

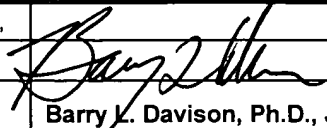
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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53 (c).

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☐ Additional inventors are being named on the _____ separately numbered sheets attached hereto					
TITLE OF THE INVENTION (500 characters max)					
MODULAR WET COMPOSITING-MULTIPLE LOT SCREENING PROTOCOLS FOR DETECTION OF PATHOGENS					
CORRESPONDENCE ADDRESS					
Direct all correspondence to:					
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ENCLOSED APPLICATION PARTS (check all that apply)					
☒ Specification Number of Pages		10		☐ CD(s), Number _____	
☐ Drawing(s) Number of Sheets				☒ Other (specify) <u>Postcard</u>	
☒ Application Data Sheet. See 37 CFR 1.76					
METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT					
☒ Applicant claims small entity status. See 37 CFR 1.27.					
☒ A check or money order for \$80 is enclosed to cover the filing fees.					
☐ The Commissioner is hereby authorized to charge filing fees to Deposit Account Number:				04-0258	
☒ The Commissioner is hereby authorized to charge any deficiency or credit any overpayment to Deposit Account Number:				04-0258	
☐ Payment by credit card. Form PTO-2038 is attached.					
The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.					
☒ No.					
☐ Yes, the name of the U.S. Government agency and the Government contract number are: _____					
Respectfully submitted,					
SIGNATURE				DATE	
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				47,309	
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**MODULAR WET COMPOSITING-MULTIPLE LOT SCREENING PROTOCOLS
FOR DETECTION OF PATHOGENS**

5 FIELD OF THE INVENTION

The present invention, in various aspects, relates generally to improving the efficiency of producing foods , and the safety and quality of food products, including but not limited to beef, pork, sheep, bison, deer, elk, poultry (*e.g.*, chicken and turkey) and fish, produce, juices, dairy products, dry goods (cereals, etc), and all manners of raw and processed foods, environmental
10 samples (water, wastewater, soil, surface samples, samples taken by impingers and filtration, etc), pharmaceuticals, and other types of samples that are to be analyzed using enrichment-detection protocols. More specifically, embodiments of the present invention relate to novel methods for pathogen testing that allow for enhanced sensitivity, enhanced cost savings, and enhanced traceability for remedial purposes.

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BACKGROUND

The ultimate goal of food producers and the regulatory agencies is to continually improve the safety and the quality of food products. In the United States, in response to the recent FSIS directives and guidelines, many food producers have adopted a sampling plan that involves
20 testing of raw (beef trims, ground beef) for *E. coli* O157:H7, and ready to eat products for *Listeria monocytogenes* and *Salmonella*. Similar trends are observed in other countries, for products detained for domestic consumption and/or export. The sampling plan is a “hold and release” plan which dictates that the food product being tested is not released by the producing facility to a customer until negative laboratory results are obtained for the presence of the
25 pathogens of concern, from each production “Lot”.

The art with respect to this testing consists of a number of different sampling plans, mostly shaped around ICMSF guidelines for testing food products, or similar statistical based sampling plans. For microbial pathogens a typical plan calls for taking anywhere from 10 to sixty pieces of samples from a given Lot, to form a composite sample for testing (depending on the outcome of infection/poisoning, the level of hazards, and potential increase in the hazard levels due to storage). For instance in testing for *E. coli* O157 the plan calls for taking 60 samples to form a single composite sample, and for *Listeria monocytogenes* 30 samples/composite sample.

Because of the cost associated with testing food manufacturer's resort to increasing the size of the "Test Lot", for instance the standard size lot for trim testing in the beef industry in the USA is five combos (of approximately 2000 pond/combo), or for *Listeria* and *Salmonella* (ready to eat products) one composite sample/production line/shift. The problem associated with the use of large size test lots is that when there is a positive finding of a pathogen a large quantity of products are downgraded or destroyed. Also because of the nature of the 'lotting' (using several independent sub-lots, or a long period of time in production), it is very hard to investigate the cause of the failure, and pinpoint the source/cause.

Once a composite sample is constructed, it is then enriched for the presence of a given pathogen by the addition of appropriate amounts of enrichment media and incubation at appropriate temperature for a given amount of time. The composite sample is then tested by any one of the available, recognized methods including enrichment followed by Immunoassay based tests or PCR based methods, or culturing of the organism of interest.

This art suffers from several deficiencies. *First*, the composite Lot sample represents several sublots (for instance 5 combo/pallets of products, or entire shift of production) and if a

positive finding is obtained the entire lot is rejected (all 5 combos/pallets, or one shift of production) and diverted to economically undesirable end uses (e.g. cooking or disposal), even though only a portion of the composite sample (corresponding to one or two combos/pallets or an hour of production) contains the pathogen of concern.

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Second, the current art does not allow for retesting the sublots mainly because microbial contaminants are unevenly spread throughout the products, and in many instances the levels of contamination are minute and may be present on very small portions of the products in each subplot. Therefore, once a composite sample is constructed if it tests positive all of the sublots are destroyed. The regulatory agencies do not allow for re-testing of the sublots, because they rightly argue that after a lot tests positive, negative test results of the sublots or a new composite sample, are meaningless since the microbial contaminants are not uniformly present throughout the products.

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Third, the current 'lotting' protocols often group together unrelated pallets of combos of products or hours of production. This makes investigating the nature of the failure, a difficult task. For instance when a combo/pallet of product can be traced through the production lines, five unrelated combos/pallets are much harder to trace, and there is no way to determine which one(s) contained the pathogen, especially that re-sampling is not a viable option since it may not yield the same result.

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Therefore, there is a pronounced need in the art to implement a testing protocol that provides a greater measure of assurance that the food product is safe, that provides economic relief to the producer, and allows for effective tracking of the contaminated lot(s) for remedial purposes.

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SUMMARY OF THE INVENTION

The present invention provides novel enrichment, testing and detection methods for detection of pathogens or other microbes in a food, water, wastewater, industrial, pharmaceutical, botanical, environmental samples and other types of samples analyzed by enrichment-detection methods. In preferred aspects, a production Lot is defined as a single unit of production,, rather than (as in the prior art) as a composite Lot (comprised of multiple production units). A number of pieces/portions of sample are collected from the single production unit and composited to form one single production unit Lot sample. Samples taken from each unit of production are enriched *separately* using appropriate enrichment protocols to allow for the levels of the target organism to reach detection levels and *uniformity of presence* in the enriched sample, thus circumventing the prior art problem of having microbial contaminants that are not uniformly present throughout the product sample. Equal portions of enrichment buffer are removed (aseptically) from several single unit production Lots (preferably 5) and pulled together to form a modular wet composite sample (MWCS). The MWCS is then tested using an appropriate test (detection assay) for the target organism. If a *negative* result is obtained from the MWCS, then all of the individual Lots are released. If a *positive* result is obtained from a MWCS, the enrichment buffers from each of the individual single production unit Lots are then separately (individually) tested, using the same test protocol. If a *positive* finding is obtained for any individual single production unit Lot sample, that particular Lot sample is diverted to cooking, disposal, or other acceptably safe endpoint. If a *negative* finding is obtained for any individual single production unit Lot sample, then that particular Lot is released. In this manner, any member lot of the MWCS that tests *negative* using the same method (about 5-fold more sensitive relative to the prior art), or the enhanced method (about fifty times more sensitive), will be released for consumption.

DETAILED DESCRIPTION OF THE INVENTION

According to preferred aspects of the present invention, a production Lot is defined as a single unit of production (*e.g.*, a single combo, bin, pallet, or segment of production, etc.), rather than (as in the prior art) as a composite Lot (comprised of multiple production units).

5

In the preferred embodiments, a number of pieces (portions) of sample are collected from the single production unit (*e.g.*, from the single combo, bin, pallet, or segment of production, etc.), and composited to form one single production unit (production Lot) sample. Enrichment buffer is added to the sample (with the exception of when dry enrichment is done, wherein no enrichment buffer is initially required).

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Significantly, in the inventive modular wet compositing method, samples taken from each unit of production are enriched *separately* using appropriate enrichment protocols to allow for the levels of the target organism to reach detection levels and *uniformity of presence* in the enriched sample (thus circumventing the prior art problem of having microbial contaminants that are not uniformly present throughout the product sample).

15

Following incubation for the target organism, equal portions of enrichment buffer are removed (aseptically) from several single unit production Lots (preferably 5) and pulled together to form a modular wet composite sample (MWCS). The MWCS is then tested using an appropriate test (detection assay) for the target organism. If a *negative* result is obtained from the MWCS, then all of the individual Lots are released.

20

If a *positive* result is obtained from a MWCS, the enrichment buffers from each of the individual single production unit Lots are then separately (individually) tested, using the same test protocol. More preferably, an augmented protocol that uses a concentration step for the

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target organism, such as immunomagnetic bead separation, affinity chromatography, etc., is used to test the enriched individual single production Lot samples.

5 In preferred aspect, the inventive method of analysis is modified by introducing a step employing antibody-coated paramagnetic beads into the procedure (*e.g.*, DNA-based detection, biosensor based detection, classical microbiological detections, etc). The beads afford an approximately 10-fold increase in sensitivity. This, combined with the fact that the individual single production unit Lot samples are not 5-fold diluted relative to the MWCS, allows for an overall 50-fold increase in sensitivity relative to prior art sampling and testing protocols. If a
10 lateral flow device is used for detection, the immunomagnetic concentration may or may not be used, in which case the detection sensitivity is increased by about 5-fold.

If a *positive* finding is obtained for any individual single production unit Lot sample, that particular Lot sample is diverted to cooking, disposal, or other acceptably safe endpoint. If a
15 *negative* finding is obtained for any individual single production unit Lot sample, then that particular Lot is released. In this manner, any member lot of the MWCS that tests *negative* using the same method (about 5-fold more sensitive relative to the prior art), or the enhanced method (about fifty times more sensitive), will be released for consumption.

20 In alternate preferred embodiments, a modular wet composite is reconstructed from the member lots that have tested *negative*, and subjected to a third layer of testing to again show (confirm) that they are negative.

The methods of the present invention provide substantial economic savings, because of
25 the precision in detecting small portions of production (single production units) which are contaminated. The inventive methods prevent unnecessary destruction of *negative* Lots that are

condemned because of guilt by association (*i.e.*, just because they were grouped with a contaminated composite prior art multiple production unit Lot in a prior art testing protocol). Where a *positive* finding is obtained, a smaller quantity of material is diverted to an economically undesirable end use.

5 Additionally, and significantly, In addition, it is easier to trace the source of the contamination, because the single production unit combo contains just one product.

EXAMPLE I

10 (The present inventive methods were shown to provide a substantial economic advantage, while providing for enhanced safety)

TABLE 1, shows the result of testing 62,919 multiple lot screening using the modular wet compositing method. Using prior art protocols, all of the production sublots that comprised the 271 positive samples would go to rendering or be downgraded for cooking (about 2,710,000 pounds of product).

15 By contrast, using the inventive methods only 391 single production unit combo's, corresponding to 782,000 pounds of the product will be sent to rendering or cooking, and the rest (1,928,000 ponds) is released to the chain of commerce). In this particular example, where the product was beef trim, this corresponds to a cost savings of about \$2,500,000, relative to prior art methods.

20 Additionally, the testing of the single production unit Lots is 5- to 50-fold more sensitive than the MWCS. This provides substantial additional assurance of the safety of the food products.

25 **Table 1: Results of modular wet composite testing of 62,919 multiple lots.**

Location	Total # Analyses	# Positives	1 Bag	2 Bags	3 Bags	4 Bags	5 Bags
Est. A, B, C.	62919	271	180	39	18	11	7

CLAIMS

ABSTRACT OF THE DISCLOSURE

The present invention provides novel enrichment, testing and detection methods for detection of pathogens or other microbes in a food, water, wastewater, industrial, pharmaceutical, botanical, environmental samples and other types of samples analyzed by enrichment-detection methods. In preferred aspects, a production Lot is defined as a single unit of production,, rather than (as in the prior art) as a composite Lot (comprised of multiple production units). A number of pieces/portions of sample are collected from the single production unit and composited to form one single production unit Lot sample. Samples taken from each unit of production are enriched *separately* using appropriate enrichment protocols to allow for the levels of the target organism to reach detection levels and *uniformity of presence* in the enriched sample, thus circumventing the prior art problem of having microbial contaminants that are not uniformly present throughout the product sample. Equal portions of enrichment buffer are removed (aseptically) from several single unit production Lots (preferably 5) and pulled together to form a modular wet composite sample (MWCS). The MWCS is then tested using an appropriate test (detection assay) for the target organism. If a *negative* result is obtained from the MWCS, then all of the individual Lots are released. If a *positive* result is obtained from a MWCS, the enrichment buffers from each of the individual single production unit Lots are then separately (individually) tested, using the same test protocol. If a *positive* finding is obtained for any individual single production unit Lot sample, that particular Lot sample is diverted to cooking, disposal, or other acceptably safe endpoint. If a *negative* finding is obtained for any individual single production unit Lot sample, then that particular Lot is released. In this manner, any member lot of the MWCS that tests *negative* using the same

method (about 5-fold more sensitive relative to the prior art), or the enhanced method (about fifty times more sensitive), will be released for consumption.

Document7

APPLICATION DATA SHEET**Application Information**

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LOT SCREENING PROTOCOLS FOR
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Country::	Application number::	Filing Date::	Priority Claimed::

Assignee Information

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Country of mailing address::	
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