures of Jarvis provide growth to levels which are suitable for detection (p. 141) and thus fully meet the limitations of the claims. Patent owner makes reference to Exhibit B, a Jarvis 2007 publication, which is a post-filing date reference. The statements are noted, but do not evidence that the prior art is non-enabling for its teachings on point to composite testing.

Ground # 4:

Claims 1 and 17 are unpatentable under 35 USC § 103(a) as obvious over Patent owner's admissions regarding the state of the art in view of Price.

This rejection was proposed by the third party requester (see pages 38-39 of the request) and was **not adopted** for the reasons set forth in the 5/26/10 office and 12/17/10 ACP. The decision to not adopt is maintained.

The consideration under 35 U.S.C. 303 of a request for reexamination is limited to prior art patents and printed publications. See *Ex parte McGaughey*, 6 USPQ2d 1334, 1337 (Bd. Pat. App. & Inter. 1988). Thus an admission, *per se*, may not be the primary basis for establishing a substantial new question of patentability or for rejecting the claims. While admissions in conjunction with prior art patents or publications may be considered in a reexamination proceeding, any rejection must be based on prior art patents or publications, as explained by the admissions. Accordingly, admissions are utilized to interpret or modify the patent or printed publication which is the basis for the SNQ and which is used as the primary reference in a ground of rejection; they are not themselves used as the primary basis for rejecting the claims. Therefore, the proposed rejection of claims 1 and 17 as unpatentable over Patent owner's admissions regarding the state of the art in view of Price is **not adopted**.

Response to arguments:

Neither the Patent owner nor the Requester presented arguments regarding non-adoption of Ground 4. Therefore, the examiner's position remains unchanged.

Ground # 5:

Claims 1 and 17 are unpatentable over Andrews in view of Price and Midura (see SNQ # 5 at pages 39-43 of the Request).

This rejection was proposed by the third party requester and **adopted** for the reasons stated in the 5/26/10 office action and 12/17/10 ACP. The proposed rejection is set forth at pages 39-43, 106-111 and 119-120 of the request. The rejection is reiterated to the claims as amended after-ACP.

Claims 1 and 17 are rejected under 35 U.S.C. 103(a) as being unpatentable over Andrews in view of Price and Midura.

Regarding ***claims 1 and 17***, Andrews teaches methods of testing foods for the presence of *Salmonella* comprising collecting at random a plurality of portions (analytical units) from the food to be analyzed to comprise a larger sample unit, which is then used to determine the acceptability of the entire lot of food (see page 358, column 2, full paragraph). Andrews teaches that because *Salmonella* organisms in foods are often present in low numbers and in a debilitated condition due to processing the food, samples should be inoculated into pre-enrichment broth cultures (with lactose broth exemplified), incubated to allow the organisms to reach a desired number and condition, transferred into enrichment medium, which is selective for the target organism, and again incubated. Andrews does not teach incubating the sample units or test lots separately, as in the patented claims.

As was set forth previously in Ground 2, Price teaches a method for testing multiple food samples for *Salmonella* comprising collecting a plurality of samples (or portions) from the food, inoculating the samples individually into pre-enrichment broth cultures (with lactose broth exemplified, as was exemplified in Andrews; see Price at page 679, column 2, first full paragraph), incubating the cultures to allow for growth of the *Salmonella*, pooling the pre-enrichment broth cultures into wet composite test lots, incubating the pooled test lots into a single enrichment broth (the "modular composite sample" of the patented claims), incubating the composite enrichment broth, and subsequently identifying any *Salmonella* organisms present in the culture. Price teaches that the procedure offers a simple, effective way to increase sample capacity in the *Salmonella* testing of foods and also permits the pre-enrichment broth cultures to be retained for subsequent individual analysis if a positive result is obtained from the pooled enrichment culture (see page 679, abstract; page 680, Fig. 1; page 681, column 1, second full paragraph and column 2, first full paragraph). Price teaches that the pre-enrichment lactose broth enables the actively growing salmonellae to compete more favorably with organisms introduced from the other samples when pooled at the enrichment stage (see page 681, column 1, second full paragraph).

Midura discloses methods for statistical-based sampling plans and testing foods for microbial contamination. Midura teaches that when products with a low incidence of the

pathogen are tested, additional samples need to be collected and tested to ensure a high probability of detecting the pathogen (p. 19-20, 2.516). The different sampling plans allow conclusions regarding whether to accept, reject or retest specific lots of food. Midura teaches collecting a plurality of samples from a food lot produced in one batch or in some specified period of time and testing the samples for the presence of microbial contaminants, with coliforms and *Salmonella* exemplified.

It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have incubated the pre-enrichment broth cultures taught by Andrews separately, as suggested by Price, in order to be able to specifically identify the contaminated food lots. The person of ordinary skill in the art at the time of the invention would also have found it *prima facie* obvious to have collected a plurality of samples, in a statistically-based sampling plan and/or to have performed multiple sampling runs, as recommended by Midura, in order to be able to detect highly pathogenic organisms present in foods at low levels.

Response to Patent owner's arguments:

Patent owner argues (pp. 91-94) essentially as set forth supra in regard to Ground 2, that Price, Midura and Andrews do not motivate to compositing separate test lot samples, and that the media is not sufficient for growth as claimed. Andrews is on point to multiple pre-enrichment and enrichment media, as well as various tests for detection (pp. 360-362, 37.123-37.126 and p. 359, Table 2). Such variables for Salmonella testing are suitable for growth to detectable levels and such that the agent or organism is uniform, substantially uniform and enriched as in art accepted standards. Thus, Andrews exemplifies suitable conditions as recited in steps c)-e). Regarding the testing of multiple lot samples via compositing, as above it is Price that is relied upon for these teachings. Midura clarifies statistical-based sampling plans as the art standard basis for separate lot testing.

Ground #6:

Claims 1 and 17 are unpatentable over Silliker in view of Price and Midura (see SNQ # 6 at pages 43-46 of the Request).

This rejection was proposed by the third party requester and **adopted** for the reasons stated in the 5/26/10 office action and 12/17/10 ACP. The proposed rejection is set forth at pages 43-46, 106-111 and 119-120 of the request. The rejection is reiterated to the claims as amended after-ACP.

Claims 1, and 17 are rejected under 35 U.S.C. 103(a) as being unpatentable over Silliker in view of Price and Midura.

Silliker discloses a method for sampling dried foods for *Salmonella* contamination comprising collecting a plurality of 25g or 100 g subsamples, incubating these subsamples in individual cultures of pre-enrichment broth, and wet compositing the pre-enrichment cultures in groups of five (for 25 g subsamples) or three (for 100 g subsamples) (see page 475, abstract and last full paragraph of the page, and page 176, column 1, second full paragraph, and column 2, last full paragraph). Silliker teaches that pooling sixty 25g or fifteen 100g pre-enrichment cultures into groups of 5 (wet compositing) followed by selective enrichment of the composites is as sensitive a detection method as analysis of individual samples (page 478, last full paragraph of the page) and is economically more feasible (page 179, first full paragraph).

The method described by Silliker differs from the claimed method in that Silliker defines the incubation step of individual cultures as "pre-enrichment", while the patented claims recite separately incubating the test lot samples in medium favorable for "enrichment" of the target microbial agent or organism or simply recite incubating the test lot samples to increase the levels of the target agent (see step "c" of patented ***claims 1 and 17***) However, as has previously been set forth herein, the '584 patent specification defines "enrichment" as "incubating a test lot sample under conditions suitable to allow levels of a target agent or organism to reach detectable levels and become uniform or substantially uniform..." (see column 6, lines 7-16). Absent evidence to the contrary, incubation of the pre-enrichment cultures disclosed by Silliker meets the limitation of step c) in patented ***claims 1 and 17***. Silliker does not teach testing individual subsamples separately upon obtaining a positive result from the composite sample.

As was set forth previously in Ground 2, Price teaches a method for testing multiple food samples for *Salmonella* comprising collecting a plurality of samples (or portions) from the food, inoculating the samples individually into pre-enrichment broth cultures (with lactose broth

exemplified, as was exemplified in Andrews; see Price at page 679, column 2, first full paragraph), incubating the cultures to allow for growth of the *Salmonella*, pooling the pre-enrichment broth cultures into wet composite test lots, incubating the pooled test lots into a single enrichment broth (the "modular composite sample" of the patented claims), incubating the composite enrichment broth, and subsequently identifying any *Salmonella* organisms present in the culture. Price teaches that the procedure offers a simple, effective way to increase sample capacity in the *Salmonella* testing of foods and also permits the pre-enrichment broth cultures to be retained for subsequent individual analysis if a positive result is obtained from the pooled enrichment culture (see page 679, abstract; page 680, Fig. 1; page 681, column 1, second full paragraph and column 2, first full paragraph).

Midura discloses methods for statistical-based sampling plans and testing foods for microbial contamination. Midura teaches that when products with a low incidence of the pathogen are tested, additional samples need to be collected and tested to ensure a high probability of detecting the pathogen (see page 19, the paragraph bridging columns 1 and 2). Midura teaches collecting a plurality of samples from a food lot produced in one batch or in some specified period of time and testing the samples for the presence of microbial contaminants, with coliforms and *Salmonella* exemplified.

It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have followed the composite testing disclosed by Silliker with individual tests of the subsample pre-enrichments cultures upon obtaining a positive result for the composite subsample, as suggested by Price. The person of ordinary skill in the art at the time of the invention would have been motivated to do so to track the contamination to a particular subsample and thus specifically identify the contaminated food. The person of ordinary skill in the art at the time of the invention would also have found it *prima facie* obvious to have collected a plurality of samples, in a statistical-based sampling plan and/or to have performed multiple sampling runs, as recommended by Midura, in order to be able to detect highly pathogenic organisms present in foods at low levels.

Response to Patent owner's arguments:

Patent owner argues (pp. 94-100) essentially as set forth *supra* in regard to Grounds 1-2, that Siliker, Price, and Midura do not motivate to compositing separate test lot samples, and that the media is not sufficient for growth as claimed. Patent owner also asserts non-obviousness based upon false negative samples within Silliker and asserts the reference teaches away as depicted in Exhibits B and D. However, Siliker clearly teaches wet compositing of different test lots (Table 1; Tests 9-10, 11, 16, 21, 19-22 directed to Egg albumen solids for lot 1 and 2, Mixture albumen solids lots 1 and 2 diluted 1:4, Meat meal lots 2 and 3 and Fish meal lots 1-3), and specifically refers to preservation of the underlying lots with use of the term subsamples. "If the samples from individual lots can be lumped as described here, the monetary and time savings are obvious. Similarly, substantial savings would result by compositing in the application of strict sampling plans such as those recommended by the NRC, Committee on Salmonella (2) and the International Commission on Microbiological Specifications for Foods (report in preparation). For example, the NRC Committee recommended sixty 25-g samples for category I foods (powdered infant foods, therapeutic diet supplements, geriatric food and drugs), which are to be used by populations having high susceptibility to a Salmonella infection. The present study indicates that these products could be as effectively controlled by analyzing three 500-g samples per lot. Category II includes high-risk foods which contain a sensitive ingredient (s), have no final pasteurization, and are capable of supporting Salmonella growth. The recommended sampling plan requires analysis of twenty-nine 25-g samples for a total of 725 g. The results of this study show that analysis of two 500-g subsamples would permit a higher measure of control over such products than analysis of twenty-nine 25-g samples, since this entails the analysis of an additional 375 g. Siliker references a previous study (page 476, first full paragraph) as showing some loss of sensitivity with pooling of wet composites (pooled broth cultures) of lumped specimens. However, Silliker also references a second study by Price, which showed that pre-enrichment broth cultures could be pooled without any loss in sensitivity. The purpose of the study conducted by Silliker in the reference of record was to compare five analytical systems: sixty 25g subsamples; (2) fifteen 100 gm subsamples; (3) three 500 gm subsamples; (4) wet compositing of 25 gm pre-enrichment cultures in groups of 5; and (5) wet compositing of 100 gm pre-enrichment cultures in groups of 5 (page 476, second full paragraph). Silliker concluded

that one or more of the analytical procedures failed to detect salmonellae at low levels (page 471, Table 1 and paragraph bridging columns 1 and 2). Silliker also concluded, however,

> Analytical results from 26 samples of naturally contaminated dried foods indicate that *Salmonella*-positive samples may be detected with equal facility by analyzing sixty 25-g subsamples, fifteen 100-g subsamples, or three 500-g subsamples. Pooling sixty 25-g or fifteen 100-g pre-enrichment cultures into groups of five (wet–compositing) followed by selective enrichment of the composites apparently results in the same assurance of detection of positive lots as the analysis of individual samples (page 478, column 2, full paragraph).

Thus, Silliker points directly toward the claimed invention by teaching that a plurality of portions may be combined into individual pre-enrichment broth cultures, as in a test lot, and subsequently wet-composited for detection of the target agent without apparent loss of sensitivity. The wet composite lots of Silliker thus correspond to the claimed composite testing in steps c)-e). The collection of plural portions in accordance with statistical-based sampling plans as in Midura is standard for any test lot. Thus, the cumulative reference teachings render the claimed invention obvious.

Ground # 7:

Claims 1 and 17 are unpatentable over Gabis in view of Price and Midura (see SNQ # 7 at pages 46-51 of the Request).

This rejection was proposed by the third party requester and **adopted** for the reasons stated in the 5/26/10 office action and 12/17/10 ACP. The proposed rejection is set forth at pages 43-46, 106-111 and 119-120 of the request. The rejection is reiterated to the claims as amended after ACP.

Claims 1 and 17 are rejected under 35 U.S.C. 103(a) as being unpatentable over Gabis in view of Price and Midura.

Gabis discloses methods for sampling and testing high moisture foods, with meat and poultry exemplified, by collecting thirteen 25g specimens, inoculating each of the specimens individually into pre-enrichment broth cultures, incubating the pre-enrichment cultures to allow for levels of the organism to increase, pooling portions of each of the pre-enrichment cultures

into a composite or pooled set of samples in a single enrichment broth culture, incubating the enrichment broth culture, and subsequently recovering and identifying the salmonellae. The subsamples described by Gabis are the equivalent of the operationally-linked product specimens. Gabis teaches that analysis of a single composite sample is as sensitive as analysis of each of the individual subsamples (see page 663, abstract and first paragraph of the page; page 664, the paragraph bridging columns 1 and 2 and column 2, first two full paragraphs; and page 668, first full paragraph).

The method described by Gabis differs from the claimed method in that Gabis defines the incubation step in individual cultures as "pre-enrichment", while the patented claims recite separately incubating the test lot samples in medium favorable for "enrichment" of the target microbial agent or organism or simply incubating the test lot samples under conditions suitable to allow levels of the target agent to increase (see step "c" of ***claims 1 and 17***). However, as has previously been set forth herein, the '584 patent specification defines "enrichment" as "incubating a test lot sample under conditions suitable to allow levels of a target agent or organism to reach detectable levels..." (see column 6, lines 7-16). Absent evidence to the contrary, incubation of the pre-enrichment cultures disclosed by Gabis meets the limitation of step c) in the instant claims. Gabis does not teach testing individual subsamples separately upon obtaining a positive result from the composite sample.

As was set forth previously in Ground 2, Price teaches a method for testing multiple food samples for *Salmonella* comprising collecting a plurality of samples (or portions) from the food, inoculating the samples individually into pre-enrichment broth cultures (with lactose broth exemplified, as was exemplified in Andrews; see Price at page 679, column 2, first full paragraph), incubating the cultures to allow for growth of the *Salmonella*, pooling the pre-enrichment broth cultures into wet composite test lots, incubating the pooled test lots into a single enrichment broth (the "modular composite sample" of the patented claims), incubating the composite enrichment broth, and subsequently identifying any *Salmonella* organisms present in the culture. Price teaches that the procedure offers a simple, effective way to increase sample capacity in the *Salmonella* testing of foods and also permits the pre-enrichment broth cultures to be retained for subsequent individual analysis if a positive result is obtained from the pooled

enrichment culture (see page 679, abstract; page 680, Fig. 1; page 681, column 1, second full paragraph and column 2, first full paragraph).

Midura discloses methods for statistical-based sampling plans and testing foods for microbial contamination. Midura teaches that when products with a low incidence of the pathogen are tested, additional samples need to be collected and tested to ensure a high probability of detecting the pathogen (see page 19, the paragraph bridging columns 1 and 2). Midura teaches collecting a plurality of samples from a food lot produced in one batch or in some specified period of time, pooling the samples to obtain a pooled or composite sample, and testing the composite sample for the presence of microbial contaminants, with coliforms and *Salmonella* exemplified.

It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have followed the composite testing disclosed by Gabis with individual tests of the pre-enrichment cultures upon obtaining a positive result for the composite subsample, as suggested by Price. The person of ordinary skill in the art at the time of the invention would have been motivated to do so to track the contamination to a particular subsample so as to specifically identify the contaminated food. The person of ordinary skill in the art at the time of the invention would also have found it *prima facie* obvious to have collected a plurality of samples, in a statistical-based sampling plan as recommended by Midura for each lot, in order to be able to detect highly pathogenic organisms present in foods at low levels.

Response to Patent owner's arguments:

Patent owner argues (pages 100-103) that Gabis is limited to a single lot, is not based on a statistically-based sampling plan and is not suitably enriched. However, Gabis is not limited to single lots as it studies multiple meat and egg product samples, each of which is tested individually and in lumped or composited samples (Tables 1-2). Further, multiple portions are tested and thus Gabis is representative to statistically-based sampling plans even though Midura is more particularly on point to such teachings. Gabis also teaches direct inoculation into enrichment media, but notes that in lumped cultures, direct enrichment gave dramatically reduced recovery. Accordingly, the alternative of direct enrichment is provided, and as disclosed for individual sample testing, such methods detected positive samples as effectively as pre-

enriched samples. Patent owner is arguing against Gabis individually, whereas the rejection was based on the combination of Gabis with Price and Midura. Price provides the teaching of validation of individual specimens or lots by teaching that the method permits the pre-enrichment broth cultures to be retained for subsequent individual analysis if a positive result is obtained from the pooled enrichment cultures (see page 679, abstract; page 680, Fig. 1; page 681, column 1, second full paragraph and column 2, first full paragraph).

Ground # 8:

Claims 1 and 17 are unpatentable as obvious over Jarvis in view of BAM (see SNQ # 8 at pages 51-52 of the Request).

This rejection was proposed by the third party requester and **adopted** for the reasons stated in the 5/26/10 office action and 12/17/10 ACP. The proposed rejection is set forth at pages 51-52, 106-111 and 119-120 of the request. The rejection is reiterated to the claims as amended after-ACP.

Claims 1 and 17, are rejected under 35 U.S.C. 103(a) as being unpatentable over Jarvis in view of BAM.

As was set forth *supra* (Ground 1), Jarvis discloses methods of sampling and testing foods for low numbers of pathogenic organisms using composite samples for testing (page 135, section 8.4, first paragraph). Jarvis teaches that testing composite samples reduces the workload on the laboratory (page 141, section 8.4.2.1). The methods described by Jarvis (methods 2 and 3) comprise collecting a plurality (10) of product samples or portions (25g size); either inoculating the samples separately into one each of a pre-enrichment medium or broth to give test lot samples, each comprising a single specimen (method 2), or alternatively, pooling a plurality (about 5) of specimens and inoculating the pooled specimens into a pre-enrichment culture medium or broth (method 3); incubating the pre-enrichment broth cultures to allow for growth of the target organisms; compositing aliquots of the pre-enrichment cultures and inoculating the composite into a second enrichment culture, incubating the composite enrichment culture, and subsequently testing for the pathogenic organism. Jarvis teaches that methods 1 and 2 both provide the same degree of statistical confidence as would be attained by separately

testing each of the 10 samples (see page 141 in its entirety). Jarvis teaches that composite testing method 2 has the advantage of allowing for verification of positive samples by retesting of the stored individual pre-enrichment cultures to provide an indication of the proportion of contaminated samples (see the paragraph bridging pages 141 and 142).

Jarvis defines the medium used for incubation of the individual test lot samples as a "pre-enrichment" medium, while the instant claims do not specify the incubation medium. The instant claims recite incubating the test lot samples under conditions suitable to allow levels of a target microbial agent to reach detectable levels and become uniform or substantially uniform throughout the test lot samples in order to provide a set of separate test lot samples enriched for the target microbial agent or organism (***claims 1 and 17***). As was set forth *supra*, the '584 patent specification defines "enrichment" as incubating a test lot sample under conditions suitable to allow levels of a target agent or organism to reach detectable levels (see column 6, lines 7-16). Since the pre-enrichment medium disclosed by Jarvis is designed to recover target organisms which have been compromised by less than optimal growth conditions and render them "actively growing" (see Price, of record, page 681, second full paragraph), the method disclosed by Jarvis provides a set of separate test lot samples wherein the levels of target organisms are increased to detectable levels, thus meeting the limitations of the instant claims.

While Jarvis teaches collecting multiple samples (10) and either incubating them individually in pre-enrichment broth cultures or pooling a portion (about 5) of the samples for incubation in the pre-enrichment broth cultures, Jarvis does not specify collecting plural portions from each of a plurality of test lots, each test lot comprising one or more specimens, and combining the portions to provide a corresponding set of separate test lot samples for testing.

BAM teaches statistically-based sampling plans for Salmonella (pages 2-5). BAM teaches that multiple analytical samples must be collected from food for detection of *Salmonella,* with the number of samples collected depending on the category of the food. The multiple samples disclosed by BAM meet the limitation of operationally-linked product specimens. BAM teaches collecting a plurality of analytical units at random from a food lot (equivalent to portions of a test lot), combining the collected samples or portions into a composite test lot sample, inoculating the composite test lot sample into pre-enrichment medium, incubating the pre-enrichment mediium, and subsequently testing for the presence of *Salmonella.* BAM teaches

that when the composite unit tests negative, all of the sample lot are considered acceptable (or validated) and when the composite sample tests positive, the food lot is rejected (see pages 3 and 5 in their entirety).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have collected a plurality of food samples in a statistically-based sampling plan, as outlined by BAM, for testing by the method of Jarvis. The person of ordinary skill in the art at the time of the invention would have been motivated to test by the Jarvis method because Jarvis teaches that enrichment of samples in separate pre-enrichment broth cultures allows for subsequent identification of individual positive samples, particularly if the composite sample tests positive for a target agent or organism. Such testing thereby negates the necessity of rejecting an entire food lot, or multiple food lots.

Response to Patent owner's arguments:

Patent owner argues (pages 103-107) that BAM is limited to a single lot, is not of a statistically-based sampling plan and thus is insufficient for validation. While BAM teaches compositing in general, BAM is not relied upon alone but is instead cited with Jarvis for composite testing. BAM is on point to statistically-based sampling plans, while Jarvis teaches testing multiple lots (Example 8.4) and further teaches validation testing via composite samples as claimed. Thus, the cumulative art renders the claimed invention obvious.

Ground # 9:

Claims 1 and 17 are unpatentable as obvious over Price in view of BAM and Davies (see SNQ #9 at pages 52-53 of the Request).

This rejection was proposed by the third party requester and **adopted** for the reasons stated in the 5/26/10 office action and 12/17/10 ACP. The proposed rejection is set forth at pages 52-53, 106-111 and 119-120 of the request. The rejection is reiterated to the claims as amended after ACP

Claims 1 and 17 are rejected under 35 U.S.C. 103(a) as being unpatentable over Price in view of BAM and Davies.

Price teaches a method for testing multiple food samples for *Salmonella* comprising collecting a plurality of samples (or portions) from the food, inoculating the samples individually into pre-enrichment broth cultures, incubating the cultures to allow for growth of the *Salmonella*, pooling the pre-enrichment broth cultures into wet composite test lots, incubating the pooled test lots into a single enrichment broth (the "modular composite sample" of the patented claims), incubating the composite enrichment broth, and subsequently identifying any *Salmonella* organisms present in the culture. Price teaches that the procedure offers a simple, effective way to increase sample capacity in the *Salmonella* testing of foods and also permits the pre-enrichment broth cultures to be retained for subsequent individual analysis if a positive result is obtained from the pooled enrichment culture (see page 679, abstract; page 680, Fig. 1; page 681, column 1, second full paragraph and column 2, first full paragraph).

Price defines the medium used for incubation of the individual test lot samples as a "pre-enrichment" medium, while the instant claims do not specify the incubation medium. As was set forth above, the instant claims recite incubating the test lot samples under conditions suitable to allow levels of a target microbial agent to reach detectable levels and become uniform or substantially uniform throughout the test lot samples in order to provide a set of separate test lot samples enriched for the target microbial agent or organism (***claims 1 and 17***). The '584 patent specification defines "enrichment" as incubating a test lot sample under conditions suitable to allow levels of a target agent or organism to reach detectable levels (see column 6, lines 7-16). Since the pre-enrichment medium disclosed by Price is designed to recover target organisms which have been compromised by less than optimal growth conditions and render them "actively growing" (see page 681, second full paragraph), the method disclosed by Price provides a set of separate test lot samples wherein the levels of target organisms are increased to detectable levels, thus meeting the limitations of the instant claims. While Price teaches collecting multiple samples and incubating them individually in pre-enrichment broth cultures which are then pooled, Price does not specify collecting plural portions from each of a plurality of test lots, each test lot comprising one or more specimens, and combining the portions to provide a corresponding set of separate test lot samples for testing.

BAM teaches that multiple analytical samples must be collected from food for detection of *Salmonella,* with the number of samples collected depending on the category of the food. The

multiple samples disclosed by BAM are equivalent to operationally-linked specimens. BAM teaches collecting a plurality of analytical units at random from a food lot (equivalent to portions of a test lot), combining the collected samples or portions into a composite test lot sample, inoculating the composite test lot sample into pre-enrichment medium, incubating the pre-enrichment medium, and subsequently testing for the presence of *Salmonella*. BAM teaches that when the composite unit tests negative, all of the sample lot are considered acceptable (or validated) and when the composite sample tests positive, the food lot is rejected (see pages 3 and 5).

Davies discloses methods of sampling and testing pooled samples taken from different pig herd (lots) for *Salmonella* contamination testing (See the abstract and page 1017 in its entirety). After sample collection and pooling, composite pooled specimens were incubated in enrichment broth (buffered peptone water) and subsequently plated onto agar plates for detection of *Salmonella* contamination. Davies teaches pooled samples for bacteriological testing as pinch-sized fecal samples, carcass swabs, and muscle swabs, among others (for a plurality of assembled portions) (see page 1017, right column, first full paragraph). Davies teaches that testing pooled samples compares well with testing individual samples (page 1023, column 2, second full paragraph).

It would have been *prima facie* obvious for one of ordinary skill in the art at the time of the invention to have collected a plurality of portions of food, as taught by either of BAM or Davies, and to have combined the portions to provide a corresponding set of composite test lot samples for testing, as taught by Price. The person of ordinary skill in the art at the time of the invention would have also found it *prima facie* obvious to have inoculated each of the test lot samples into separate enrichment broth cultures, to have incubated them to allow the target agent or organism to reach detectable levels, and then to have pooled aliquots for testing because all of Price, BAM and Davies teach that composite testing is as sensitive as individual cultures, and Price teaches that separately enriching the samples allows for individual testing upon obtaining a positive test result from the composite sample.

Response to Patent owner's arguments:

Patent owner argues (page 107-115) that Price does not teach plural portions from different lots, and that Price and BAM do not teach a composite sample, with under-lying test lots and validation of the negative test lots as claimed. However, Price as discussed *supra* teaches multiple lots. Further with respect to Davies, the testing is of multiple pig herd lots. The experimentation collects plural portions from each pig according to the disclosed statistical-based sampling plans (pp. 1017-18) but retains the separate herd lot designations throughout (Tables 1-2). While ELISAs and Sera are analyzed individually the separate herd lots are also composited and tested (Tables 1-2). As disclosed, sample incubations were sufficient for detection with positive samples testing for most farms. The samples disclosed by Price and the analytical units disclose by BAM also fully meet the limitations of the test lot or production lot of the instant claims.

Ground # 10:

Claims 2, 14, 16, 19, and 20 are unpatentable as obvious over Jarvis in combination with FSIS, Midura, and Patent owner's admissions regarding the state of the art.

This rejection was proposed by the third party requester (see SNQ #s 11, 37, 44, 50, and 52 at pages 55, 76-80, and 84-85 of the Request) but is **not adopted** for the reasons set forth in the 5/26/20 office action and 12/17/10 ACP. The decision to not adopt is maintained.

The factual inquiries set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

Requester's proposed rejection of claim 2, 14, and 19 as unpatentable over Jarvis in view of FSIS and Midura has been adopted herein. See Ground 1. Claim 20 was added to the Ground

1 rejection for the reasons set forth therein. As was discussed in Ground 1, Jarvis in view of FSIS and Midura meet all the limitations of claims 2, 14, 19, and 20. Therefore, the proposed rejection of the same claims (2, 14, 19, and 20) under 103(a) over the same combination of references taken with Patent owner's admissions regarding the state of the art is improper. In a rejection based on 35 U.S.C. 103, the teachings of the references must somehow be modified by the additional reference in order to meet the limitations of the claims. Because Jarvis in view of FSIS and Midura meet all of the claim limitations and renders claims 1, 14, 19, and 20 obvious, there are no differences between the patented claims and the combination of Jarvis, FSIS, and Midura which would require further modification by Patent owner's admissions regarding the state of the art in order to meet the limitations of the claims (see #2 of the factual inquiries set forth in *Graham v. John Deere Co.* and listed above). Requester has not pointed out any such differences in the request between the claimed invention and the combination of Jarvis, FSIS and Midura, which would require modification by Patent owner's admissions regarding the state of the art in the proposed obviousness rejection.

Response to arguments:

Neither the Patent owner nor the Requester presented arguments regarding non-adoption of Ground 10. Therefore, the examiner's position remains unchanged.

Ground # 11:

Claims 2, 14, and 15 are unpatentable as obvious over Jarvis in combination with FSIS, Midura, and BAM.

This rejection was proposed by the third party requester (see SNQ #s 12, 35, and 41 at pages 56-57 and 78-79 of the Request) but is **not adopted** for the reasons set forth above in Ground 10 (as set forth in the 5/26/10 office action and 12/17/10 ACP). The decision to not adopt is maintained.

Requester's proposed rejection of claims 2 and 14 as unpatentable over Jarvis, in view of FSIS and Midura, has been adopted herein (see Ground 1). Additionally, claim 15 was rejected in Ground 1 as obvious over Jarvis in view of FSIS and Midura. Therefore, the proposed

rejection of the same claims 2, 14, and 15 over Jarvis, in view of FSIS and Midura and further in view of BAM, is improper because Requester has failed to point to any deficiency in the combination of Jarvis, FSIS, and Midura which would require modification by BAM in order to meet the limitations of the claims.

Response to arguments:

Neither the Patent owner nor the Requester presented arguments regarding non-adoption of Ground 11. Therefore, the examiner's position remains unchanged.

Ground # 12:

Claims 2, 14, and 16 are unpatentable as obvious over FSIS in combination with Price, Midura, and Patent owner's admissions regarding the state of the art.

This rejection was proposed by the third party requester (see SNQ #s 14, 40, and 46 at pages 57, 77-78, and 81 of the Request) but is **not adopted** for the reasons set forth above in Ground 10 (as set forth in the 5/26/10 office action and 12/17/10 ACP). The decision to not adopt is maintained.

Requester's proposed rejection of claims 2 and 14 as unpatentable over FSIS, in view of Price and Midura, has been adopted herein (see Ground 2). Additionally, claim 15 was rejected in Ground 2 as obvious over FSIS in view of Price and Midura. Therefore, the proposed rejection of the same claims 2, 14, and 15 over Jarvis, in view of FSIS and Midura and further in view of BAM, is improper because Requester has failed to point to any deficiency in the combination of FSIS, Price, and Midura which would require modification by BAM in order to meet the limitations of the claims.

Response to arguments:

Neither the Patent owner nor the Requester presented arguments regarding non-adoption of Ground 12. Therefore, the examiner's position remains unchanged.

Ground # 13:

Claims 2, 14, 15, and 24 are unpatentable as obvious over FSIS in combination with Price, Midura, and BAM.

This rejection was proposed by the third party requester (see SNQ #s 15, 38, 42, and 62 at pages 57, 76-77, 79, and 91-92 of the Request) but is **not adopted** for the reasons set forth above in Ground 10 (as set forth in the 5/26/10 office action and 12/17/10 ACP). The decision to not adopt is maintained.

Requester's proposed rejection of claim 2 as unpatentable over FSIS, in view of Price and Midura, has been adopted herein (see Ground 2). Additionally, claims 14, 15 and 24 were rejected in Ground 2 as obvious over FSIS in view of Price and Midura. Therefore, the proposed rejection of the same claims 2, 14, 15, and 24 over FSIS, in view of Price and Midura and further in view of BAM, is improper because Requester has failed to point to any deficiency in the combination of FSIS, Price, and Midura which would require modification by BAM in order to meet the limitations of the claims.

Response to arguments:

Neither the Patent owner nor the Requester presented arguments regarding non-adoption of Ground 13. Therefore, the examiner's position remains unchanged.

Ground # 14:

Claims 5, 23, 29, and 30 are unpatentable as obvious over Jarvis in combination with FSIS, Midura, and Entis (see SNQ #s 20, 59, 71, and 73 at pages 60-61, 89-90, 98, and 99-100 respectively of the Request).

This rejection was proposed by the third party requester and **is adopted** for the reasons stated in the 05/26/10 office action and 12/17/10 ACP. The proposed rejection is set forth at pages 60-61, 89-90, 98, and 99-100, 113, 123 and 126 of the request. Claim 6 was added to the rejection because claim 30 depends from claim 6 (see 5/26/10 office action and 12/17/10 ACP). The rejection is reiterated to the claims as amended after ACP.

Claims 5, 6, 23, 29, and 30 are rejected under 35 U.S.C. 103(a) as being unpatentable over Jarvis in view of FSIS and Midura, as applied to claim 1 above, and further in view of Entis.

As was set forth supra in Ground 1, which is incorporated herein by reference in its entirety, Jarvis in view of FSIS and Midura teaches a method of sampling and testing food test lots for microbial contamination comprising collecting a plurality of portions from each of a plurality of test lots, according to a statistical-based sampling plan, combining the portions to provide a corresponding set of test lot samples, separately incubating the test lot samples to enrich for the target microbial agent or organism, removing portions of the test lot sample cultures, combining them into a modular composite sample and testing the composite sample for the presence of the target agent or organism. None of Jarvis, FSIS, or Midura teach concentrating the microbial agent from the modular composite sample by immunomagnetic bead separation or affinity chromatography and none teach testing the modular composite samples by a DNA based method, an immunochemistry based method or a biosensor based method.

Entis teaches rapid procedures for the detection, identification, and enumeration of microorganisms in foods (see page 89, third paragraph). Regarding ***claims 5 and 30***, Entis teaches that since food is a complex matrix which causes interference with analytical methods, some degree of separation or concentration of the microorganism of interest is required (see page 89, first paragraph). With respect to ***claim 29***, Entis teaches concentrating and separating the microorganism of interest from an enrichment culture by immunoaffinity chromatography and immunomagnetic separation (see page 91). Regarding ***claim 23***, the rapid methods disclosed by Entis comprise antibody-based methods, such as immunofluorescence, enzyme immunoassay (an immunochemistry-based method), nucleic acid based methods (DNA hybridization and PCR) and bioluminescence and limulus amebocyte lysate assay (biosensor based methods) (see pages 89, 90, 93, 94, and 99). Entis teaches that the rapid methods offer savings in both time and labor (page 89, second paragraph) and can be more sensitive than conventional culture methods (see page 89, last full sentence, page 93, second full paragraph, page 94, column, and page 95, column 1).

One of ordinary skill in the art at the time of the invention would have found it *prima facie* obvious to have concentrated the target agent from the cultures disclosed by Jarvis in view

of FSIS and Midura, as taught by Entis, in order to increase testing sensitivity and negate the interference from the food sample, as is taught by Entis. Further, the person of ordinary skill in the art at the time of the invention would have found it *prima facie* obvious to have used one of more of the methods described by Entis to identify the target agent in an enrichment culture because Entis teaches that the rapid methods can be more sensitive than traditional methods and can offer significant savings in both time and labor.

Response to Patent owner arguments:

Patent owner argues (pages 116-118) essentially as set forth *supra* with respect to Ground 1; that the prior art is limited to analysis of single lots, that the prior art does not assemble a modular composite sample (of lumped samples/cultures each attributable to separate underlying test lots) and that the testing does not validate samples for release. Patentee argues the prior art reduces work and expense with sampling while their method increases work and expense for validation. Patentee argues their composite is fundamentally different because it is of a statistically sufficient number of portions. These arguments are not persuasive as previously set forth in Ground 1 over Jarvis, FSIS and Midura. As previously discussed the rejection at hand is one of obviousness. The cumulative prior art teaches each of the respective elements, and assembles these elements according to regularly practiced principles in food science testing. Further, the instant rejection includes the teachings of Entis which are on point to the rapid and highly sensitive methodologies of concentration, immunomagnetic bead separation and affinity chromatography.

Ground # 15:

Claims 5, 23, 29, and 30 are unpatentable as obvious over FSIS in combination with Price, Midura, and Entis (see SNQ #s 21, 60, 72, and 74 at pages 61, 90-91, 98-99, and 100 respectively of the Request).

This rejection was proposed by the third party requester and **is adopted** for the reasons stated in the 5/26/10 office action and 12/17/10 ACP. The proposed rejection is set forth at pages 61, 90-91, 98-99, 100, 113, 123 and 126 of the request. Claim 6 was added to the

rejection, because claim 30 depends from claim 6 (see 5/26/10 office action and 12/17/10 ACP). The rejection is reiterated to the claims as amended after ACP.

Claims 5, 6, 23, 29, and 30 are rejected under 35 U.S.C. 103(a) as being unpatentable over FSIS in view of Price and Midura, as applied to claim 1 above, and further in view of Entis.

As was set forth *supra* in Ground 2, which is incorporated herein by reference in its entirety, FSIS in view of Price and Midura teaches a method of sampling and testing food test lots for microbial contamination comprising collecting a plurality of portions from each of a plurality of test lots, according to a statistical-based sampling plan, combining the portions to provide a corresponding set of test lot samples, separately incubating the test lot samples to enrich for the target microbial agent or organism, removing portions of the test lot sample cultures, combining them into a modular composite sample and testing the composite sample for the presence of the target agent or organism. None of FSIS, Price, or Midura teaches concentrating the microbial agent from the modular composite sample by immunomagnetic bead separation or affinity chromatography and none teach testing the modular composite samples by a DNA based method, an immunochemistry based method or a biosensor based method.

Entis teaches rapid procedures for the detection, identification, and enumeration of microorganisms in foods (see page 89, third paragraph). Regarding ***claims 5 and 30***, Entis teaches that since food is a complex matrix which causes interference with analytical methods, some degree of separation or concentration of the microorganism of interest is required (see page 89, first paragraph). With respect to ***claim 29***, Entis teaches concentrating and separating the microorganism of interest from an enrichment culture by immunoaffinity chromatography and immunomagnetic separation (see page 91). Regarding ***claim 23***, the rapid methods disclosed by Entis comprise antibody-based methods, such as immunofluorescence, enzyme immunoassay (an immunochemistry-based method), nucleic acid based methods (DNA hybridization and PCR) and bioluminescence and limulus amebocyte lysate assay (biosensor based methods) (see pages 89, 90, 93, 94, and 99). Entis teaches that the rapid methods offer savings in both time and labor (page 89, second paragraph) and can be more sensitive than conventional culture methods (see

page 89, last full sentence, page 93, second full paragraph, page 94, column, and page 95, column 1).

One of ordinary skill in the art at the time of the invention would have found it *prima facie* obvious to have concentrated the target agent from the cultures disclosed by FSIS in view of Price and Midura, as taught by Entis, in order to increase testing sensitivity and negate the interference from the food sample, as is taught by Entis. Further, the person of ordinary skill in the art at the time of the invention would have found it *prima facie* obvious to have used one of more of the methods described by Entis to identify the target agent in an enrichment culture because Entis teaches that the rapid methods can be more sensitive than traditional methods and can offer significant savings in both time and labor.

Response to Patent owner's arguments:

Patent owner argues (pages 118-121) essentially as set forth *supra* with respect to Ground 2, that the prior art is limited to analysis of single lots, that the prior art does not assemble a modular composite sample (of lumped samples/cultures each attributable to separate underlying test lots) and that the testing does not validate samples for release. Patentee argues the prior art reduces work and expense with sampling while their method increases work and expense for validation. Patentee argues their composite is fundamentally different because it is of a statistically sufficient number of portions. These arguments are not persuasive as previously set forth in Ground 2 over Price, FSIS and Midura. As previously discussed the rejection at hand is one of obviousness. The cumulative prior art teaches each of the respective elements, and assembles these elements according to regularly practiced principles in food science testing. Further, the instant rejection includes the teachings of Entis which are on point to the rapid and highly sensitive methodologies of concentration, immunomagnetic bead separation and affinity chromatography.

Ground # 16:

Claim 12 is unpatentable as obvious over Jarvis in combination with FSIS, Midura, and Mauer.

This rejection was proposed by the third party requester (see SNQ # 31 at pages 71-73 of the Request) but was **not adopted** for the reasons set forth in Ground 10 above (see 5/26/10 office action and 12/17/10 ACP). The decision to not adopt is maintained.

Claim 12 was rejected in Ground 1 as obvious over Jarvis in view of FSIS and Midura for the reasons set forth therein. Therefore, the proposed rejection of the same claim 12 over Jarvis in view of FSIS and Midura and further in view of Mauer, is improper. At pages 72-73, Requester alleges that the deficiency in the combination of Jarvis, FSIS and Midura regarding patented claim 12 lies in the fact that

> "The specification does not define or provide examples of remedial measures, but refers to 'cooking, disposal, or other acceptably safe endpoint' as ways of handling lots that test positive. Col. 5, lines 10-11; col. 8, lines 31-33. The specification does not disclose what types of testing are contemplated by the recitation of "further testing" of the remediated separate test lots."

The lack of examples in the specification, however, does not represent a deficiency under the factual inquiries which are set forth under the *Graham v. John Deere Co* obviousness analysis that would necessitate the addition of Mauer. The factual inquiries require ascertaining the differences between the prior art and the **claims** at issue (emphasis added, see the discussion of the *Graham v. John Deere Co* analysis set forth in Ground 10 *supra)*. Claim 12 generally recites application of remedial measures to positive test lots. FSIS teaches such remedial measures. The specific teaching of an exemplary remedial measure that is set forth in Mauer is not required to meet the limitations of claim 12. Therefore, the proposed rejection of claim 12 as unpatentable over Jarvis in view of FSIS and Midura, and further in view of Mauer is **not adopted**.

Response to arguments:

Neither the patent owner nor the Requester has submitted arguments directed to the non-adoption of Ground 16 of rejection. Therefore, the examiner's position remains unchanged.

Ground # 17:

Claim 12 is unpatentable as obvious over Jarvis in combination with FSIS, Midura, Mauer and BAM.

This rejection was proposed by the third party requester (see SNQ # 32 at page 73 of the Request) but is **not adopted** for the reasons set forth in Ground 10 above (see 5/26/10 office action and 12/17/10 ACP). The decision to not adopt is maintained.

Claim 12 has been rejected herein as unpatentable over Jarvis in view of FSIS and Midura for the reasons set forth supra (Ground 1). Therefore, rejection of the same claim 12 as obvious over FSIS, Midura, Mauer, and BAM is improper. Requester has not pointed to any deficiency in the combination of Jarvis, FSIS, and Midura which would necessitate the addition of Mauer and BAM in order to meet the imitations of the claim.

Response to arguments:

Neither the patent owner nor the Requester has submitted arguments directed to the non-adoption of Ground 17 of rejection. Therefore, the examiner's position remains unchanged.

Ground # 18:

Claims 14, 16, and 25 are unpatentable as obvious over Jarvis in combination with FSIS, Midura, and Silliker.

This rejection was proposed by the third party requester (see SNQ #s 36, 43, and 64 at pages 75-76, 79-80, and 93-94 of the request) but is **not adopted** for the reasons set forth in Ground 10 above (see 5/26/10 office action and 12/17/10 ACP). The decision to not adopt is maintained.

Requester's proposed rejection of claim 14 as unpatentable over Jarvis, in view of FSIS and Midura, has been adopted herein (see Ground 1). Additionally, claims 16 and 25 were rejected in Ground 1 as obvious over Jarvis in view of FSIS and Midura for the reasons set forth therein. Therefore, the proposed rejection of the same claims 14, 16, and 25 over Jarvis, in view of FSIS and Midura and further in view of Silliker, is improper because Requester has failed to point to any deficiency in the combination of Jarvis, FSIS, and Midura which would require modification by Silliker in order to meet the limitations of the claims.

Response to arguments:

Neither the patent owner nor the Requester has submitted arguments directed to the non-adoption of Ground 18 of rejection. Therefore, the examiner's position remains unchanged.

Ground # 19:

Claims 14, 16, 25 and 26 are unpatentable as obvious over FSIS in combination with Price, Midura, and Silliker.

This rejection was proposed by the third party requester (see SNQ #s 39, 45, 65 and 67 at pages 77, 80-81, 94 and 95 of the Request) but is **not adopted** for the reasons set forth above in Ground 10 (see 5/26/10 office action and 12/17/10 ACP). The decision to not adopt is maintained.

Requester's proposed rejection of claim 14 as unpatentable over FSIS, in view of Price and Midura, has been adopted herein (see Ground 2). Additionally, claims 16, 25 and 26 were rejected in Ground 2 as obvious over FSIS in view of Price and Midura for the reasons set forth therein. Therefore, the proposed rejection of the same claims 14, 16, 25, and 26 over FSIS, in view of Price and Midura and further in view of Silliker, is improper because Requester has failed to point to any deficiency in the combination of FSIS, Price and Midura which would require modification by Silliker in order to meet the limitations of the claims.

Response to arguments:

Neither the patent owner nor the Requester has submitted arguments directed to the non-adoption of Ground 19 of rejection. Therefore, the examiner's position remains unchanged.

Ground # 20:

Claim is unpatentable as obvious over FSIS in combination with Price, Midura, Jarvis, and Silliker.

This rejection was proposed by the third party requester (see SNQ #53 at pages 85-86 of the Request) but is **not adopted** for the reasons set forth above in Ground 10 (see 5/26/10 office action and 12/17/10 ACP. The decision to not adopt is maintained.

Claim 20 was rejected in Ground 2 as obvious over FSIS in view of Price and Midura for the reasons set forth therein. Therefore, the proposed rejection of the same claim 20 over FSIS, in view of Price and Midura and further in view of Jarvis and BAM, is improper because Requester has failed to point to any deficiency in the combination of FSIS, Price and Midura which would require modification by Jarvis and Silliker in order to meet the limitations of the claims.

Response to arguments:

Neither the patent owner nor the Requester has submitted arguments directed to the non-adoption of Ground 20 of rejection. Therefore, the examiner's position remains unchanged.

Ground # 21:

Claim 21 is unpatentable as obvious over Jarvis in combination with FSIS, Midura, and Cousin (see SNQ #54 at page 86 of the Request).

This rejection was proposed by the third party requester and **is adopted** for the reasons stated in the 5/26/10 office action and 12/17/10 ACP. . The proposed rejection is set forth at page 86 and 123 of the request. The rejection is reiterated to the claims as amended after ACP.

Claim 21 is rejected under 35 U.S.C. 103(a) as being unpatentable over Jarvis in view of FSIS and Midura, as applied to claim 1 above, and further in view of Cousin.

As was set forth *supra* in Ground 1, which is incorporated herein by reference in its entirety, Jarvis in view of FSIS and Midura teaches a method of sampling and testing food test lots for microbial contamination comprising collecting a plurality of portions from each of a plurality of test lots, according to a statistical-based sampling plan, combining the portions to provide a corresponding set of test lot samples, separately incubating the test lot samples to enrich for the target microbial agent or organism, removing portions of the test lot sample cultures, combining them into a modular composite sample and testing the composite sample for the presence of the target agent or organism. None of Jarvis, FSIS, or Midura teaches that the target organism is a spoilage organism.

Cousin discloses that certain bacteria (psychrotrophs) are able to grow in foods at low temperatures and are thus the primary organisms responsible for spoilage of refrigerated foods. Cousin teaches that such organisms are found in meats, poultry, fish, and seafood and include *Listeria* spp., among others (see page 169, column 2, last paragraph). Cousin teaches that such organisms can be detected by culture and microscopic methods (see section 13.21 beginning on page 160).

It would have been *prima facie* obvious to one of skill in the art at the time of the invention to have used the method disclosed by Jarvis in view of FSIS and Midura to test for a spoilage organism, such as a psychrotroph, because Cousin teaches that such organisms are responsible for spoilage of refrigerated foods and can be detected by culture.

Response to Patent Owner's arguments:

Patent owner argues (pages 122-124) essentially as set forth *supra* with respect to Ground 1; that the prior art is limited to analysis of single lots, that the prior art does not assemble a modular composite sample (of lumped samples/cultures each attributable to separate underlying test lots) and that the testing does not validate samples for release. Patentee argues the prior art reduces work and expense with sampling while their method increases work and expense for validation. Patentee argues their composite is fundamentally different because it is of a statistically sufficient number of portions. These arguments are not persuasive as previously set forth in Ground 1 over Jarvis, FSIS and Midura. As previously discussed the rejection at hand is one of obviousness. The cumulative prior art teaches each of the respective elements, and assembles these elements according to regularly practiced principles in food science testing. Further, the instant rejection includes the teachings of Cousin which are on point to spoilage organisms (psychotrophs) which grow at reduced temperatures and thus are primarily responsible for spoilage in refrigerated foods.

Ground # 22:

Claim 21 is unpatentable as obvious over FSIS in combination with Price, Midura, and Cousin (see SNQ #55 at page 87 of the Request).

This rejection was proposed by the third party requester and **is adopted** for the reasons stated in the 5/26/10 office action and 12/17/10 ACP. The proposed rejection is set forth at page 87 and 123 of the request. The rejection is reiterated to the claims as amended after ACP.

Claim 21 is rejected under 35 U.S.C. 103(a) as being unpatentable over FSIS in view of Price and Midura, as applied to claim 1 above, and further in view of Cousin:

As was set forth *supra* in Ground 2, which is incorporated herein by reference in its entirety, FSIS in view of Price and Midura teaches a method of sampling and testing food test lots for microbial contamination comprising collecting a plurality of portions from each of a plurality of test lots, according to a statistical-based sampling plan, combining the portions to provide a corresponding set of test lot samples, separately incubating the test lot samples to enrich for the target microbial agent or organism, removing portions of the test lot sample cultures, combining them into a modular composite sample and testing the composite sample for the presence of the target agent or organism. None of FSIS, Price, or Midura teaches that the target organism is a spoilage organism.

Cousin discloses that certain bacteria (psychrotrophs) are able to grow in foods at low temperatures and are thus the primary organisms responsible for spoilage of refrigerated foods. Cousin teaches that such organisms are found in meats, poultry, fish, and seafood and include *Listeria* spp., among others (see page 169, column 2, last paragraph). Cousin teaches that such organisms can be detected by culture and microscopic methods (see section 13.21 beginning on page 160).

It would have been *prima facie* obvious to one of skill in the art at the time of the invention to have used the method disclosed by FSIS in view of Price and Midura to test for a spoilage organism, such as a psychrotroph, because Cousin teaches that such organisms are responsible for spoilage of refrigerated foods and can be detected by culture.

Response to Patent Owner's arguments:

Patent owner argues (pages 124-127) essentially as set forth *supra* with respect to Ground 2; that the prior art is limited to analysis of single lots, that the prior art does not assemble a modular composite sample (of lumped samples/cultures each attributable to separate underlying

test lots) and that the testing does not validate samples for release. Patentee argues the prior art reduces work and expense with sampling while their method increases work and expense for validation. Patentee argues their composite is fundamentally different because it is of a statistically sufficient number of portions. These arguments are not persuasive as previously set forth in Ground 2 over Price, FSIS and Midura. As previously discussed the rejection at hand is one of obviousness. The cumulative prior art teaches each of the respective elements, and assembles these elements according to regularly practiced principles in food science testing. Further, the instant rejection includes the teachings of Cousin which are on point to spoilage organisms (psychotrophs) which grow at reduced temperatures and thus are primarily responsible for spoilage in refrigerated foods.

Ground # 23:

Claim 23 is unpatentable as obvious over Jarvis in combination with FSIS, Midura, and Price (see SNQ #58 at pages 88-89 of the Request).

This rejection was proposed by the third party requester and **is adopted** for the reasons stated in the 5/26/10 office action and 12/17/10 ACP. The proposed rejection is set forth at pages 88-89 and 123 of the request. The rejection is reiterated to the claims as amended after ACP.

Claim 23 is rejected under 35 U.S.C. 103(a) as being unpatentable over Jarvis in view of FSIS and Midura, as applied to claim 1 above, and further in view of Price.

As was set forth *supra* in Ground 1, which is incorporated herein by reference in its entirety, Jarvis in view of FSIS and Midura teaches a method of sampling and testing food test lots for microbial contamination comprising collecting a plurality of portions from each of a plurality of test lots, according to a statistical-based sampling plan, combining the portions to provide a corresponding set of test lot samples, separately incubating the test lot samples to enrich for the target microbial agent or organism, removing portions of the test lot sample cultures, combining them into a modular composite sample and testing the composite sample for the presence of the target agent or organism. None of Jarvis, FSIS, or Midura teaches testing the

composite modular sample by a DNA-based method, an immunochemistry-based method, or a biosensor-based method.

Price teaches identification of *Salmonella* from composite enrichment broth cultures using the standard methods of biochemical and serological testing, which is an immunochemistry based method (Figure 1; Tables 1-2).

One of ordinary skill in the art at the time of the invention would have found it *prima facie* obvious to have identified the target agent or organism from the composite sample in the method of Jarvis in view of FSIS and Midura using serological testing, which is an immunochemistry based method, as described by Price. One of ordinary skill in the art at the time of the invention would have been motivated to identify the agent by serological testing because Price teaches that this was a standard method of identification of target agents or organisms at the time of the invention.

Response to Patent Owner's arguments:

Patent owner argues (pages 127-129) essentially as set forth *supra* in Grounds 1-2; and thus submits no additional arguments which require further response to the Ground 23 rejection. As above, Price is an immunochemistry based method as it teaches serological testing.

Ground # 24:

Claims 24, 28, 32, 33 are unpatentable as obvious over FSIS in combination with Price, Midura, and Gabis (see SNQ #s 63, 70, 78, and 80 at pages 93, 97-98, and 103-105 of the Request).

This rejection was proposed by the third party requester and **is adopted** for the reasons stated in the 5/26/10 office action and 12/17/10 ACP. The proposed rejection is set forth at pages 93, 97-98, 103-105 and 123-127 of the request. The rejection is reiterated to the claims as amended after-ACP.

Claims 24, 28, 32, and 33 are rejected under 35 U.S.C. 103(a) as being unpatentable over FSIS in view of Price and Midura, as applied to claims 1 and 4 above, and further in view of Gabis.

As was set forth *supra* in Ground 2, which is incorporated herein by reference in its entirety, FSIS in view of Price and Midura teaches a method of sampling and testing food test lots for microbial contamination comprising collecting a plurality of portions from each of a plurality of test lots, according to a statistical-based sampling plan, combining the portions to provide a corresponding set of test lot samples, separately incubating the test lot samples to enrich for the target microbial agent or organism, removing portions of the test lot sample cultures, combining them into a modular composite sample and testing the composite sample for the presence of the target agent or organism. FSIS teaches that the food to be tested is beef and Midura teaches that the food to be tested is fish, vegatables, dried foods, frozen foods, milk, meats, and/or shellfish (page 18, column 1, last complete sentence). None of FSIS, Price, or Midura teaches that the food to be tested is poultry, pork, sheep or bison. Additionally, although Price teaches a pooled enrichment broth for *Salmonella* (comprising a selective agent for *Salmonella* which inhibits competing organisms); none of FSIS, Price or Midura teaches that the individual test lot sample broths comprise a selective agent for the target organism.

Gabis teaches a method of sampling, testing, and validating high moisture foods for microbial contamination comprising collecting a plurality of specimens, incubating each of the specimens individually into broth cultures, incubating the broth cultures to allow for levels of the target organism to increase, and pooling the broth cultures together to test for *Salmonella* (see page 663, abstract and first paragraph of the page; page 664, the paragraph bridging columns 1 and 2 and column 2, first two full paragraphs; and page 668, first full paragraph). Gabis teaches two alternative broth cultures for the individual samples, "preenrichment" broths not containing a selective agent and "direct selective enrichment" broth cultures containing an agent selective for *Salmonella* and inhibitory for competing bacteria (see page 664, *Enrichment Procedures* and **Results**). Gabis teaches exemplary high moisture foods suitable for testing as pork and chicken (poultry) among others (page 665, Table1).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have inoculated the test lot samples disclosed by FSIS in view of Price and

Midura into individual broths containing a selective agent for *Salmonella* because Gabis teaches that the individual enrichment broth culture may or may not contain a selective agent for the target organism. Further, it would have been *prima facie* obvious to have tested pork and /or poultry because Gabis teaches that the method is suitable for such high moisture foods.

Response to Patent Owner's arguments:

Patent owner argues (pages 129-131) essentially as set forth *supra*; and thus submits no additional arguments which require further response. As above, Gabis teaches the art recognized alternative of direct inoculation to enrichment (selective) cultures (without the need for pre-enrichment).

Ground # 25:

Claim 28 is unpatentable as obvious over Jarvis in combination with FSIS, Midura, and Andrews (see SNQ #69 at pages 96-97 of the Request).

This rejection was proposed by the third party requester and was adopted for the reasons stated in the 5/26/10 office action. As in Ground 1 above, rejection of claim 4 was **withdrawn** within the 12/17/10 ACP. Claim 28 depends directly from claim 4, and thus rejection of claim 28 is withdrawn for the same reasons set forth above in Ground 1. The rejection was proposed at pages 96-97 and 126 of the request. The decision to withdraw the rejection is maintained.

Ground # 26:

Claim 32 is unpatentable as obvious over Jarvis in combination with FSIS, Midura, and BAM and ICMSF.

This rejection was proposed by the third party requester (see SNQ #77 at pages 102-103, of the Request) but is **not adopted** for the reasons set forth in the 5/26/10 office action and 12/17/10 ACP. The decision to not adopt is maintained.

Claim 32 was included in Ground 1 as unpatentable over Jarvis, in view of FSIS and Midura, because FSIS teaches the claimed limitation of meat trim pieces (see Ground 1). Therefore, the proposed rejection of the same claim 32 over Jarvis, in view of FSIS, Midura BAM and ICMSF, is improper because the combination of Jarvis, FSIS, and Midura does not

have a deficiency which would require modification by BAM and ICMSF in order to meet the limitations of the claim. Furthermore, Requester points to page 135 of ICMSF as providing the teaching of meat trim pieces which Requester alleges is absent from the combination of Jarvis in view of FSIS and Midura (see page 103 of the request). However, this teaching does not appear to be present at page 135 of ICMSF. Therefore, for these reasons, the proposed rejection **is not adopted**.

Response to arguments:

Neither the Patent owner nor the Requester provided arguments regarding the non-adoption of the Ground 26 of Rejection. Therefore, the examiner's position remains unchanged.

Response to Declarations:

The Samadpour and Koohmarie declarations were previously addressed in the 12/17/10 ACP. At pages 134-138 patent owner refers to the declarations, but does not further comment on them. Instead, patent owner points to the declarations submitted after-ACP for further clarification. The Examiner has once again reviewed these declarations, but finds the declarations insufficient to overcome the prima facie case for the same reasons discussed within the ACP. Accordingly, no further comment is made to the previous declarations here. Instead, the previous comments addressed in the 12/17/10 ACP are allowed to stand on their own.

The declarations submitted by patent owner after-ACP are addressed as follows: *Samadpour Declarations*: Dr. Samadpour submits two separate 1.132 declarations dated 1/31/11. The first is accompanied by Exhibits A-H. The second is cased as a rebuttal of the Bailey declaration and includes various comments in response to the declaration of Dr. Bailey (submitted with the request by third party requester).

The first declaration by Dr. Samadpour makes statements consistent with the arguments presented above in traverse of the cited rejections. As expressed in ¶8, Dr. Samadpour is of the opinion that the prior art is limited to how many samples should be taken from a single lot (also ¶22), to achieve statistical confidence, or be most economically tested. However, such assertions fail to acknowledge the prior art teachings as to multiple lots; see for example Jarvis p. 136,

Example 8.4. The claimed invention is asserted to concern entire sample sets, each set representing a separate lot and each having a statistically confident number of samples dealt with as a group that allows immediate release of members of the group lots, without further sampling even when other lots test positive. However, the claims do not reference different sets. As above, the prior art is not silent as to different lots. Jarvis explicitly teaches testing for three different lots, and the skilled artisan would well recognize in the cited art of Jarvis, Price, Silliker, Gabis and Davies, separate lot samples from different foods, different herds and different levels of inoculated (or uninoculated food samples) for experimental testing purposes. The claimed method is said to be disadvantageous as it tests smaller lots and a greater number of samples, but is inventive as the cost is offset by reducing the overall product that is diverted or destroyed. However, the claimed invention in no way limits the size of individual lots. Furhter, while the claims are not directed to economic incentive, the prior art notes such identified advantages. If testing was of no economic issue, statistics directs greater testing would always be conducted for increased significance. Merely because this incentive is balanced in practice, does not disincentive more rigorous testing, even though expense is of concern. Additional testing is noted to provide for increased statistical confidence in any particular lot. The declaration (¶9-10) reviews the terms "lot" and "statistical confidence," makes assertions (¶11-12) as to what Jarvis would have known, and then concludes that Jarvis disclaims a "statistical" reading and is merely an aid for practicing microbiologists. However, these assertions are not based upon present factual knowledge and thus appears to be mere opinion. The declaration asserts (¶13) that Jarvis, Price, Silliker, Gabis and BAM fail to teach compositing a plurality of samples from a plurality of lots. However, as discussed in the rejections above, multiple samples and multiple lots are tested including via composite sampling. Dr. Samadpour continues by arguing (¶14-19) that the prior art uses prepared food samples and fails to composite different types of food; and thus cannot be a sampling of different lots. However, the artisan would recognize the different foods, herds and inoculum for example, including positive and negative samples as representative of different test lots. Dr. Samadpour ¶16 takes issue with the prior art and the stated objective of reducing workloads via composite (also referred to by the prior art and patentee as pooling and lumping) testing, which is assertedly in contrast to the claimed invention, see also ¶8, 30. However, this art recognized advantage does not teach away from,

but further motivates to the invention; the inventive steps essentially being the prior art disclosed wet composite testing procedures. Dr. Samadpour draws attention to a 2007 publication of Jarvis to show that compositing is to be assessed with various merits and limitations. Such is acknowledged, however the 2007 publication is not prior and does not teach away where the artisan was apprised via the prior art to experimentally assess such compositing protocols. Such assessments are essentially the aim of the cited prior art, and while a few false negative tests are noted, the art overwhelmingly concludes that the composite testing procedures are as sensitive as testing individually. Dr. Samadpour argues ¶20-21, and 23 that the purposes expressed in the prior art and the invention differ. However, the claimed invention does not recite the statements of purpose or intent and regardless, such issues fail to distinguish so long as motivation to the method steps is provided as noted herein. With respect to Midura and FSIS ¶22-23, the references are said to be inferior as directed to double sampling plans since regulations do not allow for such testing. However, as above, government regulation is not the standard for assessing the invention. The FSIS is a special case scenario with respect to testing for E. coli 0157:H7, a particularly lethal strain, to which the claims are not limited. With respect to FSIS it also argued ¶24 that the methodology of testing is not of the cited prior art but via the FSIS laboratory procedures (disclosed in a different reference). However, this position fails to acknowledge the basis for the obviousness rejection. The FSIS reference is not relied upon alone, but is combined with other art standard testing methodology. Dr. Samadpour argues ¶25 that the compositing as in Price, Silliker and Gabis would not be combined with non-real world comparisons as in Jarvis. However, this is a characterization which appears to be formed by opinion. Jarvis is no less real-world merely because it appears to be instructional material. Jarvis is on point to testing for Salmonella in food homogenate or culture. ¶26 notes that composting may result in decreased sensitivity (or false negative results). Such reports are acknowledged in for example Gabis. Yet even this reference confirms the utility of composite testing, with preference to transfer of pre-enrichment cultures, see Gabis Abstract. ¶27-29 discusses enrichment vs. pre-enrichment, notes that the prior art teaches the use of enrichment prior to PCR, and concludes (with reference to Appendix D and Dr. Bailey's declaration) that pre-enrichment is not encompassed by the claims. However, there are differences in the standard for assessment between the claimed invention and Dr. Samadpour's analysis. The claims are

broader than interpreted as they encompass detection, uniform or substantially uniform growth, as would be provided in cultures such as Jarvis which teach growth such that at least one organism is transferable in each aliquot. Moreover, as disclosed in the growth chart at p. 141, the pre-enrichment cultures at 24 hours are said to be at 6.9 x 10^8 cells/ml, which is an amount far greater that the 10^4 cells/ml noted to be suitable for detection as in Dr. Samadpour's analysis with respect to App. D and Dr. Bailey's analysis. Further as disclosed in Jarvis, such growth standard is a minimum, not a maximum. As set forth in the rejections supra, the position is that such growth would be sufficient for detection via PCR which is a method experimentally suitable to detect at very low levels, even of only a single gene target (organism) as noted in Entis. Jarvis is thus evidenced to provide "detectability." Dr. Samadpour's arguments also appear to assert that additional culture steps are excluded from the claims. However, the method comprises the recited steps and does not exclude multiple incubation steps, which the art recognizes may be necessary. The recitation in element e) is that there is, "no requirement for further enrichment thereof." This recitation differs from excluding a further enrichment step. As above, the scope of the claim remains open to further incubations prior to c) or subsequent to d). Multiple incubations are disclosed in several references, see for example Jarvis and Gabis. Thus, as discussed supra, the cumulative prior art renders these variations obvious. Dr. Samadpour argues ¶30 differences between an IEH protocol and that of the prior art. However, the instant claims are broader than this protocol and thus are not disparaged. Dr. Samadpour ¶31 refers to a 1969 publication by Silliker (App. F) and to a 2004 FSIS guide (App. C), with arguments that false negative results disparage wet compositing for validation. However, such results are countered by the cited art which shows that detection may be as sensitive in individual samples as in composited samples, see for example Jarvis, Price, Silliker, Gabis and Davies. Such merely cautions the artisan to assess the sensitivity of any protocol via testing. Such art recognized testing appears to be a primary focus of Price, Silliker, Gabis and Davies. Dr. Samadpour argues ¶32-33 that the prior art offered no guidance as to compositing and that no one in the prior art marketed test kits using the principles of compositing and pooling until after the Samadpour invention (also with reference to App. B). However, these positions are countered by the guidance in the above cited art, whether or not such protocols were "marketed" for sale.

Dr. Samadpour's declaration also presents secondary considerations of non-obviousness.

At ¶34-35 it is submitted that it took years to overcome skepticism from experts in the field and clients. In particular, FSIS apparently issued a letter of no objection based upon a modification that required individual testing regardless of whether composite (pooled) samples tested positive or negative. This appears, as noted by Dr. Samadpour, to be because E. coli 015:H7 is an adulterant, and regulations do not allow for retesting or the possibility of false negative results. This evidence has been fully considered but it not persuasive as it appears to be solely directed to testing for E. coli O157:H7, a special case scenario to which the claims are not limited. Moreover, the prior art already taught individual testing, and as suggested by the prior art, compositing is yet another statistical tool which provides further information as to the condition of underlying lots. In essence, FSIS required modification to art standard procedures for testing individual lots. As in statistics, further testing may always be conducted to achieve greater levels of confidence and sensitivity. While such further testing as noted in ¶36 may increase the number of positive samples, this is the goal of testing; to find contaminated lots, and thus such suggestion is not non-obvious, but consistent with the art accepted principles in statistically-based food science testing. Accordingly, such evidence is insufficient to overcome the prima facie case of obviousness.

At ¶ 37-45 Dr. Samadpour presents evidence in support of commercial success, see also App. H. As above, this evidence points to more rigorous testing than federal regulations require. Specifically, the compositing method detailed tests smaller lots, (a greater number of samples) and includes composite testing, but still tests each underlying lot individually. However as above, such special case scenarios are not the subject of the claims. The instant claims are broader, and not directed to FSIS standards, or to such specifications for any particular lot or sample. Moreover, it would appear that the FSIS standards were already known and well established. The cited prior art and statistics predicts that more rigorous testing would increase confidence, and give greater assurances, with increased detection of any positive samples present. While the inventor is of the position that such finding is new and inventive, such finding is predicted by the prior art. Further, while Dr. Samadpour is of the position that such would be averse to a business, in contrast finding positive samples is the aim in food science testing and would be considers sound basis for business decisions. As noted for example in

Davies, there is an economic incentive to assuring clean herd lots. Accordingly, such evidence is insufficient to overcome the prima facie case of obviousness.

In the second declaration, Dr. Samadpour argues (pp. 4-5) that Dr. Bailey's construct of one of skill in the art is too rigorous; is one of extraordinary skill in the art. However, the prima facie case is directed to teachings within the prior art, and thus Dr. Bailey's standard is not necessarily the basis for rejection, or for establishing a prima facie case. Dr. Samadpour asserts (p. 5) Jarvis does not test multiple lots. However Example 8.4 explicitly evidences contrary with three identified test lots A, B and C. Jarvis teaches samples of food homogenate or culture; this passage is representative of the samples to be tested in Jarvis and thus is also relevant to 8.4.2.1. Dr. Samaour submits (pp. 6-14) arguments as to pre-enrichment vs. enrichment broths (with reference to exhibits B-D) a product sheet that establishes growth in pre-enrichment broths at 24-48 hours, a sample slide purportedly used by Dr. Bailey with a prophetic example, and p. 143 of Jarvis which teaches detection with pre-enrichment cultures after further enrichment. However, exhibit B evidences uniform or substantially uniform growth with pre-enrichment and exhibit D evidences positive detection of samples with enrichment, which steps are not excluded. Exhibit C, represents a case which conveys possibilities where positive samples may be missed. Nevertheless, Jarvis's growth at 6.9×10^8 cells/ml (p. 141) far exceeds the 10^4 cells/ml noted to be suitable for detection (App. D) Thus, none of these references teaches away from the art standard procedures in the cited prior art. In contrast the references show variables known to be of consideration to the artisan in standard testing procedures. Price (p. 14-17) is asserted to be representative of single lots because it does not mix the different types of food samples. However, in Price separate test lots are experimentally prepared such that the test samples represent known positives (at different inoculum levels) or known negatives (no Salmonellae present). This represents at least two lots, even if all of the negative samples are viewed as being from a single lot. However as in Jarvis's composite method (2), the lots are separately tested, and composited with the advantage that the under-lying lot information is preserved, as for individual samples. Dr. Samadpour continues with arguments (pp. 17-21) as to pre-enrichment vs. enrichment cultures and the supposed distinction of detectability vs. dividability. As above however, multiple pre-enrichment or enrichment cultures are not excluded from the instant

claims. Further, the prior art evidences the artisan's knowledge of such variables which include pre-enrichment and/or enrichment cultures. Jarvis, Price, Silliker, Gabis and Davies exemplify positive detection with composited lot samples and thus render obvious the instant claims. While Dr. Samadpour states (pp. 21-24) that Dr. Baileys comments on Midura (Chapter 2 of the Compendium), has no bearing on the issues at hand, Midura is on point to statistical-based sampling plans which form the basis for lot allocation, and thus testing performed for different lots. Midura clarifies the difference between taking multiple samples to form a lot and testing multiple (duplicate) samples of a lot. Thus, these teachings are relevant to understanding the state of the prior art, and the differences in the cited art with respect to sampling and various testing procedures to validate any number of different lots of food. Such cumulative teachings form the basis for the obviousness rejection. While Dr. Samadpour urges a reading of the prior art which presumes the art is limited to only single lots of food, this reading is not supported by the teachings of Jarvis, Price, Silliker, Gabis and Davies which include composite testing for any number of different lots of food. In regard to the FSIS reference (pp. 24-25) Dr. Samadpour objects to the use of the reference as teaching composite testing. While FSIS does teach blending composite samples with enrichment media, FSIS is not relied upon to teach composite testing. The references of Jarvis, Price, Silliker, Gabis and Davies are relied upon as teaching composite testing of multiple lots. The comments by Dr. Samadpour at p. 25, appear to refer to background discussed in Silliker at p. 476, column 1, which is asserted to teach against dry compositing and against compositing in general due to the occurrence of false negatives. Such occurrences are recognized in the cited art, see particularly Gabis. However, each of Jarvis, Price, Silliker, Gabis and Davies teach that compositing may be utilized for validation testing and may be as sensitive as testing individual samples. Accordingly, the declaration has been fully considered but does not overcome the above grounds for rejection and present a prima facie case of obviousness.

Mirtsching Declaration:

The Mirtsching declaration states that there was substantial skepticism that Dr. Samadpour's wet compositing approach would work. Independent testing was performed which confirmed the utility of the approach, and the approach has now been instituted.

This evidence has been fully considered but is not persuasive to overcome the prima facie case. As discussed *supra*, the prior art of record predicts the utility of such testing and thus renders the claimed invention obvious. Accordingly, the declaration has been fully considered but is not persuasive.

Byron Declaration:

The Byron declaration is similar to the Mirtsching declaration discussed above. It also states that there was substantial skepticism that Dr. Samadpour's modular composite pooling method would work. There was substantial concern that the method would result in false negative results. Again, independent testing was performed which confirmed the utility of Dr. Samadpour's invention, which has now been largely instituted in the art. The Byron declaration acknowledges the contribution of the technology to food science testing. The declaration further asserts that some participants in the industry have adopted a practice of copying and using the technology without commercial agreements, permission or consent.

This evidence has been fully considered but is not persuasive to overcome the prima facie case. As discussed *supra*, the prior art of record predicts the utility of such testing and thus renders the claimed invention obvious. Accordingly, the declaration has been fully considered but is not persuasive.

Duty to Disclose

The patent owner is reminded of the continuing responsibility under 37, CFR 1.985 to apprise the Office of any litigation activity, or other prior or concurrent proceeding, involving Patent No. 7,534,584 throughout the course of this reexamination proceeding. The third party requester is also reminded of the ability to similarly apprise the Office of any such activity or proceeding throughout the course of this reexamination proceeding.

Conclusion

13. **This is a RIGHT OF APPEAL NOTICE (RAN)**; see MPEP § 2673.02 and § 2674. The decision in this Office action as to the patentability or unpatentability of any original patent

claim, any proposed amended claim and any new claim in this proceeding is a FINAL DECISION.

No amendment can be made in response to the Right of Appeal Notice in an *inter partes* reexamination. 37 CFR 1.953(c). Further, no affidavit or other evidence can be submitted in an *inter partes* reexamination proceeding after the right of appeal notice, except as provided in 37 CFR 1.981 or as permitted by 37 CFR 41.77(b)(1). 37 CFR 1.116(f).

Each party has a **thirty-day or one-month time period, whichever is longer**, to file a notice of appeal. The patent owner may appeal to the Board of Patent Appeals and Interferences with respect to any decision adverse to the patentability of any original or proposed amended or new claim of the patent by filing a notice of appeal and paying the fee set forth in 37 CFR 41.20(b)(1). The third party requester may appeal to the Board of Patent Appeals and Interferences with respect to any decision favorable to the patentability of any original or proposed amended or new claim of the patent by filing a notice of appeal and paying the fee set forth in 37 CFR 41.20(b)(1).

In addition, a patent owner who has not filed a notice of appeal may file a notice of cross appeal within **fourteen days of service** of a third party requester's timely filed notice of appeal and pay the fee set forth in 37 CFR 41.20(b)(1). A third party requester who has not filed a notice of appeal may file **a notice of cross appeal within fourteen days of service** of a patent owner's timely filed notice of appeal and pay the fee set forth in 37 CFR 41.20(b)(1).

Any appeal in this proceeding must identify the claim(s) appealed, and must be signed by the patent owner (for a patent owner appeal) or the third party requester (for a third party requester appeal), or their duly authorized attorney or agent.

Any party that does not file a timely notice of appeal or a timely notice of cross appeal will lose the right to appeal from any decision adverse to that party, but will not lose the right to file a respondent brief and fee where it is appropriate for that party to do so. If no party files a timely appeal, the reexamination prosecution will be terminated, and the Director will proceed to issue and publish a certificate under 37 CFR 1.997 in accordance with this Office action.

Correspondence

All correspondence relating to this inter partes Reexamination proceeding should be directed to:

By EFS:

Registered users may submit via the electronic filing system EFS-Web at

https://sportal.uspto.gov/authenticate/authenticateuserlocalepf.html

By Mail to:
Attn: Mail Stop "Ex Parte Reexam"
Central Reexamination Unit
Commissioner for Patents
P. O. Box 1450
Alexandria VA 22313-1450

By FAX to:
(571) 273-9900
Central Reexamination Unit

Hand-deliver any communications to:
Customer Service Window
Attn: Central Reexamination Unit
Randolph Building, Lobby Level
401 Dulany Street
Alexandria, VA 22314

Any inquiry concerning this communication or earlier communications from the examiner, or as to the status of this proceeding, should be directed to the Central Reexamination Unit at telephone number (571) 272-7705.

Signed:

/Sharon Turner/
Patent Reexamination Specialist, CRU-3991

STEPHEN J. STEIN
PRIMARY EXAMINER
CRU - AU 3991

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PRIMARY EXAMINER

Via Electronic Submission
Attorney Docket No.: 66090-008US2

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Patentee:	Mansour Samadpour
Control No.:	95/001,294
Filed:	05 April 2010
For:	MODULAR COMPOSITING-MULTIPLE LOT SCREENING PROTOCOLS FOR DETECTION OF PATHOGENS, MICROBIAL CONTAMINANTS AND/OR CONSTITUENTS
Examiner:	Sharon Turner
Art Unit:	3991
Docket No.:	66090-008US2
Date:	March 5, 2012

Attn: Mail Stop "*Inter Partes* Reexam"
Central Reexamination Unit
Commissioner for Patents
P. 0. Box 1450
Alexandria VA 22313-1450

NOTICE OF APPEAL

Dear Sir:

Applicants hereby appeal to the Board of Patent Appeals and Interferences from the decision of the Examiner dated February 8, 2012 in the Right of Appeal Notice, finally rejecting claims 1-33, all of which are being appealed on all rejections. This Notice of Appeal is filed on or before March 8, 2012 and as such is timely filed. Pursuant to 37 C.F.R. § 41.20(b)(1), the fee for this Notice of Appeal is $270.00 for a small entity.

The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. 040258. A duplicate copy of this sheet is enclosed. Timely service to the third party requester, bioMerieux, via their attorney Anthony Figg, at Rothwell, Figg, Ernst & Manbeck, P.C., 1425 K Street N.W., Suite 800, Washington, D.C. 20005 is being affected by first class mail sent on March 5, 2012.

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Respectfully submitted,
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Via Electronic Submission
Attorney Docket No.: 66090-008US2

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Patentee:	Mansour Samadpour
Control No.:	95/001,294
Confirmation No.:	1914
Filed:	05 April 2010
For:	MODULAR COMPOSITING-MULTIPLE LOT SCREENING PROTOCOLS FOR DETECTION OF PATHOGENS, MICROBIAL CONTAMINANTS AND/OR CONSTITUENTS
Examiner:	Sharon Turner
Art Unit:	3991
Docket No.:	66090-008US2
Date:	May 7, 2012

Attn: Mail Stop "*Inter Partes* Reexam"
Central Reexamination Unit
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PATENT OWNER'S APPEAL BRIEF PURSUANT TO 37 C.F.R. § 41.67

Sir:

Appellants appeal from the rejection of Claims 1-33 of the above-identified application. This Brief on Appeal is filed in support of the Patent Owner's appeal from the decision of the Examiner dated February 8, 2012 in the Right of Appeal Notice ("RAN"), finally rejecting claims 1-33. Reversal of the rejections is respectfully urged. Submitted with this brief is authorization to pay the fee for filing a brief in support of an appeal as set forth in 37 C.F.R. § 41.20(b)(2). This Appeal Brief is timely filed as 05 May 2012 fell on a Saturday.

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I. STATEMENT OF THE REAL PARTY IN INTEREST

The real party in interest in the above-identified application is the Appellant assignee (Patent owner), Institute for Environmental Health Inc., a Washington corporation with its principal place of business at: 15300 Bothell Way NE, Lake Forest Park, WA 98155.

II. RELATED APPEALS AND INTERFERENCES

While Appellant does not believe that the opposition discussed below has any effect or bearing, directly or otherwise, on the Board's decision in this appeal, Appellants are nonetheless disclosing its existence to the Board to comply with the Appellant's duty under 37 C.F.R. § 41.67(c)(1)(ii). There is an opposition in European Patent No. 1786920 known to Appellants' attorney and the assignee of the instant application, that directly affect, that would be directly affected by, or that may have a bearing on the Board's decision in this appeal. European Patent No. 1786920 is the European sister case to Patent No. 7,534,584 subjected to this current inter partes reexam. There are no other appeals known to Appellant's attorney and the assignee of the instant application, that directly affect, that would be directly affected by, or that may have a bearing on the Board's decision in this appeal.

III. STATUS OF CLAIMS

Claims 1-33 are pending. Claims 1, 2, and 17 are Amended. Claims 2-16 and 18-33 are Original. New claims 34-36 were previously Cancelled.

Claims 1-33 stand rejected under 35 U.S.C. §103(a) as allegedly being unpatentable due to obviousness.

Claims 1-33 are presently appealed under 35 U.S.C. § 134.

IV. STATUS OF AMENDMENTS

The Patent owner submitted claim amendments subsequent to the issuance of the Action Closing Prosecution. These claim amendments were filed in the Patent owner's response January 31, 2011 and May 5, 2011 (correcting informalities) and have been entered into the record according to the Examiner (RAN page 2). The claims shown in the accompanying Appendix are an accurate representation of the pending claims.

V. SUMMARY OF CLAIMED SUBJECT MATTER

This summary is presented in compliance with the requirements of 37 C.F.R. § 41.67(c)(1)(v), mandating a "concise explanation of the subject matter defined in each of the independent claims involved in the appeal." Nothing stated within this summary is to be interpreted as changing the specific language of the claims, nor is the language of this summary intended to be considered so as to limit the scope of the claims in any way.

Independent claims 1 and 17 are directed to methods of sampling, testing and validating a plurality of test lots for release into commerce. Independent claim 1 is further directed to a method of a) collecting a plurality of portions from separate test lots, 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; 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; 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; d) removing portions of each enriched separate test lot sample, and combining the removed portions to provide a modular composite sample; and e) testing of the modular composite sample, using a suitable detection assay, for the target microbial agent or organism, wherein when such testing is negative all of said separate test lots are validated for release into commerce, and 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, 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 step a).

Independent claim 17 is an embodiment of claim 1, wherein the method of collecting a plurality of portions consists of collecting the plurality of portions from each of a plurality of single-unit production lots.

VI. ISSUES TO BE REVIEWED ON APPEAL

The issues to be reviewed on appeal are the Examiner's rejections due to Obviousness – 35 U.S.C. 103

1. Whether claims 1-4, 8, 10, 11, 13, 17-19, 22, 24, 26, 27, and 31 are rendered obvious under 35 U.S.C. §103(a) over Jarvis (Exhibit U) in view of FSIS (Exhibit O) and Midura (Exhibit L);
2. Whether claims 1-4, 6-11, 13, 17-18, 22, and 31 are rendered obvious under 35 U.S.C. §103(a) over FSIS (Exhibit O) in view of Price (Exhibit Q) and Midura (Exhibit L);
3. Whether claims 1 and 17 are rendered obvious under 35 U.S.C. §103(a) over Jarvis (Exhibit U) in view of "Patent owner's admissions" regarding the state of the prior art;
4. Whether claims 1 and 17 are rendered obvious under 35 U.S.C. §103(a) over Andrews (Exhibit N) in view of Price (Exhibit Q) and Midura (Exhibit L);
5. Whether claims 1 and 17 are rendered obvious under 35 U.S.C. §103(a) over Silliker (Exhibit P) in view of Price (Exhibit Q) and Midura (Exhibit L);
6. Whether claims 1 and 17 are rendered obvious under 35 U.S.C. §103(a) over Gabis (Exhibit R) in view of Price (Exhibit Q) and Midura (Exhibit L);

7. Whether claims 1 and 17 are rendered obvious under 35 U.S.C. §103(a) over Jarvis (Exhibit U) in view of BAM (Exhibit T);
8. Whether claims 1 and 17 are rendered obvious under 35 U.S.C. §103(a) over Price (Exhibit Q) in view of BAM (Exhibit T) and Davies (Exhibit K);
9. Whether claims 5, 23, 29, and 30 are rendered obvious under 35 U.S.C. §103(a) over Jarvis (Exhibit U) in view of FSIS (Exhibit O), Midura (Exhibit L), and Entis (Exhibit V);
10. Whether claims 5, 6, 23, 29, and 30 are rendered obvious under 35 U.S.C. §103(a) over FSIS (Exhibit O) in view of Price (Exhibit Q), Midura (Exhibit L), and Entis (Exhibit V);
11. Whether claim 21 is rendered obvious under 35 U.S.C. §103(a) over Jarvis (Exhibit U) in view of FSIS (Exhibit O), Midura (Exhibit L), and Cousin (Exhibit W);
12. Whether claim 21 is rendered obvious under 35 U.S.C. §103(a) over FSIS (Exhibit O) in view of Price (Exhibit Q), Midura (Exhibit L), and Cousin (Exhibit W);
13. Whether claim 23 is rendered obvious under 35 U.S.C. §103(a) over Jarvis (Exhibit U) in view of FSIS (Exhibit O), Midura (Exhibit L), and Price (Exhibit Q); and
14. Whether claims 24, 28, 32, and 33 are rendered obvious under 35 U.S.C. §103(a) over FSIS (Exhibit O) in view of Price (Exhibit Q), Midura (Exhibit L), and Gabis (Exhibit R).

VII. ARGUMENT

Introduction

Claims 1 and 17 define a multistage method which allows simultaneous validation of multiple, separate test lots (e.g., of food) for release into commerce without having to perform a separate test upon each lot, depending upon the results of a composite test.

Upon a negative composite test, all lots are qualified for immediate release.

Upon a positive composite test, already-existing separate enrichments for each lot are immediately available for testing. Since each of these enrichments was itself prepared according to a statistical sampling plan, the underlying lot of food may be released immediately if a separate test upon the associated enrichment is negative.

Thus, the inventive concept involves a relationship between two kinds of tests: 1) a composite test comprising samples gathered from multiple, separate lots of food and, if necessary, 2) multiple, individual tests pertaining to each, separate lot of food—performed only in the event of a positive composite.

The inventive concept further involves a special attribute of the multiple, individual tests, which is this: a negative test will automatically validate the underlying separate lot of food for release into commerce. This is so because the tests are performed upon separate, enrichments comprising an appropriate number of samples from the respective test lot so that the enrichment, on a stand-alone basis, contains a statistically significant number of food portions to be representative of the lot for validation purposes according to the statistically-based sampling plan.

This invention primarily concerns a method of testing food products for microbial contamination, as, for example, a production facility such as a meat packing line. An initial step for testing is to define what batches or "lots" of product are to be tested. A lot is typically a batch of product that is related in some operational sense, as all being part of production during a certain time period, or as all loaded into a single shipping container, or some such relationship. Defining a lot for testing purposes is much like defining an independent variable in mathematics. In the first instance, the definition may be rather arbitrary and done for various reasons of convenience.

However, once a lot is defined, statistical mathematics determines how many portions must be taken from the lot in order to obtain a representative sampling. This is analogous to taking a public opinion poll. If sampling with statistical error below a certain level is required, a given number of people must be interviewed, whether the poll is being taken nationwide or pertains to just a single city. Likewise, statistical considerations dictate a that a certain number of samples must be taken from a lot of product in order to achieve a desired level of statistical accuracy, whether the lot be large, for example an entire day of production at a facility, or whether the lot be defined as a subset of that production.

Nothing in any cited art teaches the concept of simultaneous testing of multiple lots in course of a single application of a method, as is done in the patented claims.

In the foregoing context, the References cited in the Right of Appeal Notice may be divided as follows:

Group I. General references on statistical based sampling plans:

FSIS -General discussion of regulations on testing meat for *E .coli*; and

Midura-Teaches particular numbers of portions which must be taken from a lot to achieve statistical significance for a testing plan.

Group II. References that discuss whether the portions taken from the lot need to be tested individually, or whether these may be lumped or composited prior to testing:

Price -Teaches that individual portions sampled from a lot must be pre-enriched separately;

Silliker-Teaches lumping multiple portions sampled from a lot into a composite prior to sampling;

Jarvis (p.141) -Teaches three different approaches:

a) lumping all portions sampled from a lot into a composite, as in Silliker;

b) pre-enriching each portion sampled from a lot separately as in Price; and

c) forming two composites by lumping half the portions sampled from a lot into the first, and the other half of portions sampled into a second;

Andrews -Teaches lumping of portions sampled from a lot of food into a single composite (Same teaching as in Silliker);

Gabis -Teaches that individual portions sampled from a lot must be pre-enriched separately (i.e., same teaching as Price); and

BAM -Teaches lumping of samples from "a" lot into a composite (i.e., same teaching as Silliker; and Same teaching as Silliker).

Group III. References teaching how to test an individual portion sampled from a lot:

Jarvis (p. 138) -Most Probably Number (MPN) method for testing a single sample using a set of dilution tubes; and

Entis -Use of beads in concentration step when testing. Used only against dependent claims.

All of the art cited against this invention pertains to sampling of a single lot of food. Even the two references which concern "composite" (aggregated) samples contemplate that all of the samples come from the same lot of food. The only motive for compositing in these references is to permit separate samples from the same lot to be separately enriched, thus affording some enhancement of the sensitivity of the test. Nothing in cited art motivates combination of samples from separate lots of food within a single method. Nor does anything in the cited art motivate gathering together of a statistically significant number of samples at any intermediate point in the method, much less the gathering together of multiple separate enrichments, each with an independent, statistically significant number of samples. On the contrary, all of the cited art is motivated by a desire to limit the number of samples as much as possible, in order to minimize costs of testing. No art discloses the benefits of employing multiple separate enrichments, each comprising its own statistically significant number of samples, within a single method, as in claims 1 and 17.

Figures 1-4, corresponding to Exhibits A-D and already of record, are included to facilitate understanding of the distinguishing features of Patent owner's invention relative to the methods of particular prior art references.

Rejection of Claims Under 35 U.S.C. §103(a)

Ground #1: Rejection of Claims 1-4, 8, 10, 11, 13, 17-19, 22, 24, 26, 27, and 31 Under 35 U.S.C. §103(a):

The rejection of Claims 1-4, 8, 10, 11, 13, 17-19, 22, 24, 26, 27, and 31 under 35 U.S.C. §103(a) is maintained for allegedly being unpatentable over Jarvis in view of FSIS and Midura for reasons as stated in the Right of Appeal Notice (RAN) and ACP.

Patent owner respectfully traverses the rejection because the cited references have been fundamentally misconstrued and extended by hind-sight beyond their teachings.

APPLICABLE LAW

In *KSR International Co. v. Teleflex, Inc*. 127 S. Ct. 1727, 2007 (herein referred to as "*KSR*"), the Supreme Court stated that the Graham factors ((1) scope and content of the prior art, (2) difference between claimed subject matter and the prior art, (3) the level of skill in the art, and (4) where in evidence, so-called secondary considerations; Graham v. John Deere Co., 383 U.S. 1 (1966)) continue to define the inquiry that controls the obviousness analysis. Additionally, under KSR, the TSM test is valid provided that such application does not require an overly rigid or explicit application of the asserted prior art. In keeping with KSR, to establish a *prima facie* case of obviousness there must be: (i) a suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art (POSITA), to modify the reference or to combine reference teachings; (ii) a reasonable expectation of success; and (iii) the prior art reference(s) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art and knowledge generally available to POSITA, and not based on Patentee's disclosure (*In re Vaeck,* 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991); and see MPEP §§ 2143-2143.03).

Therefore, to support a conclusion that the claimed invention is directed to obvious subject matter, either the references must expressly or impliedly suggest the claimed invention or the examiner must present a convincing line of reasoning as to why the artisan would have found the claimed invention to have been obvious in light of the teachings of the references and the knowledge and skill in the relevant art. Moreover, there can be no reasonable expectation of success where the art, alone or in combination, *teaches away* from the invention.

Additionally, ***inventions and asserted references must be considered as a whole***. *Importantly, e*ven where a *prima facie* case is established, such case is rebuttable, and the proper inquiry involves consideration of inventions as a whole (*In re O'Farrell*, 853 F.2d 894, 903 (1988). In determining the differences between the prior art and the claims, the question under 35 U.S.C. 103 is not whether the differences themselves would have been obvious, but whether the claimed invention as a whole would have been obvious. *Stratoflex, Inc. v. Aeroquip Corp.*, 713 F.2d 1530, 218 USPQ 871 (Fed. Cir. 1983); *Schenck v. Nortron Corp.*, 713 F.2d 782, 218 USPQ 698 (Fed. Cir. 1983). In *Diamond v. Diehr* (450 US 175, 209 USPQ 1) (U.S. 1981), the

Diehr Court determined "[i]t is inappropriate to dissect the claims into old and new elements and then to ignore the presence of the old elements in the [35 U.S.C. § 101] analysis." Claims have a synergistic effect, so that if a claim contains elements A, B, C, and D, it is not proper to attack each of A, B, C, and D in isolation as four separate elements. The USPTO and the courts must view the claim as a whole under 35 U.S.C. § 101.

Furthermore, in combining references under Section 103, a proposed modification of a prior art reference cannot change its principle of operation (See MPEP §2143.01(VI), and references cited therein).

Inter alia, the Examiner's urged combination of the prior art changes the principle of operation of Jarvis, Price, Silliker, etc., which in the context of their assertion by the Examiner (e.g., assay (3) of Jarvis) are devoted solely to *reducing* the number of samples taken for efficiency, and in the context of sampling a single lot, and do not teach or in any way motivate Patentee's claimed methods as discussed herein above and below.

The claims are not obvious over the combination of references

In maintaining the rejection of the claims, the RAN (beginning at p. 8) makes various confused and incorrect characterizations of Jarvis.

First, the RAN refers to "Jarvis" in various places as if "Jarvis" is the description of a single technology, and then combines passages from Jarvis from different pages into a single argument. But "Jarvis" is a general textbook that summarizes many independent papers with no necessary relationship to one another. Thus, the discussion of an mean probable number (MPN) method, in Section 8.4 on pp.135-36 has no defined relationship to the discussion on p. 141 concerning "Compositing of Samples". These are separate sections of the text which are discussions of separate scientific papers cited in different footnotes. Jarvis nowhere suggests or motivates any technique for combining MPN and Compositing and does not purport to be concerned with this task.

Second, the RAN misleadingly states that "Jarvis [Page 135-136] exemplifies the methods with a plurality of separate food, explicitly lots A, B, and C. Accordingly, it is clear that Jarvis samples, tests, and validates a plurality of test lots for release into commerce." **This is simply false**. The Jarvis method validates one lot of food for each application of the method.

The only way that lots A, B, and C are related is that the Jarvis method was separately applied to each lot in a serial fashion. The only relationship between lots A, B, and C is that the results of these three separate applications of the method happen to be juxtaposed in the same table on a piece of paper. In contrast, the methods of Claims 1 and 17 take as inputs samples from separate lots of food and simultaneously deals with these samples to arrive at a single composite test result. This establishes a simultaneous relationship between separate lots that is not present in Jarvis, or in any other cited reference. Further confirmation that sampling according to Jarvis is limited to a single "lot" is taken from the first full paragraph on page 142 "the results will provide no more information about the lot than would testing of a single sample" (emphasis added).

Third, the RAN (p. 8) misleadingly characterizes Jarvis as being based upon "a statistical-based sampling plan" which directs decisions whether the "lots" should be accepted or rejected. This is misleading and incorrect because Jarvis concerns only how "a" lot of food may be validated. "Lots", as opposed to a single "lot", are involved with Jarvis only in the sense that the same method may be applied upon various occasions to different lots of food. But this is true of any testing method in the universe that is used more than once in history. In contrast, the method of Claims 1 and 17 inherently uses samples from a plurality of lots as inputs upon each occasion that it is used. It thus defines a relationship between samples from different lots of food and how these are to be eventually combined into a single, composite result.

Fourth, the RAN (p.9) is particularly misleading and incorrect when it suggests that "the basic method of Jarvis includes inoculating one or more samples in pre-enrichment or enrichment media. Jarvis also teaches further (emphasis supplied) compositing … (pp. 141-142)." This is misleading since there is no "the basic method" of Jarvis. Jarvis drew no connection between his discussion of an MPN method (at pp. 135-138) and the separate discussion of compositing of samples (at pp. 141-142). No indication, much less clear instruction, is provided as to how a compositing method from pp. 141-142 might be combined with the 7-tube MPN method of pp. 135-138, shown also in Example 8.4. In fact, the description of Example 8.4 shows that a single series of "product dilutions" is prepared from a single, initial 10E-1 dilution. Then a 10E-2 dilution is prepared from this. Then these dilutions are parceled out among 7 MPN tubes. Thus, the starting point for this method is a single 10E-1 dilution tube.

This is directly contrary to the method of Claims 1 and 17, wherein it is required that a "plurality of samples" be gathered and that a "plurality of separate test lot samples" be enriched separately.

The Action Closing Prosecution (ACP) (pp. 12-13) presents other fundamentally incorrect arguments concerning single lot versus multiple-lot pooling, which are discussed below.

First, in the ACP rejection (p. 12, first full paragraph) it was stated that:

> *Patent owner, however, is once again arguing features which are not in the claims. Patented claims 1 and 17 do not require a statistically significant number of samples. Rather the patent claims recite collecting a plurality of portions from a plurality of test lots comprising one or more specimens i.e. they encompass collecting a few as two (a plurality) from two (a plurality) of test lots, each test lot comprising as little as a single specimen.*

In response to this statement in the ACP, claims 1 and 17 were amended to further clarify that "each" test lot is separately sampled "to conform to a statistically-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..." The word "each" together with the words "separately sampled" and "the test lot" clearly require that a sufficient number of samples must be gathered from "each" test lot in order to validate that particular test lot for release. Since there is a "plurality" of test lots involved, as required by step a), the total number of samples required is the statistical requirement for each lot multiplied by the total number of separate lots, which must be at least two. The ACP's treatment of the word "specimen" is also misleading, since "specimen," as recited in the claims, may be something like a beef carcass or a 1000 pound block of cheese, where even if there is only one specimen/sample, a statistically significant number of portions are taken from that single specimen/sample.

The ACP (p.12, first full paragraph) erroneously equates the foregoing requirements with those of Jarvis, when it states:

> *Jarvis teaches collecting from 1 to 100 samples (see Table 8.9 at page 138) and exemplified collecting 10 samples (page 141). Each of the individual samples meets the claimed limitations of a test lot comprising one or more specimens.*

The language "each of the individual samples meets the claimed limitations of a test lot" makes no sense in terms of the present claim language. When Jarvis uses the word "sample" he clearly intends this to mean physical item or piece of the food product tested. The meaning is identical to the word "portion" as used in the claims. On the other hand, claim 1, step b), states that "separate test lot samples" are formed by combining "portions", and, indeed, not an arbitrary number, but rather that number which is required by a statistically-based sampling plan. Obviously, the "samples" i.e. portions of Jarvis do not meet this requirement, since they are single portions of food.

The ACP (p.12, first full paragraph) continues to mischaracterize the patent teachings:

> *There is no disclosure in the '584 patent specification of any particular number of samples required for validation to occur. There is no disclosure as to what number is a statistically significant number of samples.*

First, this is incorrect, as the patent (col. 2, ll. 13-16) teaches taking an exemplary 60 samples for a typical plan, and teaches (col. 10, ll. 2-36) other exemplary sampling numbers.

Second, even if this was not true, the Patent provides a method for simultaneously proving that multiple, particular lots of food are uncontaminated and validated for release into commerce according to government-defined criteria. The number of portions that must be gathered from each lot for testing is determined by statistical significance requirements in government regulations and industry standards, which are cited extensively in prior art i.e. FSIS, Midura, etc. This number is determined by the needs and requirements of the user of Patented method as an independent variable. Although the Patent does so, there is no reason that the Patent should specify any particular number, since the utility of the method is not bounded in this manner. The Patented method differs from the prior art in that the statistically significant number of portions, whatever it is, must be present in "each" set of "separate test lot samples" (corresponding to separate test lots), as required in step b). No such teaching, which is *sine qua non* to the unique advantages of the patented method, is present in the prior art.

The ACP ((p. 12, first full paragraph) continues to mischaracterize the art:

> *Indeed, it would appear that the terms "inter-lot" and "intra-lot" are arbitrary designations based on how one defines a "lot". The instant claims recite a test lot as an assemblage of "one or more specimens; therefore, a single specimen falls within the definition of a "lot", as is recited in the patented claims.*

The statement is irrelevant. Why is it important if a test lot consists of only a single specimen? The ACP does not explain why this is important, so we have to speculate. It appears, perhaps, that the theory is that if only a single specimen exists in a test lot, then there can be only a single portion available to put into an enrichment, therefore, the argument goes, a single portion for the Patented method is like a single portion (i.e. food particle) being put into an enrichment in Jarvis. The ACP is thus attempting to collapse the Patented method into something that fits within Jarvis method (2), which involves single portions going into separate enrichments.

If this is what the ACP intended, it is false. A single-specimen test lot might be something like a whole beef carcass, or perhaps a 1000 pound block of cheese. But the Patented Method requires that a statistically-significant number of "portions" be taken from the test lot. Therefore, if the required statistical number is, e.g., N=60, then 60 shreds of cheese would be taken from the block, or 60 pieces of tissue would be taken from the carcass. At this point, the equivalence with Jarvis terminates, since there would be N=60 portions that would become the "test lot sample" per Claim 1. The fact that these N=60 portions came from a single carcass "specimen" is not relevant.

This attempt to equate a single specimen test lot with a "sample" going into an enrichment in Jarvis is bizarre in another respect. For example, if a whole beef carcass is the single "specimen" in a lot, and the single specimen of a lot is what goes into a Jarvis enrichment, then the ACP implies that a whole beef carcass would be placed into a Jarvis enrichment. In other words, not only would a very large enrichment container be required, but also the entire single-specimen lot would be consumed by the test itself. This is an absurd construction.

The ACP (p.12, first full paragraph) also makes an <u>incorrect</u> statement about "validation":

> *Furthermore, the '584 specification defines "validation" as determining that a particular sample tests negative using a detection assay. There is no disclosure in the '584 patent specification of a particular number of samples.*

This statement is simply false. The term "sample" in the claims denotes a statistically significant number of portions placed into an enrichment, either as a "separate test lot sample" in step b) of Claim 1 or as modular composite sample in step d). <u>This use of the word "sample" is not the</u>

same as in Jarvis, the Jarvis "sample" being most comparable to a "portion" as that term is used in the Patent. A patentee is entitled to be his/her own lexicographer.

The RAN at page 14 presents rebuttals to Patent Owner's arguments concerning Ground #1 for the rejection. These rebuttals are misleading and unsupportable. For example, the RAN states "...Patent owner argues that Jarvis is limited to testing a single sample or lot or as opposed to separate test lot samples or separate test lots." The RAN then supposedly refutes the argument by pointing out that Jarvis Example 8.4 involved testing of three different lots of food.

But Patent Owner never argued that the Jarvis MPN method (Jarvis pp. 135-138) could only be used to test a single lot of food. Obviously, the Jarvis Ex. 8.4 Method, like any other testing method in the universe, may be performed an unlimited number of times upon different lots of food. A proper characterization of Patent Owner's argument is rather to say that "each particular use of the Jarvis method pertains only to samples gathered from single lot of food." Thus, in the Jarvis Example 8.4 method, when the method was being applied to lot A, only samples from lot A were involved in arriving at a result. When the method was being applied to lot B, then only samples from lot B were in play, etc. At no point in time does the Jarvis 8.4 method require that sample materials from diverse lots of food be combined into a single container for a collective test. The Jarvis test is like a simple tool, such as a hammer. A hammer can obviously independently pound multiple nails, say nails A, B, and C. In this trivial sense, a hammer is adapted to nailing multiple nails. This is analogous to what Jarvis does for multiple lots of food. However, the hammer, like Jarvis, deals with one and only one nail at a time. It must first pound nail A, then B, then C, and in this sense, a hammer deal only with a single nail at a time. Likewise, Jarvis deals only with a single lot at any one time.

In contrast, claims 1 and 17 define a method for simultaneously dealing with multiple lots of food, requiring, for a single application of the method, that samples from multiple lots be employed. This is analogous to a new kind of hammer that is capable of nailing multiple nails at one time: For example, with a single swing, nails A, B, and C would all be driven home. Likewise, multiple lots of food may be statistically tested at the same time, which is not within the teaching of Jarvis.

Another way to illustrate the difference between simultaneous-multiple-application versus serial-multiple-application is this: A common medical thermometer is placed into the

mouth of one patient at a time to read a temperature. An alternative invention, for example, an infrared camera, could simultaneously measure the body temperature of dozens of people in a crowd by taking a single picture and then looking at the pixels corresponding to each person's forehead. To use the RAN's logic, these two inventions both "apply to multiple people", because the common thermometer could be used repeatedly upon different people until the entire crowd had been covered. However, this is an application to "multiple people" only in a trivial sense. Any sensor may be used in a serial fashion to make multiple measurements upon different objects. The infrared camera approach, in contrast, covers the entire crowd in one go, thus covering "multiple people" simultaneously, and in a non-trivial sense. This is analogous to the difference between Jarvis and the method of claims 1 and 17. This might also be referred to as the "single ball musket versus machine-gun fallacy", as is explained under Ground #2, *infra*.

The RAN p. 14 contains additional confused and illogical statements on this point. For example, it is stated that:

> *The use of the terms "pre-enrichment series" and "duplicate pre-enrichment" in this passage clearly indicates tiered testing, where the tiered testing preserves the information of the underlying test lot samples. Thus the testing of composite samples is intended for single or multiple underlying lots.*

The foregoing is a complete *non sequitor*. Whether a test is "tiered" or not has nothing to do with where the samples come from. In his discussion of composite sampling Jarvis at no time makes reference to simultaneous use of samples from multiple lots or in any manner deals with the topic of multiple-lot testing. The above RAN statement is baseless and carelessness as further shown in that the "duplicate pre-enrichment" mentioned in Jarvis pertains **only** to Jarvis method (3), where the term "duplicate" means nothing except that Jarvis chose to use two enrichments, with half the portions being placed into each. However, Jarvis says that only method (2) has the advantage of preserving information concerning the underlying samples.

The RAN p. 15, first full ¶, continues:

> *Patent owner also makes statements regarding the testing in Jarvis as not for the purpose of allowing portions of the carloads to be released, but merely as allowing for additional tests, (quoting Jarvis) "... so that some indication of the proportion of the contaminated samples can be obtained." ... The totality of Jarvis is directed to testing food samples for microbial contamination. Common sense dictates that this testing is performed to ensure food safety before food is released to consumption.*

But the foregoing statement is an express admission by Jarvis that the follow-up testing would not permit release to commerce, but only "some indication of a proportion". The only reference which teaches how this follow-up testing may result in actual release to commerce is contained in the patent owner's Patent, i.e., that separate enrichments must each contain a statistically-significant number of portions, which is not taught or otherwise suggested by Jarvis, alone or in combination.

FSIS and **Midura**. The secondary references to FSIS and Midura stand only for the proposition that a statistically-based number of portions must be examined in order to release food into commerce. But there is no teaching or suggestion as to how to use this in the context of Jarvis, except what Jarvis himself does, i.e., Jarvis starts with N as the number required to validate a lot, and he either composites N in one lump in his method (1); or he uses separate enrichments in (2), or he arbitrarily divides the samples into two groups, and separately enriches these in (3). Jarvis (last sentence, p. 141) fundamentally ***teaches away*** from patent owner's Patent by stating that "only" method (2), which requires that each portion be in an individual enrichment, will allow the advantage of (limited utility) follow-up testing. The present claimed invention does not use enrichments with individual samples as in Jarvis method (2).

Accordingly, the rejection of Claims 1 and 17 and all dependent claims under Ground #1 should be reversed.

In view of the foregoing, Patent owner respectfully submits that Claims 1-4, 8, 10, 11, 13, 17-19, 22, 24, 26, 27, and 31 are patentable over the cited references in combination or alone. Withdrawal of the rejection of claims under 35 U.S.C. §103 is respectfully requested.

Ground #2: Rejection of Claims 1-4, 6-11, 13, 17-18, 22, and 31 Under 35 U.S.C. §103(a):

The rejection of Claims 1-4, 6-11, 13, 17-18, 22, and 31 under 35 U.S.C. §103(a) is maintained in the RAN for allegedly being unpatentable over FSIS in view of Price and Midura. Patent owner respectfully traverses the rejection, because Price, which is like Jarvis method (2) has been fundamentally misconstrued, and FSIS and Midura merely teach the number of portions required to achieve statistical significance in a sampling scheme for a single sampled lot.

The claims are not obvious over the combination of references

In maintaining the rejection of the claims, the RAN (p. 22) states that FSIS teaches collecting a plurality of pieces per lot, and teaches collecting additional samples after remedial or corrective action following a test result. The RAN, amazingly, states that FSIS differs in that it is silent as to the testing methods used and fails to specify foods other than ground beef products. The RAN (pp. 22-23) states that Price teaches testing multiple food samples for *Salmonella*, pooling pre-enrichment broths into composite lots, incubating the composite enrichment broth, and subsequently identifying *Salmonella*. The RAN states that Midura teaches statistical based sampling plans. Based on this, the RAN incorrectly concludes that the presently claimed invention is obvious. This is a **gross** over-extension of the extremely limited teachings of the FSIS, alone or in combination with Price and/or Midura.

Ground #2 is duplicative of Ground #1, in that FSIS and Midura are merely simple references teaching the number of portions required to achieve statistical significance in a sampling scheme. Furthermore, Price merely teaches Jarvis method (2) i.e. a single-portion per pre-enrichment scheme. Thus, patent owner's comments about Jarvis under Ground #1 directly apply to Price, and patent owner's comments under Ground #1 apply generally and directly to Ground #2.

The Examiner mistakenly argues (RAN p. 26, ¶ 2) that Price applies to "multiple lots of food" because Price shows his method being applied to various, disparate lots of food at different times. But these are mere serial applications of a single-lot testing method. The Examiner could similarly argue that a one-shot/ball musket is like a machine gun, since a musket can be fired numerous times, and thus shoots "multiple bullets". However, what the one-ball musket cannot do is fire multiple bullets in one burst, just as Price, alone or in combination with that FSIS and Midura cannot perform the task of Claims 1 and 17, which is to simultaneously operate upon "a plurality of separate test lots each to be validated for release into commerce."

To arrive at Claims 1 and 17, Price would need to be altered so that the pre-enrichments are loaded not with single portions, but rather with "a number of portions to be taken sufficient to provide for validation and release of the test lot into commerce". However, Price (p. 682, ¶ 2) ***teaches away*** from the use of anything more than a single portion in each pre-enrichment:

> *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, in the other hand, the samples are individually pre-enriched, the actively growing salmonella 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.*

(Note: Price uses the word "sample" to mean "portion", as defined in the Patent)

Furthermore, Price states that use of cultures containing only a single sample of a food product "permits small portions to be retained for subsequent analysis" whereas "direct pooling of food ***samples*** (*emphasis supplied)* into pre-enrichment results in the loss of individual sample identity". However, Claims 1 and 17 require the use of "a plurality of portions ... sufficient to provide for validation and release" go into each pre-enrichment. This is directly contrary to the quoted teaching of Price to the effect that pre-enrichments should contain only a single sample, or, to use the terminology of the Patent, single "portions".

Furthermore, The Examiner, at page 23 of the ACP, (see also RAN p. 27) states that "With respect to claim 4, which specifies that the individual test lot samples are incubated in enrichment medium, Price provides motivation to substitute selective enrichment broth for the pre-enrichment broth in the individual broth cultures at page 682, first full paragraph, wherein Price teaches,

> 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.

The present rejection is one of obviousness, not anticipation. Based on this teaching found in Price, one of ordinary skill in the art at the time of the invention would have considered selective enrichment broth to be an equivalent medium for the individual broth cultures and would have been motivated to substitute selective enrichment medium for pre-enrichment medium in, the individual broth cultures disclosed by FSIS in view of Price and Midura whenever the possible presence of interfering organisms would be of concern."

Patentee respectfully points out, however, that the above quotation from Price is in the context of discussing whether, <u>for colony plate assays</u>, "pooled pre-enrichments are less sensitive than individual tests in the presence of interfering organisms ("probably not known"), and relates

to a discussion of whether each pre-enrichment culture should be tested by plating or whether pre-enrichment cultures can be pooled for purposes of detecting *Salmonella* by plating colonies, and concludes that if numerous non-*Salmonella* colonies are detected, the retained pre-enrichment cultures should be tested individually. Price then states that:

> "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.
>
> Patentee contends that since the problem being addressed by Price is to reduce the background of competing organisms, the statements by Price about the use of direct selective enrichments, two selective enrichments, or possibly even a reduction in the incubation time of the pre-enrichment cultures would be regarded by one of skill in the art, as being limited to the case of **non-lumped individual pre-enrichment cultures**, which would be expected to have fewer background/competitive organism problems; that is, one would not ***exacerbate*** the background problem by subjecting a *lumped* test sample to direct selective enrichments or two selective enrichments. This conclusion is further supported by Price itself, as pointed out by the Examiner at page 22 of the ACP, stating that "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 salmonella might be expected to compete more favorably with organisms introduced from other samples when pooled at the enrichment stage" (emphasis added)."

Additionally, Patentee points out that contrary to the Examiner's urging, Price, alone or in combination, does NOT teach combining a plurality of samples from each of a plurality of test lots as presently claimed.

Accordingly, the rejection of Claims 1 and 17 and all dependent claims under Ground #2 should be reversed.

In view of the foregoing, Patent owner respectfully submits that Claims 1-4, 6-11, 13, 17-18, 22, and 31 are patentable over the cited references in combination or alone. Withdrawal of the rejection of claims under 35 U.S.C. §103 is respectfully requested.

Ground #3: Rejection of Claims 1 and 17 Under 35 U.S.C. §103(a):

The rejection of Claims 1 and 17 under 35 U.S.C. §103(a) is maintained for allegedly being unpatentable over Jarvis in view of "Patent owner's admissions" regarding the state of the prior art. Patent owner respectfully traverses the rejection, because the Examiner has grossly mischaracterized both Jarvis and Patent owner's alleged statement in the background section of the Patent.

The claims are not obvious over the combination of references

In maintaining the rejection of the claims, the Examiner (RAN pages 29-30) again mischaracterizes and overextends the limited teachings of Jarvis. The Examiner states that Jarvis characterizes the incubation medium for the individual test lot samples as "pre-enrichment" medium, while the instant claims do not specify the incubation medium. The Examiner states (RAN page 31) that Patent owner admits that obtaining a plurality of portions from test lots and combining portions for subsequent testing was known in the art.

The Examiner has mischaracterized the Patent owner statement in the background section of the Patent, which is merely a statement that aggregation of a plurality of portions into a combined sample was known in the art (e.g., the art that has been used, for example, in the meat fabrication business for the last 40 years). The statement referred to, however, does **not** say that the patented invention that relates to simultaneous testing of multiple individual lots (each for release into commerce) as recited in claims 1 and 17, was known in the art—which is was not.

Ground #3 is based upon the same synthetic confusion between single-lot testing methods applied in a serial fashion versus the method of Claims 1 and 17, i.e. the "single-ball musket versus machine-gun fallacy" described previously. The background section of the patent describes that multiple "lots" have been tested in the prior art based upon various sampling plans. But the patent owner does not say that multiple lots were previously tested simultaneously within the confines of a single method as in Claims 1 and 17. In terms of the fallacy, the patent's background section merely states that "single-ball muskets" were used in the past to shoot multiple bullets, albeit one at a time in a serial fashion. The remainder of Ground #3 is just a rehash of the Jarvis discussions under Ground #1, so the discussions of Jarvis under the previous Grounds are incorporated by reference here.

Accordingly, the rejection of Claims 1 and 17 and all dependent claims under Ground #3 should be reversed.

In view of the foregoing, Patent owner respectfully submits that Claims 1 and 17 are patentable over the cited references in combination or alone. Withdrawal of the rejection of claims under 35 U.S.C. §103 is respectfully requested.

Ground #5: Rejection of Claims 1 and 17 Under 35 U.S.C. §103(a):

The rejection of Claims 1 and 17 under 35 U.S.C. §103(a) is maintained for allegedly being unpatentable over Andrews in view of Price and Midura. Patent owner respectfully traverses the rejection, because Andrews merely teaches aggregation of portions from a single lot, and Price, like Jarvis, has been fundamentally misconstrued by the Examiner.

The claims are not obvious over the combination of references

In maintaining the rejection of the claims, the Examiner states (RAN page 34) that Andrews teaches testing foods for Salmonella by collecting a plurality of portions from the analyzed food and aggregating the portions to comprise a large sample unit used to determine the acceptability of the entire lot of food. The Examiner then reiterates the mischaracterizations of Price and Midura (RAN pages 34-35), and states that it would have been obvious to have incubated the pre-enrichment cultures of Andrews separately, as suggested by Price, to specifically identify the contaminated food lots, and that one of skill in the art would have found it obvious to collect a plurality of sample according to a statistically-based sampling plan and/or to have performed multiple sampling runs, as recommended by Midura to detect highly pathogenic organisms.

Andrews, like the previously discussed FSIS and Midura references, merely teaches how many samples should be taken from a particular lot of food under a sampling plan. Continuing with the single-ball musket analogy, this is like a discussion of how much powder it takes to fire a single musket ball.

The only way to arrive at Claims 1 and 17 using Andrews, would be to apply Andrews-style sample collection ("portions" in terms of the claim language) to multiple separate lots of food simultaneously. The collection of portions from each separate lot would need to be

maintained separately, and introduced into separate enrichment broths. Ground #5 relies upon Price for the concept of separate enrichment broths. However, Price ***teaches away*** from pre-enrichment broths containing more than a single portion:

> *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, in the other hand, the samples are individually pre-enriched, the actively growing salmonella 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.*

(see also discussion above at pages 20-21 herein in relation to Ground #2). This expressly de-motivates a combination of Andrews and Price. Midura, which merely discusses what number of samples might be appropriate to achieve statistical significance, adds nothing since Price would have this number spread out among many enrichment broths, and not individually provided in each enrichment as is required in Claims 1 and 17. <u>These references, alone or in combination teach nothing except testing of a single lots of food per application of the method, as opposed to multi-lot simultaneous testing as taught by the Patent</u>.

Accordingly, the rejection of Claims 1 and 17 and all dependent claims under Ground #5 should be reversed.

In view of the foregoing, Patent owner respectfully submits that Claims 1 and 17 are patentable over the cited references in combination or alone. Withdrawal of the rejection of claims under 35 U.S.C. §103 is respectfully requested.

Ground #6: Rejection of Claims 1 and 17 Under 35 U.S.C. §103(a):

The rejection of Claims 1 and 17 under 35 U.S.C. §103(a) is maintained for allegedly being unpatentable over Silliker in view of Price and Midura. Patent owner respectfully traverses the rejection.

<u>The claims are not obvious over the combination of references</u>

In maintaining the rejection of the claims, the Examiner states (RAN page 36) that Silliker teaches sampling foods for *Salmonella* contamination comprising collecting a plurality of subsamples, incubating these in individual cultures of pre-enrichment broth, and wet

compositing the pre-enrichment cultures in groups of five or three. The Examiner states that Silliker differs from the claimed method in the Silliker defines the incubation step of individual cultures as "pre-enrichment." The Examiner then reiterates the mischaracterizations of Price and Midura (RAN pages 36-37). The Examiner states that it would have been obvious to combine the composite testing of Silliker with the individual tests of subsample pre-enrichments upon obtaining a positive result for the composite sample, as suggested by Price.

Silliker, however, teaches only how to test a single lot of food by taking a 1500g sample from the lot, dividing this into various numbers of subsamples, lumping these subsamples into groups of various sizes, and optionally grouping arbitrary numbers of subsamples into pre-enrichments prior to testing. On the other hand, Price specifically taught that lumping subsamples into groups or putting more than one subsample into a pre-enrichment would be undesirable:

> *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, in the other hand, the samples are individually pre-enriched, the actively growing salmonella 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.*

The RAN (p. 38, line 5), states that "Silliker clearly teaches wet compositing of different test lots", but this assertion makes use of the "single-ball musket versus machine-gun" fallacy discussed previously, i.e., the different lots are tested with separate, serial applications of Silliker's method, and not as part of a system for validating multiple lots simultaneously. Midura is relied upon only for teachings concerning how many samples need to be taken to achieve statistical significance and validation for release in Salmonellae testing.

To arrive at the Claims 1 and 17 from these references, it would be necessary to (a) ignore Price's objections to putting multiple portions into an enrichment; (b) collect multiple sets of portions from different lots instead of a single lot, with each such set having a statistically significant number of portions; and (c) modify Silliker so that each enrichment is loaded with one of these statistically significant sets rather than the arbitrary numbers of portions from a single lot as actually used by Silliker. The mere general teaching of Midura concerning the need to collect more samples does not motivate placement of more samples into the pre-enrichments,

especially in view of Price's discouraging words on this point. Such teachings might just as well teach use of more individual pre-enrichments, consistent with Price. (See also above discussion of Price at pages 20-21 under Ground #2).

The RAN (p. 36, line 2) admits that "Silliker does not teach testing of individual subsamples separately upon obtaining a positive result from the composite sample". This is not surprising—since Silliker takes all of his samples from a single lot of food, a positive result indicates that the lot is contaminated and must be destroyed in any event; thus, there is no motive to investigate particular subsamples.

The RAN (p. 38, line 8) states that Silliker "...specifically refers to preservation of the underlying lots with the use of the term subsamples". This statement is **completely without foundation**. The term "subsamples" merely means a subdivision of a sample, containing absolutely no implication about "preservation". There is no teaching that efforts toward later testing of the "preserved" materials were made, or that these materials were maintained in environmental conditions suitable for later testing.

Accordingly, the rejection of Claims 1 and 17 and all dependent claims under Ground #6 should be reversed.

In view of the foregoing, Patent owner respectfully submits that Claims 1 and 17 are patentable over the cited references in combination or alone. Withdrawal of the rejection of claims under 35 U.S.C. §103 is respectfully requested.

Ground #7: Rejection of Claims 1 and 17 Under 35 U.S.C. §103(a):

The rejection of Claims 1 and 17 under 35 U.S.C. §103(a) is maintained for allegedly being unpatentable over Gabis in view of Price and Midura. Patent owner respectfully traverses the rejection.

The claims are not obvious over the combination of references

In maintaining the rejection of the claims, the Examiner states (RAN page 40) that Gabis discloses methods for sampling an testing high moisture foods by collecting 13 specimens, inoculating each individual specimen individually into pre-enrichment broth cultures, incubation of each pre-enrichment culture, pooling portions of each individual pre-enrichment culture into a

composite, incubating the composite enrichment culture, and subsequently recovering and identifying the *Salmonellae*. The Examiner then reiterates the mischaracterizations of Price and Midura (RAN pages 40-41). The Examiner states that it would have been obvious to follow the composite testing of Gabis with the individual tests of subsample pre-enrichments upon obtaining a positive result for the composite sample, as suggested by Price.

Gabis, however, merely teaches taking samples from a single lot of food and either putting all samples enrichment as a lump, comparable to Jarvis method (1) or putting each sample into a single enrichment, comparable to Jarvis method (2). Midura merely teaches what quantity of portions might comprise a statistically significant sample for a single lot of food. Neither of these references teach the concept of taking multiple portions of multiple lots of food and placing these into separate enrichments. Price does teach the concept of placing single samples into enrichments, but specifically teaches against placing more than single samples therein:

> *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, in the other hand, the samples are individually pre-enriched, the actively growing salmonella 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.*

Since each of the references individually teach only testing methods which test one lot of food at a time, a combination of these references teaches only as much. There is no suggestion or motivation for taking portions from multiple lots of food in a manner which can validate multiple lots as part of a single instance of a single method, as in present Claims 1 and 17.

The RAN (p.41, line 22) states that "Gabis is not limited to single lots as it studies multiple meat and egg product samples." This is another example of the "single-ball musket versus machine-gun" fallacy, since Gabis shows only serial use of a method upon one lot of food at a time, with materials from separate lots never coming into contact with each other, as in Claim 1 step d), which requires:

> *"removing portions of each separate test lot sample, and combining the removed portions to provide a modular composite sample;"*

Accordingly, the rejection of Claims 1 and 17 and all dependent claims under Ground #7 should be reversed.

In view of the foregoing, Patent owner respectfully submits that Claims 1 and 17 are patentable over the cited references in combination or alone. Withdrawal of the rejection of claims under 35 U.S.C. §103 is respectfully requested.

Ground #8: Rejection of Claims 1 and 17 Under 35 U.S.C. §103(a):

The rejection of Claims 1 and 17 under 35 U.S.C. §103(a) is maintained for allegedly being unpatentable over Jarvis in view of BAM. Patent owner respectfully traverses the rejection.

The claims are not obvious over the combination of references

In maintaining the rejection of the claims, the Examiner again fundamentally mischaracterizes Jarvis (see discussion of the limited teachings of Jarvis under Patent owner's rebuttal of Ground #1, *supra*). The Examiner combines BAM for its teachings of collecting a plurality of samples according to a statistically-based sampling plan for *Salmonella*.

Ground #8 is a merely minor variation of previous grounds based upon Jarvis, with the reference BAM standing in for Midura or FSIS as a simple teaching concerning the number of samples that need to be taken for statistical significance purposes.

It should be noted that the RAN's description of Jarvis in this instance (RAN. p.42, line 23) is misleading in the use of the word "specimen", in place of "samples", as written by Jarvis, and in place of "portions", as used in Claims 1 and 17 for this purpose. As used by in the Patent, "specimens" refer to pieces of food product which make up the underlying lot of food to be sampled; "portions" are taken from the specimen or specimens of which the lot consists. For example, the lot might consist of one or more hanging beef carcasses, which carcasses are the "specimens", as defined in the patent. Then, portions are severed from the specimens in a statistically significant quantity per Claim 1, step a) to form a test lot sample, per Claim 1, step b). This confusion between "specimen" and "portion" appears to have been introduced by the Requestor in order to support various lines of argument set forth, but which have herein again been refuted.

The RAN (p. 43, line 5) further misuses the term "test lot samples", where it is stated that "Jarvis defines the medium used for incubation of the individual test lot samples as a ...". This is misleading since Jarvis never uses the term "test lot samples". Per the language of Claim 1, there is no such thing as an "individual test lot sample", in the sense of, for example, individual particles of beef. Claim 1, step b) defines a "test lot sample" to be a collection of a plurality of "portions" taken from the specimens which comprise a test lot. In contrast to the individual particles of food that go separately into a Jarvis method (2) enrichment, each of the enrichments of Claims 1 and 17 is loaded with an entire "test lot sample", which comprises a statistically significant number of portions. Moreover, Jarvis method (2) enrichments all contain food taken from the same lot.

The RAN (p. 43, line 20) admits that:

> *Jarvis does not specify collecting plural portions from each of a plurality of test lots, each test lot comprising one or more specimens, and combining the portions to provide a corresponding set of separate test lot samples for testing.*

Given the foregoing as a starting point, BAM adds nothing more than the concept that a certain number of food portions need to be taken from a single lot in order to provide adequate testing for release into commerce. A logical combination of Jarvis and BAM would be to do what Jarvis method (2) suggests, i.e., provide a separate enrichment for each individual portion. Jarvis teaches a one-lot at a time testing method, and BAM merely teaches how many samples should be taken in order to test this one lot. Nothing in either reference motivates simultaneous gathering of samples from multiple lots; maintaining the separate character of groups of these samples on a per lot basis. Ironically, the RAN (p. 44, line 4) confirms the idea that Jarvis and BAM pertain only "a" single lot, where it is stated:

> *It would have been prima facie obvious to one of ordinary skill in the art at the time of the invention to have collected a (emphasis supplied) plurality of food samples in a statistically-based sampling plan.*

Of course, Claims 1 and 17, step a) require the collection of multiple, separate pluralities of portions from a plurality of lots of food, which is something **not** taught by Jarvis and BAM.

The RAN statement (p. 44, line 17) that "Jarvis teaches testing multiple lots" has been previously refuted under Grounds #1 and #2, *supra*, specifically in discussions of the "single-ball musket versus machine-gun fallacy".

Accordingly, the rejection of Claims 1 and 17 and all dependent claims under Ground #8 should be reversed.

In view of the foregoing, Patent owner respectfully submits that Claims 1 and 17 are patentable over the cited references in combination or alone. Withdrawal of the rejection of claims under 35 U.S.C. §103 is respectfully requested.

Ground #9: Rejection of Claims 1 and 17 Under 35 U.S.C. §103(a):

The rejection of Claims 1 and 17 under 35 U.S.C. §103(a) is maintained for allegedly being unpatentable over Price in view of BAM and Davies. Patent owner respectfully traverses the rejection.

The claims are not obvious over the combination of references

In maintaining the rejection of the claims, the Examiner reiterates the mischaracterization of limited teachings of Price. The Examiner (RAN pages 45-46) combines BAM for its teachings of collecting a plurality of samples according to a sampling plan for *Salmonella*. The Examiner states (RAN page 36) that Davies teaches sampling and testing pooled samples taken from different pig herds, incubating composite pooled samples into enrichment medium and subsequently testing from *Salmonella*.

Ground #9 is essentially duplicative of earlier Grounds #1, #5, and #6 which also involve Price. BAM merely teaches how many samples should be taken from a lot when testing for salmonella; and Davies merely teaches the taking of various samples from various pig herds. The combination proposed by Ground #9 would have the single portion pre-enrichments of Price loaded, instead, with a number of portions determined by the BAM, but is directly against the teachings of Price, who emphasizes the disadvantages of multiple portions being placed into a pre-enrichment:

> *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, in the other hand, the samples are individually pre-enriched, the actively growing salmonella 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.*

This is a clear ***teaching-away***, it is not reasonable to combine Price and BAM in them manner proposed.

Price, BAM, and Davies all concern the testing of single lots, as opposed to simultaneous testing of samples from multiple lots as taught by patented Claims 1 and 17.

Davies involves taking of multiple samples from herds of pigs, which are directly analogous to lots of food. Per the RAN (p. 47, line 6), the samples are maintained with "separate herd designations" throughout, and at no point are enriched samples from one herd/lot combined with samples from any other herd/lot as is required by Claim 1 d) where is "...combining the removed portions to form a modular composite sample."

Specifically, the teachings of Davies et al. are limited to the use of pooled samples to provide a cheaper *Salmonella* monitoring program to identify infected finishing pig herds (e.g., see Abstract of Davies et al.; page 1017, column 1, second full paragraph; Table 4; Figure 2, etc.). Specifically, Davies et al. sampled meat juice, serum, caecal contents, carcass swabs, and pooled faeces from pig pens from 20 commercial finishing farms. The aim was to determine whether pooled serum and meat juice could be used to identify finishing pig herds with a high prevalence of infection by *Salmonella*. Specifically, comparisons were made between assay results of: *Salmonella* culture, individual ELISA tests on serum and meat juice, and pooled samples of serum and meat juice. While *Salmonella* was isolated from the samples of 19 of the 20 commercial farms, the ELISA tests failed to show significant correlation with caecal carriage of *Salmonella* or contamination of carcasses. However, mean optical density (O.D.) from pools of sera (from 5, 10 or 20 sera) showed a significant correlation with the *Salmonella* status of farm pen faeces. The conclusion of Davies et al. is that while there is a generally poor correlation between serological and bacteriological results, pooled serum or meat juice samples can be used as a cheaper substitute for serological screening of farms for Salmonella than individual samples. Davies et al., alone or in combination with Price and/or BAM therefore, teaches pooling of samples to provide cheaper monitoring, but nothing more.

Davies et al. teaches only simple sample pooling to make a classical composite (lumped) sample for enrichment and then assaying the enriched sample directly. For example, as explained on page 1017 and 1018 (under "Laboratory tests"), pooled pen faeces samples are added to media and incubated (enriched) prior to assay. Specifically, Davies et al. teaches only classical sample pooling to make a composite sample for enrichment and testing. Significantly however, none of the pooled samples of Davies et al. were used after enrichment to prepare the equivalent of a "modular composite sample" as presently claimed by Patentee.

To arrive at the method of Claims 1 and 17 based upon these references, one would first need to ignore Price's teachings against putting more than one portion into a pre-enrichment broth. Then one would need to change the teaching of BAM which teaches that a given number N samples should be used in a testing method, and rather use N samples for each pre-enrichment broth, rather than using N as a total number of samples for all enrichment broths, as in Price. Then one would need to ignore the teaching of Davies that samples from each her/lot should be kept separate until the point of detection, rather than being combined at some point before the detection step.

To arrive at the Patented method from these references one must change both the objective of the work, i.e., the task of testing individual lots of food on a one-by-one basis into the different goal of testing multiple lots in a simultaneous mode. In addition to changing the objective, one must then implement physical changes in what is done, i.e., contrary to the teachings of Price, the pre-enrichments must be loaded with many portions, rather than just single portions. In addition, one must choose the correct point in the process for applying the requirements of the BAM, i.e., whereas normally BAM sets the overall number of samples which must be gathered to implement testing of a single lot, one must now use the BAM number to drive separate collections of samples from each of a plurality of lots, then maintain these collections separately, and then load each into separate enrichment. Thus, the BAM number is used multiple times in working the method, i.e., once for each separate lot of food to be tested, whereas BAM in previous methods was used only once, for the purpose of setting the total number of portions used in the overall method. This is a use of the BAM number not seen or hinted at in any prior art. The only reference which motivates this use is the Patent owner's Patent itself.

Accordingly, the rejection of Claims 1 and 17 and all dependent claims under Ground #9 should be reversed.

In view of the foregoing, Patent owner respectfully submits that Claims 1 and 17 are patentable over the cited references alone or in combination. Withdrawal of the rejection of claims under 35 U.S.C. §103 is respectfully requested.

Ground #14: Rejection of Claims 5, 23, 29, and 30 Under 35 U.S.C. §103(a):

The rejection of Claims 5, 23, 29, and 30 under 35 U.S.C. §103(a) is maintained for allegedly being unpatentable over Jarvis in view of FSIS, Midura, and Entis. Applicants respectfully traverse the rejection.

The claims are not obvious over the combination of references

In maintaining the rejection of the claims, the Examiner the Examiner reiterates the mischaracterization of limited teachings of Jarvis as set forth under Ground #1. The Examiner states that "[n]one of Jarvis, FSIS, or Midura teach concentrating the microbial agent from the modular composite sample by immunomagnetic bead separation or affinity chromatography and none teach testing the modular composite samples by a DNA based method, an immune-chemistry based method or a biosensor based method." The Examiner states that "Entis teaches rapid procedures for the detection, identification, and enumeration of microorganisms in foods (see page 89, third paragraph).

Since Ground #14 incorporates Ground #1 by reference, (RAN p. 51), rejection of the dependent claims of Ground #14 should be reversed with the reversal of Ground #1. Entis, alone or in combination, does not cure these deficiencies of the other references.

In view of the foregoing, Patent owner respectfully submits that Claims 5, 23, 29, and 30 are patentable over the cited references alone or in combination. Withdrawal of the rejection of claims under 35 U.S.C. §103 is respectfully requested.

Ground #15: Rejection of Claims 5, 6, 23, 29, and 30 Under 35 U.S.C. §103(a):

The rejection of Claims 5, 6, 23, 29, and 30 under 35 U.S.C. §103(a) is maintained for allegedly being unpatentable over FSIS in view of Price, Midura, and Entis. Applicants respectfully traverse the rejection.

The claims are not obvious over the combination of references

In maintaining the rejection of the claims, the Examiner incorporates Ground #2 by reference, (RAN. p52), rejection of the dependent claims of Ground #15 should be reversed with the reversal of Ground #2. Entis, alone or in combination, does not cure these deficiencies of the other references discussed under Ground #2.

In view of the foregoing, Patent owner respectfully submits that Claims 5, 6, 23, 29, and 30 are patentable over the cited references alone or in combination. Withdrawal of the rejection of claims under 35 U.S.C. §103 is respectfully requested.

Ground #21: Rejection of Claim 21 Under 35 U.S.C. §103(a):

The rejection of Claim 21, under 35 U.S.C. §103(a), is maintained for allegedly being unpatentable over Jarvis in combination with FSIS, Midura, and Cousin. Patent owner respectfully traverses the rejection.

The claim is not obvious over the combination of references

In maintaining the rejection of the claim, the Examiner incorporates Ground #1 by reference, (RAN. p58), rejection of the dependent claim of Ground #21 should be reversed with the reversal of Ground #1.

The Examiner (ACP) states that "Cousin discloses that certain bacteria (psychrotrophs) are able to grow in foods at low temperatures and are thus the primary organisms responsible for spoilage of refrigerated foods. Cousin teaches that such organisms are found in meats, poultry, fish, and seafood and include *Listeria* spp., among others (see page 169, column 2, last paragraph). Cousin teaches that such organisms can be detected by culture and microscopic methods (see section 13.21 beginning on page 160)."

Cousin, however, alone or in combination, does not cure these deficiencies of the other references discussed under Ground #1.

In view of the foregoing, Patent owner respectfully submits that Claim 21 is patentable over the cited references alone or in combination. Withdrawal of the rejection of claims under 35 U.S.C. §103 is respectfully requested.

Ground #22: Rejection of Claim 21 Under 35 U.S.C. §103(a):

The rejection of Claim 21, under 35 U.S.C. §103(a), is maintained for allegedly being unpatentable over FSIS in combination with Price, Midura, and Cousin. Patent owner respectfully traverses the rejection.

The claim is not obvious over the combination of references

In maintaining the rejection of the claim, the Examiner incorporates Ground #2 by reference, (RAN p. 60), rejection of the dependent claim of Ground #22 should be reversed with the reversal of Ground #2.

Cousin, discussed above, alone or in combination, does not cure these deficiencies of the other references discussed under Ground #2.

In view of the foregoing, Patent owner respectfully submits that Claim 21 is patentable over the cited references alone or in combination. Withdrawal of the rejection of claims under 35 U.S.C. §103 is respectfully requested.

Ground #23: Rejection of Claim 23 Under 35 U.S.C. §103(a):

The rejection of Claim 23, under 35 U.S.C. §103(a), is maintained for allegedly being unpatentable over Jarvis in combination with FSIS, Midura, and Price. Patent owner respectfully traverses the rejection.

The claim is not obvious over the combination of references

In maintaining the rejection of the claim, the Examiner incorporates Ground #1 by reference, (RAN p. 61), rejection of the dependent claim of Ground #23 should be reversed with the reversal of Ground #1.

Price, discussed above, alone or in combination, does not cure these deficiencies of the other references discussed under Ground #1.

In view of the foregoing, Patent owner respectfully submits that Claim 23 is patentable over the cited references alone or in combination. Withdrawal of the rejection of claims under 35 U.S.C. §103 is respectfully requested.

Ground #24: Rejection of Claim 23 Under 35 U.S.C. §103(a):

The rejection of Claims 24, 28, 32 and 33 under 35 U.S.C. §103(a), is maintained for allegedly being unpatentable over FSIS in combination with Price, Midura, and Gabis. Patent owner respectfully traverses the rejection.

The claims are not obvious over the combination of references

In maintaining the rejection of the claims, the Examiner incorporates Ground #2 by reference, (RAN p. 63), rejection of the dependent claims of Ground #24 should be reversed with the reversal of Ground #2.

The Examiner states (ACP) that "[n]one of FSIS, Price, or Midura teaches that the food to be tested is poultry, pork, sheep or bison. Additionally, although Price teaches a pooled enrichment broth for *Salmonella* (comprising a selective agent for *Salmonella* which inhibits competing organisms); none of FSIS, Price or Midura teaches that the individual test lot sample broths comprise a selective agent for the target organism."

The Examiner states (ACP) that "Gabis teaches a method of sampling, testing, and validating high moisture foods for microbial contamination comprising collecting a plurality of specimens, incubating each of the specimens individually into broth cultures, incubating the broth cultures to allow for levels of the target organism to increase, and pooling the broth cultures together to test for *Salmonella* (see page 663, abstract and first paragraph of the page; page 664, the paragraph bridging columns 1 and 2 and column 2, first two full paragraphs; and page 668, first full paragraph). Gabis teaches two alternative broth cultures for the individual samples, "preenrichment" broths not containing a selective agent and "direct selective enrichment" broth cultures containing an agent selective for *Salmonella* and inhibitory for competing bacteria (see page 664, *Enrichment Procedures* and Results). Gabis teaches

exemplary high moisture foods suitable for testing as pork and chicken (poultry) among others (page 665, Table l)."

Gabis, however, alone or in combination, does not cure these deficiencies of the other references discussed by Patent owner in rebuttal of Ground #2.

In view of the foregoing, Patent owner respectfully submits that Claim 23 is patentable over the cited references alone or in combination. Withdrawal of the rejection of claims under 35 U.S.C. §103 is respectfully requested.

Declarations

Appellant attaches six (6) Declarations, which are incorporated by reference into this Appeal Brief in their entirety in further support of the arguments presented herein:

Exhibit E: Declaration of Mohammad Koohmaraie;

Exhibit F: Declaration of Dr. Mansour Samadpour;

Exhibit G: Further Declaration of Dr. Mansour Samadpour;

Exhibit H: Declaration by Dr. Mansour Samadpour in Rebuttal of the Bailey Declaration;

Exhibit I: Declaration of Warren Mirtsching; and

Exhibit J: Declaration by Dr. James Byron.

Appellant requests that these Declarations be considered by the Board in support of the current Appeal Brief.

Appellant contends that Declaration (Exhibit E), which contains, *inter alia*, descriptions of the importance of the Patent Owner's invention in the world of food safety. In particular, Dr. Koohmaraie explains the importance and significance of wet composite inter-lot pooling, as claimed in the Patent Owner's patent, in the control of *E. coli* O157:H7 and other beef-borne pathogens.

Appellant contends that Declaration (Exhibit F), which contains, *inter alia*, descriptions of the importance of the Patent Owner's invention to control food-borne pathogens and the nexus between the claimed invention and the commercial success of the implemented procedures Moreover, Declaration (Exhibit F) is necessary to help rebut particular errors that Appellant

respectfully contends have been maintained by the Examiner in construing various issues discussed in ACP and RAN.

Appellant contends that Declaration (Exhibit G), which contains, *inter alia*, financial revenue data for particular of Patent owners' marketed tests, is necessary to further support the nexus between Patent Owner's patent claims and commercial success. Moreover, Declaration (Exhibit G) is necessary to help rebut particular errors that Appellant respectfully contends have been maintained by the Examiner in construing various issues discussed in ACP and RAN.

Appellant contends that Declaration (Exhibit H), which contains a section by section detailed rebuttal of misconception introduced into the prosecution record by the Bailey Declaration, is necessary to help rebut particular errors that Patentee respectfully contends have been maintained by the Examiner in construing various issues discussed in the Bailey Declaration.

Appellant contends that Declaration (Exhibit I), which contains a valuable industry perspective regarding initial resistance to industry acceptance of Patent Owner's methods, is necessary to further support the non-obviousness of Patentee's invention, and the nexus between Patentee's patent claims and commercial success which was questioned by the Examiner in the ACP and RAN.

Appellant contends that Declaration (Exhibit J), which contains a valuable industry perspective regarding initial resistance to industry acceptance of Patentee's methods, is necessary to further support the non-obviousness of Patentee's invention, and the nexus between Patentee's patent claims and commercial success which was questioned by the Examiner in the ACP and RAN.

Response to Declarations

The Examiner (RAN p. 65) states that "Examiner has reviewed the Declarations [of Samadpour and Koohmaraie], but finds the declarations insufficient to overcome the *prima facie* case for the same reasons discussed in the ACP," and no further comments were made in the RAN.

The Examiner then commented on Patent owner's post-ACP declarations. Various **incorrect** statements are made in the RAN Section entitled "Response to Declarations."

First, the RAN (p. 65, last line and p.66 line 21, p. 70 line 6; and also p. 70, sixth line of first full paragraph) repeats the incorrect assertion that Jarvis pertains to multiple lots. As discussed under Ground #1 and elsewhere, the proper distinction is between methods which test multiple lots in a simultaneous manner, and those which do so in a serial manner, as in Jarvis and all other cited references.

Second, the RAN (p. 66, line 1) questions the "assertion" that the claimed invention "concerns entire sample sets, each set representing an entire lot", and further states (p. 66, line 4) that "...the claims do not reference different sets". However, Claim 1, step b) refers to "separate test lots" which contribute to "a corresponding set of separate test lot samples, wherein each separate test lot sample is attributed to a corresponding separate test lot". Thus, the RAN is incorrect.

Third, the RAN (p. 69, first paragraph) disparages Dr. Samadpour's affidavit showing skepticism from experts in the field on the basis that it is "solely directed to testing for *E. coli* O157:H7, a special case scenario". However, no basis is stated for asserting that *E. coli* testing is a "special case", and no standard is applied to distinguish "special cases" from, presumably, general cases. Since millions of species of bacteria exist, any discussion concerning a particular species can be asserted to be a "special case".

Likewise, the Mirtsching and Byron declarations were improperly marginalized (page 71, "Mirtsching Declaration," carry-over paragraph to page 72, and under "Byron Declaration"). As discussed in detail in this Appeal Brief, the prior art of record does NOT predict the utility of Patent owner's patented methods.

Fourth, Patent owner reaffirms and reasserts the *secondary indicia of objective non-obviousness* discussed in Patent owner's Response to the ACP. The Examiner's marginalization and effective dismissal of the fact that it took years to overcome skepticism is improperly based on the allegation/premise that that *E. coli* testing is a "special case". Additionally, the evidence in support of commercial success was improperly dismissed on the same basis. However, no basis is stated for asserting that *E. coli* testing is a "special case", and no standard is applied to distinguish "special cases" from, presumably, general cases. Since millions of species of bacteria exist, any discussion concerning a particular species can be asserted to be a "special

case". Furthermore, there is a strikingly clear corroborated nexus between the commercial success and the present claim elements.

VIII. CONCLUSION

For the reasons provided herein, Appellant submits the Examiner has improperly rejected the claims that are the subject of the present appeal under 35 U.S.C. § 103. Accordingly, reversal of the Examiner's rejection of the claims under appeal by the Board is respectfully requested.

The Commissioner is hereby authorized to charge the fee for filing a brief in support of an appeal as set forth in 37 C.F.R. § 41.20(b)(2), and any other fees, or credit any overpayments to Deposit Account No. 04-0258.

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IX. APPENDIX OF CLAIMS INVOLVED IN THE APPEAL

1. (Twice amended) A method of sampling, testing and validating a plurality of test lots for release into commerce[[microbial contamination]], comprising:

a) collecting a plurality of portions from each of a plurality of separate test lots 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[[, according to a statistical sampling plan]] 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[[by taking said plurality of portions thereof]];

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;

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;

d) removing portions of each enriched separate test lot sample, and combining the removed portions to provide a modular [[inter-lot]]composite sample; and

e) testing of the modular [[inter-lot]]composite sample, using a suitable detection assay, for the target microbial agent or organism, wherein when such testing is negative all of said separate test lots [[samples]] are validated for release into commerce, and wherein when such testing is positive, each of the individual separate test lots [[may nonetheless yet be]]are validated[[, according to the statistical sampling plan,]] 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, 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).

2. (Currently amended) The method of claim 1, wherein each of the test lots is a single-unit production lot, comprised of operationally-linked product specimens.

3. (Original) The method of claim 1, further comprising incubating the modular composite sample of d), prior to testing in e).

4. (Original) 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.

5. (Original) 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.

6. (Original) 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.

7. (Original) The method of claim 1, further comprising, forming an additional modular composite sample from the set of enriched separate test lot samples of c) and testing the additional modular composite sample, using the same or a different suitable assay, to confirm the testing result.

8. (Original) The method of claim 1, further comprising, forming an additional modular composite sample from an additional enriched separate test lot sample along with any enriched separate test lot samples of c) that test negative in e), and testing the additional modular composite sample, using the same or a different suitable assay.

9. (Original) The method of claim 1, further comprising, where testing of the modular composite sample in e) is negative, forming an additional modular composite sample from the set of enriched separate test lot samples of c), and testing the additional modular composite sample, using a different suitable assay, to confirm the negative testing status.

10. (Original) 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.

11. (Original) The method of claim 1, wherein the number of separate test lots is from 3 to 10.

12. (Original) 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.

13. (Original) 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.

14. (Original) 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.

15. (Original) 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.

16. (Original) 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 about 60 portions.

17. (Twice amended) A method of sampling, testing and validating a plurality of test lots for release into commerce[[microbial contamination]], comprising:

a) collecting a plurality of portions from each of a plurality of single-unit production lots, 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[[, according to a statistical sampling plan for a particular microbial agent or organism,]] 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[[by taking said plurality of portions thereof]];

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;

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;

d) removing portions of each enriched separate single-unit production lot sample, and combining the removed portions to provide a modular [[inter-lot]]composite sample; and

e) testing of the modular [[inter-lot]]composite sample, using a suitable detection assay, for the target microbial agent or organism, wherein when such testing is negative all of said separate single-unit production lot samples are validated for release into commerce, and wherein when such testing is positive, individual separate single-unit production lots [[may nonetheless yet be]] 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, 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).

18. (Original) 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.

19. (Original) 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*.

20. (Original) 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.

21. (Original) The method of claim 1, wherein the target microbial agent or organism comprises a spoilage organism.

22. (Original) 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.

23. (Original) 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.

24. (Original) 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.

25. (Original) The method of claim 1, wherein the separate test lots are separate production lots of a fish or seafood product, comprising an assemblage of one or more fish or seafood specimens, respectively.

26. (Original) The method of claim 1, wherein the separate test lots are separate production lots of a ready-to-eat product.

27. (Original) The method of claim 2, wherein product specimens are synonymous with product portions.

28. (Original) The method of claim 4, wherein the medium comprises a selective agent that will inhibit a competing non-target microbial agent or organism.

29. (Original) The method of claim 5 wherein the concentration step comprises at least one of immunomagnetic bead separation, and affinity chromatography.

30. (Original) 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.

31. (Original) 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.

32. (Original) The method of claim 24, wherein the meat or poultry specimens are meat trim pieces or poultry trim pieces, respectively.

33. (Original) The method of claim 24, wherein the meat is that of beef, pork, sheep or bison.

34. (Cancelled).

35. (Cancelled).

36. (Cancelled).

X. EVIDENCE APPENDIX

Evidence required under 37 CFR § 41.67(c)(1)(ix). Appellants rely upon evidence, such as listed in the table below, submitted and of record in the inter partes reexamination case, Control No. 95/001,294.

Evidence used in this Appeal Brief include:

Exhibit A: Figure 1 attached to and labeled as "Exhibit A" in the Response after non-final action filed via EFS-Web on July 26, 2010;

Exhibit B: Figure 2 attached to and labeled as "Exhibit B" in the Response after non-final action filed via EFS-Web on July 26, 2010;

Exhibit C: Figure 3 attached to and labeled as "Exhibit C" in the Response after non-final action filed via EFS-Web on July 26, 2010;

Exhibit D: Figure 4 attached to and labeled as "Exhibit D" in the Response after non-final action filed via EFS-Web on July 26, 2010;

Exhibit E: Declaration, under 37 C.F.R. 1.132, of Mohammad Koohmaraie, Ph.D. attached to the Response after non-final action filed via EFS-Web on July 26, 2010;

Exhibit F: Declaration, under 37 C.F.R. 1.132, of Mansour Samadpour attached to the Response after non-final action filed via EFS-Web on July 26, 2010;

Exhibit G: Declaration, under 37 C.F.R. 1.132, of Mansour Samadpour attached to the Patent Owner Comments after Action Closing Prosecution filed via EFS-Web on Jan. 31, 2011;

Exhibit H: Declaration, under 37 C.F.R. 1.132, of Mansour Samadpour to rebut the Declaration of Dr. Bailey attached to the Patent Owner Comments after Action Closing Prosecution filed via EFS-Web on Jan. 31, 2011;

Exhibit I: Declaration, under 37 C.F.R. 1.132, of Warren Mirtsching attached to the Patent Owner Comments after Action Closing Prosecution filed via EFS-Web on Feb. 3, 2011;

Exhibit J: Declaration, under 37 C.F.R. 1.132, of James Byron attached to the Patent Owner Comments after Action Closing Prosecution filed via EFS-Web on Jan. 31, 2011;

Exhibit K: Reference: DAVIES et al., "Evaluation of the Use of Pooled Serum, Pooled Muscle Tissue Fluid (meat juice) and Pooled Faeces for Monitoring Pig Herds for *Salmonella*," Journal of Applied Microbiology, 2003, 95:1016-1025, attached to and labeled as "Exhibit 2" in the Request for Inter Partes Reexamination filed on Jan. 13, 2010;

Exhibit L: Reference: MIDURA, et al., *"*Sampling Plans, Sample Collection, Shipment and Preparation for Analysis*,"* Compendium of Methods for the Microbiological Examination of Foods, 4th Edition, 2001, Ch. 2, American Public Health Association, attached to and labeled as "Exhibit 3" in the Request for Inter Partes Reexamination filed on Jan. 13, 2010;

Exhibit M: Reference: MENG, et al., *"*Pathogenic *Escherichia coli,"* Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., 2001, Ch. 35, American Public Health Association, attached to and labeled as "Exhibit 4" in the Request for Inter Partes Reexamination filed on Jan. 13, 2010;

Exhibit N: Reference: ANDREWS, et al., "*Salmonella*," Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., 2001, Ch. 37, American Public Health Association, attached to and labeled as "Exhibit 5" in the Request for Inter Partes Reexamination filed on Jan. 13, 2010;

Exhibit O: Reference: US Department of Agriculture, FSIS Directive, "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," 2004, 10.010.1, Revision. 1, attached to and labeled as "Exhibit 6" in the Request for Inter Partes Reexamination filed on Jan. 13, 2010;

Exhibit P: Reference: SILLIKER et al., "ICMSF Methods Studies. I. Comparison of Analytical Schemes for Detection of *Salmonella* in Dried Foods," Canadian Journal of Microbiology, 1973, 19: 475-479, attached to and labeled as "Exhibit 7" in the Request for Inter Partes Reexamination filed on Jan. 13, 2010;

Exhibit Q: Reference: PRICE et al., "*Salmonella* Testing of Pooled Pre-Enrichment Broth Cultures for Screening Multiple Food Samples," Applied Microbiology, April

1972, Vol. 23, No. 4: 679-682, attached to and labeled as "Exhibit 8" in the Request for Inter Partes Reexamination filed on Jan. 13, 2010;

Exhibit R: Reference: GABIS, et al., "ICMSF Methods Studies. II. Comparison of Analytical Schemes for Detection of *Salmonella* in High-Moisture Foods," Canadian Journal of Microbiology, 1974, 20:663-669, attached to and labeled as "Exhibit 9" in the Request for Inter Partes Reexamination filed on Jan. 13, 2010;

Exhibit S: Reference: The International Committee on Microbiological Specifications for Foods (ICMSF), Microorganisms in Foods, Their Significance and Methods of Enumeration, 2nd Edition, 1978, University of Toronto Press, attached to and labeled as "Exhibit 10" in the Request for Inter Partes Reexamination filed on Jan. 13, 2010;

Exhibit T: Reference: "BAM: Food Sampling/Preparation of Sample Homogenate," Bacteriological Analytical Manual, 2003, Chapter 1, pp. 1-11, US FDA, attached to and labeled as "Figure 11" in the Request for Inter Partes Reexamination filed on Jan. 13, 2010;

Exhibit U: Reference: JARVIS, B., "Errors Associated with Dilution Count and Presence/Absence Methods," Statistical Aspects of the Microbiological Analysis of Foods, Progress in Industrial Microbiology, 1989. Ch. 8, Vol. 21, Elsevier Science, attached to and labeled as "Exhibit 13" in the Request for Inter Partes Reexamination filed on Jan. 13, 2010;

Exhibit V: Reference: ENTIS, et al., "Rapid Methods for Detection, Identification, and Enumeration," Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., 2001, Ch. 10. American Public Health Association, attached to and labeled as "Exhibit 15" in the Request for Inter Partes Reexamination filed on Jan. 13, 2010;

Exhibit W: Reference: COUSIN, et al., "Psychotrophic Microorganisms," Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., 2001, Ch. 13. American Public Health Association, attached to and labeled as "Exhibit 16" in the Request for Inter Partes Reexamination filed on Jan. 13, 2010;

Exhibit X: Reference: U.S. Patent 6080437, issued 06/27/2000 to Mauer, attached to and labeled as "Exhibit 14" in the Request for Inter Partes Reexamination filed on Jan. 13, 2010; and

Exhibit Y: Reference: JARVIS, B., "On the Compositing of Samples for Qualitative Microbiological Testing," Letters in Applied Microbiology, 2007, 45:592-598, attached to the Response after non-final action filed via EFS-Web on July 26, 2010.

XI. RELATED PROCEEDINGS APPENDIX

There are no decisions by a court or the BPAI pursuant the 37 C.F.R. § 41.67(c)(1)(x) that are submitted herein.

Figure 1: PRESENT INDUSTRY ART

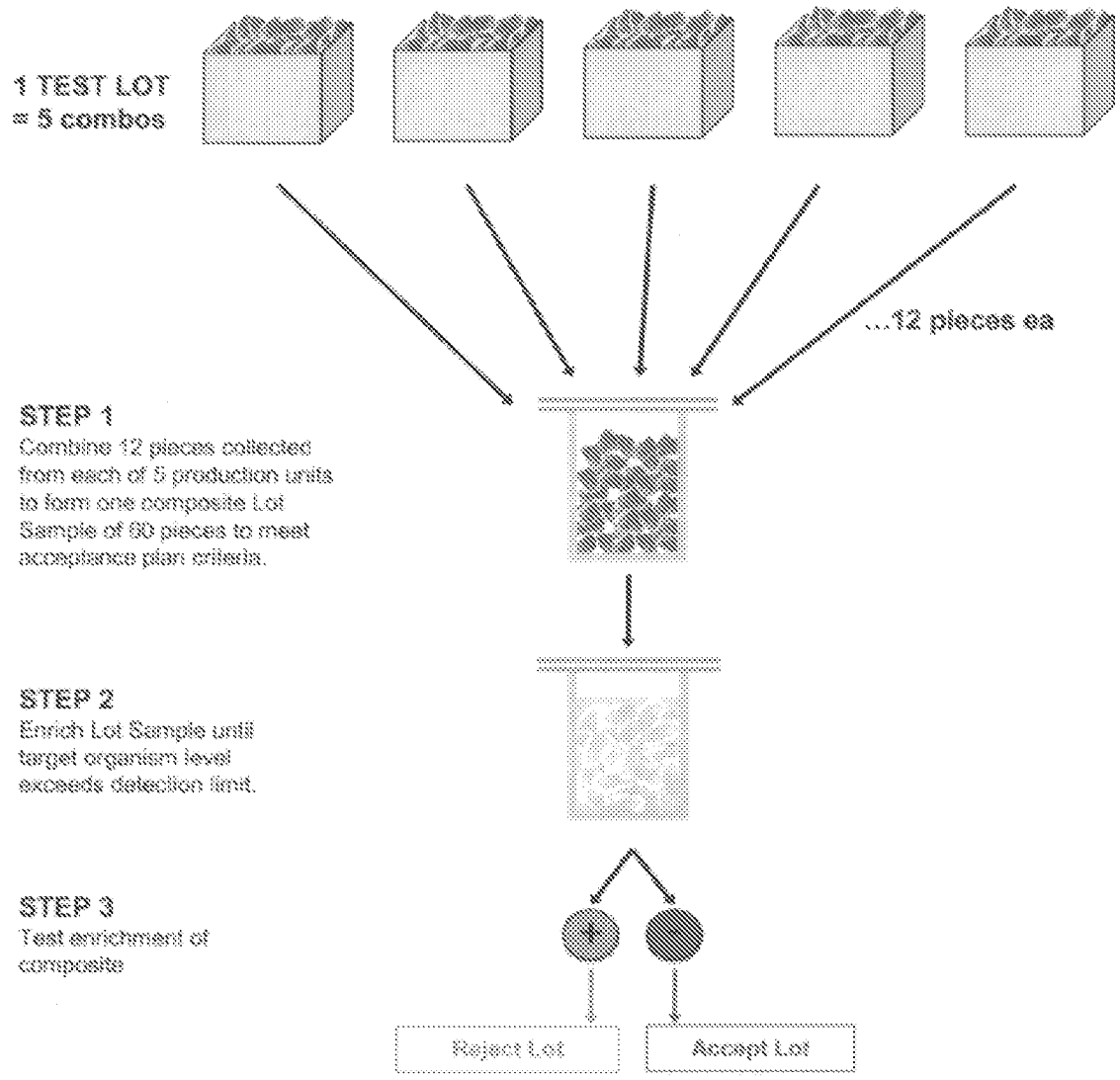


Figure 2. CURRENT INVENTION

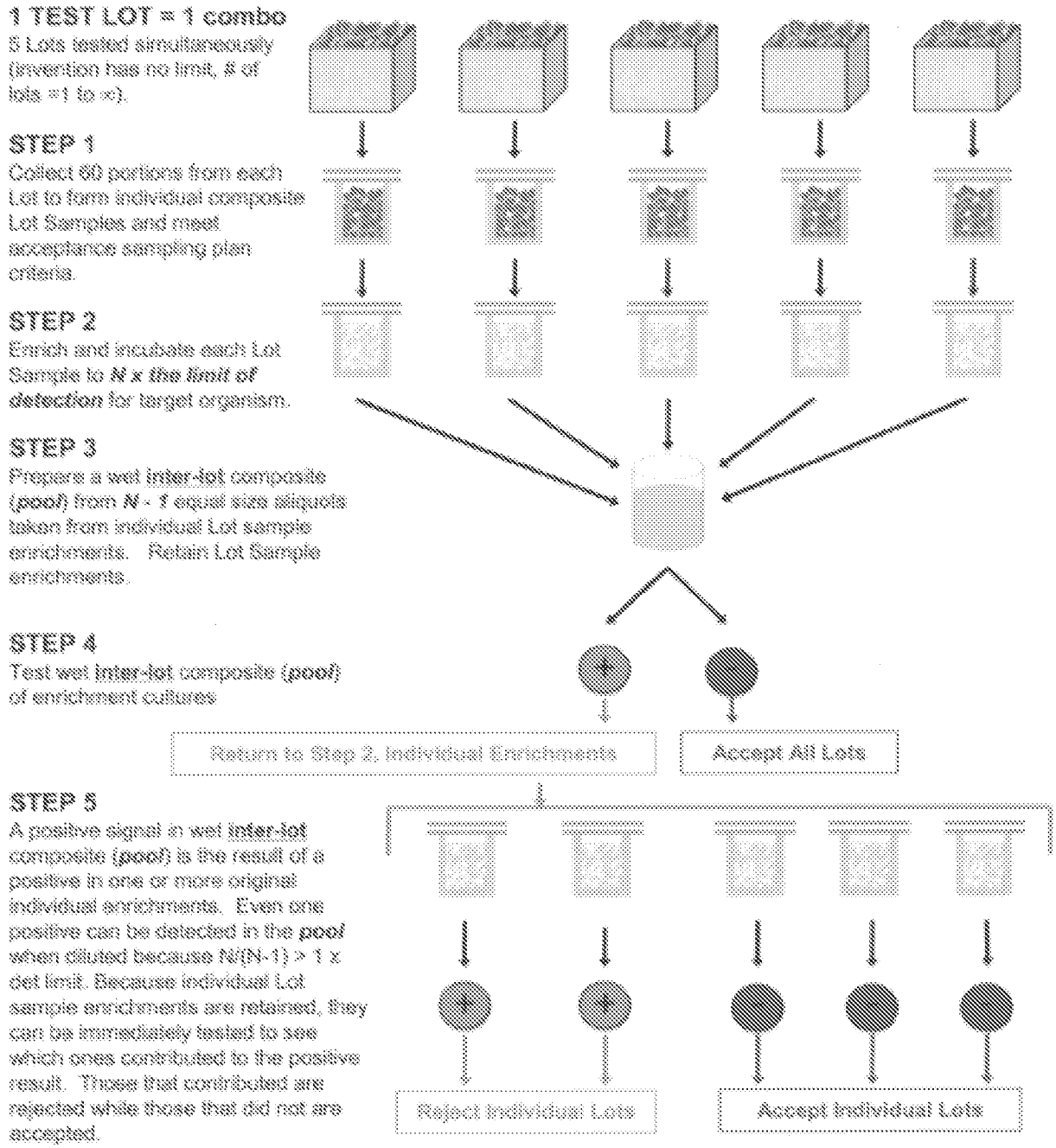


Figure 3. CITED PRIOR ART (Price et al, 1972)

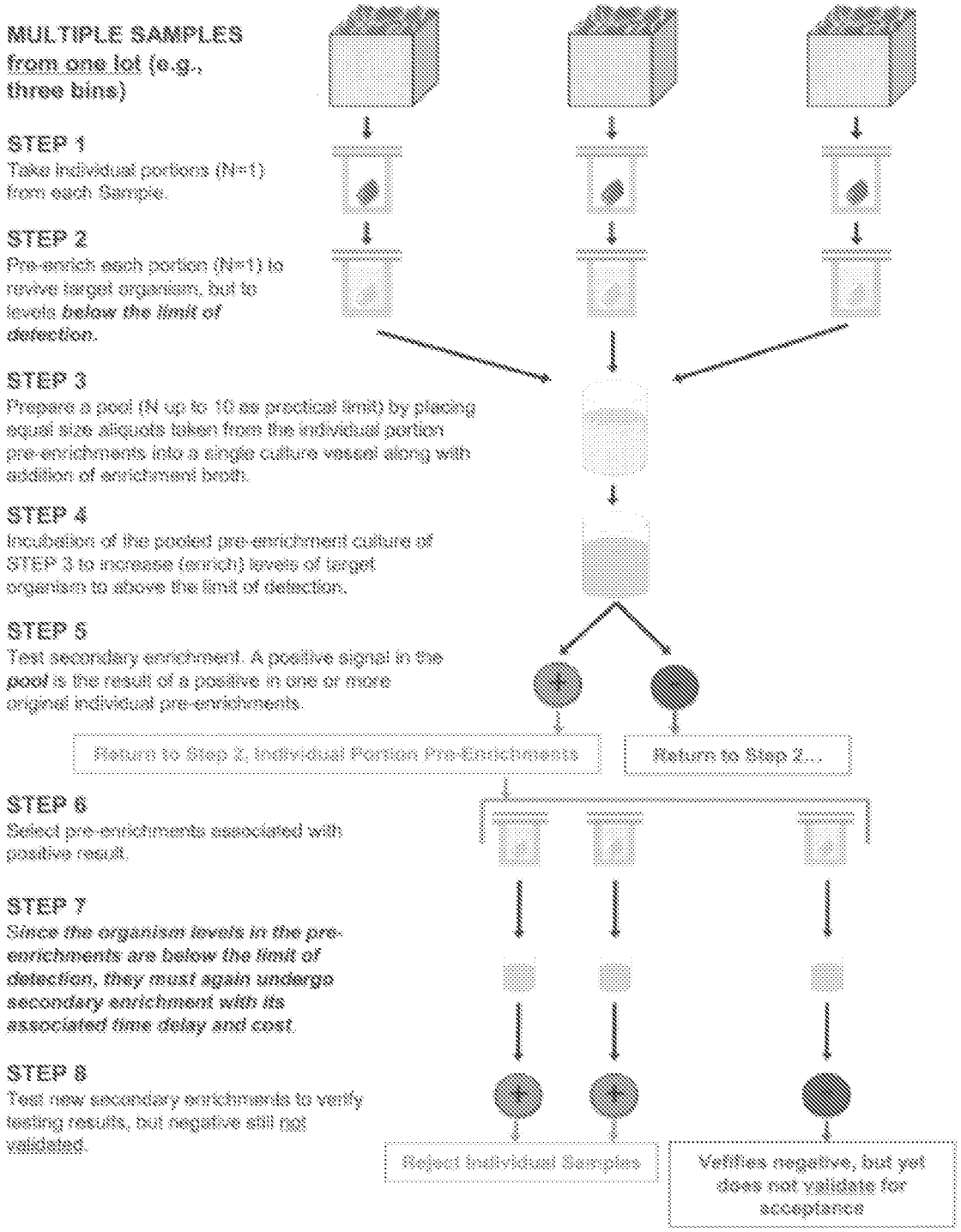
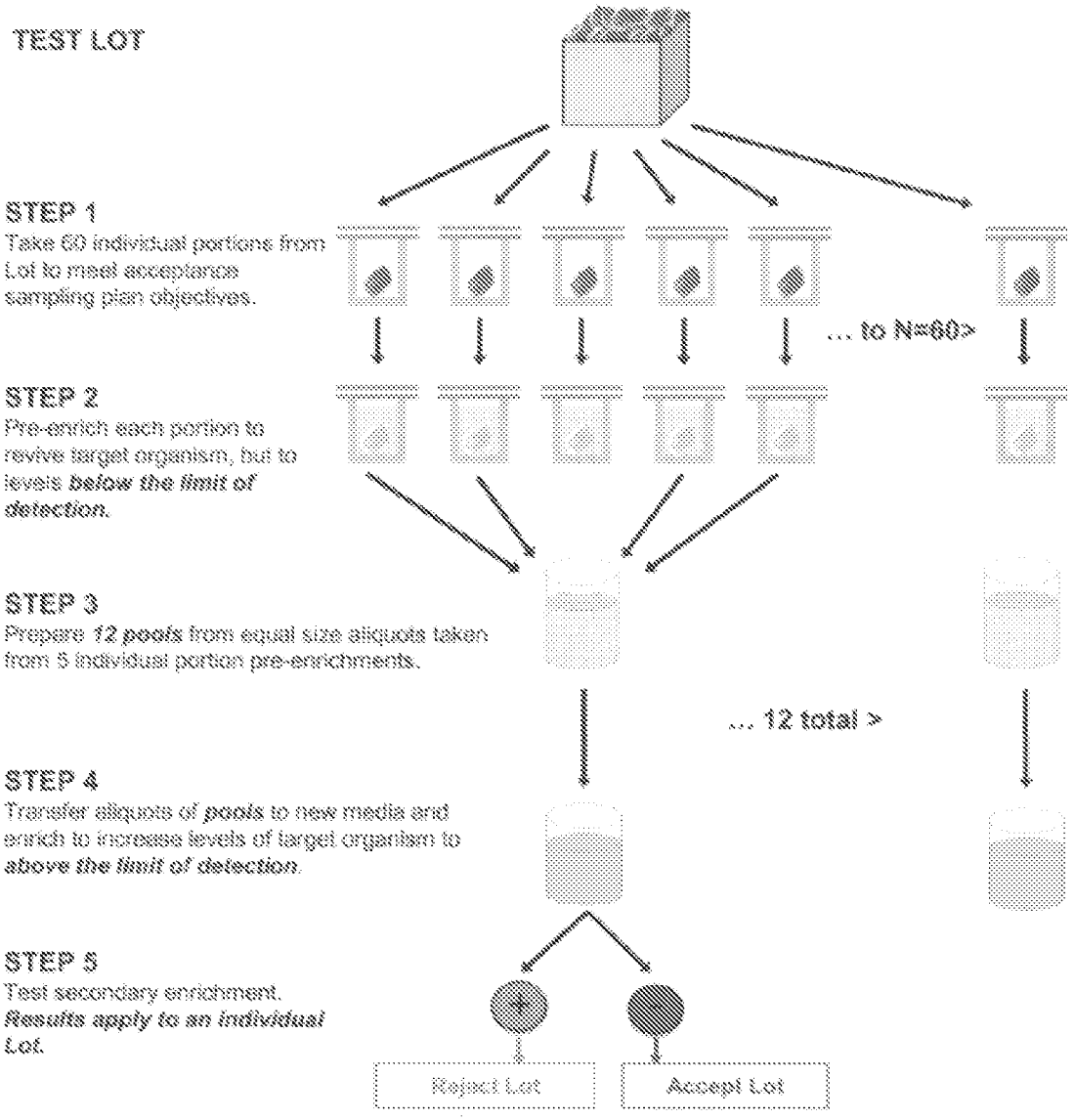


Figure 4. CITED PRIOR ART (Silliker et al, 1972)



Via Electronic Submission
Attorney Docket No.:66090-008US2

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Mansour Samadpour
Serial No.:	95/001,294
Filed:	05 April 2010
For:	MODULAR COMPOSITING-MULTIPLE LOT SCREENING PROTOCOLS FOR DETECTION OF PATHOGENS, MICROBIAL CONTAMINANTS AND/OR CONSTITUENTS
Examiner:	Brenda G. Brumback
Art Unit:	3991
Attorney Docket No.:	66090-008US2
Date:	July 26, 2010

Attn: Mail Stop "Inter Partes Reexam"
Central Reexamination Unit
Commissioner for Patents
P. 0. Box 1450
Alexandria VA 22313-1450

DECLARATION OF MOHAMMAD KOOHMARAIE, Ph.D. UNDER 37 C.F.R. § 1.132
(IN SUPPORT OF RESPONSE UNDER 37 C.F.R. § 1.111)

Madam:

I, Dr. Mohammad Koohmaraie, being duly sworn, say:

1. I am familiar with the above-identified patent/patent application, and am aware that it discloses and claims novel methods comprising sampling, testing, and validating test lots for microbial contamination. I am familiar that the claimed method comprises: collecting a plurality of portions from each of a plurality of test lots, the test lots each comprising an assemblage of one or more specimens, wherein each test lot is separately sampled by taking said plurality of portions thereof; combining the collected 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; 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; removing portions of each enriched separate test lot sample, and combining the removed portions to provide a modular composite sample; and testing of the modular composite sample, using a suitable detection assay, for the target microbial agent or organism, wherein when such testing is negative all of said separate test lot samples are validated, and wherein when such testing is positive, individual separate test lots may nonetheless yet be validated 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.

2. I am familiar with particular aspects of the Office Action from the USPTO dated 26 May 2010 in this case, and understand that one or more claims of the above-referenced patent application remain rejected under 35 U.S.C. § 103 on the alleged grounds that the claimed invention is obvious. Specifically, the Examiner appears to be alleging that several of the claims under 35 U.S.C. 103(a) are unpatentable over Jarvis in view of FSIS and Midura, amongst others.

3. I am the CEO of the Meat Division of Institutes of Environmental Health (IEH) Laboratories and Consulting Group and have been at that position since of April 15, 2008. My detailed curriculum vitae is attached hereto as APPENDIX A.

4. Prior to joining IEH, I was the Director of the U.S. Meat Animal Research Center (USMARC) of the USDA-ARS. Among other responsibilities, I had the responsibility of overseeing all pre-harvest and post-harvest food safety research.

5. From 1992 to 2005, I was the leader of all the post-harvest research conducted at USMARC.

6. For several years, I have been on the forefront of the research to assist the industry in their efforts to control *E. coli* O157, *Salmonella* and non-O157 Shiga toxin producing *E. coli*. In particular, in 1990s I conducted much of the work that enabled the USDA-FSIS to issue HACCP Rules. In my role at that USDA, I headed a team of USDA scientists that

collaborated with our Chinese counterpart.

7. I am an author or co-author of approximately 400 research articles (representative articles are attached as part of APPENDIX A) in the field of food testing, analytical sampling, meat safety and quality, and my research has been presented at more than 250 national and international meetings. In addition, I have organized several food safety sessions and meetings both nationally and internationally.

8. I am member of Board of Directors of the International Livestock Education Foundation as well as Board of Directors of the National Cattlemen Beef Association.

9. I am one of 10 members of the beef industry's Blue Ribbon Task Force on non-O157 shiga toxin producing *E. coli*.

10. I am a member of the American Meat Institute' Scientific Advisory Committee.

11. I have dedicated much of my research efforts since 1993 to the control of *E. coli* O157:H7 in our nation's red meat supply.

12. I am writing this letter to Declare and corroborate the significance of wet composite inter-lot pooling as presently claimed in the control of *E. coli* O157:H7 and other beef-borne pathogens and as such the only way to control beef-borne pathogens is an effective food safety system. Pathogen testing is an effective part of a robust food safety system.

13. For *E. coli* O157:H7 testing, prior to the present patent application, the industry common practice is to use 5 combo bins as a lot. While a smaller lot size (e.g., a single combo bin) might provide for a better chance of finding *E. coli* O157:H7 contamination, the cost of sampling and testing has strongly motivated industry retention of the 5 combo bin lot. Surprisingly, therefore, while the invention of post-enrichment inter-lot pooling, as disclosed and claimed in the current patent, requires increased sampling/testing expense (about 30% more than with the prior industry standard) it has been rapidly and widely adopted and solves a long standing problem in the art by providing for a more robust testing method with enhanced safety

for consumers.

14. At this point in time, and largely because of IEH's implementation of its modular inter-lot pooling methods, IEH provides laboratory testing for about 30% of the cattle processed in the United States and about 40% of the cattle processed in Canada. After implementation of the wet pooling, as disclosed and claimed in the current patent, all IEH clients, except for one new client, use single combo sampling and testing because we have demonstrated to their satisfaction that using single combo as the sampling unit, while more expensive for sampling and testing, provides a better opportunity of detecting and removing contaminated lots. The clients also appreciate, that the higher resolution of sampling a single combo prevents unadulterated products from being unnecessarily disposed—because of the ability, when an inter-lot composite sample tests positive, to yet validate individual enriched test lots for release into commerce. There is, therefore, a clear nexus between the benefits of the method as claimed in the patent, and industry acceptance in the context of IEH's business.

15. The data, shown in Table 1 and Figure 1 of this Declaration (Appendix A), is from an IEH conducted *E. coli* O157:H7 testing program in beef trim. This Table 1 data illustrates that due to this higher resolution associated with single combo testing (i.e., implementation of the wet inter-lot composite pooling method, as disclosed and claimed in the current patent), a representative client of the company was, in a single year, able to save 477 combo bins—each containing about 2000 lbs of beef trim.

16. The data shown in Table 1 of this Declaration (Appendix A) was used to calculate the numbers represented in Figure 1 of this Declaration (Appendix A). The results show that in the majority of instances, when a 5-combo bin lot is considered to be positive for *E. coli* O157:H7, only a single combo bin is positive. On average when a lot of 5-combo bins is rejected, only 1.65 of those 5 combos are positive, thus 3.65 combo bins are unnecessarily discarded.

17. The data presented here is the basis of the request of many customers that the trim producers conduct single combo sampling and testing by the IEH proprietary method as claimed. For example, in its latest requirement for the trim supplied for ground beef production, the National School Lunch Program is requiring single combo testing as well, according to the claimed method.

18. In conclusion, only an effective risk based beef production system can provide for the control of *E. coli* O157:H7 in raw ground beef. Testing for *E. coli* O157:H7 in trim and raw ground beef components is an integral part of such a food safety system. According to the claimed invention, and as represented by the rapid and wide-spread acceptance of the claimed methods, lot size and representative sampling of the lot are a critical part of the testing system. Industry acceptance of smaller the lot size, despite the increased costs, means a higher chance of detecting *E. coli* O157:H7 and removing the adulterated product from commerce, hence protecting public health. Wet composite inter-lot pooling, as disclosed and claimed in the current patent, is the technology that surprisingly makes single combo testing a viable option. Prior to the invention of the patent, such sampling was regarded as contra-indicated and not economically feasible.

19. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code, and may jeopardize the validity of the application or any patent issuing thereon.

M. Koohmaraie

Mohammad Koohmaraie, Ph.D.

26 July 2010

APPENDIX A

TABLE 1

Number of Combo Bins Diverted Due to a Positive Lot Finding

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec		
Traditional Lotting	185	0	17	0	53	122	99	25	147	24	52	2	726	
Single Combo	58	0	6	0	23	39	34	9	51	12	16	1	249	
													477	Saved

FIGURE 1

# of Combo Bins	% of Times Positive
1 - combo	59.2
2 - combo	24.2
3 - combo	10.8
4 - combo	4.1
5 - combo	1.7

APPENDIX B

CURRICULUM VITAE
MOHAMMAD KOOHMARAIE

IEH Laboratories and Consulting Group
15300 Bothell Way NE
Lake Forest Park, Washington
Office Phone: 206-522-5432
Fax: 206-306-8883
E-Mail: mk@iehinc.com

EDUCATION

1978	B.S., Pahlavi University, Shiraz, Iran Major: Animal Science
1980	M.S., Texas A&M at Kingsville Major: Meat Science Minors: Animal Nutrition and Statistics
1984	Ph.D., Oregon State University Majors: Meat Science and Muscle Biology Minors: Biochemistry, Microbiology, Food Science and Technology

ADDITIONAL TRAINING

1985	Short course on "Basic Cell and Tissue Culture," The Center for Advanced Training in Cell and Molecular Biology, The Catholic University of America, Washington, DC.
1990	Short course on "Recombinant DNA Methodology," The Center for Advanced Training in Cell and Molecular Biology, The Catholic University of America, Washington, DC.
1991	Seminar on "Science, Technology and Public Policy," U.S. Office of Personnel Management, at Western Executive Seminar Center, Denver, CO.
1992	Short course on "Polymerase Chain Reaction in Molecular Biology," The Center for Advanced Training in Cell and Molecular Biology, The Catholic University of America, Washington, DC.

2000	Executive Program "Leadership and Team Effectiveness," Yale School of Management, New Haven, CT.
2000	Congressional Briefing Seminar. The Government Affairs Institute at Georgetown University, Washington, DC.
2001	Seminar on "Essentials of Communicating with Diplomacy and Professionalism," SkillPath Seminars of Kansas City, MO.
2004	Seminar for executives on Legislative operations. The Government Affairs Institute - Georgetown University, Washington, DC. This course is designed for SES candidates and meets Executive Core Qualifications 1, 4, and 5.

EMPLOYMENT

1980 - 1984

Research Assistant
Clark Meat Science Laboratory, Department of Animal Science
Oregon State University, Corvallis, Oregon

1984 - 1986

Postdoctoral Research Fellow
USDA-ARS, Roman L. Hruska U.S. Meat Animal Research Center
Clay Center, Nebraska

1986 - 1987

Postdoctoral Research Fellow
Department of Food Science and Human Nutrition
Michigan State University
East Lansing, Michigan.

1987 - 1991

Research Physiologist
Meats Research Unit
U.S. Meat Animal Research Center
USDA-ARS
Clay Center, Nebraska

1991 - 2005

Supervisory Research Physiologist (Research Leader)
Meat Safety and Quality Research Unit
U.S. Meat Animal Research Center
USDA-ARS
Clay Center, Nebraska

2005 - 2008

Director
U.S. Meat Animal Research Center
USDA-ARS
Clay Center, Nebraska

2008 to present

CEO, Meat Division
IEH Laboratories and Consulting Group
Lake Forest Park, Washington

HONORS AND AWARDS

1989 Received a Certificate of Merit and cash award from USDA-ARS for "Outstanding Research on Control of Meat Tenderness"

1990 Received a Certificate of Merit and cash award from USDA-ARS for "Outstanding Research in your Field of Research for ARS"

1991 Received a Certificate of Merit and cash award from USDA-ARS for "Innovative Research and Project Leadership on Meat Tenderness"

1992 Selected as ARS's "Outstanding Early Career" Scientist of the Year

1993 Selected as Midwestern Section of the American Society of Animal Science "Outstanding Young Scientist-Research"

1993 Selected for American Meat Science Distinguished Research Award

1994 Selected for American Society of Animal Science Meat Research Award

1994 Received a Certificate of Merit and cash award from USDA-ARS for "Leadership of the Team That Developed the First Rapid Test for Bacteria on Meat and Poultry Carcasses"

1995 Secretary of Agriculture's Award for Personal and Professional Excellence

1995 Received the USDA Secretary of Agriculture's Award for Superior Service "For Development of the First Rapid Test for Generic Bacteria on Meat and Poultry Carcasses"

1997 ARS-FSIS Administrator's Certificate of Appreciation for "Excellence in the Joint FSIS/ARS Efforts to Determine the Sponge Method Parameters That Allowed the Pathogen Reduction/HACCP Rule to be Adopted Into Legislation"

1998 Received a Certificate of Appreciation and cash award from USDA-ARS for "The Transfer of Technology on Beef Tenderness to the Industry"

1999 Received a Certificate of Appreciation and cash award from USDA-ARS "In Recognition for an Outstanding Rating Based Upon an Official Performance Appraisal for the Rating Period September 1, 1998 Through October 31, 1999"

1999 Received a Certificate of Appreciation and cash award from USDA-ARS for "Demonstrating an Unusual Level of Commitment in Leading an Industry Cooperative Meat Safety Study to Support the FSIS and the Beef Industry and Playing a Key Role in Support of the USDA's Office of Inspector General"

2000 Selected as ARS "Outstanding Senior Scientist of the Year"

2001 Promoted to Super Grade Scientists in USDA-ARS (only 30 scientists from over 2000 scientists receive this recognition).

2000 Received a Certificate of Merit and cash award from USDA-ARS "In Recognition of an Outstanding Rating Based Upon an Official Performance Appraisal for the Rating Period November 1, 1999 through December 31, 2000"

2001 Received the USDA Secretary of Agriculture Award for Personal and Professional Excellence. Citation: "For Leading Team Research Resulting in Development of Technology Now Being Used by Producers and Processors to Improve the Safety and Quality of the United States Red Meat Supply"

2001 Received Area Director's Certificate of Appreciation for "Outstanding Contributions as Acting Center Director for the U.S. Meat Animal Research Center in Clay Center, Nebraska, July 2000- April 2001"

2002 Selected to chair ARS's Research Position Evaluation System Panels

2001 Received a Certificate of Merit and cash award from USDA-ARS "In Recognition of Your Exemplary Leadership by Working with the Livestock and Packing Plant Industries to Solve Food Safety and Carcass Classification Problems"

2002 Received a Certificate of Merit and cash award from USDA-ARS "In Recognition of Your Outstanding Performance in Leading Post-harvest Food Safety and Meat Quality Research and Working with the Livestock Industry to Implement New Technologies and Management Practices"

2003 Received a Certificate of Merit and cash award from USDA-ARS "For Exemplary Leadership in Identifying and Solving Food Safety Problems by Developing Hide and Trim Intervention Programs"

2004 Received a Certificate of Merit and cash award from USDA-ARS for "Outstanding Performance in Leading Post-Harvest Food Safety and Meat Quality Research and Transfer of New Technology to Industry"

2004 Selected as the recipient of an ARS Technology Transfer Award "For Exemplary Efforts to Ensure the Successful Implementation of the MARC Beef Carcass Image Analysis System by the U.S. Beef Industry"

2004 Selected for ARS Executive Professional Excellence and Knowledge (PEAK) Program

2004 Received a Certificate of Merit and cash award from USDA-ARS for "Outstanding Performance in Leading Post-Harvest Food Safety and Meat Quality Research and Transfer of new Technology to Industry"

2005 Received "2005 Professional of the Year" award presented by the National Meat Association "In Recognition of Your dedication and Valuable Service to the Meat and Poultry Industry" February 2005, Las Vegas, Nevada

2005 Received the 2005 Federal Laboratory Consortium Award for Excellence in Technology Transfer for "Beef Carcass Image Analysis Yield Classification System" March 2005, Orlando, Florida

2005 Received the American Meat Science Association "Signal Service Award' presented "In Recognition of Devoted Service and Lasting Contribution to the Meat Industry and the American Meat Science Association" August 2005, Baltimore, Maryland

2005 Received the International Meat Secretariat 2005 ICoMST (International Congress of Meat Science and Technology) Lecturer Award, Baltimore, Maryland

2005 Selected to be member of the United States' Senior Executive Service (SES) – less than 10,000 federal employees receive this level from over 2,000,000 federal employees.

2005 Member of International Meat Secretariat (IMS) Scientific and Technological Advisory Group/Committee

2006 Represented United States in U.S.-China Food Safety Research and Cooperation (ended in 2008 when Dr. Koohmaraie left federal service).

2005 to present Member of Board of Directors of the International Stockmen's Education Foundation

2007 Received the Agricultural Research Service Post-Harvest Food Safety Group Technology Transfer Award for Outstanding Effort, "For the Development and Implementation of Technology that Reduces Pathogen Contamination on Cattle Hides and Improves the Safety of the Red Meat Supply."

2008 Received the Texas A&M University at Kingsville School of Agriculture's Outstanding Alumni of the Year Award.

2008 Received "The Nebraska Cattlemen 2008 Industry Service Award" for ". . . your contributions to the Nebraska and the U.S. beef cattle industries . . . . "

INVITATIONS

Industry

1. Invited to present the paper "Relationship Between Phosphatidylinositol Metabolism and Skeletal Muscle Cell Proliferation" to the Discovery Group, American Cynamid Company, New Jersey, 1985.

2. Invited to present the paper "Tenderness and Aging: An Enzymatic Approach" to a workshop on "Sensory Aspects of Beef Quality" sponsored by USDA and National Live Stock and Meat Board, New Orleans, Louisiana, 1988.

3. Invited to make a presentation on "Factors Affecting Beef Tenderness" to the Carcass Quality Task Force of the National Cattlemen's Association, Denver, Colorado, 1992.

4. Invited to present a seminar on "Beef Tenderness and the Use of Calcium Injection to Increase Tenderness" at the Nebraska Seedstock Symposium sponsored by the Nebraska Cattlemen Purebred Council and the University of Nebraska, 1993.

5. Invited to present a seminar on "Beef Tenderness and the Use of Calcium Injection to Increase Tenderness" to the Dawson County Cattlemen, Cozad, Nebraska, 1994.

6. Invited to present a paper on "Beef Tenderness: Regulation and Prediction" at the 1994 Cattlemen's College at the 1994 National Cattlemen's Convention, Reno, Nevada.

7. Invited to chair the "Gene Markers/Calpastatin" session at the National Tenderness/Palatability Conference. Organized by the National Cattlemen's Association, Denver, Colorado, 1994.

8. Invited to speak at the 26th Annual Conference of the Beef Improvement Federation on "Advances in the Meat Processing Technology That Will Impact Beef Breeding," Des Moines, Iowa, 1994.

9. Invited to speak at the 1994 Texas A&M Beef Cattle Short Course on "Beef Tenderness: Regulation and Prediction," College Station, Texas, 1994.

10. Invited to speak on "Beef Tenderness: Regulation and Prediction" at the World South Devon Society Conference, Billings, Montana, 1994.

11. Invited to present a seminar on the "Role of Enzymes in Tenderness of Aged Beef" to the American-International Charolais Association, Louisville, Kentucky, 1994.

12. Invited to present a seminar on "The Development and Field Testing of the Microbial ATP for Detection of Generic Bacteria on Beef, Pork, and Poultry Carcasses" at the Cargill Lab Manager's Meeting, Plymouth, Minnesota, 1994.

13. Invited to present a seminar on "A Summary of Beef Quality and Meat Safety Research" at the annual meeting of the Nebraska Veterinary Medical Association, Hastings, Nebraska, 1995.

14. Invited to present a seminar on "Beef Tenderness: Regulation and Prediction" at the Muscle to Meat Symposium sponsored by the Montana Stockgrower's Association, Billings, Montana, 1996.

15. Invited to present a seminar on "Tenderness-Based Beef Classification" to the Nebraska Labelled Beef Program Fact Finding Committee of the Nebraska Cattlemen, Lincoln, Nebraska, 1996.

16. Invited to present a seminar on "Beef Tenderness: Past, Present and Future" at the Nebraska Seedstock Symposium sponsored by the Nebraska Cattlemen and the University of Nebraska, North Platte, Nebraska, 1996.

17. Invited to present a seminar on "Beef Tenderness: Past, Present and Future" at the Nebraska Cattlemen and Cattlewomen Annual Convention, Kearney, Nebraska, 1996.

18. Invited to present a talk "Tenderness-Based Beef Classification" at the National Cattlemen's Beef Association's Maximizing Retail Beef Palatability-Product Enhancement Symposium, Denver, Colorado, 1996.

19. Invited to present a seminar on "Tenderness-Based Beef Marketing" at the annual meeting of Double Muscle Inc., Denver, Colorado, 1996.

20. Invited to present a talk on the "Biological Factors Regulating Beef Tenderness" to the Nebraska Beef Task Force of the Nebraska Cattlemen, Lincoln, Nebraska, 1996.

21. Invited to present a seminar on "Beef Tenderness: Regulation and Prediction" to Cattle Capital Cattlemen, Alliance, Nebraska, 1997.

22. Invited to present a seminar titled "Beef Tenderness: Current Knowledge and Future Implications" to the Nebraska Beef Feedlot Roundtable, Grand Island, Nebraska, 1997.

23. Invited to present a talk on "Beef Tenderness: Regulation and Prediction" to Montana Stockgrowers Association, Billings, Montana, 1997.

24. Invited to present a seminar titled "Beef Tenderness: Regulation and Prediction" at the annual meeting of the Cactus Feeders, Amarillo, Texas, 1997.

25. Invited to present a seminar on "Biochemical Basis of Meat Tenderness" to the Symposium on Quality Attributes and Measurement of Muscle Foods sponsored by the American ChemicalSociety, Dallas, Texas, 1997.

26. Invited to present a seminar on "Beef Tenderness: Regulation and Prediction" to the Participants of a Beef Quality Short-Course for leaders of the Texas Beef Industry, College Station, Texas, 1997.

27. Invited to present a talk on "Beef Tenderness: Regulation and Prediction" at the Price Discovery Conference, Kansas City, Missouri, 1997.

28. Invited to present a talk on "Beef Tenderness Issues: Determination Predictability, and Marketing Opportunities" at the Industry Resource Management (IRM) Conference, Clay Center, Nebraska, 1997.

29. Invited to present a talk "Beef Tenderness" at the Range Beef Cow Symposium XV, Rapid City, South Dakota, 1997.

30. Invited to present a talk on recent advances made in tenderness research and any other pertinent carcass research information to the American-International Charolais Association Carcass and Commercial Committee, Kansas City, Missouri, 1997.

31. Invited to present a talk "Effect of Prerigor Freezing and Postrigor Calcium Chloride Injection on Tenderness of Callipyge Longissimus" at the American Sheep Industry Annual Convention, Reno, Nevada, 1998.

32. Invited to present talks "The Importance and Value of Tenderness" and the "MARC Tenderness Classification System" at the International Livestock Congress, Houston, Texas, 1998.

33. Invited to present an overview of the "Factors Influencing Beef Tenderness" and an invited presentation on "New Technologies to Improve Meat Quality" at the California Beef Cattle Improvement's Association's "Your Beef's at Stake" program, Davis, California. 1999.

34. Invited to present "Animal Genetics Update - Key Developments & Progress: Conclusions" at the Meat Industry Research Conference (MIRC), Chicago, Illinois, 1999.

35. Invited to discuss "Decontamination Methods for Small Beef Slaughter Plants" at a specialized food safety work conference on "Reducing Foodborne Illness: Advancing the Adoption of New Technologies" sponsored by the Riley Memorial Foundation, Washington, DC, 1999.

36. Invited to be a panel member to determine the research priorities of the National Cattlemen's Beef Association's for meat quality, 2001.

37. Invited to be a panel member to determine the research priorities of the National Cattlemen's Beef Association's for beef safety, 2001.

38. Invited to be a panel member to determine the research priorities of the National Pork Producers Council for pork quality, 2001.

39. Invited the Agriculture and Agri-Food Canada to be a member of the Meat Research Section Review Committee to assist in the development of future research and staffing plans, 2001.

40. Invited by the American Sheep Industry to present a seminar on "Sheep Research at the U.S. Meat Animal Research Center" to the Research & Education Policy Committee, 2001.

41. Invited to be a member of a 20-member panel of the National Cattlemen's Beef Association's BSE Blue Ribbon Task Force, 2001.

42. Invited to be a member of an 18-member committee organized by ASTM (American Society for Testing and Materials) to "Develop Standards for Carcass Evaluation Systems for Livestock," 2001.

43. Invited to be a member of a board (as the scientific advisor) that advises grocery retailers and meat suppliers (Wal-Mart, HEB, Sara Lee, Tyson, Excel, IBP, and others; these companies account for 40% of the food sold in the U.S.) on food safety matters, 2001

44. Invited by NCBA to join them in meeting with senior staff of McDonald's to communicate the industry's BSE research priorities to come up with a joint request for proposals, 2001.

45. Hosted the ConAgra's Food Safety Team to visit MARC to become better acquainted with MARC resources for future collaborations, 2001.

46. Hosted the Excel division of the Cargill Food Safety Team to visit MARC to become better acquainted with MARC resources for continued and future collaborations, 2001.

47. Represented CD and the Research Center in the NPS organized stakeholders meeting to identify biosecurity research needs, Beltsville, MD, 2001.

48. Invited by the Hawaii County Administrator to be part of a team of four to help the Island modernize its meat industry, 2001.

49. Invited by the National Pork Board to serve on a panel to evaluate the usefulness of funded and completed grants to their producers, St. Louis, MO, 2001.

50. Invited to present a lecture on "Interventions to Reduce/Eliminate Pathogens from the Red Meat Supply" to the 2002 Convention of the North American Meat Association Convention, Monterey, CA, 2002.

51. Invited to lecture on "*E. coli* O157:H7 and non-O157 Shiga Toxin *E. coli* (STECs) in Beef Processing Plants" sponsored by the Excel Corporation Food Safety and Technology Seminars for the retailer, food service, and manufacturing segment of the meat industry: Dallas, TX; Philadelphia, PA; Columbus, OH; Calgary, Alberta, Canada; Charlotte, NC; Toronto, Ontario, Canada; and Denver, CO, 2002.

52. Organized and chaired the Food Safety and Biosecurity portion of the 2002 Reciprocal Meat Conference (the annual meeting of the American Meat Science Association), East Lansing, MI, 2002.

53. Invited by the Hawaii County Administrator to be part of a team of four to help the island modernize its meat industry, 2002.

54. Invited to give a presentation on "Application of Proteomics to Meat Quality Research" to the 2002 Annual Reciprocal Meat Conference (the annual meeting of the members of the American Meat Science Association), East Lansing, MI, 2002.

55. Is a member of a committee appointed by NCBA to evaluate the feasibility and criteria for a reward system for the development of a method of tenderness-based beef classification, 2002.

56. Is a member of the NCBA *E. coli* Summit Task Force. The task force was established in the fall of 2002 by NCBA and in response to *E. coli* O157:H7 outbreaks and product recalls to develop an industry plan to regain control of the safety of beef and beef products, 2002.

57. Invited to be a member of an industry panel to develop a pathogen-testing program to replace the Salmonella-testing program, 2002.

58. Invited to give a talk on "Industry-wide Plans to Develop Hide-Interventions to Reduce Pathogens on Beef," National Cattlemen's Beef Association, Denver, CO, 2002.

59. Invited to give a talk on "Pathogens in Commercial Beef Processing Plants" to the Ground Beef Forum sponsored by Excel Corporation, Chicago, IL, 2002.

60. Invited by Elsevier Press to be the Editor for North America for Meat Science, 2002.

61. Invited to speak at the New Scientist Orientation for the Northern Plains Area on "What You Need to Know to be a Successful ARS Scientist," Fort Collins, CO, 2002.

62. Invited to present a summary of the report he prepared for FSIS on Beef Trim to the FSIS

Administrator and his staff, 2003.

63. Invited to speak on the status of the risk of *E. coli* O157:H7 in beef to the NCBA sponsored and by invitation-only *E. coli* Summit, San Antonio, TX, 2003.

64. Invited to meet with IBP/Tyson senior management to discuss the status of *E. coli* O157:H7 and potential new strategies to combat it, Dakota Dunes, SD, 2003.

65. Invited to meet with the senior staff of the American Meat Institute Foundation to update them on our recent findings and to discuss future projects, Washington, DC, 2003.

66. Brought five beef companies (IBP, Excel, Swift, National, and Harris Ranch) and two commodity groups (NCBA and AMI) together to agree to share the cost of a major project. Every company paid for the supplies in-kind, the two commodity groups covered travel costs, and ARS scientists collected and analyzed the data, 2003.

67. Invited to be a member of the National Pork Board's Quality Solutions Advisory Group to advise the organization on pork quality issues, 2003.

68. Invited to be a speaker at the Science & Technology Round Table entitled "Preventing *E. coli* O157:H7: What Works," National Meat Association MEATXPO'03. The 57th Annual Convention of the National Meat Association, Las Vegas, NV, 2003.

69. Invited to prepare an article on "Carcass Composition and Quality: Genetic Influence" for the first edition of the Encyclopedia of Animal Science (EAS), to be published by Marcel Dekker, Inc., 2003.

70. Invited to speak on "The Status and Efficacy of Post-Harvest Interventions for *E. coli* O157:H7" by the Plains Nutrition Research Council, San Antonio, TX, 2003.

71. Invited to give a presentation on "Application of Transgenic Approaches to Meat Quality Research" to the 2003 Annual Reciprocal Meat Conference (the annual meeting of the members of the American Meat Science Association), Columbia, MO, 2003.

72. Invited to give a presentation on "Sampling Protocol to Verify Beef Harvest Process and Recent Advances in Interventions to Reduce *E. coli* O157:H7 from Beef" to the 2003 Annual Reciprocal Meat Conference (the annual meeting of the members of the American Meat Science Association), Columbia, MO, 2003.

73. Invited to give two talks "Biological Basis of Beef Tenderness and Tenderization Methods" and "Recent Advances to Combat *E. coli* O157:H7 in Beef" at the Harris Ranch's "Partnership for Quality" conference, Coalinga, CA, 2003.

74. Invited by the Neogen Corporation to give a lecture on "Microbial Sampling to Verify Slaughter Process" to the Neogen Food Safety Advisory Committee, East Lansing, MI, September 2003.

75. Invited by Eli Lilly and Company to meet with their key staff and discuss the food safety challenges facing the U.S. beef and meat industry, Indianapolis, IN, December 2003.

76. Invited to meet with key Excel staff (Meat Division of Cargill) and vice president for meat and seafood of Safeway to advise them on providing tenderness guaranteed tender beef to Safeway (third largest U.S. grocer), Denver, CO, December 2003.

77. Invited to participate and give a talk on the significance of cattle hide in beef safety to "The State of New Technologies to Enhance Public Health Symposium" sponsored by the Food Safety and Inspection Service of the USDA, Omaha, NE, February 2004.

78. Invited to give a talk on "Recent Advances to Combat *E. coli* O157:H7" to the 2004 Annual Convention of the National Cattlemen's Beef Association, Phoenix, AZ, January 2004.

79. Invited to give a talk on "Effectiveness of *E. coli* O158:H7 Screening Tools," at the 58th Annual Convention of the National Meat Association and participate in the roundtable discussion "Reducing Pathogens in Raw Meat," San Antonio, TX, February 2004.

80. Invited to be one of 20 selected individuals to organize the "by invitation only" 2004 "BSE Summit" sponsored by the meat industry. I was asked to organize the section dealing with the diagnostics and surveillance section of the Summit, 2004.

81. Invited to be one of four individuals to advise Outback Steakhouse for meat quality and safety issues. Since February 2004.

82. Invited to speak on "Microbial Sampling to Verify Slaughter Process and Recent Development in Hide Interventions" to the audience at the American Meat Institute Foundation sponsored meeting on "Best Harvest Practices for the U.S. Beef Processing Plant," Kansas City, MO, April 2004.

83. Invited to meet with the Food Safety Advisory Team for the Smithfield Beef Group and give a talk on "Recent Advances in *E. coli* O157:H7 Research," Kalamazoo, MI, May 2004.

84. Invited to give a talk on "Value-based Beef Classification" to the 2004 Beef Improvement Federation Meeting, Rapid City, South Dakota, May 2004.

85. Invited to attend the Annual Convention of the Southwest Meat Association and give a talk on "The Latest Development in Pathogen Reduction Research," Albuquerque, NM, July 2004.

86. Invited to attend and make a presentation on "Postmortem Aging as Related to Tenderness and Flavor of Beef" to the Beef Food Safety Council Advisory Meeting sponsored by CTI Foods, Standard Meat Company, and Outback Steakhouse, Tampa, FL, July 2004.

87. Invited to present a talk "Recent Research Data on Status of *E. coli* O157:H7 in Beef and in Beef Processing Plants" at the National Meat Association (NMA) Summer Conference, Jackson, WY, August 2004.

88. Invited to present seminar "Biological Basis that Determines Beef Tenderness" to the decision

makers of the CTI Foods/Standard Meat Company, Fort Worth, TX, September 2004.
89. Invited to make a presentation "Post-harvest Interventions to Reduce/Eliminate *E. coli* O157:H7
in Beef" at the Meat Industry Research Conference (MIRC) of the 2004 Annual Convention of the American Meat Institute Foundation, Nashville, TN, September 2004.

90. Invited to participate in a panel discussion on "*E. coli* O157-Related Issues in Beef" at the National Meat Association's 59th Annual Convention, Las Vegas, NV, February 2005.
91. Invited to make presentation on "Food Safety Issues Related to Beef and Pork" at the Food Safety Council Advisory Meeting of CTI Foods/Standard Meat Co. /Outback Steakhouse, Las Vegas, NV, February 2005.
92. Invited to make a presentation on "Food Safety Research at MARC" at Neogen Corporation's Food Safety Advisory Committee Meeting, Lansing, MI. February 2005.
93. Invited to meet with executives of the American Food Groups and update them on our latest information of methods to predict beef tenderness. Also helped them determine the feasibility of implementing the "MARC beef classification system." Green Bay, WI. April 2005
94. Invited to meet with the Food Safety Advisory Team for the Smithfield Beef Group and advise
them on ways to control *E. coli* O157:H7 in and on beef. Fort Collins, CO. May 2005.
95. Invited to attend and make a presentation on "Postmortem Aging as Related to Tenderness and
Flavor of Beef" to the Beef Food Safety Council Advisory Meeting sponsored by CTI Foods, Standard Meat Company, and Outback Steakhouse, Tampa, FL, June 2005.
96. Invited to present "Safety Issues Related to Beef and Pork" for the Food Safety Council Advisory Meeting of officials of CTI Foods, Standard Meat Company, and Outback Steakhouse, Tampa, Florida, Febr. 2006.
97. Invited to present "Instrumentation for Beef Tenderness" in a Seminar for American Foods Group officials, Green Bay, Wisconsin, Febr. 2006.
98. Invited to present "Recent Research Data on Reducing *Escherichia coli* O157:H7 Load on Cattle" for the National Meat Association (NMA) 60th Annual Convention, San Francisco, California, Mar. 2006.
99. Invited to present "A Summary of the Results of Food Safety Projects Funded by the NCBA" at
the Beef Industry Safety Summit, Jacksonville, Florida, Apr. 2006.
100. Invited to meet with executives of Superior Farms to assist with food safety procedures, in Dixon, California, May 2006.
101. Invited to present "Instrument Grading" for the Quality Assurance Advisory Board and Quality
Assurance state coordinators of National Cattlemen's Beef Association, Kansas City, Missouri, June 2006.
102. Invited to present "Results from USMARC *E. coli* O157:H7 Research" for the Quality Assurance Advisory Board and Quality Assurance state coordinators of National Cattlemen's Beef Association, Kansas City, Missouri, June 2006.
103. Invited to speak on "Instrumentation for Beef Classification" to the Nebraska Cattlemen, June 9,
2006, Holdrege, Nebraska, June 2006.

104. Invited to make a presentation "Summary of the Last 12 Months of Food Safety Accomplishments at USMARC" for meeting of National Cattlemen's Beef Association, Reno, Nevada, July 2006.
105. Invited to participate in the Merial Igenity Roundtable, sponsored by Vance Publishing Co., Kansas City, Missouri, Aug. 2006.
106. Invited to lead group discussion on "The Research Priority of Food Safety and Quality Assurance" for the Meat Industry Research Convention, co-sponsored by AMIF and AMSA, Hollywood, Florida, Oct. 2006.
107. Invited to participate in the Foot and Mouth Disease Summit, National Cattlemen's Beef Association, Washington, DC, Oct. 2006.
108. At the request of the National Cattlemen's Beef Association (NCBA), I and four other individuals planned and organized the meeting on current and future research needs on the issue of TSEs, particularly BSE, sponsored by the National Cattlemen's Beef Association (NCBA), on behalf of the Beef Checkoff, Sonoma, California, Oct. 2006.
109. Invited to attend and chair breakout session on "Surveillance and Testing" at the invitation only
TSE Think Tank, Sonoma, California, Nov. 2006.
110. 111. Invited to present a seminar on "Sources of *E. coli* O157:H7" at the United States-Japan
Cooperative Program on Development & Utilization of Natural Resources (UJNR) 10th International Symposium on Toxic Microorganisms, FDA-CFSAN, College Park, Maryland, Nov. 2006.
112. Invited to participate in Full Proposal Review by members of AMIF Research Advisory Council,
Washington, DC, Nov. 2006.
113. Invited to present a talk on "Recent Developments in Control of *E. coli* O157:H7" for executives
of In-N-Out Burger, Baldwin Park, California, Jan. 2007.
114. Invited to meet with sheep processors to discuss data collected in sheep processing plants and
discuss future research plans, American Sheep Industry Convention, San Antonio, Texas, Jan. 2007.
115. Invited to present "Update on Food Safety Projects at USMARC" at NCBA 2007 Cattle Industry
Annual Convention, Nashville, Tennessee, Jan. 2007.
116. Invited to present "Update on Food Safety Research from the U.S. Meat Animal Research Center" at a Beef Food Safety Council Advisory Meeting organized by CTI Foods, Standard Meat Co., and Outback Steakhouse, Tampa, Florida, Febr. 2007.
117. Invited to participate in Specialty Meeting on "Kill Floor Interventions" at the National Meat
Association's Meat XPO'07, Las Vegas, Nevada, Febr. 2007.
118. Invited to participate in Round Table-Science and Technology on "Fresh Meat Pathogens" at the
National Meat Association's Meat XPO'07, Las Vegas, Nevada, Febr. 2007.
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Industry (cont'd)

119. Invited to participate in Reciprocation Session on "Transportation and Lairage Effects on Pathogen Prevalence on Beef Hides" at the NCBA Beef Safety Summit, Dallas, Texas, Febr. 2007.
120. Invited to present "Research Updates: Latest Research Data on Current Beef Safety Issues"
121. Invited to present an "Issues Update Forum: Multi Drug-Resistant *Salmonella* in the Beef Chain"
at the NCBA Beef Safety Summit, Dallas, Texas, Febr. 2007.
122. Invited to make a presentation on "Effective Controls to Reduce/Eliminate *E. coli* O157:H7 from
the Red Meat Supply" at a Symposium on Antimicrobial Interventions for Quality Control of Meat and Poultry Products, Canadian Meat Council, Toronto, Ontario, Canada, Sept. 2007.
123. Invited to speak on "How to Do Public Research for the Good of All" for the Industry Advisory
Board of the Institute for Food and Agricultural Sciences Value-Added Meat Program in Calgary, Alberta, Canada, Sept. 2007.
124. Invited to speak on "Sources of *E. coli* O157:H7 on Beef and Interventions to Control It" for
officials and scientists of GangaGen Life Sciences, Inc., Ottawa, Canada, Dec. 2007.
125. Invited to assist the Beef Industry Food Safety Council (BIFSCo) of National Cattlemen's Beef
Association in producing a video on proper sampling protocol, Thomasville, Florida, Dec. 2007.
126. Invited to make a presentation, "Update on the Latest Beef Safety Research that USMARC has
Conducted with Funds Provided by the Beef Council of NCBA" to the Joint Beef Safety Committee of NCBA, NCBA Cattle Industry Convention and Trade Show, Reno, Nevada, Febr. 2008.
127. Invited to make a presentation "Update on the Latest Beef Safety Research that USMARC has
Conducted with Funds Provided by the Beef Council of NCBA" to the Joint Product Enhancement Committee of NCBA, NCBA Cattle Industry Convention and Trade Show, Reno, Nevada, Febr. 2008.
128. Invited to moderate Round Table Session, "Whipping the *E. coli* Problem Again" at National
Meat Association's 62nd Annual Convention, Monterey, California, February 2008.
129. Invited to make a presentation on "Updates on the Latest Beef Safety Research and How the Data and Technologies Can Be Applied in Industry" to the Beef Industry Food Safety Council (BIFSCo) of the National Cattlemen's Beef Association, Dallas, Texas, March 2008.
130. Invited to make a presentation on "Best Practices: HACCP in Processing" to the Beef Industry
Food Safety Council (BIFSCo) of the National Cattlemen's Beef Association, Dallas, Texas, March 2008.
131. Invited to present a talk of the current and potential future status of the non-intact and to lead the
discussion on the regulatory status of E. coli O157. OSI Food Safety meeting, Tampa, Florida, June 2008.

132. Invited to present the baseline data on the surveillance of E. coli O157 in medium to small beef
processing plants and to lead a discussion with operators of small/medium plants to improve their dressing practices. South West Meat Association Annual Convention, Scottsdale, Arizona, July 2008.

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Industry (cont'd)

133. Invited to make a presentation "Control of *E. coli*O157 in Beef" at the National Meat Association summer convention. Telluride, Colorado. August 2008.

134. Invited to attend the 17th World Meat Congress and make a presentation entitled "Using Research to Enhance Red Meat Sustainability by Meeting Consumer Expectations." September 2008; Cape Town South Africa.

135. Invited to attend 2nd Annul Meat Meeting Congress and present a lecture on "Beef Safety and
Quality" Moscow Russia, May 2009

135. Invited to present a talk on "Slaughter Harvest Mapping: A Process Control Systems Approach"
and to participate on an industry panel on "Food Chain Management and Pathogen Control – Success and Challenges" at The Third Governor's Conference on Ensuring Food Safety: *E. coli* O157:H7 – Progress and Challenges. Lincoln, Nebraska May 2009.

136. Invited to present a talk on "Beef Tenderness: Determination, Regulation and Prediction" at the
2009 Beef Improvement Federation Conference. Sacramento, CA May 2009.

137. Invited to make a presentation on "Testing of Raw Ground Beef Components for *E. coli* O157:H7, Testing of Finished Product" at the National Meat Association Food Safety and Inspection Forum. Napa, California August 2009.

138. Invited to make a presentation on "Making it Practical for In-Plant Implementation" at the Laboratory Test Kit Validation public meeting sponsored National Meat Association, South West Meat Association and USDA-FSIS. November 4, 2009; Washington D.C.

139.

International Meetings and Congresses

1. Invited to present a seminar and write a manuscript on "The Role of Ca^{2+}-Dependent Proteases (Calpains) in Postmortem Proteolysis and Meat Tenderness" at a workshop at the University of Nottingham, United Kingdom, sponsored by the Organization for Economic Co-operation and Development, 1991.

2. Invited to present a seminar on "The Role of Proteinases in Muscle Growth and Meat Quality" to
the National Institute of Animal Science, Tjele, Denmark, 1991.

3. Invited to make an oral presentation and write a paper on "Muscle Proteases and Meat Aging, Recent Development, Present Trends and Research Needs" to the 39th International Congress of Meat Science and Technology, Clermont-Ferrand, France, 1992.

4. Invited to make a presentation and write a book chapter on "A New Insight in Genetic Selection
Possibly Based on Calpastatin" to the Congress on "Expression, Regulation and Role of

Proteinases in Muscle Development and Meat Quality" sponsored by ECCEAMST (European Consortium for Continuing Education in Advanced Meat Science and Technology) and OECD (Organization for Economic Co-operation and Development), Clermont-Ferrand, France, 1993.

5. Invited to speak on "Factors Regulating Beef Quality" at the Beef 94 World Congress, Buenos Aires, Argentina, 1994.

6. Invited to present a keynote paper on "The Biological Basis of Meat Tenderness and Potential Genetic Approaches for Its Control and Prediction" at the 41st Annual International Congress of Meat Science and Technology (ICoMST) joint meeting with American Meat Science Association, San Antonio, Texas, 1995.

7. Invited to present two keynote lectures on "Beef Tenderness: Regulation and Prediction" at the CSIRO Meat 95 International Congress, Australia, 1995.

8. Invited to give a series of lectures and seminars over a 4-day period on "Enzymology of Muscle
Protein Degradation during Animal Growth and Postmortem Storage" at the Lincoln University, Christchurch, New Zealand, 1995.

9. Invited to present a seminar on "The Role of Endogenous Proteases in Muscle Growth and Meat
Tenderness" to AgResearch-Ruakura Agricultural Research Centre, Hamilton, New Zealand, 1995.

10. Invited to present plenary lecture "Biochemical Factors Regulating the Toughening and Tenderization Processes of Meat" at the 42nd International Congress of Meat Science and Technology (ICoMST), Lillehammer, Norway, 1996.

11. Invited to present a guest lecture "Biochemical Factors Regulating the Toughening and Tenderization Process of Meat" at The Royal Veterinary and Agricultural University, Copenhagen, Denmark, 1996.

12. Invited to be one of two main lecturers in teaching a graduate level course entitled "Advanced Meat Science and Muscle Metabolism" to European students at the Swedish University of Agricultural Science, Uppsala, Sweden, 1997.

International Meetings and Congresses (cont'd)

13. Invited to be a primary lecturer in a "Meat Tenderness-Mechanisms, Industry Problems and Practical Solution" course sponsored by MATFORSK (Norwegian Food Research Institute) at the Agricultural University of Norway, 1997.

14. Invited to make a presentation entitled "Effect of Prerigor Freezing and Postrigor Calcium Chloride Injection on Tenderness of Callipyge Longissimus" at the 26th Annual Meeting of the United States-Japan Natural Resources Protein Panel (UJNR), Tsukuba, Japan, 1997.

15. Invited to chair a session at an international symposium sponsored by the European Commission on "New Developments in Guaranteeing the Optimal Sensory Quality of Meat," Madrid, Spain, May 5-7, 1999.

16. Invited to attend AnGus Association meeting (Argentine Cattle Breeders Association) to make a technical presentation on "Beef Tenderness in 2000," Rosario City, Argentina, 2000.

17. Invited to present a seminar entitled "The U.S. Beef Industry: Current Practices and Challenges" at INRA, Theix - Clermont Ferrand, France, 2001.

18. Invited to present a seminar entitled "Calpastatin: Roles in Muscle Growth and Meat

Quality" at INRA, Theix - Clermont Ferrand, France, 2001.
19. Invited to be a lecturer at the Second Annual Beef Cattle Short Course: Improvement on Reproductive Technology, Feeding Efficiency and Marbling, organized by the Wagyu F1 Council and Japan Cattleman's Association. Subject: The Biological Basis of Meat Tenderness and Its Genetic Control. Obihiro, Hokkaido, Japan, 2001.
20. Invited to present a plenary lecture "Meat Tenderness and Muscle Growth: Is There Any Relationship" at the 48th International Congress of Meat Science and Technology (ICoMST), Rome, Italy, 2002.
21. Invited to present a plenary lecture "Protein Turnover and Proteolytic Enzymes in Relation with Species, Breed and Muscle Type" at the 48th International Congress of Meat Science and Technology (ICoMST), Rome, Italy, 2002.
22. Invited to write a book chapter for "Texture in Food Volume 1: Solid Foods" edited by Dr. David Kilcast of Leatherhead Food Research Association, UK, 2002.
23. Invited to give a talk on "New Technologies for Tenderness Enhancement" at the byinvitation-
only Beef Palatability Enhancement Workshop organized by the Canadian Cattlemen, Calgary, Canada, 2002.
24. Invited to give two talks at the Consistency of Quality International Meat Symposium. Titles of the talks were "The Biological Basis and Strategies to Optimize Beef Tenderness" and "Prevalence of Foodborne Pathogens and Methods to Combat These Pathogens in Beef Processing Plants." At the invitation of the director, Dr. Koohmaraie visited ARC-ANPI meat research laboratories and facilities to advise him and the scientists on the new trends and research techniques/approaches, Johannesburg, South Africa, 2003.

International Meetings and Congresses (cont'd)
25. Invited to write a book chapter on "Biotechnology in the Meat Industry: (c) Transgenic Animals for Non-Meat Traits" in "Encyclopedia of Meat Sciences" to be published by Elsevier Science, Oxford, England, 2003.
26. Invited to speak at a meat quality symposium sponsored by the Canadian Society of Animal Science on "The Biological Basis and Strategies to Optimize Beef Tenderness," Saskatoon, Saskatchewan, Canada, 2003.
27. Invited to speak at the French Meat and Livestock Association with the sponsorship of the International Meat Secretariat at a workshop on "Control of Growth, Carcass and Meat Quality Characteristics: Beef," Paris, France, 2003.
28. Invited to present plenary lecture "Latest Advances in Meat Quality Research" at the Brazilian Nation Meeting, Santa Maria, Rio Grande De Sol, Brazil, 2003.
29. Invited by the Guelph University to present a lecture to an audience that encompassed the university, the Guelph community, and the Ontario meat industry on "Biological Factors Regulating Beef Tenderness" and "The Status of *E. coli* O157:H7 in U.S. Beef Production and Intervention Designed to Combat These Pathogens in U.S. Beef," 2003.
30. Invited to give two talks at the South African Feedlot Association Conference titled "Beef is Good for You." Titles of the talks were "The Biological Basis and Strategies to Optimize Beef Tenderness" and "Prevalence of Foodborne Pathogens and Methods to Combat These Pathogens in Beef Processing Plants," Johannesburg, South Africa, 2004.

31. Invited to make a presentation on "New Technologies for the Evaluation of Beef Carcass Quality" at the Western Nutrition Conference, Saskatoon, Saskatchewan, Canada, 2004.
32. Invited to give a talk on "Understanding and Managing Beef Quality" at the 9th World Angus Forum, Cape Town, South Africa, March 2005.
33. Invited to attend the annual Langford Food Industry Conference and present an overview talk on beef tenderness, Bristol, England, May 2005.
34. Invited to travel to New Zealand (South Island) and give a series of presentations on meat quality and meat safety to the meat industry and faculty and graduate students of Lincoln University. August 2005.
35. Invited by the International Conference of Meat Science and Technology (ICoMST) to present "Contribution of Postmortem Muscle Biochemistry to the Delivery of Consistent Meat Quality with Particular Focus on the Calpain System" for a Session on "A Science Based Approach to Assuring Consistent Meat Quality," Dublin, Ireland, Aug. 2006.
36. Invited to make a presentation on "Biology of Food-Borne Pathogens and Analysis of Bacterial Toxins" in Session on Methods of Public Health Laboratories for Detection of Human, Animal and Food Microbial Pathogens at the United States-Japan Cooperative Program on Development & Utilization of Natural Resources (UJNR) 10th International Symposium on Toxic Microorganisms, Washington, DC, Nov. 2006.
37. Invited to travel to China and make two presentations on food safety and meat quality to the faculty, graduate students and staff of the Nanjing Agricultural University. Nanjing, China. Dec 2006.
38. Invited as a Key Address Speaker for the 53rd International Conference of Meat Science and Technology (ICoMST), to present "Interventions to Reduce/Eliminate *Escherichia coli* O157:H7 in Ground Beef" in Beijing, China, Aug. 2007.
39. Invited to attend the 2008 New Zealand Institute of Food Science and Technology and make a presentation titled "Sources of E.coli 0157:H7 and Interventions to reduce / eliminate pathogens on beef." Ruakura, New Zealand, June 2008.
41. Invited to attend the 2008 New Zealand Institute of Food Science and Technology and make and participate on panel on E.coli 0157:H7 - Effect on sustainability of the beef industry - a discussion." Ruakura, New Zealand, June 2008.
42. Invited to make a plenary presentation on "Using research to enhance red meat sustainability by meeting consumer expectations" to the 17th International Meat Secretariat's World Meat Congress in Cape Town, South Africa. September 2008.
43. Invited to attend the 2008 New Zealand Institute of Food Science and Technology and make a make a presentation on "The biological basis for the variation in meat tenderness and tools for predicting beef tenderness." Ruakura, New Zealand, June 2008.
44. Invited to visit Lincoln University to meet with the faculty and students and a make a presentation on "Beef Demand." Lincoln, New Zealand. June 2008.
45. Invited to visit Lincoln University to meet with the faculty and students and a make a presentation on "Beef Demand." Lincoln, New Zealand. June 2008.
46. Invited to attend Tropical and Subtropical Beef Cattle Congres which we are organizing in Porto Alegre, Brazil and present a talk on : "The quality of beef:

Measures that can be taken by the ranchers, packers and retailers to improve it." November 2008.

47. Invited to attend the IV Congress "Del Campo al Plato" and make a presentation on "Factors that determines beef demand (beef safety and quality)." Montevideo, Uruguay, November 2008.

48. Invited to attend the final international conference in the current Pathogenic Escherichia coli Network project (FOOD-CT-026356) and present a titled "A systematic approach to control *E. coli* O157:H7 in the red meat supply." Teagasc-Ashtown Food Research Centre in Dublin, Ireland. September 2009

49. Invited to attend and make a presentation entitled " Recent Advances in Science that Have the Potential to Benefit the Meat Processing Industry" at the Australian Meat Industry Council's Meat Industry Conference. Gold Coast, Australia. September 2009

PUBLICATIONS

Refereed & Reviewed Journal Articles

1. Elgasim, E. A., W. H. Kennick, A. F. Anglemier, E. A. Elkhlifa and M. Koohmaraie. 1982. Effects of prerigor pressurization on the emulsifying capacity of muscle proteins. J. Food Sci. 47:861-863.

2. Elgasim, E. A., W. H. Kennick, A. F. Anglemier, M. Koohmaraie and E. A. Elkhlifa. 1983. Effect of pre-rigor pressurization on bovine lysosomal enzyme activity. Food Microstructure 2:91-97.

3. Koohmaraie, M., W. H. Kennick, E. A. Elgasim, R. L. Dickson and W. E. Sandine. 1983. Storage and display-life characteristics of beef as affected by prerigor pressurization. J. Food Prot. 46:878-882.

4. Koohmaraie, M., W. H. Kennick, E. A. Elgasim, R. L. Dickson and W. E. Sandine. 1983. Effect of prerigor pressurization on the retail characteristics of beef. J. Food Sci. 48:998-999.

5. Koohmaraie, M., W. H. Kennick, A. F. Anglemier, E. A. Elgasim and T. K. Jones. 1984. Effect of postmortem storage on cold-shortened bovine muscle: Analysis by SDSpolyacrylamide gel electrophoresis. J. Food Sci. 49:290-291.

6. Koohmaraie, M., W. H. Kennick, E. A. Elgasim and A. F. Anglemier. 1984. Effects of postmortem storage on muscle protein degradation: Analysis by SDS-polyacrylamide gel electrophoresis. J. Food Sci. 49:292-293.

7. Lin, C. K., W. H. Kennick, W. E. Sandine and M. Koohmaraie. 1984. Effect of electrical stimulation on meat microflora: Observations on agar media, in suspensions and on beef carcasses. J. Food Prot. 47:279-283.

8. Koohmaraie, M., W. H. Kennick, E. A. Elgasim and A. F. Anglemier. 1984. Effect of prerigor pressurization on the activity of calcium-activated factor. J. Food Sci. 49:680-684.

9. Elgasim, E. A., M. Koohmaraie, W. H. Kennick and A. F. Anglemier. 1985. The combined effects of the calcium activated factor and cathepsin D on skeletal muscle. Food Microstructure 4:55-61.

10. Koohmaraie, M., J. E. Schollmeyer and T. R. Dutson. 1986. Effect of low-calciumrequiring calcium activated factor on myofibrils under varying pH and temperature conditions. J. Food Sci. 51:28-32 & 65.

11. Koohmaraie, M., J. E. Schollmeyer and T. R. Dutson. 1986. The reversible inhibition of myoblast proliferation by -hexachlorocyclohexane. J. Anim. Sci. 63:326-333.

12. Koohmaraie, M. 1987. The relationship between cell proliferation and phosphatidylinositol metabolism in skeletal muscle cells in culture. J. Anim. Sci. 64:633-641
13. Koohmaraie, M. S. C. Seideman, J. E. Schollmeyer, T. R. Dutson and J. D. Crouse. 1987. Effect of post-mortem storage on Ca++-dependent proteases, their inhibitor and myofibril fragmentation. Meat Sci. 19:187-196.
14. Seideman, S. C., M. Koohmaraie and J. D. Crouse. 1987. Factors associated with tenderness in young beef. Meat Sci. 20:281-292.
15. Seideman, S. C., M. Koohmaraie and J. D. Crouse. 1988. The influence of muscle fiber size on tenderness in A-maturity heifers. J. Food Qual. 11:27-34.
16. Koohmaraie, M., S. C. Seideman, J. E. Schollmeyer, T. R. Dutson and A. S. Babiker. 1988. Factors associated with the tenderness of three bovine muscles. J. Food Sci. 53:407-410.
17. Koohmaraie, M., S. C. Seideman and J. D. Crouse. 1988. Effect of subcutaneous fat and high temperature conditioning on bovine meat tenderness. Meat Sci. 23:99-109.
18. Koohmaraie, M. 1988. Palatability of meat from bull and steer carcasses. ISI Atlas of Science 1:124-128. (Invited Review Paper)
19. Koohmaraie, M., A. S. Babiker, R. A. Merkel and T. R. Dutson. 1988. Role of Ca++-dependent proteases and lysosomal enzymes in postmortem changes in bovine skeletal muscle. J. Food Sci. 53:1253-1257.
20. Koohmaraie, M., A. S. Babiker, A. L. Schroeder, R. A. Merkel and T. R. Dutson. 1988. Acceleration of postmortem tenderization in ovine carcasses through activation of Ca^{2+}-dependent proteases. J. Food Sci. 53:1638-1641.
21. Koohmaraie, M. 1988. The role of endogenous proteases in meat tenderness. Proc. Recip. Meat Conf. 41:89-100. (Invited Review Paper)
22. Dickson, J. S. and M. Koohmaraie. 1989. Cell surface charge characteristics and their relationship to bacterial attachment to meat surfaces. Appl. Environ. Microbiol. 55:832-836.
23. Koohmaraie, M., J. D. Crouse and H. J. Mersmann. 1989. Acceleration of postmortem tenderization in ovine carcasses through infusion of calcium chloride: Effect of concentration and ionic strength. J. Anim. Sci. 67:934-942.
24. Crouse, J. D., L. V. Cundiff, R. M. Koch, M. Koohmaraie and S. C. Seideman. 1989. Comparisons of *Bos indicus* and *Bos taurus* inheritance for carcass beef characteristics and meat palatability. J. Anim. Sci. 67:2661-2668.

25. Crouse, J. D. and M. Koohmaraie. 1990. Effect of time of sampling postmortem on myofibril fragmentation index of meat. J. Food Sci. 55:254-256.
26. Koohmaraie, M. 1990. Inhibition of postmortem tenderization in ovine carcasses through infusion of zinc. J. Anim. Sci. 68:1476-1483.
27. Koohmaraie, M. 1990. Quantification of Ca^{2+}-dependent protease activities by hydrophobic and ion-exchange chromatography. J. Anim. Sci. 68:659-665.
28. Koohmaraie, M., G. Whipple and J. D. Crouse. 1990. Acceleration of postmortem tenderization in lamb and Brahman-cross beef carcasses through infusion of calcium chloride. J. Anim. Sci. 68:1278-1283.
29. Koohmaraie, M. and D. H. Kretchmar. 1990. Comparisons of four methods for quantification of lysosomal cysteine proteinase activities. J. Anim. Sci. 68:2362-2370.
30. Crouse, J. D. and M. Koohmaraie. 1990. Effect of freezing of beef on subsequent postmortem aging and shear force. J. Food Sci. 55:573-574.

31. Whipple, G., M. Koohmaraie, M. E. Dikeman, J. D. Crouse, M. C. Hunt and R. D. Klemm. 1990. Evaluation of attributes that affect longissimus muscle tenderness in *Bos taurus* and *Bos indicus* cattle. J. Anim. Sci. 68:2716-2728.
32. Whipple, G., M. Koohmaraie, M. E. Dikeman and J. D. Crouse. 1990. Effects of hightemperature conditioning on enzymatic activity and tenderness of *Bos indicus* longissimus muscle. J. Anim. Sci. 68:3654-3662.
33. Whipple, G., M. Koohmaraie, M. E. Dikeman and J. D. Crouse. 1990. Predicting beeflongissimus tenderness from various biochemical and histological muscle traits. J. Anim. Sci. 68:4193-4199.
34. Crouse, J. D. and M. Koohmaraie. 1990. Effect of post-cooking storage conditions on shear-force values of beef steaks. J. Food Sci. 55:858 & 860.
35. St. Angelo, A. J., M. Koohmaraie, K. L. Crippen and J. Crouse. 1991. Acceleration of tenderization/inhibition of warmed-over flavor by calcium chloride-antioxidant infusion into lamb carcasses. J. Food Sci. 56:359-362.
36. Shackelford, S. D., M. Koohmaraie, M. F. Miller, J. D. Crouse and J. O. Reagan. 1991. An evaluation of tenderness of the longissimus muscle of Angus by Hereford versus Brahman crossbred heifers. J. Anim. Sci. 69:171-177.
37. Koohmaraie, M., G. Whipple, D. H. Kretchmar, J. D. Crouse and H. J. Mersmann. 1991. Postmortem proteolysis in longissimus muscle from beef, lamb and pork carcasses. J. Anim. Sci. 69:617-624.

38. Wheeler, T. L. and M. Koohmaraie. 1991. A modified procedure for simultaneous extraction and subsequent assay of calcium-dependent and lysosomal protease systems from a skeletal muscle biopsy. J. Anim. Sci. 69:1559-1565.
39. Koohmaraie, M. and S. D. Shackelford. 1991. Effect of calcium chloride infusion on the tenderness of lambs fed a -adrenergic agonist. J. Anim. Sci. 69:2463-2471.
40. Crouse, J. D., M. Koohmaraie and S. C. Seideman. 1991. The relationship of muscle fibre size to tenderness of beef. Meat Sci. 30:295-302.
41. Crouse, J. D., M. Koohmaraie and J. S. Dickson. 1991. Storage and bacterial contamination effects on myofibrillar proteins and shear force of beef. J. Food Sci. 56:903-905.
42. Shackelford, S. D., M. Koohmaraie, G. Whipple, T. L. Wheeler, M. F. Miller, J. D. Crouse and J. O. Reagan. 1991. Predictors of beef tenderness: Development and verification. J. Food Sci. 56:1130-1135 & 1140.
43. Whipple, G. and M. Koohmaraie. 1991. Degradation of myofibrillar proteins by extractable lysosomal enzymes and m-calpain and the effects of zinc chloride. J. Anim. Sci. 69:4449-4460.
44. Koohmaraie, M., S. D. Shackelford, N. E. Muggli-Cockett and R. T. Stone. 1991. Effect of the -adrenergic agonist, L644,969, on muscle growth, endogenous proteinase activities and postmortem proteolysis in wether lambs. J. Anim. Sci. 69:4823-4835.
45. Wheeler, T. L., M. Koohmaraie and J. D. Crouse. 1991. Effects of calcium chloride injection and hot-boning on the tenderness of round muscles. J. Anim. Sci. 69:4871-4875.
46. Koohmaraie, M. 1992. The role of Ca^{2+}-dependent proteases (calpains) in *post mortem* proteolysis and meat tenderness. Biochimie 74:239-245. (Invited Review Paper)
47. Whipple, G. and M. Koohmaraie. 1992. Effects of lamb age, muscle type, and 24-hour

activity of endogenous proteases on postmortem proteolysis. J. Anim. Sci. 70:798-804.
48. Wheeler, T. L. and M. Koohmaraie. 1992. Effects of the -adrenergic agonist L644,969 on muscle protein turnover, endogenous proteinase activities and meat tenderness in steers. J. Anim. Sci. 70:3035-3043.
49. Koohmaraie, M. 1992. Effect of pH, temperature and inhibitors on autolysis and catalytic activity of bovine skeletal muscle -calpain. J. Anim. Sci. 70:3071-3080.
50. Whipple, G. and M. Koohmaraie. 1992. Freezing and calcium chloride marination effects on beef tenderness and calpastatin activity. J. Anim. Sci. 70:3081-3085.
51. Koohmaraie, M. 1992. Muscle proteinases and meat aging. ICoMST 38:61-69. (Invited Review Paper)

52. Wheeler, T. L., J. D. Crouse and M. Koohmaraie. 1992. The effect of postmortem time of injection and freezing on the effectiveness of calcium chloride for improving beef tenderness. J. Anim. Sci. 70:3451-3457.
53. Koohmaraie, M. 1992. Ovine skeletal muscle multicatalytic proteinase complex (proteasome): Purification, characterization and comparison of its effects on myofibrils with -calpains. J. Anim. Sci. 70:3697-3708.
54. Koohmaraie, M. 1992. Role of the neutral proteases in postmortem muscle protein degradation and meat tenderness. Proc. Annu. Recip. Meat Conf. 45:63-71. (Invited Review Paper)
55. Koohmaraie, M. 1992. Postmortem proteolysis and meat tenderness. Amer. Assoc. Meat Sci. Newsletter 28:6-7. (Invited Editorial Paper)
56. Whipple, G. and M. Koohmaraie. 1993. Calcium chloride marination effects on beef steak tenderness and calpain proteolytic activity. Meat Sci. 33:265-275.
57. Morgan, J. B., T. L. Wheeler, M. Koohmaraie, J. D. Crouse and J. W. Savell. 1993. Effect of castration on myofibrillar protein turnover, endogenous proteinase activities and muscle growth in bovine skeletal muscle. J. Anim. Sci. 71:408-414.
58. Kendall, T. L., M. Koohmaraie, J. R. Arbona, S. E. Williams and L. L. Young. 1993. Effect of pH and ionic strength on bovine m-calpain and calpastatin activity. J. Anim. Sci. 71:96-104.
59. Pringle, T. D., C. R. Calkins, M. Koohmaraie and S. J. Jones. 1993. Effects over time of feeding a -adrenergic agonist to wether lambs on animal performance, muscle growth, endogenous muscle proteinase activities, and meat tenderness. J. Anim. Sci. 71:636-644.
60. Morgan, J. B., T. L. Wheeler, M. Koohmaraie, J. W. Savell and J. D. Crouse. 1993. Meat tenderness and the calpain proteolytic system in longissimus muscle of young bulls and steers. J. Anim. Sci. 71:1471-1476.
61. Wheeler, T. L., M. Koohmaraie and E. T. Littledike. 1993. Effects of dietary copper and molybdenum supplementation on meat characteristics of Texel and Finnsheep rams. Sheep Res. J. 9:76-81.
62. Bishop, M. D., M. Koohmaraie, J. Killefer and S. Kappes. 1993. Rapid Communication: Restriction fragment length polymorphisms in the bovine *calpastatin gene*. J. Anim. Sci. 71:2277-2278.
63. Wheeler, T. L., M. Koohmaraie, J. L. Lansdell, G. S. Siragusa and M. F. Miller. 1993. Effects of postmortem injection time, injection level and concentration of calcium chloride on beef quality traits. J. Anim. Sci. 71:2965-2974.

64. Arbona, J. R. and M. Koohmaraie. 1993. Technical Note: Quantification of multicatalytic proteinase complex (proteasome) activity by ion-exchange chromatography. J. Anim. Sci. 71:3301-3306.
65. Koohmaraie, M. 1994. Muscle proteinases and meat aging. Meat Sci. 36:93-104.
66. Pringle, T. D., S. M. Lonergan, C. R. Calkins, S. J. Jones, P. S. Miller and M. Koohmaraie. 1994. Temporal response of rabbits to -adrenergic agonist feeding: Tissue weight, calpains and calpastatin activities, and nucleic acid and protein concentrations. J. Anim. Sci. 72:68-74.
67. Shackelford, S. D., M. Koohmaraie, T. L. Wheeler, L. V. Cundiff and M. E. Dikeman. 1994. Effect of biological type of cattle on the incidence of the dark, firm, and dry condition in the longissimus muscle. J. Anim. Sci. 72:337-343.
68. Killefer, J. and M. Koohmaraie. 1994. Bovine skeletal muscle calpastatin: Cloning, sequence analysis, and steady-state mRNA expression. J. Anim. Sci. 72:606-614.
69. Gregory, K. E., L. V. Cundiff, R. M. Koch, M. E. Dikeman and M. Koohmaraie. 1994. Breed effects and retained heterosis for growth, carcass, and meat traits in advanced generations of composite populations of beef cattle. J. Anim. Sci. 72:833-850.
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75. Wheeler, T. L. and M. Koohmaraie. 1994. Prerigor and postrigor changes in tenderness of ovine longissimus muscle. J. Anim. Sci. 72:1232-1238.
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78. Shackelford, S. D., L. V. Cundiff, K. E. Gregory and M. Koohmaraie. 1995. Predicting beef carcass cutability. J. Anim. Sci. 73:406-413.
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Postmortem injection of calcium chloride effects on beef quality traits. J. Anim. Sci. 73:1735-1740.
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83. Van den Hemel-Grooten, H.N.A., M. Koohmaraie, J. T. Yen, J. R. Arbona, J. A. Rathmacher, S. L. Nissen, M. L. Fiorotto, G. J. Garssen and M.W.A. Verstegen. 1995. Comparison between 3-methylhistidine production and proteinase activity as measures of skeletal muscle breakdown in protein deficient growing barrows. J. Anim. Sci. 73:2272-2281.
84. Shackelford, S. D., M. Koohmaraie and T. L. Wheeler. 1995. Effects of slaughter age on meat tenderness and USDA carcass maturity scores of beef females. J. Anim. Sci. 73:3304-3309.
85. Shackelford, S. D., T. L. Wheeler and M. Koohmaraie. 1995. The effects of *in utero* exposure of lambs to a -adrenergic agonist on prenatal and postnatal muscle growth, carcass cutability and meat tenderness. J. Anim. Sci. 73:2986-2993.
86. Shackelford, S. D., T. L. Wheeler and M. Koohmaraie. 1995. Relationship between shear force and trained sensory panel tenderness ratings of 10 major muscles from *Bos indicus* and *Bos taurus* cattle. J. Anim. Sci. 73:3333-3340.

87. Koohmaraie, M., S. D. Shackelford, T. L. Wheeler, S. M. Lonergan and M. E. Doumit. 1995. A muscle hypertrophy condition in lamb (callipyge): Characterization of effects on muscle growth and meat quality traits. J. Anim. Sci. 73:3596-3607.
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89. Koohmaraie, M. 1995. The biological basis of meat tenderness and potential genetic approaches for its control and prediction. Proc. Annu. Recip. Meat Conf. 48:69-75. (Invited Review Paper)
90. Koohmaraie M., T. L. Wheeler and S. D. Shackelford. 1995. Beef tenderness: regulation and prediction. CSIRO Meat '95 Proc. pp. 4A-1 - 4A-20. (Invited Review Paper)
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92. Koohmaraie, M., S. D. Shackelford and T. L. Wheeler. 1996. Effects of a -adrenergic agonist (L-644,969) and male sex-condition on muscle growth and meat quality of callipyge lambs. J. Anim. Sci. 74:70-79.
93. Koohmaraie, M. 1996. Biochemical factors regulating the toughening and tenderization processes of meat. Meat Sci. 43:S193-S201. (Invited Review Paper)

94. Wheeler, T. L., S. D. Shackelford and M. Koohmaraie. 1996. Sampling, cooking, and coring effects on Warner-Bratzler shear force values in beef. J. Anim. Sci. 74:1553-1562.
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Via Electronic Submission
Attorney Docket No.:66090-008US2

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Mansour Samadpour
Serial No.:	95/001,294
Filed:	05 April 2010
For:	MODULAR COMPOSITING-MULTIPLE LOT SCREENING PROTOCOLS FOR DETECTION OF PATHOGENS, MICROBIAL CONTAMINANTS AND/OR CONSTITUENTS
Examiner:	Brenda G. Brumback
Art Unit:	3991
Attorney Docket No.:	66090-008US2
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Attn: Mail Stop "Inter Partes Reexam"
Central Reexamination Unit
Commissioner for Patents
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Alexandria VA 22313-1450

DECLARATION OF MANSOUR SAMADPOUR UNDER 37 C.F.R. § 1.132
(IN SUPPORT OF RESPONSE UNDER 37 C.F.R. § 1.111)

Madam:

I, Dr. Mansour Samadpour, being duly sworn, say:

1. I am familiar with, and am the original inventor of the above-identified patent application, and am aware that it discloses and claims novel methods comprising sampling, testing, and validating test lots for microbial contamination. I am familiar that the detailed claimed method comprises: collecting a plurality of portions from each of a plurality of test lots, the test lots each comprising an assemblage of one or more specimens, wherein each test lot is separately sampled by taking said plurality of portions thereof; combining the collected 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; 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; removing portions of each enriched separate test lot sample, and combining the removed portions to provide a modular composite sample; and testing of the modular composite sample, using a suitable detection assay, for the target microbial agent or organism, wherein when such testing is negative all of said separate test lot samples are validated, and wherein when such testing is positive, individual separate test lots may nonetheless yet be validated 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.

2. I am the CEO and founder of Institutes of Environmental Health, the Assignee of the subject patent application. My detailed curriculum vitae is attached hereto as APPENDIX A.

3. I am an author or co-author of at least 50 peer-reviewed research articles (representative articles are attached as part of APPENDIX A) in the field of food testing, analytical sampling, meat safety and quality and my research has been presented at national and international meetings. I am an inventor on several granted United States and foreign patents and various pending domestic and international patent applications. I am an internationally recognized expert in food sampling, pathogen testing and molecular epidemiology, with over 20 years academic and industry experience in this area.

4. In my capacity as CEO at Institutes of Environmental Health, I am well versed and intimately aware of all aspects of the company and as such I am the person most acquainted with all functions of the company, including implementation of the wet inter-lot composite pooling procedure disclosed and claimed in the current patent, marketing of this procedure to others, and the surprising, rapid, and wide-spread acceptance of the claimed methods despite the fact that implementation of the methods increases the sampling by 500%, and the testing cost by about 30%.

5. I am very familiar with the rejections asserted in the Office Action issued by

the USPTO dated 26 May 2010 in this case, and understand that one or more claims of the above-referenced patent application remain rejected under 35 U.S.C. § 103 on the alleged grounds that the claimed invention is obvious. Specifically, the Examiner appears to be alleging that several of the claims under 35 U.S.C. 103(a) are unpatentable over Jarvis in view of FSIS and Midura, amongst others as described in the action.

6. I am making this Declaration to support the Response to the Office Action dated 26 May 2010, and to Declare and corroborate the inventive and non-obvious nature of wet composite inter-lot pooling, as presently claimed, in the control of food-borne pathogens and the nexus between the claimed invention and the commercial success of the implemented procedures.

7. As an initial matter, the original specification, for which I signed an inventor Oath/Declaration, of the current patent contained a working Example 1 that demonstrated the nexus between the invention as claimed and the related benefits which, despite the substantial disincentive of increased sampling and assay costs as discussed below, have led to the current rapid and wide-spread acceptance of the claimed wet composite inter-lot pooling method (see Example 1 of the patent):

> Specifically, the individual single-unit production lot samples comprising the MWCS were analyzed separately following each *positive* MWCS result. As can be seen in TABLE I, only 391 individual single-unit production lot samples yielded a *positive* result out of the cumulative total of 1,085 individual single-unit production lot samples comprising the MWCS samples which yielded a *positive* result. If only the 391 individual single-unit production lots yielding a *positive* sample result were diverted to rendering or downgraded for cooking this would represent 782,000 lbs of product. The remaining 694 individual single-unit production lots, corresponding to 1,928,000 lbs of product, are, according to preferred aspects of the present invention, validated and releasable into the chain of commerce.
>
> The product, in this Example I, being produced by this U.S. beef producer was beef trim. The release of the validated 1,9280,000 lbs of beef trim into commerce using the present invention, instead of diverting or downgrading the product, trim, represents a cost savings to the producer of about $2,500,000, relative to prior art methods. Additionally, the testing of the single-unit production lots by augmented testing protocols (representing a 5- to 50-fold increase in sensitivity relative to the MWCS testing) provides *substantial* additional assurance of the safety of the food products.
>
> ...

TABLE 1: Results of modular wet composite sampling of 62,919 multiple lots and number of Individual Production Unit Lot (IPUL) which tested positive when MWCS tested positive

Location	Total # Analyses	# Positives	1 IPUL	2 IPULs	3 IPULs	4 IPULs	5 IPULs
Est. A, B, C.	62,919	271	180	39	18	11	7

U.S. Patent No. 7,534,584, at columns 11 and 12.

8. In response to the Bailey Declaration, dated 13 January 2010, filed in connection with the re-examination request, I contend that not only the current invention, but also the cited references have been misconstrued. First, Bailey's entire Declaration is based on the presumption/assumption that prior to the current invention a person of ordinary skill would have believed that an enrichment-only step could substitute for pre-enrichment/enrichment steps. This is, however, far from the truth as outlined in the Response to the present Office Action. In fact, prior to Applicant's invention, no person of ordinary skill would have reasonably relied upon the results from a test that utilized enrichment-only, due to effects related to dilution of the target organism and competition from non-target organisms. Additionally, the prior art, including that asserted by the Examiner, *teaches away* from combining lumping and pooling in a single scheme, as discussed in the Response, and moreover pooling as generally taught and understood in the prior art was limited to the pre-enrichment level—and even in this regard, is limited to but a single method and a single organism (Salmonella). Significantly, the entire prior art is limited to intra-lot pooling to reduce the cost of Salmonella testing for release of a single lot of product. By contrast, the present invention comprises inter-lot pooling and lot validation that is applicable to all manors of lot samples as taught in the originally filed specification.

9. The Bailey Declaration cites to seven pieces of art (all of which are the same from the Office Action): Jarvis; Price; Biological Analytical Manual (BAM; exhibit 11); Compendium of Methods for the Microbiological Examination of Foods (referenced as "Compendium, chapter 2" in the declaration and as "Midura" in the OA; exhibit 3); FSIS (exhibit 6); Silliker (exhibit 7); and Gabis (exhibit 9). The opinions presented in the Bailey Declaration are not supportable as discussed in detail in the Response to the present Office Action and in this Declaration.

10. In fact, the current wide-spread adoption of the wet composite inter-lot pooling inventive methods was surprising given the points stated above under paragraph "9.",

particularly given that these methods significantly increase the sampling by 500%, and the testing cost by about 30%, and particularly in a cost-sensitive industry with narrow profit margins, where increasing such cost was a strong and long-standing significant disincentive. Surprisingly, using post-enrichment pooling (which is contra-indicated in the prior art universally taught use of pre-enrichment pooling), and more specifically, inter-lot rather than intra-lot pooling, this method has surprisingly and rapidly been widely adopted. Prior to this invention, the industry practice was still fashioned on the methods of Price and Silliker, which are nearly 40 years old, and it was the present Applicant who solved a long-standing problem in the art as described herein. I respectfully contend that the opinions of Bailey are unacceptable hindsight.

11. In fact, IEH company sales figures for the last four years have increased at an average rate of 30% per year, based on client adoption of the methods claims in the present patent application comprising inter-lot pooling of enriched samples, and the ability to yet validate and release individual lots after obtaining a positive result for an inter-lot pool sample. Implementation of the claimed methods has given rise to an increase in number of tests that Institutes of Environmental Health is contracted to conduct, and a concomitant increase in customer satisfaction and public safety. IEH's market share has also steadily increased due to acceptance of and overt demand for the claimed method.

12. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code, and may jeopardize the validity of the application or any patent issuing thereon.



Mansour Samadpour, Ph.D.

26 July 2010

APPENDIX A
CURRICULUM VITAE

MANSOUR SAMADPOUR, PH.D.
Principal

SUMMARY

Dr. Samadpour is a food microbiologist with extensive experience in food safety, food production, and epidemiology of foodborne illnesses. He is currently President of Molecular Epidemiology and IEH Laboratories & Consulting Group (IEH), who together employ upwards of 300 personnel. IEH initially focused on providing services to the beef harvesting and grinding industries, and currently IEH conducts testing for about 1/3 of the U.S. market. Since the spinach outbreak of 2006 services have expanded into the produce sector and now IEH serves major clients in fresh cut produce (lettuce, tomatoes, and green onions), apples, sprouts, and tree nuts. In this capacity Dr. Samadpour has been intimately involved in defining and developing microbiological testing programs for food safety management of *E. coli* O157, *Salmonella*, EHEC, *Shigella* and *L. monocytogenes* testing plans as well as the evaluation of the efficacy of microbial interventions. Dr. Samadpour's expertise has been sought in most of the major foodborne illnesses that have occurred since the Jack In The Box *E. coli* O157 episode of 1993, and he as served as expert witness on numerous occasions.

Currently Dr. Samadpour has over 50 scientific publications, over 50 abstracts, 12 popular press and trade articles and more than 200 research reports. He is also the inventor on over 14 patent applications involving aspects of food testing and foodborne illness risk analysis.

EDUCATION

1990	**Ph.D.**, Food Science and Technology, minor in Molecular Biology; University of Washington, Seattle, WA
1987	**M.S.**, Microbiology University of Washington, Seattle, WA
1981	**B.S.**, Microbiology University of Washington, Seattle, WA

PROFESSIONAL POSITIONS

2001-Present	**Principal**, Molecular Epidemiology, also dba IEH Laboratories & Consulting Group, Seattle, WA
1993-2003	**Assistant Professor**, Department of Environmental Health, School of Public Health and Community Medicine, University of Washington,

Seattle, WA

1991-1993 **Research Associate**, Department of Environmental Health, School of Public Health and Community Medicine, University of Washington, Seattle, WA

1990-1991 **Post-Doctoral Research Associate**, Department of Environmental Health, School of Public Health and Community Medicine, University of Washington, Seattle, WA

1988-1990 **Pre-Doctoral Fellow**, School of Fisheries, University of Washington, Seattle, WA

1978-1980 **Graduate Research Assistant**, Department of Microbiology, University of Washington, Seattle, WA

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Exhibit G

Via Electronic Submission
Attorney Docket No.:66090-008US2

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Mansour Samadpour
Serial No.:	95/001,294
Filed:	05 April 2010
For:	MODULAR COMPOSITING-MULTIPLE LOT SCREENING PROTOCOLS FOR DETECTION OF PATHOGENS, MICROBIAL CONTAMINANTS AND/OR CONSTITUENTS
Examiner:	Brenda G. Brumback
Art Unit:	3991
Attorney Docket No.:	66090-008US2
Date:	31 January 2011

Attn: Mail Stop "Inter Partes Reexam"
Central Reexamination Unit
Commissioner for Patents
P. 0. Box 1450
Alexandria VA 22313-1450

DECLARATION OF MANSOUR SAMADPOUR UNDER 37 C.F.R. § 1.132
(IN SUPPORT OF RESPONSE UNDER 37 C.F.R. § 1.111)

Madam:

I, Dr. Mansour Samadpour, being duly sworn, say:

1. I am familiar with and am the original inventor of the above-identified patent application, and am aware that it discloses and claims novel methods comprising sampling, testing, and validating test lots for microbial contamination. I am very familiar with the comments made by the Examiner in the Action Closing Prosecution (ACP) dated December 17, 2010 concerning my previous declaration dated July 26, 2010. This supplemental declaration is in response to the Examiner's comments, to explain several technical issues connected with the reasoning in the ACP, and further to corroborate the nexus between the claimed invention (modular composite testing) and commercial success. As such, according to 37 C.F.R. §

1.116(e), this declaration is necessary to respond properly to the ACP.

2. I am familiar that the detailed claimed method comprises: collecting a plurality of portions from each of a plurality of separate test lots 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; 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; incubating the set of separate test lot samples under conditions suitable to allow levels of a target microbial agent or organism if 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; removing portions of each enriched separate test lot sample, and combining the removed portions to provide a modular composite sample; and testing of the modular composite sample, using a suitable detection assay, for the target microbial agent or organism, wherein when such testing is negative all of said separate test lots are validated for release into commerce, and wherein when such testing is positive, each of the individual separate test lots may nonetheless yet be 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, wherein the enrichment in step c), prior to removing portions of step d), is sufficient to enable said validation of individual separate test lots with no requirement for further enrichment thereof, and with no requirements for further sampling in a).

3. I am the CEO and founder of the Institute of Environmental Health, the Assignee of the subject patent application. My detailed curriculum vitae is attached hereto as APPENDIX A.

4. I am an author or co-author of at least 50 peer-reviewed research articles (citations for representative articles are included as part of APPENDIX A) in the field of food testing, analytical sampling, meat safety and quality and my research has been presented at national and international meetings. I am an inventor on several granted United States and

foreign patents and various pending domestic and international patent applications. I am an internationally recognized expert in food sampling, pathogen testing and molecular epidemiology, with over 20 years academic and industry experience in this area.

5. In my capacity as CEO at Institute of Environmental Health, I am well versed and intimately aware of all aspects of the company and as such I am the person most acquainted with all functions of the company, including implementation of the statistical modular composite pooling procedure disclosed and claimed in the current patent, marketing of this procedure to others, and the surprising, rapid, and wide-spread acceptance of the claimed methods despite the fact that implementation of the methods increases the number of samples taken by about 300 to 500%, and the testing cost by about 30%. The acceptance of increased sampling is a surprising result since normally industry attempt to minimize sampling. Increasing sampling results in an increase in the number of positives found, with consequent requirements that product be diverted to an economically disadvantageous safe endpoint, such as rendering or cooking. However, my technique has actually resulted in saving of product, by permitting validation and release of smaller lots of product, even when testing of a composite enrichment encompassing several lots shows a positive result. Industry acceptance is particularly surprising in view of the increased testing cost, about 30% greater than with previous methods. Heretofore, the main goal of the industry has been to minimize testing costs, since there were viewed as a dead loss.

6. I am familiar with the rejections asserted in the Office Action issued by the USPTO dated 26 May 2010 in this case and the ACP dated 17 December 2010, and understand that one or more claims of the above-referenced patent application remain rejected under 35 U.S.C. § 103 on the alleged grounds that the claimed invention is obvious. Specifically, the Examiner asserts that several of the claims under 35 U.S.C. 103(a) are unpatentable over Jarvis in view of FSIS and Midura, amongst others as described in the action.

7. I am making this Declaration to support the Response to the Action Closing Prosecution dated 17 December 2010, to respond to the comments made by the Examiner, and to Declare and corroborate the inventive and non-obvious nature of statistical wet composite pooling, as presently claimed, in the control of food-borne pathogens and the nexus between the claimed invention and the commercial success of the implemented procedures.

8. ***It is my position*** **that my invention has nothing to do with the prior art**

references of record, which have only to do with how many samples need to be taken from a single lot to achieve a given level of statistical confidence, or else how these samples from a single lot may be most economically tested. Rather, my invention concerns how entire sample-sets, each set representing an separate lot, with each set containing a "statistically" confident number of samples, may be dealt with as a group, to achieve a surprising new result, that is, immediate release of members of the group of lots, representing product, into commerce without further sampling, even when other lots contributing to the group have tested positive. Implementation of the invention encourages sampling of smaller lots with far greater numbers of tests being performed. Though this is economically disadvantageous in terms of testing costs, this is offset by the greater advantages of obtaining more positive findings (thereby providing greater protection of public health and reducing liability risks for food producers) and reducing overall costs because the amount of positive product diverted to a safe endpoint (destroyed) is far less due to the smaller lot sizes. Nothing in any reference hints that these commercially valuable results are possible.

9. *First*, I wish to point out that the term "lot" as used in this art does not refer to an absolute number. Rather, the size of a "lot" may be selected arbitrarily in the first instance as may be gathered from the definition supplied on page 15, right hand column, first paragraph of Midura (Exhibit 3) which states:

> *"Lot – The number of sample units produced in one batch or in some specified period of time such that the units will be about the same quality".*

What is not arbitrary is application of a sampling plan to a lot. Once a lot, of whatever size, is selected then a given number of samples, defined in the plan, must be taken from that lot in order to achieve a given level of "statistical confidence" in the results. The level of statistical confidence that is given is also not fixed to any absolute in the first instance, but rather selected based upon requirements of a regulator-imposed sampling plan or other specific need. However, once the required level of statistical confidence is defined, then the science of statistics requires that at least a certain number *n* samples need to be taken from the lot to achieve the required level of statistical confidence.

10. *Second*, I wish to point out that the concept of "statistical confidence" has nothing to do with the actual, physical condition of any particular lot that is to be tested. Rather, it relates to sampling of a manufacturing batch (lot) by means of random sampling. To take a

simple example, one might think about the statistics of flipping a coin. Everyone knows that the chance of a theoretical, balanced coin flipping heads versus tails is 50-50. However, if a coin is only tossed a few times i.e. a small "sample size", then it is possible to get a "run" of results which is not 50-50. For example, it is possible to flip a coin twice or three times in a row and get all heads, which is a result completely different from 50-50. In contrast, if a large sample size is used, say, 1000 coin tosses, then the observed deviation from 50% heads-50% tails will probably be rather small. Statisticians explain this result in terms of the statistical confidence concept. The larger the sample size, the greater the 'statistical confidence" that the observed results will actually reflect the 50-50 physics of coin flipping.

11. When Jarvis (Exhibit 13) writes about "statistical confidence" in section 8.4.2.1 (page 141) with various sampling and pooling methods, he means something very specific by his use of the adjective "statistical". To an expert reading Jarvis, this adjective means that Jarvis is NOT talking about any issues of biology connected with a particular sampling method. Jarvis makes his statements **based upon no reported personal experience**. Jarvis, not having done the work himself (no data, no instructions, no references), is merely discussing the mathematical issue of statistical confidence, as it might bear upon combining particular masses or volumes of materials containing microorganism. To the extent that Jarvis does have a connection to real world microbiology, he can only be reasserting what was known to him in the only available art, which was Price (Exhibit 8), Silliker (Exhibit 7) and Gabis (Exhibit 9). In the context of the coin flipping example, Jarvis is merely making a comment about how the number of samples impacts the probability of observing a particular characteristic of a lot (i.e. whether it contains a microbial contaminant or not) ASSUMING that biological factors such as competition, dilutions, different media, etc. have introduced no bias into the procedure. This is exactly like assuming that a coin is perfectly balanced from a physics standpoint, and that the method of tossing also completely unbiased. An expert reader of Jarvis would not conclude that Jarvis was making any statement about the actual suitability or usability of any particular method of combining or lumping samples. Rather, due to the pains that Jarvis took to use the adjective "statistical", an expert reader would conclude that Jarvis specifically DISCLAIMS such a reading.

12. Jarvis himself on page 3 of his original reference from which Exhibit 13 was excerpted states:

> ***"It is not the purpose of this monograph to review the advantages and disadvantages of microbiological criteria, rather, the objective is to give an***

> ***elementary account of those statistical factors which effect "quantitative" microbiological data.*** *Examples chosen from appropriate areas of food microbiology are used to illustrate factors which affect the overall variability of microbiological counts. Reference is made not only to factors affecting results obtained by traditional methods, but also to newer concepts in automated and semi-automated microbiological methods."* ***(Emphasis added)***

The monograph considers: (a) the implication of natural variation in the distribution of microbes; (b) the effects of sampling for both quantitative (e.g. colony count and most probable number (MPN) methods) and 'presence or absence" methods; (c) the factors affecting results obtained by different methods; (d) the comparability of data obtained by different methods; and (e) the implications of statistical variation in relation to microbiological criteria for foods. Brief consideration is given also to sampling of colonies from culture plates for isolation of representative microorganisms. The monograph is intended **as an aid for practicing food microbiologists**. It assumes only minimal knowledge of statistics and references to more erudite works on statistics are cited where appropriate."

13. *Third,* I would like to furthermore point out that with respect to statistical confidence and testing Jarvis, Price, Silliker, Gabis and BAM (Exhibit/Figure 11) **do not teach compositing a plurality of samples from a plurality of lots**.

14. On this point Examiner makes the following statement concerning Jarvis: "[t]he methods described by Jarvis (methods 2 and 3) comprise collecting a plurality (10) of product samples or portions (25g size)." I respectfully submit that Examiner's statement fails to mention that the plurality of samples are from a single lot of food to be tested, as is clearly indicated by Jarvis in Section 8.4.1 (page 137):

> *In Chap. 5, the use of the binomial distribution to describe the operating characteristics (OC) curve for sample plans was described. The binomial relationship describing the probability of occurrence of defective samples can be used also to quantify the probable level of occurrence of organisms in* ***the lot*** *of food under test.*
>
> *On the assumption that the organisms in* ***a "lot"*** *of food are distributed randomly and that a number (n) of samples is tested, then the probability (P) of no defective test samples (i.e. no positive tests) is given by:*
>
> $$P = 1 - [1 - (d/100)]^n$$

where d = actual % defectives in ***the lot. (Emphasis added)***

15. Continuing on this point, Price conducts a scientific study examining pooling of pre-enrichment broth cultures for screening multiple food samples. In his Materials and Methods section (Page 679, right hand column, second paragraph, first line) he states:

> *"Salmonella-positive test samples were prepared by blending the dry test food with one of three dry inocula."*

Necessarily it follows that Price prepared individual samples of dry test food from which he took multiple portions which were subsequently pre-enriched for his investigation of pooling. **From Table 1 of Price (Page 680, middle of page) it is clear that in his investigation he examined positive enrichments pooled with various numbers of negative enrichments from the same dry test food, i.e. a single lot.** This can be seen in the column headings, which are variously labeled as P (P standing for positive), P+5 N (N standing for negative), P+9N and P+14N under each dry test food type. There is no data presented in Price where samples or portions from more than one dry test food type are combined. **Thus, contrary to any hindsight interpretations, Figure 1 of Price (Page 680, top of page) cannot be interpreted as combining samples from a plurality of test lots.**

16. Continuing on this point, Silliker conducts a scientific study examining lumping of samples from dried food for screening multiple food samples. **His objective is clearly to reduce the workload in analyzing multiple samples from the same lot**, as can be seen in his introductory remarks (Page 475, first paragraph bridging left and right columns) where he states:

> *"The sensitivity of the analytical methods is most important in the design of Salmonella control systems for foods. Increasing the amount of sample examined* ***from a particular lot*** *decreases the probability of accepting a positive lot. There has been a trend to analyzing increasingly larger samples, but the sensitivity of detecting Salmonella in large samples has not been adequately determined. The testing of 60 individual 25-g subsamples with negative results was recommended by the National Research Council (NRC) Committee on Salmonella before high risk foods could be released for consumption by aged and infirm persons and infants. It is obvious that following this recommendation* ***would not be economically feasible on a routine basis****. Examination of fifteen 100-g subsamples* ***would require one-fourth the laboratory work*** *but would be warranted only if the sampling and analytical scheme were as efficient in detecting positive lots as one involving the analysis of sixty 25-g subsamples. Similarly, if an analytical method using three 500-g* ***subsamples from the lot*** *were equally capable of detecting contamination, then* ***the laboratory cost of statistical quality control over***

Salmonella would be greatly reduced**, and more widespread application of statistical control programs would be encouraged." **(Emphasis added)

Silliker conducts his scientific inquiry in a manner consistent with his introductory remarks. He dilutes highly contaminated commercial products with Salmonella-free material of the same composition (Page 476, left column, first line of section titled Sample Preparation). He reports his results per dried food product without combinations (Page 477, bottom of page, Table 1).

17. Continuing on this point, Gabis continues the work of Silliker and conducts a scientific study examining lumping of samples from high moisture food for screening multiple food samples. **His objective is clearly to reduce the workload in analyzing multiple samples from the same lot**, as can be seen in his introductory remarks (Page 663, first paragraph bridging left and right columns) where he states:

*"Silliker and Gabis reported that Salmonella **positive lots** of low-moisture foods could be detected with equal certainty by analysis of sixty 25-g individual, fifteen 100-g, or three 500-g lumped samples. The purpose of the present investigation was to determine whether multiple 25-g subsamples could be similarly lumped to provide large sample units for the analysis of high-moisture foods, such as meats, poultry and eggs. **The economic advantage of lumping samples is obvious**; indeed, this approach makes statistical quality control over the Salmonella defect economically feasible, particularly for high-risk foods where analysis of as many as sixty 25-g subsamples has been recommended. The United States Food and Drug Administration has officially recognized the lumping of multiple 25-g samples to produce 375-g samples for analysis." **(Emphasis added)***

Gabis conducts his scientific inquiry in a manner consistent with his introductory remarks. He dilutes highly contaminated commercial products with Salmonella-free material of the same composition (Page 664, left column, section titled Sample Preparation). He reports his results per high moisture food product without combinations (Page 665, Table 1).

18. Continuing on this point, BAM also clearly refers to **food sampling and preparation of a sample homogenate within the context of a single lot.** This is clear from introductory paragraphs found in BAM (first page, first and second paragraphs) which states:

"The adequacy and condition of the sample or specimen received for examination are of primary importance. If samples are improperly collected and mishandled,

*or are not representative **of the sampled lot**, the laboratory results will be meaningless. Because interpretations about a large consignment of food are based on a relatively small **sample of the lot**, established sampling procedures must be applied uniformly. A representative sample is essential when pathogens or toxins are sparsely distributed within the food or when disposal of a food shipment depends on the demonstrated bacterial content in relation to a legal standard."*

The number of units that comprise a representative sample from a designated lot of a food product** must be statistically significant. **The composition and nature of each lot** affects the homogeneity and uniformity of the total sample mass. The proper statistical sampling procedure, according to whether the food is solid, semisolid, viscous, or liquid, must be determined by the collector at the time of sampling by using Investigations Operations Manual. Sampling and sample plans are discussed in detail in ref. 6. **(Emphasis added)

BAM recognizes that it is important to give care to the way in which a single lot sample is collected, but says nothing which teaches a plurality of lots.

19. Lastly, we would like to bring to Examiner's attention that the issue of compositing and pooling in the prior art is not as settled as the third party requester has represented. As late as 2007, in an article subsequently published by Jarvis (APPENDIX B) on the compositing of samples for qualitative microbiological testing" he stated (page 593, first column, first full paragraph):

*"The work of Silliker and Gabis (1973), has been quoted by ICMSF (1978) who claimed, 'multiple analytical units may be combined or "lumped" to provide larger units for testing purposes'. Furthermore, they stated, '60 25-g units selected from a particular product lot maybe combined ... to produce three 500-g samples for analysis. If all three 500-g samples are ... negative... then the inference is that the level of Salmonella is no greater than one in 500 g'. This statement is at best an oversimplification. Silliker and Gabis (1973) actually showed that if the test material has a high level of contamination, then compositing made no difference to the result obtained, but at lower levels of contamination the use of composited samples could give rise to false-negative results. **More recently ICMSF (2001) withdrew somewhat from their earlier position and stated, 'numerous studies since 1974 have continued to demonstrate the merits and limitations of compositing. Experience may vary for different food pathogen combinations and analytical methods, indicating a need for caution and validation that compositing is acceptable.' (Emphasis added)***

The point is that even in 2007 the status of compositing is not settled art. The very few references available could not even settle on the issue of compositing plurality of samples from a single lot.

20. *Fourth*, I wish to point out that **none of the prior art teaches re-testing of retained enrichments for the purpose of yet validating product for release into commerce**. Examiner has considered Price, Midura and FSIS (Exhibit 6) in this regard. We distinguish our approach as teaching the pooling of a plurality of enriched composite samples, each sample being an enrichment of a composite of portions taken from a single lot where the number of portions composited conforms to a sampling plan with a given level of statistical confidence, so that the pooled enrichment represents a plurality of lots and if a positive result is obtained for the pooled enrichment re-testing can reveal which of the individually enriched composite samples contributed to the positive result.

21. On this point I wish to point out that when Jarvis considers various methods of compositing **his view of the purpose of re-testing within a single lot is entirely different from the purpose of patent '584 for which the purpose of re-testing is to determine which of a plurality of test lots can yet be validated for release into commerce despite obtaining a positive result for the composite sample**. In section 8.4.2.1, page 141 Jarvis states:

> *"Method (2), at least in theory, permits the tests to be repeated on stored pre-enrichment cultures* ***so that some indication of the proportion of contaminated samples*** *can be obtained".* ***(Emphasis added)***

As Jarvis is considering only a single lot of food, the purpose of re-testing must necessarily be fundamentally different from the purpose of patent '584. For Jarvis, it can only serve one purpose which is to determine extent of the contamination within one lot of food which was tested.

22. Continuing on this point, Examiner states that Midura teaches subsequent testing of a second sample depending upon the results of the first sample as a multiple sampling attribute plans (two or three class). Examiner is misconstruing Midura in two respects. First, **when Midura discusses double sampling plans it is still within the context of testing of single lots, not a plurality of lots**. The purpose is again to reduce laboratory workload by specifying a lesser number of samples to be collected from a lot for initial testing, but if a select number of

samples **greater than one** test positive then additional samples are collected and a second test is conducted. This is clear from the language of Midura (Page 17, left hand column, first two sentences of section 2.513 Multiple Sampling (2-Class) Attribute Plans:

> *"Sampling plans of this type are designed to inspect lots based on multiple samplings.* ***The lot*** *is accepted or rejected based on sequential decisions".* ***(Emphasis added)***

Second, a double sampling plan as defined by Midura **is inferior to a single sampling plan and teaches away from re-testing.** This can be clearly seen in Midura where it is stated (P17, left column, last whole paragraph):

> *"Figure 2 shows the operating characteristic curve for the double sampling plan and one for a single sampling plan (n=7, c=0) that have the same average (5%) outgoing quality limit (AOQL).* ***Although the AOQLs are equal in the two plans, it can be seen that the probability of accepting a defective lot is lower for the single sampling plan". (Emphasis added)***

Furthermore, in the field of the art double sampling plans are only used for indicator organisms. Regulations do not allow for the use of double sampling plans for pathogen testing because inherent in the double sampling plan is the acceptance of positive results in the first stage of testing before applying the second stage of testing. **If any of the samples collected from a lot test positive for a pathogen the lot is to be diverted from commerce. There is no possibility of taking more samples and replacing the positive results.**

23. Continuing on this point, Examiner states that FSIS teaches collecting additional samples (follow-up verification) after remedial or corrective action following a positive test result (paragraph bridging pages 8-9 and page 23, last paragraph. **The Examiner has inappropriately linked the additional samples discussed in Exhibit 6 with those in the present invention because the purposes are distinctly different**. Per the cited sections FSIS will collect one or more samples from similar product, but AFTER the establishment has implemented corrective actions (such as clean-up, retraining, adjusting intervention settings, etc.) following a positive finding. Thus the resampling will not be from operationally-linked units and in fact due to the time required for the original sample to be collected, mailed to a central FSIS laboratory, analyzed to yield the test result, inform the plant and for the plant to implement

remedial or corrective actions, and then for FSIS to collect a second sample they will at a minimum be separated by several days. The FSIS purpose for resampling is to generate one or more negative results which will verify that the corrective actions implemented by the plant were successful. It has nothing to do with validating the release of operationally-linked test lots.

24. Furthermore, the sample is only analyzed by the FSIS method, which does not allow for pooling. The Examiner states that FSIS differs from the claimed invention in that it is silent as to the testing method to be used for detection of microbial contamination and does not specify testing of foods other than ground beef products. This is not true. While Exhibit 6 does not mention a method, it directs FSIS field personnel to return the samples to the FSIS laboratory (Exhibit 6, Attachment 2, Page 27, section "Sample Size"). At the FSIS Laboratory the sample would have (at that time) been analyzed according to APPENDIX C: FSIS Microbiological Laboratory Guide (MLG) 5.03. We bring to Examiner's attention that Exhibit 6 is but one portion of a complete set of rules and procedures that govern FSIS actions, and the method of analysis was clearly specified in the larger context of this set, just not explicitly within Exhibit 6.

25. *Fifth,* I wish to point out that real-world practices of lumping and compositing of samples, as dealt with by Price, Silliker and Gabis differ markedly from "statistical confidence". The analogy here is to a real-world coin toss where the coin may be bent or unbalanced due to wear and tear, or other factors. Both Price and Silliker teach that combining samples is difficult in practice and the outcome cannot necessarily be predicted by a simple, mathematical treatment such as that provided in Jarvis. The real-world discussion of microbiology found in Price, Silliker and Gabis has nothing to do with the narrow, mathematical issue discussed in Jarvis, and, in my view, a person of ordinary skill in this art would not think they could be combined to form some more generalized teaching. Indeed, the entire point of Price, Silliker and Gabis is that real-world microbiology does not allow a simple mathematical treatment of combining samples.

26. Price states that lumping of samples is very bad for sensitivity, so the only solution is to provide separate pre-enrichment broths for EACH sample. Price summarizes his findings and earlier work (Silliker, J. H. 1969. *The influence of sample size on analytical sensitivity in the analysis of dried yolk and whole egg for salmonellae*, p. 203-205. In An evaluation of the Salmonella problem. National Academy of Science publication DO 1683) as follows:

"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."

In direct contradiction Silliker and Gabis teach that samples should be lumped into a single enrichment. Silliker states as a conclusion:

"Analytical results from 26 samples of naturally contaminated dried foods indicate that Salmonella*-positive samples may be detected with equal facility by analyzing sixty 25-g subsamples, fifteen 100-g subsamples, or three 500-g subsamples."*

Gabis reaches a nearly identical conclusion for high moisture foods. Since these references teach opposite approaches, a person of ordinary skill in the art would not attempt to combine these into some unified teaching. Moreover, since Jarvis purports only to deal with a only a narrow, theoretical, mathematical issue akin to coin tossing analysis, a person or ordinary skill in the art would not seek to combine his teachings with that of Price, Silliker or Gabis in order to achieve some different result.

27. *Sixth,* I wish to offer further evidence that pre-enrichment is clearly and distinctly different from enrichment, and does not meet the specifications of patent '584. In this regard let me begin by repeating a citation from Jarvis (8.4.2.1, page 141) with a different emphasis:

"Method (2), ***at least in theory****, permits the tests to be repeated on stored pre-enrichment cultures so that some indication of the proportion of contaminated samples can be obtained".* ***(Emphasis added)***

The phrase "at least in theory" indicates that Jarvis has serious doubts that, in practice, this could actually be done. Experienced microbiologists know that the reason that Jarvis used the phrase

"in theory" is because overgrowth of pre-enrichment cultures. A pre-enrichment culture is known in the art as a non-selective culture. Since it is non-selective, it is subject to the problem of "overgrowth", that is, bacteria other than the target organism can compete with and overcome the desired target bacteria. The art considers it critical that pre-enrichment culturing be strictly time-limited, with enough time being allowed to revive the target, but not enough time being allowed for competing organisms to overgrow and overwhelm the target. The idea is to get the target revived and growing and then transfer it to selective enrichment media as soon as possible. According to Sperber (Exhibit 12, page 46, right hand column, first three lines of second whole paragraph):

> *"The preenrichment period must be long enough for effective resuscitation of stressed or injured microorganisms. If this time is too short, the target microorganism may not have recovered resulting in a false negative test result. Theoretically, if the preenrichment time is excessive, overgrowth by competing organisms may occur again resulting in a false negative test result."*

Since prolonged incubation in pre-enrichment broth can lead to overgrowth by non-target bacteria, pre-enrichment must be carefully monitored over time to strike the right balance between revival and overgrowth-competition, as opposed to being a process that may be continued at leisure as proposed by the third-party. Rather, a person of ordinary skill in the art would conclude that pre-enrichment cultures would likely overgrow with competing cultures given enough time. My patented invention avoids this problem all together by enriching to detectible levels right away, so the problem of overgrowth is avoided. The Patent, col. 6, line 7 teaches that "enrichment" must be such as to allow growth to "detectable levels".

28. Furthermore, Jarvis clearly states that the function and purpose of pre-enrichment is to allow for the resuscitation and growth of bacteria to a point that ensures that the aliquot taken for inoculating the enrichment culture has at least one viable target organism as he states in Section 8.4.2 (first paragraph, page 141):

> *"Once sufficient growth has occurred in pre-enrichment for at least 3 viable salmonella/ml medium, **the probability of transferring at least one viable organism** in an inoculum of 1 ml or more will be >0.95, assuming Poisson distribution...". **(Emphasis added)***

Simply stated, according to Jarvis the function and purpose of pre-enrichment is to take the levels of the target organisms to a point that an aliquot transferred to the enrichment would contain at least one viable cell. This is the point of **dividability, not detectability**. Since detectability is only achieved after a subsequent enrichment is performed, pre-enrichment does not meet the language of the claims, which require enrichment to detectable levels.

29. My **distinction between dividability and detectability** is generally understood by any person of skill in this art. Jarvis teaches that the objective of pre-enrichment is to reach a point where an aliquot may be removed with a high degree of assurance that it will have at least one viable organism (dividability), to Entis (Exhibit 15) who states on page 94, right column, last paragraph (detectability):

> *The appeal of PCR technology lies in the amplification of small quantities of DNA to detectible levels in a short period of time. Theoretically, a single gene target can be amplified a million fold for detection in a few hours. Thus, direct detection i.e. without the need for a long enrichment step, is possible. In practice, however, direct detection of a single gene is difficult to achieve. The small samples volumes used in PCR (microliter range) limit the sensitivity of the procedure for examining food directly. In addition, inhibitory substances that interfere with the PCR are present in many foods.* ***Thus, a preliminary enrichment culture step is common in many PCR detection protocols. (Emphasis added)***

My professional experience confirms that PCR requires an enrichment step before it is detectable in food testing protocols and that the enrichment must be far beyond the levels which Jarvis identifies as being suitable for dividability when aliquoting. As confirmation of the need for levels of concentration beyond a single cell, I draw Examiner's attention to APPENDIX D, which comprises PowerPoint slides authored by the third-party's expert, Dr. Bailey, connection with a professional meeting. Slide 57, entitled "Pooled Sample Protocol", indicates that a concentration of 10^4 cells/ml would be required for detection in the scenario described, which contradicts Dr. Bailey's previous representation that the mere fact that there were viable cells in the portion of the pre-enrichment that is transferred to the enrichment broth means that the target organisms are detectable. Thus, I conclude that **the ACP presents an improper equivalence between pre-enrichment to levels suitable for dividing the broth into aliquots vs. enrichment to detectable levels, as is required in the patent claims.**

30. *Seventh*, I wish to bring to Examiner's attention that my results are directly

against the spirit of the prior art, all of which seek to reduce the economic costs associated with single lot sampling by minimizing laboratory workload, and therefore the costs of the associated quality control or regulatory compliance sampling plan. In contrast, the current invention provides a means to reduce lot sizes while maintaining a level of statistical confidence in test results sufficient to validate individual lots for release into commerce even if a positive result is obtained for a pooled enrichment. This results in many more samples being collected for a given rate of production, since the production output is divided into smaller lots, thus enabling more effective removal of defective material. In direct contradiction to the prior art use of the invention leads to an increase in economic costs for sampling and the associated quality control or regulatory compliance sampling plan. However, in a manner which is non-obvious and contrary to the expectations of one who is skilled in the prior art, the economic costs are more than offset by savings gained by not discarding product which is not contaminated but which has been implicated by being associated with a contaminated lot in a composite sample. This is illustrated in APPENDIX E, a technical document prepared by IEH for its clients which considers the impact of changing from FSIS recommended 5 combo lots to single combo testing consistent with the present invention. Figure 3, Page 9, of APPENDIX E shows that if lots are comprised of 5 combos, as was recommended by FSIS, and each lot contains one combo contaminated at a level of 5%, then application of an N=60 statistical sampling plan will result in: (1) removal of 50% of the contaminated combos from commerce; (2) release of 50% of the contaminated combos into commerce; and (3) destruction of 40 uncontaminated combos which are implicated by association with the contaminated combos that were detected. In contrast, application of the present invention is illustrated in Figure 4, Page 9, where: (1) 95% of the contaminated combos are removed from commerce; (2) only 5% are released into commerce; and (3) NO uncontaminated combos are removed from commerce. To summarize, the producing facility is much more effective in protecting public health (95% vs. 50% removal rate for contaminated combos) and recovers the money associated with increased testing costs by preventing the destruction of uncontaminated combos implicated by association with contaminated combos (0 vs. 40 out of 100). **This is a night and day difference and shows that the current invention employs a different mode of operation from the prior art.**

31. In another illustration of how previous art taught away from my invention, I bring to Examiner's attention to information on work performed by Silliker and published as "Appendix D 'Wet Compositing' as an Approach to Control Procedures for the Detection of Salmonellae" in **An Evaluation of the Salmonella Problem**, National Academy of Sciences, Washington, D.C. (1969), included here as APPENDIX F. Silliker reports that out of 105

composites he observed 7 false negative test results, a rate of 6.7%. This rate may be evaluated in light of criteria for acceptable test results for detection of pathogens set forth in one of the primary regulatory references applicable in 2004, FSIS Microbiological Laboratory Guide (MLG) 5.03 which is included here as APPENDIX C. Section 5.4.3a. "Test Kits" of APPENDIX C specifies that one acceptable performance characteristic is that the false negative rate be ≤2%. Of the performance characteristics given the false negative rate is most important because if a test lot is truly positive but is released due to a false negative test result then there is a severe adverse impact on public health and the liability of the producing facility. In contradistinction, a false positive result only leads to the destruction of a test lot which is truly negative, an economic consequence which pales next to those for a false negative. Thus, the work of Silliker **discouraged any exploration of wet compositing as a means to validate test lots for release into commerce**.

32. **The best evidence that a person skilled in the arts would not have conceived of my invention is that they did not.** The third party petitioner has been in the business of manufacturing detection kits as an institute since 1897 and as a corporation since 1963, they did not market any kits using these principles until AFTER my invention. Coincident with the time of their filing of their petition they have begun to market products incorporating my method (please see slides 54-57 of APPENDIX D, presentation by third party petitioner's expert, Dr. Stan Bailey). A second company, Idaho Technology, has also begun to market products incorporating my invention AFTER my patent was published (see section entitled "Pooling" and Figure 2 on page 63, APPENDIX G). No one ever attempted to apply the prior art of compositing and pooling of samples from a single lot to compositing and pooling of multiple samples from multiple lots, since even compositing and pooling of samples from the same lot resulted in loss of sensitivity (e.g. Price). It was only after I successfully overcame all of the barriers that two companies (Idaho Technology and the company employing Dr. Bailey, bioMérieux) copied the invention.

33. *Eighth*, I would like to bring to the attention of the Examiner that conception of the invention was non-obvious. I had no positive guidance from the art on pooling methods. As late as 2007, when Jarvis published his follow up article on the subject of compositing covering the same issues mentioned in Jarvis 1989 (APPENDIX B), he could still only cite the work of Silliker (Exhibit 7) as an example of compositing by 'lumping'. In the same paper Jarvis presents as his preferred approach of 'wet compositing' in which, as an example for meeting a statistical confidence criterion, thirty pre-enrichments are prepared from thirty samples taken

from a single lot of food, and aliquots are transferred into enrichment media to form composite enrichment cultures on which further work (i.e. growth to detection and testing) can be done. Jarvis even goes to state (page 592 first column, 1st, 2nd and 3rd paragraphs) that:

> *"Microbiological criteria (e.g. those defined in Anon. 2005) in some instances* ***impose a need to test a significant number of sample units from individual production lots for the presence of pathogens such as Salmonellae****. Examples of such criteria are given in Table 1.*
>
> *Official agencies and manufacturers seeking to confirm that commercial products comply with the legislative criteria* ***need to obtain an adequate number of representative samples of the manufacturing batch, or 'lot'****. They need also to instruct a laboratory to undertake appropriate testing, which has significant cost and work load implications with large number of samples.*
>
> *A consequence is that both official agencies and industrial organizations are unsure regarding the validity of compositing sample units and some have used approaches that are neither statistically nor technically valid. It seems appropriate therefore to provide a brief account of the issue to be considered in respect to compositing." **(Emphasis added)***

34. While I developed the methodology in 2004, commercialization took place in 2007. I was faced with a high degree of skepticism by experts in the field, and regulatory agencies (FSIS). **It took years to overcome the skepticism of the experts in the field**. In my attempts to have USDA Food Safety Inspection Service (FSIS) accept the method and issue a letter of no objection, it became apparent that the agency was extremely apprehensive about the loss of sensitivity due to pooling, mainly masking a positive sample by a number of negative samples. At first I had to show that pooling composite samples from different lots would not result in loss of sensitivity. I had to show that the detection limit was as good or better than the FSIS method. They still remained skeptical. In a conference call with Dr. Hicks who was charged with examining my application, and his group of experts (including three FSIS microbiologist, a biostatistician, I was told that they were accepting the method in situations where the composite sample tested positive, since each enrichment was going to be tested again, individually, but they could not accepting the negative results. To gain acceptance I had to modify our test and subject each negative enrichment to individual testing to get them to accept the method. In other words, I modified the protocol so that after the pooled enrichments were tested, regardless of whether the pool tested positive or negative, each enrichment is also tested individually. This is a clear indication that the method was not obvious, and exemplifies the

high level of skepticism. Our testing protocol due to lack of acceptance by the experts from FSIS is that each set of samples from a lot is individually enriched to detection level. A modular composite sample is prepared by drawing an equal volume from each enrichment. The modular composite sample is then tested. If the test result is positive each enrichment is tested by itself to see which one(s) are positive, and which are negative, the negatives are then released into chain of commerce. If the modular composite sample is negative, each individual enrichment component is tested individually before it is released into the chain of commerce.

35. **There was a significant amount of skepticism amongst IEH's clients.** It was difficult to convince clients and other people in the industry that the approach of my inventive modular composite testing was a good idea. For example, Dr. Dean Danielson, who is Vice President of Food Safety for Tyson Foods, expressed concern that the inventive modular composite method would not work when Swift Company started using my invention. Swift is a vendor for Tyson. As conveyed to me in a conference call with Dr. Danielson, and his microbiologists and regulatory experts, they felt that combining enrichments into modular composite samples would reduce sensitivity and result in false negative result. Please note that in the beef industry presence of E. coli O157 in beef is considered to be an adulterant, therefore there is no tolerance for false negative results, since they result in outbreaks, recalls and criminal investigations. Dr. Danielson was so concerned that he requested that Swift stop using the method, since Tyson was buying product from Swift. He did not think it obvious that my invention would work. As a matter of fact they were quite sure that it didn't work. It was only after we provided them with our validation studies and their ability to follow our protocols and get the same results that they accepted the method.

36. **A major breakthrough was in overcoming the main concern of the industry, that the more you test the more products you will have to destroy.** In conducting my baseline studies, I found the unexpected result that while the number of positive findings increased due to increasing the number of lots tested (same production volume divided into more lots of smaller size) **the overall amount of products destroyed is significantly less** due to the fact that the lot sizes are much smaller,

37. *Ninth,* concerning commercial success of the current invention, my laboratory began offering this modular composite method of testing in 2007. Since that time and as can be seen in APPENDIX H – Sales by Sample Type, clients increasingly have been selecting the modular composite method as is shown by the side-by-side comparison of annual sales of the

previous classical (N=60) method (60 samples taken from 5-combo lots) with sales of the new inventive modular composite method, involving 60 per single combo production lot. The "modular composite" method as referred to in APPENDIX H is the same method described in the current claims of the patented invention. The results show a drastic increase in the sales of the modular composite over the classical method over the span of two years despite the fact that testing costs have increased.

38. It is important to note that when our clients are deciding which testing technique to select, they are given the choice of both the modular composite and the classical testing technique referred to in APPENDIX H. The classical testing technique is cheaper than the modular composite for clients to select and its lot sizes are five times bigger, wherein n = 60 samples. For example, the beef for classical lot is five combos of 2000 pounds each, with n = 60 sampling, at a price $55. For the modular composite testing, the lot is one combo, 2000 pounds, with n = 60 sampling, at a price of $15. Thus, the client pays $55 for testing 10,000 pounds with the classical, whereas the client pays $75 for the same amount with the modular. As can be seen, the increase in clients' selecting the modular composite is in spite of the increase in test cost; rather clients are selecting the modular compositing to manage their risk due to higher levels if confidence, because of decrease lot size and increase sampling. The unexpected outcome is that less material is discarded, despite the fact that there are more positives. Thus, IEH clients who used the modular composite method actually save money by allowing product to be saved, an example of which is given in col. 11, ln. 45-50 of the patent.

39. In fact, the current wide-spread adoption of the wet composite inter-lot pooling inventive methods was surprising given the points stated above under paragraph "5", particularly given that these methods significantly increase the sampling by 500%, and the testing cost by about 30%, and particularly in a cost-sensitive industry with narrow profit margins, where increasing such cost was a strong and long-standing significant disincentive. Surprisingly, using post-enrichment pooling (which is contra-indicated in the prior art universally taught use of pre-enrichment pooling), and more specifically, testing of a plurality of lots rather than pooling of samples within a single lot, this method has surprisingly and rapidly been widely adopted. Prior to this invention, the industry practice was still fashioned on the methods of Price and Silliker, which are nearly 40 years old, and it was the present Applicant who solved a long-standing problem in the art as described herein.

40. Despite the commercial success of the method, and extensive validations, there

still exists a high level of skepticism aside from the fact that FSIS has not accepted the method without the added assurance of testing each enrichment individually. Furthermore, FDA will not accept any submission in if pooling is done with their *E. coli* O157 and Listeria methods.

41. In summary, IEH company sales figures for the last four years have increased at an average rate of 30% per year, based on client adoption of the methods claims in the present patent application comprising pooling of enriched samples representing a plurality of lots, and the ability to yet validate and release individual lots after obtaining a positive result for the plurality of lots pooled sample. Implementation of the claimed methods has given rise to an increase in number of tests that Institutes of Environmental Health is contracted to conduct, and a concomitant increase in customer satisfaction and public safety. IEH's market share has also steadily increased due to acceptance of and overt demand for the claimed method.

42. IEH is an independent testing laboratory and we are in a competitive market, with numerous competitors in all areas of our market who offer testing services for beef.

43. Further, IEH does not have any long-term contracts that bind customers to use any particular method, and, importantly, no contract required or motivated customers to use the inventive method.

44. Moreover, the increase in sales for the new inventive modular composite method was not the result of advertising effort. There are no print or other media advertising, mail solicitation, telephone solicitation, etc. IEH did not employ a sales person anywhere in our laboratory business for testing methods until 2009, and even this salesperson was not involved with beef combo testing sales. Clients who use IEH for beef testing did so mostly as the result of our occasional exhibits at trade shows, where five or six major competitive labs also attend. IEH always offered both testing methods side by side and allowed the client to make the choice.

45. *Last*, the original specification, for which I signed an inventor Oath/Declaration, of the current patent contained a working Example 1 that demonstrated the nexus between the invention as claimed and the related benefits which, despite the substantial disincentive of increased sampling and assay costs as discussed below, have led to the current success of patent '584 (see Example 1 of the patent):

Specifically, the individual single-unit production lot samples comprising the MWCS were analyzed separately following each *positive* MWCS result. As can be seen in TABLE I, only 391 individual single-unit production lot samples yielded a *positive* result out of the cumulative total of 1,085 individual single-unit production lot samples comprising the MWCS samples which yielded a *positive* result. If only the 391 individual single-unit production lots yielding a *positive* sample result were diverted to rendering or downgraded for cooking this would represent 782,000 lbs of product. The remaining 694 individual single-unit production lots, corresponding to 1,928,000 lbs of product, are, according to preferred aspects of the present invention, validated and releasable into the chain of commerce.

The product, in this Example I, being produced by this U.S. beef producer was beef trim. The release of the validated 1,9280,000 lbs of beef trim into commerce using the present invention, instead of diverting or downgrading the product, trim, represents a cost savings to the producer of about $2,500,000, relative to prior art methods. Additionally, the testing of the single-unit production lots by augmented testing protocols (representing a 5- to 50-fold increase in sensitivity relative to the MWCS testing) provides *substantial* additional assurance of the safety of the food products.

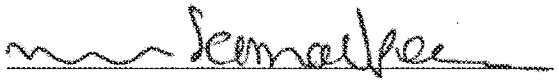
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TABLE 1: Results of modular wet composite sampling of 62,919 multiple lots and number of Individual Production Unit Lot (IPUL) which tested positive when MWCS tested positive

Location	Total # Analyses	# Positives	1 IPUL	2 IPULs	3 IPULs	4 IPULs	5 IPULs
Est. A, B, C.	62,919	271	180	39	18	11	7

U.S. Patent No. 7,534,584, at columns 11 and 12.

46. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code, and may jeopardize the validity of the application or any patent issuing thereon.



Mansour Samadpour, Ph.D.

31 January 2011

APPENDIX A
CURRICULUM VITAE

MANSOUR SAMADPOUR, PH.D.
Principal

SUMMARY

Dr. Samadpour is a food microbiologist with extensive experience in food safety, food production, and epidemiology of foodborne illnesses. He is currently President of Molecular Epidemiology and IEH Laboratories & Consulting Group (IEH), who together employ upwards of 300 personnel. IEH initially focused on providing services to the beef harvesting and grinding industries, and currently IEH conducts testing for about 1/3 of the U.S. market. Since the spinach outbreak of 2006 services have expanded into the produce sector and now IEH serves major clients in fresh cut produce (lettuce, tomatoes, and green onions), apples, sprouts, and tree nuts. In this capacity Dr. Samadpour has been intimately involved in defining and developing microbiological testing programs for food safety management of *E. coli* O157, *Salmonella*, EHEC, *Shigella* and *L. monocytogenes* testing plans as well as the evaluation of the efficacy of microbial interventions. Dr. Samadpour's expertise has been sought in most of the major foodborne illnesses that have occurred since the Jack In The Box *E. coli* O157 episode of 1993, and he as served as expert witness on numerous occasions.

Currently Dr. Samadpour has over 50 scientific publications, over 50 abstracts, 12 popular press and trade articles and more than 200 research reports. He is also the inventor on over 14 patent applications involving aspects of food testing and foodborne illness risk analysis.

EDUCATION

1990	**Ph.D.**, Food Science and Technology, minor in Molecular Biology; University of Washington, Seattle, WA
1987	**M.S.**, Microbiology University of Washington, Seattle, WA
1981	**B.S.**, Microbiology University of Washington, Seattle, WA

PROFESSIONAL POSITIONS

2001-Present	**Principal**, Molecular Epidemiology, also dba IEH Laboratories & Consulting Group, Seattle, WA
1993-2003	**Assistant Professor**, Department of Environmental Health, School of Public Health and Community Medicine, University of Washington,

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1991-1993 **Research Associate**, Department of Environmental Health, School of Public Health and Community Medicine, University of Washington, Seattle, WA

1990-1991 **Post-Doctoral Research Associate**, Department of Environmental Health, School of Public Health and Community Medicine, University of Washington, Seattle, WA

1988-1990 **Pre-Doctoral Fellow**, School of Fisheries, University of Washington, Seattle, WA

1978-1980 **Graduate Research Assistant**, Department of Microbiology, University of Washington, Seattle, WA

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APPENDIX B
Jarvis, 2007

Letters in Applied Microbiology ISSN 0266-8254

ORIGINAL ARTICLE

On the compositing of samples for qualitative microbiological testing

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Keywords
compositing, microbiological criteria, qualitative tests, sampling, statistics.

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Summary

The introduction of legislative microbiological criteria for foods [Official *J L338* (2005) 1] has increased the exposure of enforcement and industrial laboratories to the need to test multiple food samples for organisms such as salmonellae. A consequence has been an increase in the number of organizations, both official and commercial, considering whether it is permissible to composite replicate sample units for the purposes of assessing compliance with the criteria. This note summarizes the statistical and practical aspects of compositing sample units for compliance testing. Provided that the method of choice is 'fit for purpose', then there is no statistical difference between testing, say, 30 × 25 g sample units or 3 × 250 g sample units. However, compositing of sample units should be done only when the method has been demonstrated unequivocally to be sufficiently sensitive to detect potentially lower numbers of target organism(s) in the quantity of composited sample under the conditions of test. Different approaches to compositing of samples are considered.

Introduction

Microbiological criteria (e.g. those defined in Anon. 2005) in some instances impose a need to test a significant number of sample units from individual production lots for the presence of pathogens such as salmonellae. Examples of such criteria are given in Table 1.

Official agencies and manufacturers seeking to confirm that commercial products comply with the legislative criteria need to obtain an adequate number of representative samples of the manufacturing batch, or 'lot'. They need also to instruct a laboratory to undertake appropriate testing, which has significant cost and workload implications with large numbers of samples.

A consequence is that both official agencies and industrial organizations are unsure regarding the validity of compositing sample units and some have used approaches that are neither statistically nor technically valid. It seemed appropriate therefore to provide a brief account of the issues to be considered in respect of compositing.

Background

The concept of compositing of sample units is not new. It is widely used in the preparation of analytical samples for chemical and physical analyses because by efficient blending of a number of sample units, an analytical sample can be taken that is more truly 'representative' of a 'lot' (Patil 2002; Anon. 2003). The effect of compositing is to generate a sample that will be more 'typical' of a batch than will individual samples tested separately as the 'between sample' variance is reduced and the analytical result reflects more closely the true composition of the lot. Such procedures have been used widely in chemical analyses including those for trace contaminants such as mycotoxins in foods, for generic assessment of microbial populations in soil and water, for environmental studies of avian faecal microflora, etc. Note, however, that these analyses are quantitative in nature.

When a quantal analysis is used, i.e. one that is based on the presence or absence of a specific chemical or microbial contaminant, the ability of the test procedure

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Table 1 Some European legislative food safety criteria (Anon. 2005)

	Sampling plan				
Micro-organism	*n*	c	Limits	Test method	Comment
Salmonella spp*	30	0	Absence in 25 g	Anon. (2002)	Products placed on the market during their shelf-life
*Enterobacter sakazakii**	30	0	Absence in 25 g	Anon. (2006)	
Listeria monocytogenes†	10	0	Absence in 25 g	Anon. (1996)	

n, number of sample units; c, number of sample units permitted to exceed the defined limit.
*Dried infant formulae.
†Ready to eat infant foods.

to detect that contaminant at a meaningful level is paramount. When sample units have been composited, this issue becomes even more critical. For instance, if a 25-g sample unit contains a single viable target organism and that sample unit is composited with nine further sample units each of which is free from the target organism, then the overall incidence of contamination is reduced from one organism in 25 g to one organism in 250 g. So the critical issue is whether or not the test methodology can detect such a low level of contamination.

The work of Silliker and Gabis (1973) was quoted by ICMSF (1978) who claimed, 'multiple analytical units may be combined or "lumped" to provide larger units for testing purposes'. Furthermore, they stated, '60 25-g units selected from a particular product lot may be combined ... to produce three 500-g samples for analysis. If all three 500-g samples are ... negative ... then the inference is that the level of *Salmonella* is no greater than one in 500 g'. This statement is at best an over-simplification. Silliker and Gabis (1973) actually showed that if the test material has a high level of contamination, then compositing made no difference to the results obtained, but at lower levels of contamination the use of composited samples could give rise to false-negative results. More recently, ICMSF (2001) withdrew somewhat from their earlier position and stated, 'Numerous studies since 1974 have continued to demonstrate the merits and limitations of compositing. Experience may vary for different food–pathogen combinations and analytical methods, indicating a need for caution and validation that compositing is acceptable'.

Quantal analysis for the presence or absence of a specific organism is predicated on the ability of the test procedure to detect a very small number of the target organisms in a defined quantity of sample. Usually, such tests involve nonselective pre-enrichment culture followed sequentially by transfer of an inoculum to a selective enrichment medium, plating onto selective/diagnostic agars and, finally, purification of organisms giving typical reactions and their subsequent identification. Whether or not a specific organism is transferred from the pre-enrichment stage, or indeed from the enrichment stage, will depend on:

i. The initial level of contamination of the test sample unit by both the target organism and competitive organisms;
ii. The initial condition (e.g. sublethal damage) of the target organism and the competitors; and
iii. The growth kinetics (length of lag phase, specific growth rate, etc.) of target and competitive organisms in the conditions of the test (time, temperature, presence of inhibitors, etc.)

Possibly, the most important practical issue is the period of incubation of the pre-enrichment culture. Short incubation periods may not provide sufficient time for adequate recovery and growth of sublethally damaged target organisms (Jarvis 1989). Growth of the target organism must reach a critical level such that it is 'beyond reasonable doubt' that the organism will be transferred from the pre-enrichment stage to the enrichment medium. In my view, this requires at least a 99.9% probability that one or more viable organisms will be transferred in the inoculum. Assuming random distribution of organisms in the pre-enrichment culture, the probability of not transferring any organisms ($P_{x=0}$) in an inoculum of 1 ml can be derived from the Poisson distribution. Table 2 gives the probabilities for $P_{x=0}$ and $P_{x\geq 1}$ for different levels of viable organisms in a culture. To be certain of transferring at least one organism in 1 ml of the pre-enrichment culture requires an average level of occurrence of at least seven viable organisms per ml of the culture. Similar considerations apply also to the transfer of organisms from enrichment culture to a diagnostic agar plate.

Suppose there is one sublethally damaged target organism per 25 g of sample diluted into 225 ml medium; to obtain at least seven target organisms per ml of incubated pre-enrichment culture requires a total population of 1750 organisms per 250 ml culture. This will require at least 11 generations ($2^{11} = 2048 > 1750$) of the organism once the organism passes from the lag phase into the exponential growth phase. Depending upon the incuba-

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Table 2 Statistical probability of transferring less than, or greater than, one viable organism in an inoculum of 1 ml, assuming random distribution of the target organism in a pre-enrichment culture

Average level of target organisms per ml of pre-enrichment culture	Probability that 1 ml of inoculum will contain	
	0 organisms	1 or more organisms
1	0·368	0·632
2	0·135	0·865
3	0·050	0·950
4	0·018	0·982
5	0·006	0·994
7	0·001	0·999
10	<0·001	>0·999
20	<<0·001	>>0·999

tion temperature and the possible inhibitory effects of competitive organisms, this may require more than 12-h incubation of the pre-enrichment medium (Jarvis 1989). If the inoculum were smaller (e.g. one viable organism in 250 g of composited sample), the minimum incubation time could be longer than 12 h.

Similar constraints apply also to newer developments in microbiological test procedures, e.g. the use of immuno-magnetic beads as an aid to more rapid isolation of target species and even PCR techniques require a minimal amount of recoverable DNA to detect an organism.

Microbiological test procedures

Two key issues need to be considered: the sensitivity (i.e. the level at which a target organism can be reliably detected) and the specificity of the procedure. For quantal tests, both sensitivity and specificity are critical. A procedure that is capable of detecting, say, one viable cell of a specific organism in a defined quantity of a food material may not be capable to detecting one viable organism if the quantity of the test material is increased 10- or 20-fold through compositing of sample units. The more sensitive the test, the lower the level of organisms that is likely to be detected under the conditions of the test.

A test that is highly specific will detect only the chosen species or strains of an organism and will not detect closely related organisms that may be of equal importance in relation to food safety. Such a test procedure may therefore be unreliable as it is likely to lead to genuine 'false-negative' results. A procedure that has low specificity may detect related strains of organism that are not of public health concern, creating 'false-positive' results. To be of practical value in food microbiology, the method must be capable of detecting the 'most significant' strains of the target organism at a low level of contamination. A method designed to detect the presence of high levels of specific pathogens in heavily contaminated material, e.g. faeces, may be of limited value in seeking to detect very low, but possibly significant, levels of the organisms in foods.

Unfortunately, practitioners sometimes ignore such limitations and assume that a standard procedure will always detect the target organism if it is present in a sample.

Microbiological sampling plans and criteria

Criteria, such as those defined by Anon. (2005) using presence or absence tests for specific organisms, are based on two-class sampling plans. In general, sampling plans for pathogenic organisms such as salmonellae require that no target organisms should be detected in a given quantity of each of several sample units of product drawn from a lot. In a few cases (e.g. for coagulase-positive staphylococci), a less stringent plan may be used.

The stringency of the sampling plan is dependent on the number (n) of sample units of a given size (weight, volume) to be tested and on whether one or more samples (c) can be accepted if a positive result is found. A sample plan is designated as e.g. $n = 5$ (or more), $c = 0$ if no positive results are acceptable. However, there are two primary considerations that need to be understood:

i. Any result that can be classified on the basis of a presence or absence result is governed by the binomial distribution; hence, the stringency of the sampling plan will be increased if n is large (Table 1) but no matter how many sample units are tested with negative results, it can never be stated unequivocally that the lot from which the samples were drawn is totally free from contamination by the target organism, although confidence limits (CLs) can be established on the 'zero' result (see below).

ii. The typical application of the binomial distribution is predicated on the assumption that the target organisms will be distributed homogeneously (i.e. randomly) throughout the sample and that there is an equal opportunity for any sample unit to contain the target organism. In practice, such even distribution of low numbers of pathogenic organisms in food products occurs only rarely. Dried milk powders, infant formulae and chocolate are but some of the foodstuffs where there is evidence of microbial clumping in a small proportion of the lot. Thus, more accurate models that account for the non-homogenous distribution would depend on having some knowledge or understanding of the clustering, which, in most applications, is not available. However, if a homogeneous distribution of organisms throughout the material being tested is assumed, then it is possible to express the probability in terms of the assumed constant organism density, using the Poisson distribution.

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The stringency of sampling plans

The data in Fig. 1 illustrate the statistical probability for acceptance of a product (i.e. the probability that no positive tests are obtained) for different levels of 'true' prevalence of defective units in the lot (i.e. prevalence of contamination) and the number of sample units (n) tested. If negative results are obtained on 30 samples and the 'true' prevalence is 2%, the likelihood of accepting the product is 55% (conversely, the probability of rejection is 45%), but if only five samples are tested the probability of accepting the product is 90%. Similarly, if the lot contains 10% 'true' defective units, then the likelihood of product acceptance after testing 30 sample units with negative results will be only 4%, but if five samples are tested the likelihood of acceptance is 59% and if only one sample is tested, with a negative result, there is a 90% chance of accepting the lot with 10% 'true' defectives. These data are obtained from a rearrangement of the binomial distribution equation:

$$P_{(x>0)} = 1 - (1 - d)^n \qquad (1)$$

where d is the true prevalence of contamination of the lot and n is the number of sample units.

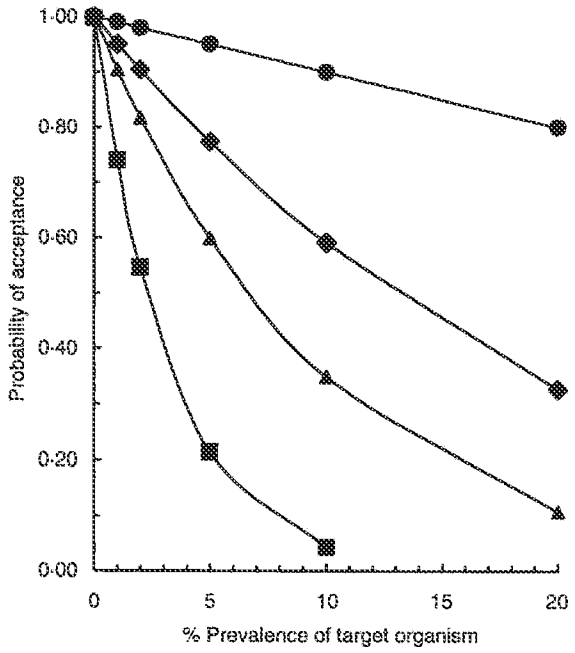


Figure 1 Operating characteristics curves for probability for acceptance of samples having a prevalence of contamination from 0% to 20% and various numbers (n) of samples examined from $n = 1$ (●), $n = 5$ (◆), $n = 10$ (▲) and $n = 30$ (■).

In setting a criterion for the number of samples to be tested, an assumption is made about the incidence of 'true' defective (i.e. contaminated) sample units that are acceptable in relation to the number of replicate tests that can reasonably be performed. For instance, a criterion having a sampling plan of $n = 30$, $c = 0$ would be likely to reject a product having 10% or more defective units with a better than 95% probability. For any given true prevalence of salmonellae in a lot and assuming the distribution of contaminated units amongst a lot is random and then by rearranging eqn (1), we can determine the number of sample units (n) that it would be necessary to test for a defined incidence of contamination and a given probability:

$$n = \log_{10}(1 - P_{(x>0)}) / \log_{10}(1 - d) \qquad (2)$$

If a lot contains 1% defectives ($d = 0{\cdot}01$) and $P_{(x>0)} = 0{\cdot}95$, we can use eqn (2) to calculate the number of sample units (n) that must be tested to have a 95% chance of obtaining at least one positive result:

$$n = \log(1 - 0{\cdot}95) / \log(1 - 0{\cdot}01) = \log 0{\cdot}05 / \log 0{\cdot}99 = 298$$

Table 3 summarizes the minimum number of samples to be tested for different levels of 'true' contamination to be able to give at least a 90% assurance that the sample units tested are not contaminated. Even if all the test results are negative, little statistical assurance of freedom from contamination can be given for small numbers of sample units tested.

If all sample units test negative, then it is possible to assign CLs for the true prevalence of contamination, reflecting the upper bound of the contamination prevalence at a given confidence level ($1 - \alpha$). These CLs are bounded by 0 and P_u, where P_u is chosen so that if the

Table 3 Number of sample units to be tested with negative results in order to be able to give a 90%, 95% or 99% assurance that the sample units comply with a criterion for freedom from a defined target organism in a given number of sample units [based on eqn (2)]

True incidence of defective sample units (%)	Number (n) of sample units required to be tested		
	$P_{(x=0)} = 0{\cdot}90$	$P_{(x=0)} = 0{\cdot}95$	$P_{(x=0)} = 0{\cdot}99$
10	22	28	44
5	45	58	90
2	114	148	228
1	229	298	458
0·5	459	598	919
0·1	2301	2994	4603
0·05	4604	5990	9208
0·01	23 025	29 956	46 049

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true prevalence were P_u, then the probability of the observation of no positive results in n samples is equal to α. Thus, P_u satisfies

$$(1 - P_u)^n = \alpha \quad (3)$$

We can solve this equation for P_u, by rearrangement:

$$P_u\,(\%) = 100(1 - \sqrt[n]{\alpha}) \quad (4)$$

For example, with $n = 30$ and $\alpha = 0{\cdot}05$, we obtain

$$P_u\,(\%) = 100(1 - \sqrt[30]{0.05}) = 9{\cdot}5\%$$

For this example, the CLs of 0–9·5% provide a 95% confidence of enclosing the true prevalence of contamination (τ%). In other words, if the same sampling and testing procedure was repeated a very large number of times, then this interval would be expected to enclose τ on 95% of occasions; but on 5% of occasions the value of τ would exceed 9·5% (by an unknown amount).

Hence, if we test 30 × 25 g sample units with negative results, we have a 95% confidence that the true incidence of defectives would not exceed 9·5%; for 25-g sample units, this is equivalent to a prevalence level of four organisms kg^{-1}. However, if we test only 1 × 25 g sample unit, then the 95% upper CL would give a possible prevalence of 120 organisms per kg. Further examples of 95% and 99% CLs are shown in Table 4.

Table 4 Percentage defective sample units and maximum contamination levels assuming random distribution of target organism and assuming 100% test effectiveness (i.e. any target organism in the sample would be detected) (modified from Jarvis 1989)

No. of sample units tested (n)	% Defectives (τ%)* at		No. of organisms per kg*† at	
	P_u = 0·95	P_u = 0·99	P_u = 0·95	P_u = 0·99
1	95·0	99·0	120	184
2	77·6	90·0	60	92
3	63·2	78·5	40	61
4	52·7	68·4	30	46
5	45·1	60·2	24	37
10	25·9	36·9	12	18
20	13·9	20·6	6	9
30	9·5	14·2	4	6
50	5·8	8·8	2	4
100	3·0	4·5	1	2

*Assuming no positives detected, the true incidence will not be more than the values shown with indicated confidence.
†Rounded to the nearest whole number and assuming sample size = 25 g.

To composite or not to composite?

Suppose the criterion requires that 30 sample units each of 25 g should be tested for the absence of salmonellae and the test procedure is capable of detecting one viable *Salmonella* in a 25-g sample unit. To carry out the test, it would be necessary to inoculate each of the 30 × 25 g sample units into a 225 ml volume of buffered peptone pre-enrichment medium and after incubation for 18 h at 37°C, to subculture 1 ml of the pre-enrichment culture into 10 ml of each of two enrichment media which are then incubated for 24 h, before subculturing onto each of two diagnostic media. After incubation, colonies showing typical characteristics need to be purified and tested to confirm, or rule out, that they are salmonellae. Hence, for a single set of 30 samples, the laboratory would need to set up 30 pre-enrichment cultures, 60 enrichment cultures, 120 diagnostic plates and possibly as many as 600 identification cultures. This is an enormous workload to provide evidence of compliance with a defined criterion for just one set of samples.

To reduce this workload, it is sometimes recommended that the initial sample units should be composited into a smaller number of larger test samples. The following scenarios describe the practical implications of compositing:

i. *An invalid approach*: Composite the 30 × 25 g sample units (i.e. 750 g) into a single well-mixed sample and test one analytical sample unit of 25 g. With this option, only one representative sample unit of 25 g is tested and a negative result is meaningless as the test has not complied with the requirements of the criterion (i.e. to test 30 × 25 g sample units). At best, the statistics of this test would merely indicate, with 95% confidence, that the average prevalence of salmonellae would likely not exceed 120 salmonellae per kg (whereas negative results from all tests on 30 samples indicate, with 95% confidence, a likely average density of no more than four salmonellae per kg).

ii. *A compromise approach*: Composite the 30 × 25 g sample units randomly into three sets each of 10 × 25 g sample units and inoculate each 250 g composite sample into a volume of 2·25 l of pre-enrichment medium. After incubation, carry out the subsequent cultivation procedure based on three pre-enrichment cultures. This approach is valid provided the ability of the method to detect one viable *Salmonella* in 250 g of product has been validated; if not, then this compromise approach is not permissible.

iii. *My preferred approach ('wet' compositing)*: Set up 30 individual pre-enrichment cultures and after incubation transfer 1 ml of each of the 30 pre-enrichment cultures into 300 ml of each of two enrichment media. This would then provide composite enrichment cultures on which further work can be done. The original pre-enrichment media can be stored, pending the outcome of the tests,

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such that if a positive result is obtained it is possible to go back to the original pre-enrichment cultures to assess by further testing how many contaminated samples are present. The benefit of this approach is that the concept of testing 30 samples has not been compromised, as compositing has taken place only after the first culture stage

Statistical considerations

Provided that the test procedure is sufficiently sensitive to detect a single *Salmonella* in the larger quantity of sample, then from a statistical perspective there is no difference in testing 1×750 p_g, 3×250 p_g or 30×25 p_g samples. But if there is any doubt as to the level of sensitivity of the test procedure, then compositing should not be done, except using the 'wet' compositing approach where each individual sample unit is pre-enriched separately.

Regarding the contention, given above: assume n sample units, each unit (i) being of size k_i grams with an organism density of δ_i organisms per g. Then, provided that the test procedure is sufficiently sensitive to detect the organism if present, the probability ($P_{x=0}$) of a negative result on any sample i is given by the Poisson probability of finding zero organisms:

$$P_{(x_i=0)} = e^{-\delta_i k_i} \tag{5}$$

where δ_i is the expected prevalence of contamination and k_i is the size of sample. If $\delta_i = 0{\cdot}1$ organisms per g and $k_i = 25$ g, then the probability of not detecting any organism in the 25-g sample (assuming the test protocol is 100% effective) is given by

$$P_{(x_i=0)} = e^{-0{\cdot}1\times 25} = e^{-2{\cdot}5} = 0{\cdot}082$$

When the probability of detecting zero organisms is 5%, the product of the density (organisms per g) and sample size (g) is equal to about three organisms. Thus, the upper 95% CL of the density, given a negative result, is 3 divided by the sample size. For example, with 25 g tested and a negative result, the upper 95% limit density is $3/25$ g $= 0{\cdot}12$ g$^{-1} = 120$ organisms per kg (Table 4).

The probability of finding a negative result on all n units is the product of the individual probabilities for a negative result in each unit:

$$P_{(x_1=0)(x_2=0)(x_3=0)\cdots(x_n=0)} = e^{-\delta_1 k_1} \times e^{-\delta_2 k_2} \times e^{-\delta_3 k_3} \times \cdots \times e^{-\delta_n k_n} = e^{-\sum \delta_i k_i} \tag{6}$$

To illustrate this effect, suppose that we have 3×250 g sample units with levels of 0·01, 0·00 and 0·02 viable organisms per g, respectively, then the total organism count in the three sample units will be 2·5, 0·0 and 5·0 organisms per 250 g, respectively. The probability of not detecting a positive result in the individual sample units will be 8·2, 100 and 0·7%, respectively. The combined probability of a negative result on the three sample units will be given by:

$$P_{(x_1=0)(x_2=0)(x_3=0)} = e^{-\sum \delta_i k_i} = e^{-\sum (2{\cdot}5+0+5{\cdot}0)} = e^{-7{\cdot}5} = 0{\cdot}00055$$

This is the same probability (0·055%) that would be found by using the mean organism count (0·01 organisms per g) and multiplying by a k value of 750 g. What these formulae show is that, when the testing is 100% effective, the probability of detection just depends on the total quantity of sample tested, not on the number of individual tests.

However, if the test procedure cannot detect the target organism at such a low level (i.e. 0·009 organisms per g in a combined 750-g sample unit) then a different situation pertains. Equation (5) is valid only if it is assumed that the viable organisms in the k grams would be recovered. If this were not the case, then another factor is needed to account for the possibility that organisms would not be recovered. For example, if there were one viable organism in the k grams, we could assume the probability of its not being recovered is r_1; if there were two organisms, we could assume that the probability of not recovering any organism is r_2; and so forth, if there were g organisms, the probability of not detecting any of them would be r_g. A simplifying assumption (just to demonstrate the mathematics) would be to assume that $r_g = r^g$, for g organisms. With this simplifying assumption, it can be shown that the probability of not detecting any organism would be

$$P_{(x=0)} = e^{-dk(1-r)} \tag{7}$$

where r is the probability of not detecting a single organism if present in the k grams. In the example given above, it was assumed that $r = 0$, i.e. that any organism would be detected if present in 25 g. However, for 750 g, r might no longer be zero, but rather some positive value. If this were the case, the probability of a negative could increase quite substantially. For example, if the density was 0·01 organisms per g and $r = 0{\cdot}25$, then the probability of a negative result is 0·361% compared with 0·055% when $r = 0$ was assumed. Hence, there would be an increased factor of 6·5 of getting a negative result.

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Hence, unless the recovery of organisms is independent of the volume being tested, testing larger quantities of sample will be less sensitive than testing individually smaller quantities of samples. This condition does not seem likely to be satisfied, particularly for large volume composite samples. Thus, it would be incorrect to indicate a possible level of contamination when the sample units are composites assuming 100% testing effectiveness; negative results might be due to the inability of the method to detect the target organism, and thus such conclusions would be erroneous.

Conclusion

Before compositing samples for testing, it is essential to validate the test procedure for each food matrix to ensure that the method sensitivity is sufficiently high to detect the presence of the target organism in the increased quantity of sample. Validation requires that the sensitivity of the method must be determined, ideally using naturally contaminated food matrices, for the lowest possible level of contamination that might be encountered in composited samples. In practice, this requires that the method is sufficiently sensitive to detect one viable *Salmonella* in the largest quantity (e.g. 250 or 500 g) of the food matrix likely to be examined. If such a level of contamination cannot be detected reliably, even though one organism in 25 g can be detected, then no certainty exists that the method is suitable for used with composited sample units. Without such certainty, the test result may not indicate the presence of contaminants even if target organisms are present in one or more of the original sample units. Such false-negative results will be grossly misleading and the test protocol will not comply with the requirements of the microbiological criterion. In such circumstances, the only reliable method is to 'wet' composite, i.e. to composite aliquots of incubated pre-enrichment cultures prepared form each individual sample unit.

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APPENDIX C
FSIS Microbiological Laboratory Guide 5.03

United States Department of Agriculture
Food Safety and Inspection Service
Office of Public Health and Science
Laboratory QA/QC Division
950 College Station Road
Athens, GA 30605

Microbiology Laboratory Guidebook
Notice of Change

Chapter new, **revised**, or archived: MLG 5.03

Title: Detection, Isolation, and Identification of *Escherichia coli* O157:H7 and O157:NM (Nonmotile) from Meat Products

Effective Date: 10/25/02

Description and purpose of change(s):

The Microbiology Laboratory Guidebook method chapters are currently under revision. The formatting is being changed to meet the requirements of the laboratory's document control system. Additional content is being added, i.e. Section 5.1.2. Limits of Detection, to meet the requirements of ISO 17025. Safety Precautions are also included in the revised chapters.

United States Department of Agriculture
Food Safety And Inspection Service, Office of Public Health and Science

MLG 5.03		Page 1 of 13
Title: Detection, Isolation and Identification of *Escherichia coli* O157:H7 and O157:NM (Nonmotile) from Meat Products		
Revision: 03	Replaces: MLG Chapter 5 Revision 2	Effective 10/25/02

Procedure Outline

5.1 Introduction
- 5.1.1 General
- 5.1.2 Limits of Detection

5.2 Safety
5.3 Quality Control Practices
5.4 Equipment, Materials, Media, Reagents and Test Kits
- 5.4.1 Equipment
- 5.4.2 Media, Reagents and Cultures
- 5.4.3 Test Kits

5.5 Detection Procedure
5.6 Isolation Procedure
5.7 Identification and Confirmation
5.8 Storage of Cultures
5.9 Selected References

5.1 Introduction

5.1.1 General

The following method is used for the analysis of raw and ready-to-eat meat products for *Escherichia coli* O157:H7 and O157:NM (O157:H7/NM). The method is based on enrichment in a selective broth medium, application of a rapid screening test, immunomagnetic separation (IMS) in paramagnetic columns, and plating on a highly selective medium.

The following definitions are used for reporting purposes. A potential positive sample causes a positive reaction on the screen test kit. A presumptive positive sample has typical colonies, observed on Rainbow Agar, and reacts specifically with O157 antiserum. A sample is a confirmed positive sample for E.coli O157:H7 or E. coli O157:NM when the isolate is confirmed biochemically and serologically, and the presence of Shiga toxin(s) or Shiga toxin gene(s) is demonstrated.

Unless otherwise stated all measurements cited in this method have a tolerance of ± 2%.

5.1.2 Limits of Detection

This test has been shown to consistently detect less than 1 colony forming unit (cfu)/g in a 65 g sample.

5.2 Safety

E. coli O157:H7/NM is a human pathogen with a low infectious dose. (Ingestion of 100 cells can cause disease.) The use of gloves and eye protection is mandatory and all work surfaces must be disinfected prior to and immediately after use. Laboratory personnel must abide by CDC guidelines for manipulating Biosafety Class II pathogens. A Class II laminar flow biosafety cabinet is recommended for activities with potential for producing aerosols of pathogens. All available Material Safety Data Sheets (MSDS) should be obtained from the manufacturer for the media, chemicals, reagents and microorganisms used in the analysis. The personnel who will handle the materials should read all MSDS sheets.

5.3 Quality Control Practices

a. Rainbow® Agar plates have a shelf life of 2 weeks.

b. All media and E-Buffer must be pre-warmed to 18-35°C prior to use.

c. The recommended fluorescent strain of *E. coli* O157:H7 must be used in this procedure to monitor for cross contamination. The protocol for the use of fluorescent strains of *E.coli* O157:H7 as positive controls follows:

Wild-type strains of *E. coli* O157:H7 transformed with pGFP produce a green fluorescent protein. As a result of this transformation, fluorescent strains of *E. coli* O157:H7 possess the unique property of expressing bright green fluorescence visible in the dark when illuminated by long-wave UV light. This property, which sets them apart from typical *E. coli* O157:H7, makes them useful positive controls for analyses of meat samples for *E. coli* O157:H7/NM. At different steps in the procedure, both test samples and (fluorescent) positive controls can be tested for the bright green fluorescence as a Quality Control measure to make sure that positive sample isolates actually came from the test sample and not from accidental contamination by the positive control cultures.

Results of studies done at the FSIS Beltsville Microbial Pathogens Laboratory showed that these fluorescent cultures can be subjected to *E. coli* O157:H7/NM isolation and identification procedures without losing their fluorescent properties. These strains retain their fluorescent properties when grown in SOB media with added ampicillin (SOB + A). These cultures must be transferred every 7 days to fresh SOB + A media, according to the protocol outlined below. The fluorescent colonies are ready to be used as positive controls on day 3 of the following protocol, and for the next 6 consecutive days without losing their fluorescent properties. If these cultures are not needed on a continuous basis, they can be stored at refrigeration temperatures on SOB + A agar plates in zip-lock bags or sealed with parafilm® for 1 month and then transferred, or started up again 2 days before needed. *Strict adherence* to the protocol described below is essential, in order to ensure that the fluorescent strains do not lose their ability to express green fluorescence.

i. Test the fluorescent *E. coli* O157:H7 strain (FSIS culture # EC 465-97 or the currently designated control strain) on SOB + A agar plate for fluorescence by illuminating colonies under long-wave UV light in the dark.

ii. Select only fluorescing colonies and inoculate into 10 ml of SOB + A broth in a tube. Incubate at 35 ± 2°C overnight.

iii. Streak the culture from the SOB + A broth onto a SOB + A agar plate. Incubate at 35 ± 2°C overnight.

iv. Examine colonies on the plate for fluorescence. The fluorescent colonies are ready to be inoculated into modified EC broth + novobiocin (mEC+n) at this stage. These cultures on SOB + A agar plates can be stored refrigerated and be used as positive controls for 6 more days. Incubate the inoculated mEC+n positive control culture at 35 ± 2°C overnight, along with the test samples.

v. Continue analysis per Sections 5.5-5.7 and test the Blood Agar Plates of the fluorescent positive controls and any positive sample cultures for fluorescence.

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5.4 Equipment, Materials, Media, Reagents and Test Kits

5.4.1 Equipment

a. Balance, sensitivity of 0.1 g
b. Stomacher™ 400 or 3500 with appropriate sizes of sterile Stomacher™ bags, with or without mesh. (Tekmar Co., Cincinnati, Ohio), or equivalent
c. Incubator, static 35 ± 2°C
d. Micropipettors to deliver 15-1000 µl with sterile disposable filtered micropipet tips
e. Mechanical Pipettor with 1.0 ml, 5.0 ml, 10.0 ml sterile pipettes
f. Inoculating loops, "hockey sticks" or spreaders, and needles
g. UV light (long-wave, e.g. VWR # 36553-124, or equivalent)
h. Filter unit, 0.2 µm, nylon, sterile
i. Infrared thermometer
j. LabQuake® Agitator (or equivalent) with clips to hold microcentrifuge tubes
k. Sterile disposable 12 x 75 mm polypropylene tubes (e.g. Fisher # 14-956-1B, or equivalent)
l. Microcentrifuge and sterile 1.5 ml microcentrifuge tubes
m. Sterile 50 ml conical tubes (e.g. Falcon® # 2070, or equivalent) or sterile bottles
n. Sterile 40 µm Cell Strainer (Falcon® # 2340, or equivalent)
o. MACS® Large Cell Separation Columns (Miltenyi Biotec # 422-02, or equivalent)
p. OctoMACS® Separation Magnet (Miltenyi Biotec # 421-09, or equivalent)
q. Multistand to support OctoMACS® Separation Magnet (Miltenyi Biotec # 423-03, or equivalent)
r. Tray, autoclavable, approximately 130 mm x 83 mm (e.g. VWR # 62663-222, or equivalent) for use with the OctoMACS®

5.4.2 Media, Reagents and Cultures

a. Modified EC broth with novobiocin (mEC+n) (or equivalent)
b. Rainbow® Agar O157 (Biolog Inc., Hayward California, 94545) containing 10 mg/L novobiocin plus 0.8 mg/L potassium tellurite, or equivalent selective medium
c. Tryptic soy agar with 5% sheep blood

d. SOB + A Medium
e. E Buffer, approximately 7 ml per sample [Buffered Peptone Water, Bovine Albumin Sigma # 7906 (or equivalent) , and Tween-20®, or equivalent]
f. Disinfectant (Lysol® I. C., 2.0%, or equivalent)
g. Dynal® # 710.04 anti-*E. coli* O157 antibody-coated paramagnetic beads (Dynal Inc., Lake Success, NY 11042), or equivalent
h. *E. coli* O157:H7 strain 465-97 (positive control used throughout method)
i. *E. coli* ATCC strain 25922 (negative control for bead capture and screen tests)

5.4.3 Test Kits

a. The screening test for the detection of *E. coli* O157:H7/NM should meet or exceed the following performance characteristics:

Sensitivity	≥98%
Specificity	≥90%
False Negative Rate	≤ 2%
False Positive Rate	≤10%

b. *E. coli* O157:H7 latex agglutination test kit (RIM® *E. coli* O157:H7 Latex Test Kit, REMEL, 12076 Santa Fe Drive, Lenexa, KS 66215, or equivalent)

c. Biochemical test kits and systems [Vitek® GNI and GNI Plus cards (bioMerieux Vitek, Inc., 595 Anglum Drive, Hazelwood, MO 63042-2395), or equivalent]

d. Shiga Toxin test kit [Premier® EHEC, cat. # 608096 (Meridian Diagnostics, Inc., 3471 River Hills Dr., Cincinnati, OH, 45244), or equivalent

5.5 Detection Procedure

a. Sample Preparation

i. Raw ground beef microbiological testing programs.
Randomly collect five 65 ± 2 g sub-samples (total of 325 ± 10 g) that are representative of the entire sample. Place each 65 ± 2 g sub-sample in a

sterile Strainer Stomacher™ bag. Add 585 ml mEC+n broth and pummel for two minutes in a Stomacher™.

ii. Cooked meat patties and semi-dry and dry fermented sausages. Randomly prepare five 65 ± 2 g sub-samples (total of 325 ± 10 g) that are representative of the entire sample. When appropriate, sample representative portions from both the outer surface (shell) and inner section (core) of RTE products, especially semi-dry and dry fermented sausages. Place each 65 ± 2 g sub-sample in a sterile Strainer Stomacher™ bag. Add 585 ml mEC+n broth and pummel for two minutes in a Stomacher™.

iii. Outbreak-related samples. Randomly collect thirteen 25 ± 1 g sub-samples (total of 325 ± 13 g) that are representative of the entire sample. Place each 25 ± 1 g sub-sample in a sterile Strainer Stomacher™ bag and add 225 ml of mEC+n broth. Pummel for 2 minutes in a Stomacher™.

b. Incubate all bags (static) with their contents for 20 to 24 h at 35 ± 2°C. Include a positive, negative, and uninoculated medium control for each group of samples tested. Use the fluorescent *E. coli* O157:H7 strain (FSIS culture # EC 465-97) as a positive control and *E. coli* ATCC strain 25922 as the negative control.

c. From the enrichment cultures in the Stomacher™ bags, perform the screening test for *E. coli* O157:H7/NM following the manufacturer's instructions. The enrichment culture may be analyzed immediately upon removal from the incubator without waiting for tempering to room temperature. To prevent clogging the pipette tip, be sure to collect the appropriate size sample from the enrichment culture outside the inner strainer bag.

d. Samples negative by the screening test can be reported as negative for *E. coli* O157:H7/NM and discarded.

e. Samples positive by the screening test should be reported as potential positives. Begin isolation procedures from the enrichment culture in the Stomacher™ bag.

5.6 Isolation Procedure

Note: Steps a.-l. may be performed in a sequence that is convenient to the laboratory personnel.

a. Prepare E Buffer by mixing 0.5 g Bovine Albumin and 50 µl Tween-20® into 100 ml Buffered Peptone Water (BPW). Filter sterilize (0.2 µm) and store at 2-8°C.

b. Remove Rainbow® Agar plates from 2-8°C storage, allowing 3 plates for each screen-positive culture and each control. Be sure that plates have no visible surface moisture at the time of use. If necessary, dry plates (e.g. for up to 30 minutes in a laminar flow hood with the lids removed) prior to use. Dried plates that are not used should be labeled "dried", placed in bags and returned to 2-8°C.

c. Remove a bottle of E Buffer from 2-8°C storage. Decant 7 ml of E Buffer for each culture and each control into a sterile tube or bottle and allow it to warm to at least 18°C. (Return the stock E Buffer to 2-8°C.)

d. For each positive control, negative control and screen-positive culture to be analyzed, order and label 50 ml conical centrifuge tubes so that the positive control is first, followed by the negative control, then all cultures. Maintain this order for subsequent steps.

e. For each positive control, negative control, and screen-positive culture, label two sterile 1.5 ml microcentrifuge tubes (for step g and step s), one 50 ml conical centrifuge tube (for step h.) and two 12 x 75 mm capped tubes (one for step p.). For each pair of 12 x 75 mm tubes, label one tube and add 0.9 ml E Buffer (for step q.).

f. Prepare the Dynal #710.04 *E. coli* O157:H7 immunomagnetic bead suspension by following Table 1 below. Be sure to include the positive and negative controls in the total number of cultures. Use the bead suspension immediately (step g), or hold at 2-8°C. Return the stock vial of Dynal #710.04 *E. coli* O157:H7 immunomagnetic beads to 2-8°C.

g. Vortex the bead solution briefly (2-3 seconds), then add 50 µl to a labeled

microcentrifuge tube (from step e), one for each control and screen-positive culture. Use immediately or hold these tubes at 2-8°C.

h. Place a 40 µm Cell Strainer on a labeled 50-ml conical centrifuge tube (from step e.). Pipet 5 ± 1 ml of each control and enrichment culture into the respective Cell Strainer and collect at least 1.0 ml of filtrate.

i. Do not proceed with more than the number of tubes that the OctoMacs® magnet(s) will hold. Transfer 1.0 ml of a filtrate (step h.) to the corresponding microcentrifuge tube containing the immunomagnetic bead suspension (step g.) and place in the clips of the LabQuake® tube agitator. Rotate the tubes for 10-15 min at 18-30°C.

j. Attach the OctoMACS® Magnet to the Multistand.

k. Position a tray on the base of the Multistand so that it will collect the filtrate passing through the columns. Add approximately 300 ml of 2% Lysol® I. C. (or equivalent) disinfectant to cover the bottom of the tray.

l. Label and place the appropriate number of Large Cell Separation columns on the OctoMACS® Magnet. Insert columns from the front making sure the column tips do not touch any surfaces. Leave the plungers in the bags at this time to maintain sterility.

m. Transfer at least 0.5ml E Buffer to the top of each column and let the buffer run through.

n. Resuspend, then transfer each culture and control from step i. to its corresponding column.

o. After a culture or control has drained through, wash the column by applying 1.0 ml of E Buffer to each column and allow to drain. Repeat 3 more times for a total of 4 washes.

p. After the last wash has drained, remove the column from the OctoMACS® Magnet and insert the tip into an empty labeled 12 x 75 mm tube (from step e.).

Apply 1.0 ml of E Buffer to the column, and using the plunger supplied with the column, *immediately* flush out the beads into the tube. Use a smooth, steady motion to avoid splattering. Cap the tubes. Repeat this for each column. If the OctoMACS® magnet is to be used for a second set of cultures, it must be decontaminated as described in step u, below. Repeat steps j.-s. for the additional cultures.

q. Vortex the tubes from step p. briefly to resuspend the beads. Make a 1:10 dilution of each treated bead suspension by adding 0.1 ml of the bead suspension to a 12 x 75 mm labeled tube containing 0.9 ml E Buffer (from step e.).

r. Vortex briefly to maintain beads in suspension and plate 0.1 ml from each tube (from step p. and step q.) onto a labeled Rainbow® Agar plate. Use a hockey stick or spreader to spread plate the beads, being careful not to spread the beads against the edge of the plate.

s. Vortex the tubes containing undiluted beads (from step p.) and transfer to a labeled microfuge tube (from step e.) and centrifuge at least one minute using a bench-top microcentrifuge to concentrate the beads. Withdraw and discard the supernatant without disturbing the beads. Add 0.1 ml of E Buffer to the beads, resuspend the beads and transfer the beads to a labeled Rainbow® Agar plate. Spread plate the beads as described in step r.

t. As soon as there is no visible moisture on the agar surface, invert plates and incubate for 24-26 h at 35 ± 2°C.

u. Decontaminate the OctoMACS® Magnet by applying 2% Lysol® I. C. (or equivalent) disinfectant directly to the surface. After approximately ten minutes, rinse with deionized or tap water. Allow the unit to air-dry or use absorbent paper towels to dry the unit.

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Table 1.

# of Cultures	*ul of Beads**	*ul of E-Buffer*	*# of Cultures*	*ul of Beads**	*ul of E-Buffer*
1	15	135	26	145	1305
2	20	180	27	150	1350
3	25	225	28	155	1395
4	30	270	29	160	1440
5	35	315	30	165	1485
6	40	360	31	175	1575
7	45	405	32	180	1620
8	50	450	33	185	1665
9	55	495	34	190	1710
10	60	540	35	195	1755
11	65	585	36	200	1800
12	70	630	37	205	1845
13	75	675	38	210	1890
14	80	720	39	215	1935
15	85	765	40	220	1980
16	90	810	41	230	2070
17	95	855	42	235	2115
18	100	900	43	240	2160
19	105	945	44	245	2205
20	110	990	45	250	2250
21	120	1080	46	255	2295
22	125	1125	47	260	2340
23	130	1170	48	265	2385
24	135	1215	49	270	2430
25	140	1260	50	275	2475

* Dynal® anti-*E. coli* O157:H7 antibody-coated paramagnetic beads (vortex briefly before use)

Approved by Phyllis Sparling, 10/17/02

5.7 Identification and Confirmation

a. After incubation, *E. coli* O157:H7 colonies have black or gray coloration on Rainbow® Agar. When *E. coli* O157:H7 colonies are surrounded by pink or magenta colonies, they may have a bluish hue. Mark colonies typical of *E. coli* O157:H7 and perform latex agglutination assays for O157, following manufacturer's instructions. Streak all latex positive colonies, up to a total of five from each sample (one per sub-sample, if possible) onto Blood Agar plates. Incubate Blood Agar plates for 16-24 h at 35 ± 2°C.

Note: If no typical colonies are present, hold the original Rainbow® plates at 20-24°C for an additional 6-24 h then re-examine for typical colonies.

b. After incubation, examine the Blood Agar plates for purity under visible light, and evidence of cross contamination with the positive control by using long wave UV light. Only the positive control culture, *E. coli* O157:H7 strain 465-97, should fluoresce. If the Blood Agar plates appear pure and uncontaminated, perform the following confirmatory tests:

i. Biochemical confirmation.
Inoculate Vitek-GNI or GNI Plus cards or use an equivalent biochemical identification testing system. The cytochrome oxidase and gram stain tests are optional.

ii. Serological confirmation.
To confirm the absence or presence of O157 and H7 antigens, use an *E. coli* O157:H7 latex test agglutination kit (RIM® *E. coli* O157:H7 Latex Test Kit, or equivalent). Use growth from the Blood Agar plate (from step b).

iii. Shiga toxin/toxin genes confirmation.
The presence of Shiga toxin(s) in a culture isolate should be confirmed by the use of a toxin assay, e.g., Meridian Premier® EHEC Kit, or equivalent. When Shiga toxin(s) is (are) not demonstrated, detection of one or more toxin genes by PCR should be used for confirmation. The positive control culture, *E. coli* O157:H7, is toxin-negative.

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c. If the isolate confirms as an *E. coli* O157:H7, or *E. coli* O157:NM (or H-indeterminate) and the Shiga toxin(s) and/or one or more toxin genes are present, the sample will be treated as positive for *E. coli* O157:H7, and regulatory action will be taken. The cultures will also be tested by pulsed-field gel electrophoresis (PFGE) for potential epidemiological association.

5.8 Storage of Cultures

For storage requirements of the fluorescent *E. coli* O157:H7 strain (FSIS culture # EC 465-97 or the currently designated control strain), refer to Section 5.3.c. of this chapter.

Store other "working" *E. coli* stock cultures on nutrient agar slants. Transfer stocks monthly onto duplicate nutrient agar slants, incubate overnight at 35 ± 1°C, and then store them at 4-8°C. Use one of the slants as the working culture. Use the other slant for sub-culturing to reduce the opportunity for contamination. Cultures may be subcultured up to 5 times. After this period the culture must be re-confirmed biochemically or a new culture initiated.

For long term storage freeze cultures using cryo-beads i.e. Cryostor™ or lyophilize.

5.9 Selected References

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APPENDIX D
Presentation of Dr. Stanley Bailey

BIOMERIEUX

New Technology for Microbiological Testing of Foods

Stan Bailey, PhD

President, International Association for Food Protection

Director Scientific Affairs - Industry, bioMerieux

stan.bailey@na.biomerieux.com

BIOMÉRIEUX

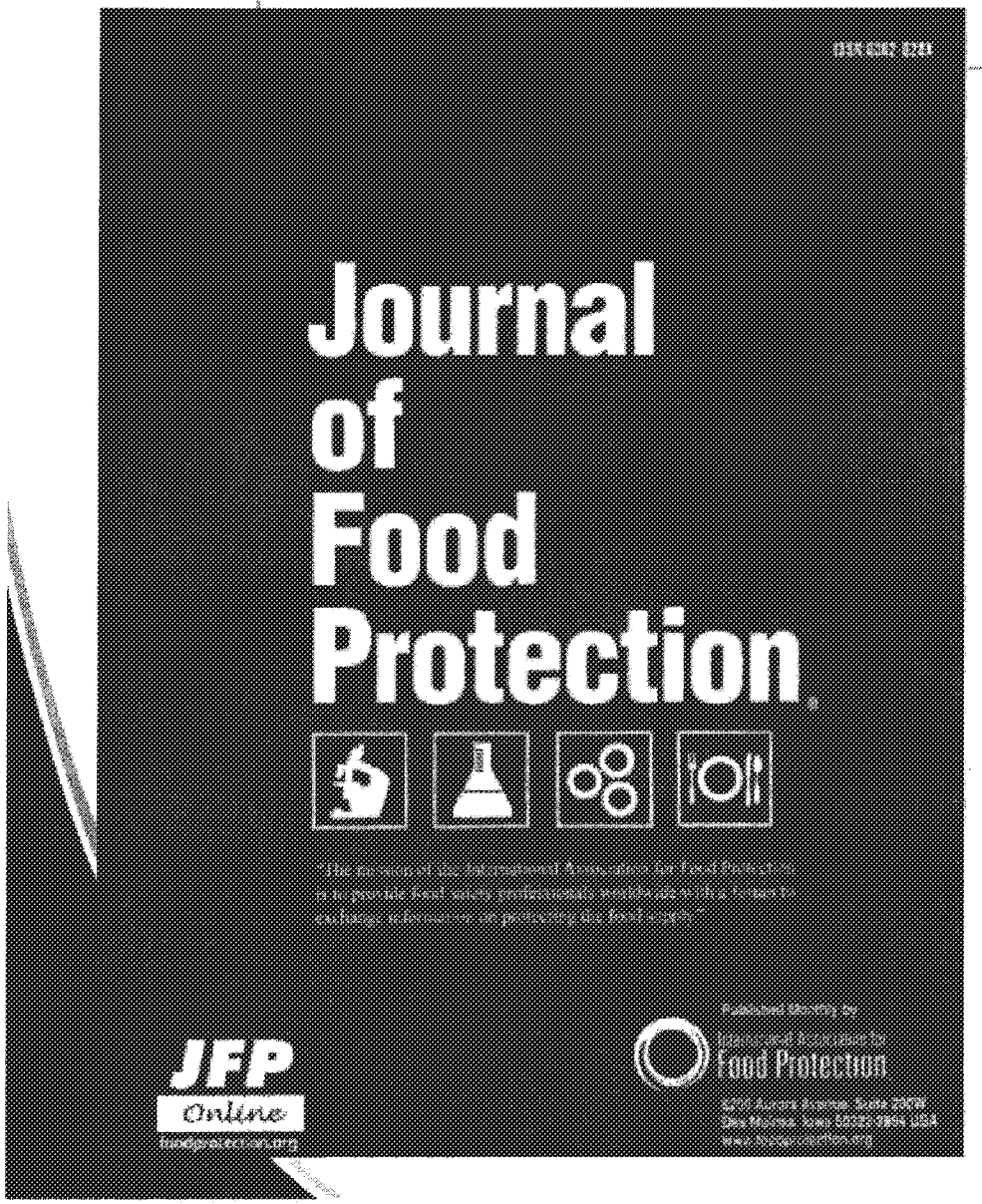
International Association for Food Protection®

Our Mission:

To provide food safety professionals worldwide with a forum to exchange information on protecting the food supply

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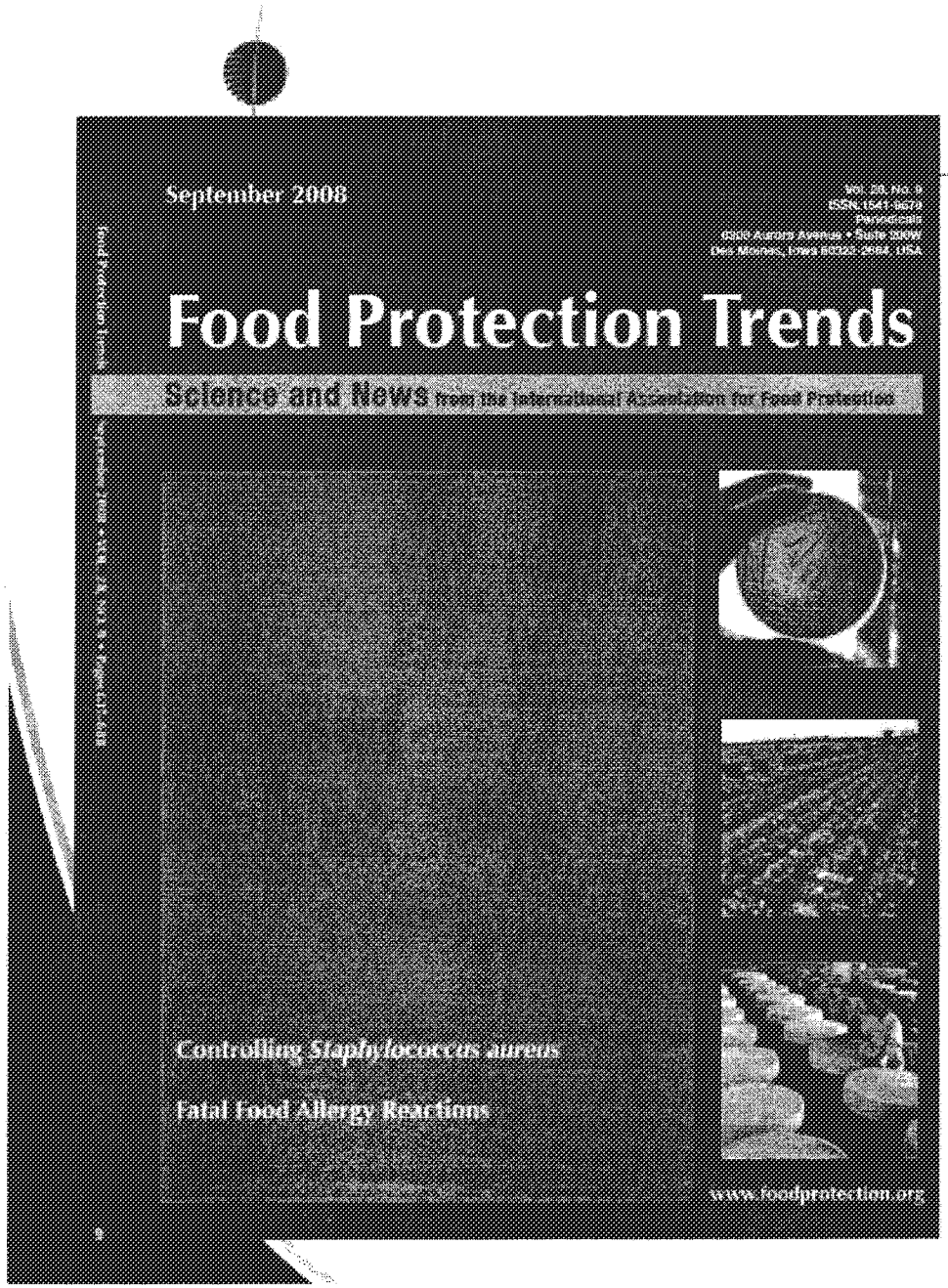
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General Membership publication for Association news, with articles on applied research and food safety applications

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IAFP Report



In this issue:

IAFP Updates

Food Safety News

Regulatory Updates

esearch & Reports

s of Interest

IAFP Report is a general Membership monthly electronic publication featuring Association updates, global news and resources, and topics of interest to food safety professionals

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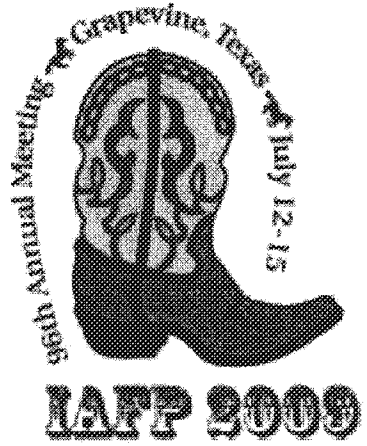
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The Leading Food Safety Conference



2009
July 12–15
Grapevine, TX

2010
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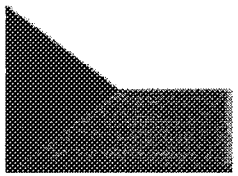
2011
July 31–August 3
Milwaukee, WI

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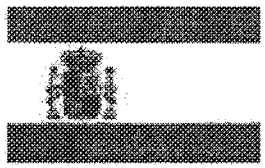
European Symposia

Berlin, Germany

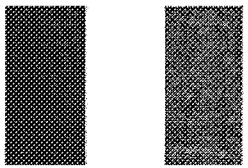
7-9 October 2009



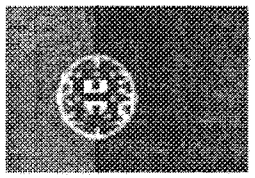
Prague
2005



Barcelona
2006



Rome
2007



Lisbon
2008

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International Symposia

Asia Pacific Symposium

Seoul, Korea

11-13 November 2009

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TEMPO, the First Automated Quality Indicator Solution and VIDAS UP – E. coli 0157:H7

June 2nd, 2009

J. Stan Bailey, PhD, Director of Scientific Affairs – bioMerieux Inc.

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BIOMÉRIEUX INDUSTRY

- **Present in over 130 countries**
  - ✓ Direct distribution through 39 subsidiairies
  - ✓ More than 100 distributors
  - ✓ 11 production sites
  - ✓ 9 R&D sites

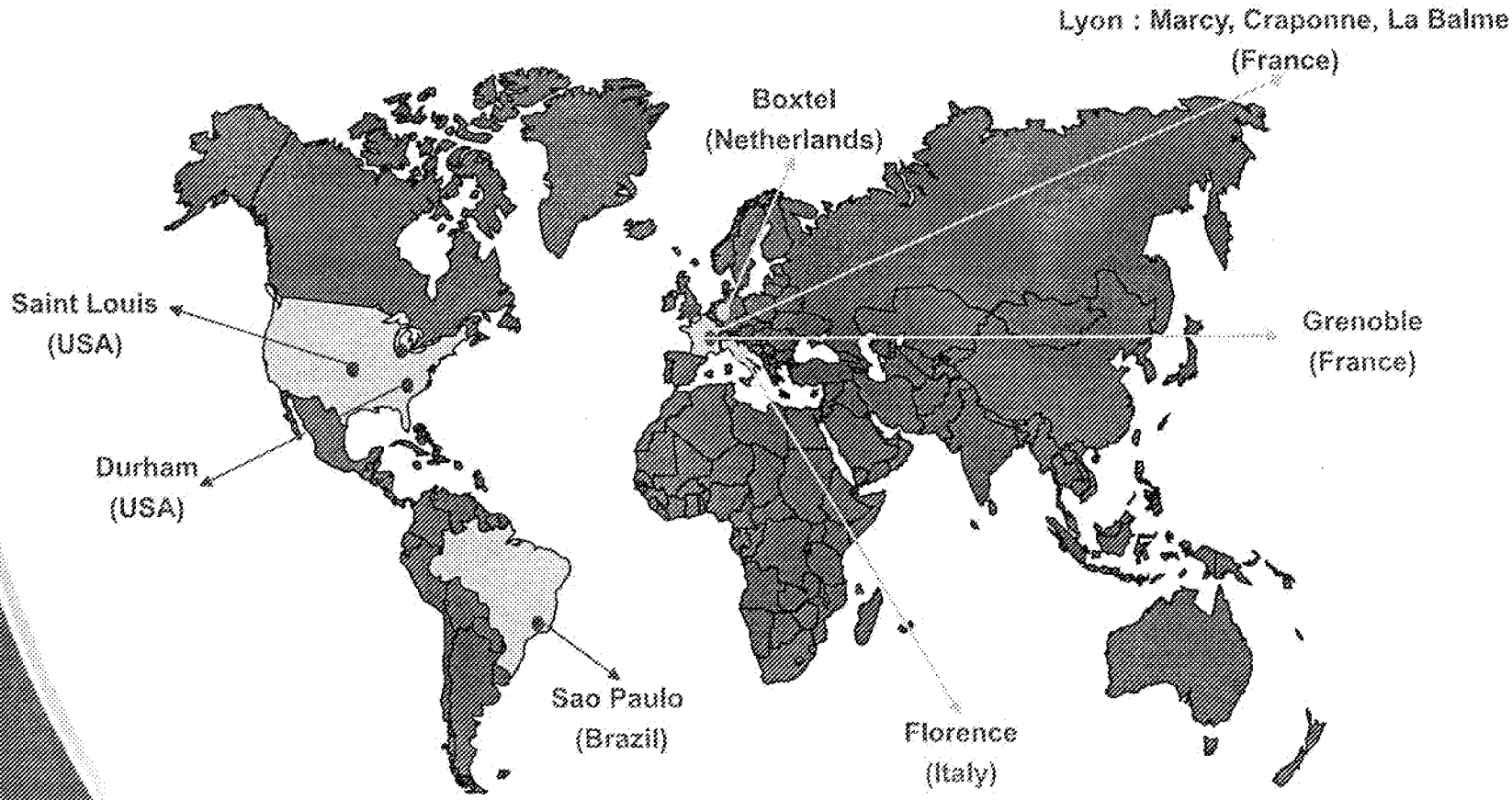
A Global Company

2008

- 1,111 M€ in sales
- 6,140 employees (+6.4% vs. 2007)
- Worldwide Installed Base: 53,000 instruments

  - Europe – 59%
  - North America – 22%
  - Asia Pacific – 12%
  - Latin America – 7%

Research & Development



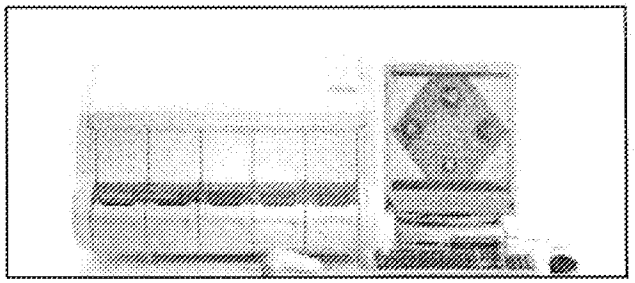
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9 R&D SITES

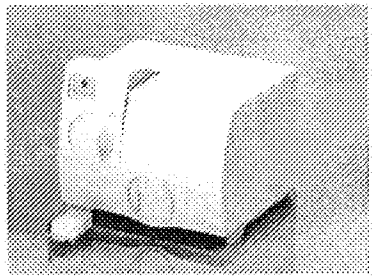
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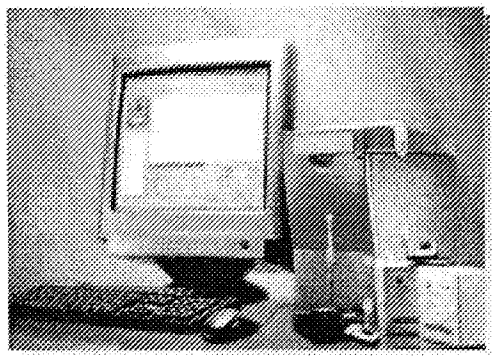
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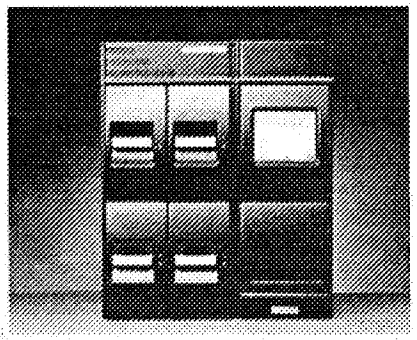
VIDAS®



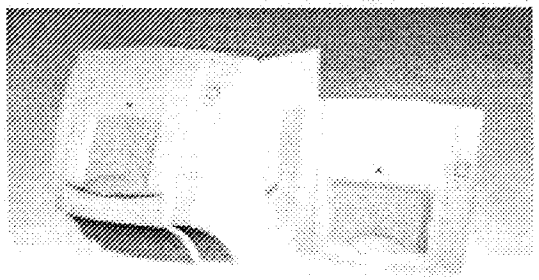
VITEK® 2 Compact



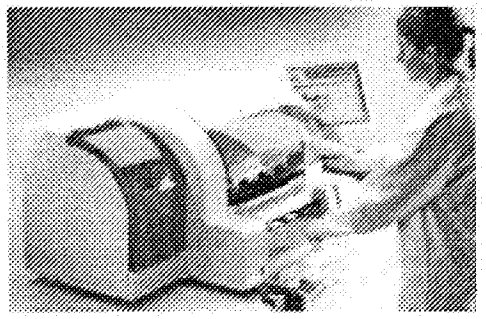
DiversiLab™ System



BacT/ALERT®



TEMPO®



easyMAG®

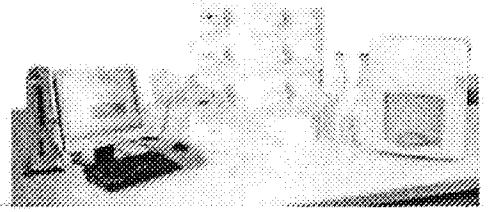
tempo

Development of TEMPO®

- 2 years of specifications
- 5 years of development
- Over 60 R&D staff involved
- Close collaboration with experts in food microbiology worldwide
  - 15 international companies and external labs
  - Involved in each phase of the development
  - Input on ergonomics
  - Involved in performance / workflow evaluations

BIOMÉRIEUX

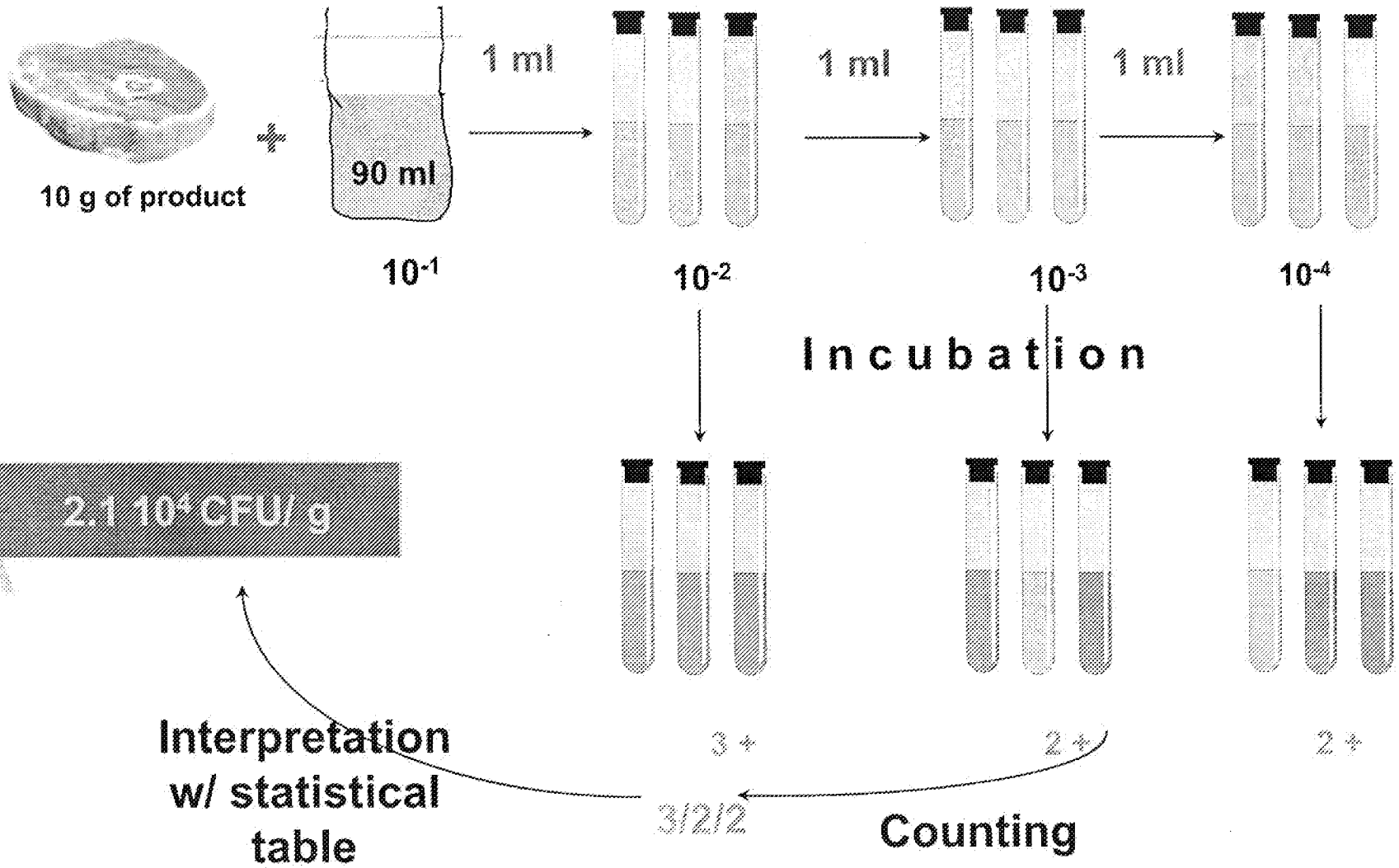
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- Concept
- Workflow
- Test Kits & Official Validations

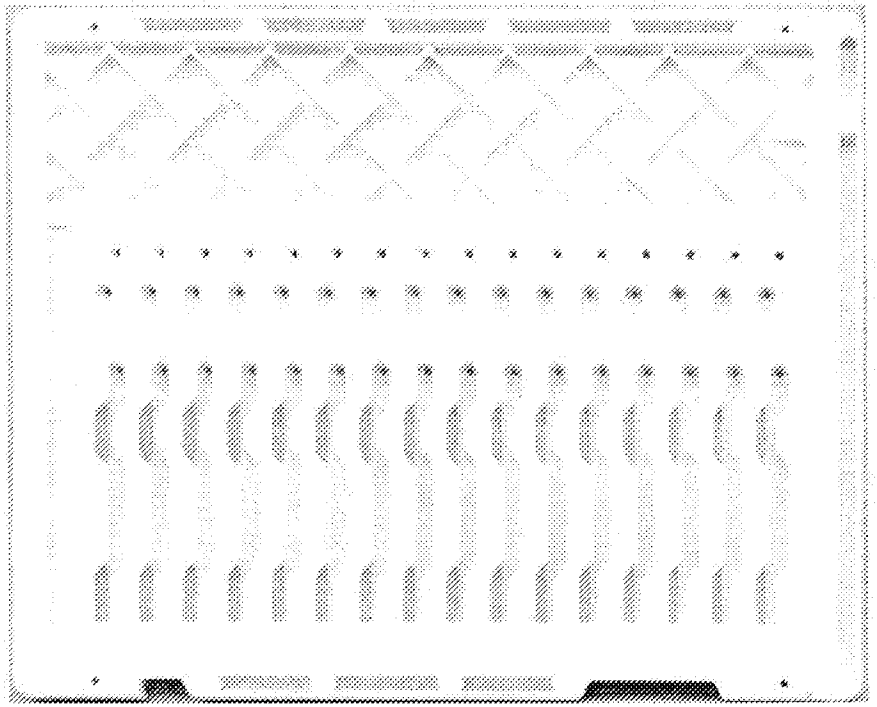
Philippe Villard, Business Development Manager

From the Traditional Approach



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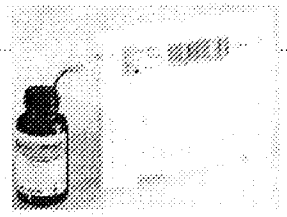
To an Innovative Solution



TEMPO Protocol



+ Diluent →



Sample + dehydrated specific culture media

↓

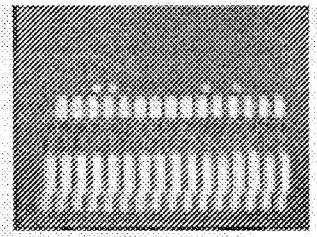
Automated Filling of the card

↓

Standard Incubation

↓

Automated Reading



Automated discrimination of positive and negative wells

Most Probable Number computing → 16 / 15 / 4

↓

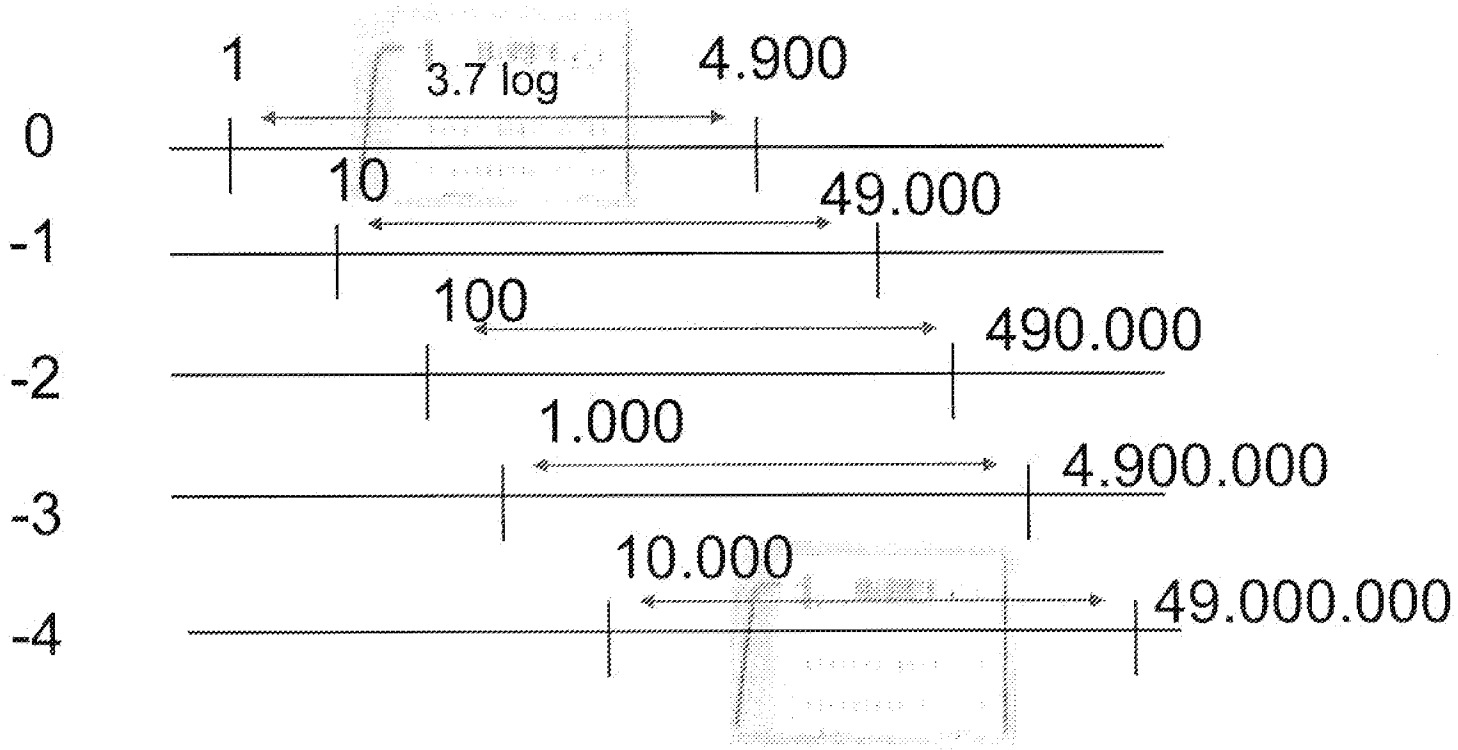
Statistic table + dilution factor

↓

Enumeration (cfu/g)

Enumeration Range

MPN 16 tubes = 3.7 log of range



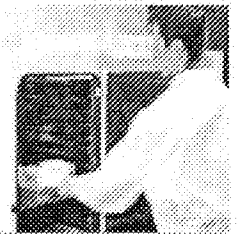
Dilutions and Enumeration ranges

Enumeration Range	TEMPO Dilution	TEMPO Vial		
		Dilution	Volume	Sterile water
1 → $4{,}9.10^{3}$ cfu/g	1 / 4	Undiluted	1 mL	3 mL
10 → $4{,}9.10^{4}$ cfu/g	1 / 40	10^{-1}	1 mL	3 mL
10^{2} → $4{,}9.10^{5}$ cfu/g	1 / 400	10^{-1}	0.1 mL	3.9 mL
10^{3} → $4{,}9.10^{6}$ cfu/g	1 / 4 000	10^{-2}	0.1 mL	3.9 mL
10^{4} → $4{,}9.10^{7}$ cfu/g	1 / 40 000	10^{-3}	0.1 mL	3.9 mL

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Incubation @ 35°C



Total Viable Count	40-48h
Enterobacteriaceae	22-27h
Coliform Count	22-27h
E.coli	22-27h
Lactic Acid Bacteria	40-48h (aerobic conditions)
Coag+ S.aureus	24-27h
Yeasts & Molds	72-76h (25 °C)

Today...

Tomorrow with TEMPO®



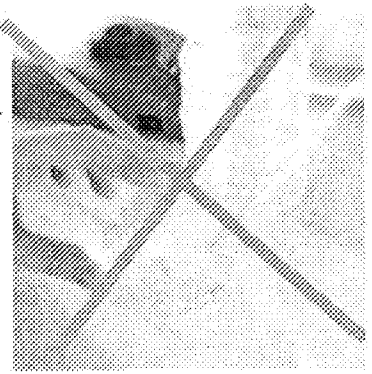
1- Media preparation



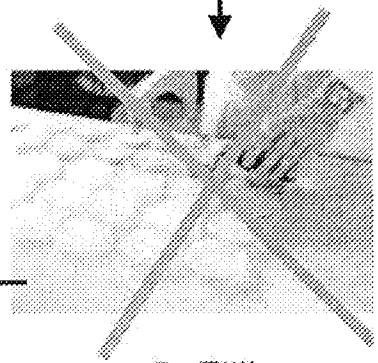
2- Sample preparation



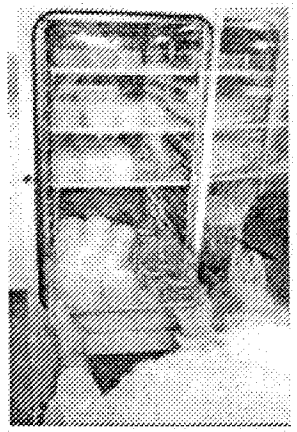
3- Dilution



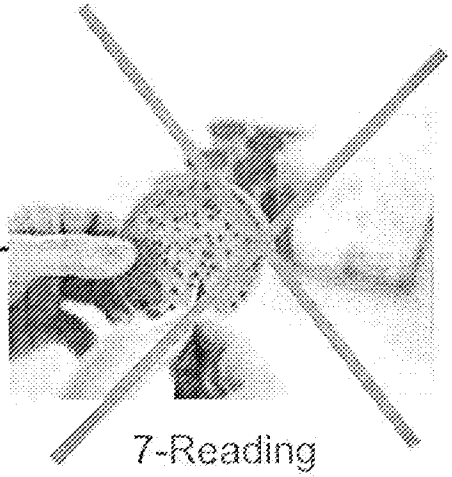
4-Inoculation



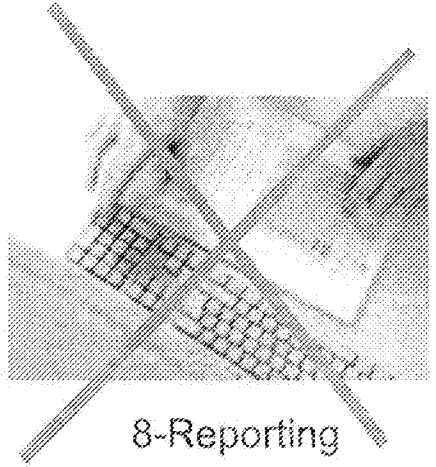
5- Filling



6-Incubation



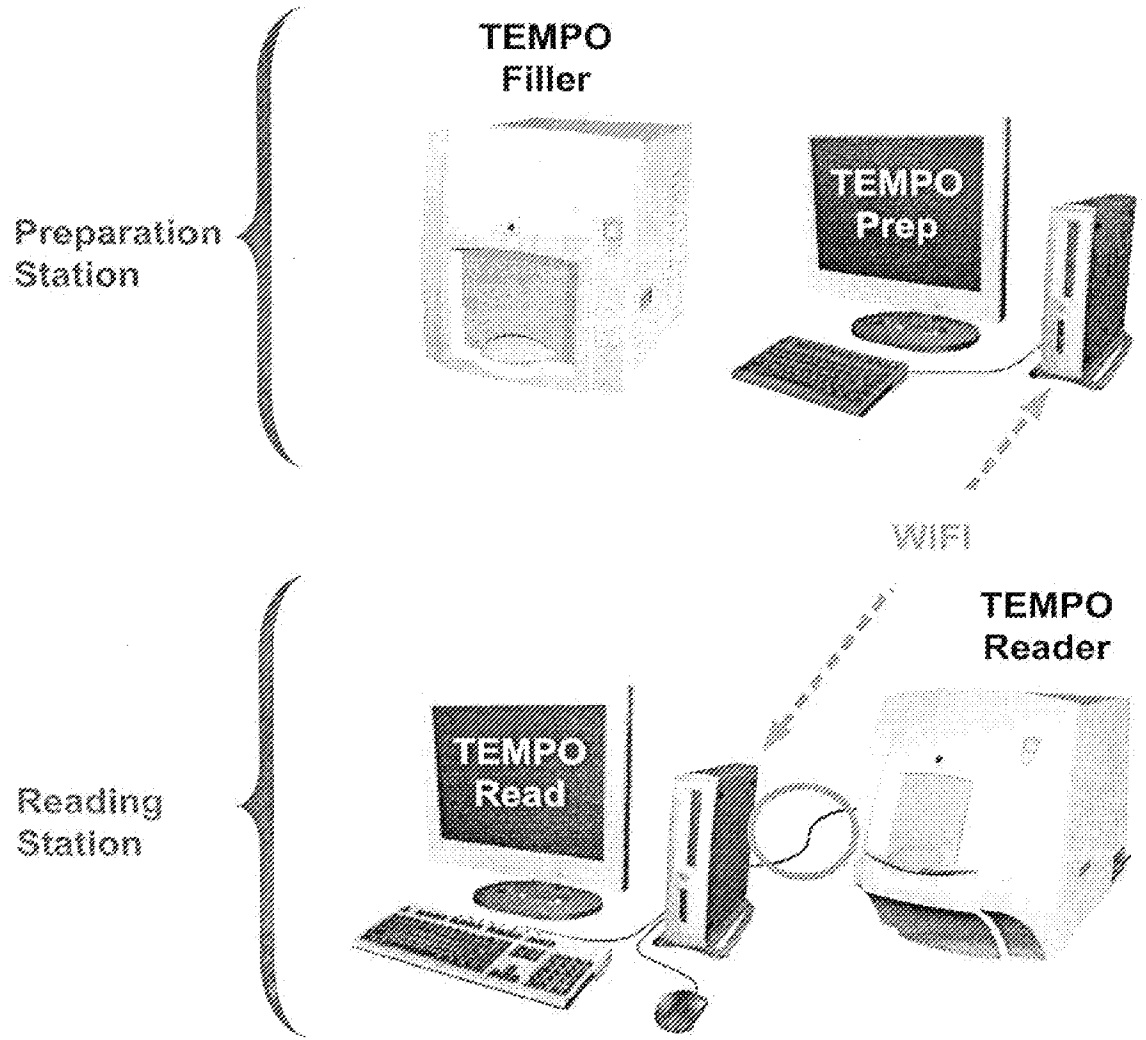
7-Reading



8-Reporting

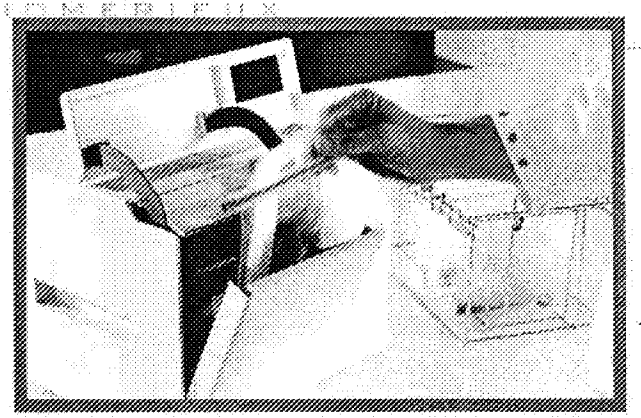
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TEMPO System

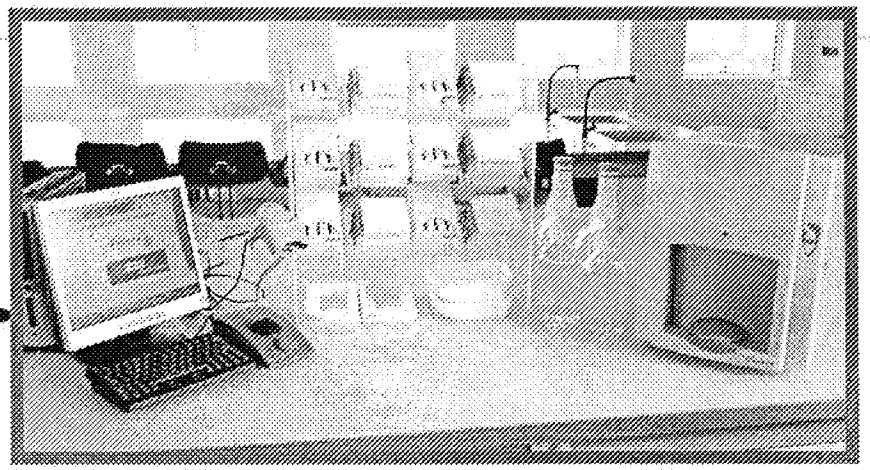


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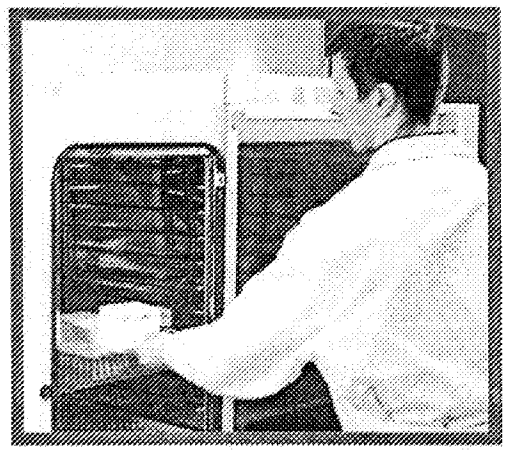
TEMPO Workflow



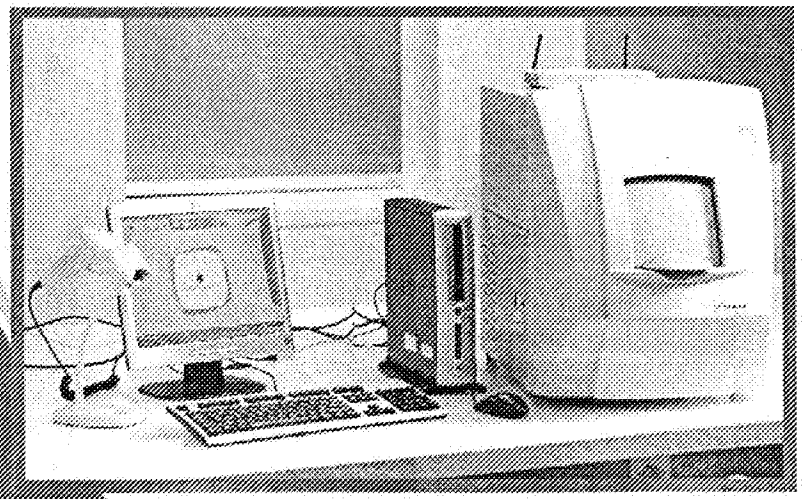
Sample preparation



Inoculation



Incubation

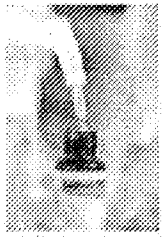


Reading & interpretation

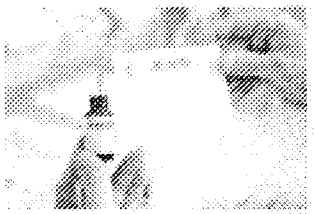
National Beef Ex. 1007
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TEMPO® Test Preparation

- Rehydrate Media Vial



- Pour the sample
- Vortex



6 Tests / 3min



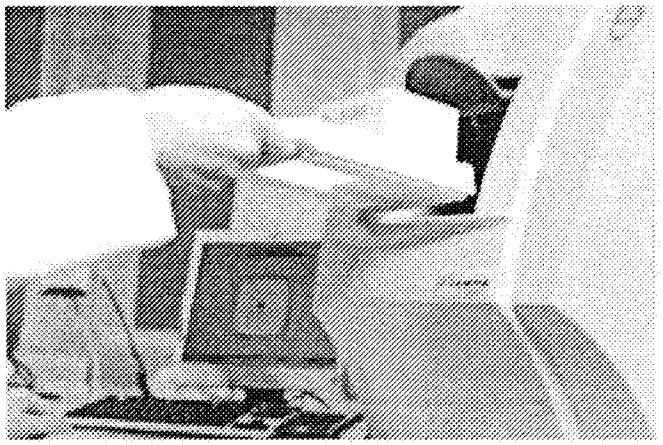
- Automatic Filling → • Incubate

National Beef Ex. 1007
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TEMPO® Test Reading

- Remove the Tests from the Incubator

20 Tests / < 3 min

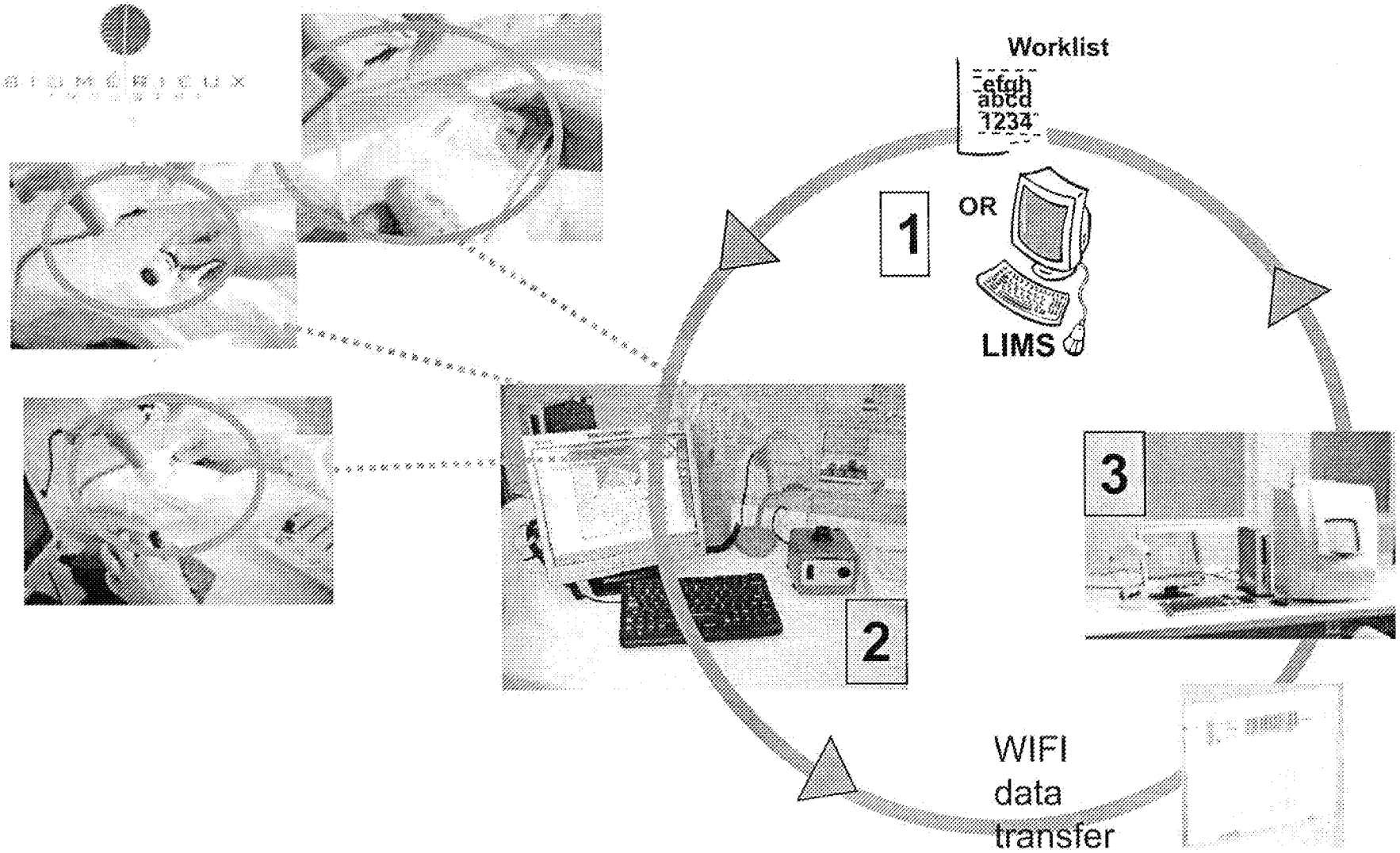


- Insert Tray in the Reader for Reading and Recording

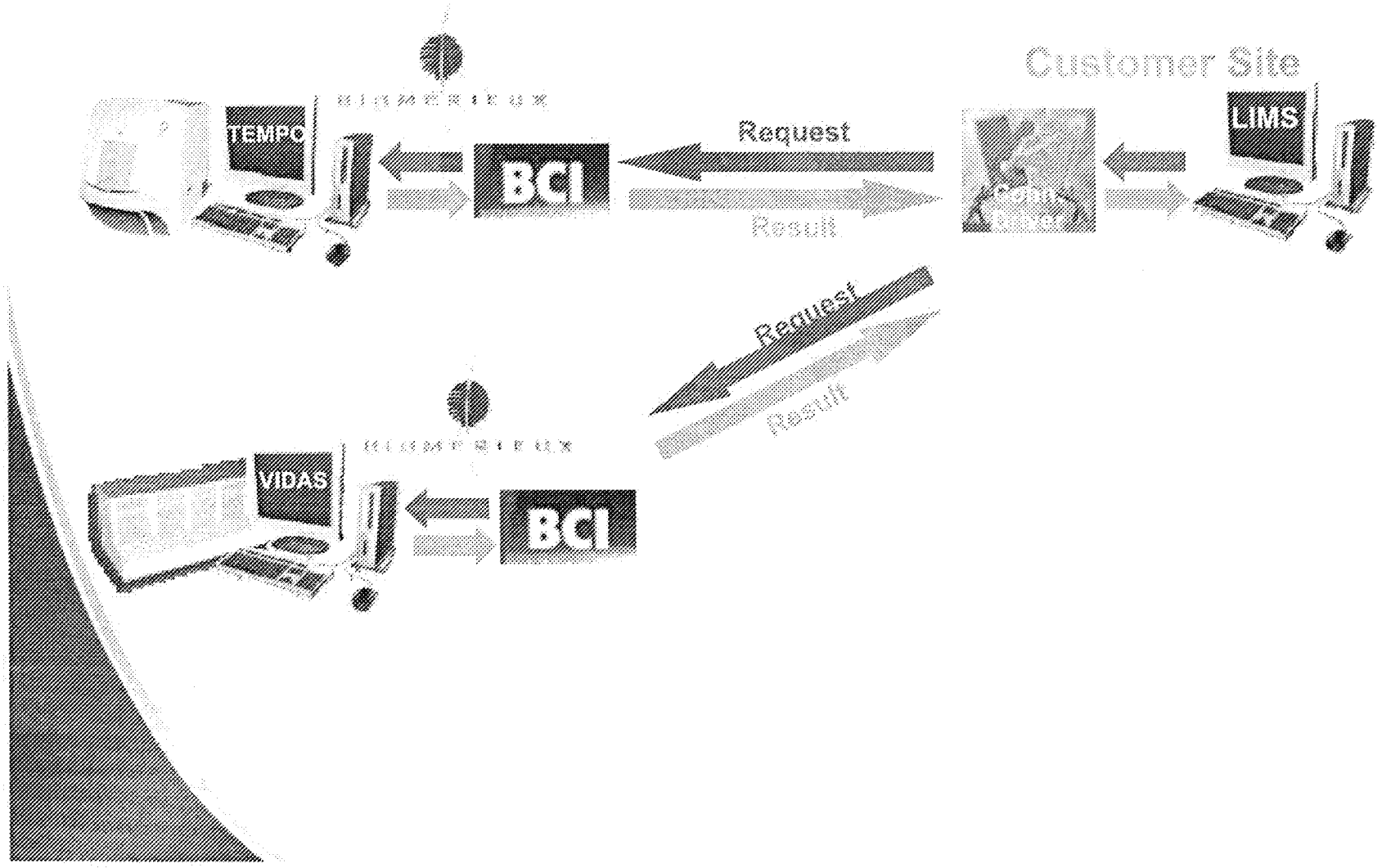
TEMPO® Test Results

Sample ID	Test	Dilution (1/)	Preparation Date	Status	CFU/G	Annotations	Comments
05\3382	TVC	40000	lun. 21/03/2005 15:23	OK	= 400000		
05\3382	CC	40	lun. 21/03/2005 15:23	OK	= 4800		
05\3382	EC	40	lun. 21/03/2005 15:24	OK	< 10		
05\3398	TVC	4000	lun. 21/03/2005 15:27	OK	= 1700000		
05\3398	CC	40	lun. 21/03/2005 15:28	OK	= 930		
05\3398	EC	40	lun. 21/03/2005 15:28	OK	< 10		
05\3617	TVC	4000	lun. 21/03/2005 15:32	OK	> 4900000		
05\3617	CC	40	lun. 21/03/2005 15:32	OK	= 1800		
05\3617	EC	40	lun. 21/03/2005 15:33	OK	= 10		
05\3618	TVC	4000	lun. 21/03/2005 15:38	OK	= 25000		
05\3618	CC	40	lun. 21/03/2005 15:38	OK	= 10		
05\3618	EC	40	lun. 21/03/2005 15:45	OK	< 10		
05\3621	TVC	4000	lun. 21/03/2005 15:47	OK	= 910000		
05\3621	CC	40	lun. 21/03/2005 15:48	OK	= 240		

Complete Traceability



TEMPO® Connectivity



TEMPO test kits

- ✓ Total Viable Count
- ✓ *Enterobacteriaceae*
- ✓ Coliform Count
- ✓ *Generic E.coli*
- ✓ Lactic Acid Bacteria
- ✓ *Staphylococcus aureus*
- ✓ *Yeasts & Molds*

Continuous Official & International Approvals

AOAC Research Institute

- Total Viable Count
- Enterobacteriaceae
- Coliforms
- *E.coli*

AOAC OMA

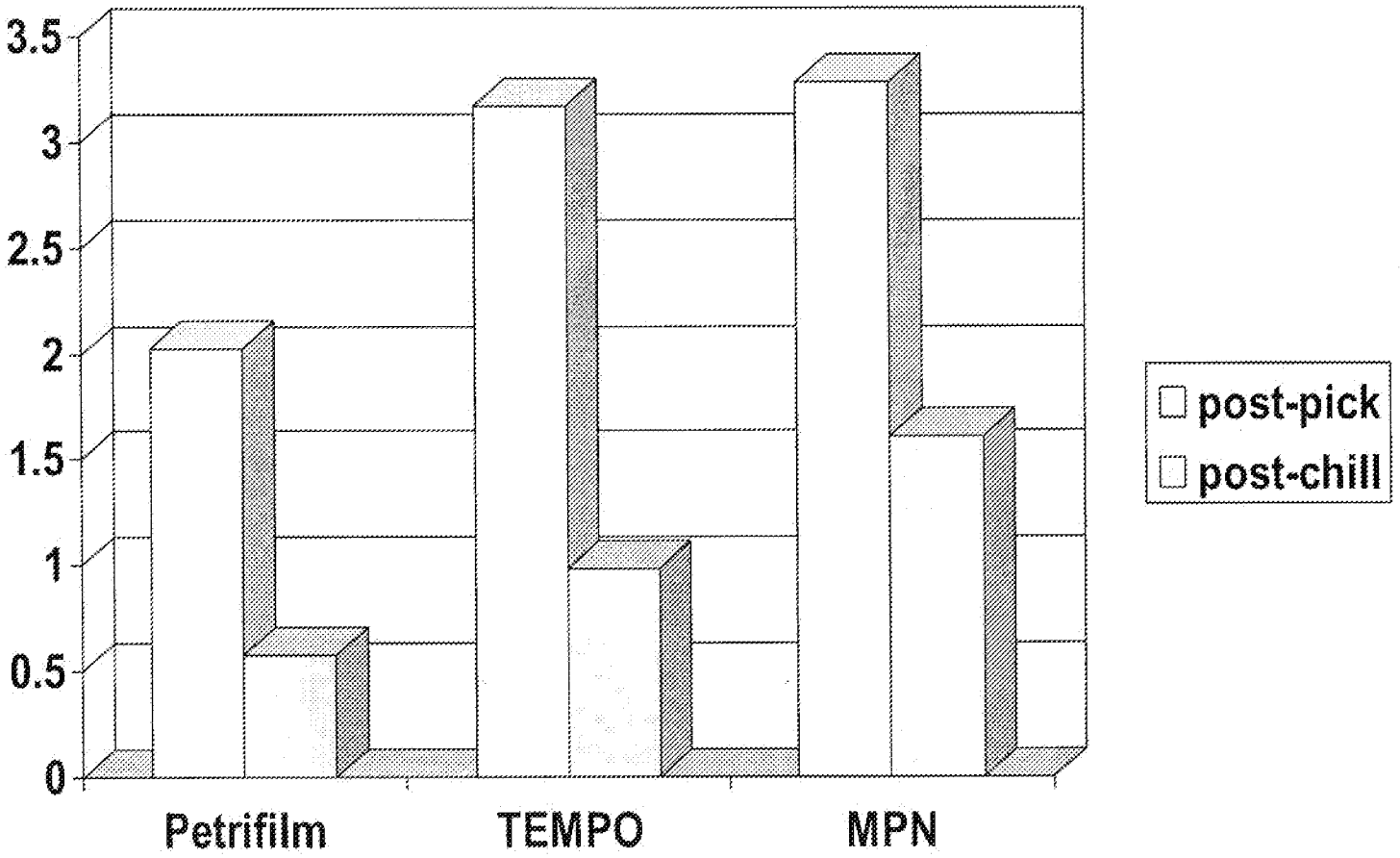
- Total Viable Count
- *E.coli* & Coliforms: In progress

ISO 16140

- Total Viable Count
- Enterobacteriaceae
- Coliforms
- *E.coli*

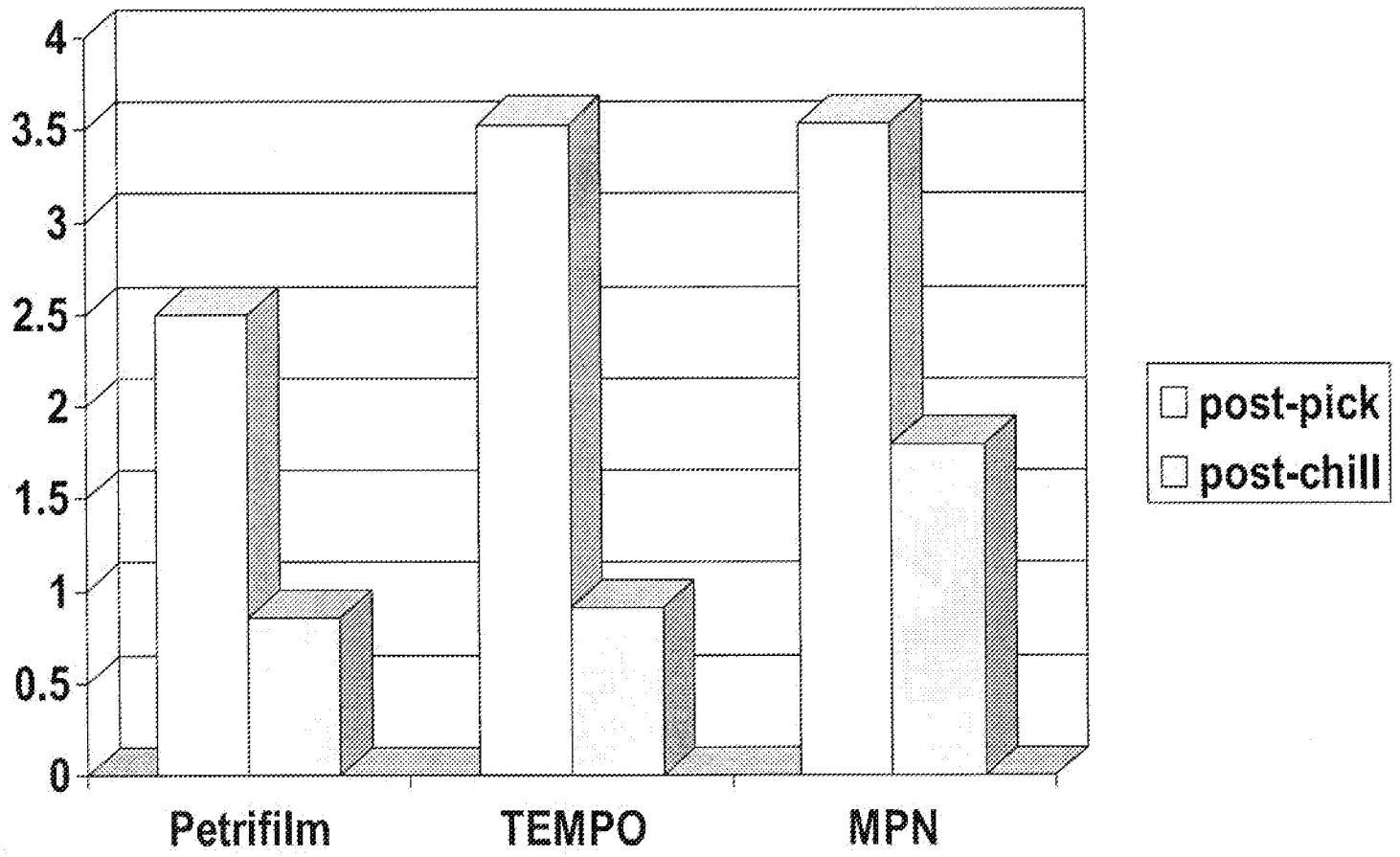
E. coli from chicken

Log_{10} cfu / mL rinse



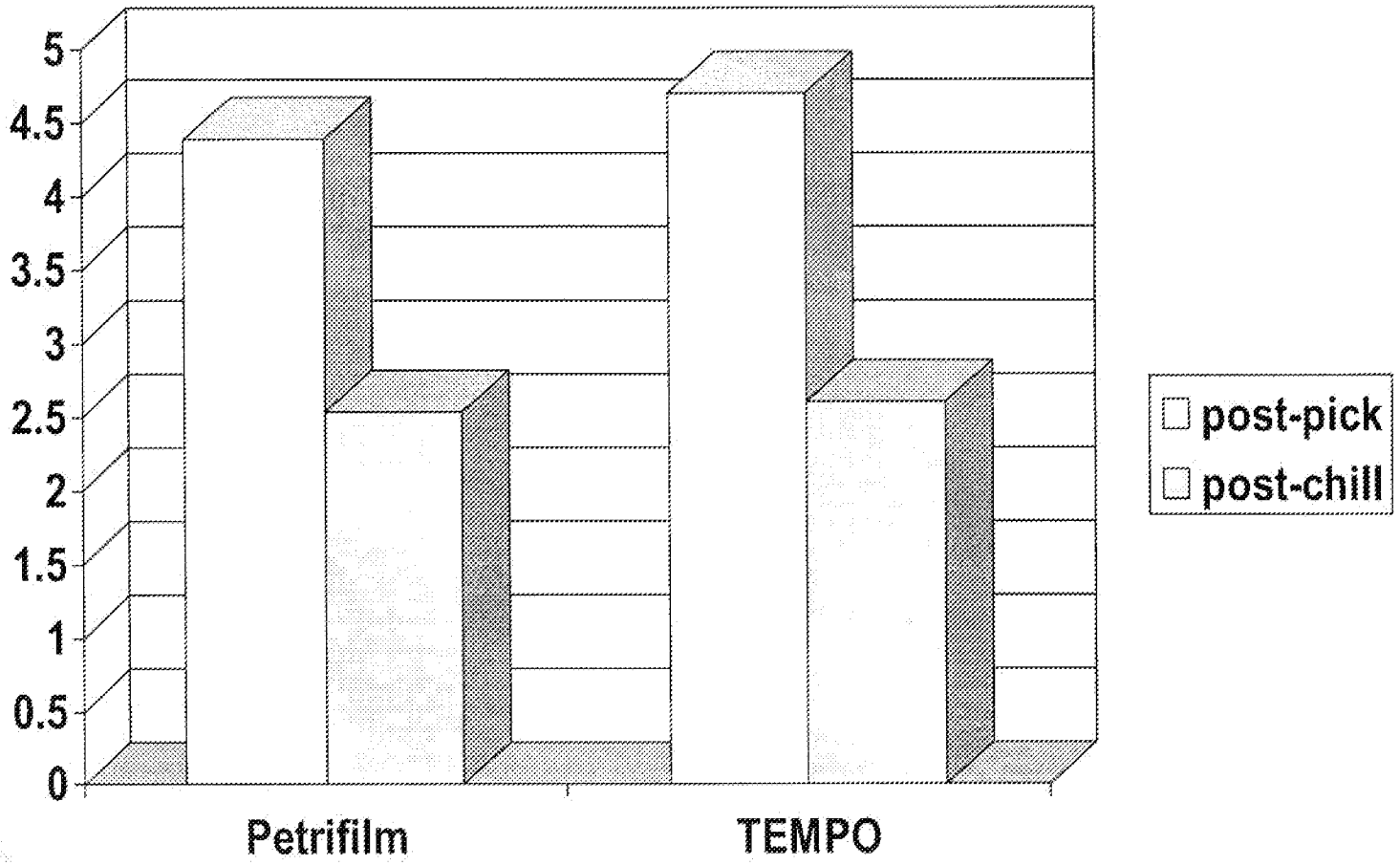
Coliforms from chicken

Log_{10} cfu / mL rinse



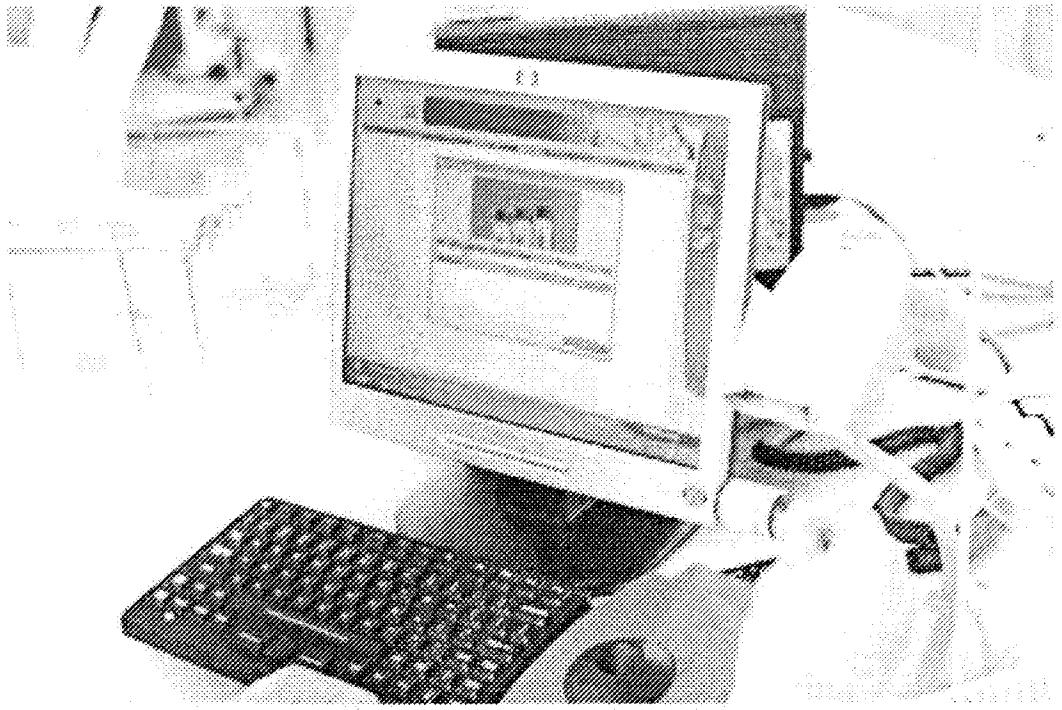
Total aerobic count from chicken

Log_{10} cfu / mL rinse



BIOMERIEUX

tempo



Thank you

Stan Bailey, Director Scientific Affairs – bioMerieux Industry

VIDAS Ultimate Performance (Phage) E. coli 0157:H7

Stan Bailey

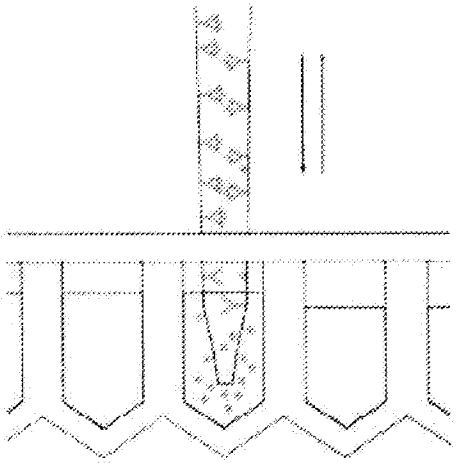
Director Scientific Affairs, bioMerieux Industry

President, IAFP

stan.bailey@na.biomerieux.com

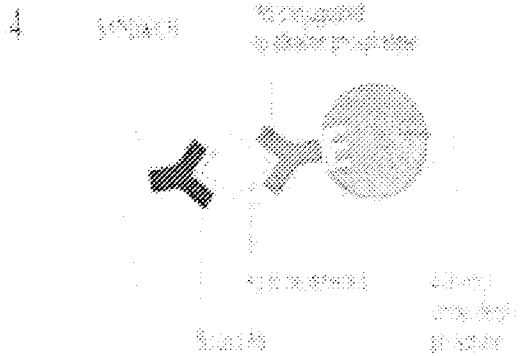
VIDAS® IMMUNO ASSAY

CAPTURE OF ANTIGENS



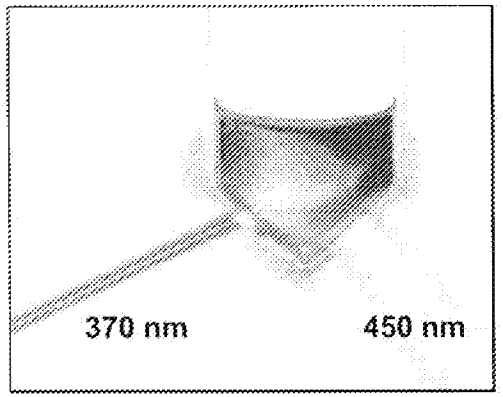
The antibody captures the target pathogens

SANDWICH TEST



A second antibody conjugated with an enzyme binds to specific antigens

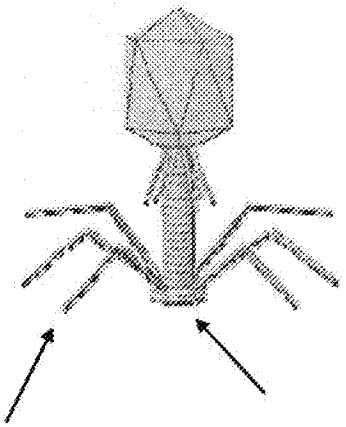
DETECTION



The intensity of the reaction is interpreted by the system

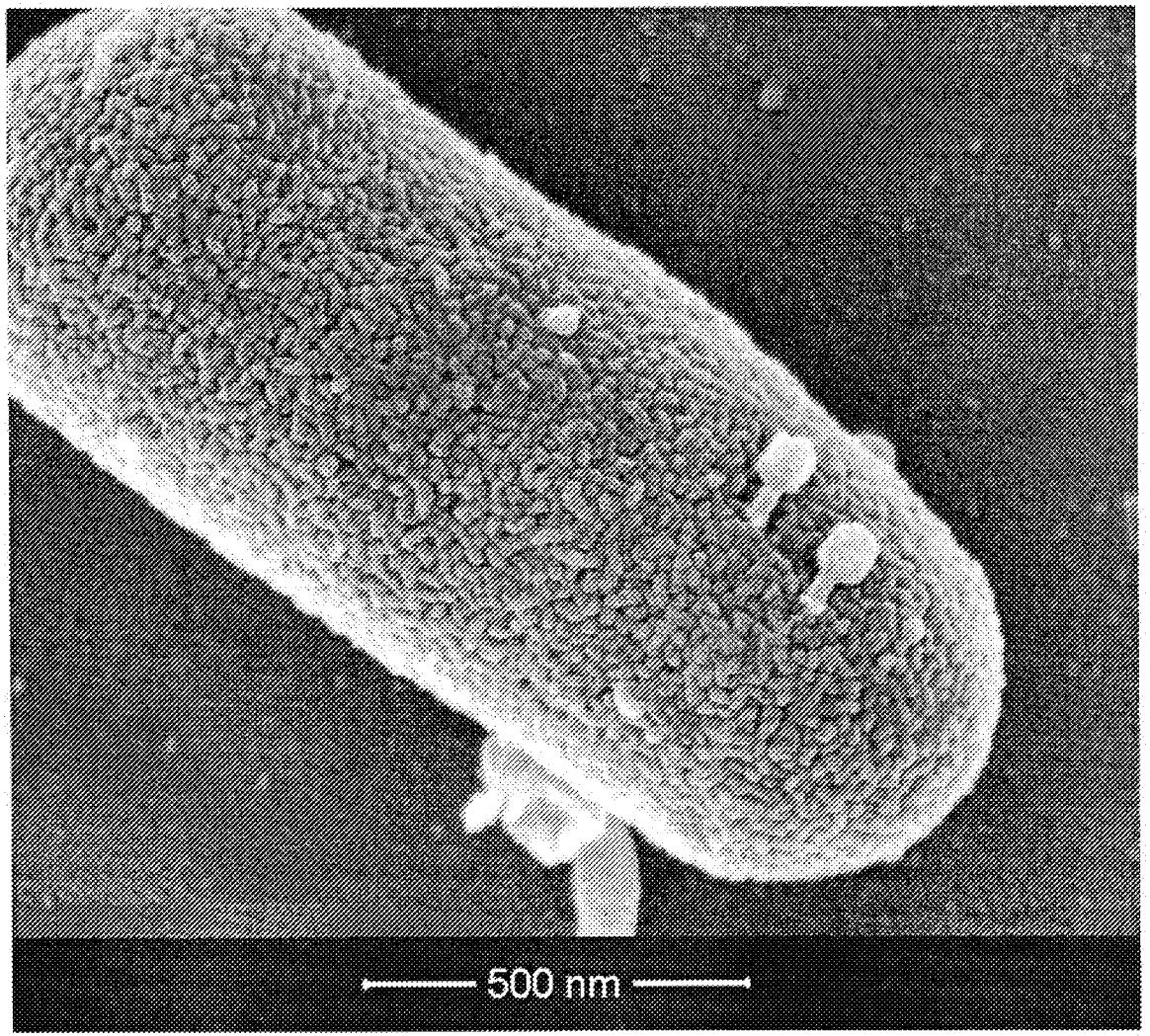
National Beef Ex. 1007
Page 1142

What is a Bacteriophage ?



"Capture" Molecules and "Lytic" Enzymes

Building blocks of the Profos technology



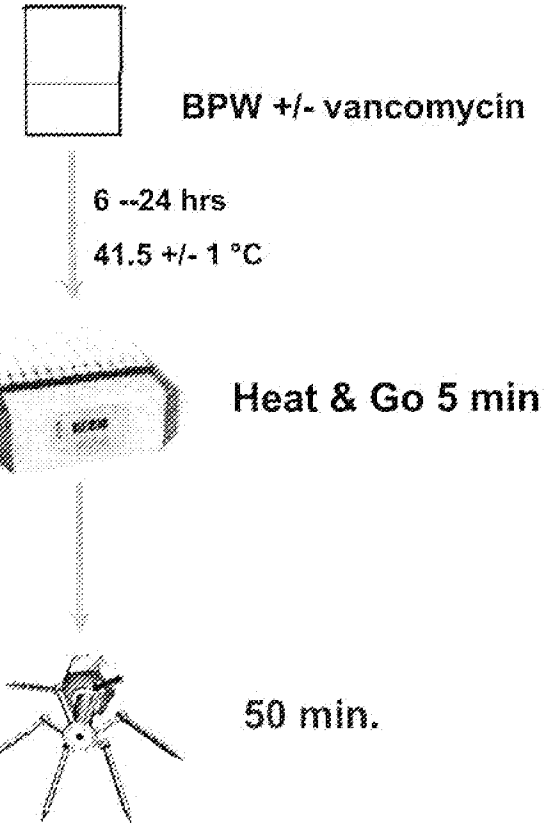
National Beef Ex. 1007
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What is a Bacteriophage ?

- **Viruses:** Exclusively infect bacteria
- **Very common:** The most abundant "life" form on earth
- **Optimized by nature:** Very specific host requirement for reproduction. Extremely stable, evolved to withstand harsh conditions (pH, temp). Phage recognition elements can be considered as a tool with unrivaled properties for bacterial detection assays with respect to specificity and sensitivity
- **Various applications:** Anti-"bacterials" (phage therapy & sanitization)
  Capture of bacteria and bacterial components

Show VIDEO

VIDAS UP E. coli O157:H7



Ecole Nationale
Vétérinaire de Lyon
Professeur Christine Vernozy-Rozand
Delphine Thevenot, Marion Bouvier

BIOMÉRIEUX

EVALUATION VIDAS UP E. coli O157:H7

FOR DETECTION OF *E. coli* O157:H7

IN FOODS & ENVIRONMENTAL SAMPLES

Inclusivity and Exclusivity

- Sensitivity : 133 *E. coli* O157:H7 strains tested
  - **VIDAS : 100 %**

- Specificity : 102 non *E. coli* O157 strains tested
  - **VIDAS : 100 %**

Results on ground beef

Sample size : 25g	Enrichment in BPW	
Detection	6h	24h
VIDAS Positive	15/15	15/15
PCR Positive	10/13	13/13

Sample size : 75g	Enrichment in BPW + Vancomycin	
Detection	8h	24h
VIDAS Positive	14/14	14/14
PCR Positive	1/15	2/15

Sample size : 375g	Enrichment in BPW + Vancomycin	
Detection	8h	24h
VIDAS Positive	9/9	9/9
PCR Positive	0/9	4/9

VIDAS Phage performance for trimmings at 75 g & 375 g

Enrichment in BPW + Vancomycin

Sample Size	75 g		375 g	
Contamination	Trimmings 3-6 CFU		Trimmings 3-6 CFU	
Incubation time	8 h	24 h	8 h	24 h
VIDAS % Positive	100	100	100	100
PCR % Positive	100	66.6	0	33.3

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FSNS®
Food Safety Net Services

EVALUATION VIDAS UP E. coli O157:H7

FOR DETECTION OF *E. coli* O157:H7

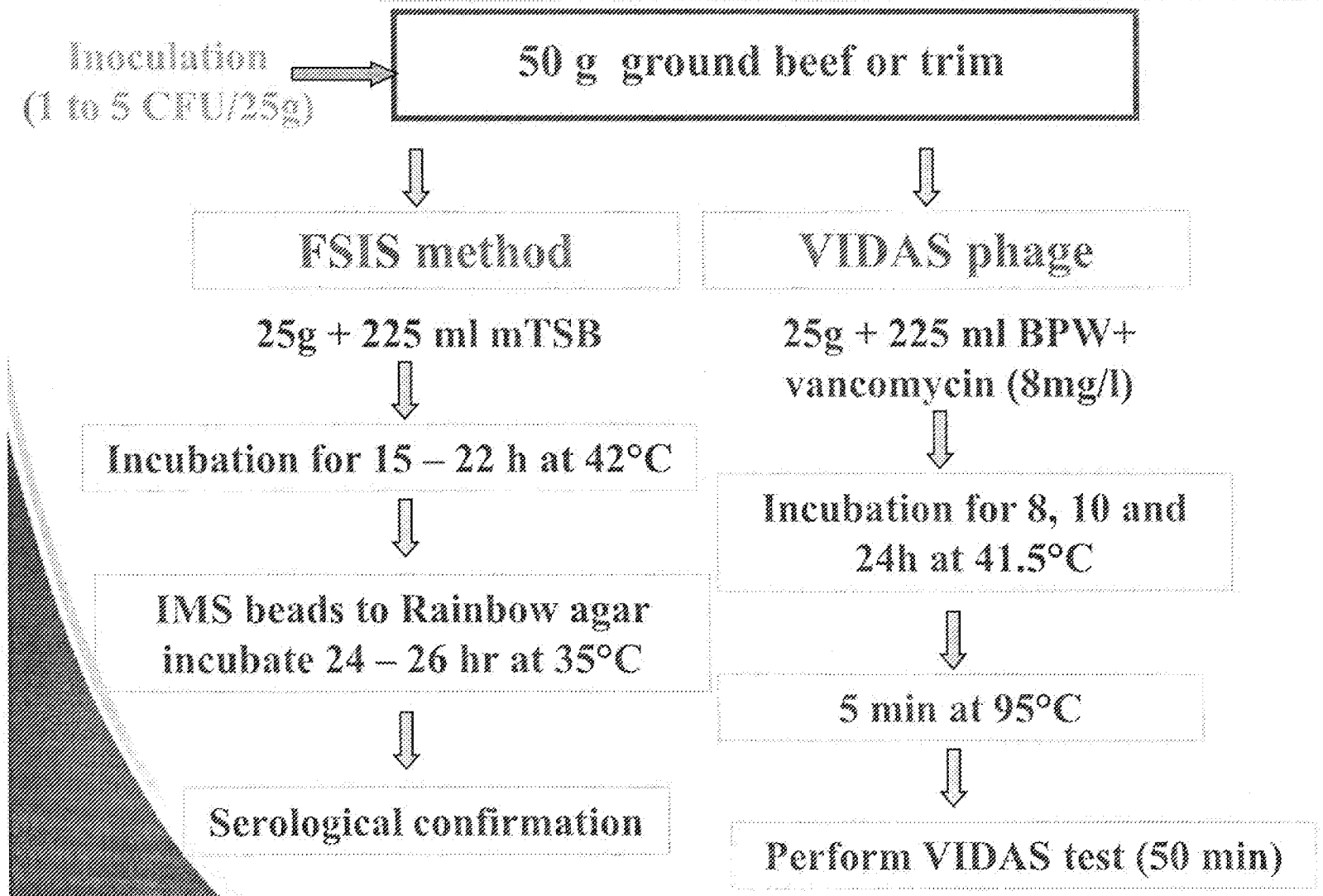
IN FOODS & ENVIRONMENTAL SAMPLES

National Beef Ex. 1007
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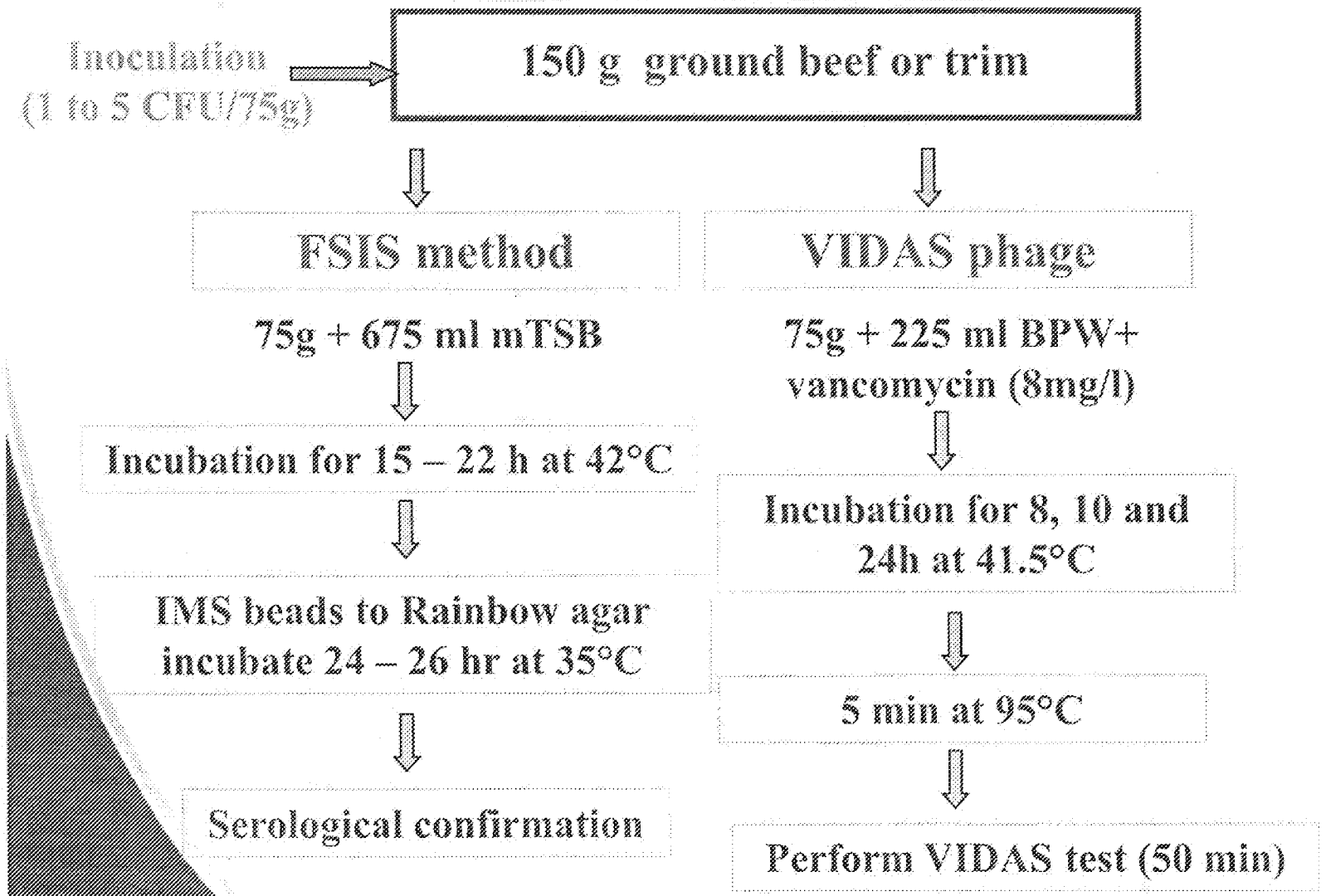
Performance Criteria for Lab Methods: **Limits of detection**

- MLG 5.04 : 0.23 cfu/g (1 cfu/4g)
- AOAC : 0.04 cfu/g (1 cfu/25g)
- Industry I : 0.015 cfu/g (1 cfu/65g)
- Industry II : 0.003 – 0.013 cfu/g (1-5/375g)
- VIDAS UP validated to Industry II
- [if inoculum more than 5 cells/375 gm then we cannot get fractional positives required by AOAC]

25 gm Validation

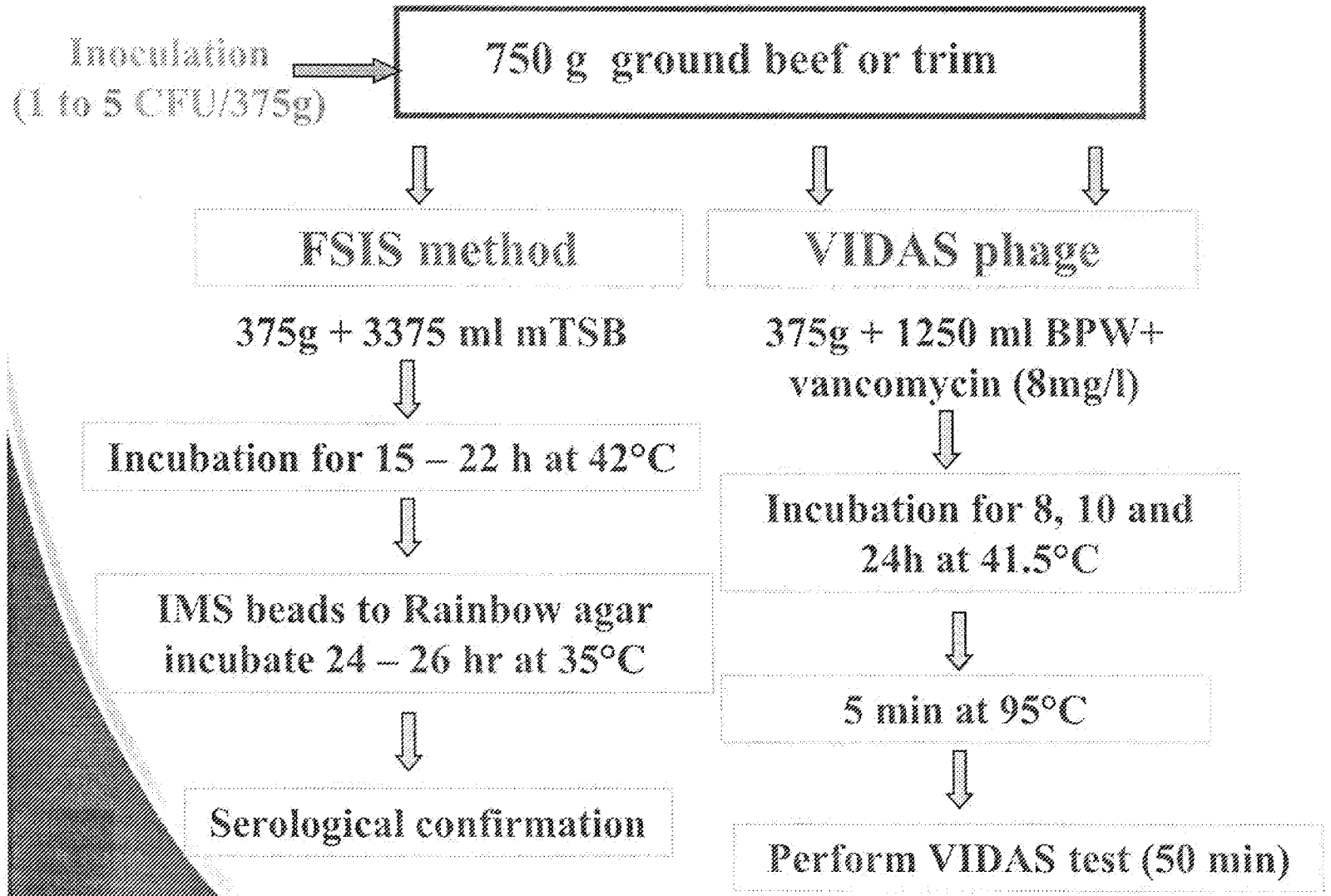


75 gm Validation



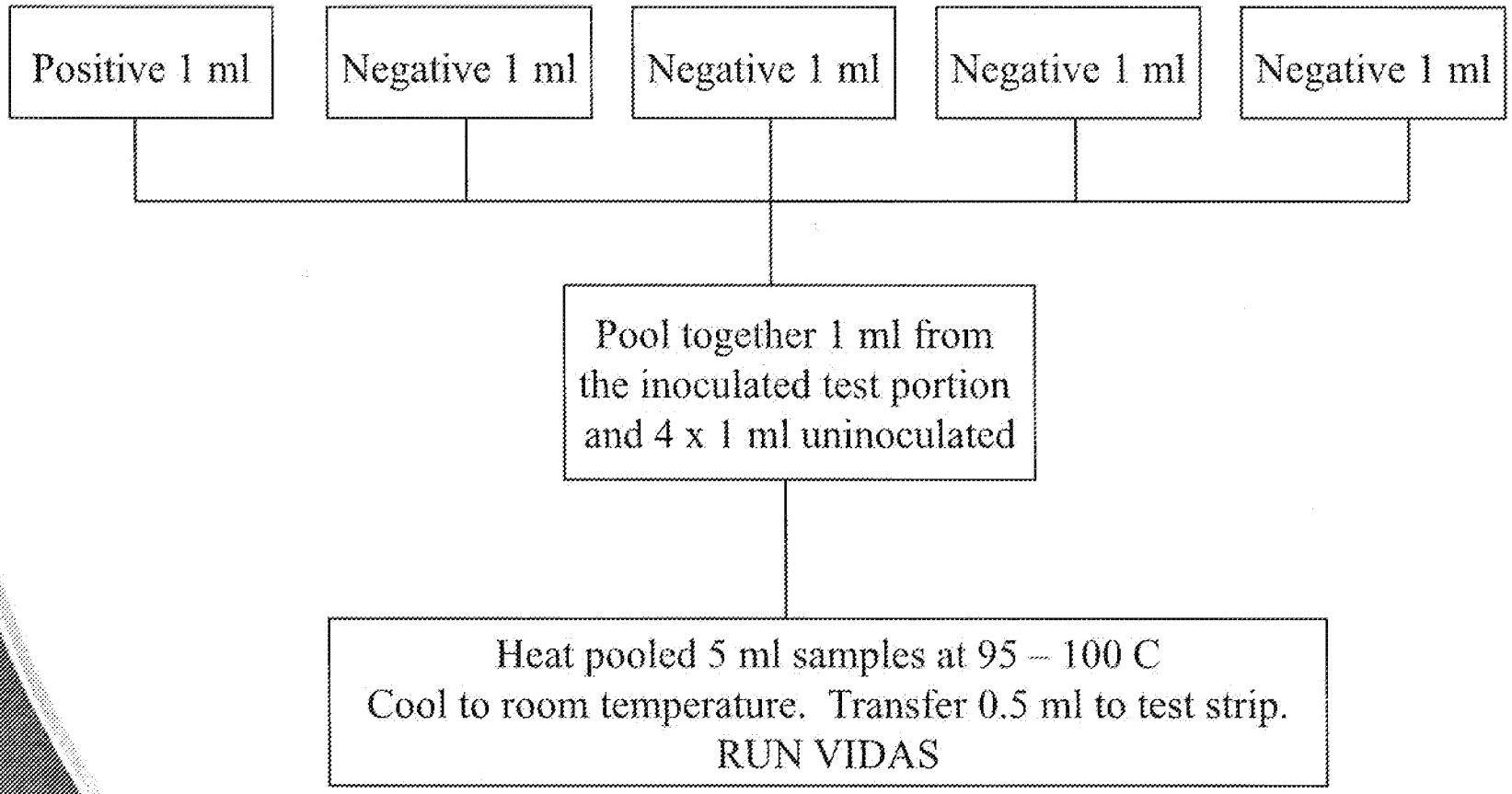
National Beef Ex. 1007
Page 1153

375 gm Validation



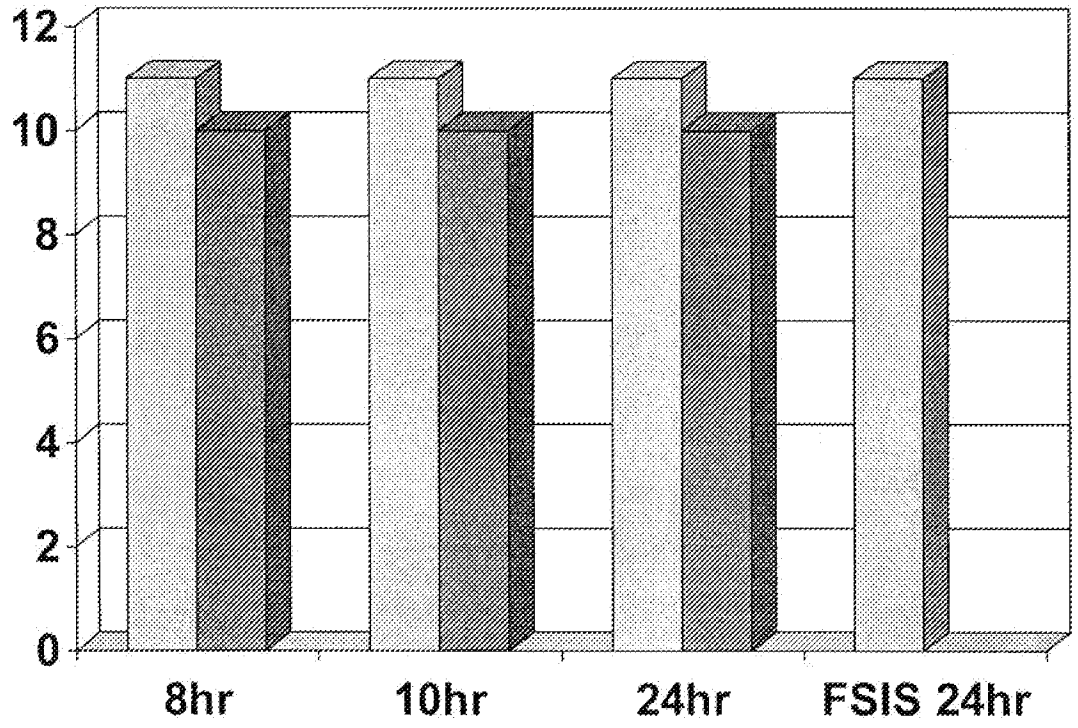
National Beef Ex. 1007
Page 1154

Pooled sample protocol



Recovery of E. coli O157:H7 from ground beef

AOAC fractional positives: inoc. = 2-4 cells/375gm

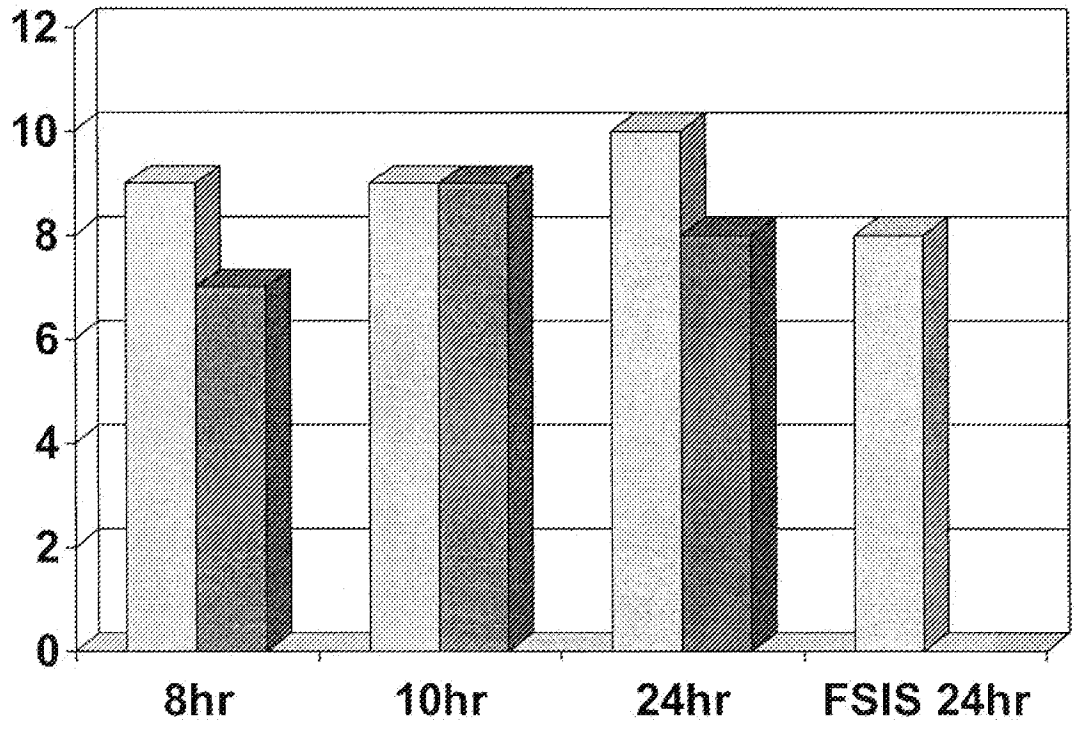


Chi Square = 0.08

n = 20 inoculated to get between 5 and 15 fractional (+)

Recovery of E. coli O157:H7 from beef trim

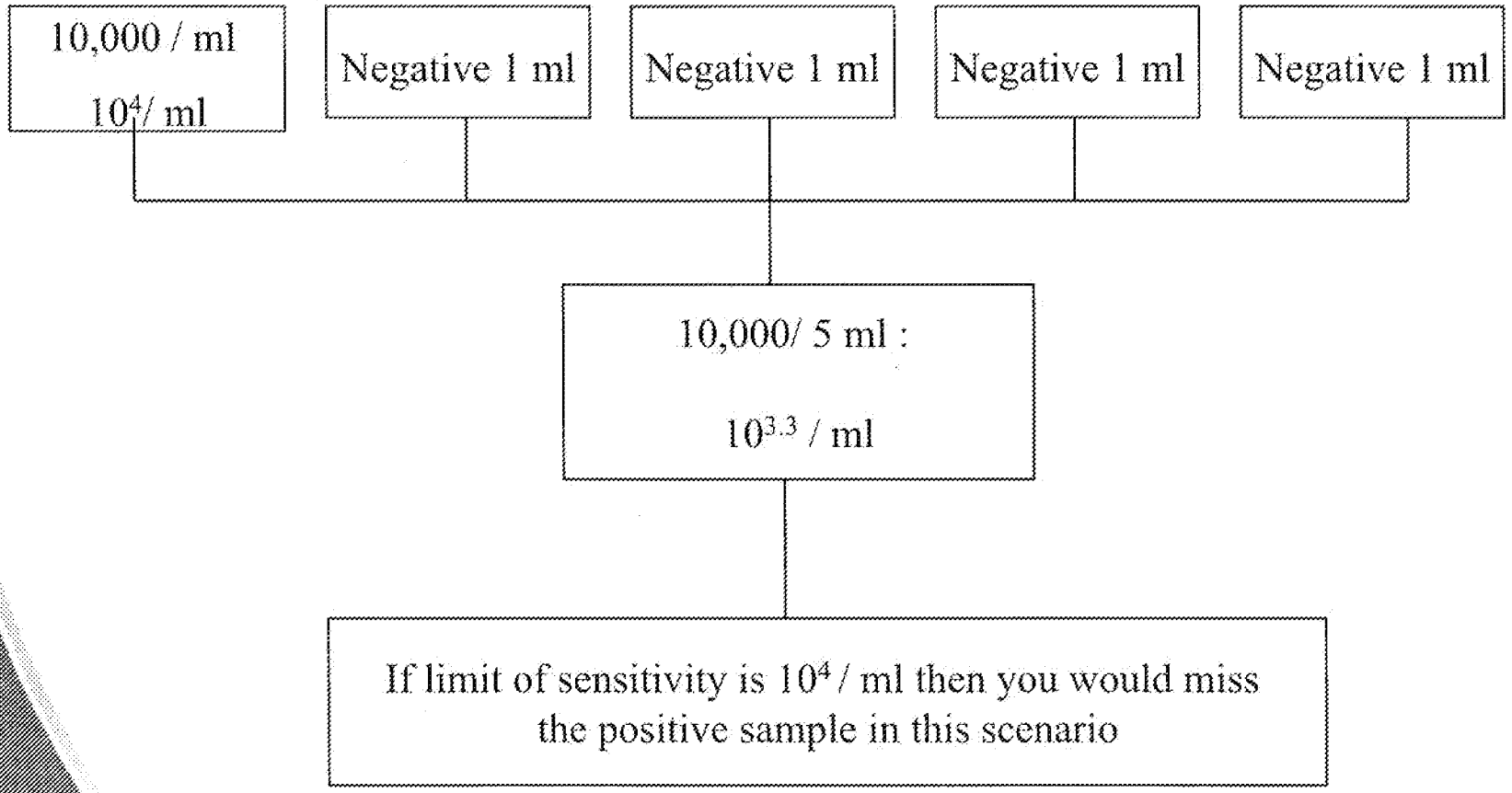
AOAC fractional positives: inoc. = 2-4 cells/375gm



Chi Square = 0.87

n = 20 inoculated to get between 5 and 15 fractional (+)

Pooled sample protocol



AOAC Evaluations
approval by July, 2009

- 25, 75 & 375 g individual samples
- 375 g wet composite
- False positives < 0.3 % (from French commercial customers)

Other Validations

- ISO and AFNOR approvals in Europe
- AOAC additional validations
  - Produce
  - Irrigation water

Why does the VIDAS UP work?

- Increased specificity due to phage protein capture instead of antibody capture
- Reduced background readings due to specificity and tight binding of phage protein – allows more efficient washing
- One to two logs more sensitive due to relatively large initial sample size: 500 ml vs 5-10 microliters

BIOMERIEUX

Questions ?

APPENDIX E
IEH Document Independence of Combos

1. ASSERTION

A widespread current practice in the beef industry is to form Lots of 5 combos, and test them using an N=60 sampling plan. IEH asserts that the preponderance of combos are independent units, and that combining such independent units into a Lot for the purpose of testing substantially reduces the statistical robustness of the N=60 sampling plan.

2. INDEPENDENCE OF COMBOS

2.1 By Sample Collection Process

One of the most common contamination events is a single carcass for which contamination is protected (e.g. behind the kidney fat) or has been transferred from the surface to a protected interior site (e.g. an incision made with a contaminated knife).

The yield from a single carcass may not be enough to bridge two single combos on a fabrication line. If part of the yield from a single carcass was deposited on the surface of one combo, the remainder would be deposited on the bottom of the next sequential combo. If it does span two combos, it is unlikely that the contaminated material would be detected by sampling. This is because current industry practice is to collect samples from the top layer of the combo, which would result in collection of contaminated material from the first combo (top layer) but not the second (bottom of the combo).

Other contamination events are described below.

2.2 By 5 Combo Lotting Process

Typically, 5 combo Lots are formed from combos which are tied together by a sales order or purchase order. Thus, at a minimum, combos are often composed of different product types desired by the client (meaning lean points for beef trim, i.e. 50/50 or 80/20). In addition, because of the tie to sales/purchase orders, Lots are often formed as trucks are loaded. The combos may be drawn from inventory produced over a period of recent hours to recent days. As a result, combos may have been produced at different times.

2.3 By Contamination Events

How beef trim becomes contaminated with *E. coli* O157 has been the subject of much inquiry and thought, and answers are still elusive. Nevertheless, it is possible to identify some potential mechanisms of contamination and consider whether or not they would span combos, thereby leading to multiple combos with related levels of contamination.

Sanitary dressing defect

A sanitary dressing defect, such as a spot of fecal contamination, may end up on a single piece of beef trim. As such, it would end up in a single combo. This combo will be an independent unit, not related to any other contaminated combos.

It is possible that the fecal contamination could contaminate a piece of equipment in the fabrication area (such as a belt or a knife). In such case, it could lead to a 'comet' effect, where it contaminates additional trim as it passes by but the levels of contamination decline as the contaminate is 'consumed'. It should be pointed out that there are sanitary practices, such as belt sprays and periodic sanitizing of knives, that would limit the extent of the spread of the contamination. In this case, the contamination might be spread through a limited number, but several, combos. Depending upon where in the fabrication area the contaminating event occurred, it is possible that the combos would consist of the same product type (the 50/50 line, for example). Thus, this situation could result in several, but not many, combos which are related by contaminating event.

Single contaminated carcass

A single contaminated carcass may be the source of contamination. Such a carcass may result if there is a disruption in the microbial intervention processes. For example, perhaps at the end or beginning of a shift a carcass passes through the hot water spray cabinet on the harvest line when the sprays are turned off or not up to the proper temperature. A single carcass would end up in several combos, probably spanning several product types. Thus, this situation could result in several, but not many, combos which are related by contaminating event.

Multiple contaminated carcasses

There may be situations which contaminate many carcasses. Two examples are (1) failure of a microbial intervention, such as a final hot water spray cabinet; and (2) unanticipated contaminant transfer mechanisms, such as external weather leading to a reversal of air flow direction such that harvest air flows to the fabrication area. These situations could lead to contamination of multiple carcasses, which would lead in turn to contamination of many combos spanning all product types. Thus, this situation would result in many combos which are related by contaminating event.

2.4 Conclusion Regarding Independence

If a 5 combo lot contains one or more combos that are contaminated with *E. coli* O157, it is highly likely that the contaminated combos are not related to each other. Even if this is not true in all cases, to the extent that it does hold true it will adversely affect the ability of lot testing to detect the contamination if present because material from a contaminated combo may be diluted with clean material from the remaining combos, thereby masking the *E. coli* O157 contamination.

3. Effect on Sampling Plans

The ICMSF N=60 sampling plan is assumed to be robust, and capable of detecting contamination at a low level. However, estimates of robustness are based in turn upon another assumption, that the average level of contaminate for which the robustness is being estimated applies to the entire lot of material being tested.

The N=60 sampling plan is an acceptance sampling plan, and its performance is described by an operating characteristic curve relating the level of contamination to the probability that the contamination will be detected. However, as a point of reference it is common to state that "the N=60 sampling plan has a 95% chance of detecting contamination if it is present at a level of 5% or more." This would be true for a 5 combo lot in which all 5 combos were contaminated to this level, or any combination of levels of contamination such that the average across all 5 combos was 5%.

However, it is NOT true that an N=60 sampling plan would have a 95% chance of detecting a single combo with a contamination level of 5% if that combo was part of a 5 combo lot in which the other four combos were clean. In that case, the average concentration rate would only be 1%, and the N=60 sampling plan would only have a 45% chance of detecting the contamination[1].

3.1 Comparison N=60 to N=12

IF 5 combo Lots are composed of combos which are independent units, such that a contaminating event may be present in one but not in the other four, then the ability of any sampling protocol to detect the level of contamination is based on the number of pieces which are taken out of the contaminated combo. If 12 pieces are being taken out of each combo, then instead of an N=60 sampling plan the true statistical robustness is equivalent to an N=12 sampling plan.

The relative performances of N=60 and N=12 sampling plans were computed using hypergeometric distributions (the most accurate mathematical model for acceptance plans). The results are tabulated in Table 1 and displayed as operating characteristic curves relating the level of contamination to the probability that the contamination will be detected Figure 1, below. In broad terms, the N=60 sampling plan can be seen to be much more robust than the N=12 sampling plan.

The relative performances of N=60 and N=12 sampling plans were also re-plotted, and tied to some specific *E. coli* O157 contamination level benchmarks, in Figure 2. The value of (1 – Probability of Acceptance) was used to show the % probability of catching a lot contaminated with *E. coli* O157 at various levels. Various benchmark contamination levels were placed on the graph: (1) the contamination incidence rate for which the plan would have a 95% probability of

[1] ICMSF acceptance plan performance calculation tool available at: http://www.icmsf.iit.edu/main/home.html

catching and rejecting the lot; (2) the 2% incidence rate reported by Elder et al., 2000[2]; (3) preliminary FSIS data regarding the contamination incidence rate in verification samples for beef trim collected by FSIS; and (4) FSIS data regarding the contamination incidence rate in verification samples for ground beef collected by FSIS.

Presentation of these contamination incidence rates is not to be construed as a statement that one or more represent what is present in beef trim. The true contamination incidence rates are not known with certainty, and are likely to be variable rather than constant. Rather, the contamination incidence rates shown are intended to give some points of reference which are reasonable, and which allow comparison of the relative performance of the N=60 and N=12 sampling plans.

At each of these benchmark contamination incident rate levels, it can be seen that the N=60 sampling plan outperforms the N=12 sampling plan by several fold.

3.2 Relative Performance of Combo Lotting Schemes

To further illuminate the consequences of contaminated combos being individual units, and not correlated or associated with other combos which might form a 5 combo lot, a situation was hypothesized in which one combo was assumed to be contaminated at a level of 2% (consistent with Elder et al., 2000), and combined with 4 uncontaminated combos to form a 5 combo lot. Again, selection of this contamination incidence rates is not to be construed as a statement that 2% represents the actual contamination level in beef trim. The value was selected for illustration purposes only.

It was assumed that twenty (20) 5 combo lots were formed. The effect of testing these using the current N=60 plan, in which only 12 pieces would be taken from the contaminated combo (resulting in an effective sampling scheme of N=12), was evaluated. This was compared to a pool of 100 combos, 20 of which were contaminated at a level of 2%, which were individually sampled using an N=60 sampling plan. The results are shown in Figures 3 and 4.

As shown in Figure 3, current procedure of using a 5 combo lotting scheme resulted in 4 lots testing positive. In these 4 lots, only 4 combos were actually contaminated, and 16 uncontaminated combos would also have been removed from commerce. Obviously, this situation represents an adverse economic consequence for the producer. Furthermore, 16 lots tested negative, yet they contained a total of 16 contaminated combos. These combos would

[2] Elder, R.O., Keen, J.E., Siragusa, G.R., Barkocy-Gallagher, G.A., Koohmaraie, M., William W. Laegreid, W.W. (2000) "Correlation of enterohemorrhagic Escherichia coli O157 prevalence in feces, hides, and carcasses of beef cattle during processing http://www.pnas.org/cgi/content/full/97/7/2999 Published online before print March 21, 2000, 10.1073/pnas.060024897; Proc. Natl. Acad. Sci. USA, Vol. 97, Issue 7, 2999-3003

have been released into commerce, and obviously would represent a risk to the health of consumers.

In contrast, as shown in Figure 4, a protocol which tests individual combos by an N=60 sampling scheme would have resulted in detection of 14 out of 20 of the contaminated lots. No uncontaminated lots would have been removed from commerce. Though 6 contaminated lots would have been released into commerce, this is nearly threefold less (6 to 16) than the current 5 combo lotting scheme.

4. Conclusion

Analysis of process variables, and the nature of potential contamination events, suggests that the preponderance of contaminated combos are independent units. If they are independent units, the consequence is that the current protocol of forming 5 combo lots and sampling by an N=60 method does not provide an N=60 level of robustness with respect to detecting the contamination. Instead, the level of robustness is N=12, the number of pieces taken from the independent combo unit, which is much lower.

A sampling protocol which uses N=60 for individual combos overcomes these problems. It would be capable of detecting and removing from commerce a much larger portion of contaminated combos, and would have the additional benefit of not implicating uncontaminated combos and causing them to be diverted to an economically disadvantageous end.

Table 1. Comparison of Acceptance Probabilities between different Sampling Plans (N=1000 pieces and c (allowable defects) =0).

Contamination Incidence Rate (IR) in Lot, (%)	# of Defective Pieces	Probability Lot Will Be Accepted		Probability of Catching Lot (1 - Acceptance)	
	Sample Size>	N=12	N=60	N=12	N=60
0.0	0.00	1.00	1.00	0.00	0.00
0.1	1.00	0.99	0.94	0.01	0.06
0.2	2.00	0.98	0.88	0.02	0.12
0.3	3.00	0.96	0.83	0.04	0.17
0.4	4.00	0.95	0.78	0.05	0.22
0.5	5.00	0.94	0.73	0.06	0.27
0.6	6.00	0.93	0.69	0.07	0.31
0.7	7.00	0.92	0.65	0.08	0.35
0.8	8.00	0.91	0.61	0.09	0.39
0.9	9.00	0.90	0.57	0.10	0.43
1.0	10.00	0.89	0.54	0.11	0.46
2.0	20.00	0.78	0.29	0.22	0.71
3.0	30.00	0.69	0.15	0.31	0.85
4.0	40.00	0.61	0.08	0.39	0.92
5.0	50.00	0.54	0.04	0.46	0.96
6.0	60.00	0.47	0.02	0.53	0.98
7.0	70.00	0.42	0.01	0.58	0.99
8.0	80.00	0.37	0.01	0.63	0.99
9.0	90.00	0.32	0.00	0.68	1.00
10.0	100.00	0.28	0.00	0.72	1.00
11.0	110.00	0.25	0.00	0.76	1.00
12.0	120.00	0.21	0.00	0.79	1.00
13.0	130.00	0.19	0.00	0.81	1.00
14.0	140.00	0.16	0.00	0.84	1.00
15.0	150.00	0.14	0.00	0.86	1.00
16.0	160.00	0.12	0.00	0.88	1.00

Assumptions:
Lot Size = 1000 pieces
Defective Rate= 0 to 16%
Number of allowable defects c=0

Conclusions:
Sampling the lot with n=60 gives higher confidence for accepting a lot compared to n=12

The probabilities were generated from **hypergeometric distribution**

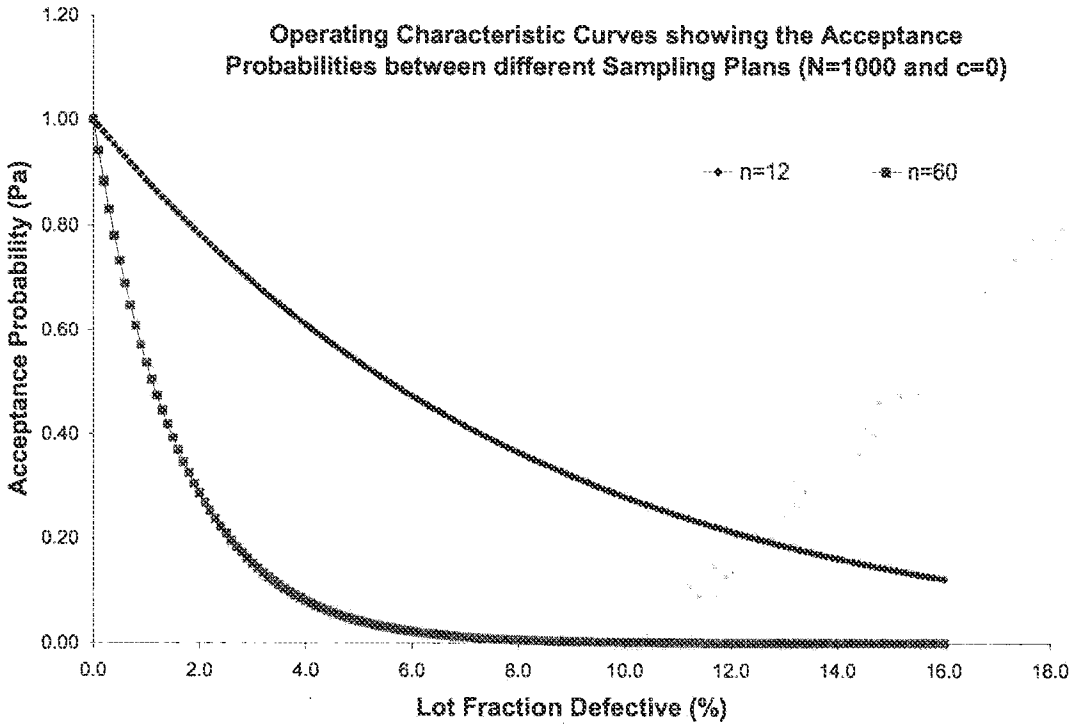


Figure 1: Comparison of operating characteristic curves for N=12 and N=60 sampling plans.

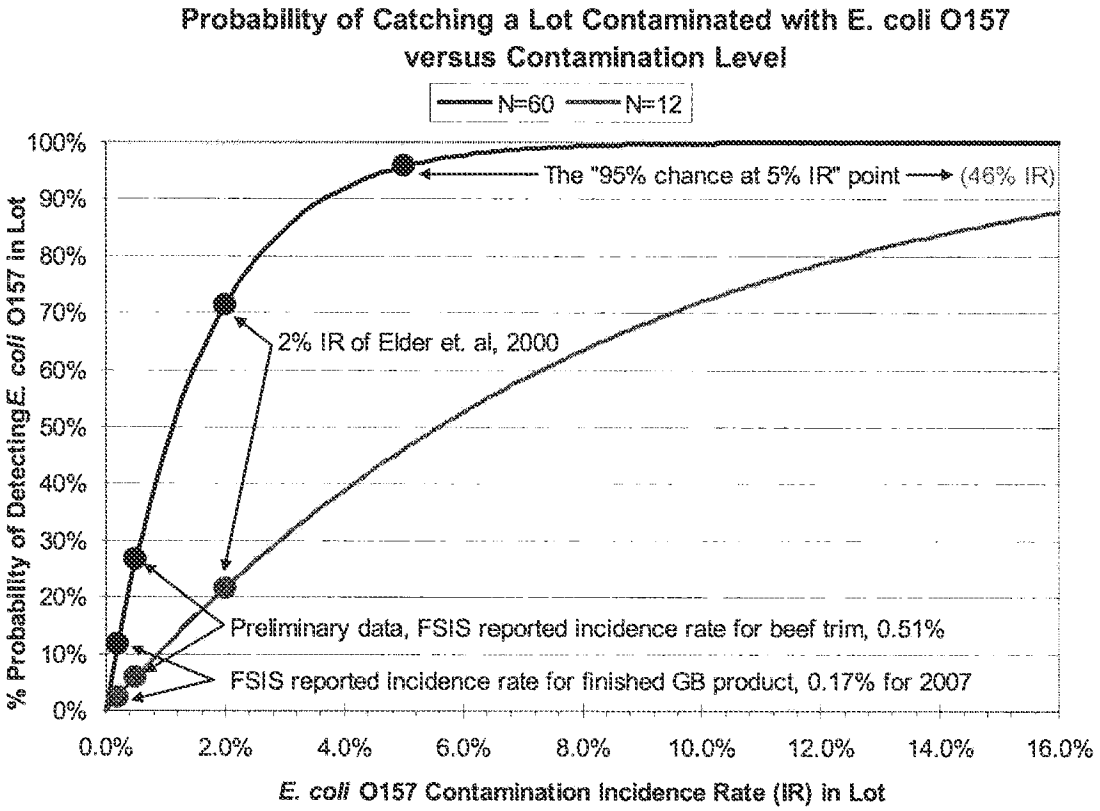


Figure 2: Comparison of chances for catching a defective Lot for N=12 and N=60 sampling plans.

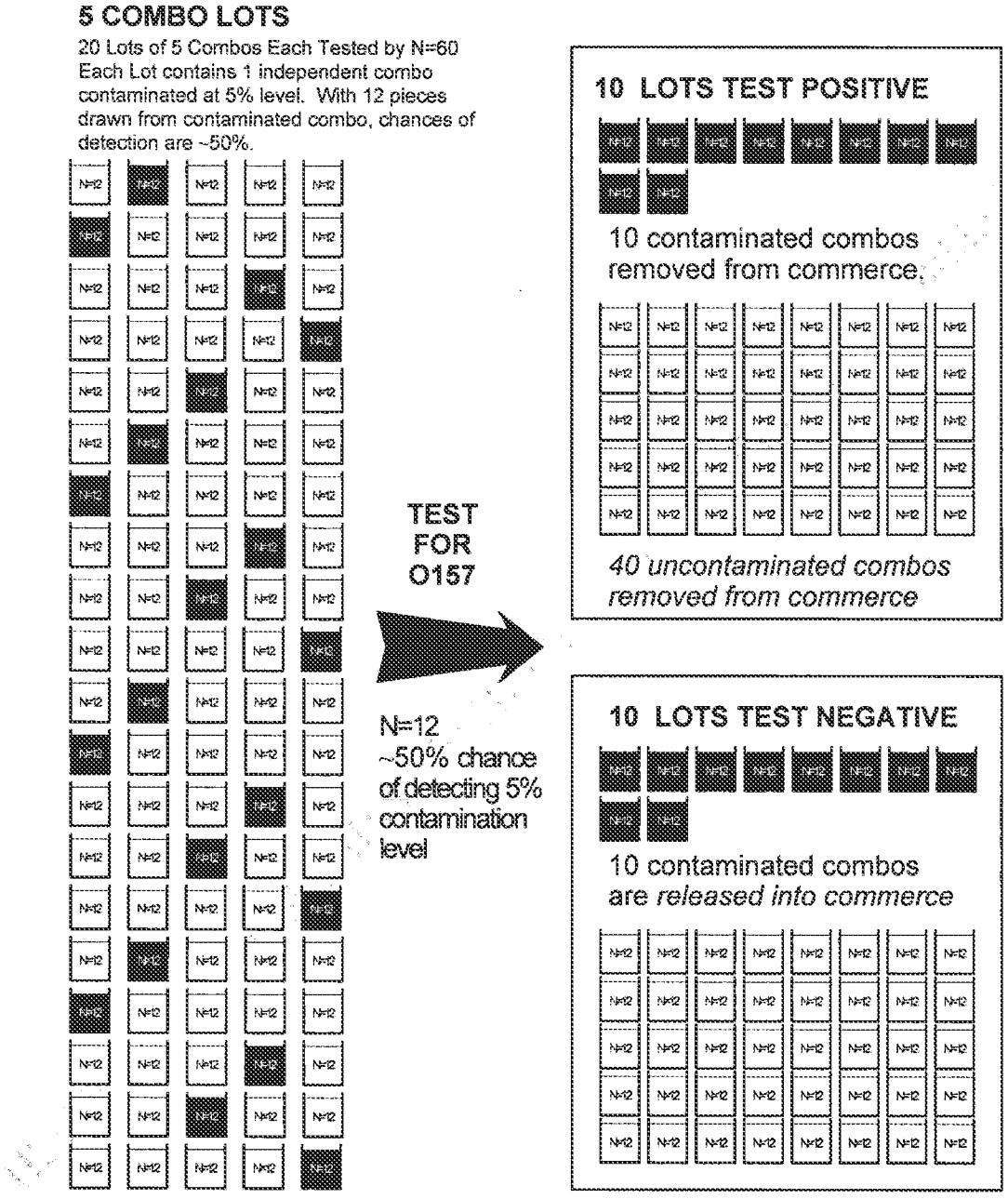


Figure 3: Illustration of detection and removal of independent combo units having a 5% level of contamination using 5 combo lotting.

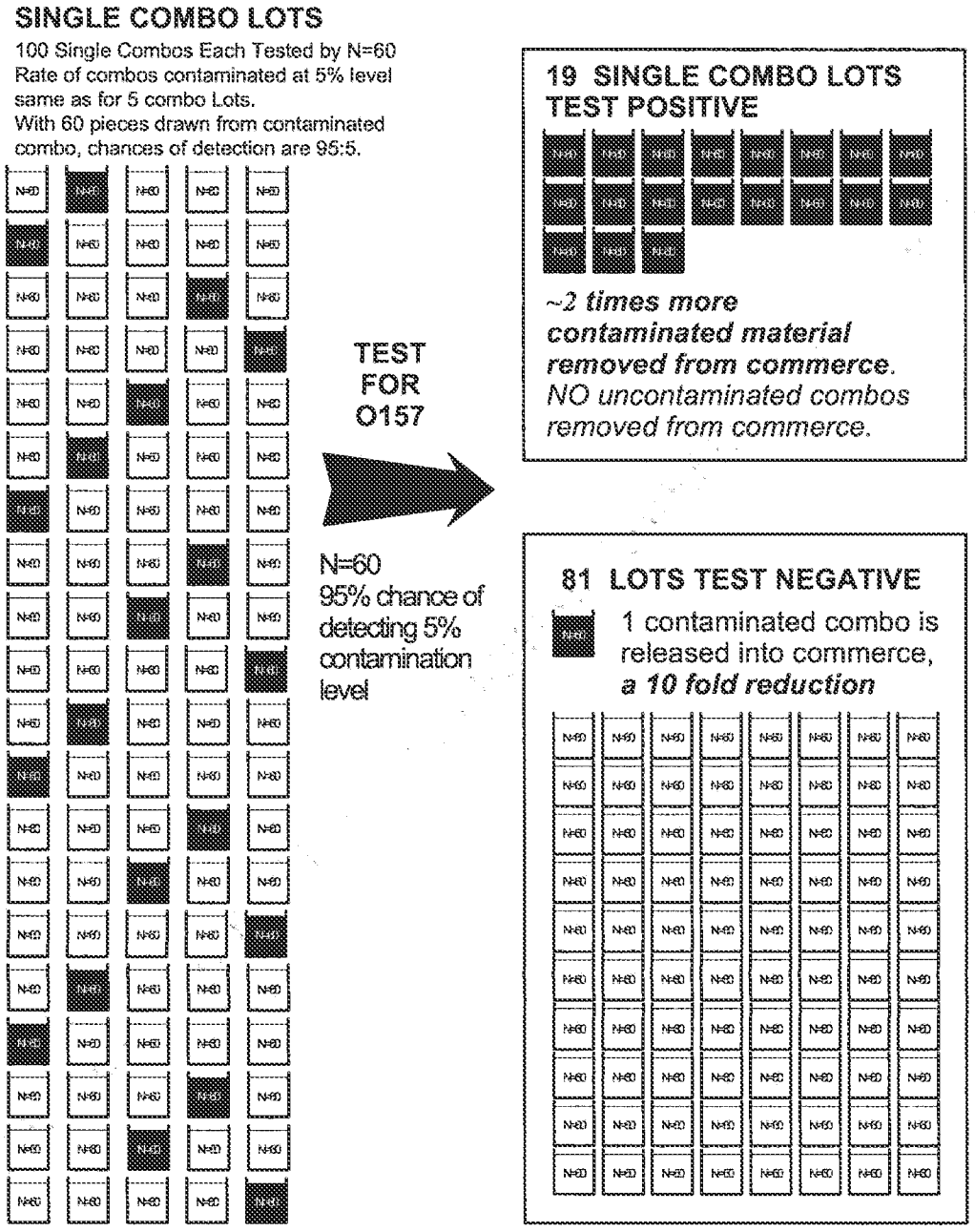


Figure 4: Illustration of detection and removal of independent combo units having a 5% level of contamination when single combo, N=60 sampling is used.