

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

NATIONAL BEEF PACKING COMPANY, LLC,
Petitioner

v.

INSTITUTE FOR ENVIRONMENTAL HEALTH, INC.,
Patent Owner

Case No. IPR2024-01156
Patent No. 9,845,486

DECLARATION OF JAMES S. DICKSON, PH.D.

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I. INTRODUCTION

I, James S. Dickson, Ph.D., declare as follows:

A. Engagement

1. I have been retained by Petitioner National Beef Packing Company, LLC (“National Beef”) to provide expert opinions in connection with a petition requesting *inter partes* review (IPR) of a patent owned by Institute for Environmental Health, Inc. (“IEH” or “Patent Owner”), U.S. Patent No. 9,845,486 (“the ’486 Patent” or the “IEH Patent”). The IEH Patent lists Mansour Samadpour as the inventor.
2. I have been asked to review relevant materials and render my opinions regarding the validity of various claims of the IEH Patent. I have used my education and decades of experience working in the fields of food science, microbiology, and food safety, and my understanding of the knowledge, creativity, and experience of a person of ordinary skill in the art of the IEH Patent in forming the opinions expressed in this declaration.
3. I am being compensated for my time in developing and rendering my opinions in this matter at my normal consulting rate for my work plus actual expenses. I have no financial interest in the outcome of this or any related proceeding. My compensation is not dependent upon the opinions that I am providing in this declaration or the results of this or any other proceeding relating to the IEH Patent.

4. Between now and any time that I may be asked to testify further, I expect to continue my review, evaluation, and analysis of evidence presented before and/or at the hearing. I also expect to review any additional declarations and other evidence submitted by IEH. I reserve the right to amend or supplement this declaration, as appropriate, after considering the opinions set forth by other experts. If additional relevant information becomes available to me, I also reserve the right to review and consider that information in further developing, rendering, or refining my opinions.

B. Background and Qualifications

5. I have personal knowledge of the facts stated in this declaration and could competently testify to them if asked to do so.

6. A copy of my latest *curriculum vitae* (CV) is submitted with this declaration as Exhibit 1021, which includes my educational background, work history, and a listing of my publications. The following paragraphs briefly summarize my relevant expertise.

7. I am currently the Morrison Endowed Chair in Meat Science and a professor in the Department of Animal Science at Iowa State University. Prior to a reorganization, I held a joint appointment as a professor in both the Department of Food Science and Human Nutrition and the Department of Microbiology, Immunology, and Preventive Medicine at Iowa State University. I primarily study

the microbiological safety of foods of animal origins with a particular focus on Gram negative bacteria, such as *Salmonella* and *E. coli*.

8. I received a B.S. in Microbiology from Clemson University in 1977, an M.S. in Dairy Science from the University of Georgia in 1980, and a Ph.D. in Food Science and Technology from the University of Nebraska–Lincoln in 1984. While working on my Ph.D., I researched the microbiology of meat and published my doctoral dissertation on the impact of certain food safety interventions on ground beef products.

9. After obtaining my doctorate, I worked for several years in industry as a food safety manager and microbiologist. Subsequently, from 1987–1993, I worked at the USDA as a research food technologist and lead scientist studying the hazards and risks of meat and poultry processing, including pathogen reduction methods.

10. In addition to my educational background and work history, I actively participate in various professional societies and organizations, including the American Society for Microbiology, the Institute for Food Technologists, and the International Association for Food Protection.

11. I have authored 150 peer-reviewed journal and conference publications and numerous books and book chapters. Some of my representative publications include:

- (1) Dickson, J.S. 1991. Attachment of *Salmonella typhimurium* and *Listeria monocytogenes* to beef tissue: Effects of inoculum level, growth temperature and bacterial culture age. Food Microbiology 8:143–151;
- (2) Dickson, J.S., G.R. Siragusa and J.E. Wray, Jr. 1992. Predicting the growth of *Salmonella typhimurium* on beef using the temperature function integration technique. Appl. Environ. Micro. 58:3482–3487;
- (3) Brodsky, M.H., C.A. Davidson, J.S. Dickson, M.J. Pettis and T.L. Tieso. 1995. Delayed incubation method for bacteriological analysis of environmental specimens and samples. Journal of Food Protection 58:879–883;
- (4) Erdmann, J.J., J.S. Dickson and M.A. Grant. 2002. A new technique for *Escherichia coli* testing of beef and pork carcasses. Journal of Food Protection 65:192–195;
- (5) Niebuhr, S. and J.S. Dickson. 2002. Impact of pH Enhancement on the Populations of *Salmonella*, *Listeria* and *Escherichia coli* O157:H7 in Boneless Lean Beef Trimmings. Journal of Food Protection 66:874–877;
- (6) Marshall, K.M., S.E. Niebuhr, G.R. Acuff, L.M. Lucia and J.S. Dickson. 2005. The identification of *Escherichia coli* O157:H7 meat processing indicators for fresh meat through the comparison of the effects of selected anti-microbial interventions. Journal of Food Protection 68: 2580–2586;

(7) Cabrera, E.T. Moseley, L. Lucia, J.S. Dickson, A. Castillo and G.R. Acuff. 2009. Fluorescent protein-marked *Escherichia coli* biotype I strains as surrogates for enteric pathogens in validation of beef carcass interventions. *Journal of Food Protection* 72:295–303;

(8) Consortium of Validation Experts (J.S. Dickson as corresponding author). 2012. Validation of Antimicrobial Interventions for Small and Very Small Processors: A How-to Guide to Develop and Conduct Validations. *Food Protection Trends* 33(2):95–104; and

(9) Redemann, M.A., J. Brara, S.E. Niebuhr, L.M. Lucia, G. R. Acuff, J.S. Dickson, M. Singh. 2018. Evaluation of thermal process lethality for non-pathogenic *Escherichia coli* as a surrogate for *Salmonella* in ground beef. *LWT – Food Science and Technology* 90:290–296.

12. In addition to my publications, I have been invited to present and discuss my work at over 150 seminars, including:

(a) Dickson, J.S. 2018. *Salmonella* and Roaster Pigs. Center for Disease Control and Prevention. Atlanta, GA 19 January 2018;

(b) Dickson, J.S. 2019. Impact of slow come up times and process deviations on bacteria on processed meats. North American Meat Institute Meat Industry Food Safety Conference. Rosemont, IL 04–05 September 2019;

(c) Dickson, J.S. 2023. Building the Scientific Support or Alternative Support for Lethality and Stabilization. USDA FSIS Food Safety Summit. Ames, IA 20 April 2023; and

(d) Dickson, J.S. 2023. Food Safety Challenges to the Meat and Poultry Industry: Where We Came From and Where We're Going. The National Provisioner. Ames, IA 11 July 2023.

13. Furthermore, I am an inventor on an issued patent related to a method of reducing contamination of animal carcasses during slaughtering.

14. I have been a member of various boards and committees, including:

- (a) Editorial Board Member, Journal of Food Protection (1988–present);
- (b) Editorial Board Member, Journal of Food Safety (1990–present);
- (c) Committee Member, National Advisory Committee on Microbiological Criteria for Foods (1998–2000, 2018–2020, 2022–2023);
- (d) Committee Member, National Advisory Committee for Meat and Poultry Inspection (2007–2010);
- (e) Chair, ISO US Mirror Committee, Technical Committee 34, sub-committee 17 (Food Safety Management Systems); and
- (f) Member, ISO US Mirror Committee, Technical Committee 34, sub-committee 9 (Microbiology).

15. Throughout my career I have received numerous honors and awards. Notably, during my time at the USDA, I was awarded several Certificates of Merit for my research contributions to the FSIS and the beef industry. I was elected as a Fellow in the American Academy of Microbiology in 1994. Additionally, in 2002 I served as the President of the International Association for Food Protection (IAFP), the leading industry organization for food safety professionals, and in 2022 I was elected as a Fellow in the IAFP. Most recently, in 2023 I received the Achievements in Microbial Research for Food Safety Award in honor of Gerhard J. Haas from the Institute of Food Technologists in recognition of my innovative research in microbial aspects of food safety assurance resulting in a positive impact and advancement of the public safety of the food supply.

16. Based on my experience, I am an expert in the fields of food science, microbiology, food safety, and the technology at issue in the IEH Patent.

C. Information Considered

17. To render my opinions in this matter, I have carefully reviewed the specification and claims of the IEH Patent as well as its prosecution history documents. These patent documents include:

- a. Exhibit 1001 – U.S. Patent No. 9, 845,486 B2;
- b. Exhibit 1003 – File History for Provisional Appl. No. 60/470,975;
- c. Exhibit 1004 – U.S. Patent No. 7,531,163 (“the ’163 Patent”);

- d. Exhibit 1005 – File History for the '163 Patent (Appl. No. 10/848,253);
- e. Exhibit 1006 – File History for the '486 Patent (Appl. No. 12/360,646);
- f. Exhibit 1007 – U.S. Patent No. 10,724,068 (“the '068 Patent”); and
- g. Exhibit 1008 – File History for the '068 Patent (Appl. No. 15/844,407).

18. My analyses are based on my years of education, research, and work experience, as well as my investigation and study of relevant materials. In my analyses, I have reviewed the publications listed below:

- a. Exhibit 1009 – Judith M. Anderson and A. C. Baird-Parker titled “A Rapid and Direct Plate Method for Enumerating *Escherichia Coli* biotype I in Food” and published in the Journal of Applied Bacteriology, Volume 39, Number 2, October 1975, pages 111-117 (“Anderson”);
- b. Exhibit 1010 – M. K. Rayman et al., “ICMSF methods studies. XIII. An international comparative study of the MPN procedure and the Anderson–Baird-Parker direct plating method for the enumeration of *Escherichia coli* biotype I in raw meats” and published in the Canadian Journal of Microbiology, Volume 25, Number 11, November 1979, on pages 1321-1327 (“Rayman”);
- c. Exhibit 1011 – “An Evaluation of the Role of Microbiological Criteria for Foods and Food Ingredients” released by the U.S. National Research Council, Food Protection Committee, Subcommittee on Microbiological Criteria and published by the National Academy Press in 1985 (“NRC”);
- d. Exhibit 1012 – European Patent Application, EP 1,253,203 A1 to Nagar, which was published on October 30, 2002 (“Nagar”);
- e. Exhibit 1013 – “Microorganisms in Foods 2: Sampling for Microbiological Analysis: Principles and Specific Applications,” Int’l Comm’n on Microbiological Spec’s for Foods, Univ. Toronto Press, Glossary (2nd ed. 1986) (“ICMSF 1986”) or (“ICMSF 2”);

- f. Exhibit 1014 – “Microorganisms in Foods 7: Microbiological Testing in Food Safety Management,” Int’l Comm’n on Microbiological Spec’s for Foods (ICMSF), Kluwer Academic/Plenum Publishers, Chapters 6, 10, 12, 15–17 (2002) (“ICMSF 2002”), (“ICMSF 7), or (“ICMSF”);
- g. Exhibit 1015 – International Publication No. WO 94/21816 to Hird, which was published on September 29, 1994 (“Hird”);
- h. Exhibit 1016 – International Publication No. WO 01/12853 A1 to Karlsen, which was published on February 22, 2001 (“Karlsen”);
- i. Exhibit 1017 – *Pathogen Reduction; Hazard Analysis and Critical Control Point (HACCP) Systems*. 61 Fed. Reg. 38806 (July 25, 1996). (“PR/HACCP Regulations,” just “PR/HACCP” or (“MegaReg”);
- j. Exhibit 1018 – U.S. Food & Drug Admin., “Bacteriological Analytical Manual,” Table of Contents, Introduction, About the Bacteriological Analytical Manual, Chapters 1, 5, 10 (June 2003) (“BAM 2003”); and
- k. Exhibit 1019 – U.S. Dept. of Agric. Food Safety and Inspection Service (FSIS), “Microbiology Laboratory Guidebook,” Table of Contents, Cover, Foreword, General Considerations, Chapters 4.02, 5.03, 8.03 (July 2003) (“MLG 2003”) or (“MLG”).

19. I may rely on these and additional materials to respond to arguments raised by IEH. My review and assessment of the materials provided in this proceeding is ongoing, and I will continue to consider any new material as it is provided. I reserve the right to review, supplement, and amend my analyses based on new information and on my continuing review of the materials already provided.

II. UNDERSTANDING OF THE RELEVANT LAW

20. For the purposes of this declaration, National Beef’s counsel has informed me about certain aspects of the law that are relevant to my opinions. My understanding of the relevant law as described below, is based on my discussions with National Beef’s counsel because I am not an attorney.

21. National Beef’s counsel informed me that patentability of each claim challenged in the Petition is assessed based on documents that predate the IEH Patent, such as other patents and printed publications, and that these documents are referred to as “prior art.” I have been informed that the IEH Patent has an effective filing date of May 16, 2003, which is the filing date of the provisional patent application from which the IEH Patent claims priority. I understand that publicly available documents, such as patents and printed publications, in existence and filed before May 16, 2003, are considered to be prior art with respect to the IEH Patent.

A. Person of Ordinary Skill in the Art

22. I am informed by National Beef’s counsel that prior art references should be understood from the perspective of a person of ordinary skill in the art (“POSA”) to which the patent is related, based on the understanding of that person at the time of the patent’s priority date. I understand that a POSA is presumed to be aware of all pertinent prior art and the conventional wisdom in the art, and is also a person of ordinary creativity.

23. The subject matter of the IEH Patent relates to a sampling, diluting, incubating, and assaying for microbial contamination. I agree with the level of skill in the art described in the Petition, in that a POSA in May 2003 would have been someone with “a working knowledge of microbiology and would have been involved in developing sampling and testing methodologies for analysis of food products (‘validation methodologies’).” Petition at § V. I also agree that “the POSA would most likely have an advanced degree in microbiology, food science, or animal science and at least two years of work experience, but may alternatively have a bachelor’s degree in those areas and at least four years of experience in developing validation methodologies.” *Id.* Additionally, I agree that “the POSA would have been familiar with relevant scientific literature and industry regulations, directives, and guidelines relating to validation methodologies.” *Id.*

24. My analyses in this declaration are from the perspective of a POSA, as described above. At the time of the priority date of the IEH Patent, May 2003, and prior to May 2003, I was an individual having at least ordinary skill in the art according to the description above.

B. Written Description

25. I understand that a patent claim is valid if the patent contains an adequate written description of the claimed invention. I understand that the test for compliance with the written description requirement is whether the disclosure of the

application relied upon reasonably conveys to a POSA that the inventor had possession of the claimed subject matter as of the filing date, even though the claims may have been changed or new claims added since that time.

26. I understand that the written description requirement does not require that the patent claims use the same terminology as the patent specification. The test instead is focused on the meaning and content of the disclosure, not any particular form of expression. In other words, a claim can use terminology different from that in the patent specification, so long as the same concepts appearing in the claim are described in the specification.

27. I understand that the level of detail required to satisfy the written description requirement varies depending on factors such as existing knowledge in the particular field, the extent and content of the prior art, the maturity of the science or technology, and the predictability of the aspect at issue. In particular, I understand that patent applications are written for a POSA and, therefore, need not include background or knowledge already held by a POSA. I also understand that courts have encouraged patent applicants to omit what is well known in the art.

C. Anticipation

28. I understand that to anticipate a claim of a patent, a prior art reference must disclose all limitations of the claim as those limitations are arranged in the claim.

29. I understand that for a reference to anticipate a claim, the reference must disclose the relevant technology in a manner such that a person of ordinary skill in the relevant art would be able to carry out or utilize the technology that the reference describes without having to undertake undue experimentation. I also understand that prior art references are presumed to be enabling.

D. Obviousness

30. National Beef's counsel informed me that a patent claim is invalid if the subject matter of the claim was considered to have been obvious to a POSA at the time the application was filed. This means that, even if all of the requirements of a claim are not found in a single prior art reference, the claim is not patentable if the differences between the subject matter in the prior art and the subject matter in the claim would have been obvious to a POSA at the time the application was filed.

31. I understand that obviousness and related issues (such as what a POSA would or would not be motivated to do) must be evaluated at the time of the invention. Even when I state things in this declaration in the present tense, that should be understood as being from the perspective of a POSA on or before May 16, 2003.

32. I have been advised and understand that a claimed invention is unpatentable if the differences between the invention and the prior art are such that the subject matter as a whole would have been obvious, at the time the invention was made, to a person of ordinary skill in the art to which the subject matter pertains.

33. It is my understanding that obviousness is a question of law based on underlying factual findings: (1) the scope and content of the prior art; (2) the differences between the claims and the prior art; (3) the level of skill in the art; and (4) objective considerations of what is referred to as nonobviousness.

34. I understand that for one or more references to render the claimed invention obvious, a POSA must have a sufficient reason to combine the teachings of the references to arrive at the challenged claims. I further understand that a basis to combine teachings from the references need not be stated expressly in any prior art reference. However, there must be an articulated reasoning with rational underpinnings to support a reason to combine the teachings.

35. I understand that when considering whether a patent claim is obvious, a POSA should consider whether a teaching, suggestion, or motivation to combine the references existed at the time of invention so as to avoid impermissibly applying an analysis based on hindsight.

36. I understand that if two or more prior art references are combined to challenge a claim, and if a proposed modification would render the prior art invention being modified unsuitable for its intended purpose, then there is no suggestion or motivation to make the proposed modification. I also understand that if the proposed modification or combination of the prior art would change the principle of operation

of the prior art invention being modified, then the teachings of the references are not sufficient to render the claims obvious.

E. Claim Construction

37. I have been informed that claim terms in IPR proceedings are construed from the perspective of a POSA at the time of the invention. I also understand that the claim terms are construed by a POSA in light of the specifications of the IEH Patent and the prosecution and litigation histories pertaining to the IEH Patent.

III. SUMMARY OF OPINIONS

38. Based on my review and analysis of the materials in this matter, as well as my experience and education, my opinions are summarized below.

- a. In Ground 1 of the Petition, Rayman discloses the four steps of claim 1 of the '486 Patent.
- b. Also in Ground 1, Rayman discloses the subject matter recited the following dependent claims: claims 4–8, 17, and 19.
- c. In Ground 2, a POSA would have modified Rayman based on the PR/HACCP. On this basis, claim 1 of the '486 Patent would have been obvious based on the combination of Rayman and the PR/HACCP.

- d. Also in Ground 2, the following dependent claims would have been obvious based on Rayman and the PR/HACCP.: claims 2–8, 17, 19 and 27 of the '486 Patent.
- e. In Ground 3, Nagar discloses the steps of the independent claims but with decimal dilution. For reasons discussed in this declaration, a POSA would have modified Nagar based on Rayman. On this basis, the independent claims of the '486 Patent would have been obvious based on the combination of Nagar and Rayman.
- f. Also in Ground 3, the following dependent claims would have been obvious based on Nagar and Rayman: claims 4–8 and 12–26 of the '486 Patent.
- g. In Ground 4, Nagar discloses the steps of the independent claims but with decimal dilution. For reasons discussed in this declaration, a POSA would have modified Nagar based on Karlsen. On this basis, the independent claims of the '486 Patent would have been obvious based on the combination of Nagar and Karlsen.
- h. Also in Ground 4, the following dependent claims would have been obvious based on Nagar and Karlsen: claims 4–8 and 12–28 of the '486 Patent.

- i. In Ground 5, Nagar discloses the steps of the independent claims but with decimal dilution. For reasons discussed in this declaration, a POSA would have modified Nagar based on PR/HACCP. On this basis, the independent claims of the '486 Patent would have been obvious based on the combination of Nagar and PR/HACCP.
- j. Also in Ground 5, the following dependent claims would have been obvious based on Nagar and Karlsen: claims 2–8 and 12–30 of the '486 Patent.
- k. In Ground 6, claims 9 and 10 of the '486 Patent would have been obvious based on Rayman in view of ICMSF.
- l. In Ground 7, claims 9 and 10 of the '486 Patent would have been obvious based on Rayman in view of AAPA.
- m. Below are tables summarizing the grounds related to my above-listed opinions regarding the claims of the '486 Patent.

Grounds 1–5				
Ground	References	§	Ind. Claims	Dependent Claims
1	Rayman	102	1	4–8, 17, and 19
2	Rayman and PR/HACCP	103		
3	Nagar and Rayman			4–8 and 12–26
4	Nagar and Karlsen			4–8 and 12–28
5	Nagar and PR/HACCP			2–8 and 12–30

Grounds 6–7 for Dependent Claims Only			
Ground	References	§	Dep. Claims
6	Rayman and ICMSF	103	9 and 10
7	Rayman and AAPA		

IV. TECHNICAL BACKGROUND — STATE OF THE ART

39. Food microbiology has always been involved in determining the safety and quality of the foods for human consumption. As the sophistication of the field increased, research was devoted to not only the analytical methods for individual samples but also the statistical aspects of sampling. In the United States as well as other countries, the regulatory agencies were actively involved in conducting or sponsoring this research, along with industry and academic partners. The International Commission on Microbiological Specifications for Foods (ICMSF) was formed in 1962 to also research these issues and make recommendations on an international basis.

40. Sampling involves collecting a representative sample or samples from a specific batch of foods. In the United States the regulatory agencies have guidelines for how to collect and transport samples to the laboratory so that the samples are not compromised. All solid foods, such as meat or cheese, are homogenized in a diluent to create a sample which can be analyzed. The homogenized samples are then diluted for a number of reasons, including but not limited to: (a) assuring that food

particles from the sample do not interfere with the assay (b) diluting out naturally-occurring inhibitory substances in the food sample, especially with spices or (c) diluting out the microbiome so that the population can be enumerated on agar plates. The diluted samples are then assayed. As part of the assay, the samples are generally incubated to produce results which can be measured. Assay methods have evolved over the years from simple plating methods to genetic assays for specific bacteria.

41. Quantitative methods are commonly used for quality assessment of foods, such as the estimated population of coliforms as an indicator of the hygienic quality of certain foods. These methods usually rely on traditional bacteriology, with various media in petri dishes.

42. Qualitative methods are more commonly associated with foodborne pathogens, such as *Salmonella* or pathogenic *Escherichia coli*. The methodology to qualitatively detect foodborne pathogens has constantly evolved over the years. Using *Salmonella* as an example, the historical method of detection involved, dating from the 1950s, involved use of media that would select for and differentiate *Salmonella* from other bacteria. It was recognized that food processes could injure these bacteria to the extent that they would not grow in or on selective media, and to overcome this limitation, a non-selective pre-enrichment was added to allow the injured bacteria to resuscitate and grow further. The traditional bacteriological method, still in use today, relies on a non-selective pre-enrichment followed by a

selective enrichment, with the selectively enriched samples transferred to several different types of selective and differential agar plates. Several different types of selective or differential agars are used because not every *Salmonella* will grow on every medium. The selectively enriched cultures are streaked onto the plates in a manner to produce individual isolated colonies, which are then further characterized by biochemical tests to confirm that they are *Salmonella*.

43. The traditional bacteriological methods are time consuming and imprecise. Each step in the process requires an incubation period of up to 24 hours, resulting in a total time to a negative sample of a minimum of three days and to a confirmed positive sample of four to five days. This does not include the time required to collect the samples and transport them to the laboratory. Many presumptive positive samples on the selective and differential agar plates would be later identified as negative samples during further characterization of the isolated colonies. For many perishable food products, this delay in obtaining results was difficult because of the limited shelf life of the foods.

44. An inherent weakness in the traditional bacteriological method is in selecting “typical” colonies from the selective and differential agar plates. This requires a thorough understanding of what a typical *Salmonella* will appear as on the different selective and differential agar plates. This is compounded by the individual colonies not being a pure culture, but occasionally a mixture of two or more bacteria, resulting

in an atypical appearance. The mixed colonies would have to be further purified before biochemical analysis could proceed.

45. Beginning in the 1960s, there was considerable research devoted to shortening the analysis time and improving efficiency in the laboratory. As the traditional methods were labor intensive, alternate screening methods were developed to reduce both the amount of labor as well as improve sensitivity and specificity. The earliest screening methods, dating from the early 1970s, involved antigen/antibody tests, where antibodies were used to determine the presence of *Salmonella* in selectively enriched samples. These screening tests were rapid, in the sense that a presumptive result could be obtained in a few minutes from a selectively enriched sample, rather than waiting 24 hours for colonies to grow on selective and differential agar plates. These methods, commonly known as enzyme-linked immunosorbent assays (ELISAs), are widely in use today. While an improvement in analysis technology, ELISAs have an inherent limitation in that some of the antibodies in the tests will cross react with other bacteria which are not *Salmonella*. *Salmonella* is a very diverse group of bacteria, and some of the antibodies that are capable of detecting all of the different serotypes of the organism will cross react with other genera that are in the same microbiological family as *Salmonella* (i.e., the *Enterobacteriaceae*), resulting in a false positive result.

46. Another inherent limitation of these assays is that they require relatively high population densities of the target bacterium to produce a visual result. Most if not all of the currently used ELISAs employ a chromogenic dye to indicate a positive result, and commonly it may require a population density of up to 1 million cells per milliliter (*see, e.g.,* Lee et al., 1990) of the target bacterium in the selective enrichment to produce a visual reaction, depending on the method format. While the pre-enrichment and selective enrichment procedures should result in this population density, it is possible that it will not, resulting in a false negative result.

47. The latest evolution of screening methods involves the use of genetic markers. These tests, which were introduced in the mid-1980s, are commonly based on the polymerase chain reaction (PCR) and use the genetic material of the bacterial cells as a means of identification. These screening methods still require the use of a pre-enrichment to recover injured bacteria but are more specific and sensitive than traditional bacteriology or ELISAs. The methods use specific primers to increase the number of copies of DNA fragments from the bacteria and then typically use a chromogenic marker to determine if the sample is positive or negative. While still requiring a certain population density of the target bacterium, the necessary population density to result in a positive result is much lower, usually in the range of 1,000 to 10,000 cells per milliliter. This results in a shorter analysis time, as there is often not a need for a selective enrichment step and in some cases the pre-

enrichment time may be shortened. For some methods, the total analysis time may be 26 to 30 hours, in comparison to approximately 72 hours for the traditional bacteriological methods. Although more precise and sensitive than other methods, genetic methods still have concerns with false positive and false negative results.

48. Sampling and laboratory analytical methods have developed further since the granting of the IEH Patent. Sampling combo bins of beef trim by removing 60 individual samples is time consuming and cumbersome. Because of this, alternative sampling methods which involve swabbing the trim have been developed which have been shown to be equivalent to the traditional sampling method. Briefly, these methods involve using a cloth or sponge to sample a large area of trim surfaces, where the microbial contamination is generally found. These methods may be performed manually or may be automated to sample continuously. The swabbing methods are much less labor intensive than other forms of sampling and have become an attractive alternative for the meat and poultry industry.

49. In addition, microbiological laboratory methods have also evolved with the further development of genetic based protocols, for example the polymerase chain reaction (PCR).

V. THE IEH PATENT

A. Disclosure and AAPA

50. The '486 Patent includes applicant-admitted prior art (AAPA). More specifically, the '486 Patent states “[p]rior art methods, as summarized herein, under the ‘Background’ section, are based on 1:10 (wt./vol.) dilutions of the sample.” Ex. 1001, 8:24-25. Accordingly, the range of dilution ratios recited in the diluting step of the independent claims is the only aspect of the independent claims that IEH has not admitted is in the prior art.

51. The '486 Patent includes AAPA admitting that, with the exception of the claimed dilution ratio range, all the steps of independent claim 1 are in the prior art. For example, the '486 Patent discloses these steps in stating “[f]or the detection of *Escherichia coli* O157:H7 in food, **25 g of ground beef is incubated in 225 ml of selective enrichment medium** for 16 to 18 h at 37° C. with agitation **Enzyme-linked immunosorbent assay (ELISA) is used to detect the pathogen from the enriched culture.**” Ex. 1001, 2:10-42; *see also id.*, 1:58-2:9, 2:49-5:34, 13:54-14:32.

52. The '486 Patent includes AAPA admitting that, with the exception of the claimed dilution ratio range, all the steps of independent claim 11 are in the prior art. For example, the '486 Patent discloses these steps in stating:

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

Ex. 1001, 2:10-32; *see also id.*, 1:58-2:9, 4:35-44.

B. Claims of the IEH Patent

53. The '486 Patent includes a first independent claim, claim 1, and a second independent claim, claim 11.

54. The independent claims differ in two respects. First, claim 1 recites that the sample is solid or semi-solid while claim 11 recites that the sample is liquid. Second, the claims differ in their recitations of the diluting step. Claim 1 recites “diluting the sample with liquid enrichment medium at a ratio of sample to diluent between about 1:0.1 to about 1:5 (wt./vol.) or lesser dilution.” Claim 11 recites “diluting the sample with liquid enrichment medium at a ratio of sample to diluent between about 1:0.1 to about 1:5 (vol./vol.) or lesser dilution, or not diluting the sample.” Other than these differences, the independent claims of the '486 Patent, recite four steps, as depicted in the diagram below.



55. In the first step, the **sampling** step, a sample is obtained that is recited as “solid or semi-solid” in claim 1 and as “liquid” in claim 11.

56. The second step, the **diluting** step, is recited in claim 1 as “diluting the sample with liquid enrichment medium at a ratio of sample to diluent between about 1:0.1 to about 1:5 (wt./vol.) or lesser dilution.” The term “lesser dilution” modifies the lower endpoint, 1:0.1, as discussed in the analysis of the prosecution history. *See* § VII-B *infra*.

57. The second step is recited in claim 11 with two differences relative to claim 1. First, the ratio is recited with (vol./vol) since the sample and medium are both

liquids. Second, claim 11 includes an alternative to diluting because claim 11 recites “or not diluting the sample.” Thus, the ratio recited in the claim 11 is merely an option that is not required.

58. The third step, the **incubating** step, requires incubating in an incubator. Both independent claims further recite an “optimal temperature for the particular bacterial pathogen or bacterial microbe to be detected for a time period sufficient to allow levels of the particular bacterial pathogen or bacterial microbe to reach levels detectable by use of an assay suitable to detect the particular bacterial pathogen or bacterial microbe.” This functional recitation of time and temperature merely recites standard incubating conditions.

59. The fourth step, the **assaying** step, generically covers, in both independent claims, use of an “assay suitable to detect the particular bacterial pathogen or bacterial microbe, whether the sample is contaminated with the particular bacterial pathogen or bacterial microbe.

1. Claim 1 of the '486 Patent

60. Claim 1 is presented below.

Claim 1 of the '486 Patent	
Preamble	A method for detection of a particular bacterial pathogen or bacterial microbe, comprising:
First Step	obtaining a test sample, the sample being solid or semi-solid;
Second Step	diluting the sample with liquid enrichment medium at a ratio of sample to diluent between about 1:0.1 to about 1:5 (wt./vol.) or lesser dilution;
Third Step	incubating, in an incubator, the diluted sample at an optimal temperature for the particular bacterial pathogen or bacterial microbe to be detected for a time period sufficient to allow levels of the particular bacterial pathogen or microbe to reach levels detectable by use of an assay suitable to detect the particular bacterial pathogen or bacterial microbe; and
Fourth Step	determining, by assaying the diluted incubated test sample, or a portion thereof, with the assay suitable to detect the particular bacterial pathogen or bacterial microbe, whether the sample is contaminated with the particular bacterial pathogen or bacterial microbe.

2. Claims Depending from Claim 1 of the '486 Patent

61. Claims 2–10, 17, 19, 21–23, and 27 depend from claim 1.

Claims Depending from Claim 1 of the '486 Patent	
2	The method of claim 1, wherein diluting is at a ratio of sample to diluent between about 1:0.1 to about 1:0.5 (wt./vol.) or lesser dilution.
3	The method of claim 1, wherein diluting is at a ratio of sample to diluent between about 1:0.1 to about 1:0.3 (wt./vol.) or lesser dilution.
4	The method of claim 1, wherein the optimal temperature is within the optimal growth temperature range of the particular bacterial pathogen or bacterial microbe in the liquid enrichment medium.
5	The method of claim 4, wherein the optimal temperature range is from about 25°C. to about 45°C.
6	The method of claim 1, wherein the assay suitable for detection of the particular bacterial pathogen or bacterial microbe contamination is selected from the assay group consisting of immunoassays, nucleic acid amplification-based assays, PCR-based assays, nucleic acid hybridization-based assays, bio-sensor assays, immunostaining-microscopy-based assays, nucleic acid-array-based assays, DNA chip-based assays, classical microbiology-based assays, and chemical or biochemical assays based on the detection of compounds associated with the particular bacterial pathogen or bacterial microbe, and combinations thereof.
7	The method of claim 1, wherein the particular bacterial pathogen or bacterial microbe is selected from the group consisting of <i>Escherichia coli</i> O157:H7 (<i>E. coli</i> O157:H7), enterohemorrhagic <i>Escherichia coli</i> (EHEC), enterotoxigenic <i>Escherichia coli</i> (ETEC), enteroinvasive <i>Escherichia coli</i> (EIEC), enteropathogenic <i>Escherichia coli</i> (EPEC), <i>Salmonella</i> , <i>Listeria</i> , <i>Yersinis</i> , <i>Campylobacter</i> , <i>Clostridial</i> species, <i>Staphylococcus</i> spp.; frank and opportunistic bacteria, indicator organisms including heterotrophes, generic <i>E. Coli</i> , total and fecal coliforms and enterococcus; spoilage organisms including <i>Pseudomonas</i> , and combinations thereof.
8	The method of claim 7, wherein the particular bacterial pathogen or bacterial microbe is <i>Escherichia coli</i> O157:H7 (<i>E. coli</i> O157:H7, or H negative), <i>Salmonella</i> , <i>Listeria</i> , EHEC, and <i>Campylobacter</i> .

Claims Depending from Claim 1 of the '486 Patent — cont'd	
9	The method of claim 1, wherein obtaining a sample is obtaining a standard Lot-unit sample.
10	The method of claim 1, wherein the sample is a composite-Lot sample, corresponding to a combination of samples selected from the group consisting of samples or subsamples taken from sublots/lots of raw or processed samples, environmental samples, industrial samples, pharmaceutical samples, bio-solid samples, samples taken by spore traps, settled dust, impingers, or filtration, and combinations thereof.
17	The method of claim 1, where in the sample is selected from the group consisting of beef, pork, sheep, bison, deer, elk, poultry, fish, produce, dairy products, dry goods, raw and processed foods, environmental samples, soil, sludge, surface samples, samples taken by impingers and filtration, pharmaceuticals, and samples analyzed using enrichment-detection protocols.
19	The method of claim 1, wherein the diluting comprises addition to the sample of a supplement selected from the group consisting of water, sugars, proteins, minerals, organics, vitamins and cofactors, antibiotics, dyes, indicators, buffers, agents to adjust the pH, water activity, nutritional contents, selective pressure to optimize the growth conditions for the particular bacterial pathogen or bacterial microbe, and combinations thereof.
21	The method of claim 1, wherein the obtaining and diluting of the solid or semi-solid sample with liquid enrichment medium is at a first location, wherein incubating the diluted sample at an optimal temperature in the incubator is during transit of the diluted sample to a second location that is not the site of sample collection and dilution, and wherein determining by assaying the diluted incubated test sample, or a portion thereof, with the assay suitable to detect the particular bacterial pathogen or bacterial microbe, whether the sample is contaminated with the particular bacterial pathogen or bacterial microbe, is at the second location.

Claims Depending from Claim 1 of the '486 Patent — cont'd	
19	The method of claim 1, wherein the diluting comprises addition to the sample of a supplement selected from the group consisting of water, sugars, proteins, minerals, organics, vitamins and cofactors, antibiotics, dyes, indicators, buffers, agents to adjust the pH, water activity, nutritional contents, selective pressure to optimize the growth conditions for the particular bacterial pathogen or bacterial microbe, and combinations thereof.
21	The method of claim 1, wherein the obtaining and diluting of the solid or semi-solid sample with liquid enrichment medium is at a first location, wherein incubating the diluted sample at an optimal temperature in the incubator is during transit of the diluted sample to a second location that is not the site of sample collection and dilution, and wherein determining by assaying the diluted incubated test sample, or a portion thereof, with the assay suitable to detect the particular bacterial pathogen or bacterial microbe, whether the sample is contaminated with the particular bacterial pathogen or bacterial microbe, is at the second location.
22	The method of claim 21, further comprising addition to the sample, prior to or during incubation, of a supplement selected from the group consisting of water, sugars, proteins, minerals, organics, vitamins and cofactors, antibiotics, dyes, indicators, buffers, agents to adjust the pH, water activity, nutritional contents, selective pressure to optimize the growth conditions for the particular bacterial pathogen or bacterial microbe, and combinations thereof.
23	23. The method of claim 21, wherein the second location is an in-house or local lab, or a remote location.
27	27. The method of claim 1, wherein diluting is at a ratio of sample to diluent between about 1:0.1 to about 1:2 (wt./vol.) or lesser dilution.

3. Claim 11 of the '486 Patent

62. Claim 11 is presented below.

Claim 11 of the '486 Patent	
Preamble	A method for detection of a particular bacterial pathogen or bacterial microbe, comprising:
First Step	obtaining a liquid sample;
Second Step	diluting the sample with liquid enrichment medium at a ratio of sample to diluent between about 1:0.1 to about 1:5 (vol./vol.) or lesser dilution, or not diluting the sample;
Third Step	incubating, in an incubator, the diluted or the not diluted sample at an optimal temperature for the particular bacterial pathogen or bacterial microbe to be detected for a time period sufficient to allow levels of the particular bacterial pathogen or bacterial microbe to reach levels detectable by use of an assay suitable to detect the particular bacterial pathogen or bacterial microbe; and
Fourth Step	determining, by assaying the diluted or the not diluted incubated test sample, or a portion thereof, with the assay suitable to detect the particular bacterial pathogen or bacterial microbe, whether the sample is contaminated with the particular bacterial pathogen or bacterial microbe.

4. Claims Depending from Claim 11 of the '486 Patent

78. Claims 12–16, 18, 20, and 24–30 depend from claim 11 and are listed below.

Claims Depending from Claim 11 of the '486 Patent	
12	The method of claim 11, wherein the optimal temperature is within the optimal growth temperature range of the particular bacterial pathogen or bacterial microbe in the liquid enrichment medium.
13	The method of claim 12, wherein the optimal temperature range is from about 25°C. to about 45°C.

Claims Depending from Claim 11 of the '486 Patent — cont'd	
14	The method of claim 11, wherein the assay suitable for detection of the particular bacterial pathogen or bacterial microbe contamination is selected from the assay group consisting of immunoassays, nucleic acid amplification-based assays, PCR-based assays, nucleic acid hybridization-based assays, bio-sensor assays, immunostaining-microscopy-based assays, nucleic acid-array-based assays, DNA chip-based assays, classical microbiology-based assays, and chemical or biochemical assays based on the detection of compounds associated with the particular bacterial pathogen or bacterial microbe, and combinations thereof.
15	The method of claim 11, wherein the particular bacterial pathogen or bacterial microbe is selected from the group consisting of <i>Escherichia coli</i> O157:H7 (<i>E. coli</i> O157:H7), enterohemorrhagic <i>Escherichia coli</i> (EHEC), enterotoxigenic <i>Escherichia coli</i> (ETEC), enteroinvasive <i>Escherichia coli</i> (EIEC), enteropathogenic <i>Escherichia coli</i> (EPEC), <i>Salmonella</i> , <i>Listeria</i> , <i>Yersinia</i> , <i>Campylobacter</i> , <i>Clostridial</i> species, <i>Staphylococcus</i> spp.; frank and opportunistic bacteria, indicator organisms including heterotrophes, generic <i>E. Coli</i> , total and fecal coliforms and enterococcus; spoilage organisms including <i>Pseudomonas</i> , and combinations thereof.
16	The method of claim 15, wherein the particular bacterial pathogen or bacterial microbe is <i>Escherichia coli</i> O157:H7 (<i>E. coli</i> O157:H7, or H negative), <i>Salmonella</i> , <i>Listeria</i> , EHEC, and <i>Campylobacter</i> .
18	The method of claim 11, where in the sample is selected from the group consisting of beef, pork, sheep, bison, deer, elk, poultry, fish, produce, dairy products, dry goods, raw and processed foods, environmental samples, soil, sludge, surface samples, samples taken by impingers and filtration, pharmaceuticals, and samples analyzed using enrichment-detection protocols.
20	The method of claim 11, wherein the diluting comprises addition to the sample of a supplement selected from the group consisting of water, sugars, proteins, minerals, organics, vitamins and cofactors, antibiotics, dyes, indicators, buffers, agents to adjust the pH, water activity, nutritional contents, selective pressure to optimize the growth conditions for the particular bacterial pathogen or bacterial microbe, and combinations thereof.

24	The method of claim 11, wherein the obtaining and diluting of the liquid sample with liquid enrichment medium is at a first location, wherein incubating the diluted sample at an optimal temperature in the incubator is during transit of the diluted sample to a second location that is not the site of sample collection and dilution, and wherein determining by assaying the diluted incubated test sample, or a portion thereof, with the assay suitable to detect the particular bacterial pathogen or bacterial microbe, whether the sample is contaminated with the particular bacterial pathogen or bacterial microbe, is at the second location.
25	The method of claim 24, further comprising addition to the sample, prior to or during incubation, of a supplement selected from the group consisting of water, sugars, proteins, minerals, organics, vitamins and cofactors, antibiotics, dyes, indicators, buffers, agents to adjust the pH, water activity, nutritional contents, selective pressure to optimize the growth conditions for the particular bacterial pathogen or bacterial microbe, and combinations thereof .
26	23. The method of claim 24, wherein the second location is an in-house or local lab, or a remote location.
28	27. The method of claim 11, wherein diluting is at a ratio of sample to diluent between about 1:0.1 to about 1:2 (vol./vol.) or lesser dilution.
29	The method of claim 11, wherein diluting is at a ratio of sample to diluent between about 1:0.1 to about 1:0.5 (vol./vol.) or lesser dilution.
30	The method of claim 11, wherein diluting is at a ratio of sample to diluent between about 1:0.1 to about 1:0.3 (vol./vol.) or lesser dilution.

5. Comparison of Claims 1 and 11 of the '486 Patent

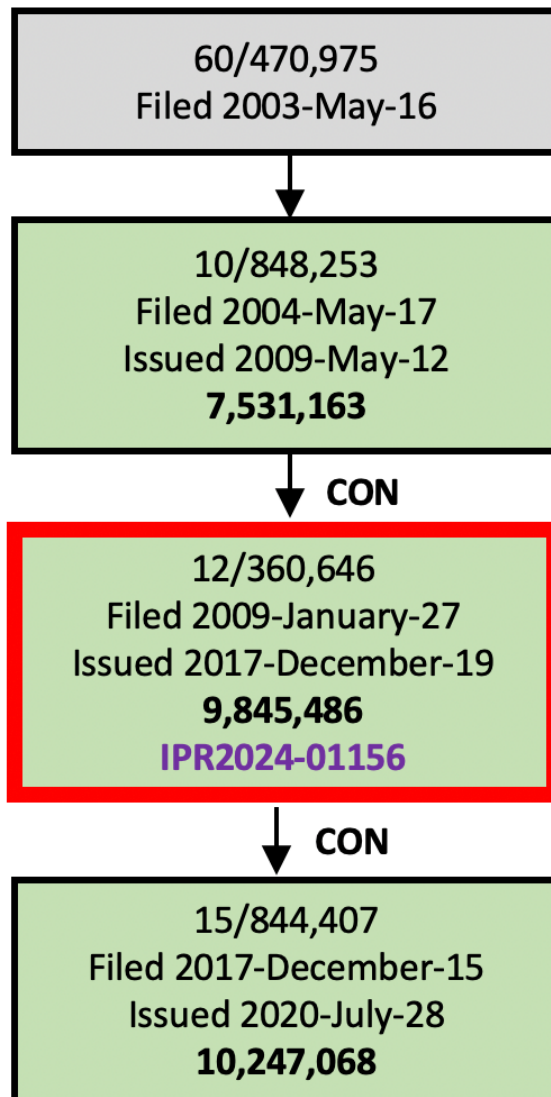
79. Claims 1 and 11 are presented below in a side-by-side comparison of the respective claim language to show the minimal differences between the claims.

	Claim 1 of the '486 Patent	Claim 11 of the '486 Patent
Preamble	A method for detection of a particular bacterial pathogen or bacterial microbe, comprising:	
First Step	obtaining	
	a test	a liquid
	sample,	
	the sample being solid or semi-solid;	
Second Step	diluting the sample with liquid enrichment medium at a ratio of sample to diluent between about 1:0.1 to about 1:5	
	(wt./vol.) or lesser dilution;	(vol./vol.) or lesser dilution, or not diluting the sample;
Third Step	incubating, in an incubator,	
	the diluted sample	the diluted or the not diluted sample
	at an optimal temperature for the particular bacterial pathogen or bacterial microbe to be detected for a time period sufficient to allow levels of the particular bacterial pathogen or microbe to reach levels detectable by use of an assay suitable to detect the particular bacterial pathogen or bacterial microbe; and	
Fourth Step	determining, by assaying	
	the diluted incubated test sample, or a portion thereof,	the diluted or the not diluted incubated test sample, or a portion thereof,
	with the assay suitable to detect the particular bacterial pathogen or bacterial microbe, whether the sample is contaminated with the particular bacterial pathogen or bacterial microbe.	

C. FAMILY TREE OF IEH'S PATENTS

80. As depicted below, IEH has three patents in this family. The '486 Patent, its parent, No. 7,531,163 ("the '163 Patent"); and its child, No. 10,247,068 ("the '068 Patent") were examined by the same examiner.

81. The prosecution history of the '486 Patent is summarized at § VI *infra*.



VI. PROSECUTION HISTORY OF '486 PATENT

A. Provisional Application No. 60/470,975

82. The '486 Patent claims priority to Provisional Application No. 60/470,975, which was filed on May 16, 2003. Ex. 1003 at 1. No. 60/470,975 is titled Dry and SemiDry Enrichment for the Detection of Foodborne Pathogens. *Id.* at 2. No. 60/470,975 includes only two paragraphs on a single page. *Id.*

Below are the two paragraphs of the provisional application with text highlighted that is relevant to independent claim 11 of the '486 Patent.:

Rapid detection of foodborne pathogens has become an essential component of food manufacturing processes. The same applies to the detection of waterborne pathogens, and in general any kind of bacterial, viral, fungal, and parasitic pathogens that are capable of in-vivo multiplication. Most regulatory agencies and customers mandate specific testing for pathogens which are common to specific food types. Many food manufacturers have had to build in-house labs to expedite the testing or lose valuable time waiting for test results when samples are shipped out to outside labs. Furthermore in many instances the cost of enrichment media can be substantial. Herein we describe a method that relies on dry enrichment or semi dry enrichment process, which would allow for incubation of the food samples without the addition of enrichment media, or with addition of small quantities of media and/or supplements. The samples will be incubated immediately at the desired incubation temperature, or they will incubate in-transit by shipping the samples in a temperature controlled shipping box that permits incubation at appropriate temperatures with ranges depending on the pathogen's optimal competitive growth range.

While with many types of foods, the food itself can be incubated with no addition of inhibitory or supplementary substances, with dry and semidry foods the water activity will be adjusted to allow for the growth of the target organism. Addition of supplements will depend on the target organism and the nature of the sample which is to be tested. The supplements include water, sugars, proteins, minerals, organics, vitamins and cofactors, antibiotics, dyes, indicators, etc. With foods or environmental samples that are in liquid form, the samples can be enriched directly with or without a concentration step with or without the addition of supplementary nutrients, inhibitors, or indicators.

The protocols as described allows for the enrichment of pathogens, or target organisms with minimal increase in volume/weight of the samples, for samples that are to be shipped out, the shipping time can be used to enrich the sample without substantial increase in the weight and volume of the product which also minimizes the chance for spill and cross contamination. Additionally the fact that sample size has not increased substantially in terms of its volume /weight, the sensitivity of the method is increased due to the elimination of dilution factor.

Id. (annotated). Claim 11 recites diluting a liquid sample “with liquid enrichment medium at a ratio of sample to diluent between about 1:0.1 to about 1:5 (wt./vol.) or lesser dilution, or not diluting the sample.” Because independent claim 11 of the ’486 Patent recites “or not diluting the sample,” the provisional application supports claim 11. The provisional application lacks other content, such as a dilution ratio, that correlates to the subject matter recited in the other claims.

83. The provisional application lacks any disclosure that supports the recitation in claim 1 of the ’486 Patent of “diluting the sample with liquid enrichment medium at a ratio of sample to diluent between about 1:0.1 to about 1:5 (wt./vol.) or lesser dilution.” Thus, priority cannot be claimed for claim 1 or its dependent claims, claims 2–10, 17, 19, 21–23, 27, 29, and 30.

B. Application No. 10/848,253 issued as Patent No. 7,531,163

84. Appl. No. 10/848,253 (“the ’253 Application”) was filed on May 17, 2004 and issued as the ’163 Patent on May 12, 2009 so it was pending for five years.

85. The only substantive rejection was based on U.S. Patent No. 6,060,266 to Naqui in view of several chapters of the FDA’s BAM Bacteriological Analytical Manual (“BAM”). IEH overcame the rejection by emphasizing that BAM only teaches decimal dilution while the claims recite a ratio of sample to diluent of between about 1:0.1 to about 1:2 (wt./vol.), or lesser dilution. The independent

claims of '163 Patent also recite subject matter like claims include claims 21–26, which are referred to herein as the transportation claims.

C. Application No. 12/360,646 issued as Patent No. 9,845,486

86. Appl. No. 12/360,646 (“the ’646 Application”) was filed on January 27, 2009 and issued as the ’486 Patent on December 19, 2017 so it was pending for nine years. The ’646 Application was handled by the same examiner who handled the parent application. The claims were not rejected based on any prior art references.

87. IEH pursued broader claims in the ’646 Application than it obtained in ’163 Patent. The claims were broader for two reasons. First, the dilution ratio range is broader because the ratio of sample to diluent is recited in claim 1 as being “between about 1:0.1 to about 1:5 (wt./vol.), or lesser dilution.” Second, the independent claims do not recite that the samples are obtained at a first location, incubating occurs in transit to a second location, and assaying at the second location. Instead the transportation content is recited in dependent claims.

D. Application No. 15/844,407 issued as U.S. Patent No. 10,724,068

88. Appl. No. 15/844,407 (“the ’407 Application”) was filed on December 15, 2017 and issued as Patent No. 10,724,068 (“the ’068 Patent”) on July 29, 2020. Again, the same examiner handled this application as its parent and grandparent and made no prior art rejections. The claims that were obtained are even broader than

those in '486 Patent because the “ratio of sample to diluent” is “between about 1:0.1 to about 1:9 (wt./vol.), or lesser dilution.”

VII. PRIOR ART REFERENCES

A. Ground References

1. Rayman

89. Rayman is focused on an earlier study by Anderson and Baird-Parker. Anderson and Baird-Parker published an article in 1975 describing a direct plating approach to the enumeration of *Escherichia coli* Biotype I. Ex. 1009. For many samples, the standard method for enumerating *E. coli* was the Most Probable Number (MPN) method. The MPN method is a means of statistically estimating a population of target bacteria with a series of tubes and dilutions. The MPN method is a relatively complex and labor-intensive procedure, and in its' simplest form suffers from a lack of accuracy. Anderson and Baird-Parker reported on a new method of direct plating, which was much less complex, labor-intensive and had a shorter time to results than the MPN. The results produced by the new method were equivalent to those of the MPN method. The direct plating assay described by Anderson and Baird-Parker also used a lower initial dilution (1:5 and not 1:10), which improved the sensitivity of the assay.

90. Rayman and co-authors performed a collaborative study of the MPN method and the direct plating method with raw meats using the method of Anderson and

Baird-Parker. Ex. 1010. This was an international study conducted through the International Commission on Microbiological Specifications for Foods (ICMSF). The results of the study demonstrated that the results obtained by the direct plating (DP) method were “less variable and gave a higher rate of detection of low numbers of *E. coli* in frozen samples.” As with the Anderson and Bair-Parker reference, the original samples were diluted 1:5 for the direct plating method, which increases the sensitivity and explains the “higher detection rate” observed with frozen samples. The study concluded that the “DP method which appears to be superior to the MPN method because of its lower variability, better recovery from frozen samples. rapidity, and decreased requirement for media and analysts' time.”

91. The U.S. National Research Council (“NRC”) published a report titled “An evaluation of the role of microbiological criteria for foods and food ingredients” in 1985. The NRC report refers to the direct plating method of Anderson and Baird-parker as well as the Rayman et al 1979 study. Ex. 1011. The context of the discussion was the usefulness of a so-called “zero tolerance”, or a standard of <1 cells per gram for an indicator bacterium. The authors of the NRC report discuss that a standard of <1 cells per gram implied the use of the MPN method, without addressing the limitations of the MPN method in terms of precision and accuracy. The report further discussed the direct plating method in terms of faster time to results and greater recovery of *E. coli* from frozen samples. The NRC report further

noted the impact of the 1:5 dilution, stating “By placing 1 ml of a 1:5 dilution of meat on duplicate filters a lower limit of detection of 2.5 cells per gram of meat could be reached (Rayman et al., 1979). With 1 ml of a 1:10 dilution, the lower limit of detection would be <10 per gram of food.” While the report did not offer a recommendation on the direct plating method, the report clearly emphasized the advantages of the procedure.

92. Taken together, these three studies emphasize the commonly understood concept that a lower dilution of a food sample results in a lower detection limit and greater sensitivity of the assay.

2. PR/HACCP

93. The USDA FSIS issued its PR/HACCP Rule with three key sections: Sanitation, Hazard Analysis and Critical Control Points (HACCP) and microbiological testing. This discussion will focus on the microbiological testing requirement.

94. The PR/HACCP Rule requires routine microbiological testing of carcasses. For beef carcasses, the documentation is on pages 38919 to 38923 and Appendix F. These sections describe the sampling and testing procedures for beef carcasses. Briefly, the PR/HACCP rule requires sampling three, 10 x 10 cm areas of a beef carcass with the same sponge, diluting the sponge into a total of 25 mls of diluent, and assaying the sample for Biotype I/II *Escherichia coli*. The text describes in

detail the sampling and assay methods, as well as the methods for trend analysis of the results.

95. The sampling method for carcass samples involves the use of a sterile sponge. Prior to the Final Rule, USDA FSIS required excision samples. The sampling locations were similar to the Final Rule but the 10 x 10 cm samples were physically cut from the carcass. After much discussion and research, it was concluded that non-destructive sponge sampling was an acceptable alternative to excision sampling. With either method, the 300 cm² sample (3, 10x10 cm samples with a single sponge) are considered representative of the carcass. While the original excision samples could be reported as populations per gram of sample, the assay described in the Final Rule is reported as population per cm². The beef carcass sampling combines 300 cm² of sampled area with 25 mls of buffer, so that 1 ml of the buffer is equivalent to 12 cm² of the carcass surface. The assay method includes an option for further diluting the samples, but the industry often found that further dilution was unnecessary. That is, the sponge with 25 ml of buffer could be directly assayed, as the *E. coli* populations did not require further dilution for quantification. Both excision and sponge sampling methods rely on the concept of transferring bacteria from a solid surface into a liquid medium for further analysis.

3. Nagar

96. Nagar discloses a method involving sampling, diluting, incubating, and assaying:

A feasibility study showed the capture of target pathogen from an artificially inoculated food sample. A 25 g aliquot of ground beef with 22% fat was artificially inoculated with different levels of *E. coli* O157:H7 pathogen. The target pathogen was captured directly from a stomacher bag via immobilized capture antibodies on a capture matrix placed inside the stomacher bag. The following protocol was used:

- (1) **25 g ground beef** with 22% fat was placed in each of five sterile Whirl-Pak stomacher bags;
- (2) 225 ml resuscitation/growth medium was added to each bag;
- (3) one sample of each of 5 levels of *E. coli* O157:H7 was added to each of the bags;
- (4) the bags were stomached/homogenized for 30 seconds;
- (5) a capture matrix (i.e., immobilon-P membrane spotted with goat anti-*E. coli* O157:H7 antibodies) was placed inside each of the bags;
- (6) the bags were incubated at 37°C for 5 hours with shaking at ~100 rpm;
- (7) the capture matrix was retrieved from each bag; and

(8) target pathogens captured on the capture matrix were detected.

Ex. 1012 ¶ [0050] (emphases added); *see also id.* ¶ [0023]. The diluting step specifies decimal dilution.

97. In addition to beef, Nagar also discloses sampling of “milk, cheese, vegetables, apple cider, etc.” *Id.* ¶ [0036].

98. Nagar provides and an additional example of a method involving sampling, diluting, incubating, and assaying:

Following the dispensing of the sample in the container, the sample can be stomached or homogenized within the bag utilizing means well known to those skilled in the art, (e.g., via Stomacher Lab-Blender, Seward Medical, London, U.K.). The sample is stomached/homogenized within the bag containing the resuscitation/growth medium. The container is incubated in the presence of the capture matrix at a temperature suitable for growth of the target pathogen. The incubation of the sample is carried out to resuscitate and grow sub-lethally injured pathogens present in the sample.

At the end of the incubation period, specific detection assays can be performed on the capture matrix to detect the presence of the target microorganism.

Id. ¶ [0044], [0045] (emphases added).

99. Nagar also describes assaying options:

At the end of the incubation period, specific **detection assays** can be performed on the capture matrix to detect the presence of the **target microorganism**.

One method of detection is through the use of **biochemical tests or assays**.

Ex. 1007 ¶¶ [0044], [0045] (emphases added).

100. Nagar's method also describes sampling at a first location, assaying in a second location, and incubating during transportation from the first location to the second location.

The method and system of the invention can also be used during transportation of a test sample (e.g., soil, etc.) from the collection site to the testing laboratory. This may be achieved by placing the capture matrix inside a sampling/transportation device containing suitable resuscitation/growth medium, thus facilitating the capture, resuscitation and growth of the target pathogen for rapid detection.

Id. ¶ [0031].

4. Karlsen

101. Karlsen discloses:

a method for detecting water-borne microorganisms, which serve as an indicator of the probable presence of pathogens, primarily of fecal origin. In a preferred embodiment, the method comprises the following steps: (1) providing a liquid or liquified sample (which may include treating the sample with a culture media to enrich microorganisms present in the sample); (2) recovering microorganisms from the treated sample;” and . . . “(7) detecting the presence of the amplicon . . . ; and (8) making a determination about the presence or absence of the microorganism in the original liquid or liquified sample based on presence or absence of the amplicon.

Ex. 1015, 10.

102. Karlsen discloses a variety of samples”

The term “liquid or liquified sample” may be used herein interchangeably with the term “test sample”. The liquid or liquified sample may comprise sewage, sludge, soil, food, feed, and water. It is therefore contemplated that the liquid or liquified sample comprise any number of sample types, both environmental and clinical. Examples of the liquid or liquified sample include but are not limited to drinking water, bathing water, cerebrospinal fluid, blood, juices, soft drinks, wine or other alcoholic beverages, milk, milk products, cheeses, and yogurt.

Id., 14.

103. Karlsen discloses diluting at a ratio of sample to diluent of 1:2 (vol./vol.):

In the first step of one version of the method “an approximately 10 ml portion of the liquid or liquified sample (as defined elsewhere) herein which is desired to be tested for bacteria mixed with approximately 10 ml of an enrichment media for enhancing growth of bacteria which may be present in the sample and is incubated.

Id., 23.

5. ICMSF

104. The International Commission on Microbiological Specifications for Foods (“ICMSF” or “the Commission”) is a non-governmental organization that sets general specifications for foods. The Commission publishes a multi-chaptered series of books titled “Microorganisms in Foods,” referred to in the industry as the ICMSF guidelines and recognized as international standards.

105. Before the priority date, a POSA would have been very familiar with the ICMSF guidelines because it is a well-known resource, one of the main guidelines for the food testing industry, and is commonly taught in academic classes on food microbiology.

106. The ICMSF guidelines are typically referenced by their series number. Exhibit 1012 includes Chapters 6, 10, 12, and 15–17 of ICMSF 7, which was published in 2002. ICMSF 7 is referred to herein simply as ICMSF for brevity. A

portion of ICMSF 2, which was published in 1986, is also included herein as Exhibit 1013.

107. The ICMSF publications vary considerably between each publication such that the different publications are not considered different editions. Rather, each publication stands on its own and reflects different but related objectives. For example, in the period between the respective publication dates of ICMSF 1 in 1978 and ICMSF 7 in 2002, the content of ICMSF changed from simply testing to prevention with content regarding tracking a consignment of lots and use of composited enrichments. In contrast, the FDA's BAM and the USDA's MLG tend to carry over more content from earlier publications such that each update is a different edition.

108. Chapter 6 is titled "Concepts of Probability and Principles of Sampling." Ex. 1012 at 113–21. Chapter 10 is titled "Experience in the Use of Two-Class Attributes Plans for Lot Acceptance." *Id.* at 183–97. Chapter 12 is titled "Sampling, Sample Handling, and Sample Analysis." *Id.* at 225–236. Chapters 15–17 respectively focus on *Salmonella*, *Listeria*, and *E. coli*. *Id.* at 273–332.

109. Chapters 6, 10, and 12 describe: (a) obtaining samples, such as sub-samples representative of meat lots in a consignment; (b) combining the sub-samples to yield composited lot samples while maintaining traceability; (c) enriching the composited

lot samples to provide a set of enriched, composited lot samples; and (d) testing to obtain a negative result. *Id.* at 113–21, 183–97, 225–36.

B. State of the Art References

1. BAM

110. FDA’s BAM provides the official methods of microbiological analysis for foods and cosmetics. The FDA has regulatory authority over most foods with the exception of meat and poultry, liquid eggs, and Siluriformes (catfish). However, as the FDA has regulatory authority over pet and animal foods, some of the methods are applicable to meat or poultry containing products. Earlier editions of the BAM were published as physical copies in a three-ring binder but transitioned to electronic and online in approximately 2000 (*see* <https://www.fda.gov/food/laboratory-methods-food/bacteriological-analytical-manual-bam>). The analytical methods described in the BAM must meet stringent scientific criteria such that the results are considered valid in legal matters. In addition, as part of the United States participation in the World Trade Organization, the methods must be made available to our trading partners as the U.S. established standards or guidelines for imported products. The BAM provides guidance on collecting and transporting samples of different types and categories of foods. The number of samples to be collected are based on scientific studies and statistical analysis from the late 1960s and early

1970s, with additional guidance from the International Commission on Microbiological Specifications for Foods (i.e., the ICMSF).

111. Exhibit 1018 includes the Table of Contents, Introduction, About the Bacteriological Analytical Manual, and Chapters 1, 4, 4A, 5, 10, and Appendix 1 of the version of the BAM published in 2003, referred to herein as “BAM 2003” or simply “BAM.” Chapter 1 is titled “Food Sampling and Preparation of Sample Homogenate.” Ex. 1018 at 10. Chapter 5 is titled “*Salmonella*.” *Id.* at 20. Chapter 10 is titled “Detection and Enumeration of *Listeria monocytogenes* in Foods.” *Id.* at 40.

112. Chapter 1 describes, at Section A, use of sampling plans to obtain a representative sample such as 60 analytical units for foods in Food Category I, which includes meat. Ex. 1018 at 11. Chapter 1, at Section E, describes mixing to overcome non-uniform distribution of microorganisms, which is the expected state of a food sample. *Id.* at 17–18.

113. Chapter 5, at Section C-15, describes steps to prepare a sample of meat for isolation of *Salmonella*, which includes placing the sample in a blender with a lactose broth. *Id.* at 27. At Section D of Chapter 5, the steps are described for isolating *Salmonella*. *Id.* at 28–31.

114. Chapter 10 describes, at Section C, collecting sub-samples to obtain a representative sample, combining the sub-samples, blending in *Listeria* enrichment

broth to obtain an enriched, pooled composite sample, and then incubating. *Id.* at 43–45. Chapter 10 also describes, at Sections D and E, an isolation procedure and an identification procedure. *Id.* at 45–48.

3. MLG

115. The Microbiology Laboratory Guidebook (“MLG”) “contains protocols for analytical tests required by FSIS regulatory activities on meat and poultry products. Specifically, microbiological methods are presented for sample preparation, isolation and identification of the major foodborne pathogenic microorganisms.” Ex. 1019 at 1. The MLG is a multi-chaptered document that is the FSIS’s equivalent to the FDA’s Bacteriological Analytical Manual (“BAM”).

116. Exhibit 1028 consists of portions of a 2003 version of MLG, referred to herein as “MLG 2003” or simply “MLG,” including chapters 4, 5.03, and 8.03, which respectively relate to *Salmonella*, *E. coli*, and *Listeria*.

a. Chapter 5.03 — *E. Coli*

117. Chapter 5.03 includes a description of procedures for *E. coli* detection in various types of foods including raw beef. Section 5.5 titled “Detection Procedure” is shown below in its entirety:

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™.

b. Incubate all bags (static) with their contents for 20 to 24 h at $35 \pm 2^\circ\text{C}$. 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.

Id. at 31–32 (highlighting added). Section 5.5 describes collecting sub-samples that are representative of the entire sample of beef. *Id.* at 31. The sub-samples are uniformly dispersed in a selective broth pool, modified EC broth with novobiocin (“mEC+n”) and incubated. *Id.* at 31–32. From the enrichment cultures, a screening test is conducted. *Id.* at 32.

118. Chapter 5.03 includes detailed information about steps to be taken to confirm a positive sample, as summarized in Section 5.1 as shown below:

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.

Id. at 27 (highlighting added).

b. Chapter 8.03 — *Listeria*

119. Chapter 8.03 includes a description of procedures for *Listeria* detection in various types of foods including chicken and beef. Section 8.5.1 titled “Sample Preparation” is shown below in its entirety:

8.5.1 Sample preparation

- a. Meat, poultry and egg products: A 25 ± 1.0 g portion is used for raw and processed red meat, poultry and egg product testing. The test portion should be a composite representative of the entire sample or available samples common to a specific lot.
 - i. Intact retail packages must be disinfected at the incision sites immediately prior to incision for sampling. Appropriate disinfectants include but are not limited to ca. 3% hydrogen peroxide, ca. 70% ethanol or ca. 70% isopropanol. If the package does not appear to be clean, scrub gently using soapy water and rinse thoroughly prior to disinfection. A sterile scalpel may be helpful for cutting the packaging. Aseptically pull the packaging away to expose the product for sampling.
 - ii. Using a sterile scalpel, knife, spoon, chisel or other tool cut small pieces from representative sites on the sample and/or a variety of same-lot samples to prepare a composite sample.
 - iii. Place 25 ± 1.0 g of the composite sample in a sterile Stomacher™ bag (i.e. filter bag recommended). If necessary, double-bag with a non-filter bag prior to stomaching (e.g. zip-lock bag).
 - iv. If analysis of the test portion is not to be initiated within 1 h, store at $\leq -10^{\circ}\text{C}$. Do not dilute the sample until ready to initiate analysis.
 - v. For analysis, proceed to Section 8.5.2.
- b. Outbreak/recall samples: Some samples or sample lots, particularly those implicated in foodborne illness outbreaks, may require analysis of up to thirteen 25 g test portions. The test portion should be a composite representative of the entire sample or available samples common to a specific lot. The need for multiple subsample analyses must be determined on a case-by-case basis, or by client requirements.

Id. at 46–47 (highlighting added).

120. After collecting sub-samples from a specific lot and combining the subsamples, the lot samples are dispersed in a selective broth pool, modified

University of Vermont broth (“UVM”), and then incubated to yield a homogenate as described at Section 8.5.2 shown below:

8.5.2 Primary Enrichment in UVM broth

- a. For all meat, poultry and egg product samples (including outbreak/recall samples):

To the 25 ± 1.0 g test portion, dispense 225 ± 5 ml (or 225 ± 5 g) of UVM broth. Stomach or blend for 2 ± 0.2 minutes. If blended, aseptically transfer the homogenate to an appropriate sterile container. Incubate the homogenate at $30 \pm 2^\circ\text{C}$ for 22 ± 2 h. Proceed to Section 8.5.3.

Id. at 47–48 (highlighting added).

121. The UVM enrichment is then transferred to a second selective enrichment broth, Fraser broth (“FB”), as described at Section 8.5.3, which is as shown below in the highlighted text here:

8.5.3 Secondary Enrichment in FB and Direct Plating of UVM

- a. Transfer 0.1 ± 0.02 ml of the UVM enrichment to 10 ± 0.5 ml of FB. As per media preparation instructions, be sure that appropriate supplements have been added to the FB prior to inoculation. Incubate inoculated FB tubes at $35 \pm 2^\circ\text{C}$ for 26 ± 2 h.
- b. Streak a MOX plate. Streak a loopful or a drop approximating 0.1 ml of the UVM over the surface of the plate. Alternatively, dip a sterile cotton-tipped applicator or equivalent into the UVM and swab 25-50% of the surface of a MOX plate. Use a loop to streak for isolation from the swabbed area onto the remainder of the plate. Incubate the MOX at $35 \pm 2^\circ\text{C}$ for 26 ± 2 h.

Id. at 48–49 (highlighting added). After selective enrichment, testing is performed for *Listeria*. *Id.* at 49–57.

VIII. PATENTABILITY

122. In my analysis below, I apply the standard discussed above at III-E to the words and phrases of the claims of the '486 Patent, unless otherwise stated. My opinions are provided for the purposes of these petitions only.

A. Grounds 1–5 and Independent Claims 1 and 11

123. The table below lists the references in each ground and the claims that are not patentable based on the grounds.

Grounds 1–5					
Ground	References	§	Ind. Claims	Dependent Claims	
1	Rayman	102	1	4–8, 17, and 19	
2	Rayman and PR/HACCP	103			2–8, 17, 19, and 27
3	Nagar and Rayman		1, 11		4–8 and 12–26
4	Nagar and Karlsen				4–8 and 12–28
5	Nagar and PR/HACCP				2–8 and 12–30

1. Independent Claims

a. Preamble

124. The preamble of the independent claims is listed below:

Claim 1	Claim 11
A method for detection of a particular bacterial pathogen or bacterial microbe, comprising:	

125. Below is the content of Rayman that is relevant to the preamble:

Preamble	Rayman in Grounds 1–3
Rayman is focused on detecting <i>Escherichia coli</i> biotype I in raw meats as indicated by its title, as shown in the section reproduced below: RAYMAN, M. K., G. A. JARVIS, C. M. DAVIDSON, S. LONG, J. M. ALLEN, T. TONG, P. DODSWORTH, S. McLAUGHLIN, S. GREENBERG, B. G. SHAW, H. J. BECKERS, S. QVIST, P. M. NOTTINGHAM, et B. J. STEWART. 1979. ICMSF methods studies. XIII. An international comparative study of the MPN procedure and the Anderson–Baird–Parker direct plating method for the enumeration of <i>Escherichia coli</i> biotype I in raw meats. <i>Can. J. Microbiol.</i> 25: 1321–1327. Ex. 1010, 1322.	

126. Below is the content of PR/HACCP that is relevant to the preamble:

Preamble	PR/HACCP in Grounds 2 and 5
PR/HACCP includes regulations for “all slaughterhouse establishments” . . . “to test carcasses for generic <i>E. coli</i> as a tool to verify process control. This document outlines the sampling and microbial testing that should be followed to meet this requirement.” Ex. 1017, 38929.	

127. Below is the content of Nagar that is relevant to the preamble:

Preamble	Nagar in Grounds 3–5
Nagar states: [0035] Target pathogens that can be detected and identified using the method and system of the present invention include Gram (-) organisms such as the <i>Enterobacteriaceae</i>, including <i>E. coli</i>, and Gram (+) organisms, including <i>Staphylococcus aureus</i> and <i>Enterococcus faecalis</i>. Ex. 1012 ¶ [0035].	

128. Below is the content of Karlsen that is relevant to the preamble:

Preamble	Karlsen in Ground 4
Karlsen describes “[a] method for the identification of the presence of <i>E. coli</i> . . . in a liquid or liquified sample. Ex. 1016, Abstract.	

129. Each reference discloses methods for detecting *E. coli*.

b. First Step — Sampling

130. The first step, the sampling step, of the independent claims is listed below:

Claim 1	Claim 11
obtaining	
a test	a liquid
sample,	
the sample being solid or semi-solid;	

131. Below is the content of Rayman that is relevant to the sampling step:

Sampling Step	Rayman in Grounds 1–3
Rayman list the types of samples that were collected under the subheading, <i>Samples</i>, which is under the main heading Materials and Methods, as shown in the section reproduced below: Materials and Methods <i>Samples</i> Raw red meats, poultry and pork, and washings and surface swabs of carcasses thereof were examined. All samples, frozen or non-frozen, were obtained in the country of the participating laboratory and were naturally contaminated. Ex. 1010, 1322.	

132. The section of Rayman copied above indicates that Rayman was focused on sampling meats including collecting pieces, washings, and swabbing carcass surfaces.

133. Below is the content of PR/HACCP that is relevant to the sampling step:

Sampling Step	PR/HACCP in Grounds 2 and 5
PR/HACCP includes “Appendix F—Guidelines for Escherichia coli Testing for Process Control Verification in Cattle and Swine Slaughter Establishments.” Ex. 1017, 38929. PR/HACCP describes “Specific Sample Collection Procedures” for cattle carcasses using “[a] sampling sponge (which usually comes dehydrated and prepackaged in a sterile bag) . . . swab the areas in the sequence indicated in this sampling protocol.” <i>Id.</i> , 38931.	

134. Below is the content of Nagar that is relevant to the sampling step:

Sampling Step	Nagar in Grounds 3–5
Nagar states: [0036] For example, in testing a food sample, the rapidly growing target pathogens that could be tested for could include, but are not limited to, <i>Salmonella</i> and/or <i>E. coli</i> . Since these pathogens which are often found in a variety of foods (e.g., ground beef, milk, cheese, vegetables, apple cider, etc.), they are often "targeted" for testing in order to determine if they are present in a food sample. Ex. 1012 ¶ [0036].	

135. The paragraph of Nagar reproduced above indicates Nagar sampled a spectrum of food samples including those that are solid such as ground beef and cheese and liquids such as apple cider.

136. Below is the content of Karlsen that is relevant to the sampling step:

Sampling Step	Karlsen in Ground 4
Karlsen states: The term “liquid or liquified sample” may be used herein interchangeably with the term “test sample”. The liquid or liquified sample may comprise . . . food , feed, and water. It is therefore contemplated that the liquid or liquified sample comprise any number of sample types, both environmental and clinical. Examples of the liquid or liquified sample include but are not limited to drinking water, bathing water, cerebrospinal fluid, blood, juices , soft drinks, wine or other alcoholic beverages, milk , milk products, cheeses , and yogurt . Ex. 1016, 14 (emphasis added).	

137. Rayman and PR/HACCP sample meat, which is a solid, thus teaching the step recited in claim 1. Nagar samples meat, cheese and apple cider thus meeting the sampling step of claims 1 and 11. Karlsen’s cheese and liquids meet the sampling step of claims 1 and 11.

c. Second Step —Diluting

138. The second step, the diluting, of the independent claims is listed below:

Claim 1	Claim 11
diluting the sample with liquid enrichment medium at a ratio of sample to diluent between about 1:0.1 to about 1:5	
(wt./vol.) or lesser dilution;	(vol./vol.) or lesser dilution, or not diluting the sample;

139. Below is the content of Rayman that is relevant to the diluting step:

Diluting Step	Rayman in Grounds 1–3
	Rayman tested the technique of Anderson and Baird-Parker. As shown in the reproduced section provided below, Rayman “solids were weighed and homogenized in 4 parts (v/w) of 0.1 % peptone water . . . [to obtain a] homogenate (1:5).” Ex. 1010, 1322. <i>Enumeration Procedure</i> Two analysts in each laboratory simultaneously but independently carried out the analyses. To avoid sampling errors, solids were weighed and homogenized in 4 parts (v/w) of 0.1% peptone water by one analyst, and the homogenate (1:5) was divided between the two analysts. Samples were examined by the direct plating (DP) and most probable number (MPN) methods for enumerating <i>E. coli</i> biotype I. Biotypes of <i>E. coli</i> are defined in <i>Microorganisms in foods</i> (ICMSF 1978).

140. The technique of Anderson and Baird-Parker, tested by Rayman, diluted food samples “1 in 5 or 1 in 10 with 0.1% (w/v) peptone water.” Ex. 1009, 113. The result reported by Rayman were discussed by NRC and the dilution was characterized as “a **1:5 dilution** of meat.” Ex. 1011, 353.

141. Below is the content of PR/HACCP that is relevant to the diluting step:

Diluting Step	PR/HACCP in Grounds 2 and 5
PR/HACCP includes “Appendix F—Guidelines for Escherichia coli Testing for Process Control Verification in Cattle and Swine Slaughter Establishments” which includes the following steps under the “Collection” heading. 1. “Carefully pour about half the contents of the sterile BPD [Butterfield's phosphate diluent] bottle (approximately 10 ml) into the sponge bag to moisten the sponge.” Ex. 1017, 38931 (Appendix F, Collection, Step 7). 2. Swab three 100 cm² areas of the carcass with the same hydrated sponge for a total area of 300 cm². <i>Id.</i> (Appendix F, Collection, Steps 14–17). 3. “Add the additional BPD (about 15 ml) to the sample bag to bring the total volume to approximately 25 ml.” <i>Id.</i> (Appendix F, Collection, Step 23). Based on the foregoing, the total buffer is 25 ml and the total area equals 300 cm². Dividing 300 cm² by 25 ml equals 12 cm² per 1 ml or 1 cm² = 0.083 ml, which equates to a 1:0.083 dilution. Some POSA only count the 15 ml added to the bag so on that basis, dividing 300 cm² by 15 ml equals 20 cm² per 1 ml or 1 cm² = 0.05 ml, which equates to a 1:0.05 dilution. In either case, the dilution is less than 1:0.1.	

142. Below is the content of Nagar that is relevant to the diluting step:

Diluting Step	Nagar in Grounds 3–5
Nagar discloses all aspects of the steps recited in the independent claims except for the dilution step because Nagar only discloses decimal dilution. For example, Nagar describes a study wherein 25 g of ground beef then 225 ml resuscitation/growth medium were added in a stomacher bag. Ex. 1012 ¶ [0050].	

143. Below is the content of Karlsen that is relevant to the diluting step:

Diluting Step	Karlsen in Ground 4
Karlsen indicates: an approximately 10 ml portion of the liquid or liquified sample (as defined elsewhere) herein which is desired to be tested for bacteria [is] mixed with approximately 10 ml of an enrichment media for enhancing growth of bacteria which may be present in the sample. Ex. 1016, 23 (emphasis added). Thus, Karlsen discloses a 1:1 ratio of sample to diluent.	

144. Rayman meets the limitation of the second step of claim 1, the diluting step, because the sample is diluted with liquid enrichment medium to 1:5 (wt./vol.). PR/HACCP meets the diluting steps of claim 1 because the sample is diluted with liquid enrichment medium to at less than 1:0.1 (vol./vol.), and based on the calculations in the '486 Patent, the dilution is also less than 1:0.1 (wt./vol.).

145. Nagar discloses decimal dilution. A POSA would have modified the teachings of Nagar for obtaining and decimal diluting samples based on the teachings of Rayman, Karlsen, or PR/HACCP to only dilute the sample with liquid enrichment medium at a ratio of sample to diluent respectively to 1:4, 1:1, or less than 1:0.1.

c. Third Step — Incubating

146. The third step, the incubating step, of the independent claims is listed below:

Claim 1	Claim 11
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incubating, in an incubator,	
the diluted sample	the diluted or the not diluted sample
at an optimal temperature for the particular bacterial pathogen or bacterial microbe to be detected for a time period sufficient to allow levels of the particular bacterial pathogen or microbe to reach levels detectable by use of an assay suitable to detect the particular bacterial pathogen or bacterial microbe; and	

147. The incubating step recites “incubating in an incubator.” The ’486 Patent states that “[s]uitable incubators are known in the art.” Ex. 1001, 13:30. On this basis, the incubator recited in the incubating step is just a suitable conventional incubator, not an innovation.

148. Below is the content of Rayman that is relevant to the incubating step:

Incubating Step	Rayman in Grounds 1–3
Rayman cited to various studies including that of Anderson and Baird-Parker, which Rayman tested, and described them as using “direct incubation at an elevated temperature of 44.0–44.5°C.” Ex. 1010, 1322. Rayman also stated that when incubating plates “in a 44.5±0.5°C water-jacketed or similarly accurate incubator for 24 h.” “this temperature range be maintained throughout the incubation period.” Ex. 1010, 1323.	

149. Because the incubator mentioned in the incubating step is not unique, the limitation is met by a conventional incubator like that in Rayman. Rayman also meets the limitation of the incubating step because the sample is incubated in an incubator at an elevated temperature throughout the incubation period.

150. As referenced in PR/HACCP, AOAC Official Methods of Analysis include sampling, diluting, incubating, and assaying to detect *E. coli*.

151. Below is the content of Nagar that is relevant to the incubating step:

Incubating Step	Nagar in Grounds 3–5
Nagar provides: <u>SUMMARY OF THE INVENTION</u> [0022] The invention solves the above-identified need by providing a method and system that rapidly detects pathogens in a sample. [0023] The method and system involve the use of a capture matrix throughout the resuscitation, growth and detection processes. In accordance with one embodiment of the present invention, the method and system include the steps of: placing a sample in a container containing a resuscitation/growth medium; optionally homogenizing the sample; placing a capture matrix in the sampling bag with the sample; incubating the sample in the resuscitation/growth medium for a pre-determined amount of time at an elevated temperature while optionally shaking; optionally removing the capture matrix from the container; performing detection assays to detect the presence of the target pathogens in the sample. Ex. 1012 ¶ [0023]. Nagar also provides: [0044] Following the dispensing of the sample in the container, the sample can be stomached or homogenized within the bag utilizing means well known to those skilled in the art, (e.g., via Stomacher Lab-Blender, Seward Medical, London, U.K.). The sample is stomached/homogenized within the bag containing the resuscitation/growth medium. The container is incubated in the presence of the capture matrix at a temperature suitable for growth of the target pathogen. The incubation of the sample is carried out to resuscitate and grow sub-lethally injured pathogens present in the sample. [0045] At the end of the incubation period, specific detection assays can be performed on the capture matrix to detect the presence of the target microorganism. <i>Id.</i> ¶¶ [0044], [0045].	

152. These paragraphs of Nagar reproduced above discuss incubating in a container. Thus, Nagar meets the step for claims 1 and 11 because the sample is incubated “in a container” at an elevated temperature for an incubation period sufficient for the “assays to detect the presence of the target pathogens in the sample.” Ex. 1012 ¶ [0023].

153. Below is the content of Karlsen that is relevant to the incubating step:

Incubating Step	Karlsen in Ground 4
Karlsen indicates: an approximately 10 ml portion of the liquid or liquified sample . . . mixed with approximately 10 ml of an enrichment media for enhancing growth of bacteria which may be present in the sample and is incubated, preferably at about 41° to about 44°C for about three to about five hours. Ex. 1016, 23 (emphases added).	

154. Karlsen does not refer to an incubator but incubation occurs at the preferable temperature of 41C to 44C. These temperatures are above ambient temperature and therefore require a mechanical incubator.

d. Fourth Step — Assaying

155. The fourth step, the assaying step, of the independent claims is listed below.

Claim 1	Claim 11
determining, by assaying	

the diluted incubated test sample, or a portion thereof,	the diluted or the not diluted incubated test sample, or a portion thereof,
with the assay suitable to detect the particular bacterial pathogen or bacterial microbe, whether the sample is contaminated with the particular bacterial pathogen or bacterial microbe.	

156. Below is the content of Rayman that is relevant to the assaying step:

Assaying Step	Rayman in Grounds 1–3
Rayman states: After review of the rapid methods for enumerating fecal coliforms and <i>E. coli</i> . . . the ICMSF selected as most promising the direct plating method of Anderson and Baird-Parker (1975). Ex. 1010, 1322.	

157. Rayman also meets the limitation of the assaying step because the incubated sample is tested with the direct plating method.

158. Below is the content of PR/HACCP that is relevant to the assaying step:

Assaying Step	PR/HACCP in Grounds 2 and 5
PR/HACCP indicates “Samples must be analyzed using one of the <i>E. coli</i> (Biotype I) quantitation methods found in the Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC), International, 16th edition.” Ex. 1017, 38933.	

159. Rayman meets the limitation of the fourth step of claim 1, the assaying step, because Rayman describes determining, by assaying with an assay suitable to detect *E. coli* in meat samples.

160. Below is the content of Nagar that is relevant to the assaying step:

Assaying Step	Nagar in Grounds 3–5
Nagar provides:	
<u>SUMMARY OF THE INVENTION</u> [0022] The invention solves the above-identified need by providing a method and system that rapidly detects pathogens in a sample. [0023] The method and system involve the use of a capture matrix throughout the resuscitation, growth and detection processes. In accordance with one embodiment of the present invention, the method and system include the steps of: placing a sample in a container containing a resuscitation/growth medium; optionally homogenizing the sample; placing a capture matrix in the sampling bag with the sample; incubating the sample in the resuscitation/growth medium for a pre-determined amount of time at an elevated temperature while optionally shaking; optionally removing the capture matrix from the container; performing detection assays to detect the presence of the target pathogens in the sample. Ex. 1012 ¶ [0023]. Nagar also lists specific types of assays “such as enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR), or manual strand displacement amplification (SDA).” Id. ¶ [0026]; <i>see also id.</i> ¶ [0045]– [0047].	

161. Nagar meets the assaying step for claims 1 and 11 because Nagar describes performing detection assays to detect the presence, in the sample, of the target pathogens such as *E. coli*.

162. Below is the content of Karlsen that is relevant to the assaying step:

Assaying Step	Karlsen in Ground 4
Karlsen indicates: The method of detection and identification preferably involves enrichment of the bacteria using selective culture media, recovery of bacteria from media, lysing of the bacteria to release DNA, polymerase chain reaction (PCR) amplification of the target sequences using the novel oligonucleotides, and specific detection of PCR products. Ex. 1016, 23 (emphases added);.	

2. Ground 1 — Claim 1 Is Not Patentable Based on Rayman

163. IEH only asserts that the dilution step differed from the prior art because the '486 Patent acknowledges the well-known usage of sampling, decimal dilution, incubating in an incubator, and assaying. However, Rayman shows that dilution of 1:5 wt./vol. was known before the priority date of the '486 Patent. Rayman also confirms that it was well known to sample meat, incubate in an incubator, and to assay to detect microorganisms. On this basis, Rayman establishes that claim 1 does not recite anything new.

3. Ground 2 — Claim 1 Is Not Patentable Based on Rayman and PR/HACCP

164. Rayman establishes it was well known to sample meat, dilute to 1:5 wt./vol., incubate in an incubator, and to assay to detect microorganisms. Claim 1 also recites a lesser dilution than 1:0.1 A POSA would have been highly familiar with PR/HACCP and recognized its teachings to use very little diluent or liquid enrichment medium. As discussed above, use of sponge to swab a carcass uses so

little diluent that the sample to diluent dilution is less than 1:0.1, which is motivating to a POSA since using less diluent is cost efficient due to lower transportation costs, disposal costs, etc. A POSA also would have been motivated to use such swabbing because Rayman also describes obtaining samples of meat including “washings and surface swabs of carcasses.” Ex. 1010, 1322. Additionally, Rayman established that the method was effective so a POSA would have considered rapid, efficient, and effective testing to be the predictable outcome of modifying Rayman based on PR/HACCP. Given the importance of rapidly receiving health-related information, a POSA would have been highly motivated to act based on Rayman’s teachings and that trajectory would lead to PR/HACCP. In conclusion, a POSA would have considered it obvious to modify Rayman based on PR/HACCP.

4. Grounds 3–5 — Claims 1 and 11 Are Not Patentable Based on Nagar and Rayman, Karlsen, or PR/HACCP

165. Nagar discloses the subject matter that is acknowledged as being in the prior art in the ’486 Patent namely, obtaining a solid or liquid sample, decimal dilution, incubating, and assaying. Before the priority date, a POSA would have been motivated to modify Nagar to use less diluent, liquid enrichment medium. Using less liquid enrichment medium would reduce costs for transportation and disposal after testing. Nagar is titled “Rapid resuscitation, growth, capture and detection of microorganisms.” Ex. 1012, 1. The emphasis of Nagar, Rayman, Karlsen, and

PR/HACCP would have led a POSA to modify Nagar and to predictably expect rapid testing to be achieved. Ex. 1010, 32; Ex. 1012, title; and Ex. 1016, 3, 5, 24. In each of Grounds 3–5, a POSA would have simply substituted the decimal dilution of Nagar with the dilution that is respectively disclosed in Rayman (1:5), Karlsen (1:1), and PR/HACCP (less than 1:0.1). Given the importance of rapidly receiving health-related information, a POSA would have been highly motivated to act based on Nagar’s teachings and that trajectory would lead to increasingly less use of liquid enrichment medium as taught by Rayman, Klarsen, and PR/HACCP. In conclusion, a POSA would have considered it obvious to modify Nagar based on Rayman, Karlsen, or PR/HACCP.

B. Grounds 1–7 and Dependent Claims 2–10 and 12-30

166. Below is a listing of Grounds 1–7, their references, and their correlation to dependent claims 2–10 and 12–30, which would have been obvious based on Grounds 1–7.

Grounds 1–7 and Dependent Claims		
Ground	References	Dependent Claims
1	Rayman	4–8, 17, 19
2	Rayman and PR/HACCP	2–8, 17, 19, 27
3	Nagar and Rayman	4–8, 12–26
4	Nagar and Karlsen	4–8, 12–28
5	Nagar and PR/HACCP	2–8 and 12–30
6	Rayman and ICMSF	9 and 10

7	Rayman and AAPA	
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167. Below is a table listing the dependent claims based on their grouping as dependent from either claim 1 or 11 and also identifying the general subject matter of the dependent claim.

Claim 1	Claim 11	Subject Matter
2, 3, 27	28–30	Dilution Ratios
4, 5	12, 13	Temperature
6	14	Assay Type
7, 8	15, 16	Pathogen Type
9, 10		Lot Sample
17	18	Sample Types
19	20	Supplement
21–23	24–26	Locations

1. Grounds 1–5 and Dependent Claims 2–8 and 12–30

a. Claims 2, 3, and 27–30 — Dilution Ratios

27	The method of claim	1	wherein diluting is at a ratio of sample to diluent between about 1:0.1 to about	1:2 (wt./vol.)	or lesser dilution.
28		11		1:2 (vol./vol.)	
2		1		1:0.5 (wt./vol.)	
29		11		1:0.5 (vol./vol.)	
3		1		1:0.3 (wt./vol.)	
30		11		1:0.3 (vol./vol.)	

168. Because Nagar as modified by Karlsen discloses diluting a solid or liquid sample at a dilution of sample to diluent of 1:1, claims 27 and 28 would have been obvious based on Ground 4.

169. Because Rayman as modified by PR/HACCP discloses diluting a solid sample at a dilution of sample to diluent of less than 1:0.1, claims 2, 3, and 27 would have been obvious based on Ground 2.

170. Because Nagar as modified by PR/HACCP discloses diluting a solid or liquid sample at a dilution of sample to diluent of less than 1:0.1, claims 2, 3, and 27–30 would have been obvious based on Ground 5.

b. Claims 4, 5, 12, and 13 — Temperature

Claim 4	Claim 12
The method of claim 1,	The method of claim 11,
wherein the optimal temperature is within the optimal growth temperature range of the particular bacterial pathogen or bacterial microbe in the liquid enrichment medium.	

Claim 5	Claim 13
The method of claim 4,	The method of claim 12,
wherein the optimal temperature range is from about 25° C. to about 45° C.	

171. Rayman discloses incubation at a temperature of 44.0–44.5°C. Ex. 1010, 1322, 1323. On this basis, claims 4 and 5 were anticipated by Rayman and claims 4 and 5 would have been obvious based on Ground 2.

172. Nagar discloses incubation at a temperature of approximately 35°C +/- 2°C, 37°C. Ex. 1012 ¶¶ [0009], [0050]. On this basis, claims 4, 5, 12 and 13 would have been obvious based on Grounds 3–5.

c. Claims 6 and 14 — Assay Types

Claim 6	Claim 14
The method of claim 1,	The method of claim 11,
wherein the assay suitable for detection of the particular bacterial pathogen or bacterial microbe contamination is selected from the assay group consisting of immunoassays, nucleic acid amplification-based assays, PCR-based assays, nucleic acid hybridization-based assays, bio-sensor assays, immunostaining-microscopy-based assays, nucleic acid-array-based assays, DNA chip-based assays, classical microbiology-based assays, and chemical or biochemical assays based on the detection of compounds associated with the particular bacterial pathogen or bacterial microbe, and combinations thereof.	

173. Rayman is using two assays, as the paper is a methods comparison paper. The assays are a modified direct plating method and a Most Probable Number (MPN) method. Ex. 1010, 1322, 1323. The Rayman paper dates from the late 1970's (published in 1979). This was before the genetic assays (PCR, etc.) were commercially available, but would definitely fit within the “classical microbiology-

based assays,” because that is exactly what direct plating and MPN assays are. On this basis, claim 6 was anticipated by Rayman and claim 6 would have been obvious based on Ground 2.

174. Nagar states:

One method of detection is through the use of biochemical tests or assays. Examples of **biochemical assays** include enzyme detection, products of bacterial metabolism, immunochemical markers, specific stains, chemiluminescence, and bioluminescence.

Ex. 1012 ¶ [0046] (emphasis added); see also *id.*, claims 3 and 4. On this basis, claims 6 and 14 would have been obvious based on Grounds 3–5.

d. Claims 7, 8, 15, and 16 — Bacterial Pathogen or Microbes

Claim 7	Claim 15
The method of claim 1,	The method of claim 11,
wherein the particular bacterial pathogen or bacterial microbe is selected from the group consisting of <i>Escherichia coli</i> O157:H7 (<i>E. coli</i> O157:H7), enterohemorrhagic <i>Escherichia coli</i> (EHEC), enterotoxigenic <i>Escherichia coli</i> (ETEC), enteroinvasive <i>Escherichia coli</i> (EIEC), enterpathogenic <i>Escherichia coli</i> (EPEC), <i>Salmonella</i>, <i>Listeria</i>, <i>Yersinia</i>, <i>Campylobacter</i>, <i>Clostridial</i> species, <i>Staphylococcus</i> spp.; frank and opportunistic bacteria, indicator organisms including heterotrophes, generic <i>E. Coli</i>, total and fecal coliforms and enterococcus; spoilage organisms including <i>Pseudomonas</i>, and combinations thereof.	

Claim 8	Claim 16
The method of claim 7,	The method of claim 15,
wherein the particular bacterial pathogen or bacterial microbe is <i>Escherichia coli</i> O157:H7 (<i>E. coli</i> O157:H7, or H negative), <i>Salmonella</i> , <i>Listeria</i> , EHEC, and <i>Campylobacter</i> .	

175. Rayman test for *Escherichia coli* biotype I in raw meats. Ex. 1010, 1321. On that basis, claims 7 and 8 were anticipated by Rayman and claims 7 and 8 would have been obvious based on Ground 2.

176. Nagar discloses a process for detecting *E. coli*. Ex. 1012 ¶ [0035]; see also *id.* ¶ [0036] (describing testing food samples for “*Salmonella* and/or *E. coli*.”). On that basis, claims 7, 8, 15 and 16 would have been obvious based on Grounds 3–5.

e. Claims 17 and 18 — Sample Types

17	The method of claim 1,	where in the sample is selected from the group consisting of beef, pork, sheep, bison, deer, elk, poultry, fish, produce, dairy products, dry goods, raw and processed foods, environmental samples, soil, sludge, surface samples, samples taken by impingers and filtration, pharmaceuticals, and samples analyzed using enrichment-detection protocols.
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18	The method of claim 11,	wherein the liquid sample is selected from the group consisting of fruit juice, vegetable juice, milk and dairy products, raw and processed liquid foods, environmental samples, water, wastewater, samples taken by impingers and filtration, botanical liquid, industrial liquids, pharmaceutical liquids, and other liquid samples analyzed using enrichment-detection protocols.
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177. Rayman describes types of samples that are tested, including red meats, poultry, and port. Ex. 1010, 1322 On that basis, claims 7 and 8 were already known based on Rayman in Ground 1 and claims 7 and 8 would have been obvious based on Ground 2.

178. Nagar discloses a process for detecting pathogens in beef, milk, cheese, vegetables, apple cider, etc.” *Id.* ¶¶ [0036], [0050]. On that basis, claims 17 and 18 would have been obvious based on Grounds 3–5.

f. Claims 19 and 20 — Supplement

Claim 19	Claim 20
The method of claim 1,	The method of claim 11,
wherein the diluting comprises addition to the sample of a supplement selected from the group consisting of water, sugars, proteins, minerals, organics, vitamins and cofactors, antibiotics, dyes, indicators, buffers, agents to adjust the pH, water activity, nutritional contents, selective pressure to optimize the growth conditions for the particular bacterial pathogen or bacterial microbe, and combinations thereof.	

179. Rayman describes diluting samples with 0.1% peptone water. Ex. 1010, 1322. On that basis, claim 19 was already known based on Rayman in Ground 1 and claim 19 would have been obvious based on Ground 2.

180. Nagar discloses the use of “a pre-enrichment broth that comprises . . . glucose.” Ex. 1010 ¶ [0016]. Nagar further discloses a medium that includes buffer,

water, and a growth medium. *Id.* ¶ [0039]. On that basis, claims 19 and 20 would have been obvious based on Grounds 3–5.

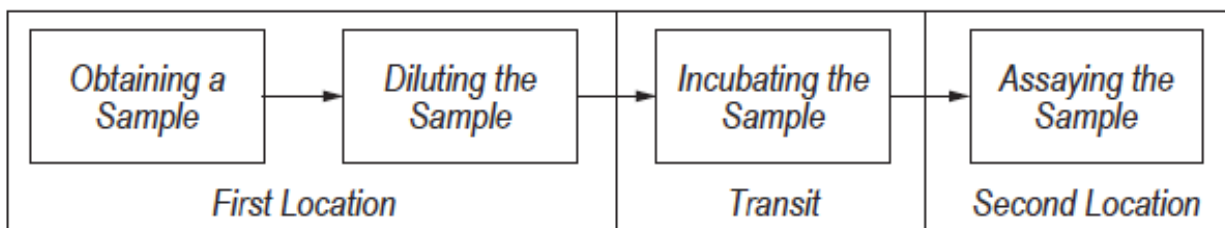
g. Claims 21–26 — Location

Claim 21	Claim 24
The method of claim 1,	The method of claim 11,
wherein the obtaining and diluting of the solid or semi-solid sample with liquid enrichment medium is at a first location, wherein incubating the diluted sample at an optimal temperature in the incubator is during transit of the diluted sample to a second location that is not the site of sample collection and dilution, and wherein determining by assaying the diluted incubated test sample, or a portion thereof, with the assay suitable to detect the particular bacterial pathogen or bacterial microbe, whether the sample is contaminated with the particular bacterial pathogen or bacterial microbe, is at the second location.	

Claim 22	Claim 25
The method of claim 21,	The method of claim 24,
further comprising addition to the sample, prior to or during incubation, of a supplement selected from the group consisting of water, sugars, proteins, minerals, organics, vitamins and cofactors, antibiotics, dyes, indicators, buffers, agents to adjust the pH, water activity, nutritional contents, selective pressure to optimize the growth conditions for the particular bacterial pathogen or bacterial microbe, and combinations thereof.	

Claim 23	Claim 26
The method of claim 21,	The method of claim 24,
wherein the second location is an in-house or local lab, or a remote location.	

181. The diagram below depicts the requirements recited in Claims 21–26, for the obtaining and diluting steps occur at a first location, incubating during transit to a second location, and assaying at the second location.



182. Nagar describes these steps:

The method and system of the invention can also be used during transportation of a test sample (e.g., soil, etc.) from the collection site to the testing laboratory. This may be achieved by placing the capture matrix inside a sampling/transportation device containing suitable resuscitation/growth medium, thus facilitating the capture, resuscitation and growth of the target pathogen for rapid detection.

Ex. 1012, ¶ [0031]. In Nagar, the first location equals a collection site and the second location is “the testing laboratory.” On that basis, claims 21–26 would have been obvious based on teachings of Nagar in Grounds 3–5.

2. Grounds 6 and 7: Obviousness of Claims 9 and 10 Based On *Rayman* in View of *ICMSF* and *Rayman* in View of *AAPA*

a. Claims 9 and 10 — Lot Samples

9		wherein obtaining a sample is obtaining a standard Lot-unit sample.
10	The method of claim 1,	wherein the sample is a composite-Lot sample, corresponding to a combination of samples selected from the group consisting of samples or subsamples taken from sublots/lots of raw or processed samples, environmental samples, industrial samples, pharmaceutical samples, bio-solid samples, samples taken by spore traps, settled dust, impingers, or filtration, and combinations thereof.

183. Rayman is not focused on collecting samples in a production environment; however, ICMSF and the AAPA describe collecting samples from lots and combining the samples in a production environment.

184. Regarding dependent claim 9, the '486 Patent states “[a] **prior art ‘Lot-unit sample’ refers to a 375 g composite sample**, comprised of seventy-five (75) gram samples from each combo-bin of the Lot-unit.” Ex. 1001, 9:66-10:1 (emphasis added). Moreover, “[a] ‘Lot-unit’ or ‘five-combo-lot unit’ refers to a composite unit, comprised of five combos (combo-bins). **In prior art sampling plans, the Lot-unit represents raw [sic] the material (composite trim) upon which sampling, testing and acceptance or rejection is based.**” *Id.*, 9:56-60 (emphasis added). Furthermore, “[a] ‘combo’ or ‘combo-bin’ refers to the trim packaging unit.” *Id.*,

9:52-53. “‘Trim’ refers to small pieces of meat and fat which are excised during the fabrication process in order to produce primal and subprimal pieces and marketable cuts.” *Id.*, 9:46-48. Thus, the “Lot-unit sample” of claim 9 is a 375 g composite sample of trim.

185. Regarding dependent claim 10, the '486 Patent states:

[a] **typical trim-testing** plan involves analysis of ‘five-combo-lot’ units, and **comprises analysis of a single composite-Lot sample of about 375 g**, prepared by combining five combo-bin samples (about 75 g each), in each case corresponding to one to five randomly-selected pieces from each combo-bin, such that, on average 5-25 pieces representing the five combo-bins are in the composite sample. The combo-bin is comprised of pieces of a plurality of carcasses, and thus the test results under **these prior art systems reflect random and incomplete sampling; that is, only a small fraction of the carcasses are represented in the test results, particularly where, as is true of many such plans, only a sub-fraction of the composite-Lot sample is used for the pathogen-testing assay (e.g., when large pieces of trim are collected).** For example, for ground beef production, final product testing comprises taking ground beef samples at given time intervals (e.g., every 10-30 minutes) and compositing a number of samples into one composite sample.

Ex. 1001, 13:61-14:11 (emphasis added). Therefore, the “composite-Lot sample” of claim 10 is also a 375 g composite sample of trim.

186. ICMSF discloses obtaining a 375 g composite sample of trim for testing:

The method can be adjusted for analysis of 15 analytical units into **a single 375 g composite sample. Research has validated that** up to fifteen 25 g analytical units of ground beef or **trimmings can be composited without significant loss in *E. coli* 0157:H7 detection sensitivity** (Silliker & Nickelson, 1995). This permits testing at a more reasonable cost and makes it more practical to screen lots to detect those of higher prevalence.

Ex. 1014, 327 (emphasis added).

187. Additionally, ICMSF states:

Many different types of **samples** are **collected** and submitted to the laboratory for analysis. Some are sample units from **lots** or **consignments** of food or ingredients for **lot acceptance** determination. . . . A **sample unit** is a small **portion of the lot** in which the distribution of both numbers and types of microorganisms should be **representative** of the whole lot.

Id., 225 (emphasis added).

188. ICMSF also describes combining samples from lots in a consignment:

To **reduce laboratory effort** while maintaining the stringency of the sampling plan, it would be helpful to

combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. . . . This finding suggests that considerable reductions may be made in the cost of laboratory analyses **by compositing (pooling) analytical units**. Such compositing offers the prospect of large increases in the number of analytical units, *n*, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort.

Id., 187 (emphasis added). Similarly, ICMSF states, “[t]here is the very great technical advantage of combining many analytical units for bacteriological examination.” *Id.*, 188; *see also id.*, 187.

189. Thus, ICMSF discloses obtaining a standard Lot-unit sample as recited by claim 9 and ICMSF also discloses a composite-Lot sample corresponding to a combination of samples selected from the group consisting of samples or subsamples taken from sublots/lots of raw or processed samples as recited by claim 10.

190. Furthermore, the AAPA refers to both the “Lot-unit sample” of claim 9 and the “composite-Lot sample” of claim 10 as “prior art”; thus, a POSA would have been familiar with obtaining samples as recited by claims 9 and 10.

b. Grounds 6 and 7: Obviousness Rationales

191. Rayman would have guided a POSA to obtain samples of meat. Ex. 1010, 1322. However, Rayman is written for a POSA who fully understands the details of collecting samples from lots and combining the samples. ICMSF provides such details and a POSA would be quite familiar with ICMSF and the procedures it describes for collecting and combining samples. Likewise, the AAPA provides these details as well to indicate that a POSA would have known how to collect and combine samples as recited by claims 9 and 10.

192. A POSA would have been motivated to combine the teachings of Rayman with ICMSF or AAPA because ICMSF indicates that combining analytical units (samples) reduces “laboratory effort” and the AAPA indicates that collecting and testing composites was known in the art. Moreover, a POSA would have improved Rayman with the similar methods of ICMSF or AAPA to yield efficient analysis, which is a predictable result. In this combination, a POSA would have simply substituted the sampling step of Rayman, as described above, with the sampling and combining steps of ICMSF or AAPA for predictably efficient analysis. The foregoing supports my conclusion that claims 9 and 10 would have been obvious based on Rayman in view of ICMSF and Rayman in view of AAPA.

IX. CERTIFICATION

193. In signing this declaration, I recognize that this declaration will be filed in support of the Petitioner in case numbers IPR2024-00183, IPR2024-00185, and IPR2024-00186 at the U.S. Patent and Trademark Office.

194. I reserve the right to supplement my opinions in the future to respond to any arguments that Institute for Environmental Health, Inc. raises and to consider new information as it becomes available to me.

195. I acknowledge that, as part of these IPRs, if an IPR trial is instituted, I may be subject to cross-examination in the United States regarding the content of this declaration. If cross-examination is required of me, I will make myself reasonably available for cross-examination within the United States during the time allotted for cross-examination.

196. I 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, further, that these statements were made with the knowledge that willful false statements and the like are punishable by fine, imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

Dated: July 31, 2024

Respectfully submitted,


James S. Dickson, Ph.D.