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

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Patentee:	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
Docket No.:	66090-008US2
Date:	05 May 2011

Attn: Mail Stop "*Inter Partes* Reexam"
Central Reexamination Unit
Commissioner for Patents
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RESPONSE TO NOTICE REGARDING DEFECTIVE PAPER

Sir:

This is a RESPONSE TO A NOTICE REGARDING DEFECTIVE PAPER, dated 27 April 2011. This Response is timely.

Kindly amend the above-identified patent application as follows:

Application No.:	95/001,294
Attorney Docket No.:	66090-008US2
First Patentee's Name:	7534584 (Mansour Samadpour)
Application Filing Date:	05 April 2010
Notice Regarding Defective Paper Dated:	27 April 2011
Date of Response:	05 May 2011
Examiner:	Brenda G. Brumback

IN THE CLAIMS:

Patentee, pursuant to 37 C.F.R. § 1.121, submits the following amendments to the claims:

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 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).

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 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 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).

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).

REMARKS

Claims 1-36 are pending and stand rejected.

Claims 1, 2 and 17 have been amended herein.

Prior added claims 34-36 have been cancelled herein.

No new matter has been added.

This is a Response to Notice of Defective Paper requesting that matter omitted from the claims be enclosed in brackets (instead of strikethrough), and where amendments are to be made relative to the patent specification, including the claims, in effect as of the date of filing the request for re-examination. Accordingly, Patentee has corrected these informalities by enclosing the matter omitted from the claims in double brackets (rather than strikethrough), and where the amendments are made relative to the patent specification, including the claims, in effect as of the date of filing the request for re-examination. Finally, the status identifiers of the amended claims have been revised to recite "Twice amended" (claims 1 and 17) and "Currently amended" (claim 2) to further clarify the amendment history relative to the patent specification, including the claims, in effect as of the date of filing the request for re-examination.

The following remainder of this Response is unchanged from Patentee's Response filed on 31 January 2011 (the referenced Declarations and cited support documents already being of record).

Additional Declarations

Patentee attaches four (4) further Declarations, which are incorporated by reference into this Response in their entirety in further support of the rebuttal arguments presented herein:

(1) Further Declaration of Dr. Mansour Samadpour;

(2) Declaration of Warren Mirtsching;

(3) Declaration by Dr. Byron; and

(4) Declaration by Dr. Mansour Samadpour in Rebuttal of the Bailey Declaration.

Patentee requests that these Declarations be considered by the Examiner in support of the current Response.

Patentee contends that Declaration (1), which contains, *inter alia*, financial revenue data for particular of Patentee's marketed tests, is necessary to further support the nexus between Patentee's patent claims and commercial success which is being questioned by the Examiner, and is being presented now because of the additional time required to assemble the financial data. Moreover, Declaration (1) is necessary to help rebut particular errors that Patentee respectfully contends have been maintained by the Examiner in construing various issues discussed in RCP, where the Examiner's premise therefore was only known to Patentee as of issuance of the RPC.

Patentee contends that Declaration (2), 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 is being questioned by the Examiner in the RCP, the premise of said Examiner's questioning being only known to Patentee as of the issuance of the RCP.

Patentee contends that Declaration (3), 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 is being questioned by the Examiner in the RCP, the premise of said Examiner's questioning being only known to Patentee as of the issuance of the RCP.

Patentee contends that Declaration (4), 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, where the Examiner's premise therefore only known to Patentee as of issuance of the RPC..

Patentee, therefore, contends that the Declarations meet the requirements of 37 CFR

1.116, and should be considered as requested herein to support Patentee's present Response.

Patentee further contends that the present claim amendments meet the requirements of 37 CFR 1.116 because they were necessitated based on what Patentee respectfully contends is continued misunderstanding of Patentee's invention by the Examiner.

In any event, Patentee herein respectfully requests that if the presently presented claims are not allowed, that prosecution of this case be reopened, where the Patentee has been prejudiced by issuance of an ACP, after a single Office Action, based on what Patentee respectfully contends is continued misunderstanding and/or error by the Examiner in interpreting of Patentee's claim elements and particular aspects of the asserted prior art, as discussed in the present Response.

Rejections under 35 U.S.C. § 103

Ground #1. The Examiner maintained the rejection claims 1-3, 8, 11, 13, 17-19, 22, 24 26, 27 and 31, under 35 U.S.C. 103(a), as being unpatentable over Jarvis in view of FSIS and Midura. The rejection was withdrawn for claim 4.

Claims 1, 17, 34 and 35:

The Examiner states, with respect to claims 1, 17, 34 and 35, that Jarvis discloses methods of sampling and testing foods for low numbers of pathogenic organisms using composite samples for testing (citing page 135, section 8.4, first paragraph), and teaches that testing *composite* samples reduces the workload on the laboratory (citing page 141, section 8.4.2.1) (technically speaking, Jarvis is not teaching compositing in the strict sense, but merely saying that one or more samples are inoculated into a suitable medium (see also page 135, section 8.4, first paragraph).

Claims 34-36 have been cancelled herein.

Jarvis method 2. The Examiner states that method 2 comprises collecting a plurality (10)

of product samples or portions (25g size) and 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 of Jarvis then composites equal portions of each pre-enrichment to inoculate a single enrichment broth).

Patentee points out, however, that with respect to method 2, Jarvis (who, technically speaking, recites "sample" not 'specimen' or 'portion') is (1) taking samples from a single underlying test lot, and (2) does NOT teach or motivate, *increasing* the work load by compositing a statistically sufficient plurality of samples from each of a plurality of separate test lots (each test lot to be independently validated for release) to form a set of corresponding separate statistically sufficient test lot samples (i.e., forming a plurality of independently validatable (for release) test lot samples)—where each separate underlying test lot can be validated for release by the corresponding statistically sufficient separate test lot sample, but rather *explicitly* teaches sampling from a single test lot, and *reducing* the work load by taking a number (e.g., 10) of samples from the single lot to be validated to form a plurality of statistically insufficient single pre-enrichment broths (one sample each)—where the underlying single test lot is NOT validated for release by any one of the single pre-enrichment broths, because individual pre-enrichment broths do not each contain a statistically sufficient plurality of samples (rather, only a single sample).

Patentee further directs the Examiner's attention to the attached further Declaration of Dr. Mansour Samadpour, and in particular paragraphs 13 though 18., relating to this point.

Jarvis method 3. The Examiner states that method 3 comprises pooling (actually "compositing" or lumping) a plurality (about 5) of specimens and inoculating the pooled specimens into a pre-enrichment culture medium or broth, 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.

Patentee points out, however, with respect to Jarvis method 3 (as in the case of Jarvis

method 2), Jarvis is (1) taking samples from a single underlying test lot, and (2) does NOT teach or motivate, *increasing* the work load by compositing a statistically sufficient plurality of samples from each of a plurality of separate test lots (each test lot to be independently validated for release) to form a set of corresponding separate statistically sufficient test lot samples (i.e., forming a plurality of independently validatable (for release) test lot samples)—where each separate underlying test lot can be validated for release by the corresponding separate statistically sufficient test lot sample, but rather *explicitly* teaches sampling from a single test lot, and *reducing* the work load by forming two statistically insufficient pre-enrichment cultures each formed by lumping a number (e.g., 5) of samples from the same/common single underlying test lot to form two separate pre-enrichment broths (5 samples each)—and where the underlying single test lot is NOT validated for release by either of two pre-enrichment broths, because the two individual pre-enrichment broths do not each contain a statistically sufficient plurality of samples (rather, only 5 samples each).

Statistical confidence. The Examiner states that 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 (citing page 141 in its entirety) (emphasis added). The Examiner states that 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* (citing the paragraph bridging pages 141 and 142) (emphasis added by the present Patentee). The Examiner appears to be implying that Jarvis teaches Patentee's claimed validation for release aspect. Patentee respectfully disagrees.

Patentee points out that even if one were to assume, *arguendo*, that Jarvis methods 1 and 2 teach validation for release of the single test lot that is sampled (not a plurality of separate test lots), and that both Jarvis methods 1 and 2, provided the same degree of statistical confidence (i.e., from the viewpoint that each method comprises taking 10 samples from the single lot), the fact remains, as explained in detail above, that any sampling of Jarvis is from a single test lot to be validated, and that even this single underlying test lot can NOT be validated for release by any one of the single pre-enrichment broths (of Jarvis methods 1 or 2), because they individually do

not contain a statistically sufficient plurality of samples; that is, any construable validation for release of the single underlying test lot would necessarily be based on testing the total collective number of samples, which in the Jarvis methods are distributed among multiple statistically insufficient pre-enrichment broths.

Patentee further directs the Examiner's attention to the attached further Declaration of Dr. Mansour Samadpour, and in particular paragraphs 20 though 24., relating to retesting in the prior art.

In summary of the above-discussed points, in fundamental contrast with both Jarvis methods 2 and 3, Patentee's claimed methods comprise a sampling hierarchy comprising sampling from a plurality of independent test lots, each to be separately validated for release, by separately compositing a statistically sufficient plurality of samples (according to a statistical-based sampling plan) from **each** of the plurality of separate test lots (each test lot to be independently validated for release) to form a set of corresponding separate statistically sufficient test lot samples (i.e., forming a plurality of independently validatable (for release into commerce) test lot samples)—where each separate underlying test lot can be validated for release by the corresponding separate statistically sufficient test lot sample (i.e., independent attribution is maintained between the respective test lots and the statistically sufficient test lot samples), and subsequently forming a modular composite sample using aliquots of each of said statistically sufficient test lot samples (and also where the collective plurality of separate test lots can be validated for release by Patentee's claimed modular composite sample, if negative). The nature of Patentee's statistically sufficient test lot samples, therefore, is **fundamentally distinguished** from the statistically insufficient pre-enrichment broths of Jarvis methods 2 and 3, and is further ***counterintuitive*** because Patentee's claimed method, by comparison with Jarvis and/or the rest of the asserted alleged prior art, substantially ***increases*** the workload by requiring independent statistically sufficient sampling from each of the plurality of test lots to form each of the respective plurality of independent test lot samples; that is, Patentee's method requires a substantial increase in sampling with attendant increase in cost.

Moreover, Jarvis does NOT teach or motivate "validation for release" in the presently

claimed sense, because it is inescapably clear that retesting, after further enrichment, of individual stored statistically insufficient pre-enrichment cultures to "verify positives" is not statistically equivalent to retesting separate test lot samples, each comprising a statistically sufficient number of sample portions to enable "validating" negative test lots for release into commerce.

Patentees respectfully contend that the Examiner' treatment of Jarvis, alone or in combination with any of the alleged asserted prior art, in the above aspects amounts to clear error and is manifestly unsupportable. Likewise, in view of any alleged motivation, in Jarvis' presentation of method 1 (which is the method of Silliker 1972, Gabis and Silliker 1974) and method (2) (which is the method of Price 1972), any compositing is of sample portions taken from a single underlying test lot, in which case retesting of any statistically insufficient pre-enrichment culture (as is also the case for any resampling of the single underlying test lot), is strictly for the purpose of determining the extent of the contamination because the lot as a whole **has already been invalidated by a positive result of the pooled enrichment broth**. The tested pre-enrichment cultures are simply not composed of a statistically sufficient number of portions based on a statistical-based sampling plan. The references, therefore, along or in combination, do not motivate, and do not enable, re-testing of any individual pre-enrichment broths (contributing to a pooled sample for which a positive result is obtained) for the purpose of release of a negative lot.

Claim 2:

With respect to claim 2, the Examiner states that since Jarvis teaches testing food for low numbers of pathogenic organisms by collecting samples from the food "the test lot" would necessarily be composed of operationally-linked product specimens. Patentee respectfully disagrees that the test lot of Jarvis would be necessarily composed of operationally-linked product specimens as defined in Patentee's specification; for example, the Jarvis test lot could be an aggregate of non-operationally linked product specimens.

In any event, Patentee respectfully points out that the Examiner's assertion is clearly misleading in the present context in view of the fact that Patentee's '584 patent is not about

testing a single test lot (e.g., of food), but rather teaches testing a plurality of separate test lots each to be independently validated for release as explained in detail here and in the prosecution record.

In view of the Examiner's comments, however, Patentee has amended claim 2 to recite "The method of claim 1, wherein each of the test lots is a single production lot, comprised of operationally-linked product specimens."

It is inescapably clear that unlike the presently claimed subject matter, the lumped samples from Jarvis, like those of FSIS, Midura, Siliker and Price, etc., are all taken from what must be effectively regarded as a **single test lot to be validated**; that is, the lumped statistically insufficient pre-enrichment cultures of the asserted prior art, alone or in combination, do NOT correspond to, and are not attributable to separate underlying test lots each to be validated for release.

In the case of Jarvis, for example, at page 137 under 8.4.1, Jarvis recites: "…to quantify the probable level of occurrence of organisms **in the lot of food under test**"; "[o]n the assumption that the organisms in **a 'lot'** of food are distributed randomly and that a number (n) of samples is tested…"; "…where d = actual % defectives in **the lot,**" etc. (emphasis added). Patentee's invention is directed to testing a plurality of test lots each to be validated for release, whereas the asserted art is directed a testing a single lot to be validated. Additionally, at page 141, under 8.4.2.1, Jarvis recites "[w]hen a number of samples is to be tested it is sometimes recommended that the samples be composited in order to reduce the work load on the laboratory" and in the method under "(3)", for example, while two groups of 5 samples were lumped/composited to form two pre-enrichment cultures, all 10 samples were attributable to a single underlying reportable lot, and thus the two pre-enrichment cultures are not attributable to two separate reportable test lots.

This is a fundamental and profound distinction, which underlies the distinguishing utility of Patentee's methods for independently "validating" each of a plurality of separated test lots for release into commerce—as opposed to merely enabling retesting, after further enrichment, of individual pre-enrichment cultures to verify positives in pre-enrichment cultures each of which

do not comprise a statistically adequate number of samples to enable validation for release of even the single underlying test lot.

Claim 3:

With respect to claim 3, the Examiner states that Jarvis teaches incubating a "modular composite" sample in enrichment broth (citing Jarvis at page 135, section 8.4). Patentee respectfully points out that this is explicitly **not true**, because Jarvis teaches only forming of a composite sample from a plurality of statistically insufficient pre-enrichment broths (i.e., from pre-enrichment broths that each do not contain a statistically sufficient number of sample portions).

By contrast, the nature of Patentee's modular composite sample is *fundamentally different* because it is formed by combining aliquots from each of a plurality of statistically sufficient test lot cultures (i.e., each comprising a statistically sufficient number of sample portions) to enable independent validation for release of each of the respective underlying plurality of separate test lots.

Patentee's claims are not obvious in view of the alleged asserted art, alone or in combination, under U.S. patent law, because the prior art, alone or in combination, does not teach or motivate a modular composite sample claim element as presently claimed. For example, Jarvis in Section 8.4 simply says:

> "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. Apart from purely technical errors, presence/absence tests are subject to a number of statistical considerations concerning the distribution of organisms"

There is no mention of modular compositing here, and certainly no mention of forming a modular composite sample as presently claimed (from a plurality of statistically sufficient separate test lot samples corresponding to a respective plurality of separate test lots each to be validated for release). He is simply restating the general knowledge of the art that a sample to be tested is inoculated in an appropriate media. Likewise, in Section 8.4.2.1, Jarvis is specifically

mentioning inoculating the composite (not a modular composite sample as presently claimed), into a lumped pre-enrichment broth culture, followed by enrichment of the pre-enrichment broth:

> "(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)."

Alternatively, Jarvis teaches pre-enriching the samples without compositing:

> "(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. (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 Examiner's continuing lack of recognition of the fundamental distinction between the classic lumping of Jarvis to form a composite sample, and forming Patentee's modular composite sample is reflected in the Examiner's comments with respect to claims 11 and 13.

Claims 11 and 13:

Specifically, with respect to claims 11 and 13, the Examiner states that Jarvis teaches "**a** test lot of 10 samples, which falls within the claimed range of 3 to 10." (emphasis added). On the one hand, based on this statement the Examiner recognizes that the 10 samples of Jarvis are attributed to a single underlying test lot. On the other hand, however, it is clear that for Patentee's independent claims, the Examiner is failing to acknowledge Patentee's **explicit** claim limitation of "a plurality of test lots," despite the fact that dependent claim 11 explicitly recites "wherein the number of separate test lots is from 3 to 10" (emphasis added). Patentee's invention relates to testing of a plurality of separate test lots each to be independently validated, and is not directed, in the sense of Jarvis, and the alleged prior art alone or in combination, to merely taking samples (e.g., 10) from a single test lot and forming a lumped sample. Patentee respectfully contends that the Examiner has erred by failing to properly recognize this distinctive limitation of Patentee's claimed subject matter. Claim 11 is not about the number of samples taken from a test lot, but rather is drawn to the number of test lots contributing to forming the

claimed modular composite sample.

Claim 13 recites a range for the number of samples taken from each one of the plurality of test lots to be validated for release. Jarvis merely teaches taking 10 samples from a **single lot** of food which is to be tested individually, and not by Patentees claimed methods, comprising forming a modular composite sample by combining aliquots from each of a plurality of statistically sufficient test lot cultures (i.e., each comprising a statistically sufficient number of sample portions) to enable independent validation for release of each of the respective underlying plurality of separate test lots.

The Examiner additionally states that Jarvis teaches particular limitations of dependent claims 18, 19, 22, 27, 31 and 36 as discussed in the ACP.

Medium:

The Examiner additionally states that "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 either 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) or they simply recite incubating the set of separate test lot samples under conditions suitable to allow levels of a target microbial agent or organism to increase in the test lot samples to provide a set of separate incubated test lot samples *(claims* 34 *and* 35)."

The Examiner further states that "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."

Patentee respectfully disagrees. Not only are the test lot samples of Jarvis fundamentally distinguishable from those presently claimed (Patentee's test lot samples each being statistically sufficient to enable independent validation for release of the respective one of the plurality of test lots), but there is a fundamental distinction between the attained levels of enrichment between Patentee's claimed methods and those of the prior art employing "pre-enrichment" incubation.

The prior art is clear 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. For example, from Jarvis (1989), Section 8.4.2:

> "Different methodological details are recommended by different authorities. Using the recommendations of ICMSF (1978) for salmonellas testing, 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. Thereafter, one 5 mm loopful (about 0.02 ml) for each selective enrichment is streaked on to one plate of each of three selective diagnostic media and, after incubation, selected colonies are picked for typing.
>
> Hence, three dilution stages occur. The first stage results in a 10-fo1d dilution of the sample. Organisms inoculated with the sample will grow during incubation, but the number growing will depend upon (a) the initial contamination level and the dispersion both of the organism being sought and of competitive organisms; (b) the condition of the organisms (i.e. whether cells have been "stressed" by the environmental conditions in the food); (c) the population doubling time for the organism sought in the conditions of the test (i.e. culture medium composition, incubation temperature and time).
>
> The second and third stages (selective enrichment) will be affected similarly, but in the third stage the individual propagules will be required to form discrete colonies on the diagnostic medium. The fourth stage (picking of colonies) is considered below (§ 8.4.3).
>
> Clearly, the possibilities for isolation of specific organisms will depend not only on the initial incidence of contamination but also on the manner in which the test is done. A too short period of pre-enrichment may result in no recovery of stressed organisms, thereby reducing the statistical probability of transferring resuscitated organisms to the enrichment broth. Too long a period in a non-selective pre-enrichment may provide opportunity for overgrowth by competitive organisms, leading to suppression of the organism sought. A hypothetical example of the effects of incubation time is shown in

Ex. 8.6 and in Fig. B.4." (emphasis added).

In the same section Jarvis goes on to say:

> "Once sufficient growth has occurred in pre-enrichment for at least 3 viable salmonellae/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, with m=3: 1'(x=O) = 0.05, therefore 1'(:c)-I) 0.95. However, at lower numbers, the chances of transferring at least 1 viable organism will be affected not only by the initial contamination level (x) but also by the volume of medium inoculated into the enrichment culture. In methods which permit only resuscitation not growth, during pre-enrichment it is essential to use the entire pre-enrichment culture to inoculate double-strength enrichment medium (Hall, 1975) although selectivity may then be affected by the food constituents.
>
> Obviously the level of salmonellae in the pre-enrichment culture after any given incubation period will depend upon the cultural conditions used, the initial contamination level. the extent of sublethal cell damage and the relative numbers and types of competitive organisms in the sample."

Simply stated, therefore, the function and purpose of pre-enrichment according to Jarvis 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 to the point of dividability (i.e., transferred pre-enrichment aliquot having at least one viable cell) not to detectability as presently claimed. Detectability is only achieved after enrichment according to Jarvis/Silliker/Price/Gabis/FDA BAM, or any other text book of microbiology, which draw a firm and clear distinction between pre-enrichment and enrichment. These are terms of art with long-established definitions.

Furthermore, Patentee points out that sampling according to a statistical-based sampling into pre-enrichment broth and growing to detection levels would be strongly contraindicated in the prior art, due to the fear of higher probability of introducing contamination and outgrowth of contaminants with increased number of lumped sample portions. This is why even Jarvis method 1 limits the pre-incubation, and relies on enrichment incubation of enriching to detection.

Patentee respectfully submits, that sufficient evidence contrary to the Examiner's position in this regard, is provided by: (1) Jarvis' own admission, that the purpose of the pre-enrichment is to make sure that in the portion which is used to inoculate the enrichment there are at least

three viable cells; (2) Patentee's patent '584, teaching that formation of the modular composite sample is done *after* the levels of the target organisms reach delectability; and (3) the long-standing canonical understanding of one of ordinary skill in the art to draw clear distinction between the dividable levels of target organisms after pre-enrichment, vs. detectable levels of target organisms after enrichment.

Patentee respectfully contends that the Examiner's conclusion that "pre-enrichment incubation" provides for detectability as presently claimed, flies in the face of art-recognized canons, is inconsistent with the teachings of the Examiner's own asserted alleged prior art, alone or in combination, and amounts to clear error and is manifestly unsupportable.

Amendments to claims 1, 17, 34 and 35:

Patentees have herein amended step a) of claims 1 and 17 to recite:

"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~~, according~~ 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~~;"

and have further amended step e) of claims 1 and 17 to recite:

"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 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 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)."

Explicit support for the amendments is found in Patentee's patent, for example, at col. 5, ll. 52-52-56 and 63-66 (number of sample is "selected so as to conform to statistical-based sampling plans"); col. 6, first carry-over paragraph; col. 1, line 62 through col. 2, line 26; original claim 3 (teaching "[t]he method of claim 1, further comprising incubating the modular composite sample of d), prior to testing in e)"); and see, for example, col. 1, last carryover paragraph through col. 2 line 26. It is clear from the specification that the modular composite sample can be assayed with no requirement for further enrichment incubation (as recited in original dependent claim 3) (see also see col. 7, line 64 teaching testing the modular composite sample with no further incubation; and col. 9, ll. 43-45, teaching optional incubation of the moduclar composite sample (original claim 3)), and with no requirement for further sampling (see, e.g., col. 3, ll. 4-16; col. 4; ll., 12-16; and wherein the specification generally teaches that additional further sampling is prohibited by regulatory agencies). Support for recitation, in step c), of "if present" in place of "that is present" is found for example in step e) of original claim 1, where it is clear that the assay provides for validation for release (i.e., a negative test result).

The amendments serve to further clarify the distinguishing features of Patentee's claimed subject matter by further clarifying that, unlike prior art methods comprising pre-enrichment incubation in pre-enrichment broth, the incubation of Patentee's step c) is sufficient, if the microbial agent or organism is present, to achieve an extent of enrichment sufficient to enable **validation** of negative test lot samples according to the statistical-based sampling plan with no required further enrichment, (i.e., such further enrichment after step c) is an alternate/optional embodiment; dependent claim 3) and with no requirement for additional test lot sampling. As recognized by the Examiner, Jarvis teaches that the pre-enrichment cultures are stored, and may be subsequently retested—but this would ***require***, as is clear from methods (1), (2) and (3) of Jarvis, and according to art-recognized practice (e.g., Jarvis, Price, Silliker, etc.), incubation of the pre-enrichment culture, or an aliquot thereof, in enrichment medium prior to said retesting. Additionally, as recognized in the art, it would increase the chance of overgrowth by any

contaminants.

In any event, Patentee's claims do not stand or fall based solely on enrichment to detection in step c), and even if one were to misconstrue the pre-enrichment art as urged by the Examiner (i.e., that pre-enrichment provides for such detectability without subsequent enrichment), Patentee contends that the currently claimed subject matter is yet patentable. As stated above, Patentee's claimed methods comprise a sampling hierarchy comprising sampling from a plurality of independent test lots, each to be separately validated for release, by separately compositing a statistically sufficient plurality of samples (according to a statistical-based sampling plan) from **each** of the plurality of separate test lots (each test lot to be independently validated for release) to form a set of corresponding separate statistically sufficient test lot samples (i.e., forming a plurality of independently validatable (for release into commerce) test lot samples)—where each separate underlying test lot can be validated for release by the corresponding separate statistically sufficient test lot sample (i.e., independent attribution is maintained between the respective test lots and the statistically sufficient test lot samples), and subsequently forming a modular composite sample using aliquots of each of said statistically sufficient test lot samples (and also where the collective plurality of separate test lots can be validated for release by Patentee's claimed modular composite sample, if negative). The nature of Patentee's statistically sufficient test lot samples, therefore, is **fundamentally distinguished** from the statistically insufficient pre-enrichment broths of Jarvis methods 2 and 3, and is further ***counterintuitive*** because Patentee's claimed method, by comparison with Jarvis and/or the rest of the asserted alleged prior art, substantially ***increases*** the workload by requiring independent statistically sufficient sampling from each of the plurality of test lots to form each of the respective plurality of independent test lot samples; that is, Patentee's method requires a substantial increase in sampling with attendant increase in cost.

Moreover, Jarvis does NOT teach or motivate "validation for release" in the presently claimed sense, because it is inescapably clear that retesting, after further enrichment, of individual stored statistically insufficient pre-enrichment cultures to "verify positives" is not statistically equivalent to retesting separate test lot samples, each comprising a statistically

sufficient number of sample portions to enable "validating" negative test lots for release into commerce.

The Examiner's reliance on secondary and tertiary references (FSIS and Midura) (claims 8, 10, 19, 20, 24, and 26):

Jarvis:

The Examiner states that "**Jarvis does not teach repeat testing of negative samples**, as in patented dependent *claim 8*) (emphasis added). **Patentee agrees**, as discussed in detail above, because there would be absolutely no motivation to do so, and it would not be possible in the sense of Patentee's patent claim 8 subject matter, where the pre-enrichment cultures of Jarvis do not represent statistically sufficient broths each attributable to separate underlying test lots (plurality of underlying separate test lots), and thus there would be no attributable separate test lots, and even the single underlying test lot of Jarvis could not be validated by repeat testing of a statistically insufficient pre-enrichment broth.

The Examiner states that Jarvis 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 or *Salmonella* (among others), as in *claims 19 and 20*, and does not specify the food to be tested, as in patented *claims 24, 26*. The Examiner states that these limitations are taught by FSIS, and Midura for reasons stated in the Office Action. Patentee points out, however, that to the extent that such limitations are taught in FSIS, and Midura as urged by the Examiner, they are taught only in the context of sampling from a single test lot, and where the pre-enrichment cultures of Jarvis do not represent statistically sufficient broths each attributable to separate underlying test lots (plurality of underlying separate test lots).

FSIS:

Claims claims 10, 19, 20, 24, and 26:

With respect to claim 20 the Examiner states that FSIS teaches combining pieces for a representative sample of a production lot for the current day's production (allegedly meeting the

limitations of *dependent* claims 10, 19, 20, 24, and 26). Again, Patentee respectfully contends that the Examiner's reliance on FSIS to support Jarvis in alleged satisfaction of the limitations of these claims is unsupportable as these are *dependent* claims in the context of Patentee's method. FSIS merely teaches taking a plurality of samples to form a single composite sample representing a day's production. Jarvis and/or FSIS do not teach forming statistically sufficient composite samples (of any type) from a plurality test lots each to be validated for release as presently claimed. The teachings of FSIS in this regard do not go beyond Jarvis. While FSIS may arguably teach testing for *E. coli O157*, FSIS is not teaching testing the presently claimed modular composite samples formed of aliquots from each of a plurality of enriched statistically sufficient test lot samples, as presently claimed for *E. coli O157*, and moreover does not cure the deficiencies of Jarvis with respect to claims 10, 19, 20, 24, and 26 (see further Declaration of Dr. Mansour Samadpour at Exhibit 6, Appendix 2, pg 27 "Representative or Randomized Sampling").

With respect to *dependent* claims 8 and 12, the Examiner states that FSIS teaches follow-up *sampling* upon identification of contaminated lots. Patentee respectfully points out that *dependent* claim 8 is in fact not drawn to follow-up *sampling* upon identification of a contaminated lot, and rather is drawn to the method of claim 1 further comprising forming an *additional* enriched test lot sample (i.e., not from a previously sampled lot), and forming an additional modular composite sample from an aliquot of the additional enriched test lot sample along with aliquots of any enriched test lot samples of d) that tested *negative*. With respect to *dependent* claim 12, while FSIS may arguably teach follow up *sampling* upon identification of a contaminated lot, claim 12 is not about such *resampling* and rather relates merely to identifying positive testing separate enriched test lots, subjecting them to remedial measures and retesting them to validate the lot.

Patentee respectfully contends, therefore, that with respect to dependent claims 8 and 12, the Examiner's position on this is unsupportable because FSIS has no such teachings.

Moreover, Patentee further points out that to the extent FSIS teaches *resampling* as urged by the Examiner, FSIS actually profoundly ***teaches away*** from the present invention because, as

taught in Patentee's patent at column 3, ll. 4-16 the current art does not allow for retesting of any production sublots for validation for release purposes, primarily because microbial contaminants are, or are presumed to be unevenly spread throughout the products, and in many instances the levels of contamination are minute and may be present on very small portions of the products in each sublot. As discussed in Patentee's patent, therefore, in the case of prior art methods such as that of attached Exhibit A, where the lot to be validated comprises 5 combi bins (sublots), once the lumped prior art composite tests positive all 5 of the combi bin sublots are destroyed. The regulatory agencies do not allow for re-testing of any such sublots, premised on the argument that after a production lot tests positive, negative test results obtained for any production sublots, or even a new production lot composite sample, are meaningless since the microbial contaminants are, or are presumed not to be uniformly present throughout the products. While, FSIS instructs it's personnel on verification testing of food establishments that have had a positive pathogen test result, and provides instructions for collecting *samples*, this has absolutely nothing to do with claim 8, which **does not involve further sampling** from any lots that have already been sampled, and rather claim 8 is about forming and testing a further modular composite sample formed in part from an "additional test lot sample" (not one that was already sampled) along with aliquots of test lot samples of step c) that tested negative in step e). Likewise claim 12 has nothing to do with the FSIS's directive. Claim 12 **does not involve resampling** at all, and rather recites retesting of test lots that have been subjected to remedial measures. FSIS can not be relied upon, alone or in combination with Jarvis to provide the elements of dependent claims 8 and 12 (which also include the elements of the respective independent claim). FSIS doesn't apply since the FSIS reference is that if an establishment sample tests positive for E. coli O157, a few days to weeks later, FSIS personnel would go in and collect a set of samples from a day's production (another day which has nothing to do with the original day that a sample has tested positive), for the purpose of verification of the process. The Third Party Requesor has misconstrued the facts, reason and logic, by framing the FSIS reference in the manner presented.

Claims 32 and 36:

With respect to dependent claims 32 and 36, the Examiner states that FSIS teaches that manufacturing trimmings may be processed for a food product. Again, however, Patentee points out that claims 32 and 36 are *dependent* claims, and that the FSIS teaches **absolutely nothing**, **alone or in combination with Jarvis and/or Midura**, that would cure the deficiencies of Jarvis and/or Midura, as described above and below, with respect providing Patentee's claimed methods (forming a modular composite sample from a plurality of statistically sufficient test lot samples each comprises a number of sample portions sufficient to validate a respective one of a plurality of underlying test lots). As such FSIS's revelation that trim is food is of no consequence.

Midura:

Claim 10:

With respect to Midura, the Examiner states that "Midura discloses methods for sampling and testing foods for microbial contamination. 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, and shell fish exemplified, as in patented *claims* 24 *and* 25); 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 (emphasis added by Patentee to point out that the teachings of Midura relate to testing of a single test lot, and not of a plurality of test lots in the presently claimed manner).

Claim 8:

Regarding *claim* 8, the Examiner states that 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) (see pages 17-20). Patentee notes in this regard that Midura actually acknowledges that the double sampling plan according to Midura is inferior to a single sampling plan (see Midura at page 17, 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"). In any event, as stated above, to the extent that Midura teaches *resampling* of a previously sampled lot, it profoundly ***teaches away*** from the presently claimed methods. Patentee respectfully contends that invoking Midura, as urged by the Examiner, is both technically and scientifically wrong

The Examiner states that 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 (see page 19, the paragraph bridging columns 1 and 2). Patentee points out, however, that any teachings of Midura to take a greater number of samples where there is a low incidence of target pathogen does not go beyond the teachings of FSIS and Jarvis, because Jarvis, FSIS and Midura teach sampling only in the context of taking multiple samples from a single test lot and either testing each sample individually or as a lumped/composite sample. For the same reasons as stated above in the case of Jarvis, therefore, FSIS and Midura cannot be combined with Jarvis to create an supportable *fiction* as presently asserted. In fact, Midura, FSIS, Silliker, Gabis, Price and Jarvis, **alone or in combination**, are about taking a plurality of samples from a single lot and analyzing the samples in the context of that **single sampled lot** to be validated. As such they cannot be asserted here, alone or in combination, as allegedly obviating Patentee's claimed invention.

Patentee further directs the Examiner's attention to the attached further Declaration of Dr. Mansour Samadpour, and in particular paragraphs 13 though 18., relating to this point.

Claims 14-16:

With respect to *claims* 14-16, Midura exemplifies collecting 30 or 60 samples or portions (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). Once again, Patentee points out that Patentee's claimed invention is not about taking 30-60 samples from a single test lot of food to be tested and validated, but rather about taking a statistically-sufficient number of samples from

each of a plurality of separate lots to be validated by Patentee's modular compositing test method as presently claimed. As such Midura and/or FSIS cannot be asserted here, alone or in combination with Jarvis, as allegedly obviating Patentee's claimed invention.

As in the case of Jarvis and FSIS, Midura teaches **absolutely nothing**, alone or in combination with Jarvis and/or FSIS, that would cure the deficiencies of Jarvis and/or FSIS, as described above and below, with respect to testing a plurality of separate test lots according to a statistical-based sampling plan and method that would provide for validation and release of separate test lots based on assaying individual test lot sample cultures that are attributable to respective separate test lots, as presently claimed. Again, the nature of Patentee's modular composite sample is *fundamentally different* because it is formed by combining aliquots from each of a plurality of statistically sufficient test lot cultures (i.e., each comprising a statistically sufficient number of sample portions) to not only enable validation of the collective plurality of underlying test lots, but also enable independent validation for release of each of the respective underlying plurality of separate test lots.

Patentee's claims are not obvious in view of the alleged asserted art, alone or in combination, under U.S. patent law, because the prior art, alone or in combination, does not teach or motivate a modular composite sample claim element as presently claimed.

Merely teaching that at specific number of samples is to be collected (whether initially, or in combination with subsequent re-sampling) does not provide for validation and release as presently claimed, because there is no relating in any of Jarvis, FSIS, and/or Midura that a plurality of samples are taken separately from each of a plurality of test lots to form a set of respective attributable test lot samples, wherein the number of samples taken in forming each test lot sample conforms to a statistical-based sampling plan to not only enable validation of the collective plurality of underlying test lots, but also to provide for validation and release of individual test lots based on testing of the test lot samples. None of the references, alone or in combination, teaches the presently claimed subject matter, and in fact, FSIS and Midura actually profoundly ***teach away***, by teaching ***reducing*** the workload by composite testing with respect to a single underlying test lot, and by teaching re-sampling.

Given the knowledge, skill and extant canons of the relevant art, a person of ordinary skill in the art would have taken samples from a singly lot for testing the single lot for a given pathogen to see if the single lot was contaminated. If the single lot was found to be contaminated, then the lot was discarded.

By contrast, as is further elaborated in the attached further **Declaration of Dr. Mansour Samadpour**, a primary impetus behind Patentee's invention was protecting consumer health. At the time of the invention, a large number of deaths were attributed to foodborne diseases and industry continued to struggle to balance the cost of testing and the cost of products that had to be destroyed if they tested positive, against profitability. Given that many retailers and food services don't always attach a premium to a food safety programs, coupled with continued industry advocacy for sampling plans based on wrong assumptions (e.g., assuming random distribution of contaminants in non-liquid foods) that would justify minimal sampling (e.g., treating a whole day's production as a single lot, and taking multiple samples from only a single lot, thus reducing the chance of finding lots contaminated with pathogens), Patentee set out to develop a test system that, in contrast to the motivation of the prior art to *reduce* the cost of testing, would actually ***increase*** the cost to the industry, and would be expected to actually ***increase*** the number of test lots testing positive, where both of these aspects would be regarded as **de-motivational** in the knowledge and canons of the prior art. As stated in the further Samadpour Declaration, Patentee's method was initially not well received in the industry, where increased cost, and the specter of having to destroy even more test lots because of increased sample statistics, were **profound deterrents** to adopting Patentee's method. Patentee's method, however, is now rapidly supplanting prior art methods because in actual practice while more test lots are found to be positive, the test lots are generally *smaller*, **resulting in destruction of a substantially smaller amount of overall product**. Additionally, because Patentee's method is substantially more statistically robust, it provides for better management of not only public health risk, but also of liability risk by managing the cost of insurance.

Patentee's claimed methods, therefore, have been remarkably and surprisingly adopted

given the profound deterrents that were initially present in the prior art and industry. As explained in the further Samapour Declaration, it took years to overcome the skepticism of the experts in the field, including FSIS, which was extremely apprehensive of the methodology because it feared that pooling composite samples from different lots would result in loss of sensitivity—a clear indication that the method was not obvious. As explained by Dr. Samadpour, he initially encountered the same skepticism from his clients. In one case, a major customer to one of Patentee's clients threatened to stop purchasing the client's products after reading the Patentee's protocols, and did not accept the method until they has actually conducted the method themselves.

Particular points discussed in the further Declaration of Dr. Mansour Samadpour are relevant to the above-described acceptance issue. Significantly, as stated above, a major concern of the industry was that the more you test, the more products you will need to destroy. As explained in the further Samadpour Declaration, in conducting Patentee's baseline studies it found an unexpected result that while it was true that the number of positive findings would increase and that more lots would need to be destroyed, the overall amount of products destroyed was significantly less because the lot sizes were generally smaller. This could not have been predicted because prior to implementation of Patentee's more statistically robust methods, one would not have known that the extant baseline contamination was such that the overall amount of product destroyed would be smaller despite an increase in the number of lots that would need to be destroyed.

In short, a person of ordinary skill in the relevant art would not have been motivated to develop or even practice Patentee's methods had they developed them. In fact a person of ordinary skill in the relevant art would not have attempted to apply the teachings of Silliker/Price/Gabis/Jarvis to even another method for *Salmonella*; where the best proof of that is that the prior art did not do so.

According to the further Samadpour Declaration, the Third Party Requestor has been in the business of manufacturing detection kits for ages, and prior to Patentee's invention, it did not

market any kits using Patentee's claimed methods and/or principles. At the time of the Requestor's Petition, however, it started marketing Patentee's method (see Dr. Stan Bailey's presentation on the subject, attached to the further Samadpour Declaration). According to Dr. Samadpour, prior to Patentee's invention, **no one** ever attempted to apply/extend compositing and pooling of samples from a single lot to compositing and pooling of multiple samples from multiple test lots as presently claimed, because it was feared in the art that merely even compositing and pooling of samples from the same lot resulted in loss of sensitivity (Price, Gabis and Jarvis). According to the further Samadpour Declaration, it was only after Patentee successfully overcame all of the skepticism and barriers in the industry and prior art, that two companies (including Dr. Bailey's company) have now copied the invention

Second, given the knowledge, skill and extant canons of the relevant art, a person of ordinary skill in the art would have taken samples from a singly lot for testing the single lot for a given pathogen to see if the single lot was contaminated. If the single lot was found to be contaminated, then the lot was discarded.

Patentee's traversal.

Patentee respectfully maintains Patentee's traversal of the rejection based on arguments of record and as presented herein above and below.

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, even 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.

Patentee respectfully contends, therefore, that the Examiner and the Third Party Requestor cannot simply arbitrarily parse a plurality of elements from each of the

respective asserted art reference, while ignoring the inventions of each reference as a whole. Patentee respectfully contends, however, that this (arbitrary parsing) is precisely the way that the asserted alleged prior art has been applied, contrary to U.S. patent law as cited above.

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).

Patentee respectfully contends that 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.

Analysis:

In response to the Examiner's and/or Third Party Requestor's allegations that Patentee has only rebutted individual asserted references (and not references in combination), Patentee contends that this is absurd based on Patentee's arguments of record. Additionally, Patentee points out that any assertion of multiple prior art references for an obviousness rejection must initially involve consideration of each reference *per se*, as a whole, so that arbitrary "cherry picking" of elements from particular reference can be avoided where such combination would be contrary to the teachings of particular individual references as a whole, as supported by the U.S. case law cited above. Accordingly, Patentee addresses references individually, as a whole, in addition to addressing them in combination in rebutting obviousness, as required under U.S. patent law.

Patentee respectfully contends that the methods disclosed in Jarvis, FSIS and Midura, differ fundamentally and significantly from one another and Patentee's claimed invention, and combining the prior art as suggested by the ACP requires the application of impermissible hindsight, and ignores established case law that the proper inquiry involves consideration of inventions as a whole.

A. **Unlike the presently claimed subject matter, the aggregated samples from Jarvis, like those of FSIS, Midura, Siliker and Price, etc., considered alone or in combination, are all taken from what must be effectively regarded as a single reportable test lot to be validated; that is, the lumped pre-enrichment cultures of the prior art do NOT correspond to, and are not intended to form a plurality of statistically sufficient test lot cultures each attributable to separate one of an underlying corresponding plurality of test lots to be validated, and therefore the asserted art, alone or in combination does not provide for or motivate validation and release according to the presently clamed methods, and moreover the art also does not teach Patentee's unique "modular composite sample" claim element, such that the asserted alleged prior art cannot obviate Patentee's invention.**

As an initial matter, claim1 has been amended to recite:

"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~~, according~~ 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 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 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)."

Conforming amendments have been made to claim 17.

Support for the amendments is discussed in detail at pages 20-22 of this Response and Amendment.

It is clear from Patentee's step b) that "each separate test lot sample is attributed to a particular corresponding separate test lot", where such is not taught or suggested in any of Jarvis, FSIS and/or Midura, alone or in combination.

It is clear from Patentee's step a) that "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 and growth conditions sufficient to provide for validation and release of the test lot into commerce," where such is not taught or suggested in any of Jarvis, FSIS and/or Midura, alone or in combination, because these references, as discussed above in detail relate to

sampling from a single underlying test lot, and do not teach applications of statistical sampling and testing of a plurality of test lots in the manner as presently claimed.

It is clear from Patentee's step e) that "…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 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)," where such is not taught or suggested in any of Jarvis, FSIS and/or Midura, alone or in combination with each other and the knowledge and skill of the art at the time of Patentee's invention and filing.

In the case of Jarvis, for example, at page 137 under 8.4.1, Jarvis recites: "…to quantify the probable level of occurrence of organisms **in the lot of food under test**"; "[o]n the assumption that the organisms in **a 'lot'** of food are distributed randomly and that a number (n) of samples is tested…"; "…where d = actual % defectives in **the lot,**" etc. (emphasis added). Patentee's invention is directed to testing a plurality of reportable test lots, whereas the asserted art is directed a testing a single reportable lot. Additionally, at page 141, under 8.4.2.1, **Jarvis recites "[w]hen a number of samples is to be tested it is sometimes recommended that the samples be composited in order to reduce the work load on the laboratory**" and in the method under "(3)", for example, while two groups of 5 samples were lumped/composited to form two pre-enrichment cultures, all 10 samples were attributable to a single underlying reportable lot, and thus the two pre-enrichment cultures are not attributable to two separate reportable test lots.

Additionally, the sampling and testing described in FSIS and/or Midura, alone or in combination with Jarvis, relate only to testing of a single lot, as evidence by the fact that the lumped test lot sample are not attributed to separate underlying test lots, etc., and are rather collectively viewed as a number of samples relating to a single underlying test lot. There is no collective testing of a plurality of separate test lots as presently claimed.

This is a fundamental and profound distinction, which underlies the distinguishing utility of Patentee's methods for "validating" test lots for release into commerce—as opposed to merely enabling retesting, after further enrichment, of individual pre-enrichment cultures to verify positives.

Patentee's claimed methods comprise a sampling hierarchy comprising sampling from a plurality of independent test lots, each to be separately validated for release, by separately compositing a statistically sufficient plurality of samples (according to a statistical-based sampling plan) from **each** of the plurality of separate test lots (each test lot to be independently validated for release) to form a set of corresponding separate statistically sufficient test lot samples (i.e., forming a plurality of independently validatable (for release into commerce) test lot samples)—where each separate underlying test lot can be validated for release by the corresponding separate statistically sufficient test lot sample (i.e., independent attribution is maintained between the respective test lots and the statistically sufficient test lot samples), and subsequently forming a modular composite sample using aliquots of each of said statistically sufficient test lot samples (and also where the collective plurality of separate test lots can be validated for release by Patentee's claimed modular composite sample, if negative). The nature of Patentee's statistically sufficient test lot samples, therefore, is **fundamentally distinguished** from the statistically insufficient pre-enrichment broths of Jarvis methods 2 and 3, and is further ***counterintuitive*** because Patentee's claimed method, by comparison with Jarvis and/or the rest of the asserted alleged prior art, substantially ***increases*** the workload by requiring independent statistically sufficient sampling from each of the plurality of test lots to form each of the respective plurality of independent test lot samples; that is, Patentee's method requires a substantial increase in sampling with attendant increase in cost (see also, the further Declaration of Dr. Mansour Samadpour, attached hereto in support of this Response and Amendment).

Moreover, Jarvis, FSIS and Midura, alone or in combination, do NOT teach or motivate "validation for release" in the presently claimed sense, because it is inescapably clear that retesting, after further enrichment, of individual stored statistically insufficient pre-enrichment cultures to "verify positives" is not statistically equivalent to retesting separate test lot samples,

each comprising a statistically sufficient number of sample portions to enable "validating" negative test lots for release into commerce.

Patentee respectfully contends that the Examiner' treatment of Jarvis, alone or in combination with any of the alleged asserted prior art, in the above aspects amounts to clear error and is manifestly unsupportable.

Finally, Jarvis, FSIS and Midura, alone or in combination, in addition to not teaching Patentee's claim limitations of: a plurality of separate test lots to be validated for release; a respective plurality of test lot samples each comprising a statistically sufficient number of sample portions; do not teach Patentee's "modular composite sample" element of step d), which is unique, and also counterintuitive as discussed in detail above (requires increased expense and sampling).

Patentee's modular composite sample is *fundamentally different* because it is formed by combining aliquots from each of a plurality of statistically sufficient test lot cultures (i.e., each comprising a statistically sufficient number of sample portions) to enable independent validation for release of each of the respective underlying plurality of separate test lots.

Patentee contends that Patentee's claims cannot be obvious in view of the alleged asserted art, alone or in combination, under U.S. patent law, because the prior art, alone or in combination, does not teach or motivate several of Patentee's presently recited claim limitations, including Patentee's unique "modular composite sample" claim element, which would be contraindicated (disincentive) by the prior art as discussed in detail herein.

B. Contrary to the Examiner's urging, one of skill in the art would NOT have "found it *prima facie* obvious to have subsequently followed up with additional testing when pre-enrichment composites test negative.

The examiner states that 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 negative because both FSIS and Midura contemplate additional testing of test lots depending on the initial testing results and Midura teaches that production lots of product with low numbers of the target pathogen requires collecting and testing additional samples."

Patentee disagrees, and respectfully points out that the teachings of Jarvis, FSIS and/or Midura, alone or in combination, are at best limited to retesting for positive samples to provide for an indication of the proportion of positive samples, as discussed herein above in detail. None of this art, alone or in combination however, contemplates or in any way motivates such retesting of negative test lot samples. ***First***, this is because the test lot samples of Jarvis, FSIS and/or Midura, alone or in combination, are not attributable to underlying separate test lots, but rather a single underlying test lot, and therefore given a positive composite test, there would be no motivation to validate a lumped test lot sample as negative, because the entire underlying test lot (not separate test lots as presently claimed) would not be releasable; that is, there would be no benefit, and so there is no such motivation. ***Second***, because the test lot sample of Jarvis, FSIS and/or Midura, alone or in combination, are not a plurality of separate test lot samples attributable to underlying respective separate test lots that have **each been sampled to statistically conform to a sampling plan to enable validation of each separate test lot**. Therefore, testing for a negative composite test lot sample would be statistically meaningless.

C. **Jarvis, alone or in combination with FSIS and/or Midura, does not disclose "pre-enrichment medium," or the incubation in same, as a medium that favors the growth of the target organisms in a way/extent that "meets the limitations of the patented claims," does not teach post-enrichment pooling as presently claimed, and further does not provide for lot validation by re-assay of prior art pre-enrichment cultures inoculated by lumped samples**.

As an initial matter, Patentee respectfully points out that Jarvis has been misconstrued in several material respects. ***First,*** contrary to the Examiner's statement, Jarvis does not disclose

"pre-enrichment medium" as a medium that favors the growth of the target organism in a way that "meets the limitations of the patented claims." As stated in Patentee's last Response, Jarvis adheres to, and does not *blur* the long-standing, art-recognized functional/operative distinction between "pre-enrichment" broth/medium and "enrichment" broth/medium, where functionally/operationally, testing for a target organism may meaningfully occur after enrichment in an enrichment broth/medium, and not in the absence thereof after a pre-enrichment incubation.

Patentee refers the Examiner to Patentee's detailed discussion of this point at pages 18 (under "Medium") through page 21 of this Response and Amendment.

See **also further Declaration of Dr. Mansour Samadpour on this point (para 27)**.

Moreover, as widely appreciated in the relevant art, including that of the presently cited Jarvis, Silliker, Price, etc., incubation in "pre-enrichment" broth/medium is a *resuscitative* culture/stage that is not subjected to organism testing absent subsequent enrichment because, while there may be some limited target organism growth during pre-enrichment, the number of target organisms achieved is not taught by any of these references, and would not be so interpreted by one of skill in the art, to be functionally/operationally sufficient for assay/testing, whereas incubation in enrichment broth/medium during an enrichment stage is recognized in the art as providing a level of target organism growth functionally/operationally sufficient for assay/testing. Jarvis, alone or in combination with FSIS and Midura, refers to and uses "pre-enrichment" and "enrichment" medium/incubation consistent with the art-recognized meanings, and nowhere, do these references alone or in combination teach or suggest testing (absent subsequent enrichment) for the organism after mere "pre-enrichment," thereby providing the "evidence to the contrary" requested by the Examiner. In further evidence of this, assays (1) through (3) described by Jarvis explicitly draw the art recognized functional/operative distinction between "pre-enrichment" and "enrichment" medium. In fact, Jarvis 1989, page141, first paragraph teaches that the function of the pre-enrichment media is to allow for multiplication of the target organism to a level that will ensure the transfer of at least one viable cell to the enrichment media. This would be reasonably understood by one of skill in the art to be different

that a level that would reasonably provide for detection without a requirement for further enrichment. Jarvis 1989 clearly states that if the pre-enrichment only resuscitates, then the entire pre-enrichment is to be transferred to the enrichment step—eliminating the possibility of later testing of the pre-enrichment sample (*Id*, "In methods which permit only resuscitation, not growth, during pre-enrichment, it is essential to use the entire pre-enrichment culture to inoculate double-strength enrichment medium (Hall, 1975), although selectivity may then be affected by the food constituents") (see also Jarvis, "On the compositing of samples for qualitative microbiological testing," Letters in Applied Microbiology, 2007, pp. 592-598, Volume 45; already of record).

In other words, growth in the pre-enrichment media of Jarvis is **not** to a level that would be understood by one of skill in the art to be detection level, and rather is either for resuscitation in which case the entire pre-enrichment is to be transferred to enrichment, or allows for limited growth to ensure that there is at least one viable organism in the portion which is transferred to the enrichment step. Moreover, the disclosure of Jarvis regarding pooling are limited to only one method, and organism (Salmonella).

Patentee's meaning and use of an "enrichment" incubation and medium is also consistent with the art-recognized meaning because, as in Patentees step c) of claims 1 and 17, enrichment incubation is under conditions suitable to expand target organisms to "to reach detectable levels and become uniform, or substantially uniform, throughout the respective one or more separate test lot samples" and organism testing is, in fact, conducted thereafter (no requirement for a subsequent incubation in an enrichment medium). This is not the case for the "pre-enrichment" medium of Jarvis or of the prior art generally, **which does not, therefore, satisfy the functional limitations of Patentee's claims**.

Patentee has, in view of the Examiner's comments, amended step e) to recite:

", 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)."

The amendment serves to further distinguish the asserted art, considered alone or in combination, by clarifying that the level of enrichment is sufficient for validation for release as presently claimed, which is NOT the case when using the pre-enrichment incubations of the asserted art. As stated above, while the levels attained in pre-enrichment incubations may be sufficient to ensure that there is at least one viable organism in the portion which is transferred to the enrichment step, as recognized by the Examiner, Jarvis teaches "stores" the pre-enrichment samples (i.e., The Examiner states that 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* (citing the paragraph bridging pages 141 and 142). That is, the art teaches storing of the pre-enrichment cultures, and does not teach continuing to incubate the pre-enrichment cultures during and after pooling and enrichment in Jarvis method 2 (i.e., for fear of overgrowth by competing organisms; increased background to noise signals), but rather that one can verify the proportion of contaminated samples by retesting of the "**stored pre-enrichment samples**." That is, Jarvis teaches storing of the pre-enrichment samples, and subsequently further enriching them prior to "retesting" for positives. Jarvis, alone or in combination with any other asserted art, therefore, does not even teach verifying a positive pre-enrichment culture by retesting stored pre-enrichment cultures, without further enriching them prior to the "retesting" for positives--and such prior art would certainly, therefore, not be reasonably construable as teaching validation of a negative test sample by retesting of stored pre-enrichment cultures without a requirement for further enrichment. Validation of a negative for release would, as appreciated in the art, be more stringent than verifying a positive (and would require having a statistically sufficient number of product portions in the sample to begin with) and if verifying a positive requires further enrichment, then validation of a negative (even if the prior art and Jarvis were to be construed as teaching such validation (which it does not)) would certainly require further enrichment of any stored pre-enrichment cultures, and such would be the understanding of a person skilled in the art. In summary of this aspect, Patentee provides methods, involving a plurality of separate test

lots and respective separate test lot samples each comprising a statistically sufficient number of sample portions sufficient for "validating" negative test lots for release into commerce—as opposed to merely enabling retesting, after further enrichment, of individual stored pre-enrichment cultures to verify positives.

Second, as stated in Patentee's last Response and consistent with the above distinction, Jarvis does not, as urged by the Examiner, teach inoculating the composite into a "second enrichment medium", and rather explicitly teaches initial inoculation of "pre-enrichment" broth, and subsequently "inoculating *the* enrichment broth" (emphasis added). Consequently, while Jarvis may teach compositing aliquots of the pre-enrichment cultures and inoculating the composite into the enrichment broth/medium, Jarvis does not teach or suggest compositing aliquots of enriched cultures as claimed by Patentee. This is an important, fundamental distinction, as the present Patentee is the first to conceive of and use post-enrichment pooling as presently claimed.

Third, it follows that even if Jarvis, alone or in combination with FSIS and/or Midura is construed to teach that composite testing method (2) has the advantage of permitting "the tests to be repeated on stored pre-enrichment cultures, so that some indication of the proportion of contaminated samples can be obtained" (see Jarvis at last sentence of page 142 carrying over to page 143), this: **(i)** would **necessarily involve delay** because of the need for inoculation of the stored individual pre-enrichment cultures (not sufficiently enriched for testing) individually into separate enrichment broth cultures, followed by enrichment incubation prior to assay/testing for a target organism; and **(ii)** while arguably allowing for *verification of positive* samples by retesting of the stored individual pre-enrichment cultures, would not provide for the fundamentally different statistical "**validation**" for release into commerce.(i.e., validation of no contamination or lack of presence of target organism) of individual pre-enrichment cultures in the context of prior art assays, such as Jarvis assay (3), comprising lumping into pre-enrichment cultures, followed by pooling thereof prior to enrichment. Again, this is because prior art statistical sampling/testing methods (including those described by Jarvis) involve sampling of a **single lot** and subsequent *intra*-lot sample pooling, where the total number of samples taken

from the single test lot, and collectively represented upon pooling of the pre-enrichment cultures, is a collective number chosen to statistically satisfy the statistical sampling plan of the single lot (e.g., 10 samples in the case of the Jarvis examples), **but where the number of samples lumped in each pre-enrichment culture (e.g., 5, as in Jarvis method (3)) is insufficient to validate/certify the lot for release even if such were possible**. Significantly, in the Jarvis method (3), for example, the samples are taken from a single lot, and even though the samples are grouped, for example, in two groups of five, any positive from the composite pre-enrichment pool must, by regulation, invalidate the lot, and accordingly there is no value, aside from curiosity to test the pre-enrichment cultures, to go back and verify a positive, and this is irrespective of whether you are lumping and pooling or not lumping and pooling, and there is certainly no motivation to go back and validate a negative, because there is effectively only one underlying test lot. In prior art assays where a single lot is sampled by forming a plurality of lumped samples, the number of samples lumped in each pre-enrichment culture is *insufficient* to provide for statistical ***validation*** of the lot (validation of no contamination) by assay of lumped pre-enrichment culture according to the statistical sampling plan, and thus no validation for release into commerce (i.e., validation of no contamination) of the pre-enrichment samples of Jarvis method (3) could be obtained upon repeated testing of stored pre-enrichment cultures. This is in *fundamental* contrast to Patentee's claimed invention comprising collective testing of multiple test lots and forming of a modular composite sample comprising aliquots of test samples that are each statistically sufficient and attributable to a separate underlying test lot, wherein, as recited in step (e) of claims 1 and 17, "when such testing is positive, individual separate test lots may nonetheless yet be **validated** for release into commerce by further testing of a portion of the respective enriched separate test lot sample… and obtaining a negative test result." Patentee's method comprises making a modular composite sample by pooling of enriched test lot samples/cultures each inoculated with a statistically sufficient number of samples to satisfy lot validation criteria, and each attributable to a respective underlying separate test lot, whereas prior art methods, including that of Jarvis, comprise pooling of pre-enrichment samples that are NOT attributable to separate underlying test lots, and are NOT formed by lumping a statistically

sufficient number of samples to satisfy lot **validation** criteria for release into commerce, as presently claimed.

Fourth, the Examiner appears to be implying that the statement in Jarvis—that "methods (1) and (2) and (3) provide the same degree of statistical confidence as would be attained by testing the 10 samples separately, in so far as a negative result indicates no sample contamination…"—indicates that one of skill in the art would consider that pooling of lumped samples (as in Jarvis assay (3)) and pooling of non-lumped samples (as in Jarvis assay (2)) would be equally reliable, and thus would be motivated to lump samples, with subsequent pooling of pre-enrichment broths. Patentee contends that such is not the case.

As stated in Patentee's last Response, the asserted Jarvis (first edition, 1989) reference contains no enabling experimental results or actual real-world assay data, and must be properly construed as the pure mathematical/statistical treatment that it is, which does not take into account the art-recognized factors of assay sensitivity, sample dilution and introduction of competitive organisms in the presented formulae and conclusion drawn thereon. For example, Jarvis (first edition) at page 117 states the premise/assumption that "[i]f each propagule is assumed to be capable of producing a growth response…," from which it would be immediately obvious to one of ordinary skill in the art that such assumption excludes such non-random variable such as assay sensitivity limits, sample dilution effects and competition from non-target organisms. Moreover, "Statistical confidence", as used by Jarvis, is a term of art describing the chance, upon repeating of a test, of getting the same result—assuming random variables. As would be immediately obvious to one of ordinary skill in the art, the art-recognized, non-statistical factors of assay sensitivity, sample dilution and introduction of/competition by competitive organisms are not random variables that can be ignored, and therefore one of ordinary skill in the art would not interpret the statement of Jarvis to mean that the methods (1) and (2) and (3) provide the same degree of real-world reliability of *negative* results—particularly where the threshold for freedom from salmonellae of "<11 organisms/kg" (at P = 0.95) (an exemplary threshold from the data in Table 8.9 of Jarvis at carryover paragraph between pages 137 and 138, where 10 samples are taken) recited in Jarvis is substantially above the real-world,

art-recognized Lot validation threshold of <1 organisms/kg in the food industry (e.g., for salmonella). **Patentee's conclusions in this regard are in fact supported by Jarvis himself**.

Specifically, Jarvis (second edition; JARVIS, "Errors Associated With Quantal Response Methods," STATISTICAL ASPECTS OF THE MICROBIOLOGICAL EXAMINATION OF FOODS, Chapter 8, pp. 143-176, 2008, Academic Press, Amsterdam, London, and San Diego; attached hereto), teaches and clarifies, as was already recognized and practiced in the art, that when variables such as assay sensitivity, sample dilution and introduction of competitive organisms are considered, wet compositing of previously lumped pre-enrichment samples into enrichment medium (e.g., as in assay (3) of Jarvis (first addition)) does not provide an acceptable degree of statistical confidence and reliability. Specifically, Jarvis (second edition) teaches that with respect to pure lumping methods (i.e., lumping all 10 samples at pre-enrichment stage, followed by inoculation of pre-enrichment broth into enrichment broth prior to assaying), sensitivity of the test method is a key issue, and that adequate control of competitive organisms must be exercised (see Jarvis second edition at page 170), and additionally teaches, with respect to wet compositing, that **"the only fully acceptable method of compositing is to inoculate 10 pre-enrichment cultures (i.e., one for each 25g sample) and, after incubation, to inoculate equal volumes of each pre-enrichment culture (i.e., 10 X 1 ml) into 100 ml enrichment broth"** and that "by retaining the original pre-enrichment samples, it is possible also to re-culture from these if the composite enrichment broth gives a positive result, so that the prevalence of positive samples can be determined" (*Id*). However, as appreciated in the art, verification of positive samples is fundamentally different that validation of negative samples.

Patentee further directs the Examiner's attention to the attached further Declaration of Dr. Mansour Samadpour, and in particular paragraphs 11, 12 and 19., relating to this point.

Patentee, therefore, maintains that Jarvis (first edition) would not be reasonably construed by one of ordinary skill in the art as teaching that pooling of lumped samples (as in Jarvis assay (3)) and pooling of non-lumped samples (as in Jarvis assay (2)) would be equally reliable, and thus would not be motivated to practice assays comprising lumped samples, with subsequent

pooling of pre-enrichment broths.

Patentee points out that the Examiner, in the present Office Action, failed to even acknowledge, discuss or respond to Patentee's comments relating to Jarvis (second edition reference), which were discussed in Patentee's last Response, and which support Patentee's position on the teachings of Jarvis.

Consistent with the teachings of Jarvis, the prior art, including the asserted prior art, generally *teaches away* from combining both lumping and pooling in a sampling and testing scheme. Specifically, Price et al (*Applied Microbiology* 23:679-682, 1972; reference "7" Exhibit 8) at page 681, left column, teaches that "Silliker (4) reported a loss in sensitivity when several samples were pooled in a large container of pre-enrichment broth as 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 salmonella 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." Additionally, at page 681, left column carry over paragraph, Price et al teach (with respect to the data of Table 2) that "[t]he results show that a higher number of positives were recovered by pooling pre-enrichments [individual pre-enrichments] rather than by pooling samples: and that "[i]n addition, pre-enrichment pooling yielded positives at lower levels of inoculation." On this point, therefore, **both Jarvis and Price** contradict the Examiner's use of the 'statistical equivalence statement of Jarvis (first edition).

Likewise, as stated in Patentee's last Response, Silliker & Gabis (*Can. J. Microbiol.* 19:475-479, 1973; reference 6; Exhibit 7) show in their data of Table 1 of page 477(compare the second and fifth column of data for 100 gm samples) that lumping combined with pooling results in false negatives, and, for example, state that "[i]n test 18, three of the fifteen 100-g subsamples were positive, but all of the wet composites were negative" and that "a minimum of one and a maximum of three positives would have been expected" (Id page 477 second column). Additionally Silliker & Gabis discuss that "Silliker (Appendix D of reference 2) reported that of

106 wet composites derived from 1060 individual sample, 39 were positive for Salmonella and 66 were negative. In seven instances negative results were obtained despite the fact that one or more of the 10 individual samples comprising the wet composite did contain salmonella. **Thus, these seven were false negative results**."

In summary of this aspect, therefore, the prior art asserted by the Examiner, and including Jarvis (first and second editions) when properly construed, does not teach or suggest post-enrichment pooling as presently claimed, and even in the case of pre-enrichment pooling generally *teaches away* from combining both lumping and pooling in a sampling and testing scheme.

Patentee maintains his contention that the teachings of Jarvis, alone or in combination with FSIS and/or Midura, **do not in fact raise any substantial new questions of patentability** beyond the teachings of alleged prior art (e.g., Price et al., and Silliker et al.,) already of record in the underlying issued patent prosecution history.

Furthermore, as discussed above (and below), one of skill in the art would recognize that prior art assays such as Jarvis (3) do not provide for statistical validation of pre-enrichment samples that tested negative, as presently claimed.

D. **Patentees's method comprises pooling of enriched test lot samples/cultures each attributable to a separate test lot, and each inoculated with a number of samples conforming to a statistical-based sampling plane to provide for validation of lots testing negative, whereas prior art methods, alone or in combination, including that of Jarvis (which is a reiteration of Silliker, Gabis, and Price), Silliker, Gabis and Price, etc., comprise pooling of pre-enrichment test lot samples/cultures that are NOT each attributable to a separate test lot, and are NOT each inoculated with a statistically sufficient number of samples such validation of lots testing negative for release into commerce is provided**.

Patentee's claimed methods comprise pooling of enriched cultures each inoculated with a number of samples sufficient to provide for statistical validation according to the demands of a

"statistically-based sampling plan." By contrast, prior art methods, including the hypothetical proposed statistical scheme of Jarvis, comprise pooling of pre-enrichment samples, where the pre-enrichment samples/lumped samples, are NOT each inoculated with a statistically sufficient number of samples to provide for validation for release as presently claimed.

As would be immediately evident to one of skill in the art from Patentee's teachings and claims, validation in step e) of a negative test lot sample (incubated sample of step c)) requires that the number of portions/samples lumped in step a), must be large enough to statistically provide for test lot validation according to step e). Such is not the case with prior art pooling regimes that comprise pooling of test lot samples that are not attributable to separate underlying test lots.

Patentee, to further emphasize this distinction, has nonetheless amended claim 1 as described herein:

"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~~, according~~ 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 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 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)."

Support for particular amendments is as discussed at pages 20-22 of this Response and Amendment. For example, support for recitation of "wherein each test lot is separately sampled, according to a statistical sampling plan for a particular microbial agent or organism" is found in the '584 patent, for example, at column 5, ll. 53-55, ll. 63-66, and see also at column 6, ll. 3-6 (stating that "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"—including the subsequent validation of negative lots for acceptance); and particularly at col. 5, ll. 63-66 (teaching that "the number of sample is selected so as to conform to statistical-based sampling plans.

As stated herein above, the *deficiencies* of Jarvis with respect to lack of teaching or motivation for formation of a modular composite sample from aliquots of enriched test lot samples/cultures that are each attributable to a respective separate underlying test lot, to enable

validation, are NOT cured by combination of Jarvis with FSIS, and Midura. Even if both FSIS and Midura are construed, as urged by the Examiner, to contemplate additional testing of test lots depending the initial test results, neither references teaches, suggests, motivates or otherwise provides for Patentee's presently claimed methods.

Moreover, Jarvis and Midura, to the extent that they teach (1) formation of lumped pre-enrichment sample that are all attributed to the same underlying test lot, and (2) resampling of the underlying test lot, actually ***teach away*** from the presently claimed subject matter as described in detail herein.

At page 12 of the ACP, the Examiner states that "Jarvis teaches collecting from 1 to 100 samples (see Table 8.9 at page 138) and exemplifies collecting 10 samples (page 141). Each of the individual samples disclosed by Jarvis meets the claimed limitation of a test lot comprising one or more specimens. Furthermore, the '584 specification defines "validation" as determining that a particular sample tests negative using a detection assay (column 7, lines 1-10). There is no disclosure in the '584 patent specification of any particular number of samples required for validation to occur. There is no disclosure regarding what number is a statistically significant number of samples." Patentees respectfully disagree.

First, with respect to the statement "Each of the individual samples disclosed by Jarvis meets the claimed limitation of a test lot comprising one or more specimens," Patentee again points out that in Jarvis only a single underlying food lot is being sampled, and while the collective number of samples taken may be statistically significant with respect to the one underlying test lot, the lumped test lot samples (e.g., of Jarvis assay (3)) are not attributable to different underlying test lots (but rather are from one common test lot), and even if they were there is not teaching or motivation in Jarvis, alone or in combination with any other asserted art, for sampling each of the lumped test lots of Jarvis to comply with a statistically-based sampling plan for the test lot as presently claimed.

Second, with respect to the statement "Furthermore, the '584 specification defines "validation" as determining that a particular sample tests negative using a detection assay (column 7, lines 1-10). There is no disclosure in the '584 patent specification of any particular

number of samples required for validation to occur. There is no disclosure regarding what number is a statistically significant number of samples", Patentee points out that claim 1 has been amended to recite "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 and growth conditions sufficient to provide for validation and release of the test lot into commerce." The specification teaches that validation refers to "determining that a particular sample tests negative" such that :the test lots can be "cleared for release" (see, e.g., column 7, defining "validation" in terms of cleared for release; also see under Example 1 at column 11, lines 40-44 teaching "validated and releasable into the chain of commerce." Additionally, the specification teaches that validation is with respect to a statistical-based sampling plan (e.g., see col. 1, ll. 56-58, ll. 62-64; col. 2, ll. 34-37; col. 4, ll. 42-44; col. 5, first carry-over paragraph; and in particular at col. 5, ll. 50-56, the carry-over paragraph from col. 5 to col. 6, and col. 7, first full paragraph ("cleared for release"), and most particularly at col. 5, ll. 63-66 (reciting that "**the number of samples is selected to conform to a statistical-based sampling plan**").

Patentee contends, therefore, that it would be clear to one of ordinary skill in the art that the number of samples is selected to conform to a statistical-based sampling plan, and that this number provides for validation of negative test lot samples for release into commerce, as presently claimed.

Patentee further contends that, contrary to the Examiner's statement, Patentee's patent does provide specific examples of the number of samples to be taken to conform to particular statistical-based sampling plans (see, e.g., col. 2, ll. 14-16, and ll. 16-18 (*E. coli* O157 testing, and *Listeria moncytogenes* testing). Moreover, Patentee's specification further teaches that the number of samples will vary depending on the particular plan, based on "the outcome of the infection/poisoning (e.g., the severity), the level of hazards, and potential increase in the hazard levels due to storage," generally reflecting the "overall sensitivity of the test method and the limit of detection that applies to the test unit,..." (see col. 2, ll. 1-26). Patentee contends therefore, based on Patentee's teachings and the general knowledge in the art regarding statistical-based

sampling plans, that one of skill in the art would know how to practice the claimed invention, without undue experimentation, by sampling to conform to a statistical-based sampling plan for a particular microbial agent or organism, such that negative test lot samples/cultures could be validated for release into commerce. There is no need therefore, for Patentee's independent claims to be limited to any specific number, as this would depend on the particular microbial agent or organism and the particular statistical-based sampling plan.

At page 13 of the ACP, the Examiner states that "Regarding the argument (pages 17-18) that the prior art generally teaches away from combining both lumping and pooling in a sampling and testing scheme, as in the instant claims, Patent owner is once again reminded that the test lot of the instant claims consists of as little as two portions of a single sample, and so encompasses test lots of as little as one or two samples "lumped" together. Even so, Jarvis directly exemplifies combining lumping and pooling in a sampling and testing scheme as option 3 (page 141), wherein 5 samples are composited into a pre-enrichment broth and subsequently, two composited pre-enrichment broths are pooled and inoculated into the enrichment medium. Jarvis teaches that the combination provides the same degree of statistical confidence as is attained by testing the samples separately."

Patentee respectfully disagrees. ***First***, Patentee's method recites "collecting a plurality of portions from each of a plurality of reportable test lots." Second, as described above in detail, Patentee contends that Jarvis has been misconstrued in this regard. As stated in Patentee's last Response, and reasserted herein, the asserted Jarvis (first edition, 1989) reference contains no enabling experimental results or actual real-world assay data, and must be properly construed as the pure mathematical/statistical treatment that it is, which does not take into account the art-recognized factors of assay sensitivity, sample dilution and introduction of competitive organisms in the presented formulae and conclusion drawn thereon.

Moreover, as stated in Patentee's last Response, and reasserted herein, **Patentee's conclusions in this regard are in fact supported by Jarvis himself**.

Specifically, Jarvis (second edition; JARVIS, "Errors Associated With Quantal Response Methods," STATISTICAL ASPECTS OF THE MICROBIOLOGICAL EXAMINATION OF

FOODS, Chapter 8, pp. 143-176, 2008, Academic Press, Amsterdam, London, and San Diego; attached hereto), teaches and clarifies, as was already recognized and practiced in the art, that when variables such as assay sensitivity, sample dilution and introduction of competitive organisms are considered, wet compositing of previously lumped pre-enrichment samples into enrichment medium (e.g., as in assay (3) of Jarvis (first addition)) does not provide an acceptable degree of statistical confidence and reliability. Specifically, Jarvis (second edition) teaches that with respect to pure lumping methods (i.e., lumping all 10 samples at pre-enrichment stage, followed by inoculation of pre-enrichment broth into enrichment broth prior to assaying), sensitivity of the test method is a key issue, and that adequate control of competitive organisms must be exercised (see Jarvis second edition at page 170), and additionally teaches, with respect to wet compositing, that **"the only fully acceptable method of compositing is to inoculate 10 pre-enrichment cultures (i.e., one for each 25g sample) and, after incubation, to inoculate equal volumes of each pre-enrichment culture (i.e., 10 X 1 ml) into 100 ml enrichment broth"** and that "by retaining the original pre-enrichment samples, it is possible also to re-culture from these if the composite enrichment broth gives a positive result, so that the prevalence of positive samples can be determined" (*Id*). However, as appreciated in the art, verification of positive samples is fundamentally different that validation of negative samples.

At page 15 of the ACP, the Examiner states that "At page 22, Patent owner argues in summary that the patented claims are more than a predictable use of prior art elements according to their established functions. As has been set forth herein, all of the elements of the patent claims are taught in the prior art and they are taught for the same established functions as in the' 584 patent claims." Patentees respectfully disagree.

Patentee contends that the teachings of Jarvis in combination with either or both of FSIS and Midura **do not in fact raise any substantial new questions of patentability** beyond the teachings of art (e.g., Price et al., and Silliker et al.) already of record in the underlying issued patent prosecution history. The art, including that cited by the Examiner, does not teach, suggest or motivate Patentee's presently claimed subject matter comprising collective testing of a plurality of test lots, using a respective plurality of attributable, lumped enriched test

samples/cultures that are each sample to conform to a statistical-based sampling plan to enable validation and release of individual test lot samples into commerce.

As previously argued, various rebuttal points above may be illustrated in the context of following beef combo testing example. Under Jarvis, a carload of beef trim consisting of 5 "combo" boxes of 2000 pounds of beef trim each, would be sampled as a whole, with a statistically valid number of samples being drawn from the overall contents of the box. Thus, 60 samples might be taken from the carload, with perhaps 12 samples taken from each combo, or perhaps more or less from each combo—Jarvis does not define or require any particular method of sampling different parts of the overall carload to be sampled.

Because Jarvis does not define how sub-domains of the overall testing space must optimally be sampled to afford the possibility for later vindication of these sub-domains by later testing, the later testing of Jarvis of retained samples is not for the purpose of allowing portions of the carload to be cleared for release, but rather merely allows for additional tests "...so that some indication of the proportion of contaminated samples can be obtained." But obtaining an "indication of the proportion of contaminated samples" from additional testing does nothing to achieve a benefit of the present invention, which provides a way for some combo boxes from the carload of beef to be immediately validated and released to commerce, by means of an additional test upon a saved pool sample that contained ONLY samples from that particular combo.

Suppose that the method proposed by Jarvis were to be used after it was found that some beef in a carload was found to be contaminated. Since, under Jarvis, there was no teaching that samples from each combo of the 5-combo carload should have been kept segregated into their own, individual enrichment containers, there would be no way to trace back the contamination from the overall pool tested back to an individual combo. But even if such tracing could be accomplished, the combo could still not be validated for release to commerce for a simple reason: nothing in Jarvis, or any other asserted art, teaches the value of sampling each combo unit by taking a number of samples sufficient to validate that combo standing on its own. For beef testing, for example, this means taking about 60 samples from each combo, since anything less will not provide the level of statistical confidence for regulatory compliance.

The present invention teaches that each combo must be sampled as a separate test lot, such that it may be validated with a number of samples that is statistically adequate according to the statistical-based sampling plan, for that combo standing alone, if need be. By starting out with this number of samples for each combo, a surprisingly useful result is achieved in the event that the larger pool comes up positive. The surprising result is that a single simple test, applied to the enrichment vessel that was dedicated exclusively to that combo, can then validate or not validate that combo for release to commerce. In other words, the re-testing suggested by Jarvis, if done, merely provides a bit of information about "the proportion of positive samples," which is of no economic value at all. On the other hand, individual enrichment broth testing in the present invention yields a profound result—boxes of beef vindicated and clear for release into commerce, and the savings of thousands of dollars.

By contrast, since Jarvis does not teach statistically significant sampling of each combo before pooling, if the teachings of only Jarvis are used, it would be necessary to go in and do additional sampling on each combo after the positive was found. For example, according to the typical rule, any lot of beef, of whatever size, must have at least 60 samples taken for validation. So, if the original Jarvis sampling plan only did 60 samples for the overall carload of 5 combo boxes, then it would be necessary to now re-sample by taking 60 samples from each combo, then enriching each separately, and testing. This means sampling would essentially start from scratch, and no immediate release from testing of an already-processed enrichment broth container would be possible. Additionally, the user of only the Jarvis approach would be confronted with a more serious issue: Current government guidelines do not allow beef, once sampled, to be re-sampled, and rather, a positive sample having been found, all of the beef would be designated for disposal.

In overall summary, Patentee's invention is **more than a predictable use of prior art elements according to their established functions**. Specifically, the formation of Patentee's modular composite sample is by combining aliquots of a plurality of lumped test lot samples/cultures, each attributable to a respective plurality of underlying test lots, and each sampled to conform to a statistical-based sampling plan, which aspects enable validation and release of individual respective test lots. The asserted prior art, alone or in

combination, has no such modular composite sample, and no such plurality of lumped test lot samples/cultures, each attributable to a respective plurality of underlying test lots, and each sampled to conform to a statistical-based sampling plan, and therefore the composite/lumped samples of the prior art do NOT provide the validatation and release function in the context of the claimed method. In fact, the lumped test lot samples/cultures of the prior art relate to a single underlying test lot, and the established function of the lumped test lot samples/cultures of asserted Jarvis, FSIS, Midura, Price, Silliker, etc., references is to reduce the work and expense of sampling, **not increase the work and expense as required to provide for validation and release of individual respective test lots as presently claimed**.

Jarvis, alone or in combination with FSIS and Midura, does not disclose "pre-enrichment medium" and incubation therein, as a medium/method that favors the growth of the target organisms in a way/extent that "meets the limitations of the patented claims," does not teach post-enrichment pooling of aliquots of test samples/cultures that are individually attributable to respective separate test lots, does not provide for real world validation of pre-enrichment cultures inoculated by lumped samples, and further does not teach forming of separate lumped enriched test lot samples/cultures—each inoculated with a number of samples to provide for validation of lots testing negative.

Finally, as acknowledged by the Examiner at page 17 of the ACP with respect to claim 4, Jarvis, FSIS, and Midura, however, lack the motivation to substitute direct enrichment for pre-enrichment," and "there is no motivation found in any of Jarvis, FSIS, or Midura to eliminate the pre-enrichment stage and directly inoculate the dry food samples into individual selective enrichment broth cultures for subsequent pooling and testing. Patentees point out, therefore, that the Examiner has effectively acknowledged that the intent of the asserted art was not to practice or motivate a method 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 required further enrichment thereof.

No teaching of Patentee's claim elements:

Finally, Jarvis, FSIS and Midura, alone or in combination, in addition to not teaching Patentee's claim limitations of: a plurality of separate test lots to be validated for release; a respective plurality of test lot samples each comprising a statistically sufficient number of sample portions; do not teach Patentee's "modular composite sample" element of step d), which is unique, and also counterintuitive as discussed in detail above (requires increased expense and sampling).

Patentee's modular composite sample is *fundamentally different* because it is formed by combining aliquots from each of a plurality of statistically sufficient test lot cultures (i.e., each comprising a statistically sufficient number of sample portions) to enable independent validation for release of each of the respective underlying plurality of separate test lots.

Patentee contends that Patentee's claims cannot be obvious in view of the alleged asserted art, alone or in combination, under U.S. patent law, because the prior art, alone or in combination, does not teach or motivate several of Patentee's presently recited claim limitations, including Patentee's unique "modular composite sample" claim element, which would be contraindicated (disincentive) by the prior art as discussed in detail herein.

Patentee, for all of the above reasons, and based on the present claim amendments, respectfully requests withdrawal of the Examiner's rejection of Patentees claims, under 35 U.S.C. 103(a) over Jarvis in view of FSIS and Midura.

Ground #2. The Examiner rejected claims 1-4, 6-18, 20, 22, 24-26, 31 and 34 -36, under 35 U.S.C. 103(a), as being unpatentable over FSIS in view of Price and Midura.

Overview. Patentee contends, as discussed below, that Price merely teaches taking multiple samples from a lot of food to be tested, pre-enriching, pooling pre-enrichments, enriching the pooled pre-enrichments, and testing—and therefore does not extend beyond Jarvis (who presents Price's work) in combination with FSIS and Midura, which Patentee contends do

not, alone or in combination, obviate Patentee's invention as discuss in considerable detail herein above under Ground #1 (moreover, Price inherently appears to already comprise all of the relevant issues of FSIS and Midura).

Claims 34-36 have been cancelled herein.

The Examiner, with respect to claims 1, 17, 18, 20, 24, and 34-36, states that FSIS discloses: methods of sampling ground beef for *E. coli* 0157:H7 contamination, comprising collecting a plurality of pieces or samples per lot and combining the pieces for a representative sample of a production lot (citing pages 4 and 27-28 in their entirety).

Patentee respectfully disagrees, FSIS teaches taking a plurality of samples from a production lot defined as a day of production, and compositing the samples by putting them in a bag. FSIS anticipated testing their composite sample with their method which is dividing the sample in to a few subsamples, and enriching and testing each sample individually. The FSIS sampling plan is a part of the FSIS testing method, there is no anticipation, nor expectation of using the sampling protocol for any other method. Both sampling and analysis in FSIS are fundamentally different than Patentee' claimed subject matter.

Moreover, the Third Party Requestor refers to Exhibit 6, Appendix 2, Page 27, middle section entitled "Randomized or Representative Sampling". This is an FSIS Directive issued to FSIS field personnel regarding collecting a sample. When the sample was returned to the FSIS, lab analysis was governed at that time by FSIS Microbiological Laboratory Guide 5.03 (FSIS MLG 5.03, provided with email). The collected composite sample weighed 1.5 lbs (Directive). In the lab five (5) subsamples of 65 g each (325 g total) were removed from the composite sample and analyzed separately (pg 5, Section 5.5) simply because the lower detection limit was believed to be 1 cfu/65 g. Thus (a) not even the entire sample was used; (b) there was no provision for going back and 'uncompositing' the composite sample for any further action.

Patentee emphasizes that FSIS does **NOT** teach a reportable test lot corresponding to a single combi bin—and rather teaches a reportable lot comprising multiple combi bins. For example, at pages 29 and 28, cited by the Examiner, under "2. Space", FSIS teaches determining the lot size (i.e., the number of "containers to be sampled") as "the square root of that number"

of "boxes or combo bins containing the requested product types" and "round up to the next whole number (i.e., if the number of boxes is 29, the square root is 5.38; the next whole number is 6). That number, and no more than 10, is the number of containers to be sampled. Moreover, **as widely appreciated in the art**, FSIS has been moving towards adaptation of ICMSF sampling plan recommendations in the testing of beef products for *E. coli* O157:H7. FSIS Notice 73-05, "NATIONWIDE RAW GROUND BEEF COMPONENT MICROBIOLOGICAL BASELINE DATA COLLECTION PROGRAM – UPDATE" dated 11/10/05, referenced computer-based training (CBT) to be provided to FSIS personnel as to procedures to be used to collect beef samples. The CBT instructions call for collection of 12 samples each at random from the top layers of trim in 5 combos of product. A lot is comprised of 5 combos, and thus a total of 60 samples are collected from a lot. The overall sampling plan follows the recommendation of ICMSF for a Case 15 sampling plan, which is the most stringent of those recommended by ICMSF and appropriate for the kind of food, degree of handling, and expected future handling of the product.

FSIS, therefore, actually follows the ICMSF sampling plan recommendations, which establish a reportable test lot of 5 combi bins (not a single combi bin). FSIS, therefore, alone or in combination with Midura and/or Price, does NOT teach, suggest, or otherwise motivate using lumped test lot samples/cultures that are attributable to a *respective* single combi bin test unit. Rather, the lumped samples of FSIS are attributable to a 5 combi bin lot, and if the lumped sample is positive—the entire 5 combi bin lot is destroyed and/or diverted.The Examiner, with respect to claims 2, 10 and 36, states that FSIS (citing page 4, last paragraph and page 27) teaches aseptic collection for current day's production, at specific times, or by specific combo bins, which the Examiner states is equivalent to a teaching of a test lot consisting of a single combo bin. Again, FSIS's description of the sampling method applies to taking a set of samples from the entire day's production, the sample is composited and per FSIS test method MLG 5.03, the sample is analyzed in 65 gram portions, which is the reverse of the pooling process. Patentee respectfully submits that the FSISs sampling has nothing to do with the above mentioned claims, which are about taking a plurality of samples, from a plurality of lots and testing them using the

claimed modular compositing method.

Patentee respectfully points out, and as discussed above, that the Examiner's position in this regard is fundamentally *erroneous* and unsupportable in view of FSIS, and as would be immediately appreciated in the art. FSIS, alone or in combination with Midura and/or Price, does NOT teach, suggest, or otherwise motivate using lumped test lot samples/cultures that are attributable to a *respective* single combi bin test unit. Rather, the lumped samples of FSIS (which generally follows the ICMSF sampling plan recommendations) are attributable to a 5 combi bin lot, and if the lumped sample is positive—the entire 5 combi bin lot is destroyed and/or diverted. FSIS, alone or in combination with Midura and/or Price, does NOT, therefore teach, suggest or otherwise motivate forming test lot samples/cultures by collecting a plurality of samples from a plurality of separate test lots, wherein each lumped test lot sample/culture is attributable to a respective separate test lot.

The Examiner, with respect to claims 8 and 12, states the FSIS (citing page 8-9 and 23) 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 (due to the turn-around time for analysis, they will at a minimum be separated by several days and sanitation breaks). For the FSIS the purpose of the sampling is to generate one or more negative results which will verify that the corrective actions were successful. It has nothing to do with Patentee's presently claimed subject matter. Furthermore, the sample is only analyzed by the FSIS method, which does not allow for pooling.

Again, re-sampling is not allowed in the context of validating test lots. The "follow-up" sampling (expected to be double the number of subsamples) discussed at pages 8-9 and 23 occurs after "the establishment has taken its corrective action," and does not change the fact that

the underlying test lot is 5 combi bins, and is thus unrelated to the presently claimed methods for sampling, testing and validating a plurality of test lots for microbial contamination.

Patentee respectfully contends that the Examiner's position regarding the above alleged teachings of FSIS is fundamentally misconstrued and unsupportable in view of FSIS, alone or in combination with Price and/or Midura.

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. Patentee points our that 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 FSIS MLG 5.03. The point is that Exhibit 6 is one slice of a complete set of rules and procedures that govern FSIS actions, and the method of analysis was clearly specified within this set, just not explicitly in this document.

The Examiner, with respect to claims 1, 17, 34 and 35, states that 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).

Patentee respectfully disagrees. Price merely teaches collecting a plurality of samples from a **single lot** of food. Price attempted to ***reduce*** the workload associated with the FDA BAM test protocol for sampling and testing foods for *Salmonella* (i.e. "Therefore, a test

procedure that gives a single negative answer for many negative samples offers an important saving in the analytical effort required for quality control." Page 679, first paragraph left column, 2nd sentence.). As such it is drastically and fundamentally different from the invention claimed in Patentee's claimed subject matter, as discussed in considerable detail in Patentees rebuttal of Ground #1, all of which rebuttals are applicable to the presently asserted rejection based on Price, FSIS and Midura.

At page 22 of the ACP, the Examiner presents a block quote of Price saying "[i]f many food samples are pooled...". From this, the Examiner jumps, without support, to the notion that Price was pooling samples from "other food lots." Patentee points out, however, that Price says that the analyses were carried out in accordance with BAM methods, where BAM teaches about how single lots are sampled, which contradicts the examiner's unsupported urging that "other" food lots were involved in the sampling of Price.

In this regard, the Examiner says that the Price samples were "powders which would be expected to have any agents or target organisms homogeneously distributed throughout..." and states that Midura teaches that for products such as these, a single sample may be considered representative of the lot but that for solids and semi-solids, where the levels of target organisms vary throughout the specimen, multiple portions should be sampled from different locations.

Patentee respectfully points out that the Examiner has misconstrued Midura at section 2.522 (cited by the Examiner). In fact, Midura states that even for bulk liquid material, multiple samples are required because of a possible lack of "adequate mixing." This would also be true where the number of pathogentic organisms was very low—no amount of mixing would homogenize them uniformly in the sample. **Patentee further directs the Examiner's attention to the attached further Declaration of Dr. Mansour Samadpour, and in particular paragraphs 13 though 18., relating to this point**.

Therefore, the Examiner's position in this regard presumes adequate mixing, and one of skill in the art faced with complying to a regulatory sampling and testing plan would not be able to presume adequate mixing to homogeneity (if such in fact exists). Therefore, the Examiner's cited support for this "expectation" actually supports that such expectation is unfounded,

particularly since powders are subject to many sources of non-homogeneous contamination, for example from agents that would not be homogeneously distributed no matter how much adequate mixing was performed (e.g., wherein the pathogen is present in very low concentrations, or external contaminated agents/foreign objects that gratuitously find there way into powders). For example, a discrete foreign object carrying pathogens would yet be a descrete foreign object carrying pathogens after mixing in a powder.

Despite the lack of support for presuming adequate mixing to homogeneity for the samples of Price, the Examiner yet attempts to equate the idea of a "representative sample" with "multiple portions from the entire lot." Regardless of whether there is a basis for the Examiner's presumption that that a portion of powder can be thought of as a plurality of smaller portions of powder, this logic does not result in supporting the Examiner's assertion that Price teaches the present step a) claim limitations, which require "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…" (emphasis added). Patentee points out that a "portion" is a an amount of material that is "apportioned," i.e., separated from the bulk, by the associated act of collecting that portion. Physically collecting a plurality of separate portions in NOT equivalent to collecting one big portion, and one of ordinary skill in the art would understand this. Patentee's language equates the concept of a plurality of "portions" with that of a plurality of "samples," and the "portions" or "samples" are THAT plurality of portions each of which was taken (collected)—and not some infinite regression of theoretical sub-portions which make up every level of a larger portion.

Patentee contends therefore, that not only is the Examiner's reliance on Midura to support that powders can be sampled with a single sample flawed because of Midura's own teaching that one cannot presume adequate mixing (as taught in section 2.522 of Midura cited by the Examiner), but also that **collecting a plurality of portions is explicitly NOT collecting one portion**.

In any event, Patentee respectfully submits that if the Examiner is relying on "common knowledge" or "taking official notice," with regard to the statement that "the powders of the

prior art are in fact homogeneous" then the Examiner must provide adequate documentary evidence if the basis of the rejection is to be maintained. *See* 37 C.F.R. §1.104(c)(2); MPEP 2144.03. Patentee further submits that if the Examiner is relying on personal knowledge to support the finding of what is known in the art, the Examiner must provide an affidavit or declaration setting forth specific factual statements and explanation to support the finding. *See* 37 C.F.R. §1.104(d)(2). Furthermore, according to MPEP 2144.03 (C), "if Patentee challenges a factual assertion as not properly officially noticed or not properly based upon common knowledge (as in the present case), the Examiner must support the finding with adequate evidence the based on the fact that the Examiner failed to provide adequate support.

Patentee, therefore, requests for this and other reasons elaborated throughout this Response, that in the event the presently presented claims are not allowed, that prosecution be reopened.

The Examiner states that "Price defines the medium used for incubation of the individual test lot samples as a '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 either 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) or they simply recite incubating the set of separate test lot samples under conditions suitable to allow levels of a target microbial agent or organism to increase in the test lot samples to provide a set of separate incubated test lot samples *(claims* 34 *and* 35). 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."

Patentee respectfully disagrees, and contend that there is a fundamental distinction between the attained levels of enrichment between Patentee's claimed methods and those of the prior art, including Price alone or in combination with FSIS and/or Midura, employing "pre-enrichment" incubation.

Patentee directs the Examiner to the Patentee's detailed rebuttal of this point at pages 18 (under "Medium") through 21, in the context of Ground #1, which rebuttal is fully applicable here.

As an initial matter and as discussed above in relation to the Examiner's assertion of Jarvis in combination with FSIS and/or Midura, Patentees have amended step a) of claims 1 and 17 to recite:

"…a number of portions to be taken and growth conditions sufficient to provide for validation and release of the test lot into commerce,"

and have further amended step e) of claims 1 and 17 to recite:

", 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).

Explicit support for the amendments has already been cited herein above, for example, in Patentee's patent at col. 5, ll. 63-66 (number of sample is "selected so as to conform to statistical-based sampling plans"); original claims 3 (teaching "[t]he method of claim 1, further comprising incubating the modular composite sample of d), prior to testing in e)"); and see, for example, col. 1, last carryover paragraph through col. 2 line 26.

The amendments serve to distinguish the asserted prior art by clarifying that, unlike prior art methods comprising pre-enrichment incubation in pre-enrichment broth, the incubation of Patentee's step c) is sufficient, if the microbial agent or organism is present, to achieve an extent of enrichment sufficient to enable **validation** of negative test lot samples according to the statistical-based sampling plan with no required further enrichment. As recognized by the Examiner, Price (like Jarvis) teaches that the pre-enrichment cultures are stored, and may be

subsequently retested—but this would ***require*** (like the methods (1), (2) and (3) of Jarvis) incubation of the pre-enrichment culture, or an aliquot thereof, in enrichment medium prior to said retesting (see Price at page 679, under "Materials and Methods"). Moreover, in any event, as stated above in the case of Jarvis, Patentee's invention is directed to collectively testing a plurality of reportable statistically sufficient separate test lots each attributable to an underlying separate test lot to be validated to release into commerce, whereas the asserted art, including Price alone or in combination with FSIS and Midura, is directed a testing a **single** test lot for release (the test lot of Price corresponds to a single lot of dried egg albumin, cocoa or nonfat dry milk, and the lumped samples of Price were effectively attributable only to such a single underlying test lot). Patentee's method further provides methods for "validating" negative test lots for release into commerce—as opposed to merely enabling retesting, after further enrichment, of individual stored pre-enrichment cultures to verify positives.

The Examiner states, regarding *claim 3*, that Price teaches incubating the composite broth culture *(i.e.,* the modular composite sample) prior to testing.

Patentee respectfully contends that the Examiner misconstrues Price. Price teaches pooling the pre-enrichment broths, after incubation in groups of up to 15, and inoculating selective enrichment broths. The intent of Price is to take the level of target organisms to detection, the intent of claim 3, is if the laboratory is not prepared to conduct the detection test, it allows for optional further incubation. The purpose of claim 3 and Price as stated are fundamentally different.

The Examiner states, with respect to ***claim 4***, that "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.)"

Patentee respectfully disagrees. Price stipulated two pre-enrichment media, one was distilled water with an inhibitor dye (Brilliant Green), which anyone with skills in the art would

agree that lacks sufficient nutrients to be an enrichment broth (would correspond to no growth, requiring transferring entire pre-enrichment without retention). The second one is 0.5% lactose broth (see prior discussion regarding fact that *Salmonella* does not ferment Lactose, the purpose of lactose broth is to allow the background flora to grow and drop the pH). Patentee objects to the Examiner's reference to the patent's description of the enrichment media, while ignoring the prior paragraph in '584 which describes the function of the enrichment media, which as specified in the patent is to take the target organisms to detectable levels—particularly where Patentee's independent claims do not recite "enrichment medium." Again, the function of incubating in Patentee's step c) is fundamentally different than the function of incubation in the pre-enrichment media as established in the art and asserted by Price/Jarvis/Gabis and/or Silliker, alone or in combination, which is to take the target organisms to a point at which an aliquot transferred to the enrichment broth would contain at least 3 viable cells.

The Examiner states that "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).

Patentee points out that the Examiner's position in this regard is inconsistent, because while the Examiner has yet failed to recognize that the both Silliker and Price actually teach against compositing before pre-enrichment, the Examiner yet tries to use Price's explanation of why compositing the samples before pre-enrichment is a bad idea, to assert that pre-enrichment allows for growth of Salmonella. The Examiner's conclusion in this regard is wrong. No one claimed that pre-enrichment in lactose broth does not allow for growth of *Salmonella*—and rather Patentee's argument is that it does not allow for the growth to detection levels as appreciated in the art. As widely appreciated in the relevant art, the reason that inoculation of pre-enrichment into the enrichment media allows for *Salmonella* to compete better is that the low pH of the spent lactose broth retards the growth of all of the 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. Price does not teach that pre-enrichment is sufficient to get the *Salmonella* levels to detection levels, **if he had, he would have called it enrichment**, and would have tested after pooling, without inoculating into a different media and incubating for an additional day!

The Examiner states that "absent evidence to the contrary, the pre-enrichment broth disclosed by Price meets the definition of enrichment medium in patented **claim 4**."

Patentee contends that the above rebuttal in combination with Patentee's detailed rebuttal of this point at pages 18 (under "Medium") through 21, in the context of Ground #1, which rebuttal is fully applicable here, provides sufficient evidence to the contrary on this subject.

Furthermore, Patentee points out that sampling according to a statistical-based sampling into pre-enrichment broth and growing to detection levels would be strongly contraindicated in the prior art, due to the fear of higher probability of introducing contamination and outgrowth of contaminants with increased number of lumped sample portions. This is why even Jarvis method 1 limits the pre-incubation, and relies on enrichment incubation of enriching to detection.

Likewise, the assertions in this regard by the Third Party Requestor are contrary to the art and science of microbiology. Their own asserted references teach the role and function of the pre-enrichment (e.g., Jarvis, Silliker-Gabis, Price, and FDA BAM explain the function of pre-enrichment).

The Examiner states, regarding *claim 6*, that 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*.

Patentee respectfully points out that Price cannot properly be used to provide teachings recited in Patentee's claim 6, since Price's testing of the pre-enrichment is not for the purpose of release of the product, rather it is for the purpose of determining the extent of contamination. Price uses a 1-class attribute with plurality of samples taken from a lot, and c=0, (per FDA BAM, and per Jarvis 1989 and Jarvis 2002). As such if Price wants to retest the pre-enrichment, Price must go to the archival samples, wherein, after 4-6 days of storage (usual amount of time to

conclude the test), the microbiological characteristics have changed—and this is why Jarvis emphasizes that method 2 at least in theory allows for retesting of the pre-enrichments for the purpose of determining the extent of contamination (Jarvis 1989). By contrast, retesting of the individual enrichments in claim 6 allows for determination of which of the enrichments are negative, which in itself allows for release of products.

The Examiner states, with respect to *claims 22 and 31*, that 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).

Patentee reaffirms and reasserts Patentee'e rebuttal argument against the assertion of Jarvis on the same point. Price is about pooling of pre-enrichments of individually enriched samples from a single lot, as such it is drastically and fundamentally different from Patentee's presently claimed invention (and that of the Patent '584).

Midura. The Examiner states that Midura "discloses methods for sampling and testing foods for microbial contamination. 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, shellfish, 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."

Patentee reaffirms and reasserts Patentee's rebuttal argument against the assertion of Midura in the context of Jarvis on these same points. As discussed above in the context of Jarvis in view of FSIS and Midura, Midura teaches **absolutely nothing**, alone or in combination with Price and/or FSIS, that would cure the deficiencies of Price and/or FSIS, as described above and below, with respect to simultaneous testing a plurality of separate test lots according to a statistical-based sampling plan and method that would provide for validation and release of separate test lots based on assaying individual test lot sample cultures that are attributable to respective separate test lots, as presently claimed.

For example, merely teaching that at specific number of samples is to be collected (whether initially, or in combination with subsequent re-sampling) does not provide for validation and release as presently claimed, because there is no relating in any of Price, FSIS, and/or Midura that a plurality of samples are taken separately from each of a plurality of test lots to form a set of respective attributable test lot samples, wherein the number of samples taken in forming each test lot sample conforms to a statistical-based sampling plan to provide for validation and release of individual test lots based on testing of the test lot samples. None of the references, alone or in combination, teaches the presently claimed subject matter, and in fact, Price, FSIS and Midura actually ***teach away***, by teaching testing with respect to a single underlying test lot, and by teaching re-sampling.

Patentee's Traversal.

Patentees respectfully traverse the Examiner's rejection, based on Patentee's rebuattal arguments under Ground #1 above (Jarvis, FSIS and Midura), and the limited teachings of FSIS, Price and Midura as follows.

FSIS Directive. As discussed above, the FSIS directive provides instructions for sampling raw beef as part of validation testing for *E. coli* O157:H7. At page 4, cited by the Examiner, paragraph 4. states that personnel collect samples from the current day's production. At pages 27-28, cited by the Examiner, FSIS describes selection of representative samples by

sampling at (1) random times during the day or (2) randomly selected boxes, according to the square root of the number of boxes rounded up to the next whole number (e.g., 6, but in no case more than 10) (FSIS page 27). FSIS additionally describes collecting small pieces using a spoon, scoop, tongs, hook, or knife and hook, depending on the size of the pieces (FSIS page 27, carry-over paragraph to page 28).

Patentee notes that the FSIS directive discusses sample for a **single lot** (the days production), and teaches nothing about forming statistically sufficient lumped test lot samples/cultures that are attributable to respective separate test lots to be independently validated for release, to enable validation of individual negative test lots where a modular composite sample is found to be positive, as presently claimed.

Price. As discussed above, Patentee respectfully contends that Price has been fundamentally misconstrued in relation to the Patentee's claimed invention, and actually ***teaches away*** from Patentee's invention in several fundamental ways. To facilitate the following discussion of Price, Patentee draws the Examiner's attention to Patentee's Exhibit A, Figures 1 and 2 (attached hereto).

Figure 1 (Exhibit A) illustrates the practiced industry art, as described in Patentee's issued '584 patent under "Background," for an exemplary embodiment involving testing of beef trim test lots. The **single test lot** to be validated for release into commerce comprises 5 'combo' bins each containing a plurality of trim portions. In **step (1)**, 12 pieces are collected from each combo bin and placed together into enrichment medium to form one composite test lot sample of 60 pieces, which is the number required to validate the single test lot to comply with the statistical based sampling plan, and there is **no** attribution of the lumped sample portions in the composite test lot to any particular combi bin of the single 5 combi bin test lot. In **step (2)**, the composite test lot sample is incubated/enriched until the target organism exceeds the detection level. In **step (3)**, the enriched composite test lot sample is tested. Where negative, the test lot (5 combo bins) is validated/accepted, and where positive the entire test lot (5 combo bins) is rejected.

Figure 2 (Exhibit B) illustrates an embodiment of the invention recited in the claims, and

described in detail in the application as filed. Five test lots each to be independently validated fro release into commerce, now each comprising one 'combo' bin containing a plurality of trim portions, are tested collectively. In **step (1)**, 60 pieces are separately collected from each test lot (combo bin) and placed together into enrichment medium to form five separate individual composite test lot samples of 60 pieces each, wherein each test lot sample comprises a number of pieces according to the statistical-based sampling plan to enable validation for release, and wherein each test lot sample is attributable to a particular underlying separate test lot. In **step (2)**, the separate composite test lot samples are incubated/enriched until the target organism exceeds the detection level (e.g., N *x* the limit of detection). In **step (3)**, a wet modular composite sample/pool is prepared by combining (N-1) equal size aliquots taken from the separate enriched composite test lot samples, with retention of the remainders of the separate enriched composite test lot samples. In **step (4)**, the enriched modular composite sample is tested *immediately* (no further enrichment is needed because even one positive separate enriched composite test lot sample can be detected in the modular composite pool because N/N-1 > 1 x the detection limit). In **step (5)**, where the modular composite sample tests negative, the five original separate test lots (combi bins) are validated/accepted. A positive modular composite sample, however, indicates that one or more of the separate enriched composite test lot samples is positive, and the retained separate enriched composite test lot samples can be separately retested *immediately* (no further enrichment or sampling is required) to determine which one(s) contributed to the positive result and should thus be rejected, and which one(s) are negative and can yet be validated according to the statistical-based sampling plan, and thus accepted.

Figure 3 (Exhibit C) illustrates the method of Price (see also Figure 1 of Price). *First*, as described on page 681 (column 1) of Price, and as appreciated in the art, *Salmonella* in a food sample must grow out in a pre-enrichment broth in competition with other organisms. Consequently, as illustrated in **steps (1) and (2)** of Figure 3 and contrary to the urging of the Examiner and Requester in this case, Price teaches that preferably individual samples (and not composited samples) from a single lot are pre-enriched, (and not enriched to detection). Price teaches that if many food samples are pooled at the pre-enrichment stage, the target organism

(e.g., *Salmonella*) face competition from the flora of all the samples in the pool (see page 681, 1st column, 2nd full paragraph). Price states at page 681, 1st column, carryover paragrapyh to 2nd column, that "the results show that a higher number of positive was recovered by pooling pre-enrichments rather than by pooling samples" (*i.e.*, pooling individual enrichments is more sensitive than lumping samples into pre-enrichment cultures). Therefore, Price is fundamentally distinguished from the present invention because Price ***teaches away*** from a composited initial test lot sample, and therefore *teaches away* from the use of such composited test lot samples in its methods.

Patentee's method (see attached Figure 2), by contrast, comprises initial formation of composited test lot samples that are enriched to above the detection limit prior to aliquot pooling ("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 required further enrichment thereof") to make the modular composite sample (wet composite sample). Price, on page 681 at column 1, discusses an earlier work by Silliker et al., "Laboratory Methods," FOOD-BORNE INFECTIONS AND INTOXICANTS," Chapter XI, pp. 455-474, 1969, Academic Press, New York and London, Edited by Hans Riemann; attached hereto) in which sensitivity was lost when several initial samples were pooled into pre-enrichment broth and then enriched and tested, instead of pre-enriching each sample individually, followed by wet composite pooling of pre-enriched broths as taught by Price. Price then goes on to compare detection in pooled samples (the method of Silliker 1969, with the method according to Price, and, as shown in Table 2,"a higher number of positives was recovered by pooling pre-enrichments rather than by compositing/pooling the initial samples followed by pre-enrichment." Thus, Price, in premise, teaching and results, *teaches away* from the use of composited initial samples in its testing regime, Price teaches away from combining lumping and pooling in a sampling/testing scheme, and in any event teaches only pooling of samples (lumped or non-lumped) at the pre-enrichment stage, **which does not meet the limitations of Patentees claimed subject matter as discussed in detail herein above**..

Second, while Price describes pooling of pre-enrichment samples (see step (3) of attached

Figure 3), it does *not* describe or teach pooling of enriched samples (to "detectable levels") each having a statistically sufficient number of sample portions and each attributable to a respective one of a plurality of underlying separate test lots each to be independently validated for release into commerce, as presently claimed. Said differently, the lumped test lot samples in the comparative tests of Price were attributable to a single underlying test lot, and the methods of Price do not include a plurality of statistically sufficient lumped test lot samples/cultures each attributable to a underlying separate test lot to be independently validated for release , as presently claimed.

As stated above, the pre-enrichment samples of Price are fundamentally distinguished from the enriched samples according to the current invention because the pre-enrichment samples of Price are not enriched to the point of detection of the target organism (even with respect to detecting individual pre-enrichment samples), whereas the enriched samples according to the current invention are enriched to the point of detection of the target organism (both in individual enriched test sample, as well as with respect to the subsequent modular composite sample) (step e) of Patentee's independent claims recite ", 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)."). By contrast, even in sampling and testing of a single underlying test lot, in forming the wet composited sample of Price (see step (3) of attached Figure 3) an aliquot of the pools of Price must be subsequently incubated in a different enrichment medium (***secondary enrichment***) and incubated to increase the levels of target organisms to above the detection limit (see step (4) of attached Figure 3).

Moreover, as shown in steps (6)-(8) of attached Figure 3, where the secondary enrichment culture of Price is positive (or negative), the individual pre-enrichment samples (with target organism levels below the detection level) must be individually subjected to secondary enrichment with substantial time delay, with concomitant increased risk of contamination of the pre-enrichment samples. Finally, even if one were to expand the individual pre-enrichment cultures of Price, while verification of positive testing or negative testing may be afforded, no

validation of the original single lot is afforded by verification of a negatively testing individual pre-enrichment cultures. This is because the scheme and sampling of Price involves sampling of a single lot and subsequent intra-lot test sample pooling, where the total number of samples taken from the single test lot, and collectively represented upon pooling of the pre-enrichment cultures, is a collective number chosen to statistically satisfy the statistical sampling plan of the single lot, but where the number of samples lumped in each pre-enrichment culture, as operationally applied in Price, is *insufficient* to provide for ***validation*** of the non-lumped (or lumped if such was the case) pre-enrichment culture according to any statistical-based sampling plan, and thus no validation (i.e., validation of no contamination) of the pre-enrichment samples of Jarvis method (3) is obtained or obtainable upon repeated testing of stored pre-enrichment cultures. This is directly analogous to the deficiencies of Jarvis in this regard, which have been discussed in detail above. Again, this is in fundamental contrast to Pantee's claimed invention as described in detail herein above.

FSIS and Midura in combination with Price do not cure the deficiencies of Price in relation to Patentee's claimed invention.

In summary for Price, alone or in combination with FSIS and/or Midura, in contrast to Patentee's claimed methods, Price does not teach: (i) initial formation of composited/lumped test lot samples/cultures each comprising a statistically sufficient number of sample portions, and that are each attributable to a separate underlying test lot; that (ii) the lumped test lot samples/cultures are enriched to above the detection limit as presently claimed *prior* to aliquot pooling; to make (iii) an enriched modular composite sample (wet composite sample) that can be tested immediately with no requirement for further enrichment, and where (iv) the individual enriched composited test lot samples can be also be tested immediately with no requirement for further enrichment, wherein "when such testing is positive, individual separate test lots may nonetheless yet be validated for release, by further testing of a portion of the respective enriched separate test lot sample…and obtaining a negative test result." Price teaches nothing about post-enrichment pooling as presently claimed, nothing about post-enrichment formation of a modular composite sample as presently claimed, and nothing about validation of negative lots where a

post-enrichment modular composite sample is found to be positive, as presently claimed.

Finally, with respect to the Examiner's statement that "absent evidence to the contrary, the pre-enrichment broth disclosed by Price meets the definition of enrichment medium," Patentee has provided evidence to the contrary as already discussed above herein with respect to Jarvis, and as widely appreciated in the art, namely that the function of the pre-enrichment media is to allow for multiplication of the target organism to a level that will ensure the transfer of at least one viable cell to the enrichment media. This would be reasonably understood by one of skill in the art to be different that a level that would reasonably provide for detection without a requirement for further enrichment—as presently claimed.

The Examiner, at page 23 of the ACP, 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, for colony plate assays, "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 does NOT teach combining a plurality of samples from each of a plurality of test lots as presently claimed. **The test lot of Price is a single food powder lot**.

Midura. Midura discusses sampling plans, collection, shipment and preparation for analysis, with the aim of providing representative samples of a food lot. A "lot" is defined at page 15 as "[t]he 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." At page 19, cited by the Examiner, Midura discusses sampling production lots having a low incidence/number of pathogen, and lists in Table 1, the number of sample units (n) needed to detect a positive result at a given level of fraction positive analytical units in a lot. Patentee notes, however, that the teachings of Midura in this respect, amount to nothing more than recommendations for the number of pieces to be

taken from the initial lot given a particular suspected contamination level, and confidence/probability target.

With respect to pages 17-20, cited by the Examiner for two-class and three class attribute plans comprising multiple samplings, Patentee points out that these plans comprise re-sampling from the original lot, which is fundamentally different from Patentees claimed subject matter, including that of Patentee's claim 8 (forming an additional modular sample from the enriched lot samples does not involve re-sampling the original respective lot).

As discussed in Patentee's last Response, Patentee does not see anywhere that Midura discusses lumping of samples (pooling of initial samples from the sampled lot), but even if Midura is construed as teaching lumping or pooling of the initial samples from the sampled lot, the teachings of Midura are limited to recommendation for sampling of a **single lot** (the days production) and handling that lot sample (NOT collective testing of a plurality of statistically sufficient lumped test lot samples/cultures each attributable to a separate underlying test lot for be independently validated for release as presently claimed), and teaches nothing about post-enrichment pooling as presently claimed, nothing about post-enrichment forming of a modular composite sample as presently claimed, and nothing about validation of negative lots where a modular composite sample is found to be positive, as presently claimed.

As stated above in the case of Jarvis, Patentee's invention is **more than a predictable use of prior art elements according to their established functions**. Specifically, the formation of Patentee's modular composite sample is by combining aliquots of a plurality of lumped test lot samples/cultures, each attributable to a respective plurality of underlying test lots, and each sampled to conform to a statistical-based sampling plan, which aspects enable validation and release of individual respective test lots. The asserted prior art, alone or in combination, has no such modular composite sample, and no such plurality of lumped test lot samples/cultures, each attributable to a respective plurality of underlying test lots, and each sampled to conform to a statistical-based sampling plan, and therefore the composite/lumped samples of the prior art do NOT provide the validatation and release function in the context of the claimed method. In fact,

the lumped test lot samples/cultures of the prior art relate to a single underlying test lot, and the established function of the lumped test lot samples/cultures of asserted Jarvis, FSIS, Midura, Price, Silliker, etc., references is to reduce the work and expense of sampling, **not increase the work and expense as required to provide for validation and release of individual respective test lots as presently claimed**.

FSIS in view of Price and Midura, does not disclose "pre-enrichment medium" as a medium that favors the growth of the target organisms in a way/extent that "meets the limitations of the patented claims," does not teach post-enrichment pooling as presently claimed, does not provide for real world ***validation*** of pre-enrichment cultures inoculated by lumped samples as presently claimed, and further does not teach formation of a modular composite sample by pooling of enriched cultures each inoculated with a number of samples according to a statistical-based sampling plan to provide for validation of lots testing negative. Rather, such prior art methods that do comprise pooling, are directed to sampling an testing a **single test lot**, and thus subsequent pooling of the lumped pre-enrichment samples/cultures represents pooling of pre-enrichment samples that are each attributable to a separate underlying test lot, and not inoculated with a statistically sufficient number of samples to each enable validation of lots testing negative—as presently claimed. FSIS in view of Price and Midura do not teach, describe, suggest, or otherwise motivate Patentee's claimed invention. Patentee's invention solves a long-standing technical problem in the art, namely (i) how to provide for a more statistically robust sample protocol for test lots, without sacrificing speed and economy so that the scheme will actually be used in commerce to save human lives, (ii) how to decrease the amount of product destruction/diversion. Moreover, the time savings, relative to the prior art methods, have profound consequences in the art in which these procedures are practiced because pre-validated food products must be stored or 'held-up' at substantial cost under preserving conditions, with shipping only after the test lots are cleared/validated. With Patentee's faster and more accurate testing procedure, test lots can be tested and cleared/validated in less time and with greater accuracy, which results in substantial savings to food processors and safety to consumers.

As explained in the further Samadpour Declaration, technically, because sampling and

testing procedures are *per se* expensive, **there is a strong incentive using prior art methods to use limited or minimal sampling protocols (limited number of samples) from relatively *large* test lots**—resulting in non-statistically robust sampling protocols. However, with Patentee's faster and more accurate testing procedure, and that fact "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…and obtaining a negative test result"—food processors accept the increase the in initial sample numbers, and attendant additional expense, taken during compositing from relatively *smaller* lots, providing for a technical advantage in that the initial sampling is more statistically *robust*, and less product must be destroyed or diverted to lower value uses.

Patentee's method, therefore, satisfies a long-standing need in the art by providing for speed, economy, a more statistically robust test lot sample protocol to save human lives, and substantial savings in terms of the amount of product must be destroyed or diverted to lower value uses.

Finally, Patentee's modular composite sample is *fundamentally different* because it is formed by combining aliquots from each of a plurality of statistically sufficient test lot cultures (i.e., each comprising a statistically sufficient number of sample portions) to enable independent validation for release of each of the respective underlying plurality of separate test lots.

Patentee contends that Patentee's claims cannot be obvious in view of the alleged asserted art, alone or in combination, under U.S. patent law, because the prior art, alone or in combination, does not teach or motivate several of Patentee's presently recited claim limitations, including Patentee's unique "modular composite sample" claim element, which would be contraindicated (disincentive) by the prior art as discussed in detail herein.

Patentees respectfully maintain that the above asserted teachings **do not in fact raise any substantial new questions of patentability** beyond those already of record in the underlying issued patent prosecution history.

Patentee, therefore, respectfully requests withdrawal of the Examiner's rejection of Patentee's claims, under 35 U.S.C. 103(a), as being unpatentable over FSIS in view of Price and

Midura.

Ground #3. The Examiner rejected claims 1, 17 and 34 and 36, under 35 U.S.C. 103(a), as allegedly being unpatentable over Jarvis in view of Patent owner's admissions regarding the state of the prior art.

Claims 34-36 have been cancelled herein.

The Examiner re-states the teachings of Jarvis, "[a]s was set forth *supra* (Ground 1), with respect to *claims 1, 17* and *34 and 35*, which are not repeated here.

Patentee, as already discussed above herein in relation to Patentee's rebuttal of Ground 1, has provided detailed rebuttal arguments relating to the limitations of Jarvis in view of Patentee's presently amended claims, and Patentee reaffirms and reasserts those arguments here.

In brief, as discussed above in relation to the Examiner's assertion of Jarvis, FSIS and Midura, it is inescapably clear that unlike the presently claimed subject matter, the aggregated samples from Jarvis, like those of FSIS, Midura, Siliker and Price, etc. are all taken from what must be effectively regarded as a single reportable test lot; that is, the lumped pre-enrichment cultures do NOT correspond to, and are not attributable to separate underlying reportable lots. In the case of Jarvis, for example, at page 137 under 8.4.1, Jarvis recites: "…to quantify the probable level of occurrence of organisms **in the lot of food under test**"; "[o]n the assumption that the organisms in **a 'lot'** of food are distributed randomly and that a number (n) of samples is tested…"; "…where d = actual % defectives in **the lot,**" etc. (emphasis added). Patentee's invention is directed to testing a plurality of reportable test lots, whereas the asserted art is directed a testing a single reportable lot. Additionally, at page 141, under 8.4.2.1, Jarvis recites "[w]hen a number of samples is to be tested it is sometimes recommended that the samples be composited in order to reduce the work load on the laboratory" and in the method under "(3)", for example, while two groups of 5 samples were lumped/composited to form two pre-enrichment cultures, all 10 samples were attributable to a single underlying reportable lot, and thus the two pre-enrichment cultures are not attributable to two separate reportable test lots.

Patentee further directs the Examiner's attention to the attached further Declaration of Dr. Mansour Samadpour, and in particular paragraphs 13 though 16., relating to this point.

This is a fundamental and profound distinction, which underlies the distinguishing utility of Patentee's methods for "validating" test lots for release into commerce—as opposed to merely enabling retesting, after further enrichment, of individual pre-enrichment cultures to verify positives.

With respect to claim 3, the Examiner states that Jarvis teaches incubating a "modular composite" sample in enrichment broth (citing Jarvis at page 135, section 8.4). Patentees respectfully point out that this is explicitly **not true**, because Jarvis teaches only lumping/compositing of a sample, and NOT forming a modular composite sample from a plurality of separate statistically sufficient enriched test lot cultures each attributable to separate reportable test lot to be validated for release—as presently claimed.

The Examiner's continuing lack of recognition of the fundamental distinction between the classic lumping of Jarvis to form a composite sample, and forming Patentee's modular composite sample is reflected in the Examiner's comments with respect to claims 11 and 13.

Specifically, with respect to claims 11 and 13, the Examiner states that Jarvis teaches "**a** test lot of 10 samples, which falls within the claimed range of 3 to 10." (emphasis added). On the one hand, based on this statement the Examiner recognizes that the 10 samples of Jarvis are attributed to a single underlying test lot. On the other hand, however, it is clear that for Patentee's independent claims, the Examiner is failing to acknowledge Patentee's **explicit** claim limitation of "a plurality of test lots," despite the fact that dependent claim 11 explicitly recites "wherein the number of separate test lots is from 3 to 10" (emphasis added). Patentee's invention relates to testing of a plurality of separate test lots each to be independently validated, and is not directed, in the sense of Jarvis, and the alleged prior art alone or in combination, to merely taking samples (e.g., 10) from a single test lot and forming a lumped sample. Patentee respectfully contends that the Examiner has erred by failing to properly recognize this distinctive limitation of Patentee's claimed subject matter. Claim 11 is not about the number of samples

taken from a test lot, but rather is drawn to the number of test lots contributing to forming the claimed modular composite sample.

Claim 13 recites a range for the number of samples taken from each one of the plurality of test lots to be validated for release. Jarvis merely teaches taking 10 samples from a **single lot** of food which is to be tested individually, and not by Patentees claimed methods, comprising forming a modular composite sample by combining aliquots from each of a plurality of statistically sufficient test lot cultures (i.e., each comprising a statistically sufficient number of sample portions) to enable independent validation for release of each of the respective underlying plurality of separate test lots.

The Examiner additionally states that Jarvis teaches particular limitations of dependent claims 18, 19, 22, 27, 31 and 36 as discussed in the ACP.

The Examiner additionally states that "Jarvis defines the medium used for incubation of the individual test lot samples as a 'preenrichment' medium, while the instant claims do not specify the incubation medium. The instant claims either 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) or they simply recite incubating the set of separate test lot samples under conditions suitable to allow levels of a target microbial agent or organism to increase in the test lot samples to provide a set of separate incubated test lot samples *(claims* 34 *and* 35)."

The Examiner further states that "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."

Patentee respectfully disagrees, and contend that there is a fundamental distinction between the attained levels of enrichment between Patentee's claimed methods and those of the prior art employing "pre-enrichment" incubation.

Patentee refers the Examiner to Patentee' detailed rebuttal of this point in relation to the Examiner's Grounds #1 and #2 above.

Again, Patentee has amended step a) of claims 1 and 17 to recite: :

"…a number of portions to be taken and growth conditions sufficient to provide for validation and release of the test lot into commerce,"

and have further amended step e) of claims 1 and 17 to recite:

", 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).

Explicit support for the amendments has already been cited herein above, for example, in Patentee's patent at col. 5, ll. 63-66 (number of sample is "selected so as to conform to statistical-based sampling plans"); original claims 3 (teaching "[t]he method of claim 1, further comprising incubating the modular composite sample of d), prior to testing in e)"); and see, for example, col. 1, last carryover paragraph through col. 2 line 26.

The amendments serve to distinguish the asserted prior art by clarifying that, unlike prior art methods comprising pre-enrichment incubation in pre-enrichment broth, the incubation of Patentee's step c) is sufficient, if the microbial agent or organism is present, to achieve an extent of enrichment sufficient to enable **validation** of negative test lot samples according to the statistical-based sampling plan with no required further enrichment. As recognized by the Examiner, Jarvis (like Price) teaches that the pre-enrichment cultures are stored, and may be subsequently retested—but this would ***require*** (like the methods (1), (2) and (3) of Jarvis) incubation of the pre-enrichment culture, or an aliquot thereof, in enrichment medium prior to said retesting (also see Price at page 679, under "Materials and Methods"). Moreover, in any event, Patentee's invention is directed to collectively testing a plurality of reportable statistically

sufficient separate test lots each attributable to an underlying separate test lot to be validated to release into commerce, whereas the asserted art, including Jarvis, alone or in combination with Price, FSIS and/or Midura, is directed a testing a **single** test lot for release (e.g, the test lot of Price corresponds to a single lot of dried egg albumin, cocoa or nonfat dry milk, and the lumped samples of Price and/or Jarvis are effectively attributable only to such a single underlying test lot). Patentee's method further provides methods for "validating" negative test lots for release into commerce—as opposed to merely enabling retesting, after further enrichment, of individual stored pre-enrichment cultures to verify positives.

The Examiner states that "**Jarvis does not teach repeat testing of negative samples**, as in patented dependent *claim 8*) (emphasis added). **Patentees agree**, as discussed in detail above, because there would be absolutely no motivation to do so, and it would not be possible in the sense of Patentee's patent claim 8 subject matter, where the pre-enrichment cultures of Jarvis do not represent statistically sufficient broths each attributable to separate underlying test lots (plurality of underlying separate test lots), and thus there would be no attributable separate test lots, and even the single underlying test lot of Jarvis could not be validated by repeat testing of a statistically insufficient pre-enrichment broth.

Jarvis refers to and uses "pre-enrichment" and "enrichment" medium/incubation consistent with the art-recognized meanings, and nowhere, does Jarvis teach or suggest testing (absent subsequent enrichment) for the organism after mere "pre-enrichment," thereby providing the "evidence to the contrary" requested by the Examiner. **See also Patentee's rebuttal arguments on this point presented above in the context of Grounds #1 and #2**.

For example, at page 135 (cited by the Examiner) Jarvis states that in the analysis of foods for low numbers of pathogenic organisms, "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." Moreover, assays (1) through (3) described by Jarvis explicitly draw the art recognized functional/operative distinction between "pre-enrichment" and "enrichment" medium. In fact, Jarvis 1989, page141, first paragraph, teaches that the function of the pre-enrichment media is to allow for multiplication of

the target organism to a level that will ensure the transfer of at least one viable cell to the enrichment media. Jarvis clearly states that if the pre-enrichment only resuscitates, then the entire pre-enrichment is to be transferred to the enrichment step—in which case retaining a portion for later validation testing would be impossible (*Id*, "In methods which permit only resuscitation, not growth, during pre-enrichment, it is essential to use the entire pre-enrichment culture to inoculate double-strength enrichment medium (Hall, 1975), although selectivity may then be affected by the food constituents") (see also Jarvis, "On the compositing of samples for qualitative microbiological testing," Letters in Applied Microbiology, 2007, pp. 592-598, Volume 45; attached hereto).

In other words, growth in the pre-enrichment media of Jarvis is not to detection level as presently claimed, and rather is either for resuscitation in which case the entire pre-enrichment is to be transferred to enrichment, or allows for limited growth to ensure that there is at least one viable organism in the portion which is transferred to the enrichment step. Moreover, the disclosure of Jarvis regarding pooling is limited to only one organism (*Salmonella*).

The Examiner additionally states that "[w]hile 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. 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)."

Patentee points out that the prior art does not teach forming a lumped test sample/culture by collecting a plurality of portions from each of a plurality of test lots, **wherein each lumped test sample/culture is attributable to a particular respective underlying separate**

test lot, and the **patent owner does NOT admit any such thing**.

Patentee's patent, as cited by the Examiner, teaches that "The operative art with respect to such product "hold and release" monitoring efforts comprises various different sampling plans, typically pursuant to International Commission on Microbial Specifications for Food (ICMSF) guidelines for testing food products, FDA and USDA or EPA guidelines, or intuitive sampling plans. The common feature of these sampling plans is that several samples representing production lots, or a number of environmental samples, are combined into a combined production lot **prior to sampling**. For example, a typical plan for microbial pathogen detection calls for taking more than one sample, for instance ten (10) to sixty (60) sample pieces from a given sample lot (combined production lot), to form a *composite sample* for testing." Specifically, this statement merely describes the art-recognized practice of sample lumping; that is, combining the samples taken from a lot to be tested.

The limitations and deficiencies of Jarvis, as discussed in detail above, are in no way cured by any statement that sample lumping was already known in the art, and Patentee respectfully contends that the Examiner's position in this regard is unsupportable and manifestly erroneous. Any alleged motivation to test by the Jarvis method would, as described above, be a contrary motivation (i.e., teaching away) from Patentee's claimed invention. Patentee contends that the teachings of Jarvis (with or without the discussion in Patentee's "Background" section of Patentee's patent) **do not in fact raise any substantial new questions of patentability** beyond the teachings of alleged prior art (e.g, Price et al., and Silliker et al.) already of record in the underlying issued patent prosecution history.

Patentee, therefore, respectfully requests withdrawal of the Examiner's rejection of claims 1, 17 and 34-36, as being obvious over Jarvis in view of Patent owner's admissions regarding the state of the prior art. Claims 34-36 have been cancelled herein.

Ground #4. The Examiner did not adopt Ground #4 for the reasons stated in the Office Action. Patentee, for the record, traverses Ground #4 even if it had been adopted, based on the

arguments discussed herein.

Ground #5. The Examiner rejected claims 1, 17 and 34-36, under 35 U.S.C. 103(a), as allegedly being unpatentable over Andrews in view of Price and Midura.

Price and Midura have been discussed above in detail, and Patentee's rebuttal arguments relating to the assertion of these reference are reaffirmed and reasserted here.

Claims 34-36 have been cancelled herein.

The Examiner states, with respect to claims 1, 17 and 34-36, that "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) and incubated to allow the organisms to reach a desired number and condition **prior to inoculation into and then incubated again in a second enrichment medium**, which is selective for the target organism (see page 360, sections 37.123 and 37.124)" (emphasis added by Patentee).

The Examiner states that "Andrews also does not teach incubating the sample units or test lots separately, as in the patented claims."

The Examiner concludes that "[i]t 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. 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, 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."

Patentee respectfully traverses this rejection.

The deficiencies and limitations of Price and Midura, in view of Patentee's claimed invention have been discussed above in detail in relation to Ground #2, said discussions respectfully deemed not to be required here to avoid unnecessary redundancy.

No where, do any of these references teach, suggest or otherwise motivate forming a plurality of separate lumped test lot samples/cultures each containing a statistically sufficient number of sample portions from a respective one of a plurality of separate underlying test lots to be validated for release into commerce, wherein each separate lumped test lot samples/cultures is attributable to an underlying separate test lot, such that validation and release of individual test lots is afforded—as presently claimed. The limited teachings of Andrews, which is a general description of methods for isolation of *Salmonella* from foods, do not go beyond or add anything to the teachings of Price and Midura in this regard.

For example, Andrews, at page 358 second column, first full paragraph, states that "Criteria for acceptance of any particular lot of food are based on the results of analysis of a required number of 25-g analytical units, the actual portions of food analyzed. Each analytical unit is taken from a larger sample unit…A series of random sample units make up the sample, and a sample that is representative of the lot is used to determine the acceptability of the entire lot of food." This merely describes the art-recognized formation of a lumped sample, and is explicitly clear that the test lot of Andrews is a single test lot, and the lumped test samples/culture of Andrews are attributable to a single underlying test lot—there is no plurality of separate lumped test lot samples/cultures formed from a respective plurality of separate underlying test lots, wherein each separate lumped test lot samples/cultures is attributable to an underlying separate test lot, such that validation and release of individual test lots is afforded. Patentee's position in this regard is also supported by Table 3 at page 361, which shows testing of a single mixed or blended test lot for each of the food categories.

Additionally, Andrews, Price, Jarvis or Midura, alone or in combination, do **not** disclose "pre-enrichment medium" and incubation therein, as a medium/method that favors the growth of the target organism in a way that meets the limitations of the presently amended claims, for reasons that have been discussed above in detail. As described at page 360, Andrews teaches a requirement for enrichment in selective medium (Andrews at 37.124) prior to testing by selective plating (Andrews at 37.125).

Patentee further directs the Examiner to Patentee's arguments in this regard presented above in rebuttal of Grounds #1, #2 and #3.

Furthermore, the multiple *re-sampling* runs of Midura cited by the Examiner, actually *teaches away* from Patentee's claimed subject matter, as discussed above in relation to the Examiner's assertion of Jarvis, FSIS and Midura (or Price , FSIS and Midura).

In summary, Andrews, alone or in combination with Price and Midura: does not disclose "pre-enrichment medium" and incubation therein as a medium/method that favors the growth of the target organisms in a way/extent that meets the limitations of the presently amended claims; does not teach post-enrichment pooling of statistically sufficient test lot samples as presently claimed; does not provide for real world validation of pre-enrichment cultures (and the respective underlying separate test lots) inoculated by a statistically sufficient lumped samples as presently claimed; and further does not teach a plurality of separate statistically sufficient lumped test lot samples/cultures formed from a respective plurality of separate underlying test lots, wherein each separate lumped test lot samples/cultures is attributable to an underlying separate test lot, such that validation and release of individual test lots is afforded. Rather, such prior art methods that do comprise pooling, are in relation to testing of a single lot where lumped pre-enrichment samples are each inoculated with a statistically insufficient number of samples such that no validation (conforming to a statistical-based sampling plan) of individual negative test lots is afforded.

Patentee respectfully maintains that the above asserted teachings **do not in fact raise any substantial new questions of patentability** beyond those already of record in

the underlying issued patent prosecution history.

Patentee, therefore, respectfully requests withdrawal of the Examiner's rejection of claims 1, 17 and 34-36, under 35 U.S.C. 103(a), as allegedly being unpatentable over Andrews in view of Price and Midura. Claims 34-36 have been cancelled herein.

Ground #6. The Examiner rejected claims 1, 17 and 34-36, under 35 U.S.C. 103(a), as allegedly being unpatentable over Silliker in view of Price and Midura.

The Examiner states that "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 Examiner states that 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 (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..." (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.

The Examiner states that 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 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, as recommended by Midura, in order to be able to detect highly pathogenic organisms present in foods at low levels.

Patentee respectfully traverses this rejection.

The deficiencies and limitations of Price and Midura, in view of Patentee's claimed invention have been discussed above in detail, and are reaffirmed and reasserted here.

As initial matters, as already discussed above herein in relation to Patentee's rebuttal of Grounds 1 and 2, Silliker, alone or in combination Price and Midura (or with Jarvis and/or Price), does not disclose "pre-enrichment medium" or incubation therein as a medium/method that favors the growth of the target organism in a way that "meets the limitations of the presently claimed subject matter."

Additionally, Silliker, alone or in combination with Price and Midura, does not teach, suggest or otherwise motivate a plurality of separate lumped test lot samples/cultures each formed from statistically sufficient number of sample portions from each of a respective plurality of separate underlying test lots, wherein each statistically sufficient separate lumped test lot samples/cultures is attributable to an underlying separate test lot, such that validation and release of individual test lots is afforded—as presently claimed. The assay methods of Silliker, like those of Jarvis, Price, Midura, FSIS, and Andrews, are based on forming lumped test lot samples/cultures that are attributable to the same, **single underlying test lot**, and therefore do not enable validation and release as presently claimed in the context of simultaneous assay of a plurality of lumped test lot samples/cultures, each attributable to separate underlying test lots.

Patentee directs the Examiner' attention to the further Samadpour Declaration in support of Patentee's position in this regard.

Moreover, Patentee respectfully maintains that one of ordinary skill in the art would not have construed Silliker (despite the statement at page 478, last full paragraph of the page) as an indication that pre-enrichment pooling of lumped samples was as reliable as pre-enrichment non-lumped samples as urged by the Examiner ("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))." Specifically, as discussed above in relation to Ground 1, Silliker show in their data of Table 1 of page 477 (compare the second and fifth column of data for 100 gm samples) that lumping combined with pooling results false negatives, and, for example, state that "[i]n test 18, three of the fifteen 100-g subsamples were positive, but all of the wet composites were negative" and that "a minimum of one and a maximum of three positives would have been expected" (*Id* at page 477 second column). Additionally Silliker discusses that "Silliker (Appendix D of reference 2) reported that of 106 wet composites derived from 1060 individual samples, 39 were positive for *Salmonella* and 66 were negative. In seven instances negative results were obtained despite the fact that one or more of the 10 individual samples comprising the wet composite did contain *Salmonella*. **Thus, these seven were false negative results**."

With respect to the Examiner's comments at page 32 of the ACP, Patentee points out that the quoted statement by Silliker is inconsistent with Sillikers own data in Table 1, as would be obvious of one of ordinary skill in the art.

In any event, Silliker, alone or in combination with Price and Midura, does not teach, suggest or otherwise motivate a plurality of separate lumped test lot samples/cultures each formed from statistically sufficient number of sample portions from each of a respective plurality of separate underlying test lots, wherein each statistically sufficient separate lumped test lot samples/cultures is attributable to an underlying separate test lot, such that validation and release of individual test lots is afforded—as presently claimed. The assay methods of Silliker, like

those of Jarvis, Price, Midura, FSIS, and Andrews, are based on forming lumped test lot samples/cultures that are attributable to the same, **single underlying test lot**, and therefore do not enable validation and release as presently claimed in the context of simultaneous assay of a plurality of lumped test lot samples/cultures, each attributable to separate underlying test lots.

Patentee directs the Examiner' attention to the further Samadpour Declaration in support of Patentee's position in this regard.

Silliker. Patentee respectfully further points out that Silliker, alone or in combination with Price and Midura, in fact ***teaches away*** and is fundamentally distinguished from the current invention.

Patentee draws the Examiner's attention to Patentee's explanatory Figures 2 and 4, attached hereto (Exhibits B and D). Figure 2, as described in detail above, illustrates an embodiment of Patentee's invention as presently claimed. Figure 4 illustrates the method of Silliker.

As an initial matter, the aim of Silliker was to determine if, rather than testing sixty 25 g samples, a fewer number larger samples (e.g., 100g or 500g) could be used to reduce the laboratory work if such schemes were as efficient in detecting *Salmonella*. Silliker, at page 476 top of column 1, refer to Silliker's own earlier work where testing of 400 g samples resulted in loss of sensitivity relative to testing of 25 g samples. Silliker also comments that over-dilution of wet compositing (106 wet composites from 1060 individual samples) can cause false negative problems, but that Price et al., had reported that 25 pre-enrichment broths could be composited. Given this background, the clear aim of Sillker was to compare different pre-enrichment sample sizes and respectively different wet compositing protocols. However, the teachings of Silliker et al are fundamentally distinguished from those of Patentee's presently claimed invention.

As in the case of Price and Jarvis, while Silliker describes pooling of pre-enrichment samples (Silliker, page 476, 2nd column) , it does not describe or teach pooling of enriched samples as presently claimed, each comprising a statistically sufficient number of sample portions from each of a respective plurality of underlying test lots to be independently validated

fore release.

Significantly, the pre-enrichment samples of Silliker are fundamentally distinguished from Patentee's enriched samples because the pre-enrichment samples of Silliker are not enriched to provide for detection of the target organism as presently claimed (see steps (2) and (3) of attached Figure 4, Exhibit B), whereas Patentee's enriched samples are enriched to provide for of detection of the target organism (see steps (1) and (2) of attached Figure 2, Exhibit B). Consequently, in forming the wet composited sample of Silliker an aliquot of the pools of Silliker must be subsequently transferred to a new enrichment medium (secondary enrichment) and incubated to increase the levels of target organisms to above the detection limit (see steps (3) and (4) of attached Figure 4, Exhibit D), and **as described in the Method section of Silliker**. Moreover, as in the case of Price and Jarvis, where the secondary enrichment culture of Silliker is positive, the individual pre-enrichment samples (with target organism levels below the detection level) must/would be subjected to secondary enrichment with substantial time delay, with concomitant increased risk of contamination of the pre-enrichment samples).

In conclusion, Silliker is fundamentally distinguished from the currently claimed subject matter. In contrast to Patentee's method (see, e.g., steps (2)-(5) of attached Figure 2, Exhibit B), Silliker does not teach: "pre-enrichment medium" and incubation therein as a medium/method that favors the growth of the target organisms in a way/extent that meets the limitations of the presently amended claims; does not teach post-enrichment pooling to form a modular composite sample as presently claimed; does not provide for real world validation of pre-enrichment cultures inoculated by lumped samples as presently claimed; and further does not teach a plurality of separate lumped test lot samples/cultures each having a statistically sufficient number of sample portions from each of a respective plurality of separate underlying test lots to be validated for release, wherein each statistically sufficient separate lumped test lot samples/cultures is attributable to an underlying separate test lot, such that independent validation and release of individual test lots is afforded. Rather, such prior art methods that do comprise pooling, comprise pooling only in the context of a single lot being sampled, wherein any pooling is of pre-enrichment samples each inoculated with a statistically **in**sufficient number

of samples such that no validation (conforming to a statistical-based sampling plan) of even the single underlying test lot is afforded by reassay of the statistically **in**sufficient pre-enrichment sample.

No teaching of Patentee's claim elements:

Finally, Silliker, alone or in combination with Price and/or Midura, in addition to not teaching Patentee's claim limitations of: a plurality of separate test lots to be validated for release; a respective plurality of test lot samples each comprising a statistically sufficient number of sample portions; do not teach Patentee's "modular composite sample" element of step d), which is unique, and also counterintuitive as discussed in detail above (requires increased expense and sampling).

Patentee's modular composite sample is *fundamentally different* because it is formed by combining aliquots from each of a plurality of statistically sufficient test lot cultures (i.e., each comprising a statistically sufficient number of sample portions) to enable independent validation for release of each of the respective underlying plurality of separate test lots.

Patentee contends that Patentee's claims cannot be obvious in view of the alleged asserted art, alone or in combination, under U.S. patent law, because the prior art, alone or in combination, does not teach or motivate several of Patentee's presently recited claim limitations, including Patentee's unique "modular composite sample" claim element, which would be contraindicated (disincentive) by the prior art as discussed in detail herein.

Patentee directs the Examiner' attention to the further Samadpour Declaration in support of Patentee's position in this regard.

In summary, Silliker, alone or in combination with Price and/or Midura, and/or the other asserted alleged prior art, *teaches away* from the present invention, and is fundamentally distinguished from claimed invention. Price and Midura do not cure the deficiencies of Silliker in view of Patentee's claimed invention. Patentees respectfully maintain that the above asserted teachings **do not in fact raise any substantial new questions of patentability** beyond those already of record in the underlying issued patent prosecution history.

Patentee, therefore, respectfully request withdrawal of the rejection of claims 1 and 17, under 35 U.S.C. 103(a), as allegedly being unpatentable over Silliker in view of Price and Midura.

Ground #7. The Examiner rejected claims 1, 17 and 34-36, under 35 U.S.C. 103(a), as allegedly being unpatentable over Gabis in view of Price and Midura.

Claims 34-36 have been cancelled

The Examiner states that "Gabis discloses methods for sampling and testing high moisture foods, with meat and poultry exemplified, by collecting thirteen 25 g 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 sample in a single enrichment broth culture, incubating the enrichment broth culture, and subsequently recovering and identifying the salmonellae. 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)." Essentially, therefore, the Examiner is stating that Gabis teaches "inoculating each of the specimens" sampled from **a single lot** into individual "pre-enrichment culture," followed by pooling of the pre-enrichment samples into enrichment medium, followed by assay.

The Examiner further states that 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 (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..." (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 patented *claims 1 and 17*.

The Examiner states that Gabis does not teach testing individual subsamples separately upon obtaining a positive result from the composite sample.

Patentee respectfully traverses the rejection.

As an initial matter, Patentee has already provided rebuttal arguments to this basis of rejection in response to the Examiner's rejections of Grounds 1 and 2, for example. Gabis, alone or in combination with Price and Midura, does not disclose "pre-enrichment medium" or incubation therein as a medium/method that favors the growth of the target organism in a way that "meets the limitations of the presently claimed subject matter."

Gabis. As acknowledged by the Examiner, the teachings of Gabis are limited "inoculating each of the specimens" sampled from a lot into individual "pre-enrichment culture," followed by intra-lot pooling at the pre-enrichment samples into enrichment medium, followed by assay. In this respect, therefoe, Gabis (Silliker is coauthor) does not differ from the teachings of Silliker (Gabis is coauthor) discussed above.

The deficiencies and limitations of Price and Midura, in view of Patentee's claimed invention have been discussed above in detail, and are reaffirmed an reasserted here.

In summary, Gabis is fundamentally distinguished from the currently claimed subject matter. In contrast to Patentee's method (see, e.g., steps (2)-(5) of attached Figure 2, Exhibit B), Gabis, alone or in combination with Price and Midura, does not teach: (i) initial formation of composited test lot samples each having a statistically sufficient number of sample portions from each of a corresponding plurality of separate test lots to be validated for release as presently claimed; that (ii) are enriched to above the detection limit *prior* to formation of the modular composite sample; to make (iii) the modular composite sample (wet composite inter-lot sample) that can be tested immediately, and where (iv) the individual statistically sufficient enriched composited/lumped individual test lot samples/cultures can be also be tested immediately; 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…and

obtaining a negative test result." Gabis, alone or in combination with Price and Midura, teaches nothing about post-enrichment pooling as presently claimed, nothing about post-enrichment formation of a modular composite sample as presently claimed, and nothing about validation of negative lots where a post-enrichment inter-lot pool is found to be positive, as presently claimed.

No teaching of Patentee's claim elements:

Finally, Gabis, alone or in combination with Price and/or Midura, in addition to not teaching Patentee's claim limitations of: a plurality of separate test lots to be validated for release; a respective plurality of test lot samples each comprising a statistically sufficient number of sample portions; do not teach Patentee's "modular composite sample" element of step d), which is unique, and also counterintuitive as discussed in detail above (requires increased expense and sampling).

Patentee's modular composite sample is *fundamentally different* because it is formed by combining aliquots from each of a plurality of statistically sufficient test lot cultures (i.e., each comprising a statistically sufficient number of sample portions) to enable independent validation for release of each of the respective underlying plurality of separate test lots.

Patentee contends that Patentee's claims cannot be obvious in view of the alleged asserted art, alone or in combination, under U.S. patent law, because the prior art, alone or in combination, does not teach or motivate several of Patentee's presently recited claim limitations, including Patentee's unique "modular composite sample" claim element, which would be contraindicated (disincentive) by the prior art as discussed in detail herein.

Patentee directs the Examiner' attention to the further Samadpour Declaration in support of Patentee's position in this regard.

Patentee respectfully maintains that the above asserted teachings **do not in fact raise any substantial new questions of patentability** beyond those already of record in the underlying issued patent prosecution history.

Patentee, therefore, respectfully requests withdrawal of the rejection of claims 1, 17 and 34-36, under 35 U.S.C. 103(a), as allegedly being unpatentable over Gabis in view of Price and Midura. Claims 34-36 have been cancelled.

Ground #8. The Examiner rejected claims 1, 17 and 34-36, under 35 U.S.C. 103(a), as allegedly being unpatentable over Jarvis in view of BAM.

Claims 34-36 have been cancelled.

The Examiner discusses Jarvis as per Ground 1.

The Examiner states that the "method described by Jarvis differs from the claimed method in that Jarvis defines incubation of the individual test lot samples as "pre-enrichment," while the patented claims recite incubating the test lot samples in medium favorable for "enrichment" of the target microbial agent or organism (see step "c" of patented *claims 1 and 17*) or they simply recite incubating the set of separate test lot samples under conditions suitable to allow levels of a target microbial agent or organism to increase in the test lot samples to provide a set of separate incubated test lot samples *(claims* 34 *and* 35). 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.

The Examiner states that "[w]hile 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.

The Examiner states that "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. 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 in their entirety).

The Examiner concludes that 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, 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* if the composite sample tests positive for a target agent or organism, thereby negating the necessity for rejecting the entire food lot" (emphasis added).

Patentee respectfully traverses the rejection.

Patentees reassert and reaffirm Patentee's rebuttal of Jarvis in view of the asserted art as under Ground #1.

BAM. As acknowledged by the Examiner, the teachings of BAM do not extend beyond the prior art methods discussed herein; namely, collecting a plurality of samples at random **from a single lot** (a single lot) and lumping (combining) the collected samples or portions into a composite test lot sample inoculated into pre-enrichment medium, prior to inoculation into enrichment and assay.

BAM teaches **absolutely nothing**, alone or in combination with Jarvis, that would cure the deficiencies of Jarvis as described above and below, with respect to the absence of teaching or motivation for simultaneous testing of a plurality of separate test lots according to a statistical-based sampling plan and method that would provide for validation and release of separate test lots based on assaying individual lumped test lot sample/cultures each comprising a statistically

sufficient number of product portions from each of a respective plurality of separate underlying test lots to be validated for relesae, as presently claimed.

The various deficiencies and limitations of Jarvis, in view of Patentee's claimed invention have been discussed above in detail under Ground 1 and elsewhere, and are reaffirmed and reasserted herein. The Examiner's attention is, however, again directed to the fact that *verification of positive* samples by retesting of the stored individual pre-enrichment cultures, according to prior art methods (including Jarvis and BAM) is fundamentally different from statistical "**validation**" (i.e., validation of no contamination by means of a number of samples sufficient for a required level of statistical confidence) of individual pre-enrichment cultures. This is, because prior art statistical testing methods (including those described by Jarvis) involve sampling of a single lot and subsequent *intra*-lot sample pooling, where the total number of samples taken from the single test lot, and collectively represented upon pooling of the pre-enrichment cultures, is a collective number chosen to statistically satisfy the statistical sampling plan of the single lot (e.g., 10 samples in the case of the Jarvis examples), but where the number of samples lumped in each pre-enrichment culture (e.g., 5, as in Jarvis method (3)), as operationally applied in the prior art, is *insufficient* to provide for ***validation*** of the lumped pre-enrichment culture according to the statistical sampling plan, and thus no validation (i.e., validation of no contamination) of the pre-enrichment samples of Jarvis method (3) is obtained or obtainable upon repeated testing of stored pre-enrichment cultures. This is in fundamental contrast to Patentee's claimed invention, wherein, as recited in step (e) of claims 1 and 17, "when such testing is positive, individual separate test lots may nonetheless yet be **validated** for releaseby further testing of a portion of the respective enriched separate test lot sample… and obtaining a negative test result." Patentee's method comprises formation of a modular composite sample from a plurality of lumped, enriched test lot samples/cultures that are each inoculated with a statistically sufficient number of samples to satisfy the statistical-based sampling plan, and are each attributable to a respective separate underlying test lot. By contrast, prior art methods, including that of Jarvis, comprise *intra*-lot pooling of lumped

pre-enrichment samples—each inoculated with a statistically insufficient number of samples such that testing of the lumped test lot samples/cultures does NOT satisfy the lot validation criteria of the statistical-based sampling plan.

In summary, Jarvis, **alone or in combination with BAM**, is fundamentally distinguished from the current set of claims. In contrast to Patentee's method (see, e.g., steps (2)-(5) of attached Figure 2, Exhibit B), Jarvis, alone or in combination with BAM, does not teach: (i) initial formation of a plurality of statistically sufficient composited/lumped test lot samples from each of a respective plurality of separate underlying test lots; that (ii) are enriched to above the detection limit as presently claimed *prior* to (iii) pooling to form the modular composite sample that can be tested immediately; wherein (iv) the individual statistically sufficient enriched composited/lumped test lot samples/cultures can be also be tested immediately; and (v) 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…and obtaining a negative test result." Jarvis, alone or in combination with BAM, teaches nothing about post-enrichment pooling as presently claimed, nothing about post-enrichment inter-lot pooling as presently claimed, and nothing about validation of negative lots where a post-enrichment inter-lot pool is found to be positive, as presently claimed.

Patentee's modular composite sample is *fundamentally different* because it is formed by combining aliquots from each of a plurality of statistically sufficient test lot cultures (i.e., each comprising a statistically sufficient number of sample portions) to enable independent validation for release of each of the respective underlying plurality of separate test lots.

Patentee contends that Patentee's claims cannot be obvious in view of the alleged asserted art, alone or in combination, under U.S. patent law, because the prior art, alone or in combination, does not teach or motivate several of Patentee's presently recited claim limitations, including Patentee's unique "modular composite sample" claim element, which would be contraindicated (disincentive) by the prior art as discussed in detail herein.

Patentee respectfully maintains that the above asserted teachings **do not in fact**

raise any substantial new questions of patentability beyond those already of record in the underlying issued patent prosecution history.

Patentee, therefore, respectfully requests withdrawal of the rejection of claims 1, 17 and 34-36, under 35 U.S.C. 103(a), as allegedly being unpatentable over Jarvis in view of BAM. Claims 34-36 have been cancelled.

Ground #9. The Examiner rejected claims 1, 17 and 34-36, under 35 U.S.C. 103(a), as allegedly being unpatentable over Price in view of BAM and Davies.

Claims 34-36 have been cancelled.

The Examiner discusses Price as already summarized herein above.

The Examiner states that "Price defines the medium used for incubation of the individual test lot samples as a "preenrichment" medium, while the instant claims do not specify the incubation medium. As was set forth above, the instant claims either 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) or they simply recite incubating the set of separate test lot samples under conditions suitable to allow levels of a target microbial agent or organism to increase in the test lot samples to provide a set of separate incubated test lot samples *(claims* 34 *and* 35). 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 preenrichment 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" (emphasis added by Patentee).

The Examiner states that "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. 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)" (emphasis added).

The Examiner states that Davies "discloses methods of sampling and testing pooled samples (test lots) taken from pig herds for *Salmonella* contamination (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)."

The Examiner, based on this, concludes that 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."

Patentee respectfully traverses the rejection.

The deficiencies and limitations of Price and BAM have already been discussed in detail herein above, are reaffirmed and reasserted here. While Price and BAM may teach forming a lumped test sample/culture by collecting a plurality of portions from a single test lot, they do not teach, suggest or otherwise motivate forming a modular composite sample, as presently claimed, from aliquots of a plurality of lumped test lot samples/cultures each formed by collecting a plurality of portions from each of a respective plurality of test lots, wherein each lumped test lot samples/culture is attributable to respective underlying separate test lot, and wherein the number of samples taken to form the lumped test lot sample is sufficient to enable validation and release of negative test lots into commerce.

Davies does not cure this deficiency.

Davies et al. Davies et al., alone or in combination with Price and/or BAM, does not teach, suggest or otherwise motivate all the elements of the presently claimed subject matter, because while Davies et al. may teach classic sample pooling and enriching thereof, Davies et al. does teach or suggest enrichment of a "test lot sample", as recited in present claim 1 steps (b) and (c), **for use in** preparing a "modular composite sample," as recited in present claim 1 (d). Rather, Davies et al. only teaches simple sample pooling (**in the absence of modular composite sample as presently claimed**) to make a classical composite sample for enrichment and then assaying the enriched sample directly (see Figure 1 of attached Exhibit A), which, in terms of Patentee's novel scheme, only amounts to preparation and enrichment of a "test lot sample," but in Davies et al, there is a complete absence of preparing and assaying a modular composite sample as presently claimed (see Figure 2 of attached Exhibit B) and which allows, in the case of a positive testing modular composite sample, for returning to assay the retained individual enriched test lot samples determine which enriched test lot samples contributed to the positive result such that positive enriched test lots are rejected while negative test lots are validated per the lot validation plan and accepted.

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. Patentee has attached Figures 1 and 2 of Exhibits A and B, respectively, to facilitate the Examiner's review. Specifically, Davies et al. teaches only classical sample pooling to make a composite sample for enrichment and testing as shown in the Figure 1 diagram of Exhibit A. Significantly however, none of the pooled samples of Davies et al. were used after enrichment to prepare the equivalent of a "modular composite sample" much less "a modular composite sample," as presently claimed by Patentee (see Figure 2 of Exhibit B, which diagrammatically reproduces an embodiment of the method according to Patentee's claims).

Specifically, the upper portion of Figure 2 of Exhibit B illustrates an exemplary preferred embodiment of Patentee's invention as applied to lot testing of food products such as beef trim, in which a plurality of portions (e.g., 60 independent portions) are collected from each test lot and are placed into a single bag (or other suitable test containers) to provide a respective set of test lot samples (corresponds to steps a) and b) of claim 1). Each test lot sample is then enriched individually (corresponds to step c) of claim 1). A portion (e.g., equal volume portions) of medium is removed from each of the enriched test lot samples and placed together into a single wet modular composite sample (e.g., test tube) (corresponds to step d) of claim 1). The wet composite is then tested (corresponds to step e) of claim 1). Not shown in the diagram is an optional incubation/enrichment step between the modular composite sample and an Enriched modular composite sample. If a negative test result form microbial agents or organisms is obtained with respect to the modular composite sample, all of the test lot members of the composite are deemed to be negative for the pathogen of interest. On the other hand, if a positive result is obtained on the modular composite sample, samples from each of the individual enriched test lot samples (retained) that were used to prepare to the wet modular composite sample are individually tested (e.g., using the same test protocol or an alternate protocol with at least the same level of sensitivity).

Davies et al., alone or in combination with Price and/or BAM, do not teach, suggest, or motivate, for example steps 3 and 4 of the Figure 2, Exhibit B diagram, which correspond to exemplary preparation and testing of a modular composite sample as in Patentee's claim 1, steps d) and e). Likewise in Davies et al., pooled serum and meat juice samples were tested individually and as pools, but these were not enriched but rather assayed directly by ELISA.

Therefore, while the method of Davies et al., provides, as intended, merely a cheaper way of assaying by making classical pooled samples (composite lumped samples), Davies et al., alone or in combination with Price and/or BAM do not make modular composite samples as presently claimed from enriched pooled samples. For example, in Davies et al., for the pens of each of 20 commercial pig finishing farms, one pooled sample was made for each specific farm pen, and the samples were separately submitted for analysis (see Davies et al., page 1017, column 2, first full

paragraph). There was, however, no formation of a overall modular composite sample comprising aliquots of the 20 farm-specific pooled pen samples (or enriched farm-specific pooled pen samples). Thus, while Davies et al. practices assay of classic pooled/lumped pen samples for efficiency, Davies et al., alone or in combination with Price and BAM, do not practice, teach or otherwise motivate the presently claimed methods.

By way of further illustration of the distinctions of the presently claimed methods, application of the present methods to the problem of Davies et al., for example, might involve statistical sampling and enrichment of each of the 20 farm-specific pooled pen samples (collectively a single lot) (and retention of the enrichments), followed by formation of an overall composite sample (corresponding to the **single underlying lot**) comprising aliquots of the 20 enriched farm-specific pooled pen samples, and assay of the composite composite sample (or assay of an optionally further enriched modular inter-lot composite sample)—resulting in a much greater efficiency than provided for by classical methods of Davies et al.

Patentee contends, therefore that the teachings of Davis do not extend beyond the teachings of Price and BAM, and does not therefore cure the deficiencies thereof as presently asserted by the Examiner. While Davies, like Price and BAM, may teach forming a lumped test sample/culture by collecting a plurality of portions from a single test lot, they do not teach, suggest or otherwise motivate forming a modular composite sample, as presently claimed, from aliquots of a plurality of lumped test lot samples/cultures each having a statistically sufficient number of sample product portions from each of a corresponding plurality of underlying test lots to be validated for release, wherein each lumped test lot samples/culture is attributable to respective underlying separate test lot, and wherein the number of samples taken to form the lumped test lot sample is sufficient to enable validation and release of negative test lots into commerce.

The Examiner at page 40 of the ACP, states that "Patent owner's arguments (page 47) directed to a "test lot sample" for use in preparing a "modular inter-lot composite sample" are noted; however, once again, the claim amendments adding "inter-lot" fail to distinguish over the combination of Price with BAM and Davies **because the '584 patent specification fails to provide a definition of 'lot" which distinguishes over the samples disclosed in the prior art**.

Each of claims 1, 17, 34, and 35 recites that the test lots (claims 1, 34, and 35) or production lot (claim 17) comprises "an assemblage of one or more specimens". The samples disclosed by Price and the analytical units disclose by BAM both fully meet the limitations of the test lot or production lot of the instant claims. The argument that the teachings of Davies are limited to the use of pooled samples to provide a cheaper monitoring program to identify infected pig herds is once again arguing against Davies individually, wherein the rejection is based on the combination of Davis with Price and BAM" (emphasis added by Patentee).

Patentee respectfully disagrees that the **'584 patent specification the presently claimed subject matter fails to provide a definition of "lot" which distinguishes over the samples disclosed in the prior art**. Patentees point out that a distinguishing aspect of Patentee's invention lies in the fact that because Patentee's methods comprise parallel analysis of a plurality of separate test lots, the specific composition/nature of Patentee's modular composite sample is ***unique***—being comprised of a plurality of lumped test lot samples/cultures, each attributable to a respective plurality of underlying test lots, and each sampled to conform to a statistical-based sampling plan, which aspects enable validation and rapid release of individual respective test lots to enhance efficiency and safety, and reduce the amount of product destroyed and/or diverted.

In overall summary, Patentee's invention is **more than a predictable use of prior art elements according to their established functions**. Specifically, the formation of Patentee's modular composite sample is by combining aliquots of a plurality of lumped test lot samples/cultures, each attributable to a respective plurality of underlying test lots, and each sampled to conform to a statistical-based sampling plan, which aspects enable validation and release of individual respective test lots. The asserted prior art, alone or in combination, has no such modular composite sample, and no such plurality of lumped test lot samples/cultures, each attributable to a respective plurality of underlying test lots, and each sampled to conform to a statistical-based sampling plan, and therefore the composite/lumped samples of the prior art do NOT provide the validatation and release function in the context of the claimed method. In fact, the lumped test lot samples/cultures of the prior art relate to a single underlying test lot, and the established function of the

lumped test lot samples/cultures of asserted Jarvis, FSIS, Midura, Price, Silliker, etc., references is to reduce the work and expense of sampling, **not increase the work and expense as required to provide for validation and release of individual respective test lots as presently claimed**.

Patentee's modular composite sample is *fundamentally different* because it is formed by combining aliquots from each of a plurality of statistically sufficient test lot cultures (i.e., each comprising a statistically sufficient number of sample portions) to enable independent validation for release of each of the respective underlying plurality of separate test lots.

Patentee contends that Patentee's claims cannot be obvious in view of the alleged asserted art, alone or in combination, under U.S. patent law, because the prior art, alone or in combination, does not teach or motivate several of Patentee's presently recited claim limitations, including Patentee's unique "modular composite sample" claim element, which would be contraindicated (disincentive) by the prior art as discussed in detail herein.

In summary, Price, alone or in combination with BAM and/or Davies, is fundamentally distinguished from the current set of claims. In contrast to Patentee's method (see, e.g., steps (2)-(5) of attached Figure 2, Exhibit B), Price, alone or in combination with BAM and/or Davies, does not teach: (i) initial formation of a plurality of composited/lumped test lot sample/cultures having a statistically sufficient number of sample portions form each of a respective pluralityof separate underlying test lots to be validated for release; that (ii) are enriched to above the detection limit, as presently claimed, *prior* to (iii) formation of the modular composite sample as presently claimed; that (iv) can be tested immediately, and where (v) the individual enriched composited test lot samples can be also be tested immediately with a requirement for further enrichment; and (vi) 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…and obtaining a negative test result." Price, alone or in combination with BAM and/or Davies, teaches nothing about post-enrichment pooling as presently claimed, nothing about post-enrichment formation of a modular composite sample as presently claimed, and nothing about validation of negative lots where a post-enrichment pool (modular composite

sample) is found to be positive, as presently claimed.

Patentee respectfully maintains that the above asserted teachings **do not in fact raise any substantial new questions of patentability** beyond those already of record in the underlying issued patent prosecution history.

Patentee, therefore, respectfully requests withdrawal of the rejection of claims 1, 17 and 34-36, under 35 U.S.C. 103(a), as allegedly being unpatentable over Price in view of BAM and Davies.

Ground #10. The Examiner did not adopt Ground #10 for the reasons stated in the Office Action. Patentee, for the record, traverses Ground #10 even if it had been adopted, based on the arguments discussed herein.

Ground #11. The Examiner did not adopt Ground #11 for the reasons stated in the Office Action. Patentee, for the record, traverses Ground #11 even if it had been adopted, based on the arguments discussed herein.

Ground #12. The Examiner did not adopt Ground #12 for the reasons stated in the Office Action. Patentee, for the record, traverses Ground #12 even if it had been adopted, based on the arguments discussed herein.

Ground #13. The Examiner did not adopt Ground #13 for the reasons stated in the Office Action. Patentee, for the record, traverses Ground #13 even if it had been adopted, based on the arguments discussed herein.

Ground #14. The Examiner rejected claims 5, 6, 23, 29, and 30, under 35 U.S.C. 103(a), as allegedly being unpatentable over Jarvis in view of FSIS and Midura, as applied to claim 1 above, and further in view of Entis.

The Examiner discusses Jarvis in view of FSIS and Midura as was set forth in 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 immunochemistry 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). 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)."

The Examiner states that "[o]ne 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."

Patentee respectfully traverses the rejection.

Patentee respectfully contends that with respect to Patentee's presently presented claims, Jarvis in view of FSIS and Midura have been rebutted and distinguished by Patentee's claim amendments and arguments in response to the Examiner's Ground 1. Entis, even as construed by the Examiner, does **not** cure this deficiency.

Patentee points out that a distinguishing aspect of Patentee's invention lies in the fact that because Patentee's methods comprise analysis of a plurality of separate test lots, the specific composition/nature of Patentee's modular composite sample is ***unique***—being comprised of a plurality of lumped test lot samples/cultures each having a statistically sufficient number of sample protions from each of a respective plurality of separate underlying test lots to be validated for release, and each sampled to conform to a statistical-based sampling plan, which aspects enable validation and rapid release of individual respective test lots to enhance efficiency and safety, and reduce the amount of product destroyed and/or diverted into lower value products.

In overall summary, Patentee's invention is **more than a predictable use of prior art elements according to their established functions**. Specifically, the formation of Patentee's modular composite sample is by combining aliquots of a plurality of lumped test lot samples/cultures, each attributable to a respective plurality of underlying test lots, and each sampled to conform to a statistical-based sampling plan, which aspects enable validation and release of individual respective test lots. The asserted prior art, alone or in combination, has no such modular composite sample, and no such plurality of lumped test lot samples/cultures, each attributable to a respective plurality of underlying test lots, and each sampled to conform to a statistical-based sampling plan, and therefore the composite/lumped samples of the prior art do NOT provide the validatation and release function in the context of the claimed method. In fact, the lumped test lot samples/cultures of the prior art relate to a single underlying test lot, and the established function of the

lumped test lot samples/cultures of asserted Jarvis, FSIS, Midura, Price, Silliker, etc., references is to reduce the work and expense of sampling, **not increase the work and expense as required to provide for validation and release of individual respective test lots as presently claimed**.

Patentee's modular composite sample is *fundamentally different* because it is formed by combining aliquots from each of a plurality of statistically sufficient test lot cultures (i.e., each comprising a statistically sufficient number of sample portions) to enable independent validation for release of each of the respective underlying plurality of separate test lots.

Patentee contends that Patentee's claims cannot be obvious in view of the alleged asserted art, alone or in combination, under U.S. patent law, because the prior art, alone or in combination, does not teach or motivate several of Patentee's presently recited claim limitations, including Patentee's unique "modular composite sample" claim element, which would be contraindicated (disincentive) by the prior art as discussed in detail herein.

Patentee respectfully maintains that the above asserted teachings **do not in fact raise any substantial new questions of patentability** beyond those already of record in the underlying issued patent prosecution history.

Claims 5, 6, 23, 29, and 30 depend, directly or indirectly from claim 1.

Patentee, therefore, respectfully requests withdrawal of the rejection of claims 5, 6, 23, 29, and 30, under 35 U.S.C. 103(a), as allegedly being unpatentable over Jarvis in view of FSIS and Midura, as applied to claim 1 above, and further in view of Entis.

Ground #15. The Examiner rejected claims 5, 6, 23, 29, and 30, under 35 U.S.C. 103(a), as allegedly being unpatentable over FSIS in view of Price and Midura, as applied to claim 1 above, and further in view of Entis.

The Examiner states that "FSIS in view of Price and Midura (Ground 2) 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, 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.

Patentee respectfully disagrees as discussed herein above.

The Examiner states that "[n]one 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."

The Examiner states that "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)."

The Examiner states that "[o]ne 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."

Patentee respectfully traverses the rejection.

Patentee respectfully contends that with respect to Patentee's presently presented claims, FSIS in view of Price and Midura have been rebutted and distinguished by Patentee's claim amendments and arguments in response to the Examiner's Ground 2. Entis, even as construed by the Examiner, does **not** cure this deficiency.

Patentee points out that a distinguishing aspect of Patentee's invention lies in the fact that because Patentee's methods comprise analysis of a plurality of separate test lots, the specific composition/nature of Patentee's modular composite sample is ***unique***—being comprised of a plurality of lumped test lot samples/cultures each comprising a statistically sufficient number of sample portions from each of a respective plurality of separate underlying test lots to be validated for release, and each sampled to conform to a statistical-based sampling plan, which aspects enable validation and rapid release of individual respective test lots to enhance efficiency and safety, and reduce the amount of product destroyed and/or diverted into lower value products.

In overall summary, Patentee's invention is **more than a predictable use of prior art elements according to their established functions**. Specifically, the formation of Patentee's modular composite sample is by combining aliquots of a plurality of lumped test lot samples/cultures, each attributable to a respective plurality of underlying test lots, and each sampled to conform to a statistical-based sampling plan, which aspects enable validation and release of individual respective test lots. The asserted prior art, alone or in combination, has no such modular composite sample, and no such plurality of lumped test lot samples/cultures, each attributable to a respective plurality of underlying test lots, and each sampled to conform to a statistical-based sampling plan, and therefore the composite/lumped samples of the prior art do NOT provide the validatation and release function in the context of the claimed method. In fact, the lumped test lot samples/cultures

of the prior art relate to a single underlying test lot, and the established function of the lumped test lot samples/cultures of asserted Jarvis, FSIS, Midura, Price, Silliker, etc., references is to reduce the work and expense of sampling, **not increase the work and expense as required to provide for validation and release of individual respective test lots as presently claimed**.

Patentee's modular composite sample is *fundamentally different* because it is formed by combining aliquots from each of a plurality of statistically sufficient test lot cultures (i.e., each comprising a statistically sufficient number of sample portions) to enable independent validation for release of each of the respective underlying plurality of separate test lots.

Patentee contends that Patentee's claims cannot be obvious in view of the alleged asserted art, alone or in combination, under U.S. patent law, because the prior art, alone or in combination, does not teach or motivate several of Patentee's presently recited claim limitations, including Patentee's unique "modular composite sample" claim element, which would be contraindicated (disincentive) by the prior art as discussed in detail herein.

Patentee respectfully maintains that the above asserted teachings **do not in fact raise any substantial new questions of patentability** beyond those already of record in the underlying issued patent prosecution history.

Claims 5, 6, 23, 29, and 30depend, directly or indirectly from claim 1.

Patentee, therefore, respectfully requests withdrawal of the rejection of claims 5, 6, 23, 29, and 30, under 35 U.S.C. 103(a), as allegedly being unpatentable over FSIS in view of Price and Midura, as applied in Ground 2 above, and further in view of Entis.

Ground #16. The Examiner did not adopt Ground #16 for the reasons stated in the Office Action. Patentee, for the record, traverses Ground #16 even if it had been adopted, based on the arguments discussed herein.

Ground #17. The Examiner did not adopt Ground #17 for the reasons stated in the Office Action. Patentee, for the record, traverses Ground #17 even if it had been adopted, based on the arguments discussed herein.

Ground #18. The Examiner did not adopt Ground #18 for the reasons stated in the Office Action. Patentee, for the record, traverses Ground #18 even if it had been adopted, based on the arguments discussed herein.

Ground #19. The Examiner did not adopt Ground #19 for the reasons stated in the Office Action. Patentee, for the record, traverses Ground #19 even if it had been adopted, based on the arguments discussed herein.

Ground #20. The Examiner did not adopt Ground #20 for the reasons stated in the Office Action. Patentee, for the record, traverses Ground #20 even if it had been adopted, based on the arguments discussed herein.

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

The Examiner discusses Jarvis in view of FSIS and Midura as set forth in the Examiner's Ground 1.

The Examiner 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)."

The Examiner states that "[i]t 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."

Patentee respectfully traverses the rejection.

Patentee respectfully contends that with respect to Patentee's presently presented claims, Jarvis in view of FSIS and Midura have been rebutted and distinguished by Patentee's claim amendments and arguments in response to the Examiner's Ground 1. Cousin, even as construed by the Examiner, does **not** cure this deficiency.

Patentees point out that a distinguishing aspect of Patentee's invention lies in the fact that because Patentee's methods comprise simultaneous analysis of a plurality of separate test lots, the specific composition/nature of Patentee's modular composite sample is ***unique***—being comprised of a plurality of lumped test lot samples/cultures, each attributable to a respective plurality of underlying test lots, and each sampled to conform to a statistical-based sampling plan, which aspects enable validation and rapid release of individual respective test lots to enhance efficiency and safety, and reduce the amount of product destroyed and/or diverted into lower value products.

In overall summary, Patentee's invention is **more than a predictable use of prior art elements according to their established functions**. Specifically, the formation of Patentee's modular composite sample is by combining aliquots of a plurality of lumped test lot samples/cultures, each attributable to a respective plurality of underlying test lots, and each sampled to conform to a statistical-based sampling plan, which aspects enable validation and release of individual respective test lots. The asserted prior art, alone or in combination, has no such modular composite sample, and no such plurality of lumped test

lot samples/cultures, each attributable to a respective plurality of underlying test lots, and each sampled to conform to a statistical-based sampling plan, and therefore the composite/lumped samples of the prior art do NOT provide the validatation and release function in the context of the claimed method. In fact, the lumped test lot samples/cultures of the prior art relate to a single underlying test lot, and the established function of the lumped test lot samples/cultures of asserted Jarvis, FSIS, Midura, Price, Silliker, Cousin etc., references is to reduce the work and expense of sampling, **not increase the work and expense as required to provide for validation and release of individual respective test lots as presently claimed**.

Patentee's modular composite sample is *fundamentally different* because it is formed by combining aliquots from each of a plurality of statistically sufficient test lot cultures (i.e., each comprising a statistically sufficient number of sample portions) to enable independent validation for release of each of the respective underlying plurality of separate test lots.

Patentee contends that Patentee's claims cannot be obvious in view of the alleged asserted art, alone or in combination, under U.S. patent law, because the prior art, alone or in combination, does not teach or motivate several of Patentee's presently recited claim limitations, including Patentee's unique "modular composite sample" claim element, which would be contraindicated (disincentive) by the prior art as discussed in detail herein.

Patentee respectfully maintains that the above asserted teachings **do not in fact raise any substantial new questions of patentability** beyond those already of record in the underlying issued patent prosecution history.

Claim 21 depends directly from claim 1.

Patentee, therefore, respectfully requests withdrawal of the rejection of claim 21, under 35 U.S.C. 103(a), as allegedly being unpatentable over Jarvis in view of FSIS and Midura, as applied to claim 1 above, and further in view of Cousin.

Ground #22. The Examiner rejected claim 21, under 35 U.S.C. 103(a), as allegedly

being unpatentable over FSIS in view of Price and Midura, as applied to claim 1 above, and further in view of Cousin.

The Examiner discusses FSIS in view of Price and Midura as set forth in the Examiner's Ground 2.

The Examiner 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)."

The Examiner states that "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."

Patentee respectfully traverses the rejection.

Patentee respectfully contends that with respect to Patentee's presently presented claims, FSIS in view of Price and Midura have been rebutted and distinguished by Patentee's claim amendments and arguments in response to the Examiner's Ground 1. Cousin, even as construed by the Examiner, does **not** cure this deficiency.

Patentee points out that a distinguishing aspect of Patentee's invention lies in the fact that because Patentee's methods comprise simultaneous analysis of a plurality of separate test lots, the specific composition/nature of Patentee's modular composite sample is ***unique***—being comprised of a plurality of lumped test lot samples/cultures, each attributable to a respective plurality of underlying test lots, and each sampled to conform to a statistical-based sampling plan, which aspects enable validation and rapid release of individual respective test lots to enhance efficiency and safety, and reduce the amount of product destroyed and/or diverted into lower value products.

In overall summary, Patentee's invention is **more than a predictable use of prior**

art elements according to their established functions. Specifically, the formation of Patentee's modular composite sample is by combining aliquots of a plurality of lumped test lot samples/cultures, each attributable to a respective plurality of underlying test lots, and each sampled to conform to a statistical-based sampling plan, which aspects enable validation and release of individual respective test lots. The asserted prior art, alone or in combination, has no such modular composite sample, and no such plurality of lumped test lot samples/cultures, each attributable to a respective plurality of underlying test lots, and each sampled to conform to a statistical-based sampling plan, and therefore the composite/lumped samples of the prior art do NOT provide the validatation and release function in the context of the claimed method. In fact, the lumped test lot samples/cultures of the prior art relate to a single underlying test lot, and the established function of the lumped test lot samples/cultures of asserted Jarvis, FSIS, Midura, Price, Silliker, Cousin etc., references is to reduce the work and expense of sampling, **not increase the work and expense as required to provide for validation and release of individual respective test lots as presently claimed**.

Patentee's modular composite sample is *fundamentally different* because it is formed by combining aliquots from each of a plurality of statistically sufficient test lot cultures (i.e., each comprising a statistically sufficient number of sample portions) to enable independent validation for release of each of the respective underlying plurality of separate test lots.

Patentee contends that Patentee's claims cannot be obvious in view of the alleged asserted art, alone or in combination, under U.S. patent law, because the prior art, alone or in combination, does not teach or motivate several of Patentee's presently recited claim limitations, including Patentee's unique "modular composite sample" claim element, which would be contraindicated (disincentive) by the prior art as discussed in detail herein.

Patentee respectfully maintains that the above asserted teachings **do not in fact raise any substantial new questions of patentability** beyond those already of record in the underlying issued patent prosecution history.

Claim 21 depends directly from claim 1.

Patentee, therefore, respectfully requests withdrawal of the rejection of claim 21, under 35 U.S.C. 103(a), as allegedly being unpatentable over FSIS in view of Price and Midura, as applied to claim 1 above, and further in view of Cousin.

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

The Examiner discusses Jarvis in view of FSIS and Midura as set forth in the Examiner's Ground 1.

The Examiner states that "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."

The Examiner states that "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."

Patentee respectfully traverses the rejection.

Patentee respectfully contends that with respect to Patentee's presently presented claims, Jarvis in view of FSIS and Midura have been rebutted and distinguished by Patentee's claim amendments and arguments in response to the Examiner's Ground 1. Price, even as construed by the Examiner, does **not** cure this deficiency.

Patentee points out that a distinguishing aspect of Patentee's invention lies in the fact that because Patentee's methods comprise simultaneous analysis of a plurality of separate test lots, the specific composition/nature of Patentee's modular composite sample is ***unique***—being

comprised of a plurality of lumped test lot samples/cultures, each attributable to a respective plurality of underlying test lots, and each sampled to conform to a statistical-based sampling plan, which aspects enable validation and rapid release of individual respective test lots to enhance efficiency and safety, and reduce the amount of product destroyed and/or diverted into lower value products.

In overall summary, Patentee's invention is **more than a predictable use of prior art elements according to their established functions**. Specifically, the formation of Patentee's modular composite sample is by combining aliquots of a plurality of lumped test lot samples/cultures, each attributable to a respective plurality of underlying test lots, and each sampled to conform to a statistical-based sampling plan, which aspects enable validation and release of individual respective test lots. The asserted prior art, alone or in combination, has no such modular composite sample, and no such plurality of lumped test lot samples/cultures, each attributable to a respective plurality of underlying test lots, and each sampled to conform to a statistical-based sampling plan, and therefore the composite/lumped samples of the prior art do NOT provide the validatation and release function in the context of the claimed method. In fact, the lumped test lot samples/cultures of the prior art relate to a single underlying test lot, and the established function of the lumped test lot samples/cultures of asserted Jarvis, FSIS, Midura, Price, Silliker, Cousin etc., references is to reduce the work and expense of sampling, **not increase the work and expense as required to provide for validation and release of individual respective test lots as presently claimed**.

Patentee's modular composite sample is *fundamentally different* because it is formed by combining aliquots from each of a plurality of statistically sufficient test lot cultures (i.e., each comprising a statistically sufficient number of sample portions) to enable independent validation for release of each of the respective underlying plurality of separate test lots.

Patentee contends that Patentee's claims cannot be obvious in view of the alleged asserted art, alone or in combination, under U.S. patent law, because the prior art, alone or in combination, does not teach or motivate several of Patentee's presently recited claim limitations,

including Patentee's unique "modular composite sample" claim element, which would be contraindicated (disincentive) by the prior art as discussed in detail herein.

Patentee respectfully maintains that the above asserted teachings **do not in fact raise any substantial new questions of patentability** beyond those already of record in the underlying issued patent prosecution history.

Claim 23 depends directly from claim 1.

Patentee, therefore, respectfully requests withdrawal of the rejection of claim 23, under 35 U.S.C. 103(a), as allegedly being unpatentable over Jarvis in view of FSIS and Midura, as applied to claim 1 above, and further in view of Price.

Ground #24. The Examiner rejected claims 24, 28, 32 and 33, under 35 U.S.C. 103(a), as allegedly being unpatentable over FSIS in combination with Price and Midura, as applied to claims 1 and 4 above, and further in view of Gabis.

The Examiner discusses FSIS in view of Price and Midura as set forth in the Examiner's Ground 2.

The Examiner states 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 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)."

The Examiner states that "[i]t 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 for poultry because Gabis teaches that the method is suitable for such high moisture foods."

Patentee respectfully traverses the rejection.

Patentee respectfully contends that with respect to Patentee's presently presented claims, FSIS in view of Price and Midura have been rebutted and distinguished by Patentee's claim amendments and arguments in response to the Examiner's Ground 2. Gabis, already discussed and rebutted in detail herein above, does not cure this deficiency.

Patentee points out that a distinguishing aspect of Patentee's invention lies in the fact that because Patentee's methods comprise simultaneous analysis of a plurality of separate test lots, the specific composition/nature of Patentee's modular composite sample is ***unique***—being comprised of a plurality of lumped test lot samples/cultures, each attributable to a respective plurality of underlying test lots, and each sampled to conform to a statistical-based sampling plan, which aspects enable validation and rapid release of individual respective test lots to enhance efficiency and safety, and reduce the amount of product destroyed and/or diverted into lower value products.

In overall summary, Patentee's invention is **more than a predictable use of prior art elements according to their established functions**. Specifically, the formation of Patentee's modular composite sample is by combining aliquots of a plurality of lumped test lot samples/cultures, each attributable to a respective plurality of underlying test lots, and

each sampled to conform to a statistical-based sampling plan, which aspects enable validation and release of individual respective test lots. The asserted prior art, alone or in combination, has no such modular composite sample, and no such plurality of lumped test lot samples/cultures, each attributable to a respective plurality of underlying test lots, and each sampled to conform to a statistical-based sampling plan, and therefore the composite/lumped samples of the prior art do NOT provide the validatation and release function in the context of the claimed method. In fact, the lumped test lot samples/cultures of the prior art relate to a single underlying test lot, and the established function of the lumped test lot samples/cultures of asserted Jarvis, FSIS, Midura, Price, Silliker, Cousin etc., references is to reduce the work and expense of sampling, **not increase the work and expense as required to provide for validation and release of individual respective test lots as presently claimed**.

Patentee's modular composite sample is *fundamentally different* because it is formed by combining aliquots from each of a plurality of statistically sufficient test lot cultures (i.e., each comprising a statistically sufficient number of sample portions) to enable independent validation for release of each of the respective underlying plurality of separate test lots.

Patentee contends that Patentee's claims cannot be obvious in view of the alleged asserted art, alone or in combination, under U.S. patent law, because the prior art, alone or in combination, does not teach or motivate several of Patentee's presently recited claim limitations, including Patentee's unique "modular composite sample" claim element, which would be contraindicated (disincentive) by the prior art as discussed in detail herein.

Patentee respectfully maintains that the above asserted teachings **do not in fact raise any substantial new questions of patentability** beyond those already of record in the underlying issued patent prosecution history.

Claims 24, 28, 32 and 33 depend, directly or indirectly, from claims 1 or 17.

Patentee, therefore, respectfully requests withdrawal of the rejection of claims 24, 28, 32 and 33, under 35 U.S.C. 103(a), as allegedly being unpatentable over FSIS in combination with Price and Midura, as applied to claims 1 and 4 above, and further in view of Gabis.

Ground #25. The Examiner rejected claim 28, under 35 U.S.C. 103(a), as allegedly being unpatentable over Jarvis in view of FSIS and Midura, as applied to claims 1 and 4 above, and further in view of Andrews.

The Examiner discusses Jarvis in view of FSIS and Midura as set forth in the Examiner's Ground 1.

The Examiner states that "[n]one of Jarvis, FSIS, or Midura teaches the enrichment medium in the individual broth cultures as comprising a selective agent for the target organism which inhibits the growth of competing bacteria."

The Examiner states that "Andrews teaches selective enrichment for *Salmonella,* wherein the enrichment medium contains an agent which is selective for the target organism and inhibitory for competing bacteria. Andrews teaches that the use of two or more selective media provides greater method sensitivity than a single enrichment medium (see page 360, column 2, lection 37.124)."

The Examiner states that "[o]ne of ordinary skill in the art at the time of the invention would have found it *prima facie* obvious to have included a selective agent in the pre-enrichment both cultures of Jarvis in view of FSIS and Midura because Andrews teaches that the use of two or more selective media improves method sensitivity."

Patentee respectfully traverses the rejection.

Patentee respectfully contends that with respect to Patentee's presently presented claims, Jarvis in view of FSIS and Midura have been rebutted and distinguished by Patentee's claim amendments and arguments in response to the Examiner's Ground 1. Andrews, also discussed in detail herein above, does not cure this deficiency..

Patentee points out that a distinguishing aspect of Patentee's invention lies in the fact that because Patentee's methods comprise simultaneous analysis of a plurality of separate test lots, the specific composition/nature of Patentee's modular composite sample is ***unique***—being comprised of a plurality of lumped test lot samples/cultures, each attributable to a respective

plurality of underlying test lots, and each sampled to conform to a statistical-based sampling plan, which aspects enable validation and rapid release of individual respective test lots to enhance efficiency and safety, and reduce the amount of product destroyed and/or diverted into lower value products.

In overall summary, Patentee's invention is **more than a predictable use of prior art elements according to their established functions**. Specifically, the formation of Patentee's modular composite sample is by combining aliquots of a plurality of lumped test lot samples/cultures, each attributable to a respective plurality of underlying test lots, and each sampled to conform to a statistical-based sampling plan, which aspects enable validation and release of individual respective test lots. The asserted prior art, alone or in combination, has no such modular composite sample, and no such plurality of lumped test lot samples/cultures, each attributable to a respective plurality of underlying test lots, and each sampled to conform to a statistical-based sampling plan, and therefore the composite/lumped samples of the prior art do NOT provide the validatation and release function in the context of the claimed method. In fact, the lumped test lot samples/cultures of the prior art relate to a single underlying test lot, and the established function of the lumped test lot samples/cultures of asserted Jarvis, FSIS, Midura, Price, Silliker, Cousin etc., references is to reduce the work and expense of sampling, **not increase the work and expense as required to provide for validation and release of individual respective test lots as presently claimed**.

Patentee's modular composite sample is *fundamentally different* because it is formed by combining aliquots from each of a plurality of statistically sufficient test lot cultures (i.e., each comprising a statistically sufficient number of sample portions) to enable independent validation for release of each of the respective underlying plurality of separate test lots.

Patentee contends that Patentee's claims cannot be obvious in view of the alleged asserted art, alone or in combination, under U.S. patent law, because the prior art, alone or in combination, does not teach or motivate several of Patentee's presently recited claim limitations, including Patentee's unique "modular composite sample" claim element, which would be

contraindicated (disincentive) by the prior art as discussed in detail herein.

Patentee respectfully maintains that the above asserted teachings **do not in fact raise any substantial new questions of patentability** beyond those already of record in the underlying issued patent prosecution history.

Claim 28 depends indirectly, from claim 1.

Patentee, therefore, respectfully requests withdrawal of the rejection of claim 28, under 35 U.S.C. 103(a), as allegedly being unpatentable over Jarvis in view of FSIS and MIdura, as applied to claims 1 and 4 above, and further in view of Andrews.

Ground #26. The Examiner did not adopt Ground #26 for the reasons stated in the Office Action. Patentee, for the record, traverses Ground #26 even if it had been adopted, based on the arguments discussed herein.

Response to the Samadpour and Koohmarie Declarations

Samadpour Declaration

The Examiner at page 57 of the ACP, states that "with respect to obviousness, Dr. Samadpour asserts that the person of ordinary skill would not have relied upon results from a test that utilized enrichment only, due to dilution of the organism and competition from non-target organisms (1l8). Dr. Samadpour additionally asserts that the prior art teaches away from combining lumping and pooling in a single scheme and that the prior. art was limited to the pre-enrichment level limited to *Salmonella* for release of a single lot of product. As has been discussed herein in response to Patent owner's arguments, with the exception of claims 4 and 28, the claims do not require a selective pre-enrichment broth, but rather simply recite incubating under conditions suitable to allow levels of the target microbial agent to increase or to reach detectable levels. The pre-enrichment broth cultures of the prior art of record (Jarvis, Price, Silliker, and Gabis, for example) fully meet this limitation, as it is recited in the claims. As for

claims 4 and 28, which specifically require incubation in an enrichment medium (described as selective enrichment medium in the '584 patent specification), while the prior art of record teaches that it was routine in the art at the time of the invention to inoculate specimens directly into an enrichment broth (see Jarvis at page 135, Section 8.4), the prior art of record is generally silent as to the merits of inoculating lumped specimens into selective enrichment medium vs. pre-enrichment medium, with the exceptions of Gabis, who recommends pre-enrichment over direct enrichment for high moisture foods (see Gabis abstract) and Price, who teaches that when problems with interfering organisms are encountered, direct inoculation of samples into selective enrichment medium or two selective enrichments, rather than preenrichment medium, should be performed (see Price at page 682, first full paragraph). The alternative method described by Price comprises inoculation of individual food samples directly into selective enrichment broths (as an alternative to pre-enrichment broth cultures) and subsequent wet-composite pooling of the enrichment broth cultures into a second enrichment broth for detection of the target organisms. It is noted that the instant claimed methods comprise the recited steps; they do not exclude additional steps, such as the additional selective enrichment step described by Price" (emphasis added by the Patentee).

Patentee, respectfully disagrees, based on the claim amendments and responsive rebuttal arguments presented in this Response to ACP.

Additionally, Patentee submits a further DECLARATION of Dr. Samadpour (attached hereto), which further clarifies the real-world differences between "pre-enrichment" incubation and "enrichment" incubations as recognized and accepted in the art.

Additionally, the further DECLARATION of Dr. Samadpour provides more insight into the non-obvious aspects of his invention, and the challenges faced in initially getting acceptance by the industry.

Additionally, with respect to the Examiner's comments at page 58 of the APC relating to multiple vs single lot sampling (inter-lot vs intra-lot), the further DECLARATION of Dr. Samadpour provides further clarification of the underlying differences. Moreover, the claims

have been amended to delete references to "inter-lot" and "intra-lot" as these concepts are implicit in the presently presented claims.

With respect to the Examiner's comments at page 58-59 relating to corroborating the nexus between the claimed subject matter and commercial success, the further DECLARATION of Dr. Samadpour provides actual accounting data corroborating the acceptance by the industry and the nexus between commercial success and Dr. Samadpour's claimed invention. Counter intuitively, clients are willing to pay substantially more for testing for reasons explained in detail in the further DECLARATION. It is clear from the further DECLARATION that customers are given a choice between the traditional assay method and the modular compositing approach, and that despite a substantial cost increase, the claimed methods have rapidly displaced the traditional testing methods, even though more test lots are found to be positive (greater sensitivity) with increased assay and sampling costs—and this is because of (1) enhanced safety, and (2), due the ability of the claimed assay to validate and release negative enriched test samples, each attributable to a respective test lot, such that there is, overall, much less product that gets destroyed and/or diverted into lower value products. Patentees contend that the revenue figures showing preference in the marketplace for the claimed method in the context of a clear customer "choice" between assays, serve to reasonably corroborate the nexus between the claimed methods and the commercial success—particularly in view of the substantially increased sampling and assay costs associated with the claimed method.

Additionally, the further DECLARATION of Dr. Samadpour describes the challenges in getting the regulatory agencies and customers to initially accept the method—because it was so counterintuitive. Such initial resistance in the marketplace further corroborates the nexus between the claimed method and commercial success, because it corroborates that the method had to win acceptance based on the merits of its claim elements, and not on marketing and/or other factors unrelated to the claim limitations.

Finally Patentee submits the Declaration of Warren Mirtsching, and the Declaration of Dr. Byron.

The Declaration of Warren Mirtsching 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.

The Declaration of Dr. Byron 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.

Koohmaraie Declaration

The Examiner states that "While it is agreed that the data presented in the Koohmaraie Declaration demonstrates a decrease in the number of bins which are unnecessarily discarded upon obtaining a positive result due to the subsequent ability to test broth cultures inoculated with the individual bins, the comparison does not speak to the obviousness of the claimed method over the prior art of record. All of the prior art rejections of record herein are based on methods of incubating samples or lots individually in broth cultures prior to pooling and identification. For this reason, the Declaration is not sufficient to overcome the *prima facie* obviousness rejections, which are set forth herein."

Patentee respectfully disagrees. As outlined in detail herein, there is no motivation provided by the asserted prior art, alone or in combination, to practice Patentee's claimed invention.

Patentee points out that a distinguishing aspect of Patentee's invention lies in the fact that because Patentee's methods comprise sanalysis of a plurality of separate test lots, the specific composition/nature of Patentee's modular composite sample is ***unique***—being comprised of a plurality of lumped test lot samples/cultures, each attributable to a respective plurality of underlying test lots, and each sampled to conform to a statistical-based sampling plan, which aspects enable validation and rapid release of individual respective test lots to enhance efficiency and safety, and reduce the amount of product destroyed and/or diverted into lower value

products.

In overall summary, Patentee's invention is **more than a predictable use of prior art elements according to their established functions**. Specifically, the formation of Patentee's modular composite sample is by combining aliquots of a plurality of lumped test lot samples/cultures, each attributable to a respective plurality of underlying test lots, and each sampled to conform to a statistical-based sampling plan, which aspects enable validation and release of individual respective test lots. The asserted prior art, alone or in combination, has no such modular composite sample, and no such plurality of lumped test lot samples/cultures, each attributable to a respective plurality of underlying test lots, and each sampled to conform to a statistical-based sampling plan, and therefore the composite/lumped samples of the prior art do NOT provide the validatation and release function in the context of the claimed method. In fact, the lumped test lot samples/cultures of the prior art relate to a single underlying test lot, and the established function of the lumped test lot samples/cultures of asserted Jarvis, FSIS, Midura, Price, Silliker, Cousin etc., references is to reduce the work and expense of sampling, **not increase the work and expense as required to provide for validation and release of individual respective test lots as presently claimed**.

Patentee's modular composite sample is *fundamentally different* because it is formed by combining aliquots from each of a plurality of statistically sufficient test lot cultures (i.e., each comprising a statistically sufficient number of sample portions) to enable independent validation for release of each of the respective underlying plurality of separate test lots.

Patentee contends that Patentee's claims cannot be obvious in view of the alleged asserted art, alone or in combination, under U.S. patent law, because the prior art, alone or in combination, does not teach or motivate several of Patentee's presently recited claim limitations, including Patentee's unique "modular composite sample" claim element, which would be contraindicated (disincentive) by the prior art as discussed in detail herein.

Rejections necessitated by Amendment

Rejections under 35 USC 112

Claims 1, 17, 34 and 35 were rejected, under 35 USC 112, second paragraph as being allegedly indefinite in view of recitation of "inter-lot" as stated by the Examiner in the ACP.

Patentee, without acquiescing to the conclusion of the Examiner, has nonetheless deleted references in the claims to "inter-lot." Patentees contend that this term is not necessary, because the inter-lot concept is already explicit and implicit to the claims, and has been further clarified by the present claim amendments, which are fully supported by Patentee's patent.

Patentee points out that a distinguishing aspect of Patentee's invention lies in the fact that because Patentee's methods comprise analysis of a plurality of separate test lots, the specific composition/nature of Patentee's modular composite sample is ***unique***—being comprised of a plurality of lumped test lot samples/cultures, each comprising a statistically sufficient number of sample portions from each of a respective plurality of separate underlying test lots to be validated for release, and each sampled to conform to a statistical-based sampling plan, which aspects enable validation and rapid release of individual respective test lots to enhance efficiency and safety, and reduce the amount of product destroyed and/or diverted into lower value products.

Patentee, therefore, respectfully requests withdrawal of this rejection.

Rejections under 35 USC 112

Claims 1, 17, 34 and 35 were rejected, under 35 USC 112, first paragraph as allegedly lacking sufficient written description in view of recitation of "inter-lot" as stated by the Examiner in the ACP.

Patentee, without acquiescing to the conclusion of the Examiner, has nonetheless deleted references in the claims to "inter-lot." Patentees contend that this term is not necessary, because the inter-lot concept is already explicit and implicit to the claims, and has been further clarified by the present claim amendments, which are fully supported by Patentee's patent.

Additionally, Patentees have amended step a) of claims 1 and 17 to recite:

"…a number of portions to be taken and growth conditions sufficient to provide for validation and release of the test lot into commerce,"

and have further amended step e) of claims 1 and 17 to recite:

", 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).

Explicit support for the amendments has already been cited herein above.

Patentee contends, therefore, that it would be clear to one of ordinary skill in the art that the number of samples is selected to conform to a statistical-based sampling plan, and that this number provides for validation of negative test lot samples for release into commerce, as presently claimed.

Patentee further contends that, contrary to the Examiner's statement, Patentee's patent does provide specific examples of the number of samples to be taken to conform to particular statistical-based sampling plans (see, e.g., col. 2, ll. 14-16, and ll. 16-18 (*E. coli* O157 testing, and *Listeria moncytogenes* testing). Moreover, Patentee's specification further teaches that the number of samples will vary depending on the particular plan, based on "the outcome of the infection/poisoning (e.g., the severity), the level of hazards, and potential increase in the hazard levels due to storage," generally reflecting the "overall sensitivity of the test method and the limit of detection that applies to the test unit,…" (see col. 2, ll. 1-26). Patentees contend therefore, based on Patentee's teachings and the general knowledge in the art regarding statistical-based sampling plans, that one of skill in the art would know how to practice the claimed invention, without undue experimentation, by sampling to conform to a statistical-based sampling plan for a particular microbial agent or organism, such that negative test lot samples/cultures could be validated for release into commerce. There is no need therefore, for Patentees independent claims to be limited to any specific number, as this would depend on the particular microbial agent or organism and the particular statistical-based sampling plan.

Patentee, therefore, respectfully requests withdrawal of this rejection.

Rejections under 35 USC 305

Claims 1-36 were rejected, under 35 USC 305, as allegedly enlarging the scope of the patent being examined in view of the new matter described above.

Patentees have deleted the reference to "inter-lot."

Patentees further contend that recitation of the phase "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" does not add new matter because it is explicitly supported by the original specification and claims, and moreover is a clarifying amendment that serves to clarify the existing claim limits, rather than broaden them.

Likewise, the step e) amendment of "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)" is not new matter as described herein above, and moreover is a clarifying amendment that serves to clarify the existing claim limits, rather than broaden them.

Other Issues

The Examiner states that "it is noted that an issue not within the scope of reexamination proceedings has been raised: On pages 46-47 of the Requester's Comments filed *08/25/10,* Requester states that new claim 36 is improper because it was previously not elected in response to a restriction requirement during the prosecution of the application which issued as the '584 patent. The issue will not be considered in a reexamination proceeding. 37 CFR 1.552(c). While this issue is not within the scope of reexamination, the patentee is advised that it may be desirable to consider filing a reissue application provided that the patentee believes one or more claims to be partially or wholly inoperative or invalid based upon the issue."

Patentees have herein cancelled claim 36 without prejudice to obviate this issue.

Application No.:	95/001,294
Attorney Docket No.:	66090-008US2
First Patentee's Name:	7534584 (Mansour Samadpour)
Application Filing Date:	05 April 2010
Notice Regarding Defective Paper Dated:	27 April 2011
Date of Response:	05 May 2011
Examiner:	Brenda G. Brumback

Duty to Disclose

Patent owner is not aware of any litigation activity, or other prior or concurrent proceeding, involving U.S. Patent 7,534,584

Level of skill in the art

As an additional matter, Patentee contends, with respect to the Examiner's asserted obviousness rejections in the ACP, that the level of skill attributed by the Examiner to a "person of ordinary skill in the art" is too high.

Patentee draws the attention of the Examiner to the arguments in this regard found in the attached Declaration by Dr. Mansour Samadpour in Rebuttal of the Bailey Declaration, at page 4:

"Dr. Bailey defines a person with ordinary skill in the field as a Bachelor's degree in microbiology, environmental health, or food science. Then he decides that the person would have had several years of experience in developing methodologies for the sampling and analysis of food products. Then he throws in comprehensive familiarity with the literature, and a working knowledge of rules, regulations, guidelines, and directives relating to sample preparations and testing methodologies. This is a fictional construct. In this field, methods are developed by molecular biologists, immunochemists, biochemists, and classical microbiologists. People involved in methods development are not the same as the people who develop sampling plans. Neither of the two groups follows FSIS and FDA directives and guidelines. Those are done by the practitioner's in the field. Dr. Bailey's construct is, therefore, a person with extraordinary skills in several arts.

It is not valid to simply assert that microbiologists or other practitioners have knowledge of statistics suitable for designing sampling plans. The preface to Jarvis (1987) states as follows: *"For many years I have been aware of a lack of understanding of statistical concepts by biologists in general and microbiologists in particular."*

In other words a person of ordinary skills in the field is either a practitioner (user of methods), a method developer (to develop PCR, immunochemical, and clinical methods), a statistician (to develop sampling plans), a regulatory compliance person (to decide how to address applicable regulations and directives) or a quality systems professional (who conducts validation studies).

Dr. Bailey's "person of ordinary skill" is necessarily a person with skills in many arts."

Patentee, therefore, contends that to the Extent that the Examiner has adopted the standard urged by Bailey, the Examiner's asserted standard for what would be obvious to a person of ordinary skill in the art is based on a fictitious abstraction that doesn't exist in this art area, and as such is erroneously applied.

Conclusion

In view of the foregoing amendments and remarks, Patentee respectfully requests entry of the present Response and Amendment and allowance of all claims as presented and amended herein. The Examiner is encouraged to phone Patentee's attorney, Barry L. Davison, to resolve any outstanding issues and expedite allowance of this application.

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Respectfully submitted,
Mansour Samadpour
Davis Wright Tremaine LLP

/Barry L. Davison, Ph.D., J.D./
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Registration No. 47,309

EXHIBIT A

Figure 1: PRESENT INDUSTRY ART

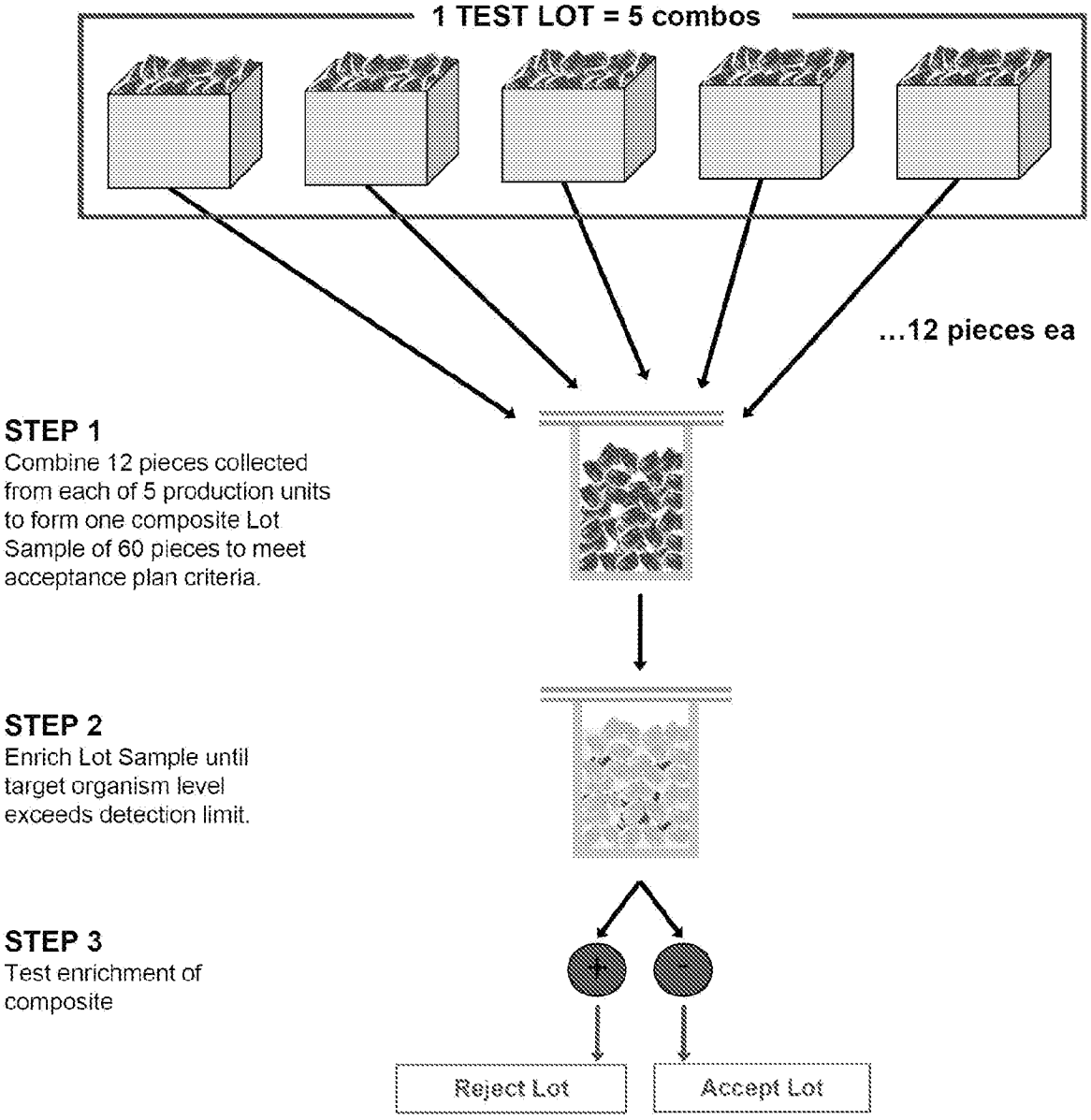


EXHIBIT B

Figure 2. CURRENT INVENTION

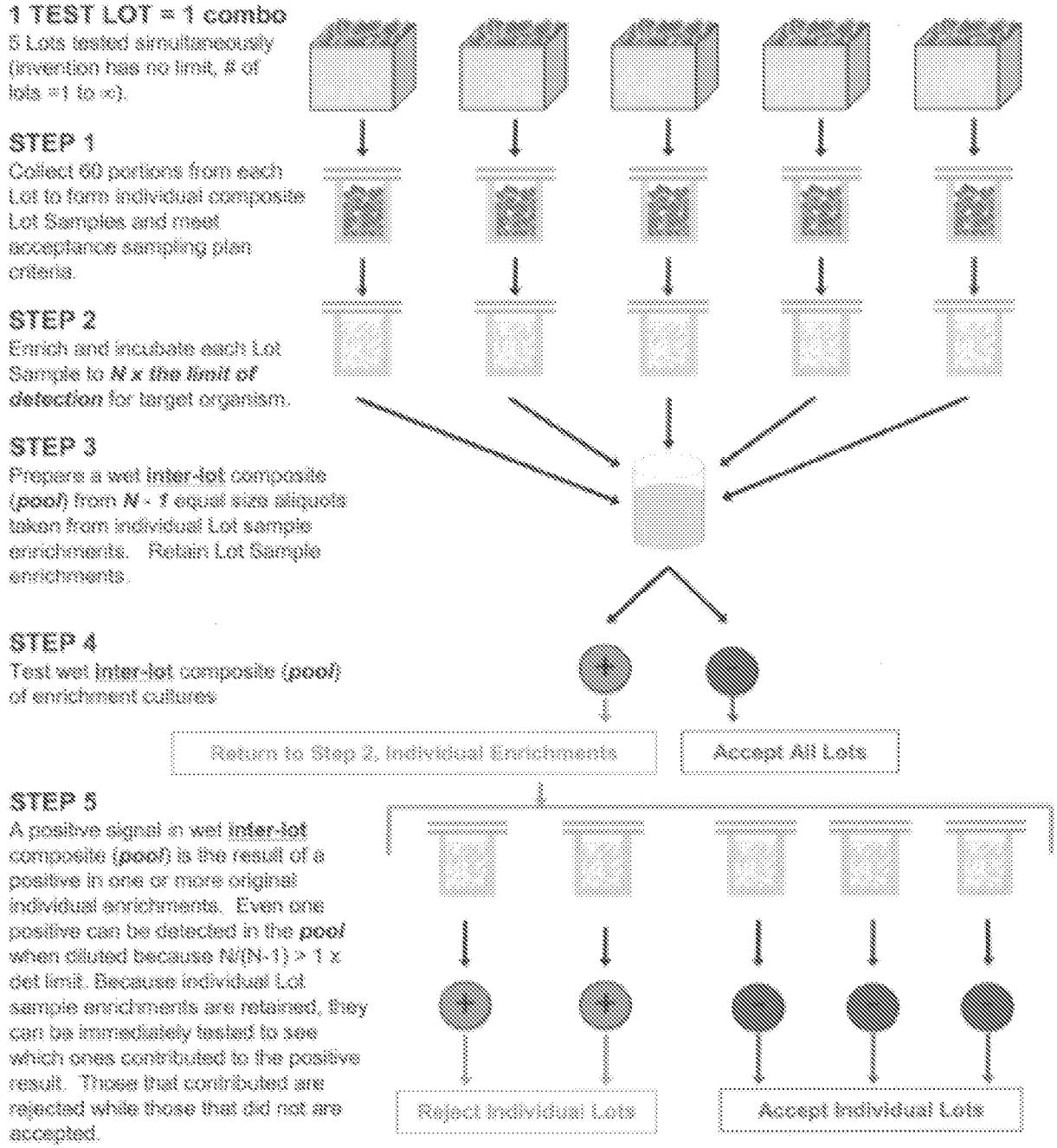


EXHIBIT C

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

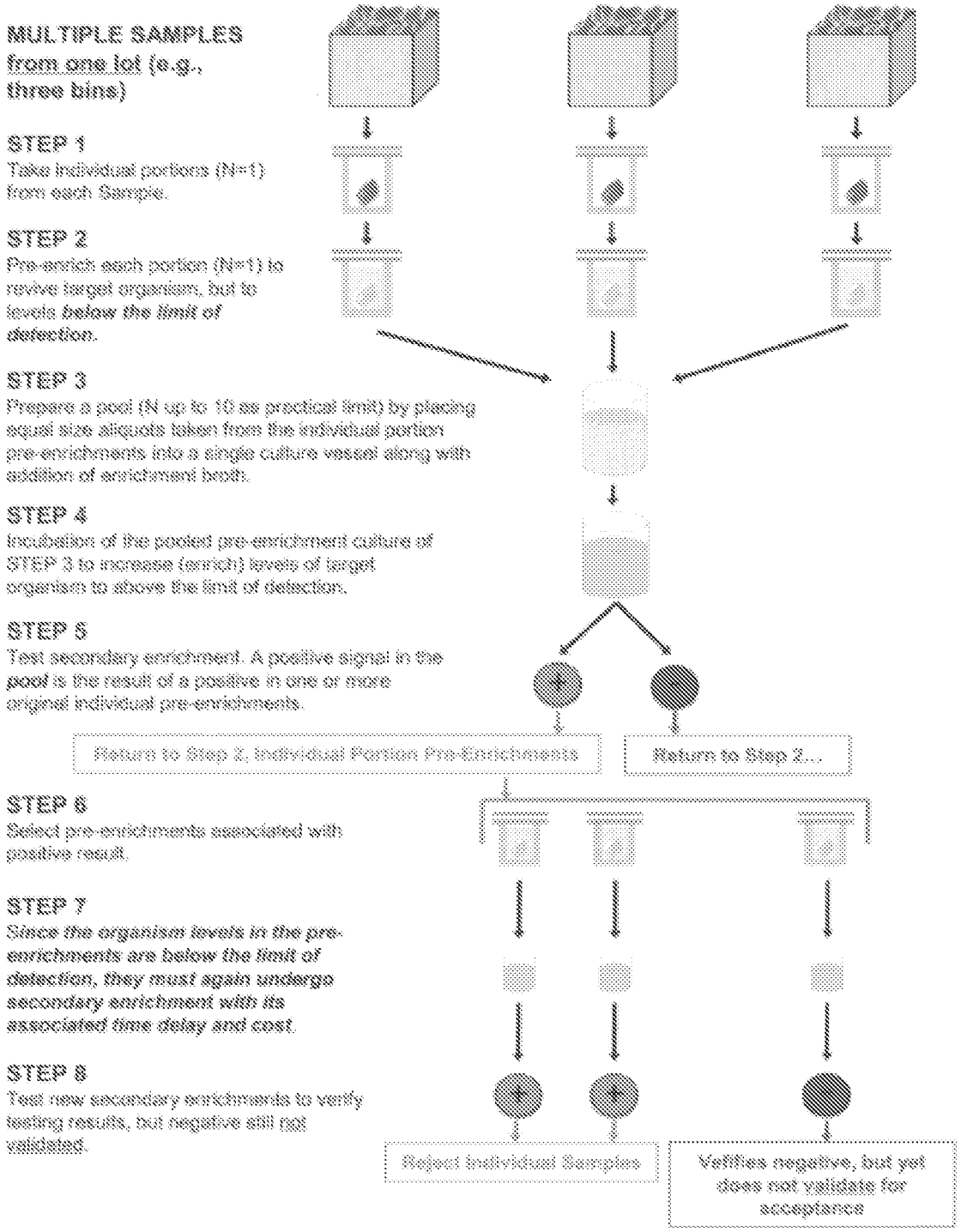
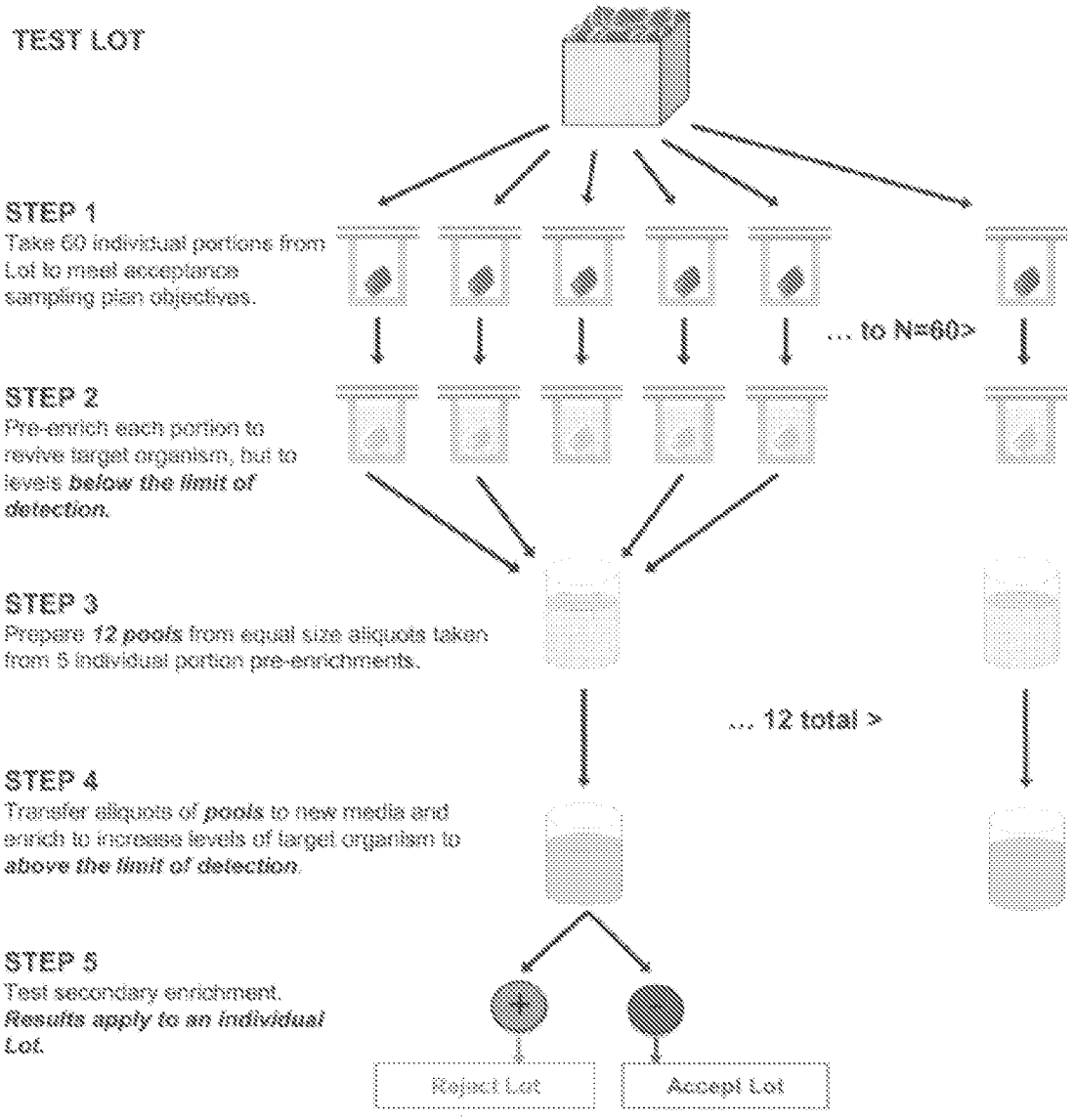


EXHIBIT D

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



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.

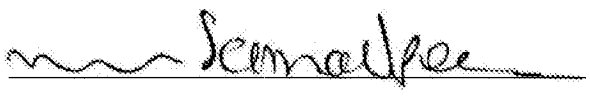
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TABLE 1: Results of modular wet composite sampling of 62,919 multiple lots and number of Individual Production Unit Lot (IPUL) which tested positive when MWCS tested positive

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,

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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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
composting, 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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Page 0653

Table 1 Some European legislative food safety criteria (Anon. 2005)

Micro-organism	Sampling plan		Limits	Test method	Comment
	n	*c*			
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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National Beef Ex. 1007
Page 0654

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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National Beef Ex. 1007
Page 0655

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.

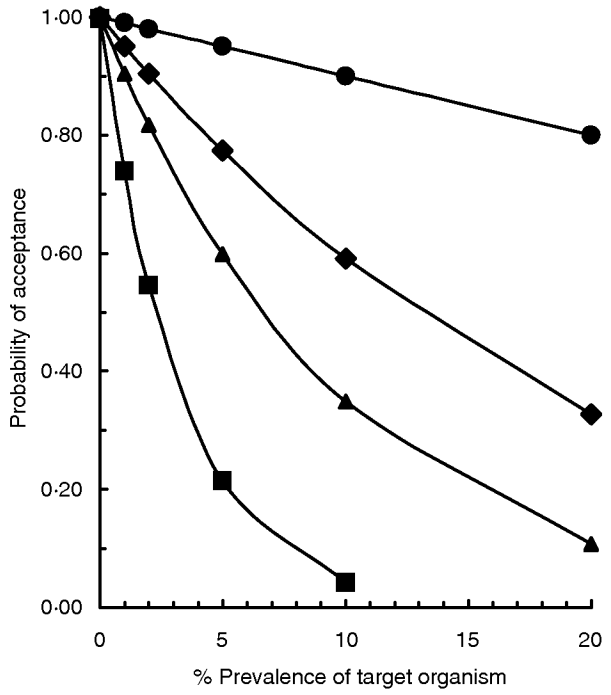


Figure 1 Operating characteristics curves for probability for acceptance of samples having a prevalence of contamination from 0% to 20% and various numbers (n) of samples examined from n = 1 (●), n = 5 (◆), n = 10 (▲) and n = 30 (■).

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 = 0·01) and $P_{(x > 0)}$ = 0·95, 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		
	$P_{(x = 0)}$ = 0·90	$P_{(x = 0)}$ = 0·95	$P_{(x = 0)}$ = 0·99
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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National Beef Ex. 1007
Page 0656

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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National Beef Ex. 1007
Page 0657

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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National Beef Ex. 1007
Page 0658

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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National Beef Ex. 1007
Page 0659

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.

National Beef Ex. 1007
Page 0661

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 18-35ºC 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.

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.

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.

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

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

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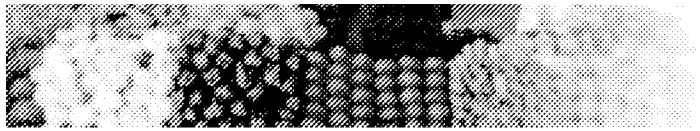
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Page 0678



General Membership publication for Association news, with articles on applied research and food safety applications

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Page 0679

IAFP Report



In this issue:

IAFP Updates

Food Safety News

Regulatory Updates

Research & Reports

Items 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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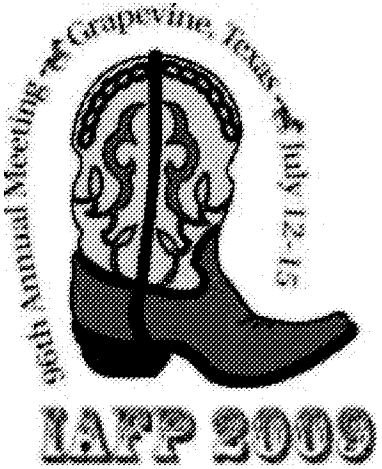
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Anaheim, CA

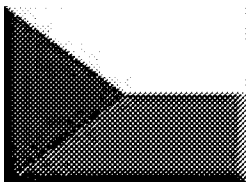
2011
July 31–August 3
Milwaukee, WI

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Page 0682

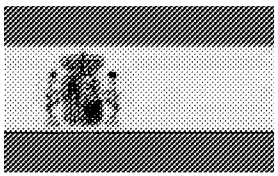
European Symposia

Berlin, Germany

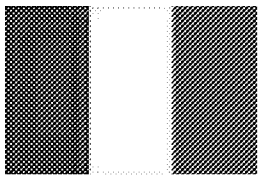
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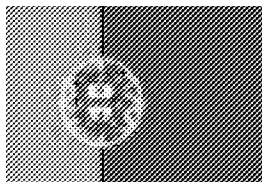
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2005



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2006



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Page 0683

International Symposia

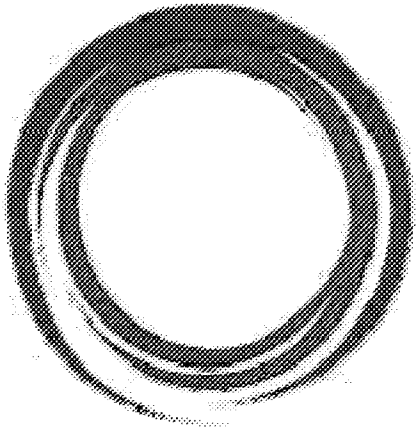
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J. Stan Bailey, PhD, Director of Scientific Affairs – bioMerieux Inc.

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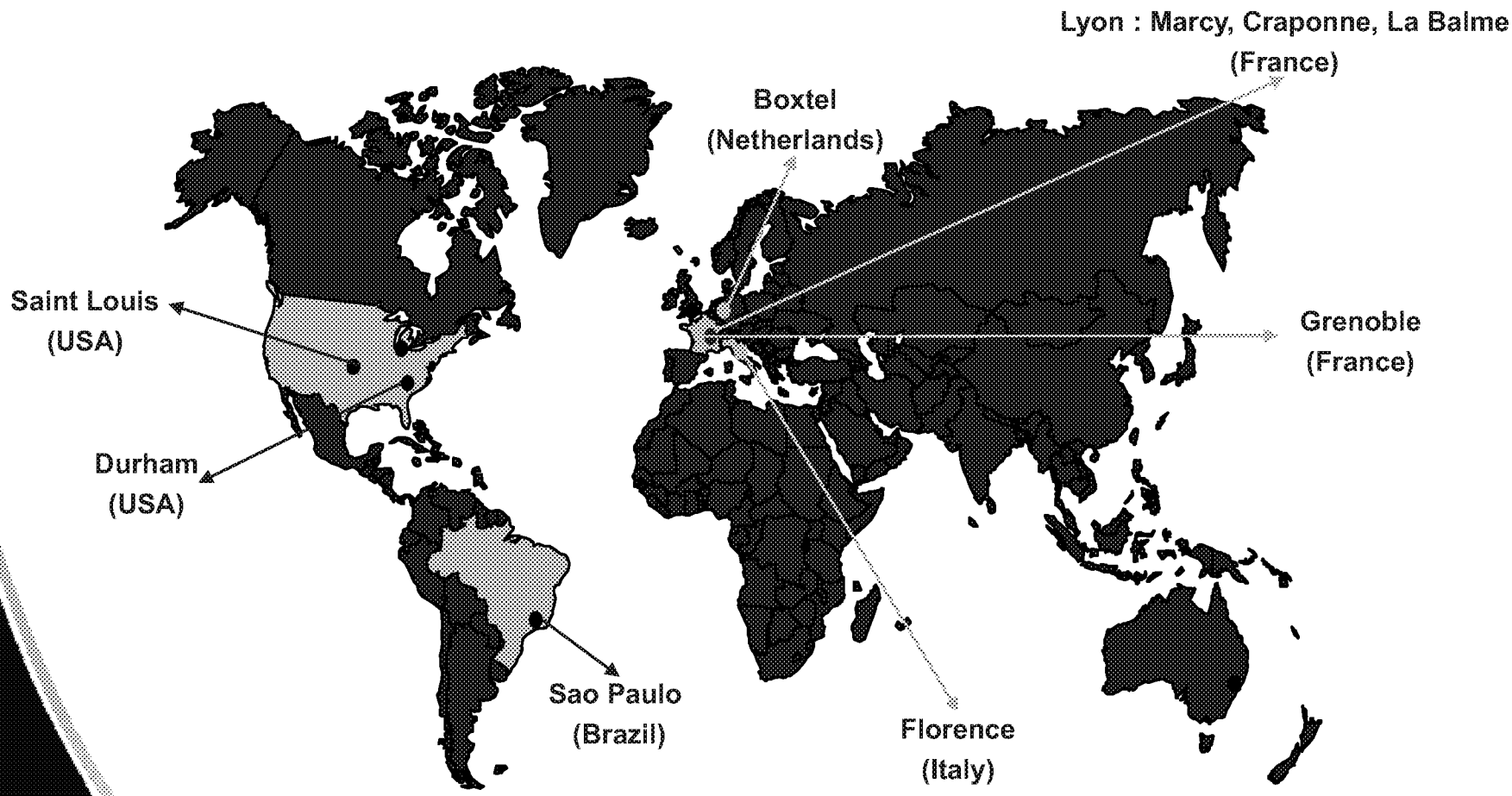
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  - Europe – 59%
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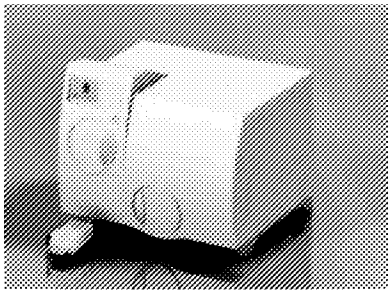
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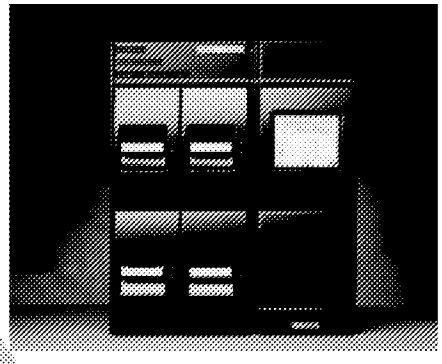
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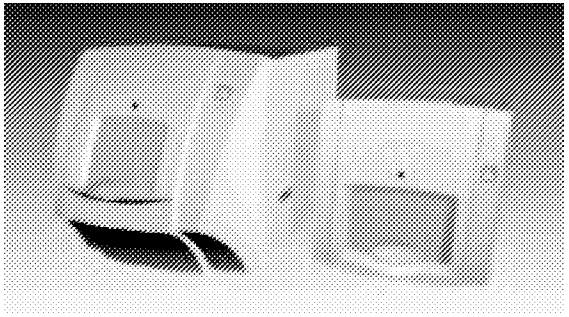
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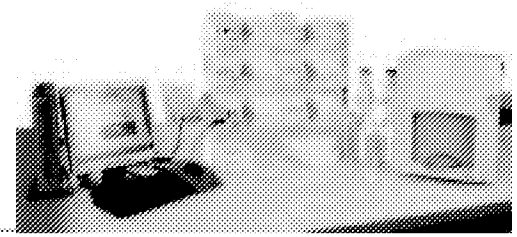


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Page 0690



tempo

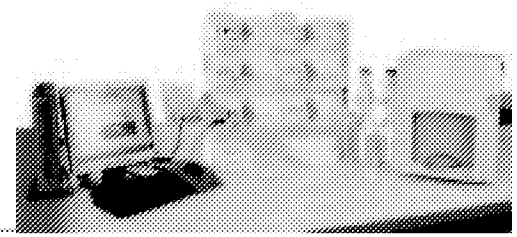
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Page 0691

Development of TEMPO®

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- 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

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Page 0692

tempo

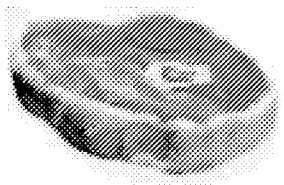


- Concept
- Workflow
- Test Kits & Official Validations

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Page 0693

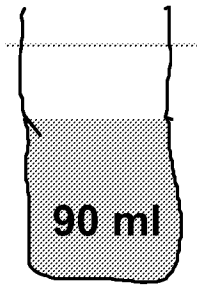
Philippe Villard, Business Development Manager

From the Traditional Approach



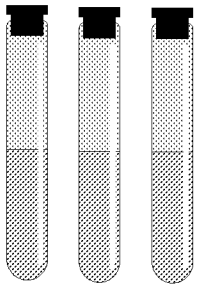
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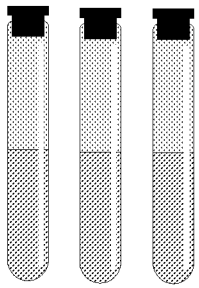
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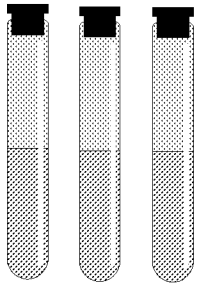
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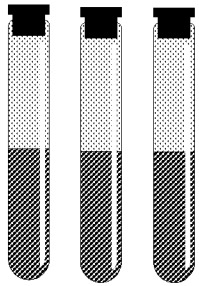
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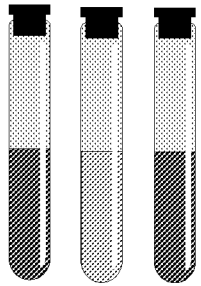


10^{-4}

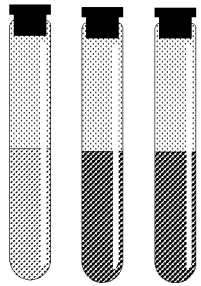
Incubation



3 +



2 +



2 +

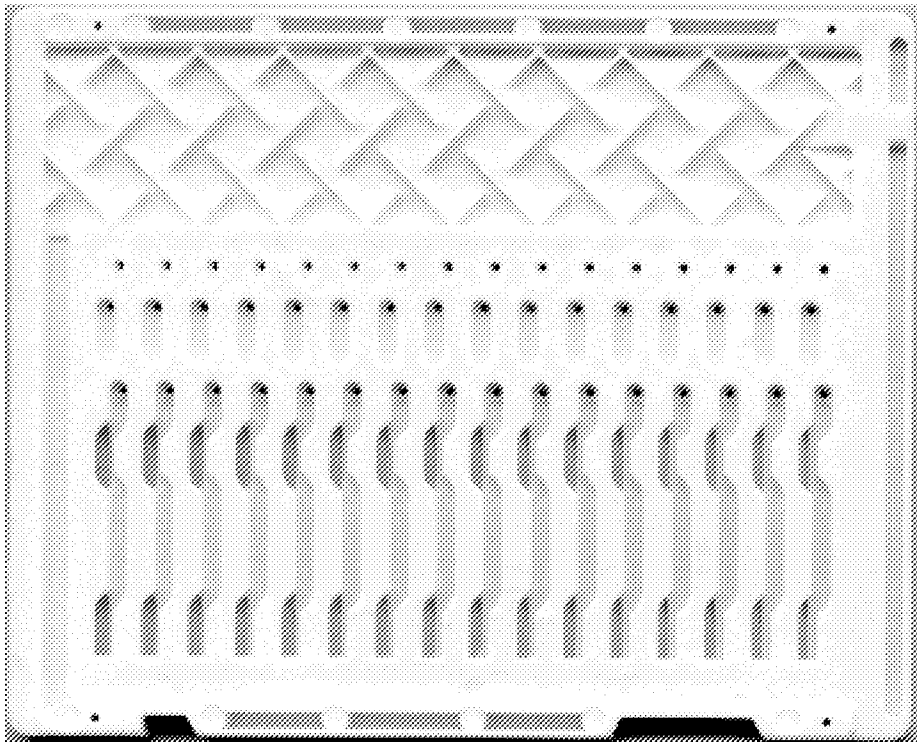
3/2/2

Counting

Interpretation w/ statistical table

2.1 10^4 CFU/ g

To an Innovative Solution

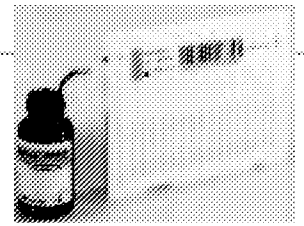


National Beef Ex. 1007
Page 0695

TEMPO Protocol



+ Diluent →



Sample + dehydrated specific culture media

↓

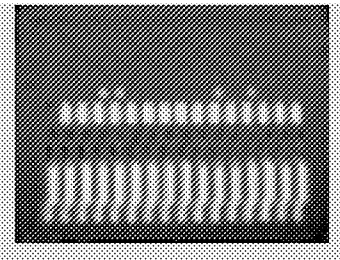
Automated Filling of the card

↓

Standard Incubation

↓

Automated Reading



Automated discrimination of positive and negative wells
Most Probable Number computing → 16 / 15 / 4

↓

Statistic table + dilution factor

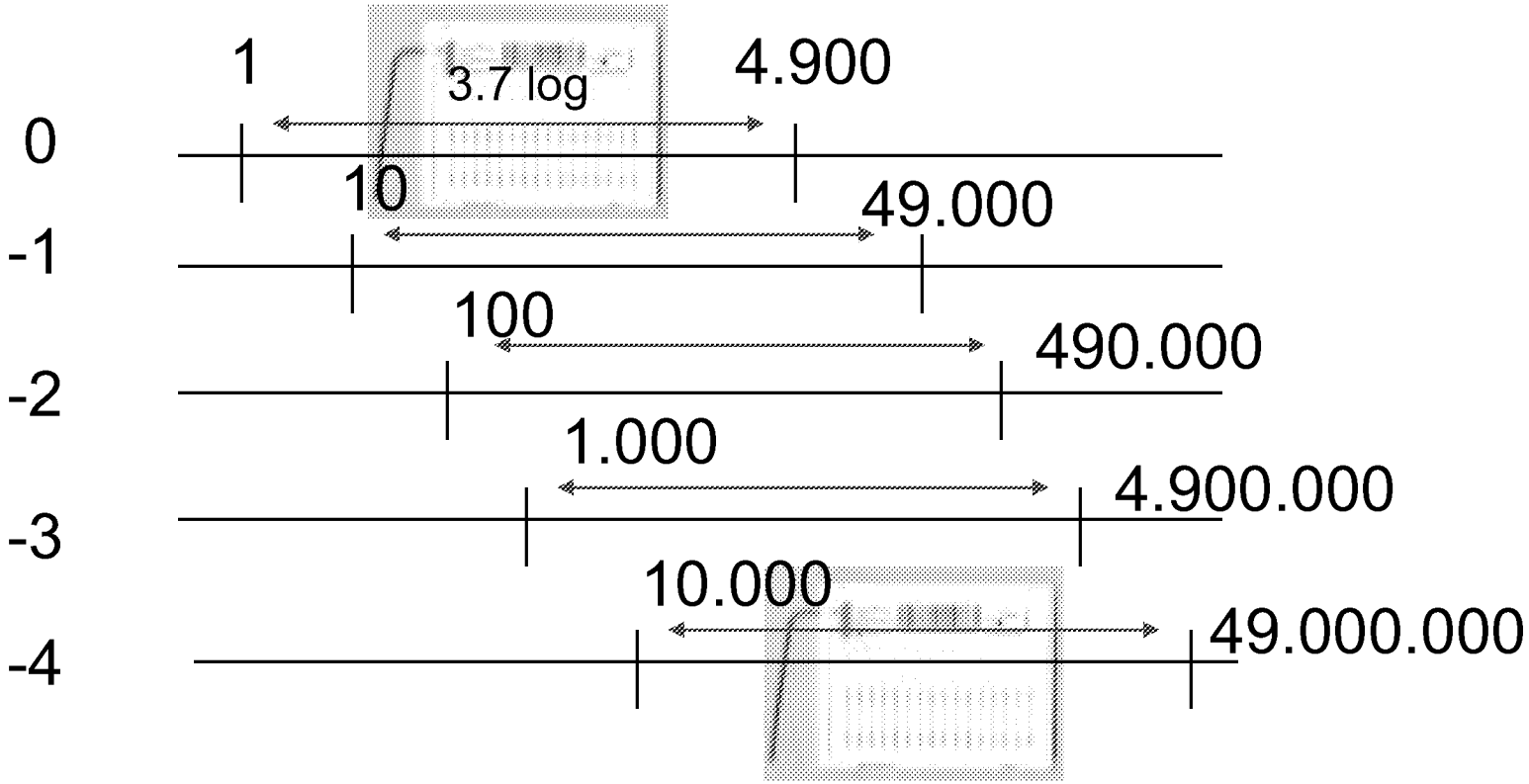
↓

Enumeration (cfu/g)

National Beef Ex. 1007
Page 0696

Enumeration Range

MPN 16 tubes = 3.7 log of range



National Beef Ex. 1007
Page 0697

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

National Beef Ex. 1007
Page 0698

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®



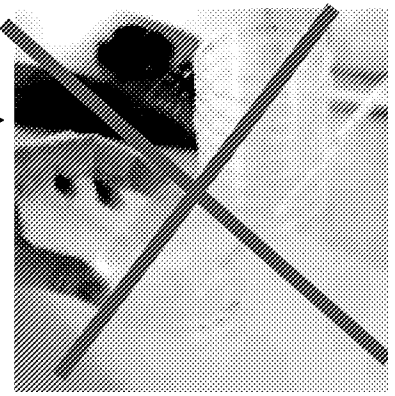
1- Media preparation



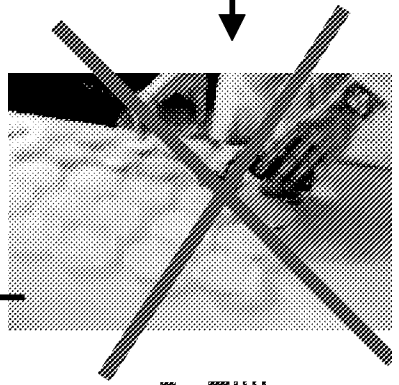
2- Sample preparation



3- Dilution



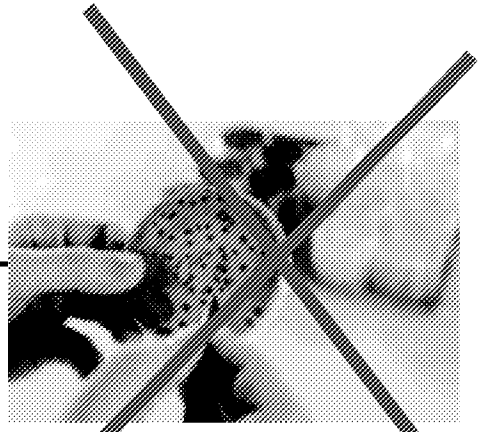
4-Inoculation



5- Filling



6-Incubation



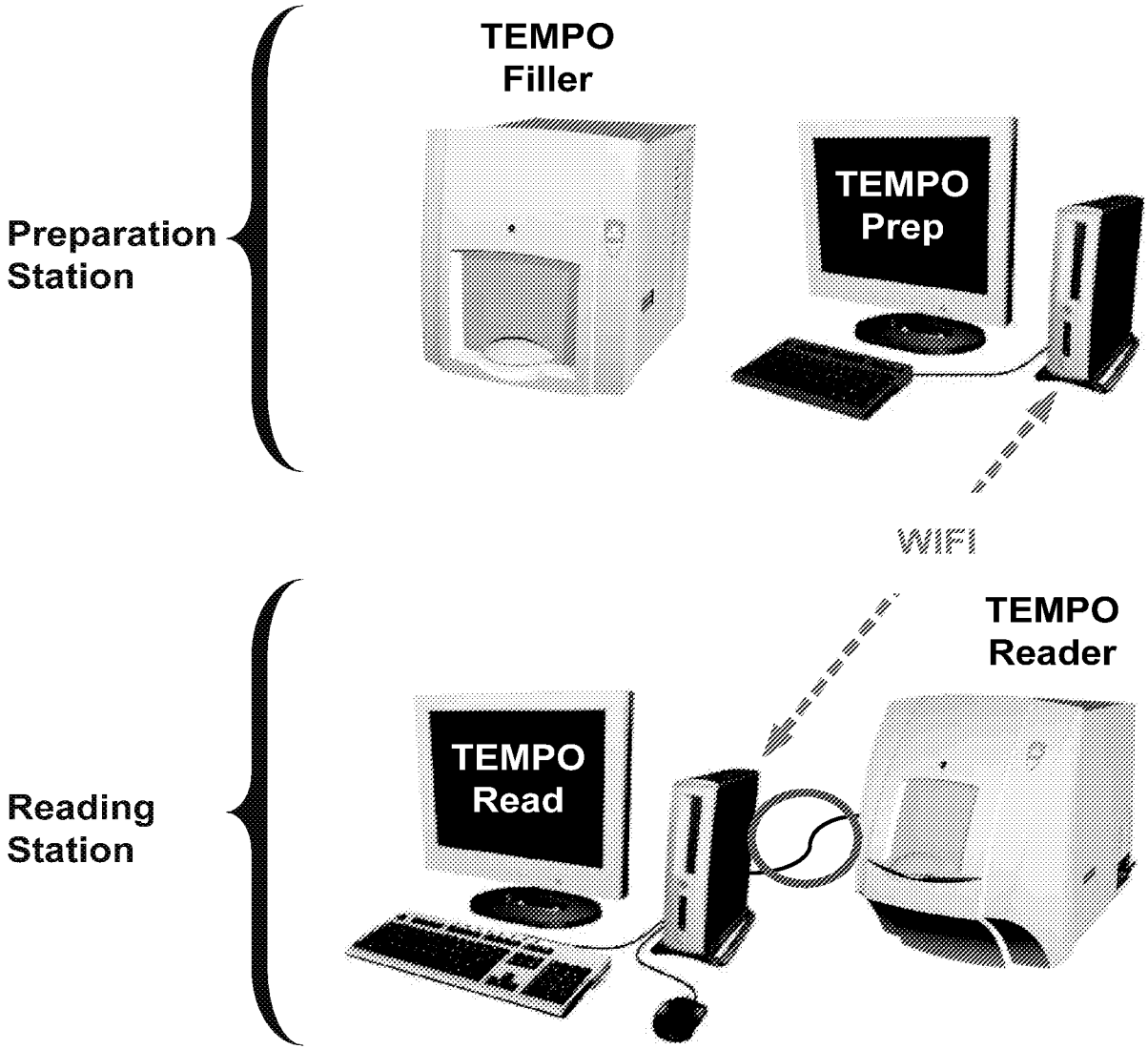
7-Reading



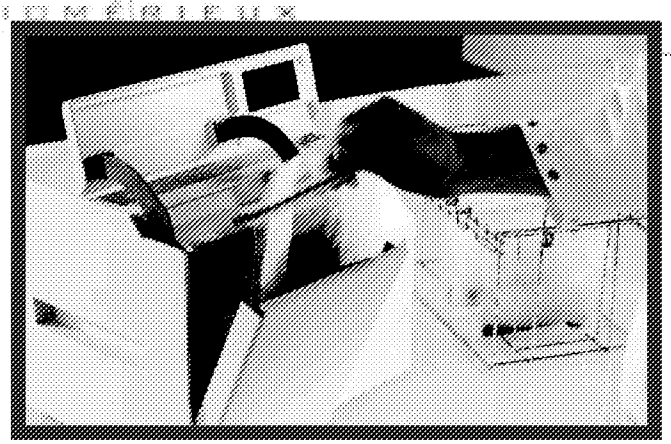
8-Reporting

National Beef Ex. 1007
Page 0700

TEMPO System



TEMPO Workflow



Sample preparation



Inoculation



Incubation

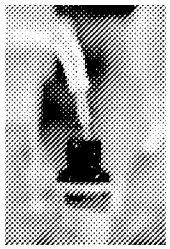


Reading & interpretation

National Beef Ex. 1007
Page 0702

TEMPO® Test Preparation

- Rehydrate Media Vial



- Pour the sample
- Vortex



6 Tests / 3min

- Automatic Filling



- Incubate

National Beef Ex. 1007
Page 0703

TEMPO® Test Reading

- Remove the Tests from the Incubator

20 Tests / < 3 min

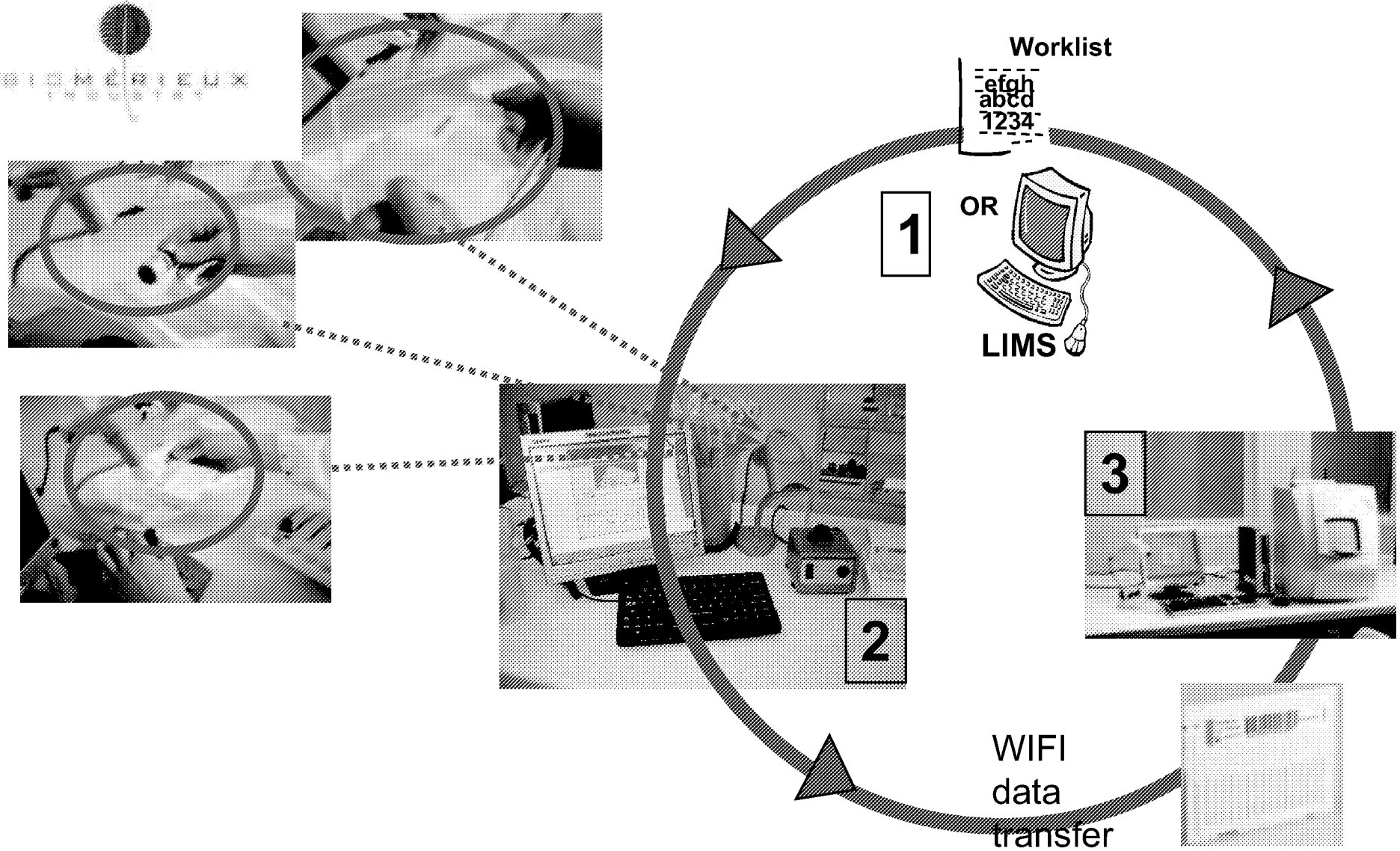
- Insert Tray in the Reader for Reading and Recording



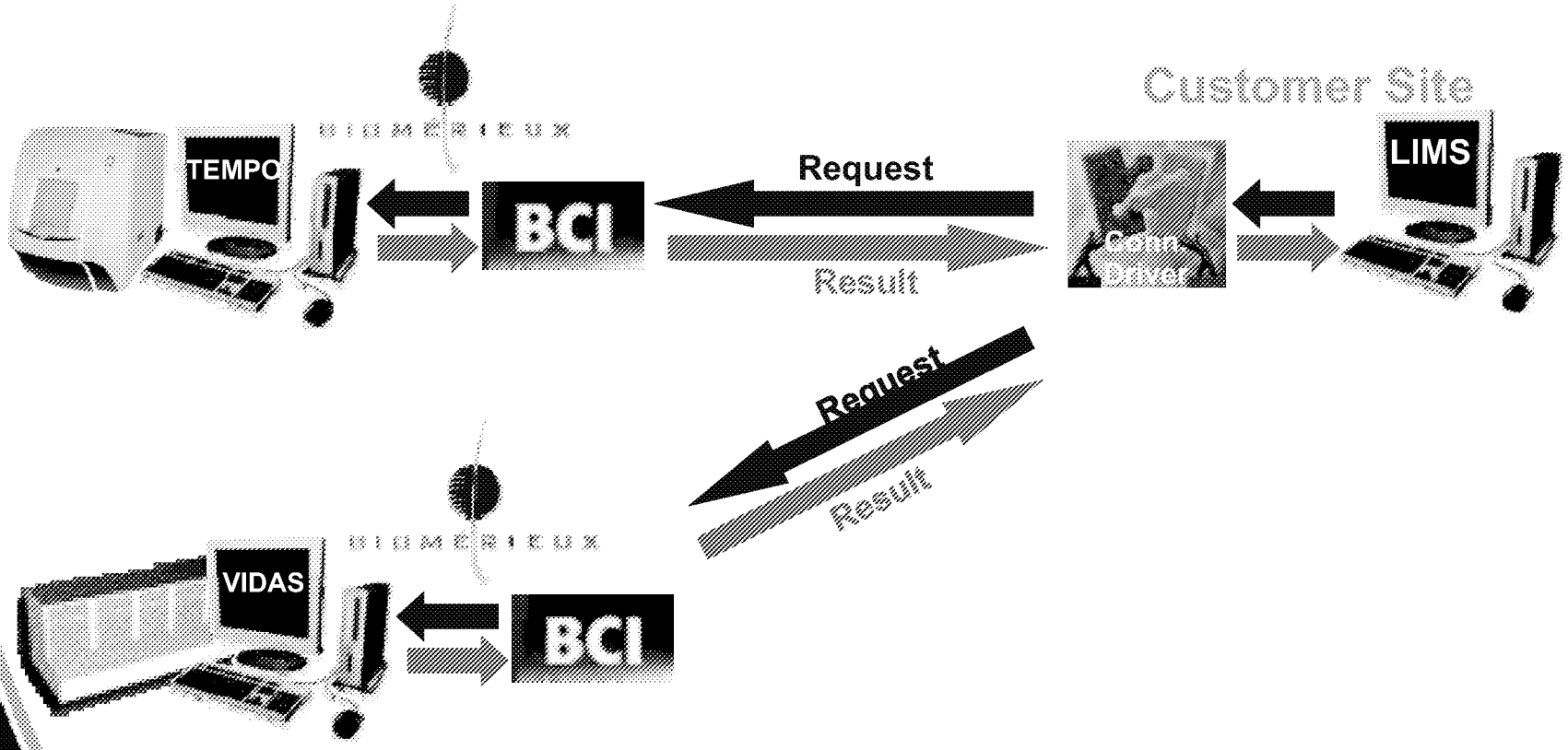
TEMPO® Test Results

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

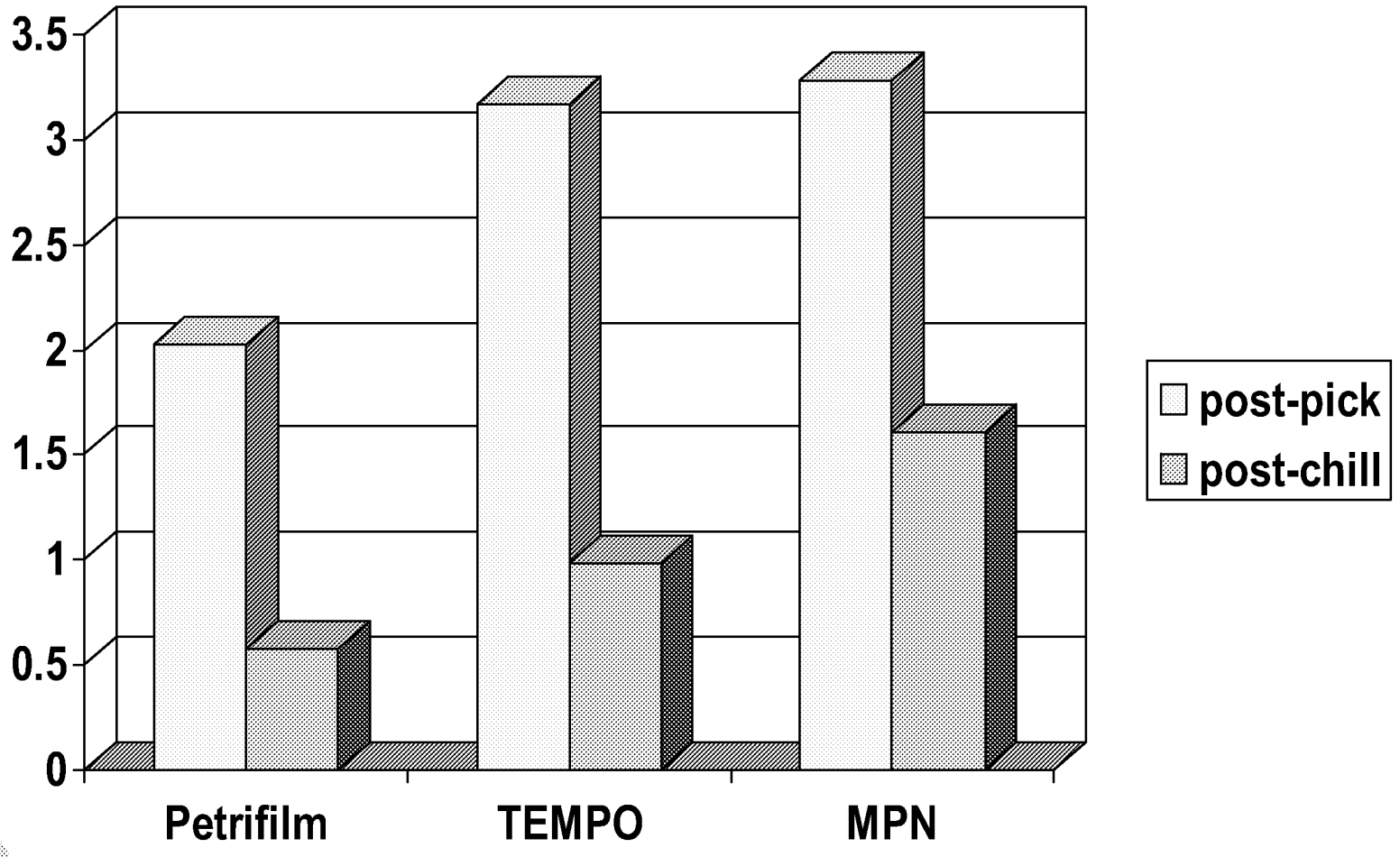
- **Total Viable Count**
- ***E.coli* & Coliforms: In progress**

ISO 16140

- **Total Viable Count**
- **Enterobacteriaceae**
- **Coliforms**
- ***E.coli***

E. coli from chicken

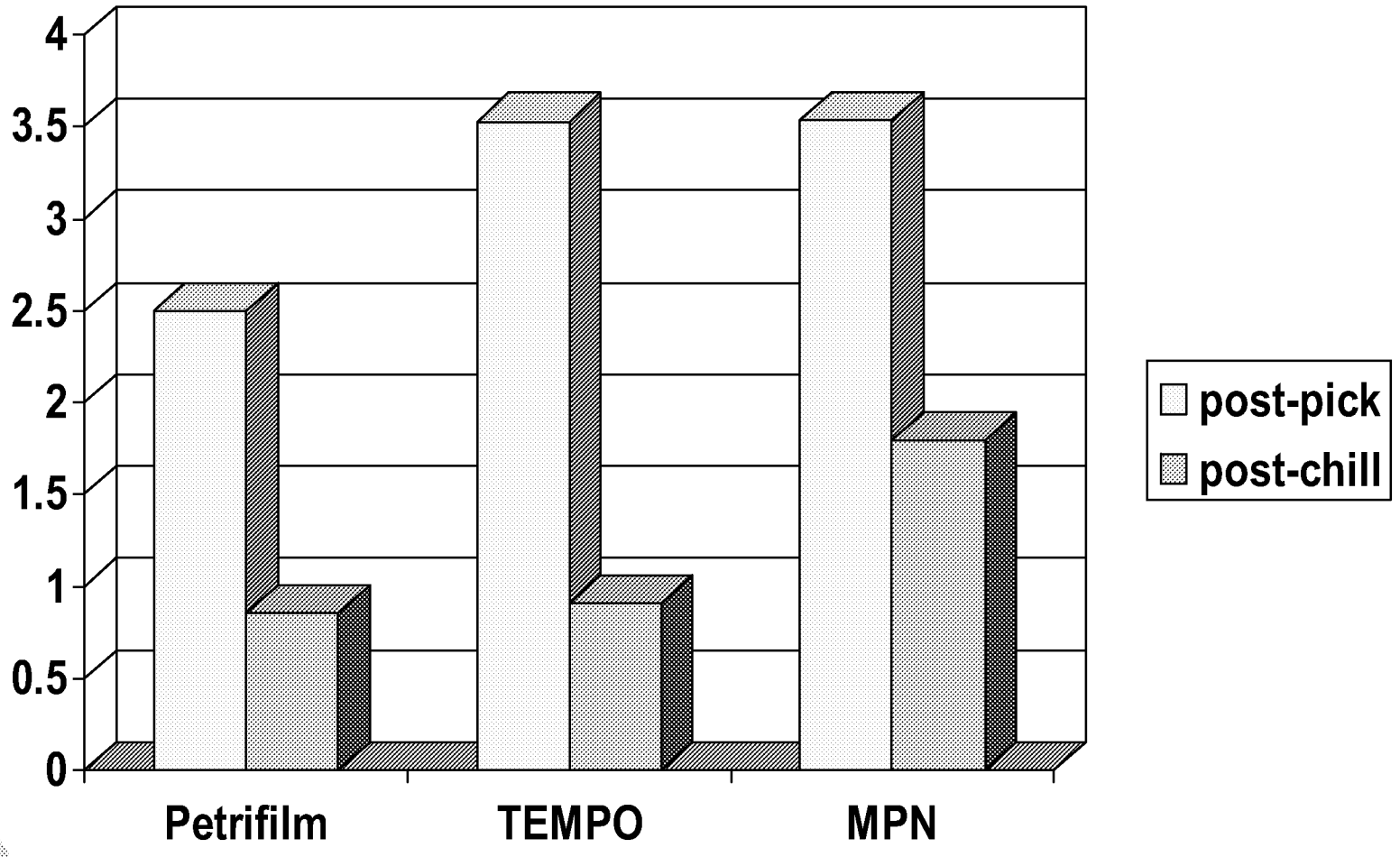
Log_{10} cfu / mL rinse



National Beef Ex. 1007
Page 0710

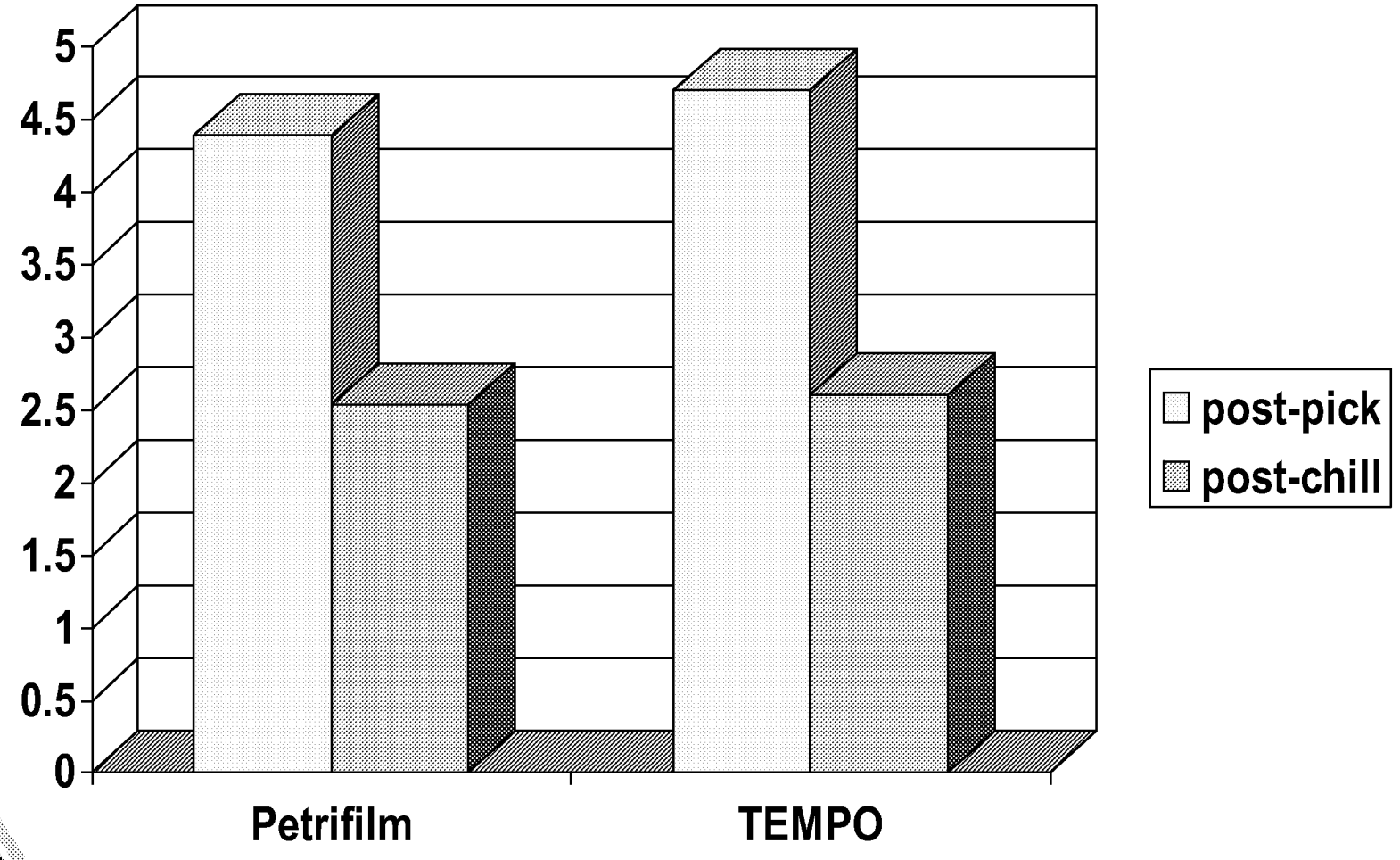
Coliforms from chicken

Log_{10} cfu / mL rinse



Total aerobic count from chicken

Log_{10} cfu / mL rinse



tempo



Thank you

Stan Bailey, Director Scientific Affairs – bioMerieux Industry

National Beef Ex. 1007
Page 0713

VIDAS Ultimate Performance (Phage)
E. coli 0157:H7

Stan Bailey

Director Scientific Affairs, bioMerieux Industry

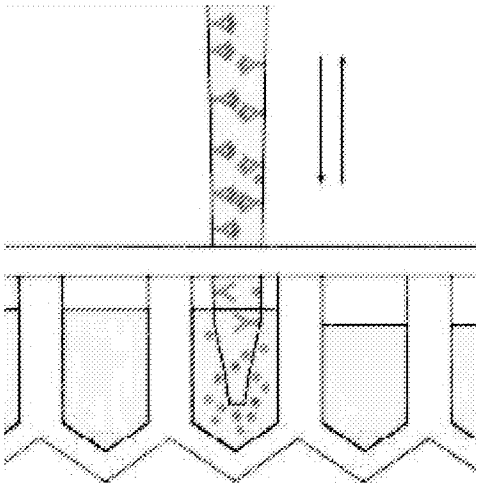
President, IAFP

stan.bailey@na.biomerieux.com

National Beef Ex. 1007
Page 0714

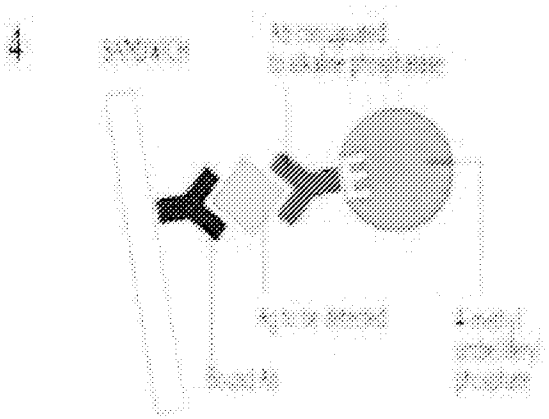
VIDAS® IMMUNO ASSAY

CAPTURE OF ANTIGENS



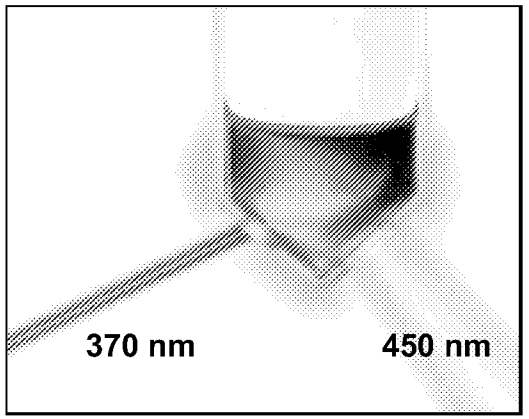
The antibody captures the target pathogens

SANDWICH TEST



A second antibody conjugated with an enzyme binds to specific antigens

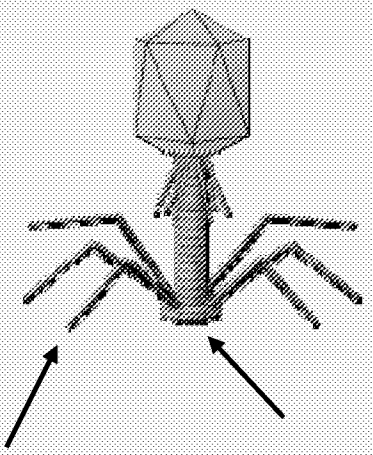
DETECTION



The intensity of the reaction is interpreted by the system

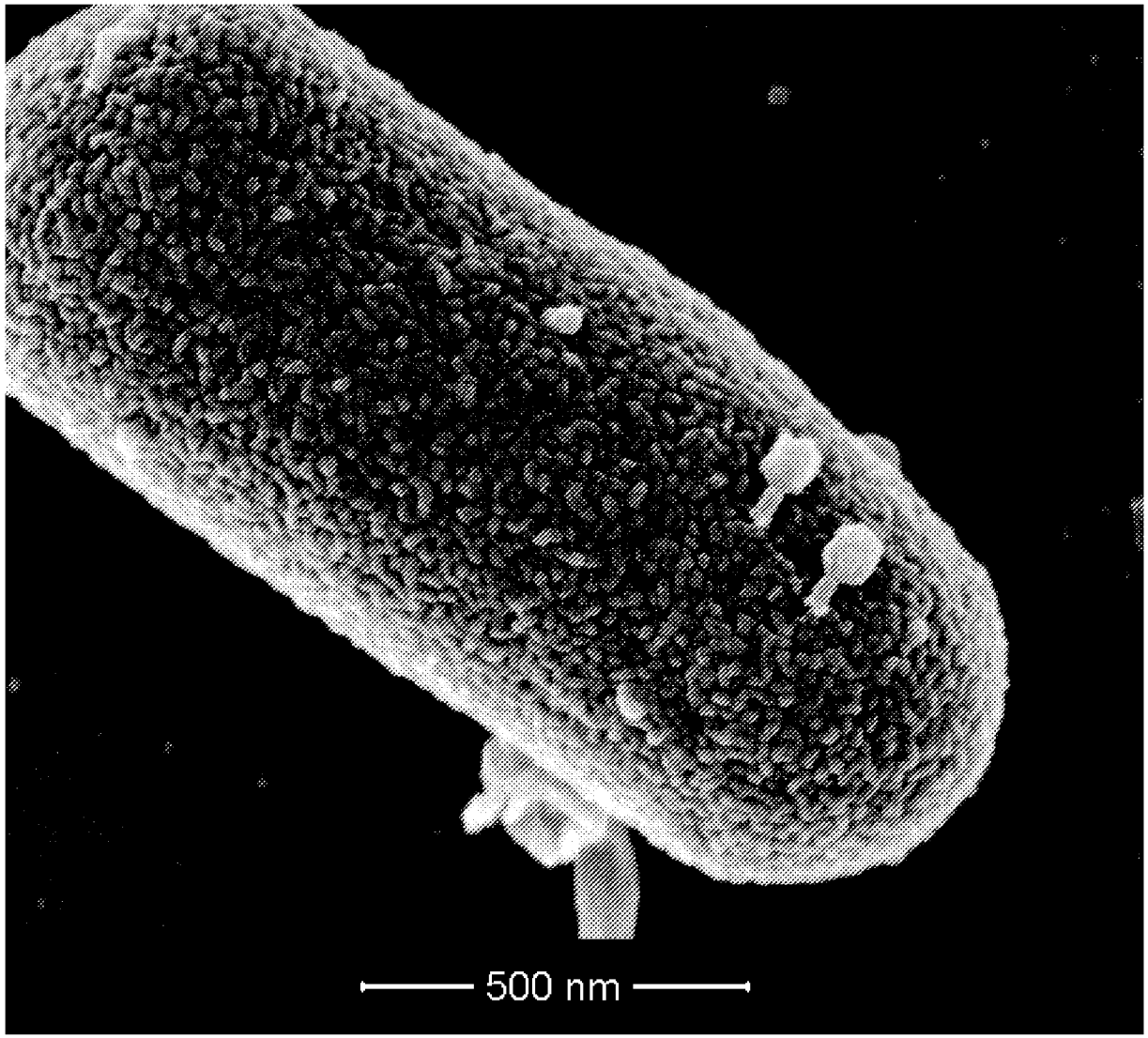
National Beef Ex. 1007
Page 0716

What is a Bacteriophage ?



"Capture" Molecules and "Lytic" Enzymes

Building blocks of the Profos technology



National Beef Ex. 1007
Page 0717

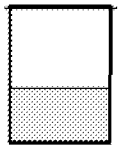
What is a Bacteriophage ?

- **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

National Beef Ex. 1007
Page 0718

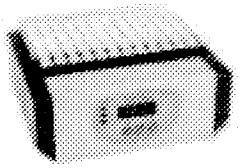
VIDAS UP E. coli O157:H7



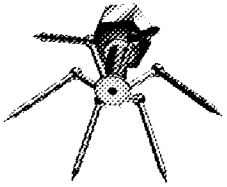
BPW +/- vancomycin

6 --24 hrs

41.5 +/- 1 °C



Heat & Go 5 min



50 min.

VIDAS UP E. coli O157:H7

National Beef Ex. 1007
Page 0719

Ecole Nationale
Vétérinaire de Lyon
Professeur Christine Vernozy-Rozand
Delphine Thevenot, Marion Bouvier

BIOMERIEUX

EVALUATION VIDAS UP E. coli O157:H7

FOR DETECTION OF *E. coli* O157:H7

IN FOODS & ENVIRONMENTAL SAMPLES

National Beef Ex. 1007
Page 0720

Inclusivity and Exclusivity

- Sensitivity : 133 *E. coli* O157:H7 strains tested
  - **VIDAS : 100 %**

- Specificity : 102 non *E. coli* O157 strains tested
  - **VIDAS : 100 %**

National Beef Ex. 1007
Page 0721

Results on ground beef

Sample size : 25g Detection	Enrichment in BPW	
	6h	24h
VIDAS Positive	15/15	15/15
PCR Positive	10/13	13/13

Sample size : 75g Detection	Enrichment in BPW + Vancomycin	
	8h	24h
VIDAS Positive	14/14	14/14
PCR Positive	1/15	2/15

Sample size : 375g Detection	Enrichment in BPW + Vancomycin	
	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

EVALUATION VIDAS UP E. coli O157:H7

FOR DETECTION OF *E. coli* O157:H7

IN FOODS & ENVIRONMENTAL SAMPLES

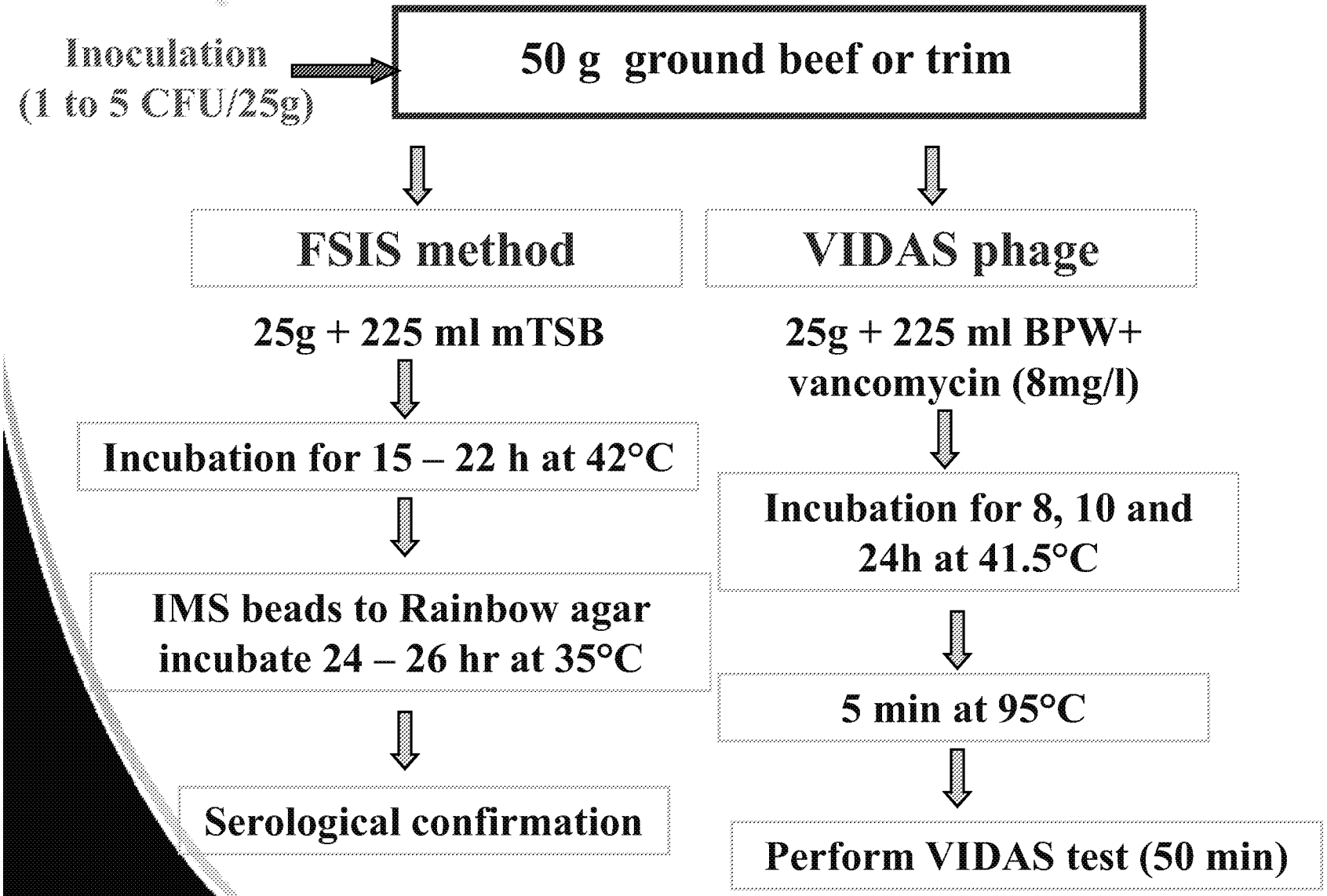
National Beef Ex. 1007
Page 0724

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]

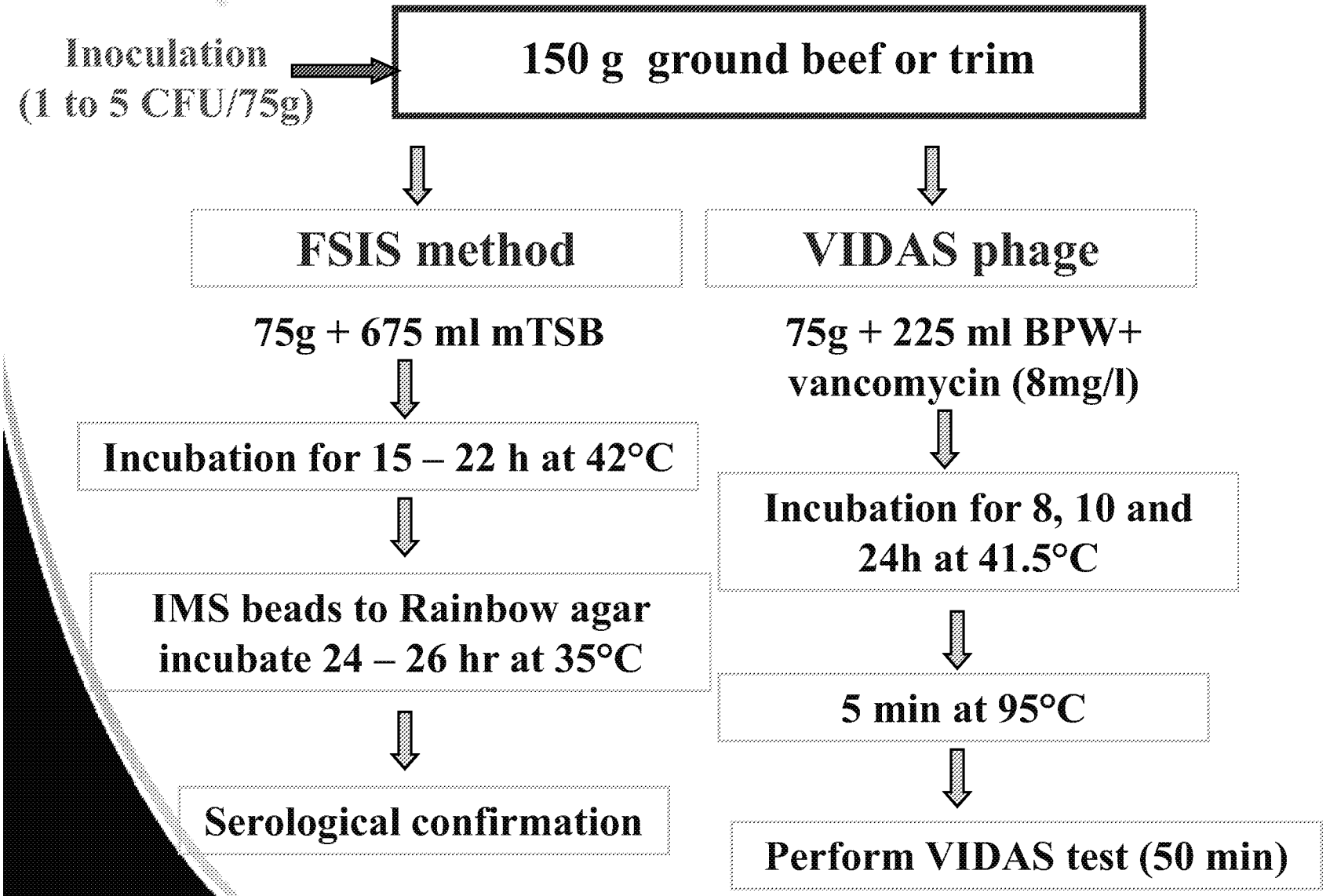
National Beef Ex. 1007
Page 0725

25 gm Validation



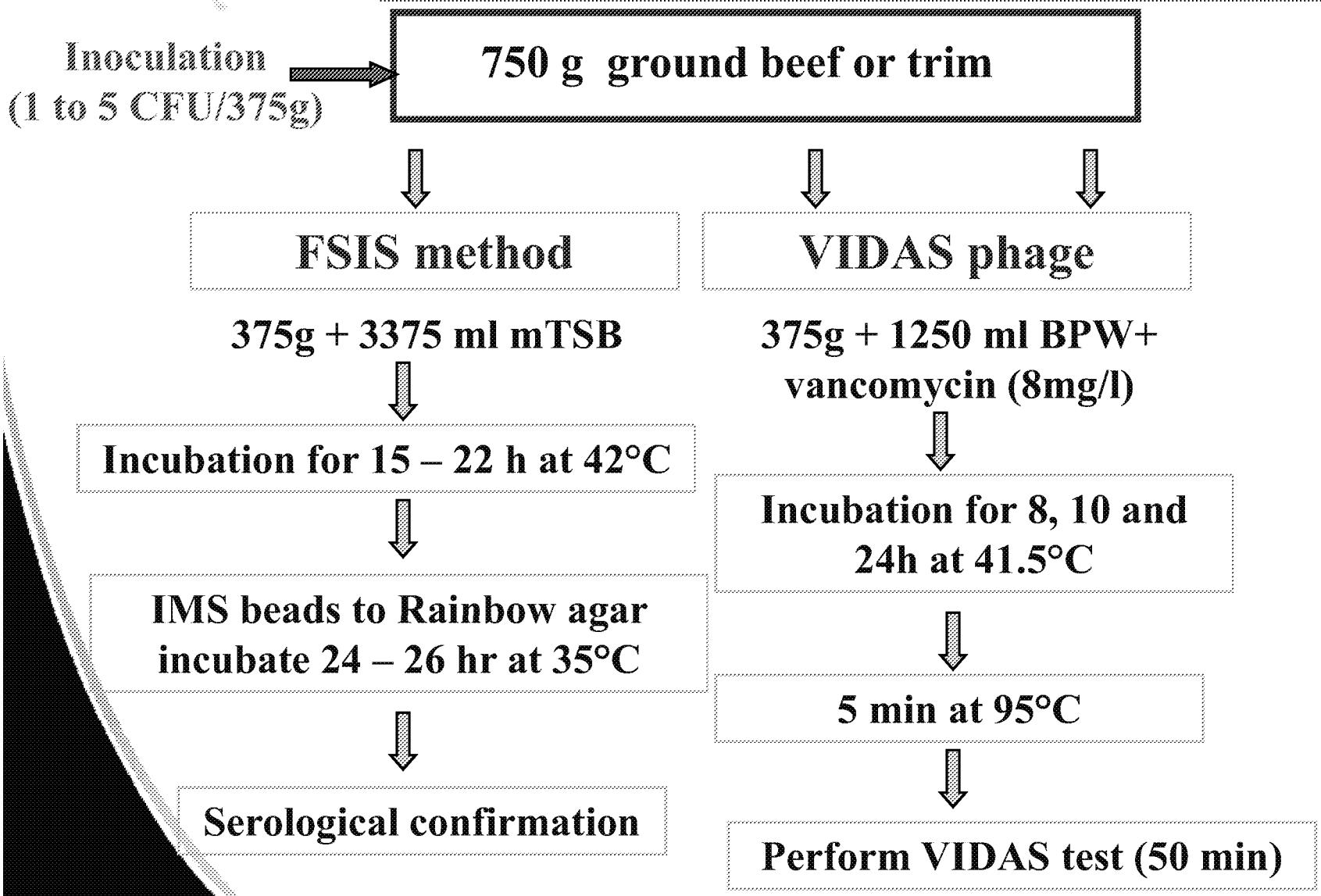
National Beef Ex. 1007
Page 0726

75 gm Validation



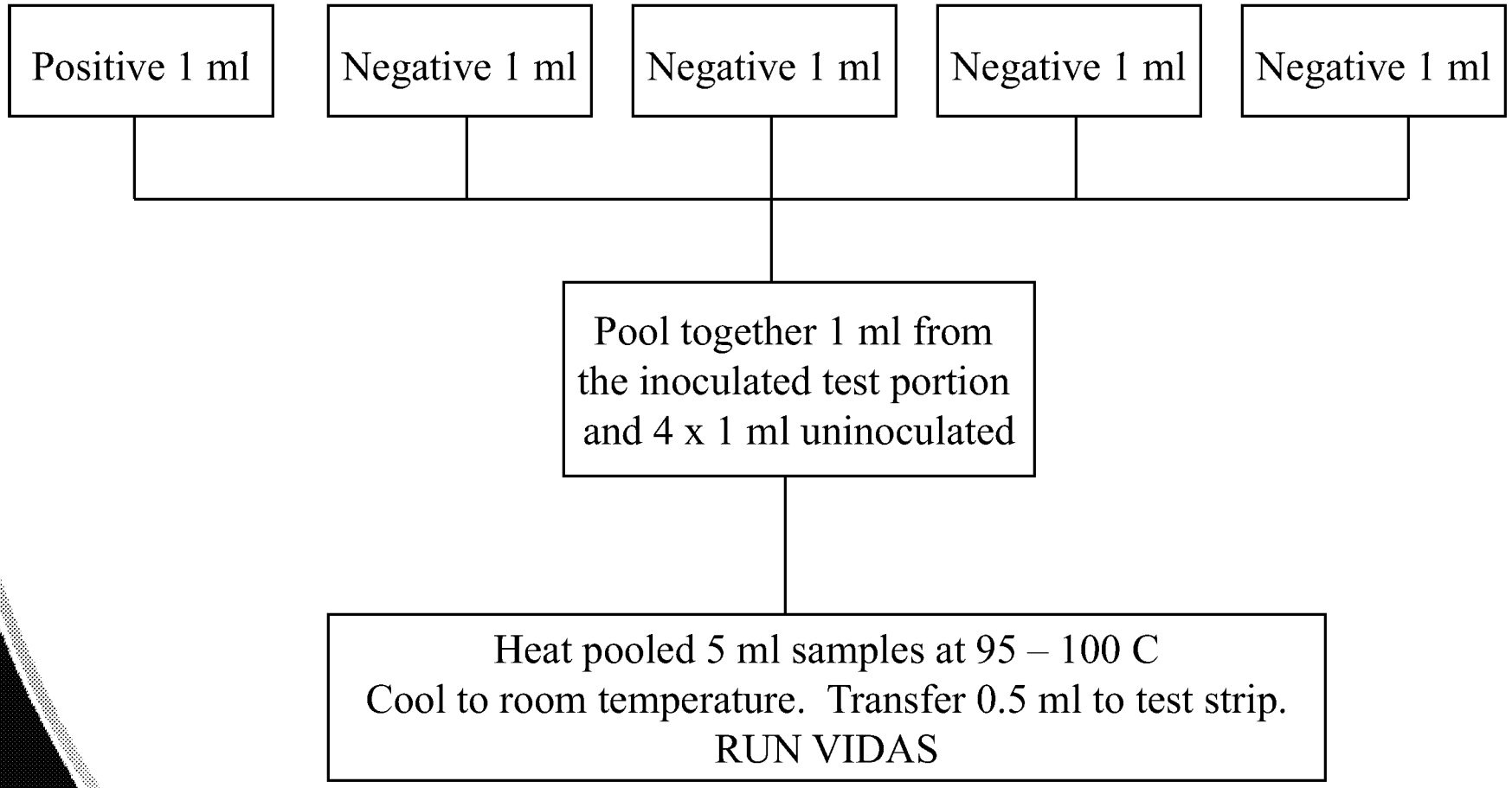
National Beef Ex. 1007
Page 0727

375 gm Validation



National Beef Ex. 1007
Page 0728

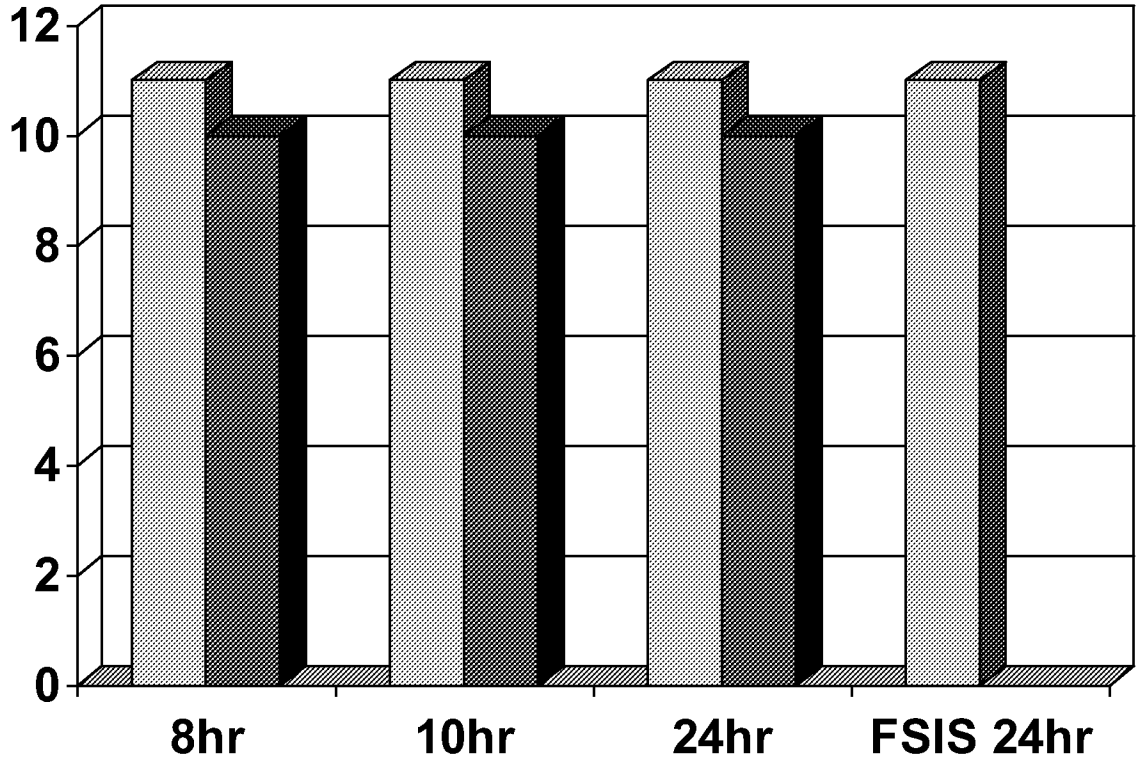
Pooled sample protocol



National Beef Ex. 1007
Page 0729

Recovery of E. coli O157:H7 from ground beef

AOAC fractional positives: inoc. = 2-4 cells/375gm

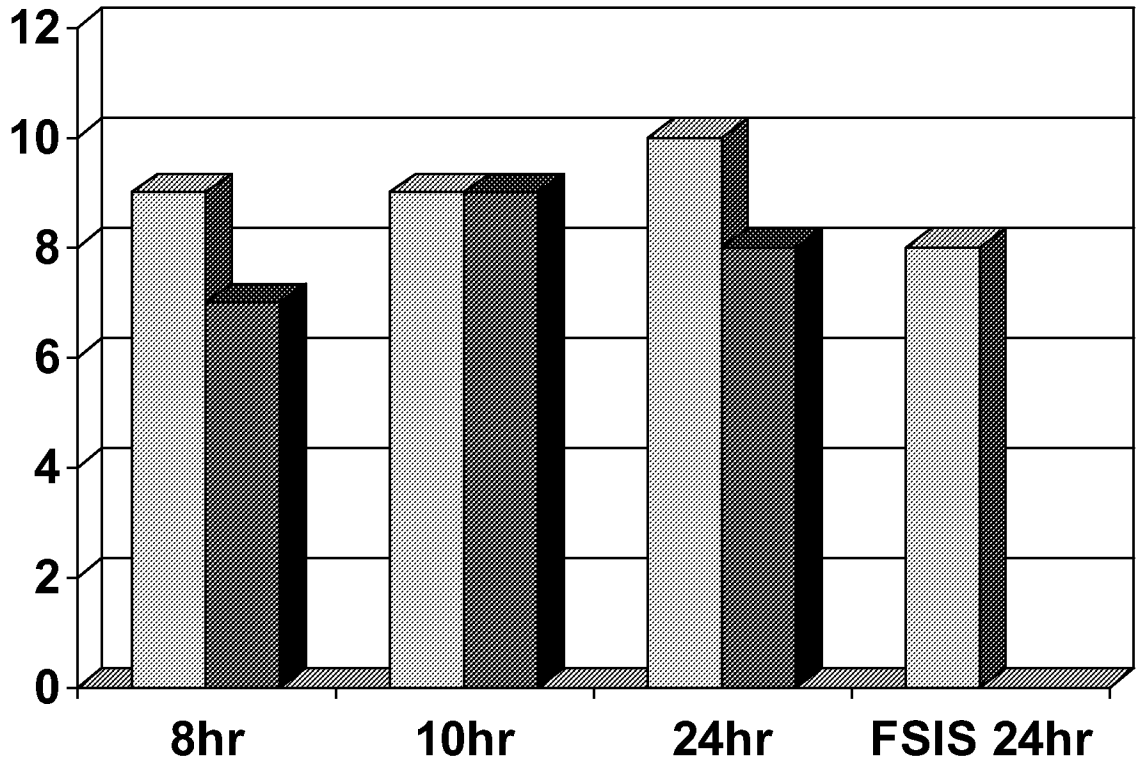


Chi Square = 0.08

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

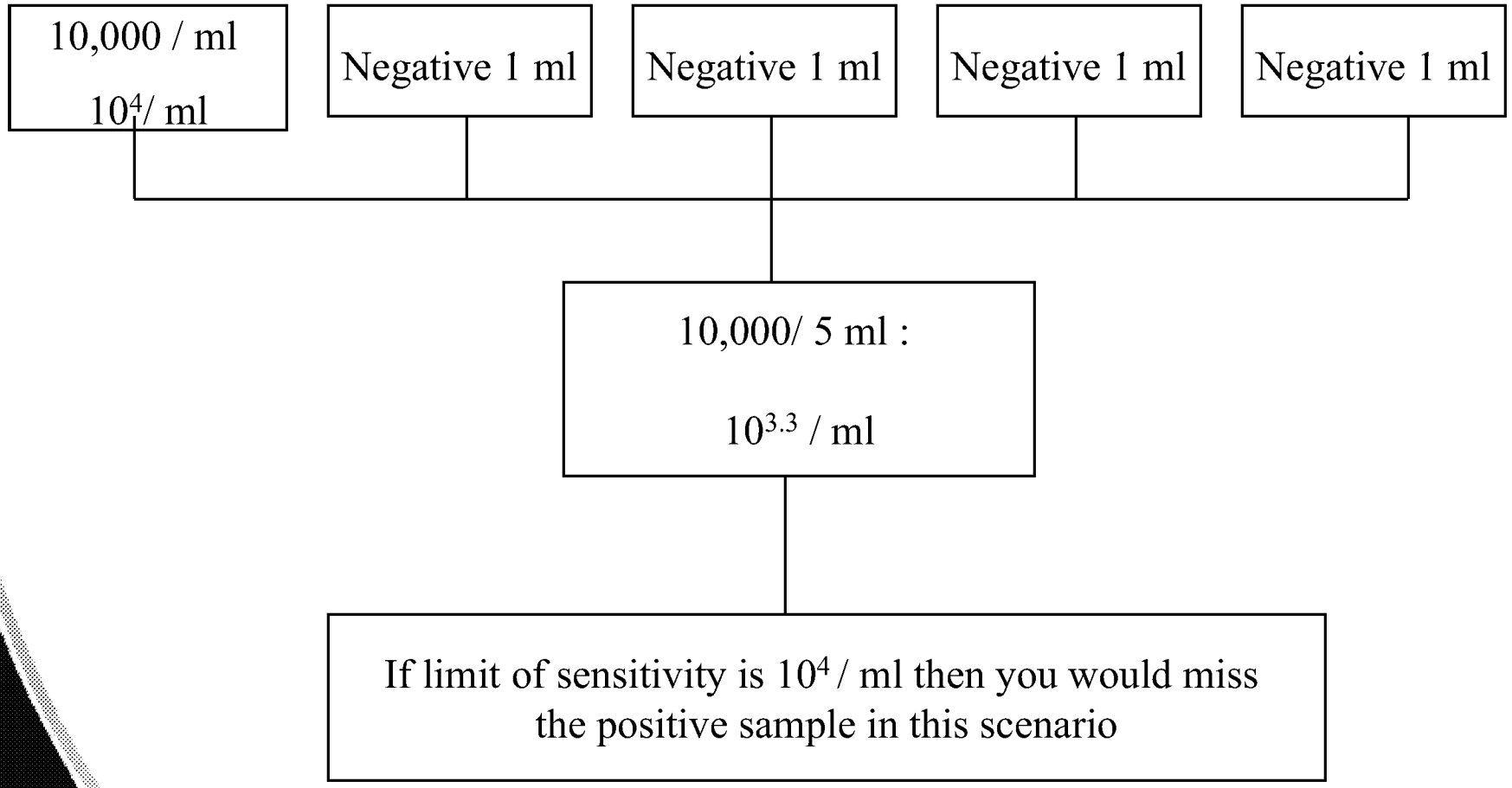


Chi Square = 0.87

n = 20 inoculated to get between 5 and 15 fractional (+)

National Beef Ex. 1007
Page 0731

Pooled sample protocol



National Beef Ex. 1007
Page 0732

AOAC Evaluations
approval by July, 2009

- 25, 75 & 375 g individual samples
- 375 g wet composite
- False positives < 0.3 % (from French commercial customers)

National Beef Ex. 1007
Page 0733

Other Validations

- ISO and AFNOR approvals in Europe
- AOAC additional validations
  - Produce
  - Irrigation water

National Beef Ex. 1007
Page 0734

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

National Beef Ex. 1007
Page 0735

Questions ?

APPENDIX E
IEH Document Independence of Combos

National Beef Ex. 1007
Page 0737

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.

CONFIDENTIAL

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.

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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**

SUBJECT TO CONFIDENTIALITY AGREEMENT - PROPRIETARY INFORMATION

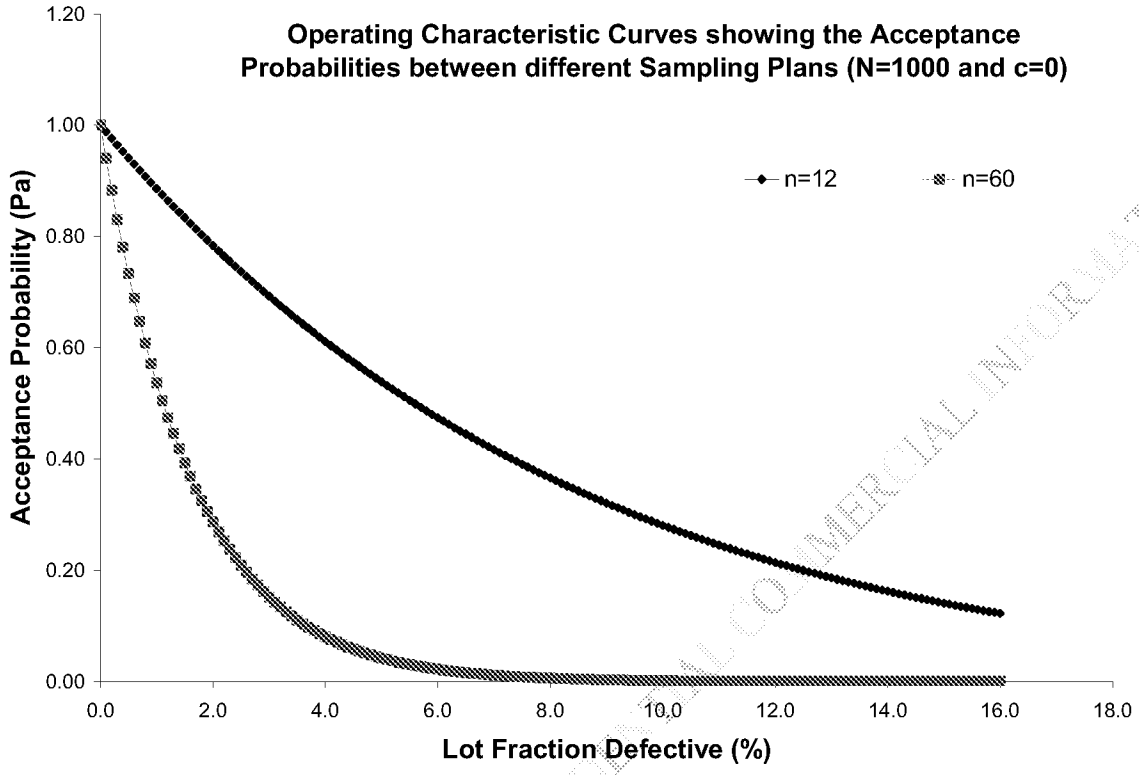


Figure 1: Comparison of operating characteristic curves for N=12 and N=60 sampling plans.

TRADE SECRET - CONTAINS CONFIDENTIAL COMMERCIAL INFORMATION

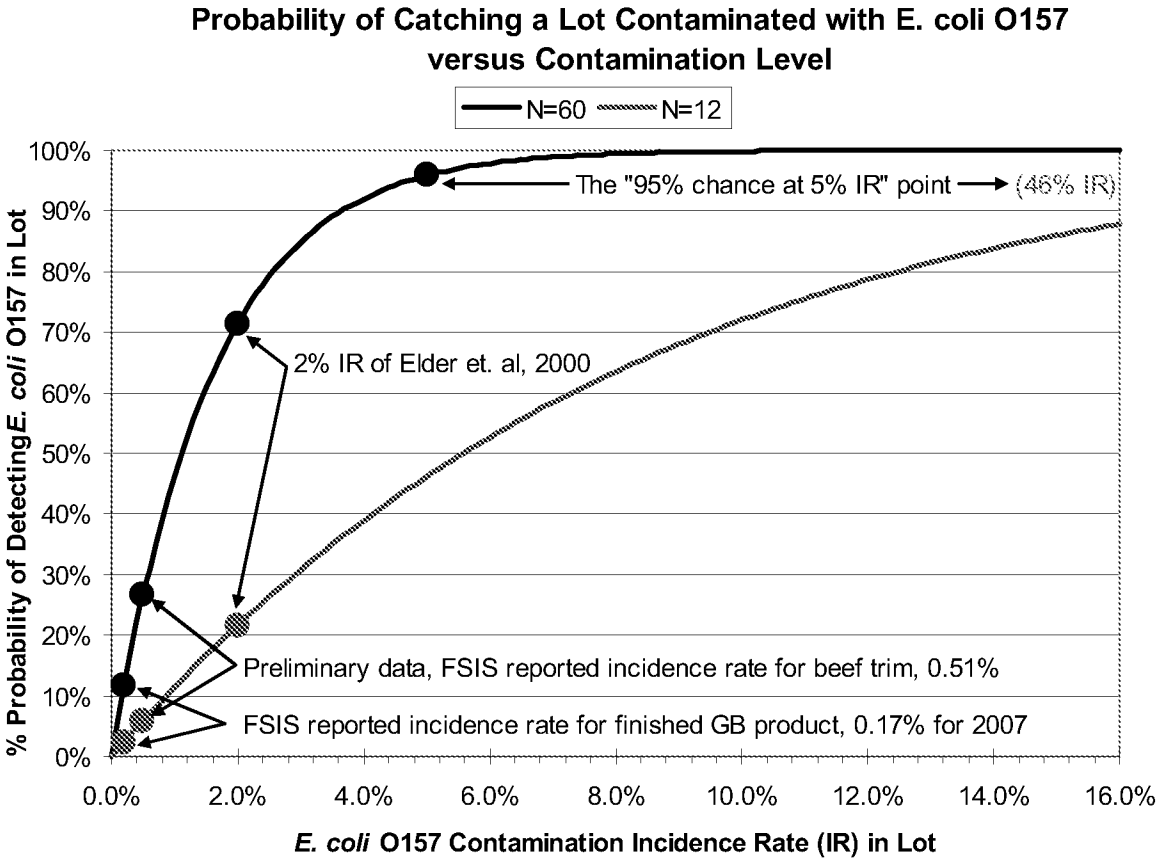


Figure 2: Comparison of chances for catching a defective Lot for N=12 and N=60 sampling plans.

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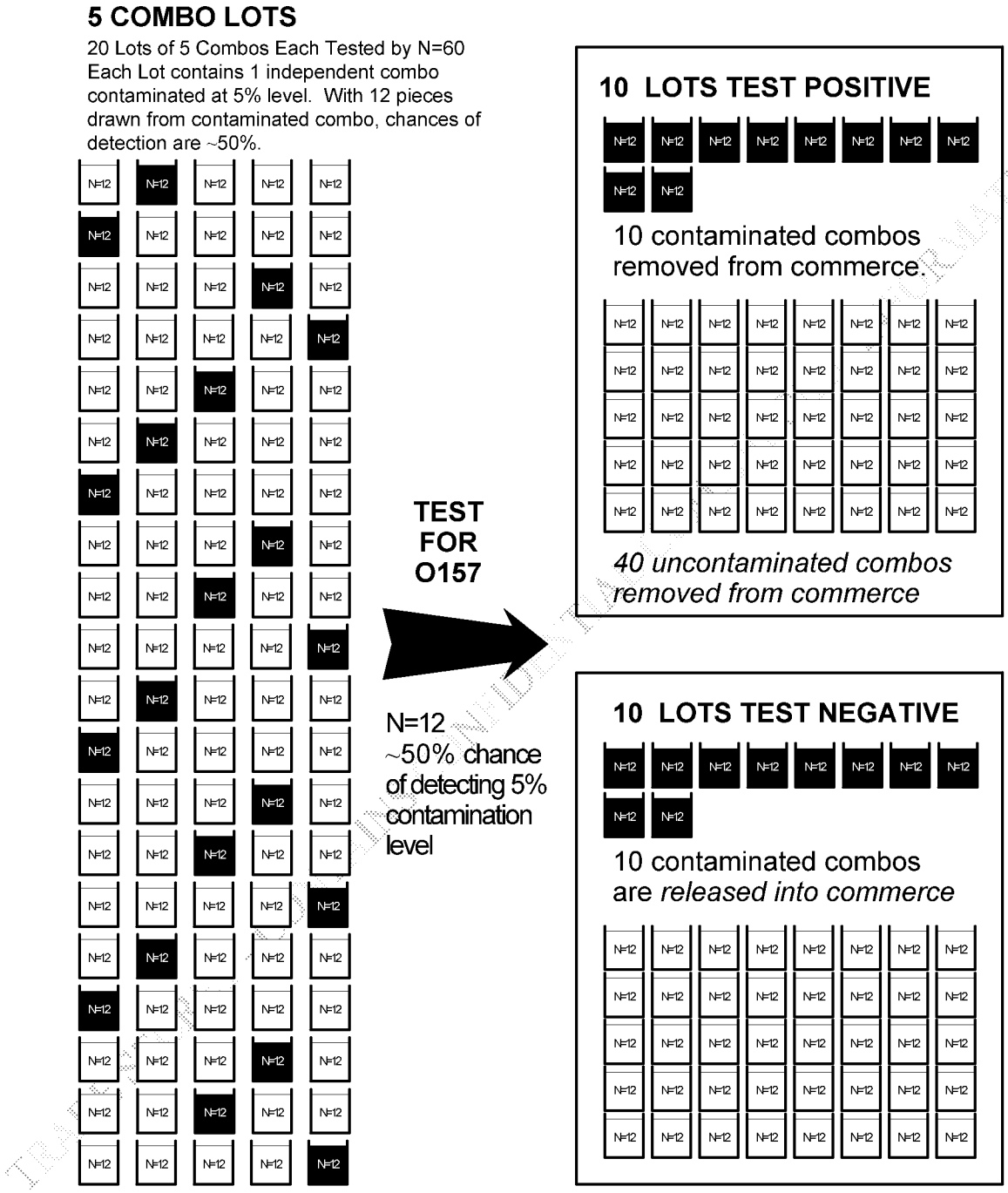


Figure 3: Illustration of detection and removal of independent combo units having a 5% level of contamination using 5 combo lotting.

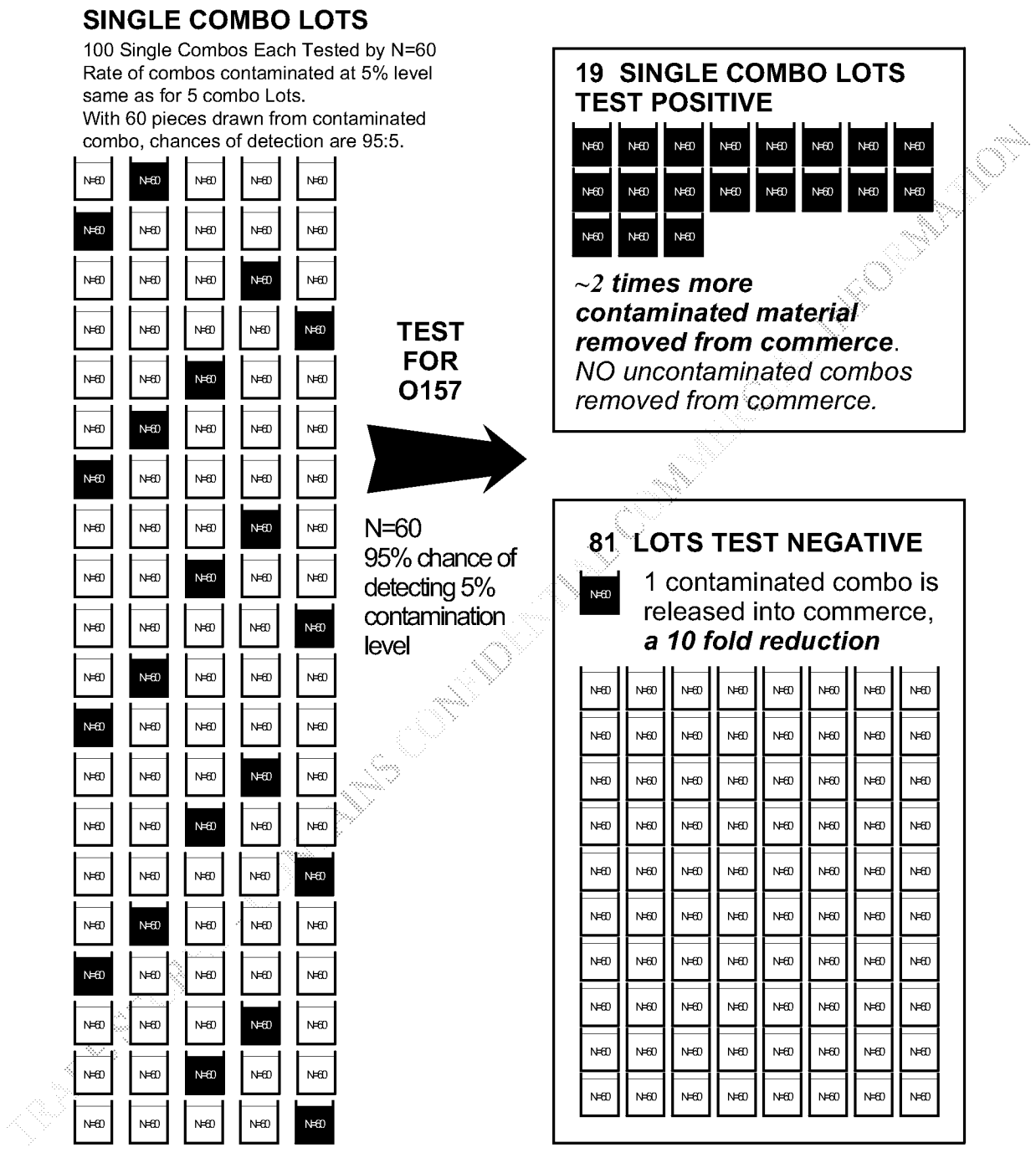


Figure 4: Illustration of detection and removal of independent combo units having a 5% level of contamination when single combo, N=60 sampling is used.

APPENDIX F
Silliker, 1969

17050

AN EVALUATION OF THE SALMONELLA PROBLEM

A Report of the
U.S. DEPARTMENT OF AGRICULTURE
and the
FOOD AND DRUG ADMINISTRATION,
U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

prepared by the
COMMITTEE ON SALMONELLA
DIVISION OF BIOLOGY AND AGRICULTURE
NATIONAL RESEARCH COUNCIL

NATIONAL ACADEMY OF SCIENCES
WASHINGTON, D.C.
1969

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FEB 16 79

Prefac

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Appendix D

"Wet Compositing" as an Approach to Control Procedures for the Detection of Salmonellae

Silliker (unpublished) has conducted a feasibility study of "wet compositing" as an approach to control procedures for the detection of salmonella. His results are summarized here.

The samples involved in the study were dried foods and feeds that were being analyzed for salmonellae as a part of quality-control programs. All samples were pre-enriched for 18–24 hours at 35°C before subculture. The nature of the pre-enrichment medium varied depending on the nature of the sample. For example, dried milk samples were enriched in water containing brilliant green, dried eggs in lactose broth, rendered animal by-products in lactose broth containing tergitol, and candy in reconstituted nonfat dried milk containing brilliant green.

The pre-enrichment cultures were subcultured in tetrathionate broth containing brilliant green. Approximately half of the samples were subcultured into cystine selenite broth, in addition to tetrathionate broth. The selective broths were incubated at 35°C for 24 hours, at which time the cultures were streaked onto brilliant green, SS, and bismuth sulfite agars.

After incubation, the selective agars were examined for suspect salmonella colonies. These were studied and identified using conventional biochemical and serological procedures.

To evaluate the "wet compositing" approach, incubated pre-enrichment cultures were randomly segregated in groups of 10. Within each group of 10, then, a variety of different samples were represented. Each of the 10 samples was subsequently analyzed according to routine procedures as described above. In the "wet compositing" technique, 1 ml from each of the 10 enrichment cultures was introduced into 10 ml of double-strength selective medium. This "wet composite," representing 10 randomly selected samples, was then analyzed as a single unit, using the procedures described above. Those samples that would ordinarily be subcultured in tetrathionate broth only were "wet composited" only in tetrathionate broth. On the other hand, from those samples that were analyzed using both tetrathionate and selenite broths, "wet composites" were prepared in both selenite and tetrathionate broths. The "wet composites" were then analyzed using the procedures described above.

Table D1 summarizes results obtained in the analysis of 106 "wet composites" representing a total of 1,060 individual samples. Thirty-nine composites were positive for salmonella, 66 negative. In seven instances (designated false negative), one or more of the individual samples comprising the "wet composite" yielded salmonellae. Interestingly, in one case (designated false positive) the "wet composite" was positive for salmonella, even though the analysis of each of the 10 samples comprising the composite was negative.

TABLE D1 Sensitivity of "Wet Compositing" for *Salmonella* Detection

No composites	105
No. composites positive	39
No. composites negative	66
No. false negative composites[a]	7
No. false positive composites[b]	1

[a] False negative indicates failure to isolate salmonellae from the "wet composite," even though one or more of the 10 components was found positive.
[b] False positive indicates "wet composite" was found positive for salmonella, even though each of the 10 components was found negative.

APPENDIX G
Sales Brochure, Idaho Technology

Idaho Technology *Salmonella* LT Food Security System Granted *Performance-Tested Method*℠ Status

AOAC RESEARCH INSTITUTE
Leading the World in Performance-Based Validation

Idaho Technology, Inc.
Salmonella LT Food Security System
for the R.A.P.I.D.® LT
Cat. No.: ASAY-ASY-0116
PTM Status: March 2008
Certificate No.: 030803

Summary

The *Salmonella* LT Food Security System (FSS; Figure 1) is a rapid detection method for *Salmonella* that combines sensitive, rapid, real-time polymerase chain reaction (PCR) with simple sample preparation. The PCR process takes only 35 min and the entire procedure only 17 h. The method involves a 16 h sample enrichment using traditional media, sample processing by cell lysis to release DNA, rehydration of freeze-dried assay reagents, DNA amplification (PCR) in the Idaho Technology (Salt Lake City, Utah, USA) R.A.P.I.D. LT instrument, and automatic result interpretation by the R.A.P.I.D. LT FSS software.

The AOAC Research Institute certified the Salmonella LT FSS as a *Performance-Tested Method*℠ in March 2008 (Certificate No. 030803). Idaho Technology, Inc. (ITI) developed the *Salmonella* LT FSS to enable fast, simple, inexpensive testing for *Salmonella*-contaminated foods. ITI utilized experience with patented rapid thermocycling technology, real-time PCR detection systems, and pathogen detection for the military to design a custom system for the food industry. The FSS is approved for both single and pooled samples. To qualify for AOAC approval, the *Salmonella* FSS was evaluated for sensitivity, specificity, ruggedness, and stability of reagents.

This report describes the evaluation of the *Salmonella* LT FSS compared to reference methods in three foods: cooked ham, raw chicken, and chocolate.



Figure 1. Salmonella LT Food Security System.

Method

See Figure 2 for an outline of the *Salmonella* LT FSS method.

Enrichment

The sensitivity of PCR allows for a shorter enrichment time than standard detection methods. One colony forming unit (CFU) of Salmonella in 25 g of food can be detected after only 16 h of enrichment using traditional media (buffered peptone water or nonfat dry milk with brilliant green) at 37 C. After enrichment, up to 5 samples can be pooled for analysis. *See* the pooling section below.

Sample Processing

DNA is extracted from the *Salmonella* bacteria by mechanical cell lysis. Bead tubes for mechanical lysis are contained in the *Salmonella* LT test kit. The bacteria are lysed either directly after enrichment or after cell concentration with the MATRIX MicroScience PATHATRIX® System. The direct method includes a dilution step to remove PCR inhibitors before amplification (referred to as PCR-only). The PATHATRIX is useful for isolating *Salmonella* from dirty food backgrounds that may interfere with detection.

Freeze-Dried Reagents

PCR is simple with the RAPID LT FSS because all PCR components are included in easy to use freeze-dried reagent vials contained in the *Salmonella* LT test kit. Freeze-dried reagents are stored at room temperature. The processed sample is used to rehydrate freeze-dried assay reagents with a buffer, and the mix is added directly to a PCR vessel.

PCR

Real-time DNA amplification (PCR) occurs in the Idaho Technology R.A.P.I.D. LT instrument. *Salmonella* LT has two levels of specificity. PCR primers specifically amplify *Salmonella* DNA that is then detected by specific fluorescent probes that only detect *Salmonella*. Thus,

National Beef Ex. 1007
Page 0754

Table 1. Method comparison results: Individual samples

Matrix	Inoculating organism	Level	MPN, CFU/25 g[a]	Samples	*Salmonella* LT positive	Confirmed positive	Reference positive (paired samples)
Raw chicken	*Salmonella* typhimurium	Low	<0.8	20	13	13	13
		Control	0	5	0	0	0
Cooked ham	*Salmonella* enteriditis	Low	<0.8	20	7	7	7
		Control	0	5	0	0	0
Cooked ham[b]	*Salmonella* typhimurium	Low	2.3	20	13	13	13
		Control	0	5	0	0	0
Chocolate	*Salmonella* senftenberg	High	10.8	20	17	17	17
		Low	0.9	20	9	9	9
		Low	<0.8	20	1	1	1
		Control	0	15	0	0	0

[a] MPN = Most Probable Number. [b] Independent laboratory results.

fluorescent detection only occurs if a *Salmonella* target is amplified. The freeze-dried reagents also contain an internal amplification control to ensure that amplification is successful. The R.A.P.I.D. LT instrument is faster than traditional PCR instruments (35 min versus 3 h) because it uses air thermocycling to heat and cool the sample.

Final Result

Results are reported by the RAPID LT FSS software as "positive" or "negative." The final result is determined within about 17 h from the beginning of enrichment.

Pooling

Post-enrichment sample pooling is an excellent way to increase throughput and decrease costs. A 5:1 pooling approach enables the cost per test to approach that of traditional methods, reducing PCR assay costs by up to 80%, when most samples are negative. Samples are individually enriched and then pooled into a composite sample, while preserving the individual samples in case of a positive. The composite sample is tested as a single sample. If the composite sample is positive, the individually enriched samples may be retested to determine which of the samples is positive. Retesting takes less than 1 h because of the short 35 min PCR run. In the *Salmonella* LT FSS evaluation, pooled samples yielded the same results as individual samples with no false negatives.

Validation Results

With both individual and pooled samples, the *Salmonella* LT FSS demonstrated 100% sensitivity and 100% specificity for the three different food types (cooked ham, raw chicken, and chocolate). Each sample was grown in standard media and tested with both the *Salmonella* LT FSS and the reference method.

The FSS also proved to be robust and reproducible. The ruggedness study demonstrated that the system produced consistent results with variability in reagent preparation time and sample volumes. Lot-to-lot and shelf-life studies demonstrated that the *Salmonella* LT produced consistent results with several lots of reagents produced at different times.

Method Comparison, Individual Samples

The *Salmonella* LT FSS is as sensitive as the USDA and FDA reference methods at low inoculum levels in raw chicken, cooked ham, and chocolate. Results are summarized in Table 1. The *Salmonella* LT FSS was compared to reference methods from the FDA *Bacteriological Analytical Manual* (BAM; 1) and USDA *Microbiology Laboratory Guidebook* (MLG; 2). A total of 25 samples per food type were prepared for primary enrichment in the

(Continued on page ??.)

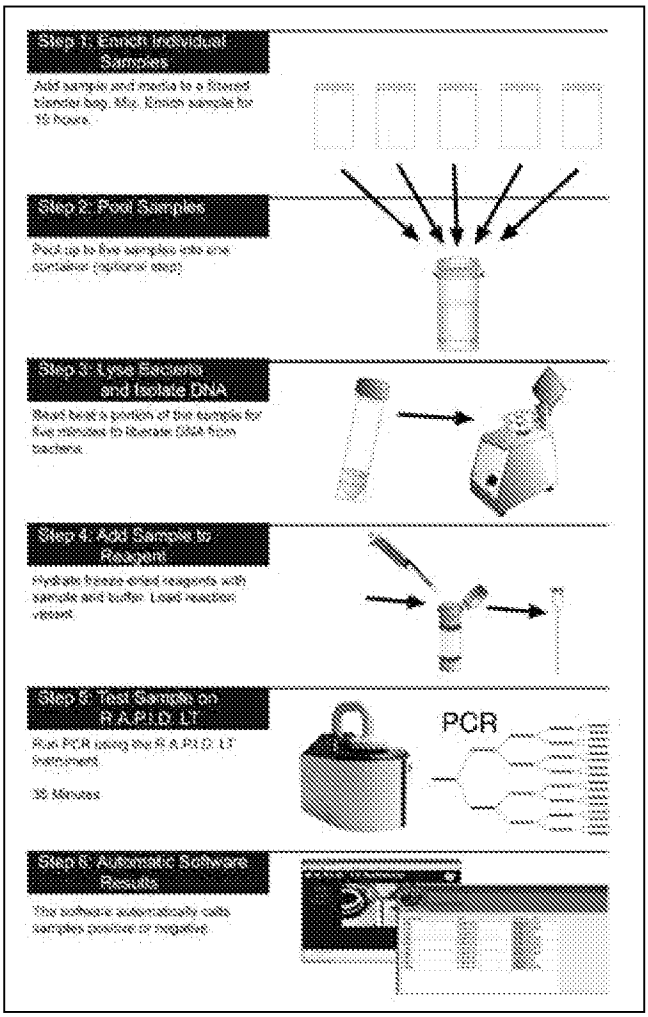


Figure 2. Salmonella LT FSS method workflow.

National Beef Ex. 1007
Page 0755

Idaho Technology *Salmonella* LT Food Security System Granted *Performance-Tested Method*℠ Status

Continued from page ??.

recommended broth according to the reference method. Twenty samples were inoculated with a low level of target organism (approximately 1 CFU per 25 g food sample), while 5 samples were not inoculated. Cooked ham and raw chicken samples were inoculated with liquid culture and allowed to equilibrate at 4°C for 48-72 h. Chocolate samples were melted, inoculated with liquid culture, allowed to harden at room temperature, and equilibrated at room temperature for 2 weeks. Each food matrix was inoculated with a different *Salmonella enterica* serovar (see Table 1). These serovars have been responsible for foodborne illness or associated with recent outbreaks. Each sample was tested with both the reference method and the *Salmonella* LT FSS PCR-only protocol (no cell concentration with the PATHATRIX) since they both use the same primary enrichment protocol. This allowed for a paired sample analysis. All presumptive positive samples were confirmed by standard plating methods.

Method Comparison, Pooled Samples

The system is sensitive even with pooled samples (5 samples combined). Results are summarized in Table 2. Ten samples were inoculated with a low level of target organism (approximately 1-10 CFU per 25 g food sample), while 40 samples were not inoculated. A 50 mL aliquot from each individual positive sample was combined with 50 mL aliquots from 4 negative samples to create a 250 mL pooled sample. A total of 10 pools comprised of the 50 individual samples were constructed. Following a 16 h incubation, the pools were analyzed with one (PCR-only) or both (PCR-only and PATHATRIX with PCR) *Salmonella* FSS protocols.

Inclusivity and Exclusivity

The *Salmonella* LT FSS was able to detect 121 out of 121 strains tested in the inclusivity panel. It did *not* detect 30 out of the 30 bacteria tested in the exclusivity panel consisting of closely related non-*Salmonella* species. Each strain was grown in buffered peptone water and nonfat dry milk with brilliant green.

Conclusions

The *Salmonella* LT FSS is as sensitive as the USDA and FDA methods at low inoculum levels in food, with individual and pooled samples. Out of a total of 230 samples evaluated in the study, 126 samples were positive with both the *Salmonella* LT and the reference method. Sensitivity and specificity were 100% for the *Salmonella* LT FSS with individual or pooled samples, and with PATHATRIX for pooled samples. False-positive and false-negative rates were 0%. *Salmonella* LT detected all 121 Salmonella serotypes evaluated. It detected none of the 30 non-Salmonella species tested.

The *Salmonella* LT FSS is a significant improvement over standard methods. It provides results significantly faster, in 17 h compared to 72 h for the USDA and FDA reference methods. The R.A.P.I.D LT performs real-time PCR and provides automated results in about 30 min (after enrichment and sample processing). Results are easier to interpret than standard methods because the software gives a "positive" or "negative" answer. The *Salmonella* LT FSS is easy to use, with very few steps and minimal sample handling. Pooled samples yield the same results as individual samples. Pooling enables five times the throughput at the same cost.

Based on results of the validation study, the *Salmonella* LT FSS for the detection of *Salmonella* in cooked ham, raw chicken, and chocolate was adopted as AOAC *Performance-Tested Method*℠ No. 030803.

—*Stephanie Thatcher, Haleigh Millward, and Mike Powers*
Contributing Writers
stephaniet@idahotech.com, haleigh@idahotech.com

Mention of trade names or commercial products is for identification only and does not constitute preference over similar ones not mentioned. If you are interested in submitting an article regarding a test kit that has been granted Performance-Tested MethodSM status, contact Deborah McKenzie at dmckenzie@aoac.org.

References

(1) U.S. Food and Drug Administration, Bacteriological Analytical Manual, http://www.cfsan.fda.gov/~ebam/bam-5.html

(2) U.S. Department of Agriculture/Food Safety and Inspection Service, Microbiology Laboratory Guidebook, http://www.fsis.usda.gov/PDF/MLG_4_03.pdf

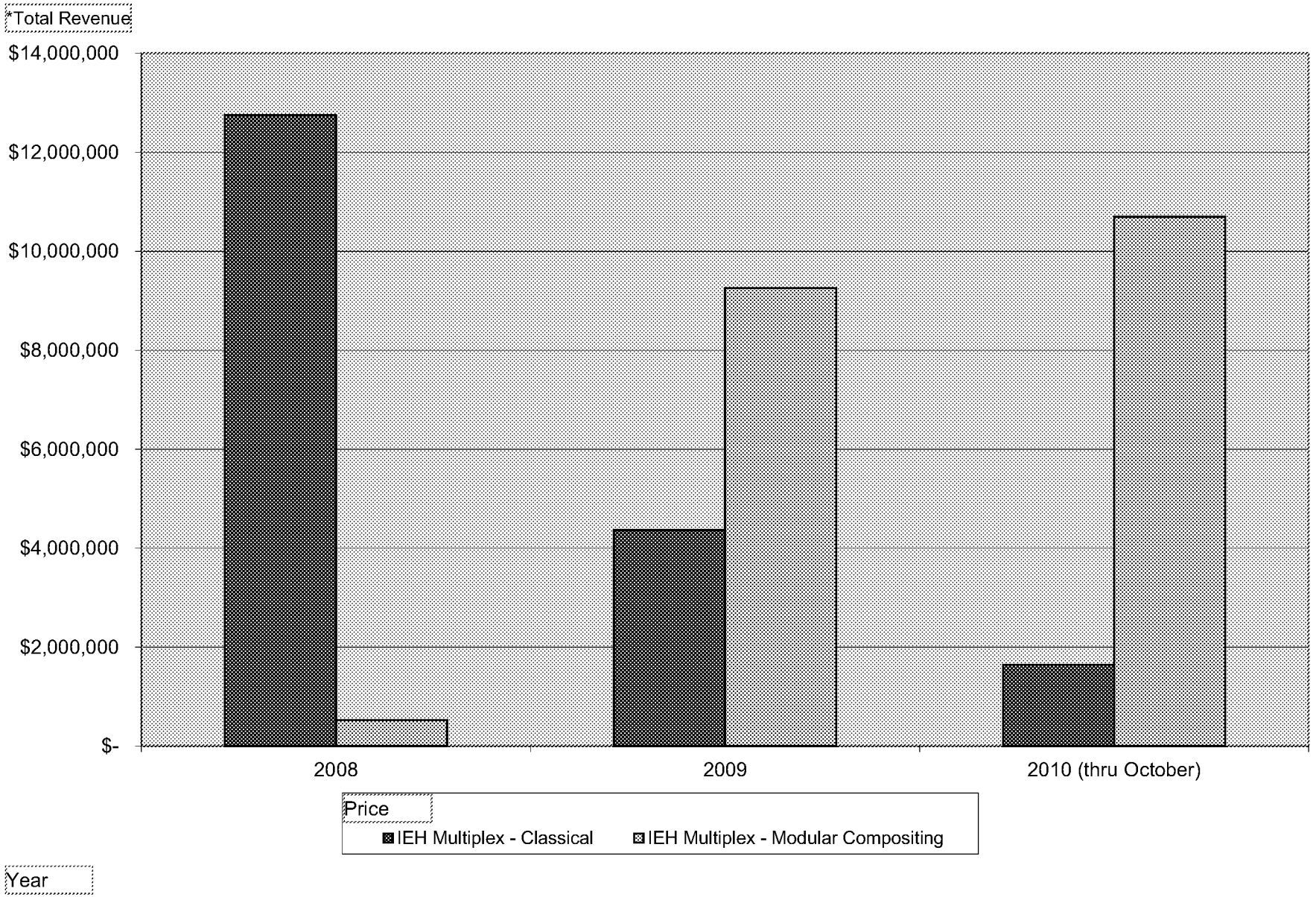
Table 2. Method comparison results: Pooled samples

Matrix	Inoculating organism	Protocol	MPN, CFU/25 g[a]	Samples	*Salmonella* LT positive	Confirmed positive	Reference positive (paired samples)
Raw chicken	*Salmonella* typhimurium		18.8	10	10	10	10
		PATH[b]	18.8	10	10	10	10
Cooked ham	*Salmonella* enteriditis		5.8	10	10	10	10
		PATH	5.8	10	10	10	10
Cooked ham[c]	*Salmonella* typhimurium		0.9	10	4	4	4
		PATH	0.9	10	4	4	4
Chocolate	*Salmonella* senftenberg		10.8	10	9	9	9
		PATH	10.8	10	9	9	9

[a] MPN = Most Probable Number. [b] PATH = Cell concentration with PATHATRIX. [c] Independent laboratory results.

National Beef Ex. 1007
Page 0756

APPENDIX H
IEH Sales Figures


*Total Revenue
$14,000,000
$12,000,000
$10,000,000
$8,000,000
$6,000,000
$4,000,000
$2,000,000
$-
2008
2009
2010 (thru October)
Price
IEH Multiplex - Classical
IEH Multiplex - Modular Compositing
Year

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 TO REBUT THE DECLARATION OF DR. BAILEY 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. "Dr. J. Stan Bailey, a citizen of the United States of America, hereby declare and state: I have over thirty-five years of work and research experience in the area of testing for microorganisms in foods.

2. "I have a Bachelor of' Science in Environmental Health Science, which was conferred upon me by the University of Georgia in Athens, Georgia, in 1974. I have a Master of Science in Food Science, which was conferred upon me by the University of Georgia in Athens,

Georgia, in 1978. I have a Ph.D. in Poultry Science, which was conferred upon me by the University of Georgia in Athens, Georgia, in 1989.

3. "I have been employed by bioMerieux, Inc., the real party in interest under 37 C.F.R. §1.915(b)(8) in the *Inter Partes* Reexamination of the above-identified patent (hereinafter "the real party in interest"), since January 2008 as the Director of Scientific Affairs. Prior to my employment with bioMerieux, Inc., I spent thirty-four years as a research microbiologist employed by the United States Department of Agriculture ("USDA") within the Agricultural Research Service ("ARS") during which time I received seventeen USDA Certificates of Merit and was named "Outstanding Senior Research Scientist of the Year" in 2002. I have taught as an Adjunct Professor in the Poultry Science Department at the University of Georgia and at the Department of Food Science at Kansas State University. I have been a Faculty Member at the "Rapid Methods and Automation in Microbiology Workshop" taught at Kansas State University where over one thousand scientists from fifty countries have received training in the area of microbiological testing.

4. "I have authored or co-authored over five-hundred scientific publications in the area of food microbiology. I am on the editorial boards of *Poultry Science, Journal of Rapid Methods and Automation in Microbiology,* and the *Journal of Applied Poultry Research.*

5. "I have received numerous honors in the area of food microbiology that include the following awards and appointments:

"a) Chairman of the Food Microbiology Division of the American Society for Microbiology (1992);

"b) Secretary of the Microbiological Methods Committee of the Association of Official Analytical Chemists ("AOAC") (1990-95);

"c) Food and Agriculture Organization of the United Nations Expert Consultant on Animal Feeding and Food Safety;

"d) Fellow of the American Academy of Microbiology (1994);

"e) Technical Advisor to the National Advisory Committee on Microbiological Criteria in Foods for poultry production (1995 and 2006);

"f) Scientific Advisor to the International Life Sciences Institute, Food Microbiology Committee (1997 -2008);

"g) Federal Laboratory Consortium Award for Technology Transfer;

"h) Laboratorian of the Year by International Association for Food Protection ("IAFP"), (2003);

"i) Fellow of the International Association for Food Protection (2005); and

"j) President of the International Association for Food Protection (2009).

6. "I am a member of the following Professional Societies: Southern Poultry Science Society, Worlds Poultry Science, A1llerican Society for Microbiology, American Academy of Microbiology, Poultry Science Society, International Association for Food Protection, Georgia Association for Food Protection, AOAC.

7. "I have a professional relationship with the real party in interest. In the course of that professional relationship, I receive compensation directly from the real party in interest for my work relating to food microbiology. I am not being separately compensated for my work in connection with this Declaration and my compensation is not dependent on the outcome of these proceedings.

8. "I have read U.S. Patent 7,534,584 which issued to Mansour Samadpour on May 19,2009 (referred to herein as the "584 patent"). Based on my experience and knowledge of the relevant literature, it is my opinion that the method of sampling, testing and validating test lots (or singleunit production lots) for microbial contamination claimed in the '584 patent is an aggregation of sample-compositing, enrichment, wet-compositing, testing, validation and optional re-testing steps that were known in the art prior to August 2004. It is my opinion that each of the steps comprising the claimed method performs its art-recognized function and that the combination of steps, as defined by the claims, yields predictable results. It is also my opinion that it would have been obvious to a person or ordinary in August 2004 to have combined the steps, as set forth in the claims, to improve efficiencies and reduce costs. The scientific field relevant to '584 patent is the field of microbiology as it relates to the microbiological analysis of food products and industrial food safety. A person of ordinary skill in that field in August 2004 would have been involving in developing methodology for sampling, testing and validating food products for microbial contamination. Such a person would have possessed either an advanced scientific degree in microbiology, environmental health and safety, or food science or would have possessed a Bachelor's degree and bad several years of experience in developing methodology for the sampling and analysis of food products. The person of ordinary skill would have been familiar with the scientific literature relevant to the

microbiological analysis of food also would have been required to possess a working knowledge of rules, regulations, guidelines and directives relating to sample preparation and testing methodology to evaluate food for the presence of absence of pathogenic microorganisms."

RESPONSE BY INVENTOR:

Dr. Bailey defines a person with ordinary skill in the field as a Bachelor's degree in microbiology, environmental health, or food science. Then he decides that the person would have had several years of experience in developing methodologies for the sampling and analysis of food products. Then he throws in comprehensive familiarity with the literature, and a working knowledge of rules, regulations, guidelines, and directives relating to sample preparations and testing methodologies. This is a fictional construct. In this field, methods are developed by molecular biologists, immunochemists, biochemists, and classical microbiologists. People involved in methods development are not the same as the people who develop sampling plans. Neither of the two groups follows FSIS and FDA directives and guidelines. Those are done by the practitioner's in the field. Dr. Bailey's construct is, therefore, a person with extraordinary skills in several arts.

It is not valid to simply assert that microbiologists or other practitioners have knowledge of statistics suitable for designing sampling plans. The preface to Jarvis (1987) states as follows:

"For many years I have been aware of a lack of understanding of statistical concepts by biologists in general and microbiologists in particular."

In other words a person of ordinary skills in the field is either a practitioner (user of methods), a method developer (to develop PCR, immunochemical, and clinical methods), a statistician (to develop sampling plans), a regulatory compliance person (to decide how to address applicable regulations and directives) or a quality systems professional (who conducts validation studies). Dr. Bailey's "person of ordinary skill" is necessarily a person with skills in many arts.

"9a. I have read at least sections 8.4.2 and 8.4.2.1 of B. Jarvis, *Statistical Aspects of the Microbiological Analysis of Foods,* 21 PROGRESS IN INDUSTRIAL MICROBIOLOGY 138·142 (1989) (hereinafter "Jarvis") and provide the following explanation of how Jarvis would have been understood in August of 2004 by one of ordinary skill in the art. By August 2004, a person of ordinary skill involved with the development of methods for the sampling, testing, and validation of food products for microbial contamination would have been aware of the importance of ensuring the statistical validity of such methods, and therefore would have

consulted a reference like the Jarvis publication, which addresses the statistical aspects of such methods."

RESPONSE BY INVENTOR:

Dr. Bailey continues on the basis of the false assumption that there is a person with ordinary skills in the art that has mastery of sampling method design, designing test systems, has expertise in statistics, and designs and conducts validation studies.

The Jarvis reference is an obscure reference that deals with statistical analysis of sampling methods. As the title of the chapter implies it is not an enabling reference. It does not teach how to conduct the methods, does not provide materials and methods, and does not report results. Furthermore, when it gets to the section that discusses compositing it does not cite the enabling references. It also misrepresents the teachings of Silliker, Gabis and Price regarding method 3 (section 8.4.2) who argue against compositing before pre-enrichment.

"9b. Section 8.4.2 describes the effect of subculture volume and incubation times in the conventional testing of samples for the presence or absence of microbes. See p. 138. As was the usual practice, the testing procedure involves both "pre-enrichment" and "selective enrichment stages." *Id.*"

RESPONSE BY INVENTOR:

Dr. Bailey relies on a series of misrepresentations of the references cited to make his case. To begin with he fails to explain that the Jarvis section cited here is about testing a single lot of food for *Salmonella*. The method in question is the FDA BAM method for *Salmonella*. The method requires the use of pre-enrichment for the samples, followed by enrichment in selective media, followed by detection using plating.

"9c. Regarding how the samples themselves are collected and prepared, in the introduction to the chapter, Jarvis simply discloses that the sample could be an "initial food homogenate (or culture)." See p. 117."

RESPONSE BY INVENTOR:

In my opinion, either Dr. Bailey either doesn't understand, or is misrepresenting, the referenced. On page 117 (APPENDIX A), Jarvis states (first paragraph): "In its simplest form, the dilution consists of serial dilution of the initial food homogenate (or culture)

followed by inoculation of an appropriate volume of each dilution into one or more tubes of culture (agar or broth)…". Within Jarvis, it is the subject of Sections 8.4.2 and 8.4.2.1 which are the prior art relevant to patent '584. Anyone with ordinary skills in the art would realize that a sample description for a given method, would not necessarily apply to a different method. In this regard, by citing page 117 Dr. Bailey is using a sample description for a dilution count method for indicators, and incorrectly implying that it is the sample description for the *Salmonella* method of Sections 8.4.2 and 8.4.2.1, which are the core of the argument (the only argument) in Jarvis.

9d. "During the pre-enrichment stage, an initial sample is dispersed in a pre-enrichment medium and then it is incubated, resulting in a "pre-enrichment culture." See Example 8.6, p. 139. The incubation of the sample in the pre-enrichment medium causes at least the target microorganism to grow. *Id.* Due to the incubation, the pre-enrichment culture has been enriched in the target microorganism, which in Example 8.6 discussed in section 8.4.2 is *Salmonellae.* In August of 2004, it was well known in the art that this type of enrichment would both resuscitate the target microorganism and cause it to grow to reach detectable levels and become substantially uniform within the liquid pre-enrichment culture."

RESPONSE BY INVENTOR:

Neither the definition, nor the function of pre-enrichment has changed from 1960s (Silliker), 1970s (Silliker, Gabis, Price), 1980s (Jarvis) to 2004 (patent '584), to 2007 (Jarvis, 2007). Pre-enrichment is a term of art that refers to an initial incubation of a sample in a media that will not allow rigorous growth but is instead designed to resuscitate and result in limited growth of the target organism to a point that when an aliquot of the pre-enrichment is taken to inoculate the enrichment media, there is 99% confidence that the aliquot taken will at least contain at least one viable cell of the target organism in the aliquot taken. In this regard I do not believe that Dr. Bailey is properly interpreting the text in Jarvis.

I have attached (APPENDIX B) a technical datasheet for lactose broth issued by PML Microbiologicals, a purveyor of microbiological media. Lactose Broth is the pre-enrichment media referenced by Jarvis, Price, Silliker and Gabis. The technical sheet for the lactose broth clearly shows that Dr. Bailey's interpretation is incorrect when he states: *"The incubation of the sample in the pre-enrichment medium causes at least the target microorganism to grow."* On the first page of the datasheet, second paragraph of the section

entitled Principle it states:

> ***This pre-enrichment medium is used to provide a higher ratio of Salmonella to non-Salmonella bacteria after incubation. Lactose is fermented by most non-Salmonella bacteria while Salmonella does not. A bactiostatic effect on competing microorganisms is created by the decrease of pH in the medium produced by the lactose fermenting bacteria. The Salmonella will have little growth during the initial 24 hours of incubation, while the non-Salmonella will be utilizing the lactose. As the products of fermentation build, the non-Salmonella are inhibited and the Salmonella begins its growth phase (24-48 hrs.) (Emphasis added)***

In my opinion Dr. Bailey is misleading the Examiner with his interpretation of pre-enrichment which does not conform to the commonly understood term of art.

9d. (continued) "Microorganisms in food products often are debilitated because of the prior handling and processing of the products, but are able to resuscitate and proliferate under favorable conditions. Pre-enrichment utilizes nutrients and conditions to effect such resuscitation and growth so that the pre-enrichment culture can be even further diluted at the collective enrichment stage. Specifically, according to Jarvis, only 1 ml of each pre-enrichment broth will be added to 100 m1 of the enrichment broth to form the composite."

RESPONSE BY INVENTOR:

Again, per the lactose broth technical sheet (APPENDIX B) the function of the lactose broth is to encourage the growth of the competitive flora in order to drop the pH, damage the acid sensitive bacteria, and provide a competitive advantage to *Salmonella* cells when they are transferred into the selective enrichment broth.

9d. (continued 2) "The table in Example 8.5, on page 141 of Jarvis, shows that it is necessary to incubate the pre-enrichment medium at least 12 hours to ensure a 99% probability that any 1 m1 of the pre-enrichment culture that is transferred to the composite includes more than one viable *Salmonella* organism. This means that each 1 ml of the pre-enrichment culture includes viable *Salmonella* organisms, i.e." the viable *Salmonella* organisms are detectable and uniformly distributed within the pre-enrichment culture."

RESPONSE BY INVENTOR:

Dr. Bailey references a table for Example 8.6 of Jarvis where Jarvis is attempting to illustrate his main point; i.e. that the function of pre-enrichment is to assure that the

portion transferred to the enrichment broth contains at least one viable cell. In this mathematical model, he assumes a concentration of 0.1 CFU/gram or 10 organisms per 100 grams.

Here, Dr. Jarvis also assumes a generation time of 30 minutes in Lactose Broth, an assumption which is inconsistent with the technical sheet of APPENDIX B, which states that Salmonella does not begin its growth phase until after 24 h. Furthermore, Jarvis does not consider the impact of the competitive organisms. Jarvis is engaged in performing theoretical mathematical calculations and reports his results in a table having a mathematical frame work. However, right under the table Jarvis explains the reality of pre-enrichment:

> ***"Once sufficient growth has occurred in pre-enrichment for at least 3 viable salmonellae/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, with m=3: 1'(x=0) = 0.05, therefore 1'(:c)-I)= 0.95. However, at lower numbers, the chances of transferring at least 1 viable organism will be affected not only by the initial contamination level (x) but also by the volume of medium inoculated into the enrichment culture. In methods which permit only resuscitation, not growth, during pre-enrichment, it is essential to use the entire pre-enrichment culture to inoculate double-strength enrichment medium (Hall, 1975), although selectivity may then be affected by the food constituents. Obviously the level of salmonellae in the pre-enrichment culture after any given incubation period will depend upon the cultural conditions used, the initial contamination level, the extent of sub-lethal cell damage and the relative numbers and types of competitive organisms in the sample."***

Dr. Bailey elects to use selective portions of the reference in an attempt to present his case and misdirect the Examiner. Dr. Bailey incorrectly states:

> ***"This means that each 1 ml of the pre-enrichment culture includes viable Salmonella organisms, i.e." the viable Salmonella organisms are detectable and uniformly distributed within the pre-enrichment culture."***

Please note that this is the same 1 ml of the pre-enrichment culture that aims to deliver at least one viable cell to the enrichment culture. Please also note that the limit of detection of food enrichments by PCR method is 10,000 CFU/ml. A presentation by Dr. Baily himself, an excerpt of which is included as APPENDIX C, shows an enrichment level of 10E4 (10,000 CFU/mL). It is common knowledge that the limit of detection for lateral flow

devices is in the range of 100,000-1000,000 CFU/ml.

9d. (continued) "Microorganisms in food products often are debilitated because of the prior handling and processing of the products, but are able to resuscitate and proliferate under favorable conditions. Pre-enrichment utilizes nutrients and conditions to effect such resuscitation and growth so that the pre-enrichment culture can be even further diluted at the collective enrichment stage. Specifically, according to Jarvis, only 1 ml of each pre-enrichment broth will be added to 100 m1 of the enrichment broth to form the composite."

RESPONSE BY INVENTOR:

Again, per the lactose broth technical sheet (APPENDIX B) the function of the lactose broth is to encourage the growth of the competitive flora in order to drop the pH, damage the acid sensitive bacteria, and provide a competitive advantage to *Salmonella* cells when they are transferred into the selective enrichment broth.

9d. (continued 2) "The table in Example 8.5, on page 141 of Jarvis, shows that it is necessary to incubate the pre-enrichment medium at least 12 hours to ensure a 99% probability that any 1 m1 of the pre-enrichment culture that is transferred to the composite includes more than one viable *Salmonella* organism. This means that each 1 ml of the pre-enrichment culture includes viable *Salmonella* organisms, i.e." the viable *Salmonella* organisms are detectable and uniformly distributed within the pre-enrichment culture."

RESPONSE BY INVENTOR:

Dr. Bailey references a table for Example 8.6 of Jarvis where Jarvis is attempting to illustrate his main point; i.e. that the function of pre-enrichment is to assure that the portion transferred to the enrichment broth contains at least one viable cell. In this mathematical model, he assumes a concentration of 0.1 CFU/gram or 10 organisms per 100 grams.

Here, Dr. Jarvis also assumes a generation time of 30 minutes in Lactose Broth, an assumption which is inconsistent with the technical sheet of APPENDIX B, which states that Salmonella does not begin its growth phase until after 24 h. Furthermore, Jarvis does not consider the impact of the competitive organisms. Jarvis is engaged in performing theoretical mathematical calculations and reports his results in a table having a mathematical frame work. However, right under the table Jarvis explains the reality of

pre-enrichment:

> ***"Once sufficient growth has occurred in pre-enrichment for at least 3 viable salmonellae/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, with m=3: 1'(x=0) = 0.05, therefore 1'(:c)-I)= 0.95. However, at lower numbers, the chances of transferring at least 1 viable organism will be affected not only by the initial contamination level (x) but also by the volume of medium inoculated into the enrichment culture. In methods which permit only resuscitation, not growth, during pre-enrichment, it is essential to use the entire pre-enrichment culture to inoculate double-strength enrichment medium (Hall, 1975), although selectivity may then be affected by the food constituents. Obviously the level of salmonellae in the pre-enrichment culture after any given incubation period will depend upon the cultural conditions used, the initial contamination level, the extent of sub-lethal cell damage and the relative numbers and types of competitive organisms in the sample."***

Dr. Bailey elects to use selective portions of the reference in an attempt to present his case and misdirect the Examiner. Dr. Bailey incorrectly states:

> ***"This means that each 1 ml of the pre-enrichment culture includes viable Salmonella organisms, i.e." the viable Salmonella organisms are detectable and uniformly distributed within the pre-enrichment culture."***

Please note that this is the same 1 ml of the pre-enrichment culture that aims to deliver at least one viable cell to the enrichment culture. Please also note that the limit of detection of food enrichments by PCR method is 10,000 CFU/ml. A presentation by Dr. Baily himself, an excerpt of which is included as APPENDIX C, shows an enrichment level of 10E4 (10,000 CFU/mL). It is common knowledge that the limit of detection for lateral flow devices is in the range of 100,000-1000,000 CFU/ml.

9d. (3) Thus, at the pre-enrichment stage in Jarvis, the sample is dispersed in a pre-enrichment medium and then incubated to form a pre-enrichment culture, and the pre-enrichment culture has been incubated under conditions suitable to allow the target microorganism to reach detectible levels and is substantially uniform throughout the pre-enrichment culture. This procedure described by Jarvis is typical of known methods for the pre-enrichment and selective enrichment of salmonella.

RESPONSE BY INVENTOR:

Dr. Bailey again is misrepresents Jarvis. We repeat here the section of Jarvis cited above as explaining the reality of pre-enrichment under the table on Page 141:

> ***"Once sufficient growth has occurred in pre-enrichment for at least 3 viable salmonellae/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, with m=3: 1'(x=O) = 0.05, therefore 1'(:c)-I)= 0.95. However, at lower numbers, the chances of transferring at least 1 viable organism will be affected not only by the initial contamination level (x) but also by the volume of medium inoculated into the enrichment culture. In methods which permit only resuscitation, not growth, during pre-enrichment, it is essential to use the entire pre-enrichment culture to inoculate double-strength enrichment medium (Hall, 1975), although selectivity may then be affected by the food constituents. Obviously the level of salmonellae in the pre-enrichment culture after any given incubation period will depend upon the cultural conditions used, the initial contamination level, the extent of sub-lethal cell damage and the relative numbers and types of competitive organisms in the sample."***

We urge the Examiner to consider this pre-amble paragraph, taken from the previous passage on page 139, fourth paragraph, of the Jarvis reference:

> ***"Clearly, the possibilities for isolation of specific organisms will depend not only on the initial incidence of contamination but also on the manner in which the test is done. A too short period of pre-enrichment may result in no recovery of stressed organisms, thereby reducing the statistical probability of transferring resuscitated organisms to the enrichment broth. Too long a period in a non-selective pre-enrichment may provide opportunity for overgrowth by competitive organisms, leading to suppression of the organism sought. A hypothetical example of the effects of incubation time is shown in Ex. 8.6 and 8.7 and in Fig. 8.4."***

This preamble establishes two things: *first* that the table on page 141 is a hypothetical model; and *second* that Dr. Bailey once again misrepresents the Jarvis reference. The growth of *Salmonella* in the Lactose Broth is not a sure event, and the outcome is not predictable, and certainly the *Salmonella* after the pre-enrichment is not detectable, according to Jarvis.

9e. "At the selective enrichment stage, a 1 ml portion of the pre-enrichment culture (which has already been incubated) is transferred to a selective enrichment medium (in this example two separate selective enrichment media). See p. 139. Each selective enrichment medium is intended to foster further growth of the target microorganism. Then, loopfuls of the

selective enrichment media are streaked on selective diagnostic media, incubated again, and then tested. *Id.* The incubation after streaking on selective diagnostic media causes the target microorganism to grow on the selective diagnostic media."

RESPONSE BY INVENTOR:

Dr. Bailey's submission of the Jarvis reference stopped at page 142. However, material on page 143 (attached as APPENDIX D) shows that pre-enrichment does not result in the levels of organisms claimed by Dr. Bailey. Page 143 shows that pre-enrichment results in only 410 CFU/ml of the pre-enrichment. Considering that the pre-enrichment is diluted 1:10 dilution into enrichment, the initial concentration of *Salmonella* in the enrichment culture at the beginning of the incubation is a mere 41 CFU/ml, which is far below detectable levels.

Only after 18 hours of further enrichment does the level of *Salmonella* in the enrichment culture reach 670,000 CFU/ml, and then after 24 hours 43,000,000 CFU/ml. Both of these *Salmonella* levels (after 18 and 24 hours) are detectable by any method. Clearly, pre-enrichment did not accomplish enrichment to detectable levels, contrary to Dr. Bailey's assertions.

9f. "Section 8.4.2.1 describes a modification of the procedure discussed in section 8.4.1 by pooling (i.e., compositing) a plurality of samples at various stages. See p. 141. Method (2) in section 8.4.2.1 discusses wet-compositing samples after incubation. *Id.* Specifically, according to method (2), multiple pre-enrichment series (one for each of a plurality of samples) are prepared, and then after incubation 1 ml portions of each of the resulting pre-enrichment cultures are composited into a 100 ml enrichment broth. *Id.* Thus, according to method (2), a plurality of samples that have first been incubated to form pre-enrichment cultures (i.e., enriched in the target microorganism) are composited. Jarvis discloses that compositing pre-enrichment cultures after incubation (1) reduces workload and, (2) if a positive test result is obtained, permits tests to be repeated on stored pre-enrichment cultures to determine which of the pre-enrichment cultures resulted in the positive test. See pp. 141 and 142."

RESPONSE BY INVENTOR:

Dr. Bailey fails to mention that Jarvis describes taking a plurality of samples from a single lot. Also, simply identifying which pre-enrichment culture(s) contributed to a pooled positive test result is not helpful in that taking additional samples from a lot after a positive finding is prohibited by government agencies for the purposes of validating a test lot for

release into commerce. My method, in contrast, puts a statistically sufficient number of samples for validation into each separate enrichment, thereby eliminating the need to take additional samples and providing a means for retesting of the separate enrichments contributing to the pool which conforms to regulatory agency expectations and which can thereby result in release of the lot of food corresponding to the separate enrichment into commerce.

Jarvis does not say that such retesting is practical, but rather talks about this issue as a "theory". Jarvis says: " Method (2), at least in theory, permits the tests to be repeated on stored pre-enrichment cultures". Jarvis qualifies his statement because what he has retained are pre-enrichments, and as is well known in the art pre-enrichments are unstable. If pre-enrichment is allowed to continue past the optimum time point the target organism can be masked by competing microflora. Even if the pre-enrichment is stored in refrigerator psychrotrophic organisms in the pre-enrichment will continue their growth and, in all likelihood, mask the *Salmonella* which is unable to grow at low temperatures. Jarvis states (page 139, fourth paragraph):

> ***"A too short period of pre-enrichment may result in no recovery of stressed organisms, thereby reducing the statistical probability of transferring resuscitated organisms to the enrichment broth. Too long a period in a non-selective pre-enrichment may provide opportunity for overgrowth by competitive organisms, leading to suppression of the organism sought."***

Since the time period between pre-enrichment and obtaining a test result in Jarvis is 3-5 days, consistent with obtaining results by cultural methods, the retesting option is purely theoretical. Jarvis clearly implied that the tests done on the stored pre-enrichment cultures would be done using the same type of test that was used to test the enrichment broth. Obviously, since the broth after initial pre-enrichment required a second enrichment step before the test could be performed, so the stored pre-enrichments would also require this second enrichment step.

Patent '584 overcomes the limitations of using pre-enrichments by using fully enriched individual broths that are stable and permit immediate testing and validation of the corresponding lot for release into commerce. Even more significant, when a positive *Salmonella* finding occurs in Price, he presents no further options and all of the lot which was tested must be destroyed. In the patented method, several of the lots forming the modular composite may yield negative results upon retesting, and because the individual

enrichments were formed on the basis of collecting a number of portions that conforms to a statistical-based sampling plan a negative re-test result validates those particular lots for release into commerce.

10a. "I have read W.R. Price et al., *Salmonella Testing of Pooled Pre-Enrichment Broth Cultures for Screening Multiple Food Samples,* 23(4) APPLIED MICROBIOLOGY 679-682 (1972 (hereinafter "Price") and provide the following explanation of how Price would have been understood in August of 2004 and 2005 by one of ordinary skill in the art. Price describes a method of pooling pre-enrichment broth cultures into a single enrichment broth prior to plating and testing. See abstract, FIG. 1, and pp. 679-682. According to the method, samples are added to pre-enrichment broths. See p. 679 and FIG. 1. Figure 1and the accompanying text indicate that the food products being tested by Price were dried products of a particulate or powdery nature. Thus, Figure 1 shows that Price began with single samples of each food product test lot."

RESPONSE BY INVENTOR:

Dr. Baily is clearly implying that Figure 1 represents compositing of single samples from a plurality of test lots, which is a misinterpretation which must be denied by the Examiner. Price clearly explains that three types of dried foods were used to conduct his experiments. He acquired undisclosed quantities of egg albumin, cocoa, and nonfat dry milk powder. As explained on page 679, materials and methods section, he "blended the dry test food with one of the three dry inocula". The inocula contained various types of *Salmonella*. 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 the interpretation of Dr. Bailey Figure 1 of Price (Page 680, top of page) cannot be interpreted as combining samples from a plurality of test lots.

Price's work is not based on real samples taken from a lot of food to be tested. He attempted to mimic a real lot of food by dividing a single batch of dry food into artificially-inoculated and non-inoculated parts, taking a plurality of samples and inoculating each sample into an individual pre-enrichment. Price contains no discussion to suggest that he

is doing anything other than attempting to find a more economical way of testing a single lot of food. Price has three lots of different product that he uses in his experiment. <u>But at no point does he attempt to combine enrichments representing these different lots of product</u>. Rather, he maintains experimental separation between the various different product types and reports the results concerning each in separate columns of his tables.

10b2. "By August 2004, a person or ordinary skill would have understood that such particulate or powdery products could be mixed or blended until homogeneous, and thus a single sample would be representative for microbiological testing."

<u>RESPONSE BY INVENTOR:</u>

Dr. Bailey is making an unwarranted extrapolation. A person with skills in the art and scientific training would read the materials and methods section of Jarvis and interpret it as the factual basis of the experiments. It is absolutely incorrect to assert that when Price takes a quantity of a dry food, divides it into two portions, inoculates one portion, and then takes multiple samples from each portion to conduct the experiment it is anything but taking a plurality of samples from a single test lot. The inoculation of a portion of the quantity of dry food was a means to ensure that the test lot had positive samples when a plurality of samples were taken.

10b3. "As discussed in Section 2.52 of the "Compendium of Methods for the Microbiological Examination of Foods," 4th Ed., Eds. Frances Pouch Downes and Keith Ito, American Public Health Association, 2001 (referred to herein as the "Compendium", for products such as liquids, the product is mixed until homogenous before drawing a sample. However, for samples that cannot be adequately mixed or bulk solids or semi-solids, the Compendium teaches that multiple samples of aliquots should be taken from several areas of the product to ensure that a representative sample is obtained."

<u>RESPONSE BY INVENTOR:</u>

Dr. Bailey correctly states that the compendium teaches taking a plurality of samples from a single lot. But Dr. Bailey fails to mention that this is standard practice, and that the method employed by Price, Gabis and referenced by Jarvis (the FDA BAM method) required that each of the samples taken were to be pre-enriched individually. This is what Price did, this is what everyone before and after him did, and this is what Jarvis referenced in 1989, and 2007 (page 596, right column, bottom, section entitled 'iii. My preferred approach ('wet' compositing)').

10b4. "Thus, in my opinion, if the Price procedure were to be used to assay lots of solid or semi-solid food products (e.g., beef trim or ground beef), a person of ordinary skill would have recognized that multiple portions of the test lot would be sampled and composited to obtain a representative sample."

RESPONSE BY INVENTOR:

Dr. Bailey's assertion is false and goes directly against the teachings of Price. Not only did Price take multiple samples and pre-enriched them individually (page 679, materials and methods), but also on page 681 he teaches against compositing the plurality of samples (second full paragraph), and asserts Silliker (1969) on the same subject.

10b5. "As discussed below, the pre-enrichment broths described by Price are individually enriched by incubation, resulting in pre-enrichment broth cultures. See pp. 679-682, and FIG. 1. At the enrichment stage, equal portions of each pre-enrichment broth culture are pooled (i.e., composited) into a single selenite enrichment broth, thereby forming a first composite of the pre-enrichment broth cultures, and composited into a single tetrathionate enrichment broth, thereby forming a second composite of the pre-enrichment broth cultures. See FIG. 1 and p. 679. Following the enrichment stage, each enrichment broth is streaked onto plates and then identified colonies are biochemically tested. See FIG. 1 and p. 681. When the test is positive, the saved portions of each pre-enrichment culture are individually tested to determine which culture or cultures contributed *Salmonella* to the composite(s). See abstract, FIG. 1, and pp. 679 and 681. The individual tests could be done using the same type of test that is used to test the enrichment broth. A person of ordinary skill in August 2004 also would have understood that negative test results for the composited enrichment cultures described by Price would validate each of the samples making up the composite as negative for the target microorganism."

RESPONSE BY INVENTOR:

Here again Dr. Bailey is making an unwarranted extrapolation. He starts by describing Price as taking a plurality of samples from a single lot, and then he makes the unwarranted extrapolation of saying that anyone reading this would have taken plurality of samples from a plurality of lots and composited the samples from each lot, and then he returns to Price and continues on. A person with skills in the art would not read Price and expand it against his teachings. Nothing in Price suggests that testing methods involving multiple lots are being addressed.

As a matter of fact a scientist with proper training reads a research paper, assimilates the "materials & methods" section which is the enabling portion of each paper, for the purpose of gaining the ability to repeat the work. Anyone reading Price will attempt to understand his teachings, will understand the limitations of his work, and will not try to selectively choose portions and misrepresent other portions to fit one's preconceived objective as Dr. Baily is so aptly doing.

10c. "Price states that *"Salmonella* analyses were carried out as described in the Bacterial Analytical Manual (BAM) except for the pooling of pre-enrichment broths in the following paragraph." Thus, in Price, the samples that are included in the pre-enrichment broths are first prepared according to BAM (see paragraph 11 below for a discussion of BAM).

10d. "One of ordinary skill in the art in August 2004 and 2005 would have understood Price to require incubation of the pre-enrichment broths for at least the following three reasons. First, on page 682, Price refers to "the incubation time of the pre-enrichment cultures," thereby indicating that the pre-enrichment cultures have been incubated. Second, Price refers to "pooled pre-enrichment broth cultures." See title, abstract, and pp. 680-682. The use of the term "cultures" implies that the pre-enrichment broths were incubated. This is consistent with how the term "culture" was used in the art as evidenced by Jarvis, discussed above. Third, in August 2004, it was common to use two separate enrichment steps: (1) a "pre-enrichment" enrichment step in which a pre-enrichment broth is incubated to cause organism growth resulting in a pre-enrichment broth "culture," and (2) a second enrichment step that causes further organism growth.

10e1. "In August 2004, it was well known that the incubation of the pre-enrichment broth cultures disclosed in Price would cause the microorganism to reach detectable levels and become substantially uniform within each pre-enrichment broth culture at least because a portion of each pre-enrichment broth culture will be further diluted at the selective enrichment stage."

<u>RESPONSE BY INVENTOR</u>:

Dr. Bailey's statements are not true. In 2004 and today the status of levels of *Salmonella* at the pre-enrichment stage have not changed. It is still at a point where when 1 ml is taken to inoculate enrichment there should be a 99% chance that there is at least one viable organism in the one ml transfer portion (the dividability stage). Today, as I write this, the BAM method still relies on pre-enrichment followed by selective enrichment. <u>The logical question that needs to be asked is "If at the end of pre-enrichment *Salmonella* levels are</u>

detectable, why did Silliker, Gabis, Price, Jarvis, BAM, the compendium, etc. require a second enrichment step?" The answer is simple, at the end of pre-enrichment there simply are not enough *Salmonella* cells to be detected by any method including PCR.

It is also my opinion that Examiner's assertion that PCR can detect one cell (per Entis) is wrong. Entis merely says that "in theory" PCR can detect a single copy of a gene in a PCR reaction, but she drastically qualifies her statement by noting that PCR involves only microliter scale portions, so that much larger concentrations are practically required to get a single cell into a PCR tube. There are also the issues of inhibitors and interfering molecules, and the Poison distribution of the cells in solutions, that put the minimum detectable limits by PCR at about 10,000 CFU/ml.

10e2. "That is, because as little as 1 ml of each pre-enrichment broth culture would be transferred to each of the enrichment broths (see p. 182), each 1 m1 of each contaminated pre-enrichment broth culture would have to have a detectable amount of *Salmonella.*"

RESPONSE BY INVENTOR:

The term "detectable" is a term of art, it implies a level of a target organism in an enrichment culture that gives a positive signal for the said organism using a test method. Dr. Bailey is misconstruing the terms of art. Jarvis 1989 is seeking dividability, not detectability, per page 141 first paragraph:

> ***Once sufficient growth has occurred in pre-enrichment for at least 3 viable salmonellae/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, with m=3: 1'(x=O) = .05….."***

10e3. "For every 1 m1 of each contaminated pre-enrichment broth to have a detectable amount of *Salmonella,* each pre-enrichment broth culture would have had to have been incubated long enough to allow any *Salmonella* therein to reach detectable levels and become substantially uniform. Otherwise, if the incubation period was not sufficient to allow *Salmonella* to reach detectable levels and become substantially uniform, it is possible that a 1 m1 portion of a contaminated pre-enrichment broth culture might not contain a viable *Salmonella* organism."

RESPONSE BY INVENTOR:

This is another circular argument confusing the 1 ml of the pre-enrichment broth having at

least one viable cell (dividability) with detectability. This is purely for the purpose of confusing the Examiner. No person with ordinary skills in the art would reasonably accept this argument.

10e4. "Furthermore, Price discloses that the saved portions of each pre-enrichment culture are individually tested to determine which culture or cultures contributed *Salmonella* to the composite(s). For the saved portions to be individually tested, any *Salmonella* therein must reach detectable levels and become substantially uniform."

RESPONSE BY INVENTOR:

Dr. Bailey conceded that: "10c. Price states that *"Salmonella* analyses were carried out as described in the Bacterial Analytical Manual (BAM) except for the pooling of pre-enrichment broths in the following paragraph."

Thus, in Price, the samples that are included in the pre-enrichment broths are first prepared according to BAM (see paragraph 11 below for a discussion of BAM). Bailey leaves out the fact that, per BAM, if anyone was going to test the saved pre-enrichments they would have had to transfer 1 ml to each of the two selective enrichment broths, followed by incubation, plating, etc. Again Dr. Bailey misleads the Examiner by confusing the fact that the pre-enrichment would have needed to contain only one viable target cell, whereas the patent requires detectable levels.

10f. "In August 2004 and 2005, it was well known in the art that enrichment broths (which could be selective enrichment broths) would be incubated to foster organism growth and that plates would also be incubated to foster organism growth so that colonies of the microorganism could be identified for testing.

10g. "In Price, two different enrichment broths (selenite and tetrathionate) are used (1) because one may be more effective than the other at fostering *Salmonella* growth in a particular sample, (2) because one may be more effective at inhibiting competing organism growth in a particular sample, and/or (3) for confirming the test results. Because the different enrichment broths are more/less effective at fostering *Salmonella* growth and inhibiting competing organism growth, when inoculated with the same material, the two broths will have different concentrations of *Salmonella* following incubation."

RESPONSE BY INVENTOR:

Here Dr. Bailey admits that two separate enrichment broths are needed, and are to be inoculated with the pre-enrichment broth. His problem lies in the fact that if the levels of *Salmonella* in the pre-enrichment broth were detectable why did Price, and the rest of the references (Silliker, Gabis, Jarvis, BAM, The Compendium) continue using the selective enrichment, a practice that has continued up to the present day? Dr. Bailey's third assertion, (3), is false. It is very simple. If you inoculate two enrichments, incubate for prescribed time, and then plate the purpose is for detection, not confirmation.

10h. "Price discloses that the pooling of pre-enrichment broth cultures "offers a simple, yet effective way to increase sample capacity in the *Salmonella* testing of foods," and that a portion of each pre-enrichment culture can be retained for later reference so that, if the test of the pooled pre-enrichment broths is positive, individual tests can be performed on the retained pre-enrichment cultures to determine which culture or cultures contributed *Salmonella* to the pool. See abstract, FIG. 1, and pp. 679 and 681."

RESPONSE BY INVENTOR:

Price claims that pooling of pre-enrichments of a plurality of samples from a single lot for testing for *Salmonella* in types of foods that have incidents of contamination, would result in reducing the number of enrichment cultures and detection plates. The results presented by Price in his paper *teach away* from using pooling in that a person with ordinary skills in the art would immediately recognize that false negative rates for pooling pre-enrichments of up to 28% (Table 2 pooled pre-enrichments for egg albumin) and 50% for pooling samples before pre-enrichment, are not acceptable for validating test lots for release into commerce by any regulatory agency or accreditation body. As an example the USDA FSIS Microbiological Laboratory Guide (MLG) 5.03 (in effect at the time the patent was submitted) specifies an upper acceptable limit of false negatives as less than 2%). Anyone with skills in the art recognizes, and Jarvis (1989, 2007) confirms the fact that the retesting of the pre-enrichment is to establish the extent of contamination, unlike patent'584 where the goal is to statistically enable release the negative lots according to a statistical-based sampling plan.

10i. "Thus, Price at least teaches that a plurality of pre--enrichment broth cultures (each corresponding to a sample) which have been incubated under conditions suitable to allow any target microorganism to reach detectible levels and become substantially uniform, are composited prior to testing and a portion of each pre-enrichment broth culture is saved and is individually tested, if the test of the composite is positive."

RESPONSE BY INVENTOR:

Dr. Bailey misrepresents Price. Price does not teach that pre-enrichment allows for *Salmonella* to reach detectable levels, for he would have plated the pre-enrichments and bypassed the selective enrichments.

11. "Food Sampling and Preparation of Sample Homogenate." BACTERIAL ANALYTICAL MANUAL (U.S.D.A. 2003), http://www.fda.gov/Food/ScienceResearch/http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalytucalManualBAM/ucm063335.htm (hereinafter "BAM") was a well-known FDA publication that describes a method of preparing samples (and particular food homogenates) for microorganism testing."

RESPONSE BY INVENTOR:

This has no bearing on the issues at hand.

12. "In August 2004, in view of Price's disclosure that *"Salmonella* analyses were carried out as described in the Bacterial Analytical Manual (BAM)," the skilled artisan would have looked to the most recent version of BAM available at that time, which was the 2003 version of BAM."

RESPONSE BY INVENTOR:

This has no bearing on the issues at hand.

13. "In August 2004 and 2005, serological testing would have been considered an "immunochemistry-based" testing method. See J. Coombs, DICTIONARY OF BIOTECHNOLOGY 310 (2nd ed. 1992)."

RESPONSE BY INVENTOR:

This has no bearing on the issues at hand.

14. "In August 2004 and 2005, it was well known that a "selective enrichment" broth fostered the growth of a particular target organism (e.g., *Salmonella)* while inhibiting the growth of competing organisms."

RESPONSE BY INVENTOR:

This has no bearing on the issues at hand.

15. "In August 2004 and 2005, it was well known that "selective plating" fostered the growth of a particular target organism (e.g., *Salmonella)* while inhibiting the growth of competing organisms."

RESPONSE BY INVENTOR:
This has no bearing on the issues at hand.

16. "By August 2004, the Compendium was a standard reference textbook for food microbiologists. Chapter 2 of the Compendium describes general concepts and procedures for sampling food products for microbiological testing. I am familiar with '584 patent claims 1, 2 and 17 (and claims dependent on them) which specify that the claimed method be applied to a plurality of "test lots" or "single-unit production lots." I also am aware that the term "test lot" is defined in the '584 patent as

> 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., proximitytime, 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, col. 5, lines 21-32. I am also aware that the terms "single production unit" or "single-unit production lot" are described in the '584 patent as a form of test lot, and "refers to a single unit of production (e.g., for beef trim a 'combo' or for almonds a 'bin') rather than as in the prior art, to a composite comprised of multiple, non-operationally linked single production units (production sublots)." '584 patent, co1. 5, lines 33-38. By August 2004, it was common practice for microbiologists to sample operationally linked production lots for microbiological testing. The statement in the above quotation from the '584 patent implying that the prior art employed a "composite comprised of multiple, non-operationally linked single production units (production sublots)" is an overstatement. Regardless of whether some processors combined production lots before samp1ing for microbiological testing, it is also clear that the use of single production units or lots for sampling was also commonplace. This is evidenced by Chapter 2 of the Compendium. In that chapter the authors discuss the importance of developing a "sampling plan" and of

obtaining 'representative samples" for microbiological testing. In Section 2.511, "Sampling Plan" is defined as "[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." "Lot" is defined as,

> [t]he 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.

"Thus, Chapter 2 of the Compendium makes clear that, prior to August 2004, microbiologists utilized sampling plans that involved sampling a "number of units to be collected from each lot" and that a "lot" was understood to be a production batch consisting of operationally linked units. Accordingly, a person of ordinary skill in the art was aware that a "test lot" or "single-unit production lot" consisted of an assemblage of units or specimens operationally linked by "type, grade, class, size and composition" and "manufactured under the same conditions and at essentially the same time." Based on the teachings of Compendium Chapter 2 and my general experience, it is my opinion that the sampling of "test lots" or "single unit production lots" by taking a plurality of portions and combining (compositing) them, as specified in steps a) and b) of claims 1, 2 and 17 (and claims dependent upon them) was known and obvious to persons of ordinary skill in the art at the time.

RESPONSE BY INVENTOR:

Dr. Bailey equates the taking a plurality of samples from one lot to taking a plurality of samples from a plurality of lots. This is an unwarranted extrapolation that strikes at the heart of the difference between patent '584 and the prior art. To make this extrapolation is simply an effort to cause confusion and mask the difference between patent '584 and the prior art.

17. "I am familiar with the USDA FSIS Microbiology Guidebook Protocol for the sampling and analysis of raw ground beef for *E. coli* contamination, as reproduced as Section 35.243 of the Compendium ("the FSIS Protocol"). In my opinion, by August 2004 a person of ordinary skill in the art would have understood that the method described in the FSIS Protocol would be applied to a "test lot" or "single unit production lot" of ground beef, as those terms are defined in the '584 patent."

RESPONSE BY INVENTOR:

This observation by Dr. Bailey continues the effort to cause confusion and mask the difference between patent '584 and the prior art noted in the prior answer to issue 16.

18. "The FSIS Protocol refers to blending composite samples of ground beef with an enrichment medium defined as "mEC with novobiocin." In August 2004, a person of ordinary skill in the art would have understood that m.EC broth was a modified form of EC broth, a well-known culture medium for the detection of *E. coli* and that "mEC broth with novobiocin" was a selective enrichment medium for *E. coli."*

RESPONSE BY INVENTOR:

Dr. Bailey does correctly represent the FSIS testing protocol as stated in MLG 5.03, with effective implementation date of 10/25/02. The FSIS test method for the samples taken from a lot of food is exactly the reverse of the pooling/compositing process. FSIS teaches to take a plurality of samples from a single lot, and composite them into one composite sample. However, at the time of analysis, per MLG 5.03, Revision 03, Section 5.5, five 65 gram sub-samples are taken out of each bag, and each is individually enriched and tested. This is without any pooling. In other words, FSIS teaches de-compositing and teaches away from pooling.

19. "The FSIS Protocol describes a procedure for analyzing a batch or test lot of raw ground lots on a regular basis. The FSIS Protocol describes sampling, enriching and analyzing each lot of product being tested. In other words, there is no suggestion in the FSIS Protocol would be applied to multiple separate production lots or test lots of raw ground beef to produce a set of separate test lot samples, particularly in large operations producing multiple production lots on a regular basis. The FSIS Protocol describes separately sampling, enriching and analyzing each lot of produc.t being tested. [n other words, there is no suggestion in the FSIS Protocol that non-operationally linked production lots should be combined before sampling."

RESPONSE BY INVENTOR:

This is a misrepresentation of the FSIS Directive. The directive is aimed at FSIS inspectors, and prescribes a specific manner of collecting one set of sample from a day's production. No one with ordinary skill in the art would extend the protocol. This is a specified regulatory sampling for FSIS. The sample concept of taking multiple samples from a single lot is in Price, Jarvis, Gabis, BAM and Compendium. Thus, the FSIS reference merely overlaps the other references, rather than showing the way toward any modification or extension or combination. References which completely overlap one

another cannot be combined to teach something additional.

20. "I am familiar with two articles published J.H. Silliker and D.A. Gabis in the early 1970s: Silliker, J.H. et al., 1973. ICMSF Method . I. Comparison of Analytical Schemes for Detection of *Salmonella* Dried Foods. *Can. J. Microbiol., 19*:475 and Gabis, D.A. et al, 1974. ICMF Method Studies. II. Comparison of Analytical Schemes for Detection of *Salmonella* High-Moisture Foods. *Can.J. Microbiol., 20:663-669.* Silliker et al. and Gabis et al. describe methods for analyzing dried food products and high-moisture food products (such as meat, poultry and eggs) respectively for contamination with *Salmonella.* of these publications described taking multiple samples from the lot of food product being tested, those samples in pre-enrichment media, incubating those media, transferring aliquots to a selective enrichment media and testing for the presence of *Salmonella.* my , each of these publications described the sample-compositing and incubation steps a), b) c) of the claims of the '.584 patent. As discussed above in connection with Jarvis and Price, pre-enrichment procedures used by Silliker et al. and Gabis et al. were effective in growing target microorganism to detectable levels. In each instance, 1 ml aliquots of the pre-enrichment were transferred to selective enrichment broths for further enrichment and by conventional plating techniques. It is thus apparent that each 1 ml aliquot had to a detectable amount of the microorganism in positive samples. A person of ordinary skill by August 2004 would have understood that the Silliker et al. and Gabis et al. procedures would be applied to "test lots" or "single-unit production lots" of food products and that such "test lots" or "single-unit production lots" would consist of combinations or assemblages of separate pieces or specimens of the food product. A person or ordinary skill also would have understood that the Silliker et al. and Gabis et al. procedures would be applied to a plurality of separate "test lots" or "single-unit production lots," especially in large facilities producing a number of production lots on a regular basis."

RESPONSE BY INVENTOR:

Here, Dr. Bailey revives the argument that the presence of at least one viable cell in the 1 ml of the pre-enrichment media, as detectable level. Per my earlier comments I believe that this is a misrepresentation of the art and science. He also fails to mention that the Silliker 1969 reference used by Price in his article, which was reaffirmed by Price, specifically prohibits and teaches against dry compositing samples into a pre-enrichment. Price reports up to 50% false negatives and reaffirms Silliker on this issue. Furthermore, there is nothing that Dr. Bailey has presented which shows that the Price and Jarvis's has ever been extended to any other method in microbiology, or to plurality of samples taken from a plurality of lots in the manner of my patent claims 1 and 17.

21. "Silliker et al. and Gabis et al. describe testing for *Salmonella* using conventional plating technology. By August 2004 so-called "Rapid" methods for the detection of microorganisms had been developed and were being widely used. These Rapid methods were based on immunochemical or nucleic acid-based technology (such as PCR and nucleic acid probes). These methods were faster and often more sensitive than the conventional microbiological techniques utilized in the preceding decades. By August 2004, it would have been obvious to a skilled person to utilize these Rapid methods in combination with the sample compositing and wet-compositing procedures described, for example, by Jarvis, Price, Silliker et al. and Gabis et al. A person of ordinary skill in the art would have understood that pre-enrichment and enrichment procedures described in those publications could be streamlined because of the greater sensitivity of the Rapid methods. A number of Rapid methods are described in Chapter 10 of the Compendium."

RESPONSE BY INVENTOR:

This is irrelevant, none of these arguments pertain to the claim language.

22. I have read U.S. Patent No. 7,534,584 (584 Patent). The term "production assemblage" is not defined in the specification of the 584 Patent. There is no special industry definition of this term that would have applied in August 2004 and 2005. Thus, to construe the term production assemblage, the skilled artisan would have employed the common definition of "assemblage," which is "a collection of things." AMERICAN HERITAGE COLLEGE DICTIONARY 82 (3rd ed. 1998) *(Exhibit B)*. This common definition is consistent with the use of the term "production assemblage" in the specification."

* * *

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.



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31 January 2011

statistical aspects of the microbiological analysis of foods

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CHAPTER 8

ERRORS ASSOCIATED WITH DILUTION COUNT AND PRESENCE/ABSENCE METHODS

In its simplest form, the dilution count method consists of serial dilution of the initial food homogenate (or culture) followed by inoculation of an appropriate volume of each dilution into one or more tubes of culture medium (agar or broth). Following incubation, the number of tubes showing evidence of growth (e.g. colonial growth in agar; turbidity in broth; production of acid and gas in MacConkey's Bile Salt broth in the test for presumptive coliforms, etc.) are recorded. The cultures showing no evidence of growth are assumed not to have received a viable inoculum ($p = 1$), i.e. to have received < 1 viable cell or cell aggregate; an assumption which may not always be correct. Similarly, any culture showing growth is assumed to have received at least one viable propagule ($p = 1$). If each viable propagule is assumed to be capable of producing a growth response, then the proportion ($P_{(x=0)}$) of cultures at any one dilution which receive no viable inoculum after inoculation with a mean inoculum level of m propagules is given by the first value of the Poisson expansion, i.e.:

$$P_{(x=0)} = e^{-m} \quad \text{(Table 3.2)}$$

Similarly, the proportion becoming turbid:

$$P_{(x>1)} = 1 - P_{(x=0)} = 1 - e^{-m}$$

8.1 Tube dilution series and Most Probable Number counts

When more than one culture is inoculated at each dilution level, and several dilution levels are inoculated, one would expect a graded response with all tubes positive at the lowest dilution and none, or only a few tubes, positive at the highest dilution tested. This is illustrated in Table 8.1 for dilutions from 10^{-3} to 10^{-8} of culture initially containing 2×10^5 viable cells/ml, and 10, 5 or 1 replicate tubes at each dilution level.

Figure 8.1 illustrates the shape of the expected dose-response curve, based on Poisson distribution. It has been pointed out by several workers (e.g. Eisenhardt and Wilson, 1943) that the response curve may be less steep. This could arise through systematic technical error (Meynell and Meynell, 1965) and as a result of contagious distribution, reflecting clumping, sublethal damage, etc.

LACTOSE BROTH
INDUSTRIAL APPLICATIONS

PRODUCT DESCRIPTIONS:
Bottled Media: B8445 (225 mL), B8457 (90 mL, wide mouth), B8458 (100 mL, wide mouth), B8460 (500 mL, Boston Round)

PURPOSE:
Lactose Broth is used for the detection of lactose-fermenting, Gram-negative coliforms, as a pre-enrichment broth for *Salmonella* species, and in the study of lactose fermentation of bacteria in general. It is used extensively in testing water, foods, dairy and pharmaceutical products.

Sublethally injured salmonellae may be present in low numbers in dried or processed foods. The recovery of *Salmonella* may be hindered by the presence of other bacteria as well as components of the food sample. Lactose Broth, a nonselective medium, supplies a pre-enriched environment, which allows for repair of cell damage while diluting toxic or inhibitory substances, and providing nutritional advantage to *Salmonella* over other bacteria.

PRINCIPLE:
Beef Extract and Peptone provide carbon and nitrogen sources for general growth requirements, while lactose provides a carbohydrate source.

This pre-enrichment medium is used to provide a higher ratio of *Salmonella* to non-*Salmonella* bacteria after incubation. Lactose is fermented by most most non-*Salmonella* bacteria while *Salmonella* does not. A bactiostatic effect on competing microorganisms is created by the decrease of pH in the medium produced by the lactose fermenting bacteria. The *Salmonella* will have little growth during the initial 24 hours of incubation, while the non-*Salmonella* will be utlizing the lactose. As the products of fermentation build, the non-*Salmonella* are inhibited and the *Salmonella* begins its growth phase (24-48 hrs.)

FORMULAS:

Approximate, per liter of deionized filtered water.

(1) Lactose Broth

Enzymatic Digest of Gelatin	5.0 g
Beef Extract	3.0 g
Lactose	5.0 g

Final pH: 6.9 ± 0.2 at 25° C

PRECAUTIONS:*
For laboratory use only. Observe approved biohazard precautions.

Storage: Upon receipt store at 2-25°C. Media should not be used if there are signs of contamination, deterioration (discoloration), or if the expiration date has passed. Media can be inoculated up to the expiration date and incubated for the appropriate incubation period.

Limitations:
Due to nutritional variation, some strains may grow poorly or fail to grow in this medium

PROCEDURE:*

Method of Use:
Lactose Broth is used for the pre-enrichment phase for the testing of food and other materials, to enhance the recovery of *Salmonella.* Consult the appropriate references for specific procedures for each type of material being tested.[1-4,6]

1. Transfer a 25 g or 25 ml sample of test material into a container. Add 225 ml of sterile Lactose Broth. Mix as necessary to make
a homogeneous suspension. Incubate at 35°C for 24 hours.

2. Transfer 1 ml of suspension to appropriate enrichment broths such as Tetrathionate Broth and Selenite Cystine Broth. Incubate at 35°C for 24 hours.
3. Transfer a loopful of suspension to appropriate selective agar media, such as Hektown Enteric Agar, XLD Agar and Bismuth Sulfite Agar. Incubate at 35°C for 24 hours

Material Required but Not Provided: Standard microbiological supplies and equipment such as loops, needles, incubator, and incinerator are not provided.

QUALITY CONTROL:

Microorganism	ATCC® #	Response
Enterococcus faecalis	29212	Good Growth
Escherichia coli	18739	Good Growth
Pseudomonas aeruginosa	9027	Good Growth

User Quality Control: Check for signs of contamination and deterioration.

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*For more detailed information, consult appropriate references.

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New Technology for Microbiological Testing of Foods

Stan Bailey, PhD

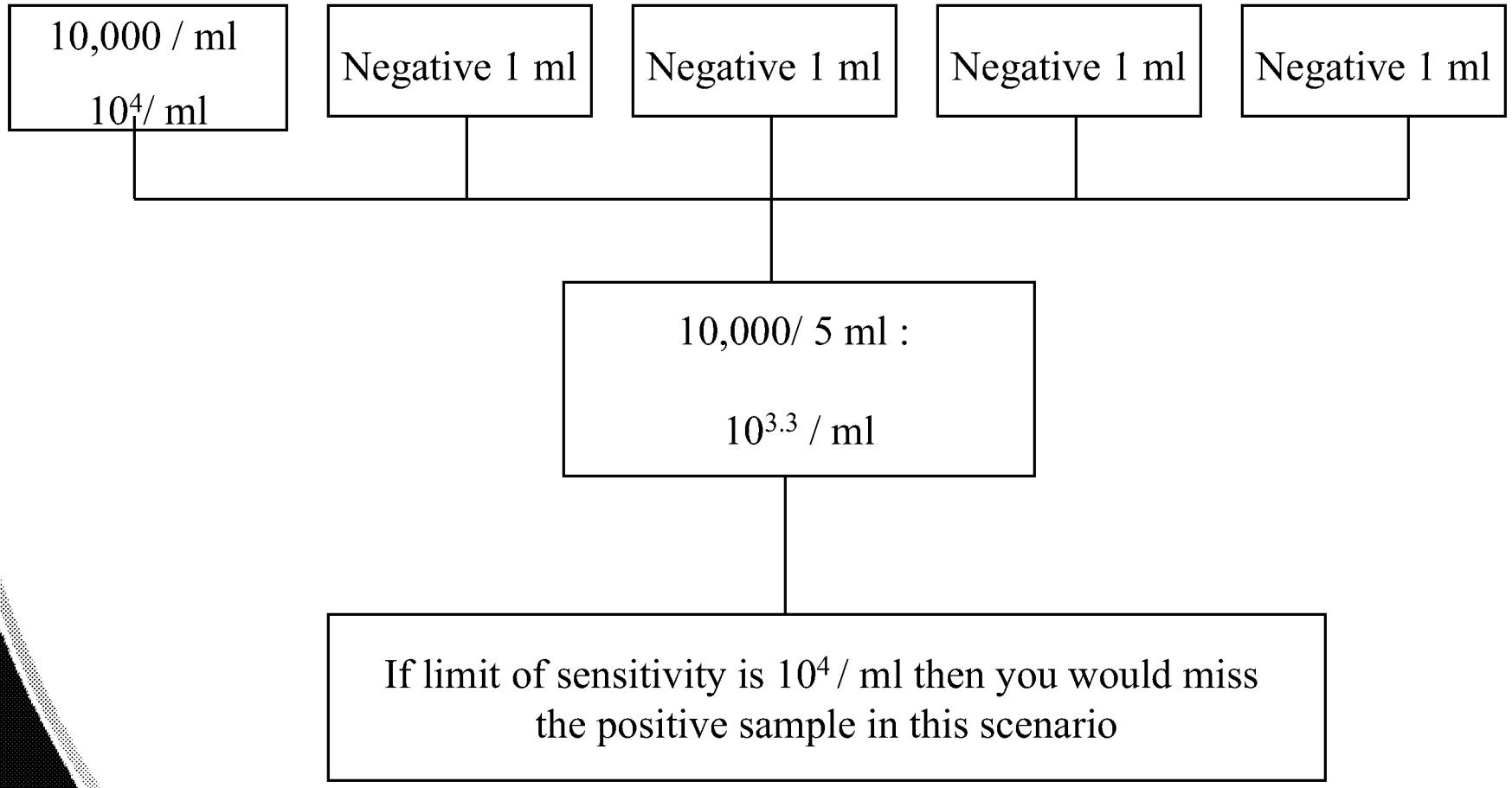
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National Beef Ex. 1007
Page 0789

Pooled sample protocol



National Beef Ex. 1007
Page 0790

statistical aspects of the microbiological analysis of foods

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Technical Director, HP Bulmer Holdings plc, Hereford HR4 0LE, United Kingdom

progress in industrial microbiology

volume 21



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Assume:

(1) initial mean contamination level = μ salmonellae/g sample;
(2) pre-enrichment for 12 h (n Ex. 8.6), yielding 410 μ salmonellae/ml;
(3) 1 ml pre-enrichment medium inoculated into 10 ml selective enrichment medium;
(4) lag time (L) in enrichment medium = 4 h; doubling time = 60 min = 1 h;
(5) enrichment period 18 or 24 h and
(6) 0.02 ml (1 loopful) inoculated onto diagnostic medium.

Then, from Ex. 8.6, after 12 h pre-enrichment the medium would contain 410 μ salmonellae/ml. Since pre-enrichment is diluted 1 in 10 into selective enrichment medium the initial level of salmonellae would be $410\ \mu/10 = 41\ \mu/ml = \mu_0$.

Then after 18 h, assuming synchronous growth and other factors as above:

$$\mu = 41\mu_0\ 2^{(18-4)/1} = 41\mu_0\ 2^{14} = 6.72 \times 10^5 \text{ salmonellae/ml}$$

Similarly, after 24 h:

$$\mu = (41\mu_0)\ 2^{(24-4)/1} = 41\mu_0\ 2^{20} = 4.30 \times 10^7 \text{ salmonellae/ml}$$

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Now if initial contamination level (μ_0) = 0.04 (i.e. 1 salmonella/25 g sample), a 0.02-ml loop would be expected to transfer 536 or 34,400 salmonellae after 18 and 24 h incubation, respectively. Hence the probability of transferring at least one viable salmonellae to the isolation medium would be ≥ 0.99.

However, had the initial mean inoculum level after 8 h pre-enrichment, with recovery but no growth, been only 0.01μ_0 (Ex. 8.6, 8 h incubation), then for $\mu = 0.04$/ml the expected numbers transferred with a 0.02 ml loop after 18 h and 24 h enrichment culture would be about 0.01 and 0.86 salmonellae, respectively. Hence, the probability of transferring at least one viable salmonella would be = 0.01 and = 0.58, respectively.

It is essential, therefore, to ensure that an adequate inoculum is transferred from the pre-enrichment culture; otherwise the probability of detecting salmonellae will be low. These effects are illustrated diagrammatically in Fig. 8.4.

Example 8.8. Probability for selection of a specific colony from a diagnostic plate culture

What is the probability for picking at least one *E. coli* colony from a diagnostic plate containing 20 typical coliform colonies if numbers of non-*E. coli* colonies are (a) 10 and (b) 200?

Since the population is finite and (relatively) small, removal of any one colony will reduce the residual number from which to select another colony. Hence, the hypergeometric distribution is the most appropriate. The probability of selecting no *E. coli* colonies is described by the equation:

$$P_{(x=0)} = [s!\ (g-s)!]/[n!\ (g-s-n)!\ g!]$$

where:

g = number of typical coliform colonies
s = number of colonies picked for identification (= integer of $\sqrt{g}$)

and,

n = number of non-*E. coli* colonies on the plate.

Since:

$$P_{(x\geq 1)} = 1 - P_{(x=0)}$$

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 JAMES BYRON UNDER 37 C.F.R. § 1.132
(IN SUPPORT OF RESPONSE UNDER 37 C.F.R. § 1.111)

Madam:

I, Mr. James Byron, being duly sworn, say:

1. I am familiar with 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. This declaration is to corroborate the declaration of M. Samdapour concerning skepticism amongst clients and others in the art for the validity, accuracy and safety of Dr. Samadpour's method. As such, according to 37 C.F.R. § 1.116(e), this declaration is necessary to respond properly to the action closing prosecution.

2. 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.

3. As an executive in the food industry I had been a competitor of IEH from November 2005 until I joined IEH in February 2010. From 2005 until June 2009, I was Executive Director of Sales for SDI -- a biotech company and manufacturer of food safety test kits with annual food safety test kits sales of $5 million. From June 2009 until January 2010, I was Vice-President of Sales & Marketing for Food Safety-Net Services of San Antonio Texas. I currently serve on the American Meat Institute Scientific Advisory Committee and I am involved in various food industry trade associations. At IEH my role is Vice-President of Technology Transfer & International Business. Prior to my involvement in the food industry, my career advanced through positions of increasing responsibility serving 25 years working in the industrial chemicals, electric utilities, and Nuclear power industries. Previous employers include Nalco Company of Naperville IL and The President & Fellows of Harvard University, Cambridge Massachusetts. My detailed curriculum vitae is attached hereto as APPENDIX A.

4. While in my Executive Director of Sales at SDI one of my responsibilities was to be aware of all emerging technology associated with food testing, especially new technology being developed either by food testing laboratories or by competitor test kit manufacturers. In December 2007, during my tenure with SDI, I received a phone call from Dr. Samadpour, IEH to inform me of his patent application related to his inventive testing method, wet modular

composite pooling, and to offer to make the technology available to SDI under a commercial agreement. At the time I took the call and related the information to a Marketing Manager responsible for SDI Food Safety business, Dr. Orla Cloak, PhD Microbiology, University of Ulster, BSc(hons) Major Microbiology, Minor Chemistry University College Dublin, for further review. At the time, the 'idea' of wet modular composite pooling was not an obvious testing strategy, in fact it was scoffed by Dr. Cloak when presented by IEH as she believed that detection limits would be compromised because of the small amount of enriched sample from a 'positive' sample when combined with 'negative' samples would lead to false negative results. Because of the lack of understanding related to the potential benefits to be gained from the technology the opportunity was ignored. Training of new food safety kits sales people at SDI included discussion of the very small amount of enriched sample used in PCR analysis and it was stated that the small aliquot of sample made it possible to 'miss' or 'not transfer' E. coli cells to thermocycle step and hence a false negative would be possible. This training was provided by Dr. James Stave, Chief Science Officer, VP Research & Development, Ph.D. in Microbiology from the University of Maryland and his B.S. in Biology from Michigan Technological University. It was considered by SDI scientists that the perceived challenges of PCR technology would be further challenged by dilution associated with wet modular composite pooling.

5. Since 2007, the industry has learned that testing using Dr. Samadpour's Wet modular composite pooling technique does not compromise or reduce the accuracy of testing. Wet modular composite pooling *increases* the number of lots that are tested, by reducing (by a factor of up to 5) the 'lot size' of product tested and thus incurs an increase in the associated testing cost. The outcome is that while the test lots are smaller (1/5), and more lots are tested (5x), increased cost of testing is insignificant relative to the overall savings from having to destroy or divert less total product. This breakthrough has significant economic impact for the food production industry, both in the value of product preservation (increase number of lots, smaller lot sizes, reduces product loss from positive test results), and in the additional benefit of allowing up to 5 times (500%) more testing of product while only increasing the total cost of testing by as little as ~15%.

6. The following is a summary of my accounting of the industry response to Dr. Samadpour's discovery, IEH's development of validation data related to the discovery, and the response from the food industry, food testing laboratories, and food test kit manufacturers.

7. The IEH discovered advantages from wet modular composting pooling methods

were initially met with strong skepticism and open ridicule from the food testing market, including but not limited to trade associations, food manufacturers, food testing laboratories, and test kit manufacturers. The idea of making one single composite from several enrichments was a complete departure from industry practice and not a single test kit or food testing laboratory was using this approach. I experienced the skepticism and obvious disapproval of Dr. Samadpour's inventive technique first hand due to my position at both a leading test kit manufacturing company and at a leading food testing laboratory.

8. In fact, prior to IEH developing this wet modular composite pooling technology, the idea of such wet pooling of such enriched samples (each corresponding to a separate underlying test lot) was not used within the food testing industry. As recently as January 2008, the prevailing industry behavior was to challenge and cast doubt upon the science behind the IEH methods, a behavior that is still perpetuated to this day among some personalities within the industry. Eventual recognition of the scientific validity of Wet Pooling was achieved only after years of effort by IEH wherein they were able to demonstrate the superiority of wet modular composite pooling, satisfying both industry leaders and government regulators that the method had acceptable sensitivity, specificity, and limits of detection as compared with conventional methods.

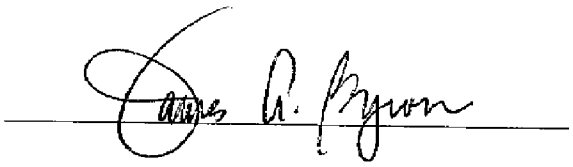
9. Food manufacturers that have adopted the use of Wet Modular Pooling as provided within US Patent 7534584 have benefitted from substantial economy improvement in overall costs (less product being destroyed/diverted) in addition to efficiency gains in their production operation and enhanced food safety program performance (statistically more robust testing).

10. It has long been my personal view, as both a former-executive for a Test Kit manufacturing company and as a former-executive in a Food Safety Testing Laboratory, that reaching a commercial agreement with IEH to gain access to this protected inventive technology is important for food safety in addition to being a wise business decision for a test kit or food testing laboratory company. Not surprising, and fully consistent with the novelty and efficacy of IEH's modular composite pooling methods, there are those in the industry that want to practice this method, but wish that it wasn't patented (e.g., the present Third Party Requestor in this re-examination). In fact, I am aware that in 2009, representatives from particular entities held a meeting in Colorado in an attempt to coordinate efforts to avoid legal complications associated with implementing modular wet composite pooling. While this is understandable, this merely illustrates not only the novelty and non-obviousness of the method, but also the importance and

promise of the method, and in no way detracts from the validity of U.S. Patent 7,534,584. This is precisely the type of invention that I understand the patent laws are designed to protect.

11. I am aware that in 2009 some participants in the industry adopted a practice of copying and using the technology encompassed within US Patent 7534584 without the benefit or protection of having a commercial agreement in place with the patent owner; and without the permission or consent of the patent owner. I also know, from public documents that Food Safety-Net Services of San Antonio in 2009 copied Dr. Samadpour's inventive technique by engaging and performing validation studies of Wet Pooling for the Vidas test kit manufactured by BioMerieux (the Third Party Requestor in this inter partes re-examination).

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.



James Byron

31 January 2011

APPENDIX A
CURRICULUM VITAE

JIM BYRON

405 Trolley Way, West Chester, PA 19382
jimbyron828@gmail.com 484.356.7283

Business professional effective in leading organizational re-direction strategic planning, including 'boots on the ground' leadership and coaching skills used to establish and lead a sales & marketing organization in a challenging, technical and growth driven environment. As a strong collaborator, this candidate possesses a creative talent for establishing measurable goals and metrics that inspire loyalty and high performing professionalism in teams. Consistent history of team leadership delivers results that exceed (or reverse negative) established revenue and profit trends while meeting business objectives for team development and business growth. Proven track record as an effective Contributor, Leader, Coach, Teacher, Manager and Professional Salesperson are indicators of future performance in your company.

Professional Experience

IEH Laboratories & Consulting, Seattle Washington – February 2010 to Present

Vice President of Technology Transfer & International Business - Consulting directly with the company owner, responsible for strategy formulation and business plan execution to support company growth goals exceeding 30% CAGR. Directly engaged within the market to facilitate plan execution, collaborating with IEH organization at the "C" level to establish performance metrics and facilitate alignment of company resources.

Food Safety Net Services, San Antonio Texas – July 2009 to February 2010

Vice President of Sales & Marketing - Food Safety Net Services is a leading provider of laboratory testing, consulting, auditing and specialized services to the food industry; operating a nationwide network of strategically located laboratories in six US cities. Exceptionally well networked and recognized as a leader within the industry, VP of Sales & Marketing is responsible for developing and driving impactful marketing strategies, including development of key client and strategic supplier-partner relationships. Responsible for leading company into new market segments and increasing compound annual growth rate to exceed 30%.

Strategic Diagnostics Inc, Newark Delaware – December 2005 to July 2009

Executive Director of Sales

- Global accountability for 4 diverse sales teams serving focus markets of Antibodies Life Science and Food Safety, in addition to Water Quality & Environmental, and Agricultural markets
- 100% retooling of company sales organization and sales management processes including hiring, metrics for performance evaluation, recognition programs, and direct coaching and development of selling skills

- Led & Managed reversal of long established low/no/negative sales trends; 2007 sales exceed $27 million
- Revenue Growth in focus markets 5.7% (2006), 27.7% (2007)
- Gross Profit Increased 2.99% (2006) and 18.84% (2007)

Nalco Company, Naperville Illinois – January 1990 to November 2005

2004-2005 **National Accounts Executive**

- Closed major deals (multi-year/multi-million dollar) including GSK, Exelon Energy, Alliant Energy
- Directed local sales/service resources to assure 100% post-sale customer satisfaction

2002-2004 **Services Development Manager, North America**

- Key Contributor to Strategic Planning associated with Global Corporate Reorganization
- "Change Leader" for Corporate Re-Organization – Led implementation of global sales/service restructuring
- Reorganization transitioned $440 million dollars annual US sales revenue to new business model
- Established 8 direct report managers in US geographic regions with >150 individual contributors
- Success became the model for transitioning worldwide organization impacting > $2.7 billion dollars

2001-2002 **District Sales Manager, St. Louis Missouri**

- 16 direct sales reports, $13.5 million dollar annual sales, 10 states
- 2001 Revenue Increase 8%, 2002 Revenue Increase 13%
- 2001 Gross Margin Improvement 4%, 2002 Gross Margin Improvement 9%

1997-2001 **Area Sales Manager, Chicago Illinois**

- Revitalized poorly performing area to increase sales from $3.6 to $5.8 million in 3 years (61% growth)
- Increased workforce retention, reduced representative turnover rate from 72% to <16%
- Business focus prioritizes personnel development, customer satisfaction, sales growth, margin improvement.
- Personnel responsibilities include hiring, promotion, terminations, performance appraisals, policy & salary administration, coaching & training sales personnel.

1990-1997 **Account Manager, Minneapolis Minnesota**

- Election to Nalco Chairman's Club Membership, Salesman of the Year 1995
- Percent Sales Increase Club 1992, 1993, 1994, 1995, 1997, 1998, 1999, 2000, 2001
- Total Dollar Sales Increase Club 1992, 1993, 1994, 1995, 1997, 1998, 1999, 2000, 2001
- New Accounts Club 1991, 1993, 1994, 1995, 1997, 1998, 1999, 2000, 2001
- Council of Sales Professionals 1995, 1998, 1999
- Focus industries include Nuclear & Fossil Power, Chemical Production, Food Production, Paper, Refining, and Aluminum & Steel.

President & Fellows of Harvard University, CMC, Boston Massachusetts – 1983 to 1990

1985-1990 **Engineering Coordinator**

- Developed, Implemented, & Managed systems for project management including schedule, weekly/monthly status/progress publication, supervision of trades people (electricians, mechanics, laborers), facilitated advancement of 'stuck' milestones to assure successful project completion
- Major projects included:
  - $ 15 million underground electric utility distribution installation

- $ 4 million steam boiler upgrade/retube & water polishing projects
- $ 2 million diesel generator protection project – lube oil management

1983-1985 Maintenance Coordinator

Associations & Professional Development Programs

- AMI American Meat Institute, Scientific Advisory Council
- Leadership & Coaching: Situational Leadership, Delivering Customer Advantage through Advanced Coaching, Becoming a Leader Manager
- Sales: Target Account Selling, SPIN Selling, Versatile Sales Person, Counselor Sales Person (Certified Trainer)
- Quality: Six Sigma Qualitrak Quality Training

Strategic Account Manager Association Annual Conference, SAMA

Compete Through Service Symposium, Arizona State University, WP Carey School of Business

Commonwealth of Massachusetts Licensed Boiler Operator, Fireman 2nd Class

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Education

Boston College, Chestnut Hill Massachusetts. B.A., Business Administration, 1989, Cum Laude, AΣN.

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. O. Box 1450
Alexandria VA 22313-1450

DECLARATION OF WARREN MIRTSCHING UNDER 37 C.F.R. § 1.132
(IN SUPPORT OF RESPONSE UNDER 37 C.F.R. § 1.111)

Madam:

I, Mr. Warren Mirtsching, being duly sworn, say:

1. I am familiar with 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. This declaration is to corroborate the declaration of Dr. Samdapour concerning showing a nexus between the inventive method and commercial success and additionally to corroborate skepticism amongst clients and others in the art for the validity, accuracy and safety of Dr. Samadpour's method. In addition, this declaration provides evidence of copying by competitors and others in the field. As such, according to 37 C.F.R. § 1.116(e), this declaration is necessary to respond properly to the action closing prosecution.

2. 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.

3. ~~My position~~I am the Chief Operating Officer in the Meat Division at Institutes of Environmental Health, which I have held since May 2008~~, relates to the Safety and Quality at the Greely Laboratory~~. I have a Bachelor of Science Degree in in Animal Science from Texas A&M University. My detailed curriculum vitae is attached hereto as APPENDIX A.

4. Previously, in 2004, I was employed by Swift and Company in charge of food safety. At this time Swift and Company decided to investigate whether the wet composting technique developed by Dr. Samadpour could be used for testing meat products produced by Swift.

5. Intially, I was concerned that Dr. Samadpour's approach would not give the accuracy of testing to reach a sensitivity to identify the pathogen, due to a "dilution effect". Gerome Lawler, then the Director of Food Safety at Swift, was also highly skeptical of Dr. Samadpour's wet compositing approach. He questioned whether it was sensitive enough to meet Swift's requirements.

6. To confirm that Dr. Samadpour's wet compositing approach would work, we spent a great deal of effort conducting side-by-side emparsions at Swift's Greely plant for about six months, from early to mid-year 2004. The tests were accomplished by comparing the standard old manner that we had been using with Dr. Samadpour's method. During the test period we ran about 100 tests per day by the standard old method, and about 400 tests of individual combos that went into about 100 composites as described in Dr. Samadpour's method.

7. Our extensive, detailed testing found that Dr. Samadpour's technique, in fact, worked. That is, it was sensitive enough to meet the requirements prescribed by Swift. In addition, Dr. Samadpour's technique allowed individual combo bins to be validated for release to commerce even if composite testing of the overall production lot tested positive. The techniques used prior to Dr. Samadpour's technique obligated that a positive test results in the entire production lot of 10,000 pounds be diverted to a low-value use, but implementing Dr. Samadpour's approach allowed 2000 pound single-lot units to be saved.

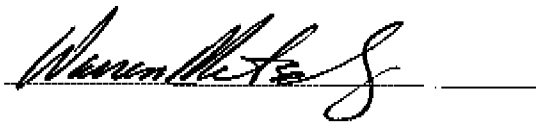
8. Major customers of Swift and Company initially refused to accept product that was tested using Dr. Samadpour's methods. In particular, Tyson Company, and their Director of Safety Dr. Dean Danielson, were highly skeptical. Dr. Danielson challenged the entire concept of wet compositing as proposed by Dr. Samadpour. Tyson initially stated they would not accept product from Swift that had been qualified with Dr. Samadpour's new form of testing. We were required to supply them with actual results from the Swift tests which proved that the process was sufficiently sensitive. In addition to Tyson Company, Cargill initially would not accept Dr. Samadpour's technique. To convince Cargill that Dr. Samadpour's technique was a valid and accurate method for testing production lots, Swift had to put a substantial amount of effort into proving to them that Dr. Samadpour's techinque worked.

9. After Dr. Samadpour's technique was tested internally, Swift and Company decided to implement the method at its major processing facilities, including the plants in Grand Island, NE; Greely, CO; Dumas, TX; Hyrun, UT; Omaha, NE; and Nampa, ID. These implementations were completed in 2007, and Swift has been using Dr. Samadpour's invention since then.

10. 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.

5



Warren Mirtsching

31 January 2011

APPENDIX A
CURRICULUM VITAE

Warren Mirtsching
Safety and Quality

Warren Mirtsching

5221 Keystone Creek Court
Ft. Collins, CO 80528
Home: (970) 206-4512 **Fax: (970) 206-1415** **Cell: (970) 692-4061**

CAREER SUMMARY

A proven food processing executive, leader, and strategist. Demonstrated ability to change organizational structures and lead others in new creative directions. Well versed in and capable of implementing food safety and quality systems. Experienced in consumer package items for both retail and foodservice trades. Designed and implemented multiple product and packaging changes to processed and fresh food for global distribution customer base. Comfortable and able to establish working relationships at all levels from the manufacturing floor to executive management. Able to adapt quickly to new environments and challenges. Experienced at evaluating facilities, organizational effectiveness and skill sets, driving productivity improvements through "Lean" process systems and methodology. Working as a leader and participant across functional lines for the betterment of the organization, acute at assessing competitive best practices scenarios due to network of peers, industry, and scientific fields.

PROFESSIONAL SUMMARY

IEH Inc. **May 2008 to present**
COO Meat Division
Laboratory and Consulting Services

IEH Inc is a laboratory and consulting service located in Seattle, WA. (www.iehinc.com) Current responsibilities are focused on client/customer service for prevention and crisis management related to the food industry. Development of consistent operating practices to improve revenue generation while enhancing and broadening customer service. Grow the customer base through the use of industry knowledge for the improvement of Food Safety Systems. Collaborate with associated companies to leverage the services provided.

JBS SWIFT & COMPANY **July 2007 to March 2008**
Oversee Food Safety, Quality, & Regulatory Compliance

JBS of Brazil purchased Swift & Company in July 2007 and redefined the organization, making JBS the world largest beef processor and a true global entity. I was retained to oversee all Food Safety, Quality, and Regulatory Compliance issues for the Beef group in North America.

- Implemented manpower reduction which generated a $1.7 million savings annually.
- Initiated deep dive into company expenses to assist in a 5% reduction in vendor cost of doing business.
- Supported the integration of new Brazilian management with business concepts in order to reduce their learning curve.
- Completed the licensing of a patented micro sampling device.
- Implemented USDA Grading trial on vision technology for improvements to consistency and value enhancement in the total supply chain.
- Completed the communication of the new single combo testing methods which will reduce the industry's product loss/devaluation by 80%.

SWIFT & COMPANY **1997 to July 2007**

Senior VP, Food Safety & Quality
North American Red Meat 2006 to July 2007

Senior Food Safety and Quality Executive for Swift & Company's $8 Billion revenue generating business in North America, 2nd largest red meat company in the world. Responsible for Food Safety, Quality, Regulatory and Trade Compliance, planning (annual and strategic), budgeting, capital spending, and interfacing with Suppliers, Customers, and Consumers.

- Implemented vision grading of beef carcasses to improve revenue opportunities identifying $5 million annually.
- Solidified Swift's Food Safety Leadership role through the reduction of E. coli incident rates by 80%.
- Managed the development of Swift's Beef Export Verification through vendor and customer relationships using Quality System Assessment process.
- Pioneered the implementation of single combo lot for trimming saving $750,000 from testing cost and logistics.

VP, Food Safety and Quality
North American Red Meat 2002 to 2006

Selected to fill the combined position of both Beef and Pork VP Food Safety and Quality. Accountable to bring the Beef and Pork separate organizations together under a consolidated and effective direction. Integrate varied practices into a business strategy which supported Sales, Marketing, Operations, and other support groups. Develop best practices and revenue generating ideas with sustainable growth and continuous improvement processes.

- Developed "SwiftTrace" traceback & traceforward systems integrating existing IT framework to capture an annual $4.0 million net revenue return with a major foodservice company. Applicable to domestic and international markets for a competitive advantage. Expandable system to implement Country of Origin Labeling Laws.
- Implemented Matrix system for the consolidation of multiple management programs into one; consistency of specifications and new product development system for all species.
- Implemented best practices/guidelines/policies which reduced product/process claims by $1.4 million annually.
- Implemented long term contracts for lab support and sanitation practices with vendors recognizing a $1.2 million cost reduction.
- Using best practices and consolidation of efforts, gained $1.5 million reduction of recall insurance over a three year period.
- Identified and implemented industry's first "Double pasteurization system" in conjunction with "Multiple Hurdles linked to Standard Operating Procedures".

VP, Food Safety & Quality
ConAgra Beef Company/Monfort 1998 to 2002

Implement continuous improvement processes and develop best practices in multiple functions within ConAgra Beef Company/Monfort. Direct P&L accountability for Cow plant Operations, Trim & Grind Sales, Research & Development, Food Safety & Quality, Logistics and Scheduling.

- Implemented a strategy to maximize trim/grind complex by $4.2, $2.8, and 2.3 million annually through aggressive customer and product management.
- Developed "Strongest Chain of Quality" and "Strongest Chain of Tenderness" marketing programs which brought beef business into a positive customer perception.
- Identified the use of "Operational Critical Control Points" for the consistency of education, communication, and production of ground beef over four different facilities.
- Instrumental in Customer Confidence related to product quality perception.

Director, Beef Quality Assurance
Monfort 1997 to 1998

Responsible to convert the product and processing quality of Monfort Beef into a competitive advantage. Implementation of consistency and detailed execution to change the customers' perception of beef quality and services. Develop a culture within the organization which eliminated waste and rework, thereby improving competitive costs.

- Drove consistency of product specifications and disciplines into the facilities eliminating $1.5 million in waste and rework.
- Earned the respect of customers and competitors who believed that Monfort's beef was not acceptable and had to be priced low.

- Managed the organization and development of personnel to be accountable and responsible for their decisions.
- Earned the respect of the organization and therefore promoted to VP over the entire Beef group.

EXCEL CORPORATION (Div of Cargill) **1977 – 1997**

General Manager, Cargill Processed Meat Products – Ft. Branch, IN 1992-1997

Chief executive for Excel's $100 million revenue generating processed meats facilities, supplying a variety of processed meat items for foodservice and retail markets. Responsibilities included P&L for the facilities, customer and consumer satisfaction, planning (annual & strategic), budgeting, capital spending, employee retention, safety, transportation, and daily operating issues.

- Negotiated the purchase of a family owned brand name consumer packaged goods company in Indiana.
- Successfully integrated two facilities into the Cargill system.
- Implemented reduction in supply vendors through consignment inventory and consolidated pricing.
- Harmonized raw material supply to match finished product values.
- Developed and implemented formulation & packaging changes to enhance the value of hams, bacon, sausage, roast beef, lunch meats, and hotdogs.
- Integrated new product formulations to maximize the consumer preference of finished packaged goods.
- Implemented technology to enhance shelf life of the packaged goods by 50% through sound science applications and detailed execution.
- Trained the management staff to engage in responsibilities which allowed for total employee growth of accountability.

General Manager
Excel Pork Customer Service & Processed Meats – Wichita, KS 1988-1992

Selected to fill a newly created dual role accountable for developing and implementing Excel's Fresh Pork Customer Service program and growing Excel's market share in the processed meat business. P&L responsibility for the processed meat division, including sales, marketing, manufacturing, engineering, and administration.

- Chaired the Corporate Total Quality Management Committee.
- Negotiated a multi-year contract with major Processed Products marketer for 40 million pounds annually.
- Linking supply chain management to leverage product position and revenue.
- Oversaw the development and operations of packaged consumer goods to retail and foodservice accounts such as: hams, bacon, and fresh sausage.

General Manager

Beef Slaughter and Fabrication – Plainview, TX 1983-1988

P&L accountability for the $900 million Beef slaughter and fabrication business unit with 1400 employees, including operations, engineering, quality assurance, distribution and administration. Oversaw the implementation of double shifting the plant, adding ground beef, and waste water revenue program in record setting time.
Simultaneously, oversaw Cargill's Brazilian operations in Maringa and Jose Bonafacio.

Plant Fabrication Manager – Dodge City, KS 1982 - 1983
(construction of fabrication and startup)
Plant Fabrication Manager – Wichita, KS 1980-1982
MBPXL Corporation – Plainview, TX 1977- 1980
Meat packing – acquired by Cargill in 1979
Various positions including: Assistant Plant Manager; Fabrication Superintendent; General Foreman; Packing Supervisor; and Technical Services Coordinator

EDUCATION

Texas A&M University, College Station, TX – BS in Animal Science 1977
Commercial Option

PERSONAL DEVELOPMENT

ISO Quality Systems – 2004 - present
Team Building – 1999 - 2001
Sportsmind – Team Building 1995
Total Quality Management 1988 – 2008
Minnesota School of Management for Marketing 1989

COMMITTEES AND ASSOCIATIONS

Beef Industry Food Safety Council (BIFSCO) 1998 – present
North American Meat Association 1998 – present
National Cattlemen's Beef Association 1998 – present
American Meat Institute 1997 – present

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In *Inter Partes* Reexamination of	:	Examiner: Brenda G. Brumback
Mansour SAMADPOUR	:	Group Art Unit: 3991
Patent No.: 7,534,584 B2	:	**Control No.: 95/001,294**
Issued: May 19, 2009	:	

Reexam Request Filed: April 5, 2010

For: MODULAR COMPOSITING-MULTIPLE LOT SCREENING PROTOCOLS FOR DETECTION OF PATHOGENS, MICROBIAL CONTAM INANTS AND/OR CONSTITUENTS

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

THIRD-PARTY REQUESTER COMMENTS ON THE ACTION CLOSING PROSECUTION AND PATENTEE'S RESPONSE THERETO

These Comments are submitted in response to the Patentee's *Response to an Action Closing Prosecution* filed and served January 31, 2011. Accordingly these Comments are timely filed if submitted on or before March 2, 2011. The Comments being timely filed and served, entry and consideration thereof is requested. On February 11, 2011, Third-Party Requester filed a petition under 37 C.F.R. §181(a)(3) objecting to the patentee's 192-page response and requesting the Director to invoke his supervisory authority to limit the response to the 50 pages authorized by the rules. On February 16, 2011, the patentee filed a petition under 37 C.F.R. § 183 to suspend the page-limit rule. The Director has not ruled on either petition as of the date of submission of these Comments. Third-Party Requester has been substantially prejudiced by

the unreasonable length of Patentee's response and its belated submission of four new declarations on issues that should have been addressed fully before the Action Closing Prosecution. As explained in greater detail herein, Third-Party Requester respectfully requests that Patentee's four new declarations be expunged from the record.[1]

COMMENTS ON CLAIM AMENDMENTS

Claim amendments following an Action Closing Prosecution ("ACP") are subject to the strict standards of 37 CFR § 1.116. 37 CFR 1.951; MPEP §2672 (III). Section 1.116 requires that an amendment filed after an ACP and that addresses the merits of the patent under reexamination may be admitted only upon a showing of good and sufficient reasons why it was necessary and was not earlier presented. 37 CFR §1.116 (b)(3). The only justification that the Patentee offers for his belated claim amendments is that "they were necessitated based on what Patentee respectfully contends is continued misunderstanding of Patentee's invention by the Examiner." (Response, p. 10). Requester disagrees and objects to the present claim amendments as not complying with the strict standards of 37 CFR. §1.116.

The Patentee has offered no explanation of what the Examiner's alleged misunderstanding was or which amendments are being presented to rectify that misunderstanding. The rationale for the Examiner's rejection has not changed since the initial rejection. Therefore, if the Patentee believed that claim amendments were necessary to overcome any "misunderstanding" by the Examiner, those amendments should have been presented before the issuance of an ACP. Accordingly, a "good and sufficient" reason as to why the claims could not have been entered earlier has not been presented, and Requester respectfully requests that the amendments not be entered. Moreover, even if the amendments were entered, for the reasons stated below, they do not overcome the outstanding rejections.

[1] In the event that any of the new declarations are allowed in the record, Third-Party Requester reserves the right to challenge or submit rebuttal evidence.

1. The Limitation of a "Statistical-Based" Sampling Plan Does Not Overcome the Examiner's Rejection.

The Patentee has introduced a limitation into step (a) of claims 1 and 17 reciting that single-unit test lots should be sampled "to conform" to a "statistical-based" sampling plan "specifying...a number of portions to be taken sufficient to provide for validation and release of the test lot into commerce." Step (e) has also been amended to recite that validation should be according to the "statistical-based" sampling plan. This limitation is virtually identical to the previous amendment specifying the use of a "statistical sampling plan." The Examiner did not find that limitation sufficient to overcome the Rejections, and it is submitted that the present amendment requiring a "statistical-based" sampling plan also does nothing to overcome the Rejections, because the use of statistical sampling plans for the validation of food lots was well known in the art.

As addressed below (*see*, for example, Ground 1), the Jarvis and Midura references both describe use of statistical sampling plans to validate food lots for release into commerce. Accordingly, it would have been obvious to a person of ordinary skill in the art to have employed a statistically valid sampling plan in the testing of food lots for microbial contamination. Indeed, the principal thrust of Midura is to emphasize the importance of developing a valid sampling plan that will result in obtaining a representative sample. Therefore, Patentee's inclusion of this limitation does not overcome the Examiner's rejection of the claims as obvious.

While the claims have been amended to require a statistical-based sampling plan, it is noteworthy that the specification contains no teaching concerning the details required for such a plan. The absence of such details indicates that the Patentee recognizes that the design and implementation of such plans was well within the abilities of the ordinary skilled artisan. The Midura reference, for example, provides this guidance, and the specification of the '584 patent provides no relevant teachings beyond what was described by Midura.

2. The Newly Added Language "for Release into Commerce" Does Not Limit the Invention

The Patentee attempts to amend the claims to require that the validation be "for release into commerce." That language only recites an intended purpose of the claimed method and does not limit the invention. *Bristol-Myers Squibb Co. v. Ben Venue Labs.*, 246 F.3d 1368, 1375 (Fed. Cir. 2001). The body of the claims define the steps of an operable method, and a statement of an intended purpose of the method ("for release into commerce") is not limiting and cannot serve to overcome the clear teachings of those steps in the prior art cited by the Examiner.

3. The Negative Limitation "No Requirement For Further Enrichment" Does Not Overcome the Examiner's Rejection.

The Patentee has introduced a limitation into step (e) of claims 1 and 17 reciting "wherein the enrichment of 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)." As a preliminary matter, the proposed negative limitation, "no requirement for further enrichment" raises new issues under 35 U.S.C. § 112, because it is not supported by the specification.[2] The patentee points to col. 7, lines 64 and col. 9, lines 43-45 as "support" without further explanation. Neither of these passages make any reference to, much less support, avoiding further enrichment. "Enrichment" is defined in the specification as "incubating a test lot sample (e.g., single-unit production lot sample) under conditions suitable to allow levels of a target agent/organism that is present to reach detectable

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[2] The Patentee failed to include a sufficient explanation of where the proposed amendments are supported by the specification, as required by 37 C.F.R. §1.530(e). *See*, M.P.E.P. 2250 ("Also in accordance with 37 CFR 1.530(e), each claim amendment must be accompanied by an explanation of the support in the disclosure of the patent for the amendment (i.e., support for the changes made in the claim(s), support for any insertions and deletions). The failure to submit an explanation will generally result in a notification to the patent owner that the amendment prior to final rejection is not completely responsive since the failure to set forth the support in the disclosure goes to the merits of the case (see MPEP § 2266.01). Such an amendment submitted after final rejection will not be entered.") The conclusory statements appearing in the Response (pp. 22-23) fall short of the explanation required by Rule 1.530(e).

levels and become uniform or substantially uniform throughout the enriched test lot sample (e.g., single-unit production lot sample)." Col. 6, lines 7-12. The specification does not limit the number of steps or number of enrichment media required, but only requires that the composite sample be "enriched using suitable enrichment protocols" to allow the target agent/organisms to reach detectable levels. *See e.g.*, col. 4, lines 9-10 and lines 61-62. Accordingly, the new limitation should not be entered, because it does not have § 112 support.

In addition, this limitation does nothing to overcome the Examiner's rejections, because these additional limitations are taught by the prior art. As discussed above in connection with the proposed limitation to "statistical-based" sampling plan, both Jarvis and Midura describe the use of a statistical sampling plan to validate food lots. Accordingly, adding a limitation that the enrichment is sufficient to enable validation does not overcome the Jarvis or Midura reference.

The limitation that the validation is possible "with no requirement for further enrichment" also does not overcome the Examiner's rejections. The media disclosed in the prior art references are sufficient to meet the definition of enrichment given in the '584 patent specification. The prior art teaches that, with appropriate detection procedures, samples may be validated without further enrichment. To the extent Patentee is attempting to argue the limitation obviates the prior art because the prior art makes mention of storing the pre-enrichment cultures, that argument is also unavailing. As discussed below, Patentee's argument , for example, overlooks the use of rapid detection methods that are well known in the art and that are capable of detecting pathogenic organisms that are present in low numbers. (*see,* for example, Entis). Such rapid detection methods prevent the necessity of storing a retained culture under conditions that would require further enrichment prior to testing.

The proposed limitation that there is "no requirement for further sampling in a)," like the previous limitation, finds no support in the written description. For this reason and Patentee's

failure to include an explanation of where support can be found, the amendment should not be entered. Moreover, the added language fails to overcome the Examiner's rejections. As discussed in detail below, the Jarvis and Price references both teach that the initial composited samples should be retained. Accordingly, there is no need for further sampling to test individual test lots for contamination following a positive result in the modular composite sample. In addition, Patentee repeatedly asserts that current guidelines do not allow test lots to be resampled. A person of ordinary skill in the art of testing food lots for release into commerce would know this and so would not design a sampling plan that required resampling.

COMMENTS ON THE DECLARATIONS

The declaration evidence that the Patentee seeks to introduce should be excluded for failure to comply with 37 C.F.R. §1.116. Declaration evidence presented after an Action Closing Prosecution "may be admitted upon a showing of good and sufficient reasons why the affidavit or other evidence is necessary and was not earlier presented." 37 C.F.R. §1.116(e). However, the Patentee does not provide such "good and sufficient reasons" as to why the declarations are being submitted at this late stage of the prosecution.

Declarations (1), (2) and (3) are submitted to "support the nexus between the Patentee's patent claims and commercial success." (Response, page 9). Patentee states these declarations are now being submitted because "the premise of said Examiner's questioning being only known to Patentee as of the issuance of the RCP [*sic*]." (Comments, page 9-10). This is not a sufficient reason to support inclusion of these declarations under 37 CFR §1.116.

A basic tenet of patent law is that "[a]n applicant who is asserting commercial success to support its contention of nonobviousness bears the burden of proof of establishing a nexus between the claimed invention and evidence of commercial success." MPEP §716.03. Accordingly, the burden was on the Patentee to establish the nexus between the claimed

invention and any alleged commercial success prior to the Action Closing Prosecution. This is especially true given the submission of two declarations by Patentee in the first response filed July 26, 2010. These two declarations purported to show the commercial success of the claimed invention, but failed to establish the necessary nexus. As the Patentee bears the burden, it was incumbent on him to establish the nexus when the evidence of commercial success was initially submitted. For the Patentee to suggest that such evidence could only now be submitted, because Patentee did not know the Examiner would question this nexus is specious. Accordingly, Requester submits declarations (1), (2), and (3) should not be admitted or considered, because their submission does not comport with the "strict standards of 37 CFR 1.116." There are no "good and sufficient reasons" that they could not have been presented earlier, and Requester submits that they should be expunged from the record. 37 C.F.R. §1.116(e); MPEP §2672 (III).

Declaration (4) should also be excluded for failure to comply with 37 C.F.R. §1.116. That declaration is said to be submitted for the purpose of rebutting the declaration of Dr. Bailey. (Comments, page 10). Dr. Bailey's declaration was submitted with the request for *inter partes* reexamination. If the Patentee felt it was necessary to rebut the testimony of Dr. Bailey, then it was incumbent on him to do so at an appropriate, earlier time such as when the first Patentee response was filed. Patentee only offers a general assertion that the declaration is now being submitted because it is necessary to rebut "the Examiner's premise therefore only known to Patentee as of issuance of the RPC [*sic*]." (Comments, page 10). Again, this general assertion is not sufficient to meet the "strict standards of 37 CFR §1.116" because it does not provide a "good and sufficient reason" as to why the declaration was not presented earlier. 37 C.F.R. §1.116(e); MPEP §2672 (III). Accordingly, declaration (4) should not be considered and should be expunged from the record.

COMMENTS ON THE REJECTIONS

Ground 1 of the Rejections (Response at pages 10 – 60)

The Examiner has maintained the rejection of claims 1 – 3, 8, 10-20, 24-27, 31 and 32 under Ground 1 based on the combined teachings of Jarvis, Midura, and FSIS.

1. **Patentee Continues to Argue the References Individually**

Despite the Examiner's admonitions to the contrary, the Patentee again attacks the references (particulary Jarvis) separately and fails to consider the combined teachings of the references as applied by the Examiner. In fact, the Patentee only considers the FSIS and Midura references as they pertain to certain dependent claim rejections.

a. The Prior Art References Teach Testing a Plurality of Test Lots

At the core of Patentee's entire argument for patentability is the erroneous contention that Jarvis (as well as other references) teach only the compositing of multiple samples of a single lot. Based on this unsupported and erroneous interpretation of the prior art, the Patentee tries to distinguish his claims on grounds that they require taking samples from a plurality of separate test lots. (*See, e.g.*, Response, pp. 11, 35). As an initial matter, this argument is nothing more than a rehash of the inter-lot v. intra-lot argument the Patentee advanced in his First Response. The Examiner found this argument unpersuasive because the '584 patent "fails to set forth any demarcation of a 'lot' that renders the claimed methods distinct from the method disclosed by Jarvis in view of FSIS and Midura." (ACP, p.12-13). This analysis does not change simply because the Patentee adds the word "separate" in front of the claim term "test lots." A "test lot" is still "an assemblage of 'one or more' specimens" and thus "a single specimen [still] falls with the definition of a 'lot' as recited in the patented claims." (ACP, p.13). Specifying that the "test lots" are "separate" does not alter the definition of the claim term.

Moreover, the Patentee's argument relies on an unsupported an illogical reading of Jarvis and other references. Jarvis method (2) involves incubating 10 separate samples in 10 separate pre-enrichment media, and then compositing the pre-enrichment media in an enrichment medium. A negative test on the enrichment medium validates all 10 samples. If the enrichment medium tests positive, then the stored pre-enrichment media can be tested separately "so that some indication of the proportion of contaminated samples can be obtained." Jarvis at 141-42. If, as the Patentee argues, all 10 of Jarvis' samples were from a single lot of food product, then Jarvis' teaching that the stored pre-enrichment media can be tested separately would make no sense, because if any one of the samples from a single lot tested positive for contamination, then the entire lot would have to be destroyed or otherwise disposed of. The more logical reading of Jarvis – and the reading that would be apparent to a person of ordinary skill at the time – is that the 10 samples are from individual lots, such that retesting of individual pre-enrichment media would identify those lots that are contaminated (and thereby must be destroyed) and those that are not. A person of ordinary skill would understand, for example from Midiura, that each of Jarvis' 10 "samples" would have to be representative of the entire lot of material. Midura teaches the importance of obtaining representative samples and also teaches how such samples are obtained in a statistically significant manner. The Patentee distorts the teachings of Jarvis in an illogical way so as to support his argument for patentability.[3]

Properly viewed as a whole, Jarvis is a reference teaching statistical sampling methods for food validation. Accordingly, the methods laid out in Jarvis are intended to be applied to real world methods. An example of such a real world method is the FSIS protocol. The FSIS

[3] The Patentee repeatedly points to a separate passage on page 137 of the Jarvis reference where the singular term "lot" is used. (Response, p. 15; *see also* Samadpour Dec., ¶14). However, the only logical reading of this passage is that the author is describing the importance obtaining a representative sample of a lot by taking "n" number of samples. This teaching is similar to that of Midura, which teaches the importance of obtaining a representative sample, which, for solids and semi-solids, involves multiple samples from different sites in the lot of material to be tested. This excerpt from Jarvis does not refer or relate to his method (2) compositing method, but deals only with procedures for taking a "sample".

protocol is a protocol for the sampling and analysis of raw ground beef for *E. coli* contamination. The FSIS protocol describes separately sampling, enriching, and analyzing each lot of product being tested. A person of ordinary skill in the art, in 2004, would have understood that the FSIS Protocol would be applied to multiple separate test lots of raw ground beef to produce a set of separate test lot samples. (Bailey Dec., ¶19). Accordingly, the FSIS protocol demonstrates that the method of Jarvis, when applied to a real world scenario, teaches a "plurality of separate test lots" should be tested.

Midura teaches application of statistical sampling plans to a plurality of separate test lots. Midura is directed to sampling plans and provides multiple examples. One example is the Three-Class Attribute Plan. (Midura, p. 17-18). The Three-Class Attribute plan is intended to be applied "to lots presented for acceptance at ports or other similar points of entry." (Midura, p. 17). However, the plan is "also useful for inspecting lots within a country or corporation." (Midura, p.18). Midura also exemplifies a Multiple Sampling (2-Class) Attribute Plan that is "designed to inspect lots based on multiple samplings." (Midura, p.17). Thus, Midura also describes statistical methods for testing "a plurality of separate test lots" and is not simply limited to sampling a single test lot.

In addition to the teachings of FSIS and Midura the Jarvis reference itself teaches sampling a plurality of separate test lots. Jarvis Example 8.4 describes a method for determining if food product test lots should be accepted or rejected. (Jarvis, p.136). In this example, Jarvis clearly states "[s]uppose the following results are obtained on three lots of product." (*Id.*). Example 8.4 then lists theoretical data for each test lot and indicates that a decision to reject the lot, repeat the test, or accept the lot can be made. (*Id.*). Thus, the Jarvis reference itself teaches the testing of a plurality of separate test lots.

It is clear from the cited prior art references that it was well known in the art to test a plurality of separate test lots. All three references provide guidance on this topic. Patentee's attempt to show otherwise by pointing to two instances in Jarvis where, in a completely different context, the author used the singular term "lot" instead of the plural term "lots" is unavailing. As the Patentee admits, prior art references must be considered as a whole. (Response, p. 35), and those teachings must be considered in proper context. Thus, taken as whole in combination with each other, the references teach sampling a plurality of separate test lots.

Patentee raises this same argument in comments submitted in specific rebuttal of the Examiner's rejection of claims 2, 11 and 13. (Response, p. 15-16). Requester believes that Patentee's arguments with respect to claims 11 and 13 have already been fully addressed by the above response.

However, with respect to claim 2, Patentee makes an additional argument. Patentee asserts that the test lots of Jarvis may not be composed of operationally –linked specimens. (Response, p. 15). However, Patentee does not point to any evidence to support this assertion. Therefore, such a statement amounts to no more than unsupported attorney argument that cannot take the place of evidence in a proceeding before the Patent Office. (MPEP § 2145). Requester points out that Jarvis is also viewed in combination with FSIS. A person of ordinary skill in the art in reading these references in combination would know not to combine non-operationally linked specimens because there is no suggestion in FSIS to do so. (Bailey Dec., ¶19).

b. <u>The Prior Art References Teach Taking a Statistically Significant Number of Samples</u>

Patentee continues to assert that the prior art references, and the Jarvis reference in particular, do not teach a statistically significant sampling plan. (Response, p.13-14). This argument is made despite the fact that both the Jarvis and Midura references explicitly and extensively describe statistical sampling methods. Moreover, Patentee does not explain why

these references do not teach statistical sampling plans. Instead Patentee points only to the Jarvis reference's disclosure of testing 5 samples and asserts that this proves Jarvis does not teach a statistical sampling plan. The Patentee also ignores the Examiner's finding that the independent claims of the '584 patent do not contain a limitation specifying the number of samples to be taken. (ACP, p. 12). Amending the claims to recite that the tests lots must be sampled "to conform to a statistical-based sampling plan" would not change this fact.

The whole point of Jarvis is to teach statistical methods to improve testing accuracy and confidence levels for food testing. Patentee asserts that Jarvis fails in this teaching, based on his flawed interpretation of Jarvis methods (2) and (3). The Patentee argues that these methods do not utilize a statistically significant number of samples (Response, p. 45). In describing methods (2) and (3), Jarvis merely refers to placing "samples" separately into 10 pre-enrichment media. A person skilled in the relevant art would understand that each of these "samples" would have to be representative of the lot of material being tested. As Midura teaches, if the material being tested were a liquid or powdered material mixed to homogeniety, then a single sample from each lot would be representative. But Midura also teaches that for non-homogeneous solids or semi-solids, the representative "sample" is itself a composite of several samplings from different locations in the lot. *See* Midura at 19 (§§ 2.522 and 2.533). To support his argument, the Patentee must ignore what was well known to those skilled in the art regarding the obtaining of representative samples of material for testing. The Patentee only comes to grips with Midura when addressing dependent claims. (Response, p. 27-30). The Patentee's approach of dissecting (and distorting) the references individually is improper, because the independent claim rejections are based on the combined teachings of the references. Importantly, Midura teaches the importance of developing statistical sampling plans and provides several examples. For example, Midura specifically states that "[s]ampling can be applied in a way that ensures that

results obtained will be statistically valid." (Midura, p.14). A person of skill in the art of designing methods for the sampling, testing and validation of food products would have been well aware of the importance of employing a statistically significant sampling plan that is representative of the product being tested. This point is highlighted by Midura's admonition to consult a statistician before implementing a sampling plan. (Midura, p. 14).

In connection with dependent claims 8, 10, and 14-16, the Patentee argues that Midura only teaches sampling from a single lot. (Response, p. 27-30 -- emphasizing the Examiner's use of the singular term "lot" in the rejection). This Argument misses the point of the rejection. Midura teaches the importance of developing statistically valid sampling plans for obtaining a representative composite sample of a lot of material (e.g., solid or semi-solid food) to be tested. This sampling correspomds to the dry compositing steps of the '584 patent claims. Jarvis (and other references) describe incubating samples in separate media and then preparing a wet composite, while preserving the separate incubation media for re-testing in the event of a positive result on the wet composite. These procedures correspond to the wet-compositing, detection and re-testing steps of the '584 patent claims. The Patentee's arguments ignore the combined teachings of the references and assume that the skilled person would perform the Jarvis method in an illogical and meaningless way.

Patentee raises an additional argument with respect to dependent claim 8. (Response, p. 28). Patentee argues that Midura's teaching of resampling teaches away from the allegedly inventive methods of the '584 patent. (*Id.*). As Patentee does not clearly articulate why this is so, it is assumed that Patentee is asserting that resampling is not permitted because of current food sampling guidelines. A person of ordinary skill in the art would be aware of current food sampling guidelines and so would not knowingly violate them. In fact, Midura also states that prior to implementing a sampling plan the reason for sampling and testing should be considered

so that the results will be significant for the intended use. (Midura, p.20). Accordingly, a person of ordinary skill would not resample the test lot, and instead would resample from the stored cultures, as taught by Jarvis.

c. The Prior Art References Teach Validation of Food Lots for Release

Patentee asserts the prior art references, alone or in combination, do not allow for validation of samples for release.[4] (Response, p.39) The '584 patent defines validation as "determining that a particular sample tests negative." ('584 patent, col. 7, ll. 2-3). In other words, the Patentee is stating that the prior art methods do not teach that samples testing negative are free from contamination. (Response, p. 45). This argument is wrong, because it eviscerates the very purpose of all three references.

As an initial matter, the '584 patent does not disclose validation in statistical terms. (ACP, p.12). Rather, it only discusses use of a "statistical based sampling plan." There is no basis for Patentee's argument that a reference to a "statistical-based sampling plan" is sufficient to enable a person of ordinary skill in the art to "validate" a negative result, but a prior art reference devoted solely to statistical methods cannot . In any event, the prior art references do in fact teach validation of a negative result within the meaning of the '584 patent.

The Patentee's argument appears to be based on the idea that Jarvis and Midura, two references that explicitly teach statistically significant sampling plans, do not actually teach taking a statistically significant number of samples. (Response, p. 45 -46). Further, although Patentee states that the combination of references does not teach validation for release, the Patentee really only applies this argument to the Jarvis reference. The Patentee's approach is improper, as it is the combination of references that renders the claims invalid.

[4] As discussed above, this statement of the intended purpose does not limit the operative method steps and cannot be a basis for distinguishing prior art that teaches or discloses those steps.

Interestingly, Patentee asserts Jarvis only teaches collecting samples from a single test lot. (Response, p.13). Patentee then asserts that the number of samples taken is statistically insufficient for validation of release. (Response, p.14). However, Patentee later asserts that the "asserted art [Jarvis] is directed a [*sic*] testing a single lot to be validated." (Response, p.16). Accordingly, Patentee admits Jarvis teaches validation of food lots.

Jarvis, in combination with FSIS and Midura, do in fact teach validation for release. The FSIS reference is a protocol for sampling ground beef for bacterial contamination. The purpose is to determine which food lots are safe for release and consumption. A person of ordinary skill, knowing the importance of food safety, would ensure that the results of the testing are statistically significant, for example, by applying Midura's statistical plans for real world validation of samples. For example, the Three-Class Attribute plan of Midura specifically states an intended use of the plan is to test lots presented for acceptance at ports or other similar points of entry. (Midura, p. 17). It would be of no value to test lots for contamination at a port if the results of that test could not be used to release the lot upon receipt of a negative result. Thus, at least one purpose of the Midura reference is to validate lots that test negative for "release into commerce."

The Midura reference also further describes validation for release by describing a double sampling plan. Midura defines double sampling plan as "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." (Midura, p. 16). The inspection of the second sample, when required, then leads to a decision to accept or reject. This is the process of validation. Moreover, as discussed above, a person of ordinary skill in the art would not interpret the step of inspecting a second sample, or resampling, to mean that another sample of product is taken from the lot under test. Rather, a person of ordinary skill would know from Jarvis that the sample should be taken from the stored cultures.

2. **Patentee Improperly Reads Limitations into the Claims**

The Patentee repeatedly argues that the claimed method increases the workload whereas the prior art methods, in particular Jarvis, reduce the workload. (*see,* for example, Response, p. 39). The implication is that this is somehow a patentable distinction. However, it must be pointed out that there is nothing in the claims of the '584 patent that address workload requirement. In addition, Patentee's own method also reduces workload. Under Patentee's interpretation of the '584 patent claims, samples are composited from a single lot. These samples are then enriched and an aliquot of each combined test lot sample is combined to form a second composite sample (the second composite sample being a composite of a plurality of separate test lots). The second composite sample is then tested for contamination. If negative, all the test lots are released, thereby avoiding the necessity of testing each lot individually. If the second composite sample is positive then the first composite samples are individually tested and those that test negative are approved.

The salient point is that the samples will not always test positive, and, in fact, in the vast majority of cases will test negative. When the second composite sample tests negative, then a number of individual lots will be released without having to carry out a contamination test on each composited sample. Even when the second composite sample tests positive, and each first composited sample is tested individually, this does not result in more work than the prior art, because the prior art references, such as Jarvis and Price, teach the same method as the '584 patent.

3. **Patentee Continues to Misconstrue Jarvis**

a. Jarvis Teaches Modular Composite Samples

Patentee asserts that the Jarvis reference does not teach Patentee's "unique modular composite sample." (Response, p.16-17). Patentee further asserts that Patentee's modular

composite sample is fundamentally different, because it is formed from a plurality of statistically sufficient test lot cultures. (Response, p. 16). As discussed above, Patentee misconstrues Jarvis and, as a consequence, is incorrect that the claimed method is fundamentally different from the prior art. Indeed, the Patentee's modular composite sample is the same as the prior art composite samples described by Jarvis and other references.

The '584 patent defines modular composite sample as a sample formed by removing, preferably aseptically, equal portions of each of a plurality of enriched single-unit production lot samples, and combining the removed portions. However, as the Examiner notes, Jarvis does in fact teach a modular composite sample as defined in the '584 patent. (ACP, p.13). In addition, Midura teaches the importance of statitistically valid sampling plans and describes the preparing a composite sample made up of "aliquots from several areas of the food under examination." Midura at 20. Jarvis method 1 clearly teaches compositing samples from a test lot into a single culture. Jarvis method 2 clearly teaches that a plurality of these test lot cultures can be composited together by taking equal portions of the culture. The Jarvis method is the same type of modular composite sample as that claimed in the '584 patent.

The Patentee's assertion that Jarvis does not teach a plurality of separate test lots has been addressed above. Patentee's restrictive reading method (2) of Jarvis would render it meaningless (or, as Patentee suggested in his response to the previous office action, merely to satisfy a "curiosity"). To the extent the Patentee argues the pre-enrichment broth does not provide sufficient enrichment, this argument is meritless, because the claims do not require the use of an enrichment broth. Moreover, as discussed below, Jarvis also teaches the use of an enrichment broth.

Patentee also asserts that the '584 patent teaches formation of the modular composite sample is done after the levels of target organism reach detectability. (Response, p.21).

However, the Jarvis reference explicitly teaches this limitation as well. As discussed below, and previously acknowledged by the Examiner, the pre-enrichment broth of Jarvis allows for sufficient "enrichment" under the meaning of the '584 patent. Therefore, the modular composite sample of Jarvis is formed after the target organisms are enriched.

b. The Pre-Enrichment Medium of Jarvis Supports Growth to Detection Levels Within the Meaning of the '584 Patent

Patentee again tries to distinguish the '584 patent claims from the prior art based on the attained levels of enrichment. Patentee essentially argues that the use of "pre-enrichment" incubation does not achieve a level permitting detection of the microorganisms. (Response, p. 19). The Examiner did not previously find this argument persuasive. (ACP, p. 11). Nonetheless Patentee argues that the pre-enrichment medium of Jarvis is only capable of taking the "levels of the target organisms to a point that an aliquot transferred to the enrichment [medium] would contain at least one viable cell." (Response, p. 20-21). Patentee asserts that one cell is insufficient for detectability which can only be achieved following incubation in enrichment medium. (Response, p. 21).

In making this assertion Patentee seizes on an extreme example of Jarvis. Jarvis does not say that that pre-enrichment media only allows three viable bacteria to be present in the pre-enrichment culture. Rather, Jarvis' point is to ensure that at least three viable organisms are present in each milliliter of medium. It is clear that Jarvis teaches that in most instances, the cell count will be considerably higher. It should also be pointed out that Patentee acknowledges that the prior art teaches there may be some "limited target organism growth during pre-enrichment [but] the number of target organisms achieved is not taught by any of [the] references." (Response, p.41-42). Patentee's later argument that a person of ordinary skill in the art would not consider growth in the pre-enrichment culture to be suitable for assaying is unsupportable.

Moreover, even in the case of a low cell count, Jarvis teaches growth of organisms sufficient to meet the limitations of the '584 patent claims. As the Examiner has already found, diagnostic methods such as PCR are capable of detecting the target organism at levels as low as one organism. (ACP, p.9). The Berri reference previously submitted by the Requester and considered by the Examiner exemplifies this method. Berri discloses the detection of a single *C. burnetii* cell in 1 mL of liquid. (Berri, p. 292). Berri's detection method involves the use of common laboratory techniques, such as PCR, equally applicable to any DNA-containing sample. (*Id.*). Thus, even in the most extreme case, the pre-enrichment medium of Jarvis is sufficient to grow bacteria to detectable levels within the meaning of the '584 patent claims.

In addition, Jarvis does not require the use of pre-enrichment media. Rather, Jarvis specifically states that "when samples are tested for presence/absence of specific microbes, the analytical procedure may involve the use of pre-enrichment and selective enrichment stages." (Jarvis, p. 138) (emphasis added). As the Examiner noted, the protocols embodied in Jarvis section 8.4.2.1 are by way of example only and should not be construed to be limiting. (ACP, p. 14). Accordingly, simply because those examples are written with reference to pre-enrichment media followed by selective enrichment does not mean that use of the Jarvis method is restricted to that protocol. Jarvis even states that "it is normal to inoculate one or more samples into a suitable selective or enrichment medium prior to streaking on a diagnostic medium." (Jarvis, p.135). Accordingly, Jarvis teaches and suggests that normal methods involve incubating samples directly into an enrichment medium without the necessity of pre-enrichment.

As the Examiner notes the claims of the '584 patent do not recite an enrichment medium. (ACP, p. 11). The claims only recite incubating the test lots samples under conditions suitable for the target agent to increase and/or reach detectable levels. (*Id.*). As the examiner agreed, the

pre-enrichment media of Jarvis meet these limitations. (ACP, p.9). The amendments offered by the Patentee after the ACP, even if entered, do nothing to change this fact.

Finally, Patentee asserts that there is a long standing canonical distinction between dividable levels of pre-enrichment vs. detectable levels of target organisms after enrichment. (Response, p.21). However, as stated in Dr. Bailey's Declaration it was well known that pre-enrichment media would both resuscitate the target microorganism and cause it to grow to detectable levels and become substantially uniform within the liquid pre-enrichment culture. (Bailey Dec, ¶9d). Further, as discussed above, it is possible to detect single organisms in culture through the use of modern immunochemical and nucleic acid detection procedures, such as PCR. Accordingly, the distinction that the Patentee attempts to draw is not relevant to the present claims as the pre-enrichment medium of Jarvis meets the '584 patent claim limitations.

c. Jarvis Accounts for Real World Scenarios

Patentee also insists that Jarvis does not teach "real world" methods for statistical validation. (Response, p.47). Not true. The Jarvis reference repeatedly warns of the dangers of "technical error" or other problems such as "contagious distribution, reflective clumping, sublethal damage, dilution effects, etc." These are all random events that may impact the results of the tests. (Jarvis, p. 117, 119, 135). The first Jarvis edition also notes other real world factors such as "incubation time, competition from other organisms, environmental stress, initial contamination level, etc." (Jarvis, p. 139). Thus Jarvis clearly acknowledges the effect of "real world" factors and cautions a person utilizing the Jarvis principles to guard against their effect.

Patentee relies on a later published edition of Jarvis as "evidence" that the first edition of Jarvis did not take into account these "real world" factors. It is unclear why Patentee would believe that a person of ordinary skill in the art at the time of the alleged invention could have possibly been influenced by a reference published years later. Second, Patentee admits that the

second Jarvis reference did nothing more than teach and clarify what was already practiced in the art. Accordingly, even assuming the first Jarvis reference did not explicitly teach "real world" factors, Patentee admits that such factors were already well-known. Thus, a person of ordinary skill in the art would have known to guard against them.

d. Jarvis Teaches Verification of Negative Samples for Release

Patentee asserts that the stored Jarvis samples are only used to verify positives. Patentee further asserts retesting of the stored cultures in Jarvis is solely for "determining the extent of the contamination." (Response, p.14). However this conclusion undermines the purpose of Jarvis.

Jarvis states that the testing of the stored samples is to gain "some indication of the proportion of contaminated samples." (Jarivs, p. 142). The practical value of such testing would be to identify the individual samples that were contaminated and to verify those that were not. Common sense supports this interpretation, as there would be no purpose in storing cultures to only determine the extent of the contamination. Any lot testing positive would be destroyed, and so the extent of the contamination is irrelevant. Therefore, a person of ordinary skill would have understood that Jarvis' purpose of testing stored cultures was to verify the negative samples.

4. Withdrawal of the Claim 4 Rejection

Third-Party Requester respectfully requests reinstatement of the rejection of claim 4 over the combined teachings of Jarvis in view of FSIS and Midura. In withdrawing the rejection, the Examiner pointed out that claim 4 requires the use of an enrichment medium that is selective, i.e., one that preferentially favors the growth of the target organism over other organisms in the sample. (ACP, p.16.) The Examiner concluded that 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. (*Id.*). In response to Requester's previously submitted arguments, as they pertain to claim 4, the Examiner indicated that there is no motivation in

Jarvis, FSIS, or Midura to eliminate the pre-enrichment stage and directly inoculate the dry food samples of Jarvis into individual selective enrichment broth cultures. (*Id.*). Requester respectfully submits that the prior art did provide such motivation.

Jarvis does not require the use of pre-enrichment media. Rather, Jarvis specifically states that "when samples are tested for presence/absence of specific microbes, the analytical procedure may involve the use of pre-enrichment and selective enrichment stages." (Jarvis, p. 138) (emphasis added). As the Examiner noted, the protocols embodied in Jarvis section 8.4.2.1 are by way of example only and should not be construed to be limiting. (ACP, p. 14). A person of ordinary skill in the art would have known that the method of Jarvis could be modified to provide for direct inoculation into a selective enrichment.

The FSIS protocol provides motivation for modifying the Jarvis method in this manner. The FSIS protocol describes a method for the detection of *E.coli* in raw ground beef utilizing direct incubation in mEC + novobiocin broth. A person of ordinary skill in the art would have understood that mEC + novobiocin broth is a selective enrichment medium for *E. coli*. (Bailey Dec., ¶18). Accordingly, a person of ordinary skill in the art would know from the combination of Jarvis and FSIS that the separate individual composited test lots may be initially incubated in selective enrichment medium prior to the subsequent wet compositing step. For these reasons, Requester requests that the rejection of claim 4 under the Ground 1 rejections be reinstated.

In summary, Patentee has failed to consider the teachings of the references as a whole, and has misconstrued the Jarvis reference. Accordingly, the Patentee has failed to rebut the Examiner's *prima facie* case of obviousness. Therefore, it is respectfully requested that the rejection of claims 1-3, 8, 10 – 20, 22, 24 – 27, 31, and 32 be maintained. It is further requested that in light of the above arguments, the rejection of claim 4 be reinstated.

Ground 2 of the Rejections (Response at pages 60 – 83)

The Examiner has maintained the rejections of claims 1 – 4, 6-18, 20, 22, 24-26, and 31 as unpatentable over FSIS in view of Price and Midura. In responding to this rejection, the Patentee misconstrues the teachings of the references and fails to consider their combined teachings.

1. Patentee Misconstrues the FSIS Reference

a. FSIS teaches sampling from separate test lots

Patentee asserts the FSIS reference teaches taking a plurality of samples from a production lot and then defines a production lot as a day's production. (Response, p. 60). Under this interpretation, Patentee thus asserts FSIS does not teach taking a plurality of samples from separate test lots, but rather teaches taking a plurality of samples from a single test lot, i.e. the day's production. (Response, p.61). As evidence Patentee cites to an FSIS Microbiological Guide and states that it demonstrates that "subsamples of the collected composite sample are removed and analyzed separately." (Response, p. 61). This argument misses the point of the FSIS reference and the rejection based on it.

The FSIS protocol is cited for teaching sampling, enriching and analyzing each lot of sample that is tested. As the Examiner notes "FSIS teaches the samples are collected from the 'current day's production,' at specific times, or by specific boxes or combo bins." (ACP, p.18). Patentee assumes that this must mean that the samples are taken from a number of different production lots. However there is no support for such an interpretation in the FSIS protocol. Indeed, a person of ordinary skill in the art would have known that the FSIS protocol would be applied to multiple separate test lots to produce a set of separate test lot samples. (Bailey Dec., ¶19). Patentee's interpretation of the FSIS protocol distorts its meaning.

This interpretation is also flawed because it focuses on the FSIS protocol in isolation from the Midura and Price references. As discussed below, Midura and Price both teach taking samples from separate test lots. Therefore, there is ample reason for a person of ordinary skill in the art to understand that the FSIS protocol teaches sampling from separate test lots.

b. The FSIS Notice is not relevant

Patentee also cites an FSIS Notice dated November 10, 2005 as further support for its assertion that FSIS only teaches compositing for a single test lot. (Response, p.61). The Notice is dated more than a year after the filing date of the provisional application from which the '584 patent claims priority. Accordingly, irrespective of its teachings, the Notice is simply irrelevant to the patentability of the '584 patent claims.

c. The FSIS protocol teaches sampling from operationally linked test lots

Patentee asserts that the FSIS protocol teaches collecting samples from a similar product, but after the institution has implemented corrective action after a positive finding. (Response, p. 63). Patentee thus concludes that the resampling will be from non-operationally linked units, because of the turn-around time for analysis. (*Id.*). However, the '584 patent defines operationally linked to mean linked in a manner, such as 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. ('584 patent, col. 5, ll. 26-30). As is clear from the '584 patent definition, time is not the only factor for determining what constitutes an operationally linked test lot. For instance, process step is an equally valid determinate. Thus, even under Patentee's assumption that the sample collection is repeated several days after a positive finding is correct, those test lots would still be operationally linked under the '584 patent definition because they are still produced by the same process.

d. FSIS teaches "follow-up" sampling in combination with Price and Midura

Patentee further argues that the "follow-up" sampling is not allowed in the context of validating test lots. However, in making this assertion, the Patentee ignores the teachings of Price that individual composited test lots may be retested following a positive test result for the enriched composite sample formed from incubating aliquots of the individual composite test lots in enrichment media. Further, Patentee ignores the teachings of Midura that such individual composite test lots should be composed of a statistically significant number of samples taken from the lot to be tested.

2. Patentee Misconstrues the Price Reference

a. The stored Price samples do not require further enrichment

Patentee asserts that the samples in the Price method must be stored and thus are required to be incubated in enrichment medium prior to retesting. (Response, p. 68). However, this assertion overlooks the use of rapid detection methods that were well known in the art at the time of the alleged invention of the '584 patent. For example, methods such as PCR detection allow for much more rapid detection of bacteria than was possible when the Price reference was published in 1971. By August of 2004, it would have been obvious for a person of skill in the art to use these rapid detection methods in conjunction with the method of Price. (Bailey, ¶21). Accordingly, a person of ordinary skill in the art would not have stored the samples in an environment where subsequent enrichment would be required prior to retesting as Patentee claims.

b. Price teaches testing of separate test lots

Patentee asserts that Price is directed to testing a single lot for release, whereas the '584 patent claims testing a plurality of separate test lots. (Response, p. 68). There is no basis for such a restrictive interpretation of Price. In fact, interpreting Price to teach that testing is limited to single lot would defeat the purpose of the enrichment culture-pooling (wet compositing)

procedure of Price. Pooling of pre-enrichment cultures of multiple samples derived from a single lot would be of no value, because a positive result would invalidate the entire lot in any event. However, Price teaches that, if the composited enrichment culture tests positive, then the retained samples are to be tested individually. (*see* Price Fig. 1 and text at 681). Such re-testing would only make sense if a negative result on an individual retained culture would allow validation of the test lot from which the sample corresponding to that retained culture was derived. If the enrichment cultures were all derived from the same lot, a positive result for the composited culture would require rejection of the lot, regardless of whether individual pre-enrichment cultures tested negative. Thus, interpreting Price (as Patentee does) to require that all samples be taken from the same lot is irrational and would defeat the purpose of the method. The reference would not have been so interpreted by person of ordinary skill. Patentee's unduly restrictive reading of Price ignores these practical considerations and is unreasonable.

c. The pre-enrichment medium of Price meets the definition of enrichment media in the'584 patent

The Patentee argues that the pre-enrichment medium of Price does not meet the '584 patent definition of enrichment media. (Response, p. 69). Patentee objects that the Examiner does not take into account the function of enrichment medium which is to "take target organisms to detectable levels." (*Id.*) The argument is misplaced, because as was well known in the art in August of 2004, the pre-enrichment cultures of Price would cause the microorganisms to reach detectable levels. (Bailey, ¶10e). In fact, Patentee states that "no one claim[s] that pre-enrichment in lactose broth does not allow for growth of *Salmonella*." (Response, p.70). Thus, Patentee admits that pre-enrichment broth allows for growth of bacteria.

Patentee asserts that the Price procedure does not allow it to grow to detectable levels. However, Patentee's "evidence" in this regard is limited to an argument about what Price may or may not have meant when he chose his terms. (Response, p. 70). More to the point, as

previously discussed, modern detection methods allow for rapid detection of as little as one cell in 1 ml of liquid. (Berri, p. 292). The independent claims of the '584 patent merely recite use of a "detection assay" without limitation. Accordingly, a person of ordinary skill in the art using a modern rapid detection method would be able to detect a target organism in the pre-enrichment culture of Price. Further, as the Examiner noted, "Price provides motivation to substitute selective enrichment broth for the pre-enrichment broth in the individual broth cultures." (ACP, p.23). Thus, even if one were to ignore the availability of sensitive rapid detection methods and conclude that the pre-enrichment medium of Price should be construed not to meet the '584 patent definition of enrichment medium, the Price teaching to use actually use enrichment medium certainly does.

d. Direct selective enrichment of Price is not limited to non-lumped pre-enrichment cultures

Patentee argues that the Price reference's teaching of substituting selective enrichment for pre-enrichment is only in an attempt to reduce the background of competing organisms. Patentee thus argues that the discussion in Price regarding direct selective enrichment "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 have fewer background/competitive organism problems." (Response, p. 79). Patentee supports this by citing to a passage in Price that cautions against lumping many food samples at the pre-enrichment stage. Patentee's argument is again incorrect.

Price cautions against the lumping of many separate test lots into a single pre-enrichment culture. However, that is not what Price is discussing while suggesting direct selective enrichment media may be used. In teaching the use of direct selective enrichment, Price teaches that in cases in which background organisms are interfering in a pre-enrichment broth, then an enrichment medium may be used. In other words, the pre-enrichment medium may be replaced at the first stage with enrichment medium. Price also makes clear that the samples incubated in

each individual jar should be drawn from same individual test lots. In other words, Price teaches only that samples from separate test lots should not be mixed in an initial composite sample – which is precisely what Patentee contends is required by his claims. Therefore, Price does not teach away from using selective enrichment media by incubating in a lumped sample as the Patentee suggests.

e. Price in combination with FSIS and Midura teaches a method for releasing product into commerce

Patentee asserts that the purpose of Price is not to release product, but only to determine the extent of contamination. (Response, p. 71). Once again, Patentee overlooks the combination of Price with FSIS and Midura. FSIS provides guidelines specifically to test food products for bacterial contamination. The purpose of this is to determine if the food is safe for release to the general public for consumption. Midura teaches that statistical sampling plans should be followed so that results are statistically significant to achieve the purpose for which the samples are being taken. (Midura, p.20). Price provides the methodology necessary to carry out the teachings of FSIS and Midura. Therefore, contrary to the Patentee's assertion, the combination of all three references does in fact teach retesting of negative samples to release the product.

Patentee also argues that the Price reference only shows a single portion from each sample being tested and thus it cannot be representative. This argument ignores the fact that the substances being tested by Price were powdered products that could be mixed to homogeneity. However, even if one were to make the irrational assumption that Price would have tested non-representative samples because of inadequate mixing (Response, p. 65), Midura teaches that multiple samples of bulk solid or semi-solid products should be taken. (Midura, p.20). Accordingly, the Price method in conjunction with Midura teaches taking a statistically significant number of samples so that the results of the presence/absence test are representative of the entire sample. Thus, the individual test lots depicted in the Price reference may be

validated for release upon retesting assuming that the enriched composite sample (also depicted in Figure 1 of Price) tests positive.

f. Price does not teach that the lots must be resampled

Patentee asserts that the combination of references teaches that the test lots must be resampled and so the references teach away from the alleged invention of the '584 patent. (Response, p.73). Patentee's assertion is belied by Figure 1 of Price. Price clearly illustrates that the pre-enrichment samples are retained and that these samples are retested. (Price, p. 680). Price does not teach or suggest that the underlying test lots must be resampled. Further, if, as Patentee asserts, it was well known in the art that the FSIS guidelines preclude resampling, then a person of ordinary skill in the art would not look to the cited FSIS protocol and violate it.

g. Price teaches the use of a representative sample

Patentee asserts the Examiner has misconstrued the Price reference in combination with the Midura reference. (Response, p. 64-65). Patentee asserts that Midura teaches that for bulk liquids multiple samples are required because of a possible lack of mixing. (Response, p. 65). Patentee further asserts that because Price only depicts one sample from each test lot, then Price does not provide a representative sample.

As discussed above, Patentee rests his argument on the non-sensical conclusion that a person of ordinary skill in the art would not ensure that the sample is mixed adequately. (Response, p. 65). Moreover, Midura teaches that if adequate mixing is not possible then multiple samples should be taken. (Response, p.65). Thus, Patentee disproves his own argument.

Patentee's argument also overlooks the nature of the products that Price used to prove the viability of his sampling and compositing procedures. In Price, the tested food products were of a particulate or powdered nature capable of being blended to homogeneity. As a consequence, a

single sample (illustrated in Price Figure 1 as a strip of product) was representative. (This sample would, of course, consist of numerous individual particles, which can be viewed as separate portions as defined in step (a) of the '584 patent claims.) In addition, Midura teaches that in testing bulk solids (like the powders of Price) "[a]liquots from several areas of the food under examination should be taken to ensure a representative sample." (*Id.*). Thus, Patentee's assertion that the combination of Price and Midura do not teach taking a plurality of samples from a test lot is incorrect.

Ground 3 of the Rejections (Response at pages 83-90)

The Examiner has maintained the rejections of claims 1 and 17 as unpatentable over Jarvis in view of the Patentee's Admissions. Specifically, the Examiner relies on Jarvis as in the Ground 1 rejection, but states Jarvis does not teach collecting plural portions from 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. As discussed in Requester's comments regarding Grounds 1 and 2, a person of ordinary skill would have read Jarvis and Price as teaching pooling of pre-enrichment cultures from samples derived from multiple test lots. Otherwise the procedures described in these references would not serve the purposes for which they were designed - namely to validate one or more uncontaminated lots by re-testing retained enrichment cultures.

The Examiner cites to portions of the Background section of the '584 patent for the teachings that Patentee argues to be missing from the reference. Specifically, in the discussion of well known sampling plans, the patent includes the statement that:

> The common feature of these sampling plans is that several samples representing production lots, or a number of environmental samples, are combined into a combined production lot prior to sampling. For example, a typical plan for microbial pathogen detection calls for taking more than one sample, for instance ten (10) to sixty (60) sample pieces from a given sample

> lot (combined production lot), to form a composite sample for testing.

('584 patent, col. 1, line 67 – col. 2, line 7). Patentee again asserts that the passage in the '584 patent "merely describes the art-recognized practice of sample lumping; that is, combining the samples from a lot to be tested." (Response, p.89). This is the same unpersuasive argument put forth by the Patentee in the previous response on pages 32-33.

As an initial matter, the Patentee ignores the plain language of the first quoted sentence, which clearly states "several samples representing production lots" (emphasis added). Therefore, samples of multiple production lots are correctly admitted to have been taught in the prior art. Second, the Patentee ignores the remaining passages cited by the Examiner including sentences stating, "[w]ith respect to *E. coli* O157 testing, for example, a typical plan calls for taking 60 samples (e.g., sample pieces) from a production lot to form a composite sample. With respect to *Listeria monocytogenes* thirty (30) samples are typically taken from a production lot to form a composite sample." ('584 patent, col.2, lls. 13-18). These sentences acknowledged that the prior art included taking multiple samples from a single production lot to form a composite sample. Single production lot is defined in the '584 patent as a form of test lot. ('584 patent, col.5, lls. 33-34.) A test lot is defined as an assemblage of one or more specimens of a medium or process…where such assemblage can be sampled by taking portions of the one or more specimens thereof…" ('584 patent, col. 5, lls. 21-23).

Accordingly, the plain language of the'584 patent admits that the prior art included combining multiple portions from a single test lot, each test lot consisting of an assemblage of one or more specimens, to form a composite sample. The Background section of the '584 patent further admits that the prior art included combining multiple production lots together to form another composite sample for testing. Thus, the Patentee's admissions in combination with the

Jarvis reference render claims 1 and 17 obvious and the Patentee has not refuted this combination.

With respect to Jarvis, Patentee essentially repeats the arguments that have been addressed in the Ground 1 rejection above. Specifically, the Patentee alleges that 1) Jarvis does not teach sampling from separate test lots, 2) Jarvis does not teach methods for validating test lots for release into commerce, 3) Jarvis does not teach a modular composite sample, and 4) the pre-enrichment medium of Jarvis is not sufficient to enrich the target organism within meaning of the '584 patent. These arguments have already been addressed under the Ground 1 rejection, and so they will not be repeated here. However, Requester does submit that in making these arguments Patentee is again distorting and improperly limiting the Jarvis reference.

Ground 5 of the Rejections (Response at pages 90 – 93)

The Examiner has maintained the rejection of claims 1 and 17 as unpatentable over Andrews in view of Price and Midura. Specifically, the Examiner states that the Andrews reference teaches methods of testing foods comprising collecting a plurality of portions 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. Andrews also teaches that samples should be inoculated into pre-enrichment broth and incubated to permit microorganisms to reach a desired number. Following this incubation, the pre-enrichment broth should be inoculated into a second enrichment medium selective for the target organism. The Examiner indicates that Andrews does not teach incubating the test lots separately. The Examiner also indicates that Midura teaches collecting a plurality of samples and/or performing multiple test runs.

1. **Patentee Argues the References Individually**

Patentee asserts that the combination of references does not teach or suggest the claims of the '584 patent. (Response, p. 91). With respect to the Price and Midura references, Patentee

asserts that they have been discussed in detail under Ground 2 and will not be repeated. (*Id.*). Patentee then states that the Andrews reference only demonstrates testing of a single test lot, and does not teach a growth medium that favors the growth of a target organism in a way that meets the limitations of the current claims. (Response, p. 92).

In asserting these arguments, the Patentee is again arguing the references individually. It is the combination of references that form the basis for the Ground 5 rejection. As discussed in the comments above regarding Ground 2, Price discloses the separate incubation the sample lots. Figure 1 of the Price reference explicitly shows the incubation of individual samples and the subsequent compositing of those cultures. Accordingly, Price in combination with Andrews teaches initially incubating the test lots separately.

The Patentee also asserts the "re-sampling runs" of Midura actually teach away from the '584 patent claims. Patentee notes that this has been discussed above under either the Ground 1 or Ground 2 rejection. (Response, p. 92). Again, the Patentee fails to argue the references in combination. Midura in combination with Price and Andrews clearly teaches the "re-sampling" is to be done with the saved pre-enrichment cultures as taught by Price.

2. The Patentee Misconstrues the Andrews Reference

Patentee asserts that the Andrews reference, alone or in combination with Price and Midura, does not disclose "pre-enrichment medium" and incubation therein as a medium that favors the growth of the target organism in a way that meets the limitations of the presently amended claims. (Response, p. 92). The pre-enrichment medium disclosed by the Price reference has been discussed in detail under the Ground 2 rejection and that discussion will not be repeated here. With respect to the Andrews reference, Patentee only states that "Andrews teaches a requirement for enrichment in selective medium (Andrews at 37.124) prior to testing by selective plating (Andrews at 37.124)."

However, Andrews also suggests using pre-enrichment medium. (Andrews, p.360, §37.123). Moreover it is clear that the pre-enrichment medium of Andrews meets the limitations of the '584 patent claims which, even taking into account the amendments presented after the ACP, only require growth to detectable levels. Specifically, Andrews discloses that "*Salmonella* organisms in foods are often present in low numbers and in a debilitated condition. As indicated previously, pre-enrichment in a non-selective medium obviates this problem..." Here Andrews implies that it may be difficult to detect *Salmonella* because of low numbers. However, Andrews then provides a solution by teaching that growth in pre-enrichment medium will fix this problem. In other words, Andrews indicates that growth in pre-enrichment will result in greater numbers of *Salmonella*.

Andrews continues by stating, "North [citation omitted] first noted enhanced recovery of *Salmonella* from egg albumen as a result of pre-enrichment in lactose broth." (Andrews, p.360). For North to note the enhanced recovery of *Salmonella* as a result of pre-enrichment, North necessarily had to have been able to detect the *Salmonella*.

Finally, Andrews also states that, "wet compositing (combining pre-enrichment cultures of individual analytical units) [citation omitted]substantially reduces the analytical workload without compromising method sensitivity." (Andrews, p.360). This statement clearly indicates that analysis for the presence of a target microorganism, in this case *Salmonella*, is possible by testing pre-enrichment broth. Therefore, the pre-enrichment broth of Andrews meets the limitations of the '584 patent by growing bacteria to "detectable levels."

Ground 6 of the Rejections (Response at pages 93 – 99)

The Examiner has maintained the rejection of claims 1 and17 as unpatentable over Silliker in view of Price and Midura. The Examiner states that it would have been *prima facie* obvious to follow the composite-testing procedures disclosed by Silliker with individual tests of the pre-

enrichment cultures upon obtaining a positive result for the composite subsample, as suggested by Price. The Examiner also states that a person of ordinary skill in the art would have found it obvious to collect a plurality of samples, as recommended by Midura, to detect highly pathogenic organisms present at low levels.

1. Patentee Simply Repeats Unpersuasive Arguments

The Patentee asserts that Silliker, alone or in combination with Price and Midura, does not teach assaying separate test lots, and instead teaches taking samples from a single underlying test lot. (Response, p.95.) However, the Patentee does not offer any evidence to support this assertion. In fact, the combined teachings of Price and Midura do teach taking samples from separate test lots as discussed above.

As in his response to the first Office Action (First Response, p. 37), Patentee again argues that Silliker does not teach that pre-enrichment pooling of lumped samples is as reliable as pre-enrichment of non-lumped samples. The Examiner has already rejected this argument. (ACP, p.32.) The Patentee attempts to rebut the Examiner's rejection by arguing that the quoted statement by Silliker is inconsistent with Silliker's own data in Table 1. (Response, p.96.) However, Patentee has offered no explanation as to how or why the data is inconsistent.

Silliker's purpose was to compare various analytical methods for detecting *Salmonella* contamination. The results of this comparison are shown in Table 1. (Silliker, p. 477.) The data in this table shows that sensitivity is not lost when separate pre-enriched test lots are composited. For example, Test 1 illustrates that when subsamples are composited into a pre-enrichment culture 59 out of 60 pre-enrichment cultures test positive for *Salmonella*. Test 1 further demonstrates that when these separate test lots composed of subsamples are composited into a wet composite 12 out of 12 composited cultures test positive. Thus, Silliker's statement that "pooling sixty 25-g or fifteen 100-g samples 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" is consistent with the data in Table 1. (Silliker, p. 478.)

2. The Patentee Misconstrues the Siliker Reference

The Patentee again asserts that the pre-enrichment medium of Silliker is not a medium that favors the growth of the target organism in a way that meets the '584 patent claim limitations. (Response, p.95.) Patentee provides nothing beyond the argument that he previously submitted. (First Response, p. 38-39.)

Silliker does, in fact, disclose a pre-enrichment medium that meets the limitation of the '584 patent claims. First, it is well known that a pre-enrichment broth is capable of growing a target organism to detectable levels. (Bailey Decl. ¶9d.) Second, Silliker indicates that following incubation, 1 mL of the pre-enrichment growth is transferred into a selective enrichment broth. After incubation in the selective broth, the organisms are detected via conventional plating techniques. For this detection by conventional plating procedures to occur, the original pre-enrichment broth would have had to contain a detectable number of organisms.

Patentee further argues that Patentee's modular composite sample is fundamentally different from the prior art references. (Response, p.98.) Patentee asserts that the modular composite sample embodied by the '584 patent claims is "formed by combining aliquots from each of a plurality of statistically significant test lot cultures to enable independent validation for release of each of the respective underlying plurality of separate test lots. (*Id.*) However, Patentee is incorrect in this assertion.

Patentee's modular composite sample is nothing more than an aggregation of steps that was already known in the prior art. Silliker teaches that aliquots can be combined from a plurality of separate test lot cultures enriched for the target organism within the meaning of the

'584 patent. Price teaches retesting of saved individual pre-enrichment cultures to validate negative test lots for release. Midura teaches that statistically significant numbers of samples should be taken. Accordingly, these three references teach the modular composite sample of the '584 patent.

Ground 7 of the Rejections (Response at pages 99 – 102)

The Examiner has maintained the rejection of claims 1 and 17 as unpatentable over Gabis in view of Price and Midura. The Examiner concluded that it would have been *prima facie* obvious to follow the composite testing disclosed by Gabis with the individual tests of pre-enrichment broths following a positive result for the composite subsample as disclosed by Price. Midura is cited because it would direct a person of ordinary skill in the art to collect a plurality of samples to detect low levels of target organisms.

In his efforts to rebut this rejection, Patentee relies primarily on previously asserted arguments concerning Price and Midura as stated in the Ground 1 and 2 rejections. (Response, p. 100.) Requester believes the deficiencies of these arguments have already been addressed.. Patentee's additional arguments concerning the Gabis reference are addressed below.

1. **The Combination of References Teaches Sampling from Separate Test Lots**

The Patentee characterizes the Examiner's rejection as based on a teaching by Gabis that each of the specimens sampled from a single lot is inoculated into individual pre-enrichment cultures. (Response, p. 100.) Patentee distorts the basis for the rejection and once again fails to address the Examiner's reliance on the combined teachings of the references.

Price teaches the use of individual samples as explicitly demonstrated in Figure 1. Midura teaches that when working with solid or semi-solid foods multiple aliquots from several areas of the food under examination should be taken. Accordingly, it is clear from these two references that the initial samples used in Gabis protocol would be the product of compositing

several samples from each individual lot. Thus in combination, Gabis, Price, and Midura teach initially compositing test lot samples. Midura also teaches that sampling plans should be developed for obtaining representative composite samples from "each lot," i.e. separate lots, of product. (Midura at 16.) Further, a person of ordinary skill in the art would understand that these procedures were to be applied to a plurality of separate test lots. (Bailey Decl., ¶20).

2. Patentee Misconstrues the Gabis Reference

a. The Gabis pre-enrichment medium sufficiently enriches to provide detection of target organisms

The Patentee asserts that the pre-enrichment samples of Gabis are not sufficiently enriched to provide detection of the target organism. (Response, p. 100.) Patentee has previously asserted this argument. (First Response, p. 41.)

Gabis discloses a pre-enrichment medium that meets the limitation of the '584 patent claims. First, it is well-known in the art that pre-enrichment broth is capable of growing a target organism to detectable levels. (Bailey Decl. ¶9d). Cultures in such pre-enrichment broths are also substantially uniform. (*Id.*). In addition, Gabis indicates that following incubation, 1 mL of the pre-enrichment culture is transferred into a selective enrichment broth. After incubation in the selective broth, the organisms are detected via conventional plating techniques. For this detection to have occurred, the original pre-enrichment broth would have had to contain a detectable number of organisms. (Bailey Decl., ¶20.)

b. Gabis teaches post-enrichment pooling

The Patentee asserts that the Gabis reference does not teach post-enrichment pooling. (Response, p.101). However, as discussed above, the pre-enrichment cultures of Gabis are sufficient to detect organism growth and are substantially uniform. Therefore, the inoculation of individual pre-enrichment cultures into enrichment broth corresponds to post-enrichment pooling within the meaning of the '584 patent claims.

c. Gabis teaches the modular composite sample of the '584 patent

Patentee further argues that Patentee's modular composite sample is fundamentally different from the prior art references. (Response, p.101-2). Patentee asserts that the modular composite sample embodied by the '584 patent claims is "formed by combining aliquots from each of a plurality of statistically significant test lot cultures to enable independent validation for release of each of the respective underlying plurality of separate test lots. (*Id.*). However, Patentee is incorrect in this assertion.

Patentee's modular composite sample is nothing more than an aggregation of steps that was already known in the prior art. Gabis teaches that aliquots can be combined from a plurality of separate test lot cultures. Price teaches retesting of saved individual pre-enrichment cultures to validate negative test lots for release. Midura teaches that statistically significant numbers of samples should be taken. Accordingly, these three references teach the modular composite sample of the '584 patent.

Ground 8 of the Rejections (Response at pages 102 – 106)

The Examiner has maintained the rejection of claims 1 and 17 as unpatentable over Jarvis in view of BAM. The Examiner states that it would have been *prima facie* obvious to collect a plurality of food samples as taught by BAM to test by the method of Jarvis. The Patentee traverses this rejection by repeating his previous erroneous arguments regarding Jarvis.

1. The Patentee Argues the References Individually

The Patentee argues that Jarvis does not teach statistical validation of individual pre-enrichment cultures. Patentee asserts that statistical verification means validation of no contamination by means of a number of samples sufficient for a level of statistical confidence. (Patentee's Resp. at 104). The Patentee then argues the exact same points, in the exact same words, as in the Patentee's First Response. (First Response, p. 43-44.) The Examiner has already

found this argument unavailing. (ACP, p.38). Patentee fails to address the BAM and Jarvis references in combination. The references teach collecting a sufficient number of samples to verify individual pre-enrichment cultures statistically. The BAM reference cautions that if the samples are "not representative of the sampled lot, the laboratory results will be meaningless." (BAM at 1). BAM further states, "[t]he number of units that comprise a representative sample from a designated lot of a food product must be statistically significant." (*Id.*). Accordingly, a person of ordinary skill in the art would have known from BAM that for the individual pre-enrichment cultures retested in Jarvis to have statistical significance, collection of a statistically significant number of samples would be required.

Patentee further argues that BAM does not teach beyond collecting a plurality of samples at random from a single lot and lumping the collected samples or portions into a composite test lot sample. (Response, p.104). Patentee offers no evidence to support this assertion. In addition, Patentee fails to acknowledge the contributions of Jarvis to the BAM reference. As discussed above under the Ground 1 rejection, Jarvis clearly teaches the sampling of separate test lots.

The Patentee also asserts that Jarvis in combination with BAM does not teach: (i) initial formation of a plurality of statistically sufficient composited/lumped test lot samples from each of a respective plurality of separate underlying test lots; that (ii) are enriched to above the detection limit as present claimed prior to (iii) pooling to form the modular composite samples that can be tested immediately; wherein (iv) the individual statistically sufficient enriched composited/lumped test lot samples/cultures can be also be tested immediately; and (v) 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...and obtaining a negative test result." The Patentee continues that Jarvis and BAM do not teach post-enrichment pooling, post-enrichment inter-lot pooling, or validation of negative results. The Patentee offers

no argument as to why the combination of Jarvis and BAM fail to teach these combinations. Presumably the Patentee is relying on previous arguments offered to refute the Jarvis reference. These arguments are unavailing for the reasons discussed above in connection with Ground 1.

2. Patentee Misconstrues the Jarvis Reference

The Patentee asserts that even if Jarvis teaches verification of positive samples by retesting stored individual pre-enrichment cultures, it does not provide for statistical validation of no contamination of individual pre-enrichment cultures. According to the Patentee, this is because Jarvis (and all other prior art methods) involve sampling of a single lot and intra-lot pooling. Under Patentee's theory, samples are taken from a lot and lumped in a non-statistically significant way into pre-enrichment cultures. These cultures are then combined and tested with the result being statistically significant only for the entire lot as a whole. As discussed in greater detail above in connection with Ground 1, this reading of Jarvis is inconsistent with the purpose of the Jarvis reference.

The Jarvis reference is directed to methods to prove the statistical validity of food testing procedures. Thus, Patentee's assertion that Jarvis does not provide for the statistical validation of individual pre-enrichment cultures is clearly erroneous. There would be no purpose for Jarvis to suggest retesting of the stored pre-enrichment cultures if such retesting would be solely for the purpose of "curiosity." To suggest otherwise eviscerates the very purpose of the Jarvis teachings.

Ground 9 of the Rejections (Response at pages 106 – 114)

The Examiner has maintained the rejection of claims 1 and 17 as unpatentable over Price in view of BAM and Davies. The Examiner found that it would have been *prima facie* obvious to collect 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 Examiner further concluded that a person of ordinary skill would have also

found it obvious to inoculate each of the test lot samples into separate enrichment broth cultures, to incubate them to allow the target organism to reach detectable levels and then pool the aliquots for testing.

Patentee argues that the references, in particular Davies, do not teach a modular composite sample. (Response, p.109). The Patentee asserts the unique feature of the modular composite sample disclosed in the '584 patent is that it is comprised of a plurality of lumped test lot samples each attributable to respective plurality of underlying test lots. (Response, p.112). Patentee's assertion is erroneous. Setting aside that Price, as discussed above, also teaches a modular composite sample matching the '584 patent description, it is clear that Davies explicitly discloses taking samples from a plurality of test lots.

Davies discloses sampling 20 different pig herds and collecting a plurality of samples from each pig sampled. (Davies, p. 1017). Davies also indicates that each herd was being treated as a unit rather than as individual animals, and so confirms the use of the term "test lot" as defined in the '584 patent. (Davies, p. 1018). BAM teaches that the number of samples should be representative and statistically significant. (BAM, p. 1). Once these samples are taken, the method of Price teaches the samples from each individual pig should be incubated in pre-enrichment medium separately from the samples taken from other pigs. Following incubation, an aliquot of each pre-enrichment broth correlating to an individual pig is then combined into one selective enrichment broth. This procedure corresponds to the modular composite sample that Patentee asserts is not made in this combination of references. In addition, if the sample tests positive, then Price teaches to retest each individual pre-enrichment test. The results of these numbers will be statistically significant due to the BAM reference's teaching to take sufficient samples for statistical significance.

Patentee asserts that Patentee's alleged invention is unique because it describes a modular composite sample that is not found in the prior art. (Response, p. 112). However, as discussed above (*see,* for example, Ground 1 §4(a)), Patentee's modular composite sample is no more than an aggregation of steps that was already known in the prior art. Davies teaches aliquots can be combined from a plurality of separate test lot cultures enriched for the target organism within the meaning of the '584 patent. Price teaches retesting of saved individual pre-enrichment cultures to validate negative test lots for release. Midura teaches that statistically significant numbers of samples should be taken. Accordingly, these three references teach the modular composite sample of the '584 patent.

Patentee again asserts that the cited combination of references decreases the work and expense of testing whereas the method of the '584 patent increases them. (Response, p. 113). Patentee is again arguing features of the claims that are not present. That is there is no requirement for increased cost or work in the '584 patent claims. Patentee's assertion is further addressed under the Ground 1 rejection.

Ground 14 of the Rejections (Response at pages 115 – 118)

The Examiner has maintained the rejection of claims 5, 6, 23, 29, and 30 as unpatentable over Jarvis in view of FSIS and Midura and further in view of Entis. The Examiner asserts that it would have been *prima facie* obvious to concentrate the target agent from the cultures described by Jarvis in view of FSIS and Midura, as taught by Entis, in order to increase testing sensitivity and negate interference from the food sample. The Examiner also found that a person of ordinary skill in the art would have also found it *prima facie* obvious to have used one or more of the rapid detection methods described in Entis to identify the target agent because they are more sensitive and more efficient.

The Patentee does not address the Ground 14 rejections directly. Instead, the Patentee asserts that it has already effectively rebutted Jarvis in view of FSIS and Midura with respect to independent claims 1 and 17. The Patentee further asserts that Entis does not cure the previously alleged deficiencies. Accordingly these rejections are not traversed and should be maintained. Requester believes the Patentee's arguments with respect to Jarvis in view of FSIS and Midura have already been addressed with respect to claims 1 and 17. Moreover, Requester Entis provides additional rationale for those rejections.

Under the Ground 1 rejection, Patentee asserts that the pre-enrichment broth of Jarvis does not meet the '584 claim limitations, because it does allow target organism growth to detectable levels. In addition to the defects in this argument set forth above, Requester submits that Entis provides further support for the rejections. Entis is directed to rapid methods for the detection of microorganisms. Entis, for example, discloses the use of PCR. Entis notes:

> The appeal of PCR technology lies in the amplification of small quantities of DNA to detectable levels in a short amount 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 lengthy enrichment step is possible. In practice, however, direct detection of a single gene target is difficult to achieve. The small sample volumes used in PCR (microliter-scale) limit the sensitivity of the procedure for examining foods directly. In addition, inhibitory substances that interfere with the PCR are present in many foods. Thus, a preliminary enrichment culture step to increase target levels is common in PCR detection protocols. Enrichment culture also provides some assurance that the DNA detected is derived from viable cells. The superior sensitivity of the PCR compared to conventional culture methods may allow for shorter enrichment times, however.

(Entis, p. 94). This passage indicates that upon enrichment of the target sample in pre-enrichment culture, the target organism is detectable by the PCR technique. The '584 patent specifically contemplates the use of DNA based detection methods. ('584 patent, col. 8, line 14). Thus, despite the Patentee's arguments to the contrary, the pre-enrichment cultures of Jarvis are sufficient to allow for detection within the meaning of

the '584 patent. This also means that Jarvis teaches the pooling of enriched cultures within the meaning of the '584 patent.

The Patentee's remaining arguments regarding the deficiencies of Jarvis are further addressed by application of Midura and FSIS as discussed in Ground 1. Accordingly, Patentee has not rebutted the Examiner's *prima facie* case of obviousness and the rejection of claims 5, 6, 23, 29, and 30 as unpatentable over Jarvis in view of FSIS and Midura and further in view of Entis should be maintained.

Ground 15 of the Rejections (Response at pages 118 – 121)

The Examiner has maintained the rejection of claims 5, 23, 29, and 30 as unpatentable over FSIS in view of Price, Midura, and Entis. The Examiner states that it would have been *prima facie* obvious to have concentrated the target agent from the cultures as disclosed by FSIS in view of Price and Midura, as taught by Entis, in order to increase testing sensitivity and negate interference from the food sample. The Examiner further found that a person of ordinary skill in the art would have found it prima facie obvious to have used one or more of the rapid detection methods described in Entis to identify the target agent because they are more sensitive and more efficient.

The Patentee does not address the Ground 15 rejections directly. Instead, the Patentee asserts that it has already effectively rebutted FSIS in view of Price and Midura with respect to independent claims 1 and 17. The Patentee then asserts that Entis does not cure the previously alleged deficiencies. Accordingly, these rejections are not traversed and should be maintained.

Requester believes the Patentee's arguments with respect to FSIS in view of Price and Midura have already been addressed with respect to claims 1 and 17. However, Requester asserts the addition of Entis adds additional rationale for the rejections

discussed in connection with the Ground 14 rejection above. Requester will not repeat those arguments here, but notes they are equally applicable to the Ground 15 rejection.

Ground 21 of the Rejections (Response at pages 122 – 124)

The Examiner has maintained the rejection of claim 21 as unpatentable over Jarvis in combination with FSIS, Midura, and Cousin. The Examiner states that it would have been *prima facie* obvious to have used the method disclosed by Jarvis in view of FSIS and Midura to test for a spoilage organism, such as a psychrotoph, because Cousin teaches that such organisms are responsible for spoilage of refrigerated foods and can be detected by culture.

The Patentee asserts that is has already effectively rebutted Jarvis, in view of FSIS and Midura with respect to independent claims 1 and 17. The Patentee then asserts that Cousin does not cure these deficiencies. Requester believes the Patentee's arguments with respect to Jarvis in view of FSIS and Midura have already been addressed with respect to claims 1 and 17 under the Ground 1 Rejection.

Patentee does not address the specific rejection of dependent claim 21 as set forth in Ground 21. Accordingly, this rejection is not traversed and should be maintained.

Ground 22 of the Rejections (Response at pages 124 – 126)

The Examiner has rejected claim 21 as unpatentable over FSIS in combination with Price, Midura, and Cousin. The Examiner states it would have been *prima facie* obvious to have used the method disclosed by FSIS in view of Price and Midura to test for a spoilage organism, such a psychrotoph, because Cousin teaches that such organisms are responsible for spoilage of refrigerated foods and can be detected by culture.

The Patentee asserts that is has already effectively rebutted FSIS in view of Price and Midura with respect to independent claims 1 and 17. The Patentee then asserts that

Cousin does not cure these deficiencies. Requester believes the Patentee's arguments with respect to FSIS in view of Price and Midura have already been addressed with respect to claims 1 and 17 under the Ground 2 Rejection.

Patentee does not address the specific rejection of dependent claim 21 as set forth in Ground 22. Accordingly, this rejection is not traversed and should be maintained.

Ground 23 of the Rejections (Response at pages 126 – 128)

The Examiner has maintained the rejection of claim 23 as unpatentable over Jarvis in view of FSIS, Midura, and Price. The Examiner states that it would have been *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 immunochemical method as described by Price.

The Patentee asserts that he has already effectively rebutted Jarvis, in view of FSIS and Midura with respect to independent claims 1 and 17. The Patentee then asserts that Price does not cure these deficiencies. Requester believes the Patentee's arguments with respect to Jarvis in view of FSIS and Midura have already been addressed with respect to claims 1 and 17 under the Ground 1 Rejection.

Patentee does not address the specific rejection of dependent claim 23 as set forth in Ground 23. Accordingly, this rejection is not traversed and should be maintained.

Ground 24 of the Rejections (Response at pages 128 – 131)

The Examiner has maintained the rejection of claims 24, 28, 32, and 33 as unpatentable over FSIS in combination with Price, Midura, and Gabis. The Examiner states it would have been *prima facie* obvious 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 taught that the individual enrichment broth

culture may or may not contain a selective agent for the target organism. The Examiner also found that 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.

The Patentee asserts that is has already effectively rebutted FSIS in view of Price and Midura with respect to independent claims 1 and 17. The Patentee then asserts that Gabis does not cure these deficiencies. Requester believes the Patentee's arguments with respect to FSIS in view of Price and Midura have already been addressed with respect to claims 1 and 17 under the Ground 2 Rejection.

Patentee does not address the specific rejection of dependent claims 24, 28, 32, and 33 as set forth in Ground 24. Accordingly, this rejection is not traversed and should be maintained.

Ground 25 of the Rejections (Response at pages 131 – 133)

The Examiner has rejected claim 28 as unpatentable over Jarvis in view of FSIS, Midura, and Andrews. The Examiner states that it would have been *prima facie* obvious to have included a selective agent in the pre-enrichment broth cultures of Jarvis in view of FSIS and Midura, because Andrews teaches that the use of two or more selective media improves sensitivity.

The Patentee asserts that is has already effectively rebutted Jarvis, in view of FSIS and Midura with respect to independent claims 1 and 17. The Patentee then asserts that Andrews does not cure these deficiencies. Requester believes the Patentee's arguments with respect to Jarvis in view of FSIS and Midura have already been addressed with respect to claims 1 and 17 under the Ground 1 Rejection. It should also be noted that the teachings of Andrews, as set forth under the Ground 5 response, further supports the position that the pre-enrichment broth of Jarvis allows for organism growth to detectable levels.

Patentee does not address the specific rejection of dependent claim 28 as set forth in Ground 25. Accordingly this rejection is not traversed and should be maintained

Conclusion

In view of the above comments, it is respectfully requested that the rejection of claims 1-33 be maintained. It is also requested that the Examiner exclude the newly submitted Declarations for failure to comply with 37 CFR §1.116.

Date: 3-2-2011

Respectfully submitted,

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	95001294
Filing Date	2010-04-05
First Named Inventor	Mansour Samadpour
Art Unit	3991
Examiner Name	Brenda G. Brumback
Attorney Docket Number	66090-008US2

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Art Unit	3991
Examiner Name	Brenda G. Brumback
Attorney Docket Number	66090-008US2

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached certification statement.

☐ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Barry L. Davison, Ph.D., J.D./	Date (YYYY-MM-DD)	2011-05-31
Name/Print	Barry L. Davison, Ph.D., J.D.	Registration Number	47309

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**