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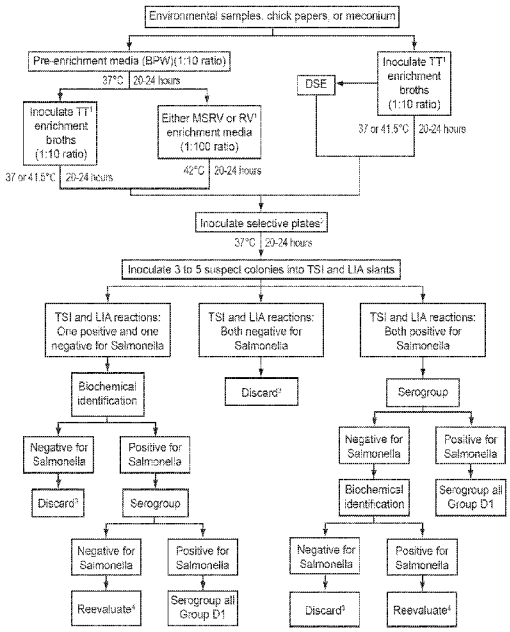
(54) Title: METHODS AND KITS FOR DETECTION OF SALMONELLA ENTERITIDIS AND RELATED SEROVARS



1. Tetrathionate enrichment broth, e.g., Rappaport-Vassiliades (RV) or modified semisolid RV (MSRV).
2. Selective plates such Brilliant Green Novobiocin (BGN) or xylose-lysine tergitol 4 (XLT 4).
3. Reevaluate if epidemiologic, necropsy, or other information indicates the presence of an unusual strain of Salmonella.
4. If biochemical identification and serogroup procedures are inconclusive, restreak original colony onto non-selective plating media to check for purity. Repeat biochemical and serology tests.

Fig. 1

(57) Abstract: Methods and kits for reducing false-positive results in testing target microorganisms in a sample comprising said target microorganisms and at least one contaminant, using immunomagnetic separation are disclosed, especially where the target microorganisms are Salmonella serogroup D1 serotypes and the contaminant is another Salmonella serogroup. Methods and kits for selectively isolating target microorganisms in testing target microorganisms in a sample comprising said target microorganisms and at least one contaminant, using immunomagnetic separation are also disclosed. The invention provides an improved method for isolating, detecting, characterizing, and confirming microorganisms relevant to human health and food safety from environmental surveillance samples and food specimens particularly, but not limited to those contaminated with other microorganisms, either per se or as indicator organisms, using enrichment and purification on magnetic beads coated with specific antibodies that recognize such microorganisms of concern to human health and food safety, and subsequently amplifying thus recovered microorganisms using cultural media, and applying relevant serologic tests for their identification.

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METHODS AND KITS FOR DETECTION OF *SALMONELLA* ENTERITIDIS AND RELATED SEROVARS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 61/369,413 filed July 30, 2010 and U.S. Provisional Application 61/493,069 filed June 3, 2011, the entire disclosure of both applications incorporated herein by reference.

FIELD OF THE INVENTION

[0002] The present invention generally relates to methods and kits for detecting microorganisms. More particularly, the invention relates to methods and kits for detecting microorganisms, particularly *Salmonella* enteritidis and serogroup D1 serovars among other *Salmonella* and non-*Salmonella* contaminants, in food samples and environmental samples, particularly samples from poultry flocks.

BACKGROUND OF THE INVENTION

[0003] *Salmonella* is a gram-negative, facultative anaerobic bacterium of the *Enterobacteriaceae* family. There are over 2,400 known *Salmonella* serotypes. Various *Salmonella* serotypes are native to the digestive tract of many different animal species and are important human pathogens. *Salmonella* has been implicated as a major cause of human foodborne illnesses worldwide. Each year, this organism is responsible for approximately 1.4 million cases of illness in the United States, 95% of which are contracted through foodborne transmission. From 400 to 600 of these typically result in death. Clinical symptoms of *Salmonella* poisoning vary, ranging from mild gastroenteritis to more serious conditions, including septicemia and death. A variety of foods have been identified as vehicles of infection for salmonellosis, including raw meat and poultry, fresh produce, processed meats, dairy products, and fruit juices. Raw meat and poultry are recognized as two of the most important vehicles for the transmission of *Salmonella.* Several control

measures have been implemented along food production lines in meat and poultry abattoirs to reduce the levels of *Salmonella* in these matrices; however, microbiological testing retains a key role in preventing foodborne salmonellosis.

[0004] Most immunochemical methods for the detection of *Salmonella* in food rely on antibody recognition of somatic O antigens associated with lipopolysaccharides (LPSs) or flagella present on the cell surface of the bacteria as the targets. Although highly sensitive and widely used, these methods are complicated by the fact that several closely related *Enterobacteriaceae,* such as *Citrobacter* spp. and *Escherichia coli,* share some of the same antigenic factors as *Salmonella.* Furthermore, these same bacteria may occur in the same food sample as *Salmonella.* **Table A** shows several examples of *Salmonella* somatic O antigens (LPSs) that are found in other gram-negative bacteria.

Table A:
Salmonella *somatic O antigen factors and associated cross-reactive non*-Salmonella

Salmonella LPS factor	*Salmonella* serogroup	Cross-reactive organism
3	E1, E2, E3, E4	*Citrobacter* spp.
4	B	*Aeromonas* spp., *Citrobacter* spp., *E. coli, Pasteurella* spp.
6	C1, C2, C4, H	*Citrobacter* spp., *E. coli*
7	C1, C4	*Citrobacter* spp.
8	C2, C3	*Aeromonas* spp., *Citrobacter* spp.
9	D1, D2, D3	*Citrobacter* spp.
10	E1, E4	*Citrobacter* spp.
11	F	*E. coli*
12	B, D1, D3	*Citrobacter* spp., *Pasteurella* spp.
16	I	*E. coli*
17	J	*Citrobacter* spp., *E. coli*
18	K	*Citrobacter* spp.
21	L	*Citrobacter* spp.

Salmonella LPS factor	*Salmonella* serogroup	Cross-reactive organism
28	M	*Citrobacter* spp.
30	N	*E. coli* (O157)
35	O	*E. coli*
38	P	*Citrobacter* spp., *E. coli*
40	R	*Citrobacter* spp., *E. coli*
41	S	*Citrobacter* spp.
42	T	*E. coli*
43	U	*E. coli*
44	V	*Citrobacter* spp.
46	D2	*Pasteurella* spp.
48	Y	*Citrobacter* spp.

Citrobacter spp. and *E. coli* are widely represented because of their close relatedness to *Salmonella.* If present in the sample at sufficiently high concentrations, these nontarget bacteria may be detected by the *Salmonella* immunoassay, producing a false-positive result. This outcome can be problematic in some sample types that have a relatively high microbial load, such as raw meat and poultry. For the end user, false positive results can lead to additional costs in confirmation, labor, lost time, and food product quarantine, thereby disrupting the release of product. Immunoassay methods are typically coupled to a sample enrichment procedure in order to grow the target organism(s) to detectable levels, *e.g.,* 10^5 to 10^6 CFU/ml. For the detection of low-level contamination of stressed *Salmonella,* a nonselective, primary enrichment step is used, followed by a transfer of an aliquot to a selective enrichment broth. Typical selective enrichment broths, such as tetrathionate and Rappaport-Vassiliadis R10, employ selective agents in the medium to suppress the growth of non-*Salmonella* bacteria. However, this approach is not always effective in eliminating immunoassay cross-reactivity, particularly if these nontarget organisms are initially present at relatively high concentrations in the sample and grow to detectable levels during the nonselective primary enrichment phase. Furthermore, overgrowth of these same nontarget

organisms may suppress *Salmonella* growth in the medium. Control of closely related non-*Salmonella* bacteria in the primary enrichment phase is complicated by the fact that the same bioactive agents (antibiotics, bioactive dyes, and other chemical agents) that may be effective against nontarget organisms, *e.g., Citrobacter* spp. and *E. coli,* may also suppress the growth of low levels of stressed *Salmonella.* Therefore, as described herein as a part of the invention, the use of specific bacteriophages (bacterial viruses) as selective agents for the control of cross-reactive non-*Salmonella* bacteria during the primary sample enrichment is described.

[0005] Bacteriophage specificity is attributed to receptor binding structures found on the tail proteins that recognize specific receptors on the bacterial cell surface. These bacterial receptor molecules include somatic O antigens (LPSs), membrane proteins, and peptidoglycans. Some bacteriophages are highly selective for specific host bacterial strains, while others exhibit relatively broad host ranges. Bacteriophages have recently been employed for the control of pathogenic bacteria in food and as specific diagnostic reagents. In the current application, bacteriophages were employed as selective agents in the primary enrichment phase of the immunochemical-based detection method.

[0006] *Salmonella* Enteritidis (SE) is the second most commonly isolated *Salmonella* serotype from humans according to the Centers for Disease Control, responsible for over 200,000 illnesses a year in the U.S. About 64% of those illnesses are attributed to eggs or egg containing products. *Salmonella* enteritidis egg contamination begins in the layer house environment.

[0007] First, there is contamination of the hen house environment through rodents, flies, and the like that have been exposed to SE. Hens then ingest SE through feed or insects that have been contaminated. SE then multiplies in the organs, such as the ovaries, of the hen, while not affecting the health of the hen. SE is then available to potentially contaminate developing eggs within the hen. Thus, if contamination of the hen house environment is found via routine monitoring, it can be potentially eliminated or reduced therefore halting or

reducing SE exposure to the hen and ultimately to the egg. If SE is kept from the environment, the eggs will ultimately be kept SE free.

[0008] Monitoring and control of *Salmonella* serogroup D1 serotypes is a primary concern for both the National Poultry Improvement Plan (NPIP) as well as state-sponsored Egg Quality Assurance Programs (EQAP's) and more recently the US Food and Drug Administration under 21 CFR Parts 16 and 188 "Prevention of *Salmonella* Enteritidis in Shell Eggs During Production, Storage and Transportation" (the Final Rule). These programs involve testing the poultry house environment for *Salmonella* spp. (cultural method), serogroup testing all positives, serotype testing all serogroup D1 isolates, and in some cases phage-type testing all *Salmonella* Enteritidis (SE) isolates. If found in either live birds or egg products, loss of SE-free certification, loss or diversion of product can occur and result in a significant economic loss to the producer.

[0009] The primary concern of the NPIP is to certify that poultry flocks are free of 1) *Salmonella* Pullorum (Pullorum disease), 2) fowl typhoid (*Salmonella* Gallinarum), 3) avian mycoplasmas, 4) *Salmonella* Enteritidis, and 5) avian influenza. Approximately 95% of all US poultry breeders participate in NPIP. The NPIP SE testing program involves testing the poultry house environment for *Salmonella* spp., serogroup testing all positives, serotype testing all serogroup D1 isolates, and phage-type testing of all SE isolates, as shown in **FIGURE 1**.

[0010] If SE is not found, then the flock is considered SE-free certified which is a requirement for some customers purchasing birds for processing or laying eggs. If SE is found, 25 birds from the flock are tested for SE. If SE is found in more than 1/50 birds in the flock, then the flock is not eligible for SE-free classification. Reinstatement of a SE-infected flock into the program requires 100% blood testing (pullorum antigen and or SE antigen) and slaughtering of every infected bird. In September 2007, the SDIX RapidChek Select *Salmonella* was approved by the NPIP as the first immunoassay-based method for use in environmental monitoring of *Salmonella* spp.

[0011] In July 2009, the US Food and Drug Administration Department of Health and Human Services published the first mandated testing program for SE in shell eggs: 21 CFR Parts 16 and 118 "Prevention of *Salmonella* Enteritidis in Shell Eggs During Production, Storage and Transportation" (the Final Rule). This was in response to the perceived relatively high incidence of SE contamination of shell eggs. There are about 47 billion shell eggs consumed annually in the US and the FDA and USDA-FSIS estimates that 2.3 million of those are contaminated with SE. In 2008, SE was the leading reported cause of *Salmonella* infections; accounting for 20.1% of the serotypes isolated and these were primarily associated with the consumption of shell eggs.

[0012] Under this Final Rule all egg farms operating with 3,000 or more laying hens must comply. That accounts for 99% of the total shell egg production. Egg farms who sell all of their eggs directly to consumers and those farms which do not produce shell eggs to be used directly for the table market are exempt from the Final Rule. Today there are 4,000 farms across the US that will be required to comply with the Final Rule. For farms with greater than 50,000 laying hens (66%), the compliance date is July 9, 2010 and for farms with 3,000 to 50,000 laying hens (34%), the compliance date is July 9, 2012.

[0013] The Final Rule requires egg farms to implement a written SE prevention plan, procure SE free pullets or raise pullets under SE monitored conditions (*e.g.* NPIP certified SE-monitored), implement a biosecurity program to control pests and cross contamination, clean and disinfect poultry houses before new laying hens are added if an environmental or egg test was positive for SE during the life of the previous flock, refrigerate eggs during storage and transportation no later than 36 hours after the eggs have been laid, and most importantly, it requires farms to register with the FDA and implement a SE environmental monitoring/testing program.

[0014] The poultry house environment is tested using established sampling protocols when the pullets are 14 to 16 weeks of age and, if negative, then again at 40 to 45 weeks. In

addition, if the flock undergoes an induced molt, the poultry house is tested 4 to 6 weeks after the molt is induced. These time periods in the life of chicken have been shown to be when SE is most likely to be shed into the environment.

[0015] A diagram outlining the SE monitoring/testing program as required by the US FDA Final Rule is shown in **FIGURE 4**.

[0016] Under the Final Rule, if the environmental test is positive, the producer must divert eggs to processing for the lifetime of the flock or begin egg testing within 14 days of egg laying. Egg testing results must be reported within 10 days of notification of a positive environmental test. During the period of time before an egg test result is obtained (7-9 days for the prescribed US FDA method), producers are likely to hold eggs or divert to processing in order to avert a shell egg recall if a positive is ultimately found. If a positive egg test is found, the producer is required to divert eggs until 4 egg tests in a row, at 2 week intervals, are negative. This scenario is significant impetus for the use of a rapid test method that would give a result for the egg pool in the shortest period of time thereby allowing the producer to release product as quickly as possible.

[0017] For environmental testing under the Final Rule, the US FDA recommends "Environmental Sampling and Detection of *Salmonella* in Poultry Houses" October 2008 (http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/ucm114716.htm) as outlined in **FIGURE 2**. This method requires 72 h in order to obtain a negative result and up to 120 hours for a confirmed positive result. As written, a positive sample could carry 4 selective agar plates and 20 selective agar slants prior to serotyping.

[0018] For shell eggs, the US FDA recommends BAM Chapter 5, December 2007 ((http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm070149.htm) as outlined in **FIGURE 4**. The method requires 168 h (7 days) in order to obtain a negative result and up to 216 hours (9 days) for a confirmed

positive result. As written, a positive sample could carry 6 selective agar plates and 12 selective agar slants prior to serotyping.

[0019] The NPIP and US FDA-recommended methods are laborious with long time-to-result. For shell egg producers, this can be very costly since they would most likely hold eggs pending testing results. A rapid, high throughput method would be useful for both applications. What is needed are methods and kits for detecting microorganisms (such as *Salmonella* enteritidis and other serogroup D1 serovars among non-serogroup D1 *Salmonella,* as well as other cross-reactive contaminants). The invention is directed toward these, as well as other, important ends.

SUMMARY OF THE INVENTION

[0020] The invention relates generally to methods and kits for detecting microorganisms, particularly *Salmonella* enteritidis and other serogroup D1 serovars in food samples and environmental samples, particularly samples from poultry flocks (including, but not limited to, poultry house drag swab, shell egg pool, and chicken carcass rinsate), especially where there are other *Salmonella* serogroups present.

[0021] Accordingly, in one embodiment, the invention is directed to methods for selectively isolating target microorganisms from a sample comprising said target microorganisms and at least one contaminant, comprising:

providing a plurality of magnetic particles coated with antibodies;

wherein said antibodies bind an antigen substantially unique to said target organisms;

optionally, enriching said target microorganisms in said sample;

mixing said sample with said plurality of magnetic particles coated with said antibodies to bind said antigens of said target microorganisms with said antibodies;

wherein said sample is relevant for human health, for food safety, as an indicator from environmental surveillance, as an indicator from a food specimen, or a combination thereof;

wherein said microorganisms are at least one *Salmonella* serogroup D1 serotypes; and

wherein said contaminant is selected from the group consisting of at least one non-serogroup D1 *Salmonella*, non-*Salmonella* bacteria cross-reactive with said *Salmonella* serogroup D1 serotypes, and mixtures thereof.

[0022] In other embodiments, the invention is directed to methods for reducing false-positive results in testing target microorganisms in a sample comprising said target microorganisms and at least one contaminant, comprising:

enriching said target microorganisms in said sample;

selectively isolating target microorganisms from said sample to obtain isolated target organisms, comprising:

providing a plurality of magnetic particles coated with antibodies; and

wherein said antibodies bind an antigen substantially unique to said target organisms;

mixing said sample with said plurality of magnetic particles coated with said antibodies to bind said antigens of said target microorganisms with said antibodies;

cultivating said isolated target organisms; and

optionally, confirming the identity of said organism using at least one serological test.

[0023] In yet other embodiments, the invention is directed to kits for reducing false-positive results in testing target microorganisms in a sample comprising said target microorganisms and at least one contaminant, comprising:

at least one container;

at least one enrichment medium;

a plurality of magnetic particles coated with antibodies; and

wherein said antibodies bind an antigen substantially unique to said target organisms;

at least one cultivating medium; and

serological test components;

wherein said microorganisms are at least one *Salmonella* serogroup D1 serotypes; and

wherein said contaminant is selected from the group consisting of at least one non-serogroup D1 *Salmonella*, non-*Salmonella* bacteria cross-reactive with said *Salmonella* serogroup D1 serotypes, and mixtures thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[0024] The accompanying drawings, which are included to provide a further understanding of the invention and are incorporated in and constitute a part of this specification, illustrate embodiments of the invention and together with the description serve to explain the principles of the invention. In the drawings:

[0025] **FIGURE 1** is a flow diagram of the NPIP cultural method for the isolation of SE in poultry houses.

[0026] **FIGURE 2** is a flow diagram of the SE environmental monitoring program as required under the Final Rule.

[0027] **FIGURE 3** is a flow diagram of the US FDA recommended method for poultry drag swabs

[0028] **FIGURE 4** is a flow diagram of the US FDA recommended method for shell egg pools.

[0029] **FIGURE 5** is a flow diagram of a *Salmonella* Enteritidis drag swab protocol.

[0030] **FIGURE 6** is a flow diagram of a *Salmonella* enteritidis shell egg pool protocol.

DETAILED DESCRIPTION OF THE INVENTION

[0031] As employed above and throughout the disclosure, the following terms, unless otherwise indicated, shall be understood to have the following meanings.

[0032] As used herein, the singular forms "a," "an," and "the" include the plural reference unless the context clearly indicates otherwise.

[0033] As used herein, the term "about," when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of ± 20%, preferably ± 10%, more preferably ± 5%, even more preferably ± 1%, and yet even more preferably ± 0.1% from the specified value, as such variations are appropriate to perform the disclosed methods.

[0034] As used herein, the term "drag swab" refers to a device used to sample microbiological specimens in a large area, such as a chicken house. Typically, a drag swab contains a cotton gauze swab attached to a cord, moistened with double strength skim milk, and dragged across the surface of the area to be tested as the sampler walks through the area.

[0035] As used herein, the term "egg pool" refers to the liquid portion of an egg, particularly a group of eggs that is tested.

[0036] As used herein, the term "rinsate" refers to liquid, generally water-based, containing low concentrations of contaminants, resulting from the cleaning of items, including containers and the carcasses of animals, such as poultry.

[0037] The invention relates generally to methods and kits for detecting microorganisms, particularly *Salmonella* enteritidis and other serogroup D1 serovars in food samples and environmental samples, particularly samples from poultry flocks (including, but not limited to, drag swab, egg pool, and chicken rinsate), especially where there are other *Salmonella* serogroups (other than D1 serogroups) present.

[0038] Accordingly, in one embodiment, the invention is directed to methods for selectively isolating target microorganisms from a sample comprising said target microorganisms and at least one contaminant, comprising:

providing a plurality of magnetic particles coated with antibodies;

wherein said antibodies bind an antigen substantially unique to said target organisms;

optionally, enriching said target microorganisms in said sample;

mixing said sample with said plurality of magnetic particles coated with said antibodies to bind said antigens of said target microorganisms with said antibodies;

wherein said sample is relevant for human health, for food safety, as an indicator from environmental surveillance, as an indicator from a food specimen, or a combination thereof;

wherein said microorganisms are at least one *Salmonella* serogroup D1 serotypes; and

wherein said contaminant is selected from the group consisting of at least one non-serogroup D1 *Salmonella*, non-*Salmonella* bacteria cross-reactive with said *Salmonella* serogroup D1 serotypes, and mixtures thereof.

[0039] In other embodiments, the invention is directed to methods for reducing false-positive results in testing target microorganisms in a sample comprising said target microorganisms and at least one contaminant, comprising:

enriching said target microorganisms in said sample;

selectively isolating target microorganisms from said sample to obtain isolated target organisms, comprising:

providing a plurality of magnetic particles coated with antibodies; and

wherein said antibodies bind an antigen substantially unique to said target organisms;

mixing said sample with said plurality of magnetic particles coated with said antibodies to bind said antigens of said target microorganisms with said antibodies;

cultivating said isolated target organisms; and

optionally, confirming the identity of said organism using at least one serological test.

[0040] In yet other embodiments, the invention is directed to kits for reducing false-positive results in testing target microorganisms in a sample comprising said target microorganisms and at least one contaminant, comprising:

at least one container;

at least one enrichment medium;

a plurality of magnetic particles coated with antibodies; and

wherein said antibodies bind an antigen substantially unique to said target organisms;

at least one cultivating medium; and

serological test components;

wherein said microorganisms are at least one *Salmonella* serogroup D1 serotypes; and

wherein said contaminant is selected from the group consisting of at least one non-serogroup D1 *Salmonella*, non-*Salmonella* bacteria cross-reactive with said *Salmonella* serogroup D1 serotypes, and mixtures thereof.

[0041] In preferred embodiments, the methods of the invention have a sensitivity/specificity of no greater than about 4% false positives and no greater than about 2% false negatives.

[0042] In certain embodiments of the methods and kits of the invention, the sample is a specimen selected from the group consisting of an environmental surveillance specimen, a food specimen, or a combination thereof. The methods and kits of the invention are particularly useful when the sample is from a drag swab application, egg pool, or a chicken rinsate.

[0043] The methods and kits of the invention may be applied to any microorganism, particularly bacteria, especially those that are found where there are contaminants that are cross-reactive. The methods and kits of the invention are particularly useful where the microorganism is at least one *Salmonella enterica* serotype, particularly *Salmonella enterica* sub. *enterica* ser. Enteritidis, especially when samples are contaminated with other *Salmonella* serotypes.

[0044] In certain embodiments of the methods and kits of the invention, the contaminant is selected from the group consisting of at least one non-serogroup D1 *Salmonella*, non-*Salmonella* bacteria cross-reactive with said *Salmonella* serogroup D1 serotypes, and mixtures thereof. The methods and kits are particularly useful when the contaminant is another non-serogroup D1 *Salmonella*.

[0045] In certain embodiments of the methods and kits of the invention, the non-*Salmonella* bacteria comprise a cross-reactive feature selected from the group consisting of a somatic O antigen associated with lipopolysaccharides, flagella on a surface of said non-*Salmonella* bacteria, and combinations thereof.

[0046] In certain embodiments of the methods and kits of the invention, the contaminant is at least one organism selected from the group consisting of *E. coli*, *Citrobacter* spp, *Aeromonas* spp., *Pasteurella* spp., non-serogroup D1 *Salmonella,* and combinations thereof.

[0047] In certain embodiments of the methods and kits of the invention, the antibody, preferably a monoclonal antibody is generated against whole cells.

[0048] In certain embodiments of the methods and kits of the invention, the enriching step comprises at least one bacteriophage selective for control of cross-reactive non-*Salmonella* bacteria. Selection of suitable bacteriophages is described in the literature. See, for example, Muldoon, *et al.*, *Journal of Food Protection*, Vol. 70, No. 10, 2007, Pages 2235–2242, incorporated herein by reference in its entirety. In other embodiments, the enriching step includes treatment with two different media, a primary media and a secondary media, especially where the bacteriophage is present in the primary media.

[0049] In certain embodiments of the methods and kits of the invention, the serological test component is an immunochromatographic strip. In other embodiments, the serological test component is a selective agar.

[0050] The use of magnetic particle technology, particularly antibody-coated magnetic beads (immunomagnetic beads), for the selective isolation of microorganisms in microbiology in general and in food and environmental microbiology in particular is becoming more widely used. Different systems and individual pieces of equipment have been developed to assist in the use of magnetic particles.

[0051] Many systems have been developed for collecting magnetic beads from small scale volume samples. Such systems typically handle samples of volumes from 1 ml (Eppendorf tubes, *e.g.* MagneSphere Technology Magnetic Separation Stand, Promega Catalog No. Z5331, Z5332, Z5333 (two-position), Z5341, Z5342 and Z5343 (twelve-position) up to about 50 ml (Falcon tubes, *e.g.* PolyAtract System 1000 Magnetic Separation Stand, Promega Catalog No. Z5410). Magnets are used to concentrate the magnetic beads at the side of the tubes and a pipette is used to either remove supernatant liquid or remove the beads directly. Magnetic pipettes, for example the PickPen™ product, may be used to remove the magnetic beads directly.

[0052] Automated systems, for example the Pathatrix™ system from Matrix MicroSciences, peristaltic pumps, tubes and in-line filters to minimize human handling of samples. Such systems are very expensive, have problems with bead loss on the filters due to the formation of bio-films, and are prone to spillage when transferring the beads from the system.

[0053] Other systems, for example the Kingfisher™ system, are based on the use of electromagnetic pins for capturing magnetic beads from an array of small-sized tubes (<2 ml) and transferring the beads to new tubes for further processing. The electromagnetic pins may be used to hold beads while exchanging tubes, and then to release the beads into the new tubes. Their applications are limited to purification of DNA from PCR products or from gels.

Various other systems use magnets in various ways to process magnetic beads. For example, US-A-2005/0013741 discloses a device for immobilizing and re-suspending magnetic particles during washing and elution steps. The device comprises two permanent magnets which are movable along the side of a tube containing the magnetic particles in a liquid.

[0054] The magnetic particles added to the medium are adapted to bind the microorganisms of interest, preferably by means of specific antibodies conjugated to the magnetic particles. Preferably, the magnetic particles are added in an amount of about 50-100 µl per 250 ml of medium, for example about 100 µl per 250 ml. Preferably, the magnetic particles are added to the medium with mixing and then the medium incubated to allow the microorganisms to bind to the magnetic particles. Incubation time is preferably about 15-30 minutes. The magnetic particles may be spherical or non-spherical. Spherical particles are preferred as non-spherical particles may kill microorganisms. Some examples of magnetic particles include Cortex Megacell™-Streptavidin magnetic particles, Cortex Megabeads™-Streptavidin CM3454 (8.8 µm particle size and coated with magnetizable polystyrene/iron oxide particles), Cortex Megabeads™-Streptavidin CTM-C M019 (15.6 µm particle size and coated with polystyrene copolymer/iron oxide particles), Dynabeads™ M-280-Streptavidin (3-4 µm particle size), and Genpoint BugTrap™ magnetic beads.

Genpoint BugTrap™ magnetic beads, which are universal for capturing gram positive and negative bacteria, have diameters in a range of about 15 μm. These are preferred over the non-spherical Cortex Megacell™-Streptavidin magnetic particles. More preferable yet are the BugTrap™ binding beads from Genpoint AS, Oslo, Norway, which have diameters in a range of about 2.5-15 μm. The Genpoint BugTrap™ binding beads can be used even when a bio-film is present in the medium, and these beads are in a ready to use kit and are coated with a ligand for capturing Gram positive as well as Gram negative pathogenic bacteria (Canadian Patent Publication 2,397,067).

[0055] Preferably, the particles are immunomagnetic particles, more preferably immunomagnetic beads, comprising one or more monoclonal and/or polyclonal antibodies that specifically bind to an antigen on the microorganisms of interest. A mixture of immunomagnetic particles comprising different antibodies specific for different species of the microorganism genus of interest may be used. Antibodies to other specific species may be raised by known methods and incorporated into an immunomagnetic particle. Immunomagnetic particles typically comprise a core magnetic particle coated with an avidin (*e.g.* streptavidin), in turn coated with biotin. The biotin is in turn coated with the antibody or antibodies. Methods for constructing immunomagnetic particles are generally known in the art (*e.g.* Safarik, I. and Safarikova, M. "Magnetic techniques for the isolation and purification of proteins and peptides.: *BioMagn. Res. Technol.* 2 (**2004**) 7).

[0056] The magnetic particles with bound microorganisms are then recovered by magnetically collecting the particles at a bottom of the container, magnetically concentrating the particles at a localized region on the bottom of the container, and retrieving the particles from the localized region with a magnetically assisted pipette. To accomplish this, the system and apparatus described in WO 2008/131554, which is incorporated herein by reference in its entirety, may be employed. As described therein, the system comprises a magnetic particle collector, a magnetic particle concentrator and a magnetic particle pipette that cooperate to recover the magnetic particles from the medium.

[0057] After recovering the magnetic particles with bound microorganisms, the magnetic particles may be washed. Washing is preferably accomplished with TALON™ binding and washing buffer in a small volume container (*e.g.* an Eppendorf tube). Preferably, a buffer having a pH in a range of from about 7.5 to about 8.0 is used to wash the particles. The wash solution may be removed, for example with a pipette, after collecting the particles using the magnetic particle collector of the present invention or any other magnetic particle separation technology (*e.g.* MagneSphere Technology Magnetic Separation Stand, Promega Catalog No. Z5331, Z5332, Z5333 (two-position). The magnetic particles with bound microorganisms may be assayed directly or frozen for storage at -80°C until later analysis for downstream needs, *e.g.* isolation, serology, ELISA, DNA extraction, PCR, hybridization, etc. Freezing may be accomplished using, for example, a CryoStor™ (Innovatek Medical Inc., Vancouver, British Columbia, Canada).

[0058] Any suitable analytical technique may be used to detect and/or measure the microorganisms of interest that have been bound to the particles. For example: the particles may be plated on a medium (*e.g.* agar) and the microorganisms cultured; DNA may be extracted from the microorganisms on the particles and amplified with PCR; serology may be performed directly from the beads (*e.g.* add *Salmonella* serogroup D1 serotype specific antibody and observe clumping); or an assay (*e.g.* ELISA) may be performed directly from the beads. Such techniques are well known in the art.

[0059] The present invention is further defined in the following Examples, in which all parts and percentages are by weight, unless otherwise stated. It should be understood that these examples, while indicating preferred embodiments of the invention, are given by way of illustration only. From the above discussion and these examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.

EXAMPLES

***Salmonella* Monoclonal Antibody Production**

[0060] During the development of the SDIX RapidChek Select *Salmonella* spp. Test Kit, a panel of monoclonal antibodies was developed to important *Salmonella* serogroups associated with food contamination (**Table 1**). Monoclonal antibodies were produced against heat-killed whole cell fractions of the various *Salmonella* serotypes (Table 2) by standard methods. See, for example, Harlowe, E., and D. Lane. 1988. Antibodies: a laboratory manual. Cold Spring Harbor, New York. Briefly, overnight broth cultures were grown in tryptic soy broth (37°C) from a single colony isolate (tryptic soy agar containing 0.6% yeast extract). Total viable cell enumeration was accomplished by dilution plate counting. The cultures were centrifuged at 7500 x g for 10 min. The cell pellet was reconstituted in 5 mL of phosphate-buffered saline (10 mM, pH 7.4) (approximately 1 x 10^9 CFU/mL) and then boiled 10 min. BALB/c mice were immunized intraperitoneally with 1 x 10^7 CFU of antigen in incomplete Freunds adjuvant. Mice were boosted at 7 day intervals for a total of 7 injections after the initial immunization. Splenocytes were harvested and fused with P3 X63-Ag8.653 myeloma cells. Hybridomas were screened on antigen-coated plates. Positive clones were expanded and IgG purified from cell culture supernatants (initially) and in some cases ascites fluid on Protein A/G. Cell lines were stored frozen at -80°C pending further production.

Table 1:
Monoclonal Antibodies Developed for Various *Salmonella* Serogroups

Monoclonal Antibody Designation	***Salmonella* Serovar**	**Strain**	**Serogroup**	**O-Factors**
283B4	Typhimurium	ATCC 15277, ATCC 19585	B	1, 4, [5], 12
	Brandenburg	ARS 20	B	1, 4, 12
278B3	Infantis	ARS 22	C1	6, 7

Monoclonal Antibody Designation	*Salmonella* Serovar	Strain	Serogroup	O-Factors
254D12	Kentucky	ATCC 9263	C3	8, 20
269P17	Javiana	ATCC 10221	D1	1, 9, 12
418B24	Strasbourg	DSM 62804	D2	9, 46
418D5	Strasbourg	DSM 62804	D2	9, 46
257B12	Abaetetuba	ATCC 35640	F	11
273B3	Cubana	ATCC 12007	G2	1, 13, 23

Immunochromatographic test strips

[0061] Serogroup-specific test strips were made as follows: serogroup-specific monoclonal antibodies were conjugated to colloidal gold (40 nm; British Biocell International, Cardiff, UK) by established techniques (Beesley, J. 1989. *Colloidal gold. A new perspective for cytochemical marking.* Royal Microscopical Society Handbook no. 17. Oxford Science Publications. Oxford University Press, Oxford). Anti-Salmonella polyclonal antibody (Strategic Diagnostics, Inc., Newark, Delaware) was striped onto the nitrocellulose membrane (Millipore Inc., Billerica, Mass.) at the test line and anti–immunoglobulin G antibody at the control line with a Biodot XY platform sprayer (Biodot, Irvine, Calif.). A polyester conjugate pad (Ahlstrom, Helsinki, Finland) was impregnated with monoclonal antibody-gold conjugate. A nitrocellulose membrane was mounted on plastic backing material (G&L Precision Die Cutting, Inc., San Jose, California), followed by the conjugate pad that overlapped the membrane at its distal end. A cellulose acetate filter pad (Filtrona Richmond Inc., Colonial Heights, Virginia) was mounted below and overlapped the conjugate pad. Cellulose sink material (Whatman, Inc., Middlesex, UK) was mounted above and overlapped the nitrocellulose membrane. Test strips were cut with a Kinematic guillotine cutter (Kinematic, Twaine Harte, Calif.). Multiserogroup reactive test strips were RapidChek Select Salmonella test strips (Strategic Diagnostics). Test strips were run by immersing the filter pad into the liquid sample and allowing 10 minutes for

development. The appearance of one line indicated a Salmonella-negative sample, whereas the appearance of two lines indicated a Salmonella-positive sample.

Isolation and characterization of cross-reactive non-*Salmonella* bacteria

[0062] Cross-reactive bacteria were isolated from tetrathionate broth (Hajna) following preenrichment in either buffered peptone water or RapidChek Salmonella nonselective primary media (Strategic Diagnostics). Non-Salmonella typical colonies from negative brilliant green sulfa or xylitol-lysine tergitol 4 agar plates from test strip–positive samples were resuspended in 0.2 ml of peptone water in microplate wells and tested with multiserogroup-reactive test strips. If the test strip was positive, the colony was restruck on nonselective agar (TSA) plates. Single colonies were retested with multiserogroup-reactive test strips. If test strip was positive, a portion of the colony was expanded into TSB, incubated overnight, and tested with a panel of Salmonella serogroup-specific monoclonal antibody test strips. Another portion of the colony was used for identification by the API 20E
Bacterial Identification kit (bioMérieux, Hazelwood, Missouri) and DNA sequence analysis (16S and 28S) (MIDI Inc., Newark, Delaware). Bacterial isolates were stored frozen at -80°C.

Isolation and characterization of bacteriophage against cross-reactive non-*Salmonella* bacteria

[0063] Bacteriophages were isolated from raw sewage, surface water, agricultural environments, and food by established methods (Lu, Z., F. Breidt, V. Plengvidhya, and H. P. Fleming. 2003. Bacteriophage ecology in commercial sauerkraut fermentations. *Appl. Environ. Microbiol.* 69:3192–3202). Briefly, microplate wells containing 20 μl of 10X TSB were inoculated with an overnight culture (10 μl) of host bacteria (cross-reactive non-*Salmonella*) and then with filter-sterilized (0.45-μm) samples (170 μl). Following overnight incubation (37°C), the microplate was centrifuged (3,000 X g, 10 min), and 10 μl of the supernatant was spotted onto a second microplate to which a lawn of host bacteria was

prepared. The second microplate was incubated overnight and evaluated the next day for the formation of lytic plaques at the zone of crude phage application. Plaque-positive wells were cloned by dilution plating the crude phage supernatant in a soft agar overlay technique (Carlson, K. 2005. Working with bacteriophages: common techniques and methodological approaches, p. 437-494. In E. Kutter and A. Sulakvelidze (ed.), *Bacteriophages: biology and applications.* CRC Press, Boca Raton, Fla.). Individual phage plaques were removed with a sterile micropipette tip and subcultured in 1 ml of TSB containing approximately 1 X 10^6 CFU/ml of the host bacteria. Following an overnight incubation (37°C), the broth culture containing the phage clone was passed through a 0.45-μm filter, and the phage titer was determined by the soft agar overlay method. Bacteriophages were further tested for lytic activity against an expanded panel of cross-reactive non-*Salmonella* bacteria as well as 352 serovars of *Salmonella* representing 23 serogroups.

Salmonella Serogroup D1 Lateral Flow Test Strip

[0064] Lateral flow test strips were constructed using the various monoclonal antibodies developed and listed in **Table 2**. A description of the components used in the RapidChek Select *Salmonella* Enteritidis test strip is listed in **Table 3**. The test uses monoclonal antibody 269P17 on both sides of the sandwich assay.

Table 2:
Materials used in the *Salmonella* Serogroup D1 Lateral Flow Test Strip

Material	Description
Sprayed membrane	
Nitrocellulose membrane	Membrane, NC M-90 20 mm (1048544)
Test line antibodies	Mouse anti-D1- group 269P17 (1064140, 1.0 mg/mL)
Control line antibody	Goat anti-Mouse IgG (SD50384, 1 mg/mL)
Gold Pad	
Gold conjugates	Mouse anti-D1- group 269P17 (1064140, 5 µg/OD, 2.0 OD/mL)
Gold conjugate buffer	2% Skim milk, 5% sucrose, 0.2% Triton X-100 in 5 mM phosphate, pH 7.4
Filter pad (buffer pad)	Filter Pad, Glass Grade 8964 30 mm (1047027)
Buffer Pad Buffer	0.15 M Tris, pH 8.5, 50 mM EDTA ($K_3 \cdot 4H_2O$), 1% (w/v) Tetronic 1307
Backing	82 mm x 100 m (1047034)
Sink material	Sink, 470 26 mm (1020010)
Clear Cover	Cover Tape, *Salmonella* (1510167CT)

Immunomagnetic Separation (IMS) Magnetic Particle Conjugation Procedure

[0065] Monoclonal antibody 269P17 was conjugated to amine-terminated magnetic particles (Polysciences PN 84100) at a loading rate of 20 mg antibody per 100 mg of magnetic particle (GaR IgG Coupled Magnetic Particle Stock Solution). The particles were diluted 1:10 in particle diluent and this was used as the working stock supplied in the kit.

Specificity Studies

[0066] *Salmonella* bacteria can be taxonomically-classified or serogrouped based on the serological detection of specific polysaccharides or O-factors found on the cell surface. Antisera made against these gram negative bacteria are typically dominated by antibodies reactive toward these O-factors. For example, serogroup B *Salmonella*e exhibit O-factors 4, and 12 as well variable exhibition of O-factors 1, 5, and 27 (O-factor designation: 1, 4, [5], 12, 27), whereas serogroup D1 *Salmonella*e exhibit O-factors 9 and 12 and variably O-factor

1 (O-factor designation: 1, 9, 12). Monoclonal antibodies (mAbs) were isolated from mice that were immunized using heat-killed *Salmonella* bacteria from various serogroups. The mAbs were used singly in immunochromatographic test strip devices. Reactivity of these monoclonal antibodies against a panel of *Salmonella* was determined and segregated according to the presence of specific O-factors associated with the particular *Salmonella* serovar (**Table 3**).

Table 3:
Specificity of Various *Salmonella* Monoclonal Antibodies in the Lateral Flow Test Strip Format

Salmonella	Serogroup	O-Factors	Monoclonal antibody designation								Multi-serogroup
			283 B4	278B 3	254D1 2	269P1 7	418B2 4	418D 5	257B1 2	273B 3	
Paratyphi A	A	1, 2, 12	-	-	-	-	-	-	-	-	+
Heidelburg	B	1, 4, [5], 12	+	-	-	-	-	-	-	-	+
Typhimurium (2)	B	1, 4, [5], 12	+	-	-	+/-	-	-	-	-	+
Saintpaul (2)	B	1, 4, [5], 12	+	-	-	+/-	-	-	-	-	+
Brandenburg (2)	B	1, 4, [5], 12	+	-	-	+/-	-	-	-	-	+
Stanley	B	1, 4, [5], 12	+	-	-	-	-	-	-	-	+
Montevideo	C1	6, 7	-	+	-	-	-	-	-	-	+
Infantis	C1	6, 7	-	+	-	-	-	-	-	-	+
Hadar	C2	6, 8	-	-	+	-	-	-	-	-	+
Blockley	C2	6, 8	-	-	+	-	-	-	-	-	+
Albany	C3	8, 20	-	-	+	-	-	-	-	-	+
Kentucky	C3	8, 20	-	-	+	-	-	-	-	-	+
Jerusalem	C4	6, 7, 14	-	+	-	-	-	-	-	-	+
Enteritidis	D1	1, 9, 12	-	-	-	+	+	-	-	-	+
Enteritidis	D1	1, 9, 12	-	-	-	+	+	-	-	-	+
Maars2en	D2	9, 46	-	-	-	-	+	+	-	-	+
Ouakam	D2	9, 46	-	-	-	-	+	+	-	-	+
Anatum	E1	3, 10	-	-	-	-	-	-	-	-	+
London	E1	3, 10	-	-	-	-	-	-	-	-	+
Drypool	E2	3, 15	-	-	-	-	-	-	-	-	+
Illinois	E3	3, 15, 34	-	-	-	-	-	-	-	-	+
Senftenburg	E4	1, 3, 19	-	-	-	-	-	-	-	-	+
Senftenburg	E4	1, 3, 19	-	-	-	-	-	-	-	-	+
Rubislaw	F	11	-	-	-	-	-	-	+	-	+
Abaetetuba	F	11	-	-	-	-	-	-	+	-	+
Poona	G1	1, 13, 22	-	-	-	-	-	-	-	-	+
Cubana	G2	1, 13, 23	-	-	-	-	-	-	-	+	+
Worthington	G2	1, 13, 23	-	-	-	-	-	-	-	+	+

[0067] Monoclonal antibody 269P17 showed low reactivity against serogroup D2 (O-factor designation: 9, 46) as well as serogroup B *Salmonella*. Both of these serogroups share a common O-factor with serogroup D1 *Salmonella*; O-factor 9 for serogroup D2 and O-factor 12 for serogroup B. Further studies conducted at the New Bolton Center, University of Pennsylvania, using an expanded panel of 351 *Salmonella* strains including 141 from serogroup D1 and 173 from serogroup B showed 100% sensitivity and 93% specificity for *Salmonella* serogroup D1 strains (**Table 4**).

Table 4:
Specificity Study of the *Salmonella* Serogroup D1-Specific test Strip

Serogroup	O-Antigens	# Tested	Positive	Negative	Result
A	1,2,12	1	1	0	+
B	1,4,[5],12,27	173	3	170	Weak +
C1	6,7,14	4	2	2	Weak +/+
C2	6,8	1	0	1	-
C3	8	1	1	0	Weak +
D1	1,9,12	141	141	0	+
D2	9,46	2	0	2	-
E1	3,{10},{15},{34}	3	0	3	-
E2	3,15	1	1	0	Weak +
E4	1,3,10,19	5	0	5	-
F	11	1	1	0	Weak +
G2	1,13,23	6	2	4	Weak +/medium +
H	[1],6,14,[25]	1	1	0	Weak +
I	16	1	0	1	-
K	6, 14,18	3	0	3	-
N	30	1	0	1	-
O	35	1	1	0	Weak +
R	1,40	3	0	3	-
S	41	1	0	1	-
V	1,44	1	1	0	Weak +

[0068] All of the serogroup D1 strains were detected and none of the serogroup B or D2 strains were detected in that study. There is no evidence to date in the literature that

serogroup D1 *Salmonella*e possess a unique antigen not found in either serogroup B or D2 *Salmonella*e. To our knowledge, this is the first report of such a unique antibody.

[0069] A large panel of non-*Salmonella* bacterial isolates, primarily isolated from food at SDIX were tested with the strip. Results of this study are shown in **Table 5**.

Table 5:
Results from the Exclusivity Study of Non-*Salmonella* Bacterial Strains

				Phage		
				FF 35-2	STR14-6	LP16-1
				Host		
Sample #	Bacteria	Source	LFD CCU	BPI 43	S66	78A2
1	A. hydrophila # 8	Chicken	-			
2	A. hydrophila # 10	Beef	-			
3	A. veronii 150-1	Chicken Rinse	-		-	-
4	A. veronii 197-1	Chicken Rinse	-		-	-
5	A. veronii 242-2	Chicken Rinse	-		-	-
6	A. veronii ATCC 9071	ATCC	-		-	-
7	A. veronii ATCC 51106	ATCC	-		-	-
8	A. hydrophila 151-1	Chicken Rinse	-			
9	C. freundii 30-3	Ground Beef	-	+	++	+ w
10	C. freundii BPI 43	Ground Beef (BPI)	-	++	++	++
11	C. freundii 41-1	Ground Beef	-	+	++	+ w
12	C. freundii # 1	Ground Beef	-	+	++	+ w
13	C. freundii 3F9	Ground Beef	-	+	++	+ w
14	C. freundii 3E4	Ground Beef	-	+	++	+ w
15	C. freundii # 4	Ground Beef	-	+	++	+ w
16	C. freundii 5F8	Ground Beef	-	+	++	+ w
17	C. freundii # 6	Ground Beef	-	-	-	-
18	C. freundii 68A1	Boneless Turkey	-	++	+ spi's	++
19	C. freundii 70A1	Boneless Turkey	-	+ w	+	++
20	C. freundii 70A2	Boneless Turkey	-	++	+ spi's	++
21	C. freundii 70A3	Boneless Turkey	-	+	+ spi's	++
22	C. freundii 7A12	Fish	-	++	+	+
23	C. freundii S66	Chicken Rinse	-	+	++	+ w
24	C. freundii T67	Chicken Rinse	-	+	+	+
25	C. freundii S68	Chicken Rinse	0.5	++	++	++
26	Citrobacter sp. 80C7	Ground Chicken	11	++	++	++
27	Citrobacter sp. 80C2	Ground Chicken	11	++	++	++
28	Citrobacter sp. 79B3	Ground Chicken	11	++	++	++
29	Citrobacter sp. 79B2	Ground Chicken	11	++	++	++

				Phage		
				FF 35-2	STR14-6	LP16-1
				Host		
Sample #	Bacteria	Source	LFD CCU	BPI 43	S66	78A2
30	Citrobacter sp. 78A7	Ground Chicken	11	++	++	++
31	Citrobacter sp. 78A2	Ground Chicken	11	++	+	+
32	C. freundii G1	Chicken Rinse	-	+	+ w	++
33	C. freundii 314-1	Ground Beef	-	+	++	+
34	C. freundii 132-1	Chicken Rinse	-	+	++	+
35	C. freundii BPI 2-3	Ground Beef (BPI)	-	-	-	++
36	E. cloacae # 2	Beef	-			
37	E. coli R7-32C4	Ground Beef	-		-	-
38	E. coli 96C5	Ground Beef	-		-	-
39	E. coli 99G1	Ground Beef	-		-	-
40	E. coli 100D4	Ground Beef	-		-	-
41	E. coli #7 BPI	Ground Beef (BPI)	-		-	-
42	E. coli #8 BPI	Ground Beef (BPI)	-		-	-
43	E. coli O44	ATCC	-		-	-
44	E. coli O62	ATCC	-		-	-
45	E. coli O68	ATCC	-		-	-
46	E. coli O70	ATCC	-		-	-
47	E. coli O73	ATCC	-		-	-
48	E. coli O99	ATCC	-		-	-
49	E. coli O106	ATCC	-		-	-
50	E. coli O129	ATCC	-		-	-
51	E. coli 74-1	Chicken Rinse	-			
52	E. coli 74-2	Chicken Rinse	-			
53	E. coli 111-1	Chicken Rinse	-			
54	E. coli 111-2	Chicken Rinse	-			
55	E. coli 164-2	Chicken Rinse	-			
56	E. coli ATCC 4157	ATCC	-			
57	E. coli ATCC 11775	ATCC	-			
58	E. coli ATCC 35218	ATCC	-			
59	E. coli ATCC 35421	ATCC	-			
60	E. coli ATCC 51446	ATCC	-			
61	E. coli ATCC 51755	ATCC	-			
62	E. coli ATCC 8739	ATCC	-			
63	E. coli ATCC 10536	ATCC	-			
64	E. coli ATCC 11229	ATCC	-			
65	E. coli ATCC 13706	ATCC	-			
66	E. coli ATCC 13762	ATCC	-			
67	E. coli ATCC 33605	ATCC	-			
68	E. coli ATCC 51813	ATCC	-			
69	E. coli O75	ATCC	-		-	-
70	E. coli 16	Chicken Rinse	-		-	-

				Phage		
				FF 35-2	STR14-6	LP16-1
				Host		
Sample #	Bacteria	Source	LFD CCU	BPI 43	S66	78A2
71	E. coli 54-1	Chicken Rinse	-		-	-
72	E. coli 103-2	Chicken Rinse	-		-	-
73	E. coli 185-1	Chicken Rinse	-			
74	E. coli 186-1	Chicken Rinse	-			
75	E. coli Smithfield #4	Pork Carcass Swab	-			
76	P. mirabilis # 68	Turkey	-			
77	P. mirabilis # 70	Turkey	-			
78	P. vulgaris #19R7	Beef	-			
79	P. mirabilis M72	Ground Pork	-			
80	Vibrio 62A1	Salmon	-			
81	Vibrio 62A2	Salmon	-			
82	Vibrio 62A3	Salmon	-			
83	Vibrio 62A11	Salmon	-			
84	Vibrio 62A12	Salmon	-			
85	K. pneumoniae #9	Beef	-			
86	K. oxytoca 1055-1	Ground Beef	-			
87	K. pneumoniae 67-3	Chicken Rinse	-			
88	Unknown 104-1	Chicken Rinse	-			
89	P. aeruginosa 112-1	Chicken Rinse	-			
90	Serratia species 168-1A	Chicken Rinse	-			

[0070] Of 90 non-*Salmonella* strains tested, 6 reacted strongly with the strip. It is very likely that this is in fact a single cross-reactive *Citrobacter* strain since all 6 strains were isolated from a single lot of ground chicken in a single experiment. In any event, this reactivity is not likely to be a problem for the test method since these cross-reactive *Citrobacter* isolates are controlled by several bacteriophages used in the RapidChek Select *Salmonella* Primary Media.

Enrichment Protocol Development

Poultry House Drag Swabs

[0071] Poultry house drag swabs were obtained from a commercial egg production facility in Maine. They were stored at 4°C until used (within 1 month) and used for the

development of the drag swab protocol. The RapidChek Select *Salmonella* Enteritidis drag swab protocol is outlined in **FIGURE 5**.

[0072] The time to a negative result for the SDIX method is 40- 48 h versus 72 h for the US FDA cultural reference method. The confirmation essentially follows the US FDA protocol with some exception. Analysis of samples with a complex *Salmonella* population requires the use of an immunomagnetic separation (IMS) step prior to streaking the selective agar plate. The time to a positive confirmation is approximately 4 days. For the purpose of these studies, for both the SDIX method and the cultural reference methods, 5 typical colonies (if available) were carried to serogroup confirmation.

Immunomagnetic Separation (IMS) Procedure

[0073] Resuspend the working stock of monoclonal antibody-magnetic particle reagent (MAb-MP) by repeated inversion. Transfer 1 mL of liquid broth sample to a 1.5 mL microcentrifuge tube. Add 0.05 mL of MAb-MP working stock per 1 mL of liquid broth sample. Vortex briefly to mix. Incubate sample tube at room temperature with rocking for 15 min. Place sample tube on magnetic separation rack for 5 minutes. Remove liquid from sample tube being careful not to touch the MAb-MP on the side of the tube closest to the magnetic source. Remove sample tubes from the magnetic source. Add 1.0 mL phosphate-buffered saline containing 0.05% (v/v) Tween 20 (PBS-T) to the sample tube. Remove, vortex to briefly mix. Place sample tube on magnetic separation rack for 5 min. Remove liquid from sample tube being careful not to touch the MAb-MP on the side of the tube closest to the magnetic source. Repeat steps 6-9 for a total of 5 washes. After the final wash and removal of liquid, reconstitute sample with 0.1 mL of PBS-T. Vortex to briefly mix. Streak selective agar plates (*e.g.* XLT4 and BGN) with 10 µL loop of the purified sample. Follow US FDA BAM confirmation procedure. For the purposes of method development, typical colonies were transferred directly to a microtiter plate well containing 150 µL peptone-water. The test strip was inserted into the well and read after 10 min to verify the presence of serogroup D1.

Method Comparison Results for Poultry House Drag Swab Samples

[0074] Drag swab samples were individually spiked with SE alone or in combination with *S.* Kentucky, a commonly occurring *Salmonella*e associated with poultry, at 5 CFU/drag each and tested using either the SDIX method of the US FDA method (**FIGURE 3**). The results are presented in **Table 6**.

Table 6:
Method Comparison Results for Poultry House Drag Swab Samples

Method	*Salmonella* Spike	# of Samples	# of Presume Positives*	# with D1 Isolates Before IMS	# with D1 Isolates After IMS
FDA	None	5	3	0	0
	SE	10	10	9	10
	SE + SK	10	10	2	9**
SDIX	None	5	1***	0	0
	SE	10	10	10	10
	SE + SK	10	10	4	10

*FDA=Positive Plates, SDIX=Positive Test Strip
** 1 sample was SE negative but S. Kentucky positive
***False Positive

[0075] The study showed the SDIX method gave similar performance as the FDA method before and after IMS. Three (3) samples tested using the FDA method gave "typical" colonies that did not confirm as SE. For the SDIX method, one sample gave a positive strip result but did not confirm. Furthermore, the study clearly demonstrated the usefulness of the IMS procedure for the reproducible recovery of SE from mixed cultures. Since cultural confirmation is based on picking "typical" colonies from the selective agar plates, the likelihood of picking SE in a mixed *Salmonella* culture is reduced compared to when it is the only serovar present. It is believed that in the egg industry, particularly when testing first begins, mixed *Salmonella* populations in the environment will be the norm rather than the exception.

[0076] An additional 60 drag swabs were tested without spiking in order to understand the false positive rate of the SDIX method with this matrix. The results are presented in **Table 7**.

Table 7:
Results from the Analysis of Unspiked Poultry House Drag Swab Samples

				# with D1 Isolates	
Method	*Salmonella* Spike	# of Samples	# of Presume Positives*	Before IMS	After IMS
SDIX	None	60	0	0	0

[0077] No false positive results were found in this study. In combination with the results from the analysis of spike samples, the method gave a 100% sensitivity and 98% specificity.

Effect of Incubation Time on the Recovery of SE from Poultry House Drag Swab Samples

[0078] Experiments were carried out that tested the effect of incubation time on the recovery and isolation of SE (**Table 8**). The incubations times tested were the outer boundaries of the incubation times claimed in the User Guide (16-22 hours).

Table 8:
Effect of Incubation Time on the Recovery of SE from Poultry House Drag Swab Samples

	Incubation Condition					
	A	B	C	D	E	F
Spike Level, CFU/swab SE & SK	0	0	5	5	5	5
Primary Enrichment Time, h	16	22	16	16	22	22
Secondary Enrichment Time, h	16	22	16	22	16	22
Test Strip Positives	0	0	8	8	8	8
Confirmed SE Positives Before/After IMS	0/0	0/0	3/8	4/8	1/8	3/8

[0079] The recovery of SE from drag swabs using the SDIX method was independent of incubation time within the 6 h tolerance for each enrichment step. Both extreme incubation time regimes (33 and 44 h total enrichment) gave no false positive results. With spiked samples, all incubation time regimes gave 8 presumptive positive results with the test strip and all 8 of these samples confirmed.

IMS Bead Lot-to-lot Performance

[0080] Three (3) lots of IMS beads were prepared and compared for SE confirmation from the sample broth. Poultry house drag swabs were tested either unspiked or spiked with 5 CFU of SE or SE and S. Kentucky. For the purposes of this experiment, 1 mL of primary media was transferred to 10 mL of secondary media. After secondary enrichment, IMS was performed on a 1 mL aliquot from each sample using each lot of IMS bead. Selective agar plates were struck before and after conducting the IMS procedure. Following incubation, typical colonies were tested with the test strip in order to enumerate serogroup D1 isolates. The results are presented in **Table 9**.

Table 9:
IMS Bead Lot-to-lot Performance

IMS Bead Lot	*Salmonella* Spike	Replicates	# of Presume Positives*	# with D1 Isolates	
				Before IMS	After IMS
1	None	5	0	0	0
	SE	5	4	2	5
	SE + SK	5	4	0	4
2	None	5	0	0	0
	SE	5	4	2	5
	SE + SK	5	4	0	4
3	None	5	0	0	0
	SE	5	4	2	5
	SE + SK	5	4	0	4

[0081] The three lots of IMS beads gave identical performance. Before IMS, only two samples gave serogroup D1 isolates whereas after IMS all presumptive positives confirmed. In addition, serogroup D1 isolates were isolated using all three lots of IMS beads from one sample that was test strip-negative.

Alpha Testing

[0082] A further study was carried out. For the analysis, 25 mL of BPW was added to the environmental swab. The sample was allowed to stand at room temperature for 15-30 minutes and then stomached for 20-30 seconds. Ten (10) mL of the sample was added to 2 individual sterile bags. To one of the bags, 90 mL of RapidChek Select *Salmonella* primary media was added and to the second bag, 90 mL of TT broth. The samples were then processed according to the respective methods. The results of the study are shown in **Table 10**.

Table 10:
Results from the Alpha Study using NPIP Drag Swabs

	Total Positives	Total Negatives	Confirmed Positives	Confirmed Negatives	False Positives	False Negatives
RC S SE	26	75	32	69	0	6*
NPIP	6	99	--	--	--	26**

*Two (2) samples were positive on direct streak. Four (4) samples were positive after IMS
**Based on all D1 positives for all methods

[0083] 101 drag swab samples were tested. Of these, 26 were found to be D1 positive by the SDIX method whereas only 6 were found positive using the NPIP cultural reference method. Six (6) additional samples were found positive either by direct plating of the RapidChek Select *Salmonella* secondary broth (2) or after IMS and then plating (4). This represented 6 false negative results found with SDIX method. On the other hand, 26 samples were found to be D1 positive that were not detected by the NPIP method. In many samples that were found positive by the SDIX method, the NPIP detected only non-serogroup D1 *Salmonella*. The study clearly demonstrated the superior sensitivity and reliability of the RapidChek Select SE test method.

Egg Pools

Method Detection of SE in Liquid Eggs (25 mL subsample)

[0084] Initial development efforts focused on a 24 h method for the detection of SE in liquid eggs. The method was able to detect low contamination (3 CFU) in a 25 mL analytical unit using a 2-step 24 h protocol (16-22 h primary plus 6-8 h secondary enrichment) (Table 11).

Table 11:
Method Comparison Results for the Detection of SE in Liquid Eggs (25 mL subsample)

Matrix	Spike Organism	Spike Level, CFU/25 mL	Number of Samples	Number of Positives: FSIS/USDA Reference Method	Number of Positives: RapidChek Select SE Test System	False Positive	False Negative
Liquid Eggs	*Salmonella* Enteritidis ATCC 13076	0	5	0	0	0	0
		3	20	12	15	0	0

RapidChek Select *Salmonella* Enteritidis Shell Egg Pool Protocol

[0085] Application of this method to the analysis of 20 egg pools (the sample type designated by the Final Rule) required additional development since it was not able to detect low level SE contamination in a pool (*e.g.* 5 CFU/pool (~1L)). At this level of contamination, the likelihood of a single bacterial cell in a 25 mL subsample of the pool is 1-in-8. In order to obtain this level of sensitivity in a 20 egg pool, the SDIX method utilizes the entire egg pool as the test unit (20 egg pool plus 200 mL primary media, incubate room temperature 40-48 h). A similar approach is taken with the US FDA method where the entire pool is incubated at room temperature for 96 h prior to subsampling 25 mL for further enrichment (**FIGURE 2**). The SDIX method is outlined in **FIGURE 6**.

[0086] The time to a negative result for the SDIX method is approximately 40-48 h versus 168 h (9 days) for the US FDA cultural reference method. The confirmation procedure follows the US FDA protocol. There is no IMS step involved since it is unlikely that other *Salmonella* serovars (non-SE) would be present in eggs.

[0087] Various protocols were tested during the development of the current method once it was clear that the entire egg pool must be considered the analytical unit. One protocol that was carried used a 24 h enrichment but required incubation of the primary enrichment sample at 37°C. This was not the preferred protocol if there are large sample volumes. For example, each sample set will consist of fifty (50) containers of 20 egg pool (~1L) plus 200

mL of primary media (~1.2 L). The incubator space required to support such large sample volumes would be prohibitive except perhaps in some very large testing labs. Furthermore, the primary incubation of 18-24 h appeared to be near an edge of reproducibility (**Table 12**).

Table 12:
Results from the Evaluation of Several Incubation Conditions on the Recovery of SE from Shell Egg Pools

Media added to pool	200		200		200		200		200		200	
Temperature (°C)	RT	RT	RT	RT	35	35	35	35	35	35	35	35
Pool tested	P16 to P20		P6 to P10		P11 to P15		P11 to P15		P1 to P5		P11 to P15	
Whole pool incubation	40 H	40H	48H	48H	16H	16H	18 H	18H	24 H	24H	40 H	40H
RCSS 2ry incubation	6H	8H	6H	8H	6H	8H	6H	8H	6H	8H	6H	8H
Positive Pools	4	4	5	5	1	1	1	1	4	4	5	5
Positive sub-samples	12	12	15	15	3	3	3	3	12	12	15	15
Average ccu	11	11	11	11	11	11	11	11	11	10.8	11	11
False Positive*	0	0	0	0	0	0	0	0	0	0	0	0
False Negative*	0	0	0	0	0	0	0	0	0	0	0	0
Method False Negative**	0	0	0	0	4	4	4	4	0	0	0	0

[0088] The 48 hour enrichment method was further pursued. The SDIX 48 hour method was compared to the FDA reference method for the analysis of egg pools (n=10) spiked to 5 CFU/pool. The results are shown in **Table 13**.

Table 13:
Results from the Analysis of Egg Pools Using the SDIX and US FDA Methods

Matrix	*Salmonella* Spike	Spike Level CFU/Pool (20 eggs)	# of Samples each Method	Confirmed Positives		False Positives	False Negatives
				FDA Cultural Method	RapidChek Select SE		
Egg Pools	None	0	1	0	0	0	0
	Enteritidis	5	10	7	8		

[0089] The SDIX method compared very favorably to the FDA cultural method. There were no false positives or false negatives found.

[0090] As part of a method evaluation, 20 egg pools (~200 mL each) were received from Wabash Valley Produce, Dubois, IN, a very large shell and processed egg producer. The samples were tested by both the SDIX and FDA methods. No SE-positive samples were found with either method (data not shown). No false positives were found.

Chicken Rinsates

[0091] A method comparison study was conducted with chicken rinsates between the SDIX method and the USDA-FSIS cultural reference method. Both the RapidChek Select SE and *Salmonella* spp. test strips were used. Two (2) chicken parts were added to 25 stomacher bags and 20 of these were inoculated with 6.6 CFU SE. For the enrichment, 100 mL BPW was added to the bag and the bag shaken vigorously. One 30 mL aliquot was used for the USDA-FSIS cultural reference method and another 30 mL was used for the SDIX method. For the SDIX method, the AOAC-approved RapidChek Select *Salmonella* procedure was followed. Briefly, 30 mL of 2X RapidChek Select Primary media was added to each sample and incubated for 16-22 hours at 42°C. Then, 0.1 mL of the primary enrichment broth was transferred to 1 mL of RapidChek Select secondary media and this was incubated for 16-22 hours at 42°C. A RapidChek Select SE test strip was added to the sample and developed 10 min and read followed by a RapidChek Select *Salmonella* spp. test strip that was developed 10 min and read. All samples for both methods were confirmed to serogroup. The results from this study are shown in **Table 14**.

Table 14:
Results from the Analysis of Chicken Rinsates Using the SDIX and USDA-FSIS Methods

				Confirmed Positives				
Matrix	*Salmonella* Spike	Spike Level CFU/sample	# of Samples each Method	USDA-FSIS Cultural Method	RapidChek Select SE	RapidChek Select	False Positives	False Negatives
Chicken Rinsate	None	0	5	0	0	1*	0	0
	SE	6.6	20	15	16**	17*		

*Serogroup B isolates were found in 2 RapidChek Select SE-negative samples
**One sample required IMS for confirmation.

[0092] The RapidChek Select SE method gave equivalent performance as the USDA-FSIS cultural reference method for the analysis of SE in chicken rinsates. One (1) sample tested using the RapidChek Select SE method required IMS for confirmation. As a side note, some of the samples were naturally-contaminated with serogroup B *Salmonella* as evidenced by the serological confirmation of 2 samples that were positive by RapidChek Select *Salmonella* spp. but not RapidChek Select SE.

[0093] The SDIX RapidChek Select *Salmonella* Enteritidis Test System is an improvement over the NPIP and US FDA cultural reference methods. For poultry house drag swabs, the SDIX method showed clear superiority over both cultural methods regarding sensitivity, time-to-result, and ease of use. The SDIX method also demonstrated superior performance over a competitive rapid immunochemical-based method.

[0094] Currently, most egg production poultry houses are not under SE surveillance. It is anticipated that once environmental testing begins under the Final Rule a large proportion of these facilities will be SE-positive and be subject to egg testing. It is known that these facilities invariably contain populations of other non-serogroup D1 *Salmonella* in the environment and this is a major complication for cultural reference methods that rely on serotyping individual bacterial isolates from selective agar plates. This task requires a high level of training and experience on the part of the technician to be able pick a SE colony among the potentially overwhelming number of non-serogroup D1 *Salmonella*. This was in fact found in the alpha study described. To circumvent this issue, the method of the

invention was developed that improved the ability to confirm D1 positive samples from presumptive positive samples.

[0095] The test methods developed herein are currently undergoing NPIP and AOAC-PTM (RI) approval and should provide valuable tools to the poultry industry for the control of SE in the poultry house environment at the level of poultry breeders, layer and broiler houses, poultry processors, and for egg testing for SE under the Final Rule.

Validation Studies

[0096] Salmonella Enteritidis (including other Group D1 serovars) in poultry house drag swabs, shell egg pool samples and chicken carcass rinsate samples. The test kit permits the presumptive detection and identification of the target pathogen in 40 or 48 hours, dependent on sample type, when present at levels as low as 1-5 organisms per sample.

[0097] This immunoassay test uses a double antibody sandwich format in a lateral flow test strip. It utilizes a murine monoclonal antibody specific for Salmonella Group D1 including Salmonella Enteritidis (SE). The antibody is sprayed and immobilized on the surface of a nitrocellulose membrane comprising a "test line". The same monoclonal antibody is also labeled with colloidal gold and is contained within a reagent pad upstream from the test line on the membrane. As the sample moves by capillary action from the filter pad into the antibody–gold pad, the antibody–gold reagent specifically binds to the target organism and moves with the liquid sample onto the test membrane. The sample passes through the test line where the immobilized antibody captures the antigen–antibody–gold complex, causing the formation of an antibody–antigen "sandwich" and development of red color at the test line. Antibody–antigen sandwiches are not formed in the absence of the Salmonella Group D1 including SE, resulting in no red color development at the test line. Anti-mouse antibody immobilized at the control line captures excess monoclonal antibody-gold reagent passing through the test line. The presence of red color at the control line indicates that the strip has flowed correctly. Therefore, the presence of only one line

(control line) on the membrane indicates a negative sample and the presence of two lines indicates a positive sample.

[0098] The immunomagnetic confirmation kit utilizes the same monoclonal antibody described above attached to magnetic particles for the purification of SE and other Group D1 serovars from a complex enriched liquid media sample. The antibody-coated magnetic particles are used to concentrate Salmonella Group D1 bacteria present within an enriched sample making confirmation of the presumptive positive result much more robust and easier to interpret. Essentially, the coated magnetic particles are added to a presumptive positive enrichment. If SE is present, it will bind to the magnetic particles via the coated antibody. A magnet is then used to concentrate the bound, coated magnetic particles and the remaining enrichment is discarded leaving only magnetic particles bound to the Salmonella Group D1serovars present in the enrichment. Confirmation procedures are then continued with the concentrated sample.

Standard Reference Materials

[0099] Salmonella Enteritidis ATCC 13076, Salmonella Enteritidis ARS 11, Salmonella Enteritidis ARS 12, and Salmonella Kentucky ATCC 9263 were used in various aspects of the study below. Salmonella Enteritidis ATCC 13076 and Salmonella Kentucky ATCC 9263 were obtained from the American Tissue Culture Collection (ATCC, Gaithersburg, MD). Salmonella Enteritidis strains ARS 11 and ARS 12 were obtained from the Eastern Regional Research Center, USDA-ARS, Wyndmoor, PA.

Sample Preparation

[0100] Poultry house drag swabs were obtained from an egg facility in Maine. The hen house was occupied by approximately 20,000 pullets (16 weeks old) at the time of sampling. The drag swab samples were collected from the manure pits below the cage banks according to methods described in "Environmental Sampling and Detection of Salmonella In Poultry

Houses" US FDA, October 2008, incorporated herein by reference. One lot of one hundred and twenty-five (125) drag swab samples were collected and shipped to SDIX for next day delivery. Upon receipt, 60 drags were subsequently shipped to the independent laboratory for next day delivery. The remaining swabs were used for the internal studies. Drag swabs were used within 48 h of sampling and maintained at approximately 4 °C throughout shipping and storage. Shell eggs were obtained from a local wholesale food provider. Chicken broiler carcasses were obtained from a local grocery store.

Interpretation and Test Results

[0101] The Mantel-Haenszel chi-square test for unmatched test portions was used to determine whether two methods were equivalent. A Chi-Square value of less than 3.84 indicates no significant difference ($p<0.05$) in the numbers of positive test portions given by the two methods being compared. The formula for determining the Mantel-Haenszel chi-square value is:

$$X^2 = \frac{(N-1)(AF - (B+C+D)E)^2}{(A+B+C+D)(A+E)(B+C+D+F)(E+F)}$$

Where $N = A + B + C + D + E + F$

Data Table for Chi Square Analysis of Independent Samples

		Confirmed+	Confirmed -
Alternative Method	Presumptive +	A	B
	Presumptive -	C	D
Reference Method		E	F

[0102] Only presumptive positive results that confirm positive are considered as positive for the alternative method. All other results (presumptive positives that confirm negative, presumptive negatives that confirm negative and presumptive negatives that confirm positive) are considered as negative for the alternative method.

Internal Validation Studies

Poultry House Drag Swab Method Comparison Study

[0103] Poultry house drag swabs were collected using standard procedures outlined in "Environmental Sampling and Detection of Salmonella In Poultry Houses" (US FDA, October 2008) from an egg layer facility with no history of SE contamination. For spiking, a single colony isolate of Salmonella Enteritidis ARS 11 (originally isolated from poultry feces) was grown in trypticase soy broth incubated at 37°C for 24 h. Viable cell enumeration of the inoculum was accomplished by dilution plate counting on trypticase soy agar plates supplemented with 0.6% yeast extract. Individual drag swabs were inoculated at a target level of 3 CFU/swab. Two (2) sets of 5 unspiked and 20 spiked drag swab samples were acclimated at 4°C for 48 h, tested by either the SDIX method or the FDA BAM reference method, and the results compared.

Methodology

FDA Method – Environmental Sampling and Detection of Salmonella in Poultry Houses (US Food and Drug Administration. Environmental Sampling and Detection of Salmonella in Poultry Houses, October 2008. http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/ucm114716.htm)

[0104] One hundred (100) mL of buffered peptone water (BPW) was added to the sterile whirl-pak bag containing the drag swab sample. The sample was shaken and incubated 35 ± 2°C for 24 ± 2 h. Then, 1 mL of the sample was transferred into 10 mL TT broth and incubated at 43.0 ± 0.2°C for 24 ± 2 h in a circulating water bath. In addition, 0.1 mL was transferred into 10 mL RV broth and incubated at 42.0 ± 0.2°C for 24 ± 2 h in a water bath. Following the secondary enrichment, a 10 µL loop of each secondary enrichment broth was struck to XLT4 and BGN selective agar plates. The plates were incubated at 35°C and examined 24 ±2 h. Up to 5 typical colonies from each set of plates were transferred to TSI and LIA slants and incubated 35 ± 1°C for 24 ± 2 h. A minimum of 1 positive TSI slant was

subjected to slide agglutination serology testing for Salmonella Group D1. If positive, then the isolate was identified biochemically using BioMerieux API20E (Official Method 978.24).

RapidChek SELECT™ Salmonella Enteritidis Test System

[0105] One hundred (100) mL of pre-warmed (42°C) supplemented RapidChek SELECTTM primary media was added to each sample bag containing the swab, hand massaged for 30 sec, and incubated at 42±2°C for 16-22 hr. Then, 0.2 mL of the primary enrichment broth was transferred to a tube containing 2 mL pre-warmed (42°C) RapidChek secondary media and incubated 16-22 h at 42°C. Then, the tubes were gently shaken, the lateral flow test strip was inserted into the tube and developed for 10 minutes. The results were recorded (Positive – two red lines, Negative – one red line). All samples were confirmed according to the FDA-BAM method described above using the RapidChek SELECT™ secondary enrichment broth.

[0106] In addition, all samples positive by the RapidChek SELECT™ Salmonella Enteritidis test strip were tested with the RapidChek CONFIRM Salmonella Enteritidis IMS method. For this, the working stock of monoclonal antibody-magnetic particle reagent was re-suspended by repeated inversion of the vial. One (1) mL of enriched sample was transferred to the a 2 mL polypropylene centrifuge tube. Then, 0.05 mL of the IMS beads was added to the enriched sample in the tube, vortexed briefly to mix and incubated at room temperature with rocking for 15 minutes. The sample tube was placed on a magnetic separation rack for 5 minutes after which time the liquid was removed from the sample. The sample tubes were removed from the magnetic rack and 1 mL of wash solution (PBS-T) was added to the tube. It was vortexed briefly to mix and placed on the magnetic separation rack for 5 minutes after which time the liquid was removed from the sample. The wash step was repeated for a total of 5 washes. After the final wash step, the sample was reconstituted with 0.1 mL of wash solution and vortexed briefly to mix. Selective agar plates (XLT4 and BGN) were struck with a 10 µL loop of the purified sample. Plates were incubated at 35°C

and examined after 24 ±2 h. Typical Salmonella colonies were confirmed as described above.

Results

[0107] The results from the study are shown in **Table 15**. Thirteen (13) samples were positive with the RapidChek SELECT™ SE test strip. All of the presumptive positives samples confirmed as Salmonella Group D1 positive. None of the test strip-negative samples (12) were culturally-positive by the FDA-BAM confirmation method. The FDA-BAM reference method detected 4 positive samples. This gave an accuracy of 325% indicating that 3.25 times more positives were found with the RapidChek SELECT™ SE method than with the reference method. The chi-square value was 8.08, indicating the RapidChek method is significantly more sensitive than the reference method for the detection of SE in poultry house drag swabs.

Table 15:
Results from the Poultry House Drag Swab
Method Comparison Study-Internal Validation

Matrix	Poultry House Swabs	
Analyte	S. Enteritidis ARS 11	
Method	Rapid Chek SELECT	
Number of samples	5	20
Inoculation level (CFU/samples)	0	3
Presumptive positives	0	13
Confirmed positives	0	13
Reference method positives	0	4
Chi square[a]	8.070	
Sensitivity rate[b]	100.0%	
False negative rate[c]	0.0%	
Specificity rate[d]	100.0%	
False positive rate[e]	0.0%	
Accuracy[f]	325.0%	

[a]Mantel-Haenszel Chi-square analysis.
[b]Sensitivity Rate = (No. of test method presumptive positives)/(No. of test method confirmed positives) x 100.
[c]False Negative Rate = 100 - Sensitivity Rate.
[d]Specificity Rate = (No. of test method negatives)/(No. of confirmed test method negatives) x 100.
[e]False Positive Rate = 100 - Specificity Rate.
[f]Accuracy = (No. of test method positives)/(No. of reference method positives) x 100.

Egg Pool Method Comparison Study

[0108] Shell eggs were purchased from a local wholesale food distributor. Approximately 1200 eggs were disinfected using established FDA-BAM procedures (70% ethanol/iodide/iodine solution). Nine hundred (900) eggs were cracked and the contents pooled. For spiking, a single colony isolate of Salmonella Enteritidis ATCC 13076 (a clinical isolate) was grown in trypticase soy broth incubated at 37°C for 24 h. Viable cell enumeration of the inoculum was accomplished by dilution plate counting on trypticase soy agar plates supplemented with 0.6% yeast extract. The egg pool was spiked at a target level of 2.4 CFU/L (approximate volume of 20 egg pool). For the negative control samples, 300 disinfected eggs were cracked and pooled. One (1) liter pools were aliquoted into sterile bags and acclimated for 48 h at 4°C prior to analysis by the SDIX and FDA-BAM cultural reference methods. At the time of analysis, following acclimation, an aerobic plate count was conducted on the negative pool and most probable number (MPN) analysis was conducted on the spiked pool.

Methodology

FDA-BAM Cultural Reference Method
(US Food and Drug Administration, Center for Food Safety and Applied Nutrition. Bacteriological Analytical Manual. Chapter 5: Salmonella. http://www.fda.gov /Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/default. htm)

[0109] For the FDA-BAM method, following acclimation at 4°C for 48 h, eggs pools were held at room temperature (20-24°C) for 96 ± 2 h. After that time, a 25 mL portion from each sample was removed and added to 225 mL of sterile trypticase soy broth (TSB) supplemented with ferrous sulfate (35 mg/L) and mixed well by swirling. This sample was incubated 60 ± 5 minutes at room temperature. The sample was mixed by swirling and the pH was adjusted to 6.8 ± 0.2. The sample was incubated 24 ± 2 h at 35°C. Then, 0.1 mL was transferred to 10 mL Rappaport-Vassiliadis (RV) broth (prepared from individual ingredients) and 1 mL to 10 mL TT broth. The RV was incubated at 42 ± 0.2°C for 24 ± 2 h

and the TT broth at 35 ± 0.2°C for 24 ± 2 h. After this incubation, the samples were mixed and then struck (10 µl) onto BS, XLD and HE agars. The plates were incubated at 35°C for 24 ± 2 h. Up to 5 typical colonies were transferred to TSI and LIA slants and incubated at 35°C for 24 ± 2 h. A minimum of 1 positive TSI slant was subjected to slide agglutination serology testing for Salmonella Group D1. If positive, then the isolate was identified biochemically using BioMerieux API20E (Official Method 978.24).

RapidChek SELECT™ SE Test System

[0110] Following acclimation at 4°C for 48 h, to each egg pool was added 200 mL pre-warmed (42°C) supplemented RapidChek SELECT™ primary media. The sample was mixed well and incubated at room temperature (23°C) for 40 h. At that time, 0.1 mL of the primary enrichment broth was added to a tube containing 1 mL pre-warmed (42°C) RapidChek SELECT™ secondary media. The tubes were incubated 6-8 h at 42°C. The tubes were gently shaken and a RapidChek SELECT™ SE test strip was added. The test strip was developed for 10 minutes and the result recorded. The results were recorded (Positive – two red lines, Negative – one red line). All samples were confirmed according to the FDA-BAM method described above using the RapidChek SELECT™ secondary enrichment broth.

Results

[0111] The results from the study are shown in **Table 16**. Seventeen (17) samples were positive with the RapidChek SELECT™ SE test strip. All of the presumptive positives samples confirmed as Salmonella Group D1 positive. None of the test strip-negative samples (8) were culturally-positive. The FDA-BAM reference method detected 15 positive samples. This gave an accuracy of 113% indicating that the RapidChek Select SE method gave nearly the same number of positives as the reference method. The chi-square value was 0.609, indicating the RapidChek method is equivalent to the FDA-BAM method for the detection of SE in egg pools. Furthermore, the RapidChek Select SE method gave a negative

result after 48 h (2 days) whereas the FDA-BAM method required 168 h (7 days) for a negative result.

Table 16:
Results from the Egg Pool Method Comparison Study-Internal Validation

Matrix	Egg Pools	
Analyte	S. Enteritidis ATCC 13076	
Method	Rapid Chek SELECT	
Number of samples	5	20
MPN (CFU/samples)	0	<3
Presumptive positives	0	17
Confirmed positives	0	17
Reference method positives	0	15
Chi square[a]	0.609	
Sensitivity rate[b]	100.0%	
False negative rate[c]	0.0%	
Specificity rate[d]	100.0%	
False positive rate[e]	0.0%	
Accuracy[f]	113.3%	

[a]Mantel-Haenszel Chi-square analysis.
[b]Sensitivity Rate = (No. of test method presumptive positives)/(No. of test method confirmed positives) x 100.
[c]False Negative Rate = 100 - Sensitivity Rate.
[d]Specificity Rate = (No. of test method negatives)/(No. of confirmed test method negatives) x 100.
[e]False Positive Rate = 100 - Specificity Rate.
[f]Accuracy = (No. of test method positives)/(No. of reference method positives) x 100.

Chicken Carcass Rinsate Method Comparison Study

[0112] Whole (broiler) chicken carcasses (25) were obtained from a local grocery store. They were aseptically drained of excess liquid and transferred to a large sterile bag. For spiking, a single colony isolate of *Salmonella* Enteritidis ARS 11 (originally isolated from poultry feces) was grown in trypticase soy broth incubated at 37 °C for 24 h. Viable cell enumeration of the inoculum was accomplished by dilution plate counting on trypticase soy agar plates supplemented with 0.6% yeast extract. Individual carcasses were inoculated at a target level of 13.3 CFU/carcass or 1 CFU/30 mL rinsate (analytical unit). Four hundred (400) mL of BPW was poured into the cavity of the carcass contained in the bag. The bird was rinsed inside and out with a rocking motion for one minute.

Methodology

USDA-FSIS Cultural Reference Method

(US Department of Agriculture, Food Safety and Inspection Service. Microbiological Laboratory Guidelines. Chapter 4: Isolation and identification of Salmonella from meat, poultry and egg products. http://www.fsis.usda.gov/Science/Microbiological_Lab_Guidebook/)

[0113] Thirty (30) mL of the sample rinse fluid obtained above was transferred to a sterile stomacher bag followed by 30 mL of sterile BPW and mixed well. The sample was incubated at 35 ± 2°C for 20-24 h. Then, 0.5 ± 0.05 mL of this was transferred into 10 mL TT (Hajna) broth and 0.1 ± 0.02 mL into 10 mL mRV broth and incubated at 42 ± 0.5°C for 22-24 h. Following incubation, the broths were struck (10 µL) to XLT4 and BGS agar plates. The plates were incubated at 35 ± 2°C for 18-24 h at which time typical colonies were picked for confirmation. If negative, plates were re-incubated for an additional 18-24 h and re-examined. Up to 5 typical colonies were transferred to TSI and LIA slants and incubated at 35°C for 24 ± 2 h. A minimum of 1 positive TSI slant was subjected to slide agglutination serology testing for Salmonella Group D1. If positive, then the isolate was identified biochemically using BioMerieux API20E (Official Method 978.24).

RapidChek SELECT SE Test System

[0114] Thirty (30) mL of the sample rinse fluid obtained above was transferred to a sterile stomacher bag followed by 30 mL of pre-warmed (42°C) 2X RapidChek SELECT™ primary media containing 2X supplement and mixed well. The sample was incubated at 42±2°C for 16-22 hr. Then, 0.1 mL of the primary enrichment broth was transferred to a tube containing 1 mL pre-warmed (42°C) RapidChek SELECT™ secondary media and incubated 16-22 h at 42°C. Then, the tubes were gently shaken, the lateral flow test strip was inserted into the tube and developed for 10 min. The results were recorded (Positive – two red lines, Negative – one red line). All samples were confirmed according to the

USDA-FSIS method described above using the RapidChek SELECT™ secondary enrichment broth.

Results

[0115] The results from the study are shown in **Table 17**. Eleven (11) samples were positive with the RapidChek SELECT™ SE test strip. All of the presumptive positives samples confirmed as Salmonella Group D1 positive. None of the test strip-negative samples (14) were culturally-positive. The USDA-FSIS reference method detected 13 positive samples. This gave an accuracy of 85% indicating that the RapidChek SELECT™ SE method gave nearly the same number of positives as the reference method. The chi-square value was 0.406, indicating the RapidChek method is equivalent to the USDA-FSIS cultural reference method for the detection of SE in chicken carcass rinsates.

Table 17:
Results from the Chicken Carcass Rinsate Method Comparison Study-Internal Validation

Matrix	Chicken Carcass Rinsates	
Analyte	S. Enteritidis ARS 11	
Method	Rapid Chek SELECT	
Number of samples	5	20
Inoculation level (CFU/samples)	0	1
Presumptive positives	0	11
Confirmed positives	0	11
Reference method positives	0	13
Chi square[a]	0.406	
Sensitivity rate[b]	100.0%	
False negative rate[c]	0.0%	
Specificity rate[d]	100.0%	
False positive rate[e]	0.0%	
Accuracy[f]	84.6%	

[a]Mantel-Haenszel Chi-square analysis.
[b]Sensitivity Rate = (No. of test method presumptive positives)/(No. of test method confirmed positives) x 100.
[c]False Negative Rate = 100 - Sensitivity Rate.
[d]Specificity Rate = (No. of test method negatives)/(No. of confirmed test method negatives) x 100.
[e]False Positive Rate = 100 - Specificity Rate.
[f]Accuracy = (No. of test method positives)/(No. of reference method positives) x 100.

Test Strip Inclusivity Study

Methodology

[0116] *Salmonella* single colony isolates were cultured in 10 mL of RapidChek SELECT™ primary enrichment media for 16-22 hr at 42°C. Then, 0.1 mL of the primary broth was transferred to 1 mL of RapidChek SELECT™ secondary enrichment media. This was incubated at 42°C for 16-22 hr. Following enrichment, a test strip was inserted into the tube and developed for 10 min after which time the test strip was read and interpreted as previously described.

Results

[0117] The results from the test inclusivity study are shown in **Table 18**. Eighty-two (82) Salmonella Group D1 strains including 63 SE strains were grown in the complete RapidChek SELECT™ Salmonella media system and tested with the test strip. All of the *Salmonella* Group D1 strains gave a positive result with the method. This indicated a method sensitivity of 100%.

Table 18:
Results from the Test Strip Inclusivity Study

Sample Number	Serovar	Strain Number	RapidChek Select SE Test Strip Result
1	Salmonella Enteritidis	ARS 11	+
2	Salmonella Enteritidis	ARS 12	+
3	Salmonella Enteritidis	M1 BGA 164-93	+
4	Salmonella Enteritidis	Tyson 22	+
5	Salmonella Enteritidis	ATCC 13076	+
6	Salmonella Enteritidis	ATCC 8391	+
7	Salmonella Enteritidis var. Jena	ATCC 49221	+
8	Salmonella Enteritidis var. Jena	ATCC 49222	+
9	Salmonella Enteritidis var. Jena	ATCC 49223	+
10	Salmonella Enteritidis var. Essen	ATCC 49218	+
11	Salmonella Enteritidis var. Essen	ATCC 49219	+
12	Salmonella Enteritidis var. Essen	ATCC 49220	+
13	Salmonella Enteritidis var. Danysz	ATCC 49217	+
14	Salmonella Enteritidis var. Chaco	ATCC 49214	+
15	Salmonella Enteritidis var. Chaco	ATCC 49215	+
16	Salmonella Enteritidis	ISU-1-2P	+
17	Salmonella Enteritidis	ISU-1-4K	+
18	Salmonella Enteritidis	ISU-1-6J	+
19	Salmonella Enteritidis	ISU-1-38s	+
20	Salmonella Enteritidis	ISU-1-78t	+
21	Salmonella Enteritidis	ISU-5-4j	+
22	Salmonella Enteritidis	ISU-6-19i	+
23	Salmonella Enteritidis	ISU-7-2i	+
24	Salmonella Enteritidis	ISU-7-6f	+
25	Salmonella Enteritidis	ISU-8-27e	+
26	Salmonella Enteritidis	ISU-8-13a	+
27	Salmonella Enteritidis	ISU-9-13e	+
28	Salmonella Enteritidis	ISU-10-3e	+
29	Salmonella Enteritidis	ISU-10-9g	+
30	Salmonella Enteritidis	ISU-10-13d	+
31	Salmonella Enteritidis	ISU-10-13p	+
32	Salmonella Enteritidis	ISU-11-2a	+
33	Salmonella Enteritidis	ISU-11-2f	+
34	Salmonella Enteritidis	ISU-12-39s	+
35	Salmonella Enteritidis	ISU-12-42e	+
36	Salmonella Enteritidis	ISU-12-53p	+
37	Salmonella Enteritidis	ISU-13-10f	+
38	Salmonella Enteritidis	ISU-13-11e	+
39	Salmonella Enteritidis	ISU-14-8g	+
40	Salmonella Enteritidis	ISU-15-2h	+
41	Salmonella Enteritidis	ISU-17-43h	+
42	Salmonella Enteritidis	ISU-18-3b	+
43	Salmonella Enteritidis	ISU-18-4h	+
44	Salmonella Enteritidis	ISU-18-5d	+
45	Salmonella Enteritidis	ISU-18-6n	+
46	Salmonella Enteritidis	ISU-18-9f	+
47	Salmonella Enteritidis	ISU-18-10g	+
48	Salmonella Enteritidis	ISU-19-11g	+
49	Salmonella Enteritidis	ISU-20-19i	+
50	Salmonella Enteritidis	ISU-20-32m	+
51	Salmonella Enteritidis	ISU-20-33n	+
52	Salmonella Enteritidis	ISU-20-36p	+
53	Salmonella Enteritidis	ISU-20-35q	+
54	Salmonella Enteritidis	ISU-20-36r	+
55	Salmonella Enteritidis	ISU-21-5f	+
56	Salmonella Enteritidis	ISU-22-5a	+
57	Salmonella Enteritidis	ISU-22-6b	+
58	Salmonella Enteritidis	ISU-23-5e	+
59	Salmonella Enteritidis	ISU-23-5h	+
60	Salmonella Enteritidis	ISU-24-3a	+
61	Salmonella Enteritidis	ISU-24-4b	+
62	Salmonella Enteritidis	ISU-24-5c	+
63	Salmonella Enteritidis	ISU-25-1f	+
64	Salmonella Dublin	ISU-2-1a	+
65	Salmonella Dublin	ISU-3-1a	+
66	Salmonella Dublin	ISU-4-1a	+
67	Salmonella Berta	ISU-16-2b	+
68	Salmonella Berta	ISU-16-3i	+
69	Salmonella Berta	ISU-16-7j	+
70	Salmonella Berta	ISU-16-10f	+
71	Salmonella Berta	ISU-16-12k	+
72	Salmonella Javiana	ATCC 10721	+
72	Salmonella Panama	Tyson 3	+
73	Salmonella Pullorum	ATCC 9120	+
74	Salmonella Pullorum	ATCC 19945	+
75	Salmonella 9,12:nonmotile	ISU-10-3a	+
76	Salmonella 9,12:nonmotile	ISU-10-5b	+
77	Salmonella 9,12:nonmotile	ISU-10-9c	+
78	Salmonella 9,12:nonmotile	ISU-10-19i	+
79	Salmonella 9,12: poorly motile	ISU-10-5"o"	+
80	Salmonella 9,12: poorly motile	ISU-10-9n	+
81	Salmonella 9,12: poorly motile	ISU-10-13h	+
82	Salmonella 9,12: poorly motile	ISU-10-15m	+

Test Strip Exclusivity Study

Methodology

[0118] Single colony isolates of non-Salmonellae as well as non-Group D1 *Salmonella* bacteria were cultured in 10 mL of Tryptic soy broth (TSB) that was incubated at 37°C for 24 h. One (1) mL of the broth culture was transferred to a 12 x 75 mm tube and a test strip inserted into the tube. The test strip was read and interpreted as previously described.

Results

[0119] The results from the test exclusivity study are shown in **Table 19**.

Table 19:
Results from the Test Strip Exclusivity Study

Bacteria	Strain Number	RapidChek Select SE Test Strip Result
Salmonella Typhimurium (B)	ATCC 14028	-
Salmonella Heidelberg (B)	WVU 5F114	-
Salmonella Montevideo (C1)	ARS 32	-
Salmonella Thompson (C1)	ARS 15	-
Salmonella Hadar (C2)	ATCC 51956	-
Salmonella Kentucky (C3)	ATCC 9263	-
Salmonella Albany (C3)	ATCC 51960	-
Salmonella Maarsen (D2)	ATCC 15793	-
Salmonella Muenster (E1)	WVU 5F22	-
Salmonella Illinois (E3)	ATCC 11646	-
Salmonella Senftenberg (E4)	WVU 6F11	-
Salmonella Abaetetuba (F)	ATCC 35640	-
Salmonella Poona (G1)	DSM 109	-
Salmonella Cubana (G2)	ATCC 12007	-
Salmonella Pomona (M)	ATCC 10729	-
Bacillus subtilis	ATCC 6633	-
Aeromonas veronii	ATCC 51106	-
Citrobacter koseri	ATCC 27026	-
Citrobacter freundii	ATCC 8090	-
Enterobacter cloacae	ATCC 27508	-
Enterobacter aerogenes	ATCC 15038	-
Escherichia coli	ATCC 35218	-
Escherichia coli	ATCC 51755	-
Escherichia hermannii	ATCC 55236	-
Escherichia hermannii	ATCC 33650	-
Klebsiella pneumoniae	ATCC 29018	-
Klebsiella pneumoniae	ATCC 35596	-
Proteus vulgaris	ATCC 8427	-
Proteus mirabilis	ATCC 4630	-
Serratia liquefaciens	ATCC 27592	-
Vibrio parahaemolyticus	ATCC 17802	-
Vibrio parahaemolyticus	ATCC 27519	-

[0120] Thirty-two (32) non-*Salmonella* Group D1 bacteria from 10 genera were tested in the exclusivity study. This included 15 *Salmonella* from serogroups other than D1. None of the test strains gave a positive response in the test indicating a specificity of 100%.

Test Strip Ruggedness Studies

Test Strip Read Time

Methodology

[0121] *Salmonella* Enteritidis ATCC 13076 and *Salmonella* Enteritidis ARS 11 single colony isolates were cultured in 10 mL of RapidChek SELECT™ primary enrichment media for 16-22 hr at 42°C. Then, 0.1 mL of the primary broth was transferred to 1 mL of RapidChek SELECT™ secondary enrichment media. This was incubated at 42°C for 16-22 hr. The samples were then diluted into RapidChek SELECT™ secondary media to approximately 1 x 10^6 CFU/mL (approximately 1 $\log_{10}$ above the detection limit of the method). *Salmonella* Kentucky ATCC 9263 was grown in non-selective media (TSB) (approximately 1 x 10^9 CFU/mL). Test strips were placed into replicate samples (5), removed from the sample at various time points (5, 10, 20 min) and the test strip was read and interpreted.

Results

[0122] The results are shown in **Table 20**.

Table 20:
Results from the Test Strip Read Time Ruggedness Study

		Test Strip Result, # of Positives		
		Read Time, min		
	Test Level, CFU/mL	5	10	20
S. Enteritidis ATCC 13076	1 x 10^6	5/5	5/5	5/5
S. Enteritidis ARS 11	1 x 10^6	5/5	5/5	5/5
S. Kentucky ATCC 9263	~1 x 10^9 (Neat)	0/5	0/5	0/5

[0123] There were no differences in the number of test strip positives found when test strips were interpreted after 5, 10 (designated read time), or 20 min in the sample. In addition, there were no false positives found with the non-Salmonella Group D1 strain. This suggested that the impact of test strip read time on the test strip result is minimal.

Test Strip Sample Temperature

Methodology

[0124] *Salmonella* Enteritidis ATCC 13076 and *Salmonella* Enteritidis ARS 11 single colony isolates were cultured in 10 mL of RapidChek SELECT™ primary enrichment media for 16-22 hr at 42°C. Then, 0.1 mL of the primary broth was transferred to 1 mL of RapidChek SELECT™ secondary enrichment media. This was incubated at 42°C for 16-22 hr. The samples were then diluted into RapidChek SELECT™ secondary media to approximately 1 x 10^6 CFU/mL (approximately 1 $\log_{10}$ above the detection limit of the method). *Salmonella* Kentucky ATCC 9263 was grown in non-selective media (TSB) (approximately 1 x 10^9 CFU/mL). Replicate (5) 1 mL aliquots were left at room temperature or placed in an incubator for 1 hr in order to further acclimate the sample at various test temperatures (room temperature (20-25°C), 42°C, and 44°C). Test strips were placed into the samples while maintained under the various conditions (*i.e.,* in the respective incubator). They were developed for 10 min, removed and interpreted.

Results

[0125] The results are shown in **Table 21**.

Table 21:
Results from the Test Strip-Sample Temperature Ruggedness Study

		Test Strip Result, # of Positives		
		Test Temperature, °C		
	Test Level, CFU/mL	RT[a]	42	44
S. Enteritidis ATCC 13076	1×10^6	5/5	5/5	5/5
S. Enteritidis ARS 11	1×10^6	5/5	5/5	5/5
S. Kentucky ATCC 9263	~1×10^9 (Neat)	0/5	0/5	0/5

[a]RT, room temperature, 23-25°C

[0126] There were no differences in the number of test strip positives found when test strips were tested on samples that varied in temperature from room temperature (20-25°C) to 44°C. In addition, there were no false positives found with the non-Salmonella Group D1 strain. This suggested that the impact of sample temperature on the test strip result is minimal.

Test Strip Sample Volume

Methodology

[0127] *Salmonella* Enteritidis ATCC 13076 and *Salmonella* Enteritidis ARS 11 single colony isolates were cultured in 10 mL of RapidChek SELECT™ primary enrichment media for 16-22 hr at 42°C. Then, 0.1 mL of the primary broth was transferred to 1 mL of RapidChek SELECT™ secondary enrichment media. This was incubated at 42°C for 16-22 hr. The samples were then diluted into RapidChek Select secondary media to approximately 1×10^6 CFU/mL (approximately 1 $\log_{10}$ above the detection limit of the method). *Salmonella* Kentucky ATCC 9263 was grown in non-selective media (TSB) (approximately 1×10^9 CFU/mL). Replicate (5) aliquots of each sample type varying in sample volumes (0.75, 1.0, 2.0, and 2.25 mL) were tested. Test strips were placed into the samples, developed for 10 min, removed and interpreted.

Results

[0128] The results are shown in **Table 22**.

Table 22:
Results from the Test Strip-Sample Volume Ruggedness Study

		Test Strip Result, # of Positives			
		Test Volume, mL			
	Test Level, CFU/mL	0.75	1.0	2.0	2.25
S. Enteritidis ATCC 13076	1×10^6	5/5	5/5	5/5	5/5
S. Enteritidis ARS 11	1×10^6	5/5	5/5	5/5	5/5
S. Kentucky ATCC 9263	~1×10^9 (Neat)	0/5	0/5	0/5	0/5

[0129] There were no differences in the number of test strip positives found when test strips were tested on samples varying in sample volume. In addition, there were no false positives found with the non-Salmonella Group D1 strain. This suggested that the impact of sample volume on the test strip result is minimal.

Test Strip Stability Study

Methodology

[0130] Three lots of test strips in their final packaging (50 test strips/desiccant canister) were placed into storage at various temperatures (4°C, room temperature (20-25°C, recommended storage condition), 37°C (accelerated stability), and 45°C (accelerated stability)). At 7 day intervals, 15 test strips from each lot were removed from each storage condition and tested in replicate (5) with replicate samples (5) of *Salmonella* Enteritidis ATCC 13076 and *Salmonella* Enteritidis ARS 11 at 1×10^6 CFU/mL (approximately 1 $\log_{10}$ above the detection limit of the test strip) in RapidChek Select secondary media and *Salmonella* Kentucky ATCC 9263 grown in non-selective media (TSB) and tested neat (approximately 1×10^9 CFU/mL).

Results

[0131] The results from this study are shown in **Table 23**.

Table 23:
Results of the Accelerated Test Strip Stability Study

			Temperature, °C											
			4			Room Temperature			37			45		
			Lot											
Time, weeks	Test Strain	Test Level, CFU/mL	1	2	3	1	2	3	1	2	3	1	2	3
0	Salmonella Enteritidis ATCC 13076	1×10^6	-	-	-	5/5	5/5	5/5	-	-	-	-	-	-
1			5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
2			5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
3			5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
4			5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
5			5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
6			5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
7			5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
8			5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
0	Salmonella Enteritidis ARS11	1×10^6	-	-	-	5/5	5/5	5/5	-	-	-	-	-	-
1			5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
2			5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
3			5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
4			5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
5			5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
6			5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
7			5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
8			5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
0	Salmonella Kentucky ATCC 9263	1×10^9 (Neat)	-	-	-	0/0	0/0	0/0	-	-	-	-	-	-
1			0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
2			0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
3			0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
4			0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
5			0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
6			0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
7			0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
8			0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0

[0132] There were no differences in test strip results for up to 8 weeks (compared to week 0) for any of the storage conditions. This demonstrated that the test strip was very stable under accelerated stability conditions (up to 45°C).

Immunomagnetic Separation (IMS) Reagent Inclusivity Study

Methodology

[0133] Single colony isolates of various Group D1 *Salmonella* were cultured in 10 mL of RapidChek SELECT™ primary enrichment media for 16-22 hr at 42°C. Then, 0.1 mL of the primary broth was transferred to 1 mL of RapidChek SELECT™ secondary enrichment media. This was incubated at 42°C for 16-22 hr. Following enrichment, 1 mL of the enriched sample was subjected to IMS as described in Section 13.1.1.2. The samples (10 µL) were struck to BGN and XLT4 plates. The plates were incubated at 35 ± 2°C for 18-24 h at which time 1 typical colony was picked for confirmation. Typical colonies were transferred to TSI and LSI slants and incubated at 35°C for 24 ± 2 h. The positive TSI slant was subjected to slide agglutination serology testing for Salmonella Group D1.

Results

[0134] The results of this study are shown in Table 8. Eighty-two (82) *Salmonella* Group D1 strains including 63 SE strains were grown in the RapidChek Select *Salmonella* media and tested with the IMS method followed by independent serological colony confirmation. All of the *Salmonella* Group D1 strains gave a positive result with the IMS confirmation procedure.

Immunomagnetic Separation (IMS) Reagent Exclusivity Study

Methodology

[0135] Single colony isolates of non-Salmonellae as well as non-Group D1 *Salmonella* bacteria were cultured in 10 mL of Tryptic soy broth (TSB) incubated at 37°C for 24 h. Following enrichment, 1 mL of the enriched sample was subjected to IMS as described in Section 13.1.1.2. The samples (10 µL) were struck to BGN and XLT4 plates. The plates were incubated at 35 ± 2°C for 18-24 h at which time 1 typical colony was picked for confirmation. Typical colonies

were transferred to TSI and LSI slants and incubated at 35°C for 24 ± 2 h. The positive TSI slant was subjected to slide agglutination serology testing for Salmonella Group D1.

Results

[0136] The results of this study are shown in **Tables 24 and 25**.

Table 24:
Results from the Immunomagnetic Separation (IMS) Method Inclusivity Study

Sample Number	Serovar	Strain Number	RapidChek Confirm SE IMS Result	Sample Number	Serovar	Strain Number	RapidChek Confirm SE IMS Result
1	Salmonella Enteritidis	ARS 11	+	43	Salmonella Enteritidis	ISU-18-4h	+
2	Salmonella Enteritidis	ARS 12	+	44	Salmonella Enteritidis	ISU-18-5d	+
3	Salmonella Enteritidis	M1 BGA 164-93	+	45	Salmonella Enteritidis	ISU-18-6n	+
4	Salmonella Enteritidis	Tyson 22	+	46	Salmonella Enteritidis	ISU-18-9f	+
5	Salmonella Enteritidis	ATCC 13076	+	47	Salmonella Enteritidis	ISU-18-10g	+
6	Salmonella Enteritidis	ATCC 8391	+	48	Salmonella Enteritidis	ISU-19-11g	+
7	Salmonella Enteritidis var. Jena	ATCC 49221	+	49	Salmonella Enteritidis	ISU-20-19i	+
8	Salmonella Enteritidis var. Jena	ATCC 49222	+	50	Salmonella Enteritidis	ISU-20-32m	+
9	Salmonella Enteritidis var. Jena	ATCC 49223	+	51	Salmonella Enteritidis	ISU-20-33n	+
10	Salmonella Enteritidis var. Essen	ATCC 49218	+	52	Salmonella Enteritidis	ISU-20-36p	+
11	Salmonella Enteritidis var. Essen	ATCC 49219	+	53	Salmonella Enteritidis	ISU-20-35q	+
12	Salmonella Enteritidis var. Essen	ATCC 49220	+	54	Salmonella Enteritidis	ISU-20-36r	+
13	Salmonella Enteritidis var. Danysz	ATCC 49217	+	55	Salmonella Enteritidis	ISU-21-5f	+
14	Salmonella Enteritidis var. Chaco	ATCC 49214	+	56	Salmonella Enteritidis	ISU-22-5a	+
15	Salmonella Enteritidis var. Chaco	ATCC 49215	+	57	Salmonella Enteritidis	ISU-22-6b	+
16	Salmonella Enteritidis	ISU-1-2P	+	58	Salmonella Enteritidis	ISU-23-5e	+
17	Salmonella Enteritidis	ISU-1-4K	+	59	Salmonella Enteritidis	ISU-23-5h	+
18	Salmonella Enteritidis	ISU-1-6J	+	60	Salmonella Enteritidis	ISU-24-3a	+
19	Salmonella Enteritidis	ISU-1-38s	+	61	Salmonella Enteritidis	ISU-24-4b	+
20	Salmonella Enteritidis	ISU-1-78t	+	62	Salmonella Enteritidis	ISU-24-5c	+
21	Salmonella Enteritidis	ISU-5-4j	+	63	Salmonella Enteritidis	ISU-25-1f	+
22	Salmonella Enteritidis	ISU-6-19i	+	64	Salmonella Dublin	ISU-2-1a	+
23	Salmonella Enteritidis	ISU-7-2i	+	65	Salmonella Dublin	ISU-3-1a	+
24	Salmonella Enteritidis	ISU-7-6f	+	66	Salmonella Dublin	ISU-4-1a	+
25	Salmonella Enteritidis	ISU-8-27e	+	67	Salmonella Berta	ISU-16-2b	+
26	Salmonella Enteritidis	ISU-8-13a	+	68	Salmonella Berta	ISU-16-3i	+
27	Salmonella Enteritidis	ISU-9-13e	+	69	Salmonella Berta	ISU-16-7j	+
28	Salmonella Enteritidis	ISU-10-3e	+	70	Salmonella Berta	ISU-16-10f	+
29	Salmonella Enteritidis	ISU-10-9g	+	71	Salmonella Berta	ISU-16-12k	+
30	Salmonella Enteritidis	ISU-10-13d	+	72	Salmonella Javiana	ATCC 10721	+
31	Salmonella Enteritidis	ISU-10-13p	+	72	Salmonella Panama	Tyson 3	+
32	Salmonella Enteritidis	ISU-11-2a	+	73	Salmonella Pullorum	ATCC 9120	+
33	Salmonella Enteritidis	ISU-11-2f	+	74	Salmonella Pullorum	ATCC 19945	+
34	Salmonella Enteritidis	ISU-12-39s	+	75	Salmonella 9,12:nonmotile	ISU-10-3a	+
35	Salmonella Enteritidis	ISU-12-42e	+	76	Salmonella 9,12:nonmotile	ISU-10-5b	+
36	Salmonella Enteritidis	ISU-12-53p	+	77	Salmonella 9,12:nonmotile	ISU-10-9c	+
37	Salmonella Enteritidis	ISU-13-10f	+	78	Salmonella 9,12:nonmotile	ISU-10-19i	+
38	Salmonella Enteritidis	ISU-13-11e	+	79	Salmonella 9,12: poorly motile	ISU-10-5"o"	+
39	Salmonella Enteritidis	ISU-14-8g	+	80	Salmonella 9,12: poorly motile	ISU-10-9n	+
40	Salmonella Enteritidis	ISU-15-2h	+	81	Salmonella 9,12: poorly motile	ISU-10-13h	+
41	Salmonella Enteritidis	ISU-17-43h	+	82	Salmonella 9,12: poorly motile	ISU-10-15m	+
42	Salmonella Enteritidis	ISU-18-3b	+				

Table 25:
Results from the Immunomagnetic Separation (IMS) Method Exclusivity Study

Bacteria	Strain Number	RapidChek Confirm SE IMS Result
Salmonella Typhimurium (B)	ATCC 14028	-
Salmonella Heidelberg (B)	WVU 5F114	-
Salmonella Montevideo (C1)	ARS 32	-
Salmonella Thompson (C1)	ARS 15	-
Salmonella Hadar (C2)	ATCC 51956	-
Salmonella Kentucky (C3)	ATCC 9263	-
Salmonella Albany (C3)	ATCC 51960	-
Salmonella Maarsen (D2)	ATCC 15793	-
Salmonella Muenster (E1)	WVU 5F22	-
Salmonella Illinois (E3)	ATCC 11646	-
Salmonella Senftenberg (E4)	WVU 6F11	-
Salmonella Abaetetuba (F)	ATCC 35640	-
Salmonella Poona (G1)	DSM 109	-
Salmonella Cubana (G2)	ATCC 12007	-
Salmonella Pomona (M)	ATCC 10729	-
Bacillus subtilis	ATCC 6633	-
Aeromonas veronii	ATCC 51106	-
Citrobacter koseri	ATCC 27026	-
Citrobacter freundii	ATCC 8090	-
Enterobacter cloacae	ATCC 27508	-
Enterobacter aerogenes	ATCC 15038	-
Escherichia coli	ATCC 35218	-
Escherichia coli	ATCC 51755	-
Escherichia hermannii	ATCC 55236	-
Escherichia hermannii	ATCC 33650	-
Klebsiella pneumoniae	ATCC 29018	-
Klebsiella pneumoniae	ATCC 35596	-
Proteus vulgaris	ATCC 8427	-
Proteus mirabilis	ATCC 4630	-
Serratia liquefaciens	ATCC 27592	-
Vibrio parahaemolyticus	ATCC 17802	-
Vibrio parahaemolyticus	ATCC 27519	-

Thirty-two (32) non-*Salmonella* Group D1 bacteria from 10 genera were tested in the IMS exclusivity study. This included 15 non-Group D1 *Salmonella*. None of the test strains gave a positive response with the IMS confirmation procedure.

Immunomagnetic Separation (IMS) Reagent Ruggedness Study

Incubation Time

Methodology

[0137] *Salmonella* Enteritidis ATCC 13076 and *Salmonella* Enteritidis ARS 11 single colony isolates were cultured in 10 mL of RapidChek SELECT™ primary enrichment media for 16-22 hr at 42°C. Then, 0.1 mL of the primary broth per mL of RapidChek SELECT™ secondary enrichment media required was transferred to secondary enrichment broth. This was incubated at 42°C for 16-22 hr. The samples were then diluted into RapidChek Select secondary media to approximately 1 x 10^6 CFU/mL (approximately 1 $\log_{10}$ above the detection limit of the method). *Salmonella* Kentucky ATCC 9263 was grown in non-selective media (TSB) (approximately 1 x 10^9 CFU/mL). Following enrichment, 1 mL aliquots of the enriched sample was subjected to IMS as described in Section 13.1.1.2 while varying the sample-IMS bead mixing time (10, 15, and 20 min). Five (5) replicate aliquots were tested per bacterial strain per mixing time. The samples (10 µL) were struck to BGN and XLT4 plates. The plates were incubated at 35 ± 2°C for 18-24 h at which time 1 typical colony was picked for confirmation. Typical colonies were transferred to TSI and LSI slants and incubated at 35°C for 24 ± 2 h. The positive TSI slant was subjected to slide agglutination serology testing for Salmonella Group D1.

Results

[0138] The results of this study are shown in **Table 26**.

Table 26:
Results from the Immunomagnetic Separation (IMS) Method Ruggedness Study-Incubation Time

Test Strain	Test Level, CFU/mL	No. Serogroup D1 Positives/ 5 Replicates		
		Incubation Time, min		
		10	15	20
Salmonella Enteritidis ARS 11	1 x 10^6	5/5	5/5	5/5
Salmonella Enteritidis ATCC 13076	1 x 10^6	5/5	5/5	5/5
Salmonella Kentucky ATCC 9263	1 x 10^9 (Neat)	0/5	0/5	0/5

The IMS confirmation procedure effectively detected SE at all sample incubation times tested (10-20 minutes).

Sample Volume

Methodology

[0139] *Salmonella* Enteritidis ATCC 13076 and *Salmonella* Enteritidis ARS 11 single colony isolates were cultured in 10 mL of RapidChek SELECT™ primary enrichment media for 16-22 hr at 42°C. Then, 0.1 mL of the primary broth per mL of RapidChek SELECT™ secondary enrichment media required was transferred to secondary enrichment broth. This was incubated at 42°C for 16-22 hr. The samples were then diluted into RapidChek Select secondary media to approximately 1 x 10^6 CFU/mL (approximately 1 $\log_{10}$ above the detection limit of the method). *Salmonella* Kentucky ATCC 9263 was grown in non-selective media (TSB) (approximately 1 x 10^9 CFU/mL). Following enrichment, aliquots of the enriched sample was subjected to IMS as described in Section 13.1.1.2 while varying sample aliquot volumes (0.8, 1.0, and 1.2 mL). Five (5) replicate aliquots were tested per bacterial strain per sample volume. The samples (10 µL) were struck to BGN and XLT4 plates. The plates were incubated at 35 ± 2°C for 18-24 h at which time 1 typical colony was picked for confirmation. Typical colonies were transferred to TSI and LSI slants and

incubated at 35°C for 24 ± 2 h. The positive TSI slant was subjected to slide agglutination serology testing for Salmonella Group D1.

Results

[0140] The results of this study are shown in **Table 27**.

Table 27:
Results from the Immunomagnetic Separation (IMS) Method Ruggedness Study-Sample Volume

Test Strain	Test Level, CFU/mL	No. Serogroup D1 Positives/ 5 Replicates		
		Sample Volume, mL		
		0.8	1	1.2
Salmonella Enteritidis ARS 11	1×10^6	5/5	5/5	5/5
Salmonella Enteritidis ATCC 13076	1×10^6	5/5	5/5	5/5
Salmonella Kentucky ATCC 9263	1×10^9 (Neat)	0/5	0/5	0/5

The IMS confirmation procedure effectively detected SE at all sample volumes tested (0.8-1.2 mL).

Immunomagnetic Separation (IMS) Reagent Stability Study

Methodology

[0141] Three lots of IMS reagents, aged approximately 2 months since manufacturing, and stored at 4°C in final packaging (10 mL polypropylene bottle), were placed into storage at various temperatures (4°C (recommended storage condition), room temperature (20-22°C), 37°C (accelerated stability), and 45°C (accelerated stability)). At 7 day intervals, an aliquot of each reagent lot was removed from each storage condition and tested in replicate (5) with

samples of *Salmonella* Enteritidis ATCC 13076 and *Salmonella* Enteritidis ARS 11 at 1 x 10^6 CFU/mL (approximately 1 $\log_{10}$ above the detection limit of the method) grown in the RapidChek Select media system and *Salmonella* Kentucky ATCC 9263 grown in non-selective media (TSB) and tested neat (approximately 1 x 10^9 CFU/mL). Following enrichment, 1 mL aliquots of the samples were subjected to IMS as described in Section 13.1.1.2. Five (5) replicate aliquots were tested per bacterial strain per reagent lot per storage temperature. The samples (10 µL) were struck to BGN and XLT4 plates. The plates were incubated at 35 ± 2°C for 18-24 h at which time 1 typical colony was picked for confirmation. Typical colonies were transferred to TSI and incubated at 35°C for 24 ± 2 h. The positive TSI slant was subjected to slide agglutination serology testing for Salmonella Group D1.

Results

[0142] The results from this study are shown **Table 28**.

Table 28:
Results of the Accelerated Immunomagnetic Separation (IMS) Reagent Stability Study

			No. Serogroup D1 Positives/ 5 Replicates											
			4C			Room Temperature			37C			45C		
Time, weeks	Test Strain	Test Level, CFU/mL	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3
0	Salmonella Enteritidis ARS 11	1 x 10^6	5/5	5/5	5/5	-	-	-	-	-	-	-	-	-
1			5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
2			5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
4			5/5	5/5	5/5	5/5	5/5	5/5						
0	Salmonella Enteritidis ATCC 13076	1 x 10^6	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
1			5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
2			5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
4			5/5	5/5	5/5	5/5	5/5	5/5						
0	Salmonella Kentucky ATCC 9263	1 x 10^9 (Neat)	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
1			0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
2			0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
4			0/5	0/5	0/5	0/5	0/5	0/5						

The IMS reagent showed good stability for up to 2 weeks at 37°C and 45°C and up to 4 weeks at 4°C to room temperature (20-25°C). This data supports a shelf-life of up to 1 year at 4°C.

Independent Validation Studies

[0143] Validation studies conducted by the independent laboratory were under the direction of the AOAC Research Institute.

Poultry House Drag Swab Method Comparison Study

[0144] Poultry house drag swabs were collected using standard procedures ("Environmental Sampling and Detection of Salmonella in Poultry Houses" US FDA, October 2008) from an egg layer facility with no history of SE contamination. For spiking, a single colony isolate of *Salmonella* Enteritidis ARS 12 (originally isolated from poultry house soil) was grown in non-selective broth. Viable cell enumeration was accomplished by dilution plate counting on non-selective agar plates. Individual drag swabs were inoculated at a target level of 5 CFU/swab. Two (2) sets of 5 unspiked and 20 spiked drag swab samples were acclimated at 4°C for 48 h, tested by either the SDIX method or the FDA BAM reference method, and results compared.

Methodology

[0145] Methods used for the Independent study were the same as those used for the Internal study described above. The Independent method comparison study used poultry house drag swab samples collected at the same time as the Internal method comparison study.

Results

[0146] The results from the study are shown in **Table 29**. Eleven (11) samples were positive with the RapidChek SELECT™ SE test strip. All of the presumptive positives samples confirmed as Salmonella Group D1 positive. None of the test strip-negative samples (14) were culturally-positive. The FDA-BAM reference method detected 6 positive samples.

This gave an accuracy of 183% indicating that 1.83 times more positives were found with the RapidChek SELECT™ SE method than with the reference method. The chi-square value was 2.49, indicating the RapidChek method was statistically equivalent to the reference method for the detection of SE in poultry house drag swab.

Table 29:
Results from the Poultry House Drag Swab Method Comparison Study-Independent Laboratory Study

Matrix	Poultry House Swabs	
Analyte	S. Enteritidis ARS 12	
Method	Rapid Chek SELECT	
Number of samples	5	20
Inoculation level (CFU/samples)	0	5
Presumptive positives	0	11
Confirmed positives	0	11
Reference method positives	0	6
Chi square[a]	2.490	
Sensitivity rate[b]	100.0%	
False negative rate[c]	0.0%	
Specificity rate[d]	100.0%	
False positive rate[e]	0.0%	
Accuracy[f]	183.0%	

[a]Mantel-Haenszel Chi-square analysis.
[b]Sensitivity Rate = (No. of test method presumptive positives)/(No. of test method confirmed positives) x 100.
[c]False Negative Rate = 100 - Sensitivity Rate.
[d]Specificity Rate = (No. of test method negatives)/(No. of confirmed test method negatives) x 100.
[e]False Positive Rate = 100 - Specificity Rate.
[f]Accuracy = (No. of test method positives)/(No. of reference method positives) x 100.

[0147] The RapidChek SELECT™ *Salmonella* Enteritidis Test Method was validated for the detection of *Salmonella* Enteritidis (SE) in poultry house drag swab samples, shell egg pools, and carcass rinsate samples. For the detection of SE in poultry house drag swab samples, a immunomagnetic separation (IMS) method was used to aid in the isolation and confirmation of SE from those samples. The test method showed equivalency to both reference methods used for the detection of SE in poultry house drag swabs and shell egg pools (FDA-BAM) as well as carcass rinsates (USDA-FSIS). The test method gave a sensitivity of 100% and a specificity of 100% across all sample types. There were no false positives or false negatives found in the study. The overall accuracy was 137%, indicating that, in general, the test method gave more positives (52) than the reference methods (38). The overall Chi square was 4.95, indicating that the test method was overall more sensitive than the reference method in this study.

[0148] The test method was highly selective for *Salmonella* Enteritidis and other *Salmonella* Group D1 serotypes and did not cross-react with other commonly occurring bacteria spanning 10 bacterial genera including several non-Group D1*Salmonella*. Both the lateral flow test strip and the IMS reagent demonstrated very good accelerated stability at elevated temperatures.

Conclusions of Validation Studies

[0149] The RapidChek SELECTTM Salmonella Enteritidis Test System was validated for the detection of Salmonella Enteritidis (SE) in poultry house drag swabs, shell egg pools, and chicken carcass rinsates. The method utilizes RapidChek SELECTTM Salmonella (AOAC PTM License Number 080601) proprietary primary and secondary enrichment media. Following enrichment, an immunochromatographic test strip is inserted into the tube containing the secondary enrichment broth, developed for 10 minutes and interpreted. Salmonella Enteritidis-inoculated samples (1 to 5 CFU SE/analytical unit) were tested by the test method as well as the appropriate cultural reference method (FDA-BAM (drag swabs and egg pools) or USDA-FSIS (chicken carcass rinsates)). A total of 80 samples were tested by both methods in the study. Fifty-two (52) samples were positive by the RapidChek SELECT™ Salmonella Enteritidis method and 38 were found positive by the respective reference method. The sensitivity of the method was 100% and the specificity was 100%. The accuracy of the test method was 137%, indicating that the method was more sensitive than the reference method. The RapidChek SELECT™ Salmonella Enteritidis method was tested with 82 Salmonella Group D1 strains including 63 Salmonella Enteritidis strains as well as 32 non-Salmonella Group D1 strains representing 10 bacteria genera. The test method detected all 82 Group D1 strains (100% sensitivity). None of the non-Salmonella Group D1 or other genera of bacteria were detected, indicating a specificity of 100%. The method was shown to be highly robust and stable under control and accelerated stability conditions.

[0150] When ranges are used herein for physical properties, such as molecular weight, or chemical properties, such as chemical formulae, all combinations, and subcombinations of ranges specific embodiments therein are intended to be included.

[0151] The disclosures of each patent, patent application, and publication cited or described in this document are hereby incorporated herein by reference, in their entirety.

[0152] Those skilled in the art will appreciate that numerous changes and modifications can be made to the preferred embodiments of the invention and that such changes and modifications can be made without departing from the spirit of the invention. It is, therefore, intended that the appended claims cover all such equivalent variations as fall within the true spirit and scope of the invention.

What is claimed is:

1. A method for selectively isolating target microorganisms from a sample comprising said target microorganisms and at least one contaminant, comprising:

providing a plurality of magnetic particles coated with antibodies;

wherein said antibodies bind an antigen substantially unique to said target organisms;

optionally, enriching said target microorganisms in said sample;

mixing said sample with said plurality of magnetic particles coated with said antibodies to bind said antigens of said target microorganisms with said antibodies;

wherein said sample is relevant for human health, for food safety, as an indicator from environmental surveillance, as an indicator from a food specimen, or a combination thereof;

wherein said microorganisms are at least one *Salmonella* serogroup D1 serotypes; and

wherein said contaminant is selected from the group consisting of at least one non-serogroup D1 *Salmonella*, non-*Salmonella* bacteria cross-reactive with said *Salmonella* serogroup D1 serotypes, and mixtures thereof.

2. The method of claim 1,

wherein said contaminant is said at least one non-serogroup D1 *Salmonella*.

3. A method of claim 1,

wherein said microorganisms are at least one *Salmonella enterica* serotype.

4. A method of claim 1,

wherein said microorganisms are *Salmonella enterica* sub. *enterica* ser. Enteritidis.

5. A method of claim 1,

wherein said non-*Salmonella* bacteria comprise a cross-reactive feature selected from the group consisting of a somatic O antigen associated with

lipopolysaccharides, flagella on a surface of said non-*Salmonella* bacteria, and combinations thereof.

6. A method of claim 1,

wherein said contaminant is at least one organism selected from the group consisting of *E. coli*, *Citrobacter* spp, *Aeromonas* spp., *Pasteurella* spp., non-serogroup D1 *Salmonella,* and combinations thereof.

7. A method of claim 1,

wherein said sample is a specimen selected from the group consisting of an environmental surveillance specimen, a food specimen, or a combination thereof.

8. A method of claim 1,

wherein said sample is from a drag swab application, egg pool, or a chicken rinsate.

9. A method of claim 1,

wherein said antibody is generated against whole cells.

10. A method of claim 1,

wherein said enriching comprises at least one bacteriophage selective for control of cross-reactive non-*Salmonella* bacteria.

11. A method for reducing false-positive results in testing target microorganisms in a sample comprising said target microorganisms and at least one contaminant, comprising:

enriching said target microorganisms in said sample;

selectively isolating target microorganisms from said sample to obtain isolated target organisms, comprising:

providing a plurality of magnetic particles coated with antibodies; and

wherein said antibodies bind an antigen substantially unique to said target organisms;

mixing said sample with said plurality of magnetic particles coated with said antibodies to bind said antigens of said target microorganisms with said antibodies;

cultivating said isolated target organisms; and

optionally, confirming the identity of said organism using at least one serological test.

12. The method of claim 11,

wherein said contaminant is selected from the group consisting of at least one non-serogroup D1 *Salmonella*, non-*Salmonella* bacteria cross-reactive with said *Salmonella* serogroup D1 serotypes, and mixtures thereof.

13. The method of claim 11,

wherein said contaminant is said at least one non-serogroup D1 *Salmonella*.

14. A method of claim 11,

wherein said microorganisms are at least one *Salmonella enterica* serotype.

15. A method of claim 11,

wherein said microorganisms are *Salmonella enterica* sub. *enterica* ser. Enteritidis.

16. A method of claim 11,

wherein said non-*Salmonella* bacteria comprise a cross-reactive feature selected from the group consisting of a somatic O antigen associated with lipopolysaccharides, flagella on a surface of said non-*Salmonella* bacteria, and combinations thereof.

17. A method of claim 11,

wherein said contaminant is at least one organism selected from the group consisting of *E. coli*, *Citrobacter* spp, *Aeromonas* spp., *Pasteurella* spp., non-serogroup D1 *Salmonella,* and combinations thereof.

18. A method of claim 11,

wherein said sample is a specimen selected from the group consisting of an environmental surveillance specimen, a food specimen, or a combination thereof.

19. A method of claim 11,

wherein said sample is from a drag swab application, egg pool, or a chicken rinsate.

20. A method of claim 11,

wherein said antibody is generated against whole cells.

21. A method of claim 11,

wherein said enriching comprises at least one bacteriophage selective for control of cross-reactive non-*Salmonella* bacteria.

22. A method of claim 11,

wherein said enriching comprises treating said sample with at least one growth-discriminating media.

23. A method of claim 11,

wherein said serological test is immunochromatographic strip-based detection.

24. A method of claim 11,

wherein said serological test is a selective agar-based detection.

25. A method of claim 11,

wherein said cultivating step is carried out in the presence of at least one material selected from the group consisting of at least one growth-discriminating media, agar, and combinations thereof.

26. A method of claim 11,

wherein said sample is a specimen selected from the group consisting of an environmental surveillance specimen, a food specimen, or a combination thereof.

27. A method of claim 11,

wherein said sample is from a drag swab, egg pool, or a chicken rinsate.

28. A kit for reducing false-positive results in testing target microorganisms in a sample comprising said target microorganisms and at least one contaminant, comprising:

at least one container;

at least one enrichment medium;

a plurality of magnetic particles coated with antibodies; and

wherein said antibodies bind an antigen substantially unique to said target organisms;

at least one cultivating medium; and

serological test components;

wherein said microorganisms are at least one *Salmonella* serogroup D1 serotypes; and

wherein said contaminant is selected from the group consisting of at least one non-serogroup D1 *Salmonella*, non-*Salmonella* bacteria cross-reactive with said *Salmonella* serogroup D1 serotypes, and mixtures thereof.

29. A kit of claim 28,

wherein said contaminant is said at least one non-serogroup D1 *Salmonella*.

30. A kit of claim 28,

wherein said microorganisms are at least one *Salmonella enterica* serotype.

31. A kit of claim 28,

wherein said microorganisms are *Salmonella enterica* sub. *enterica* ser. Enteritidis.

32. A kit of claim 28,

wherein said non-*Salmonella* bacteria comprise a cross-reactive feature selected from the group consisting of a somatic O antigen associated with

lipopolysaccharides, flagella on a surface of said non-*Salmonella* bacteria, and combinations thereof.

33. A kit of claim 28,

wherein said contaminant is at least one organism selected from the group consisting of *E. coli*, *Citrobacter* spp, *Aeromonas* spp., *Pasteurella* spp., non-serogroup D1 *Salmonella,* and combinations thereof.

34. A kit of claim 28,

wherein said antibody is generated against whole cells.

35. A kit of claim 28,

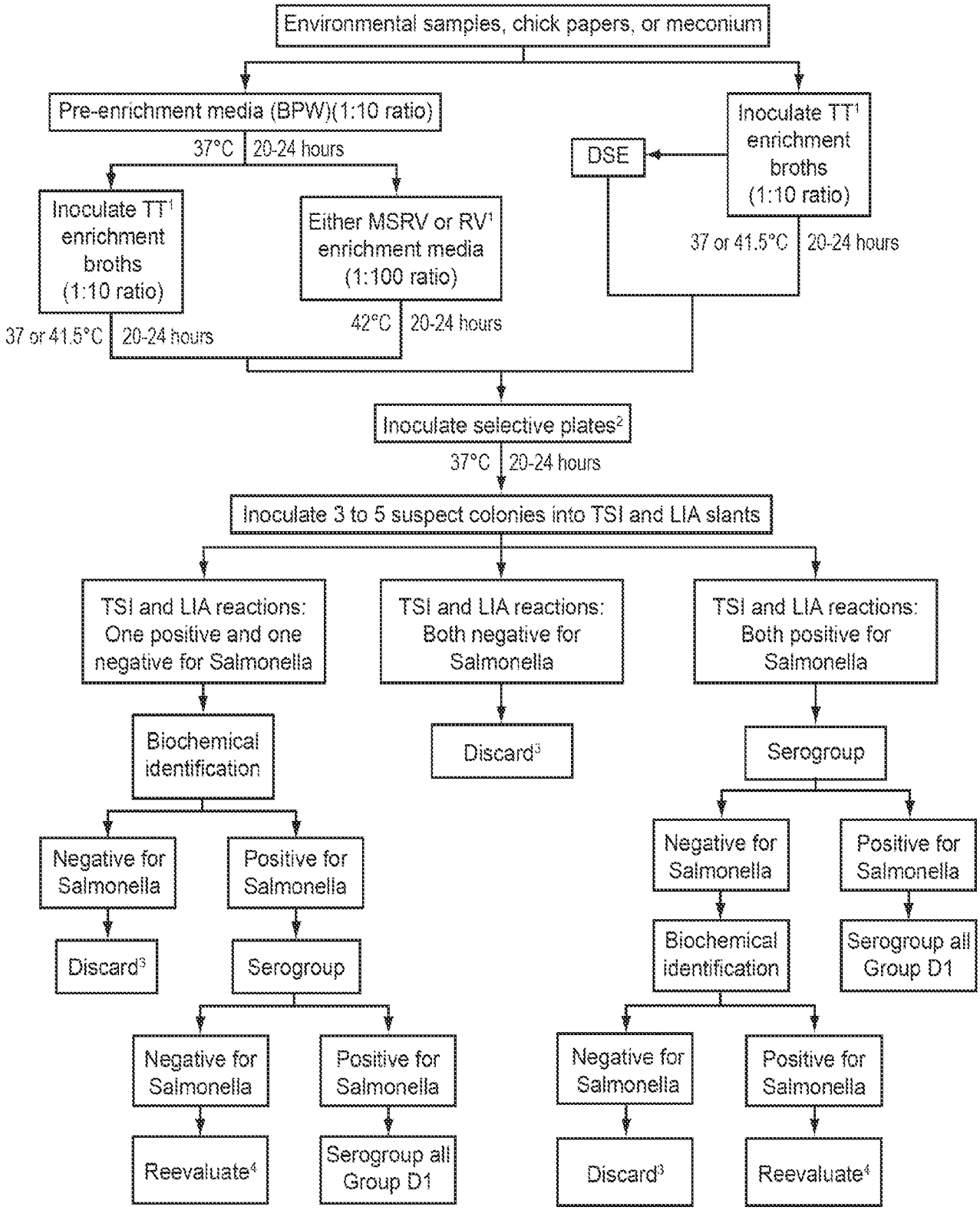
wherein said serological test component is an immunochromatographic strip.

36. A kit of claim 28,

wherein said serological test component is a selective agar.

37. A kit of claim 28,

wherein said enrichment medium comprises at least one bacteriophage selective for control of cross-reactive non-*Salmonella* bacteria.



1. Tetrathionate enrichment broth, e.g., Rappaport-Vassiliades (RV) or modified semisolid RV (MSRV).
2. Selective plates such Brilliant Green Novobiocin (BGN) or xylose-lysine tergitol 4 (XLT 4).
3. Reevaluate if epidemiologic, necropsy, or other information indicates the presence of an unusual strain of Salmonella.
4. If biochemical identification and serogroup procedures are inconclusive, restreak original colony onto non-selective plating media to check for purity. Repeat biochemical and serology tests.

Fig. 1

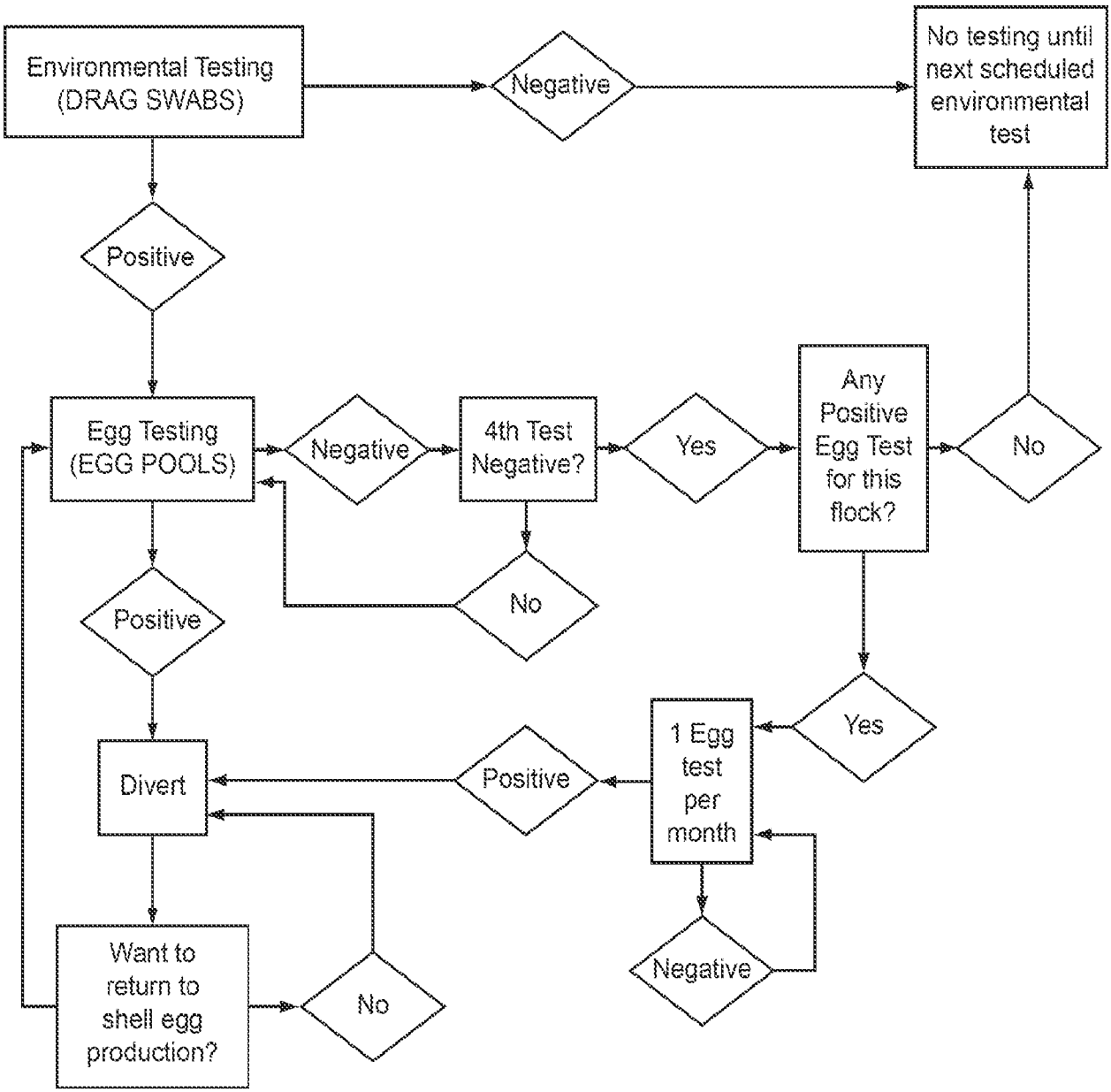

Environmental Testing (DRAG SWABS)
Negative
No testing until next scheduled environmental test
Positive
Egg Testing (EGG POOLS)
Negative
4th Test Negative?
Yes
Any Positive Egg Test for this flock?
No
No
Positive
Yes
1 Egg test per month
Positive
Divert
Negative
Want to return to shell egg production?
No

Fig. 2

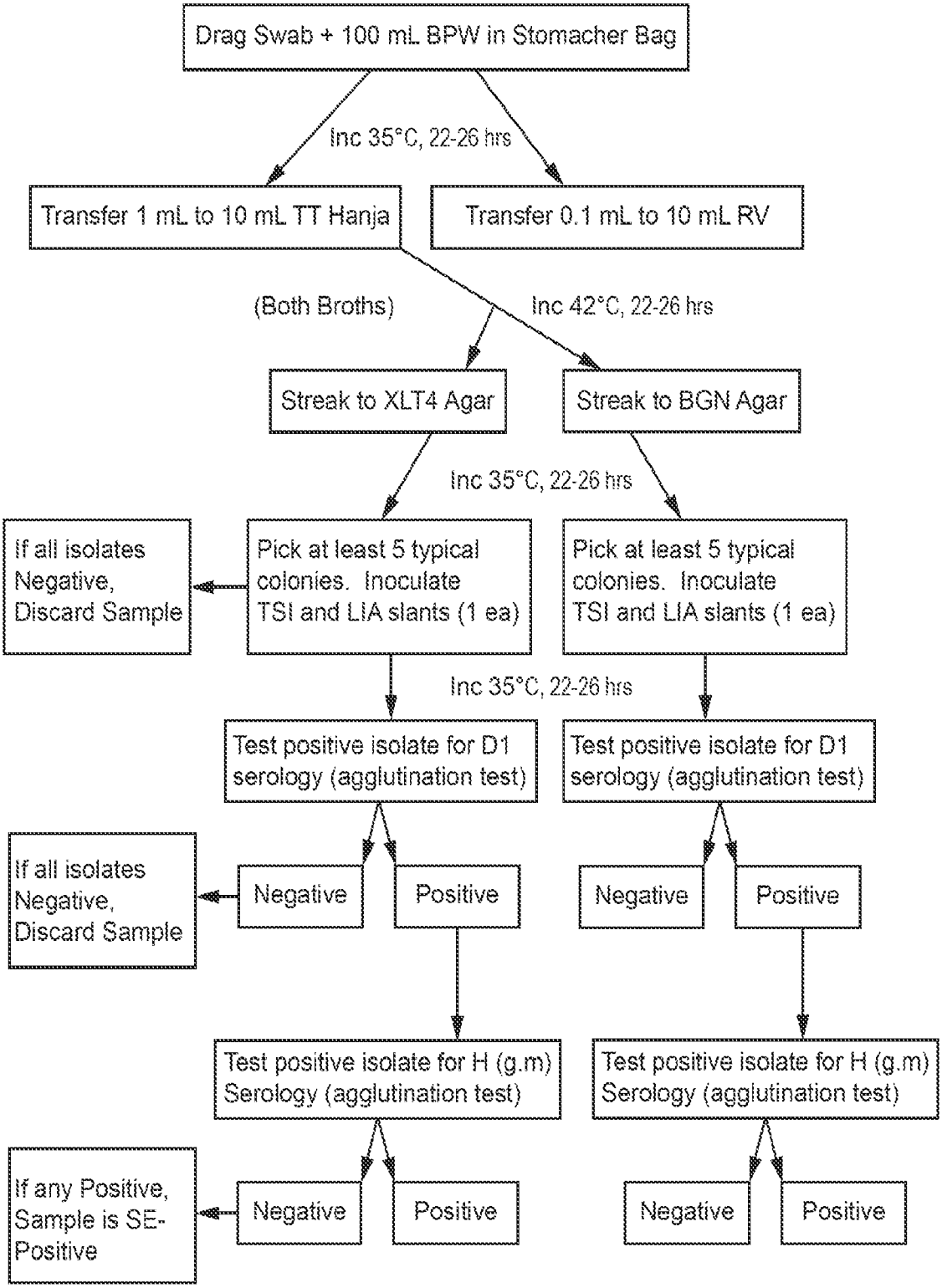

Drag Swab + 100 mL BPW in Stomacher Bag
Inc 35°C, 22-26 hrs
Transfer 1 mL to 10 mL TT Hanja
Transfer 0.1 mL to 10 mL RV
(Both Broths)
Inc 42°C, 22-26 hrs
Streak to XLT4 Agar
Streak to BGN Agar
Inc 35°C, 22-26 hrs
If all isolates Negative, Discard Sample
Pick at least 5 typical colonies. Inoculate TSI and LIA slants (1 ea)
Pick at least 5 typical colonies. Inoculate TSI and LIA slants (1 ea)
Inc 35°C, 22-26 hrs
Test positive isolate for D1 serology (agglutination test)
Test positive isolate for D1 serology (agglutination test)
If all isolates Negative, Discard Sample
Negative
Positive
Negative
Positive
Test positive isolate for H (g.m) Serology (agglutination test)
Test positive isolate for H (g.m) Serology (agglutination test)
If any Positive, Sample is SE-Positive
Negative
Positive
Negative
Positive

Fig. 3

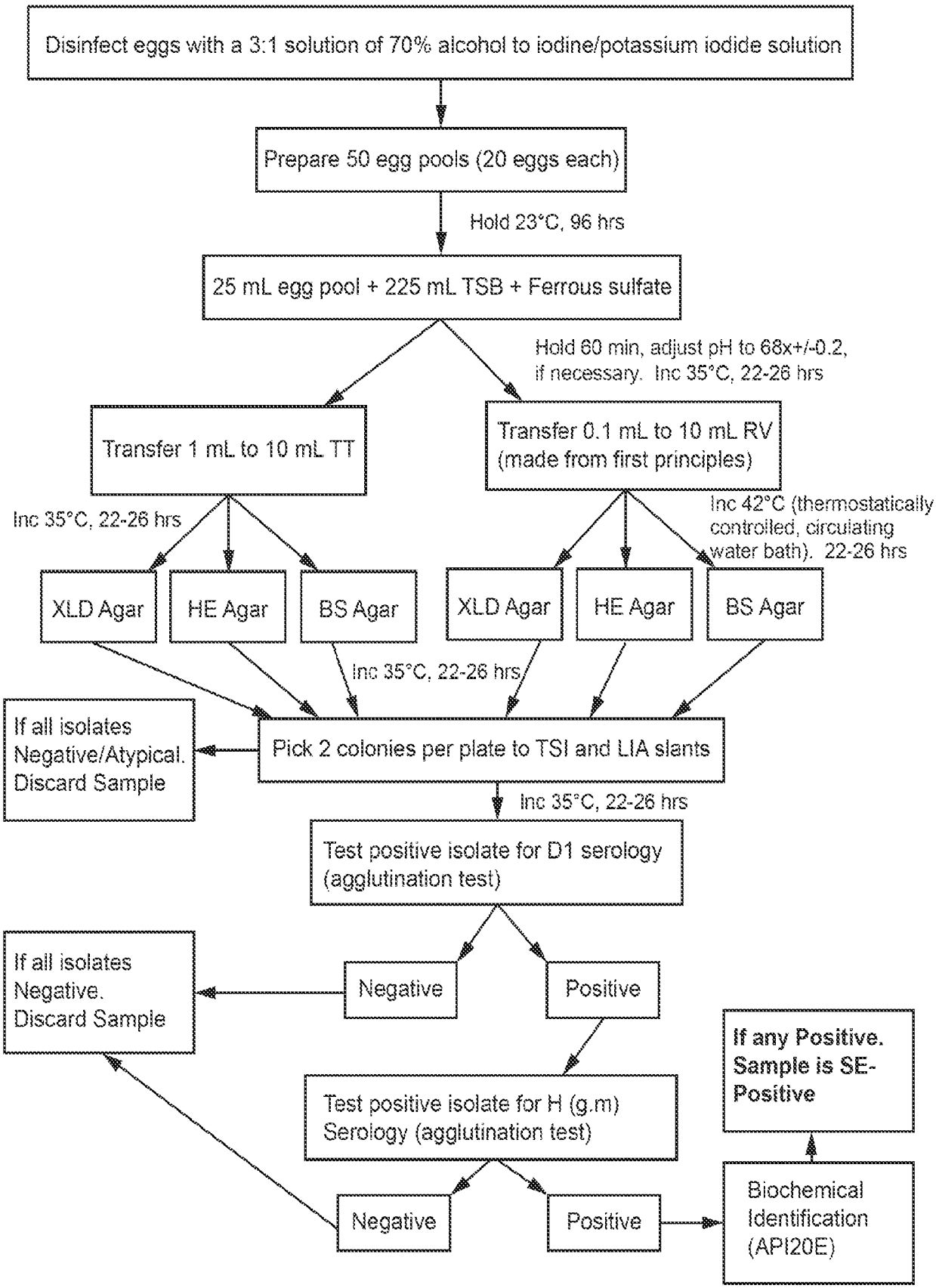

Disinfect eggs with a 3:1 solution of 70% alcohol to iodine/potassium iodide solution
Prepare 50 egg pools (20 eggs each)
Hold 23°C, 96 hrs
25 mL egg pool + 225 mL TSB + Ferrous sulfate
Hold 60 min, adjust pH to 68x+/-0.2, if necessary. Inc 35°C, 22-26 hrs
Transfer 1 mL to 10 mL TT
Transfer 0.1 mL to 10 mL RV (made from first principles)
Inc 35°C, 22-26 hrs
Inc 42°C (thermostatically controlled, circulating water bath). 22-26 hrs
XLD Agar
HE Agar
BS Agar
XLD Agar
HE Agar
BS Agar
Inc 35°C, 22-26 hrs
If all isolates Negative/Atypical. Discard Sample
Pick 2 colonies per plate to TSI and LIA slants
Inc 35°C, 22-26 hrs
Test positive isolate for D1 serology (agglutination test)
If all isolates Negative. Discard Sample
Negative
Positive
If any Positive. Sample is SE-Positive
Test positive isolate for H (g.m) Serology (agglutination test)
Negative
Positive
Biochemical Identification (API20E)

Fig. 4

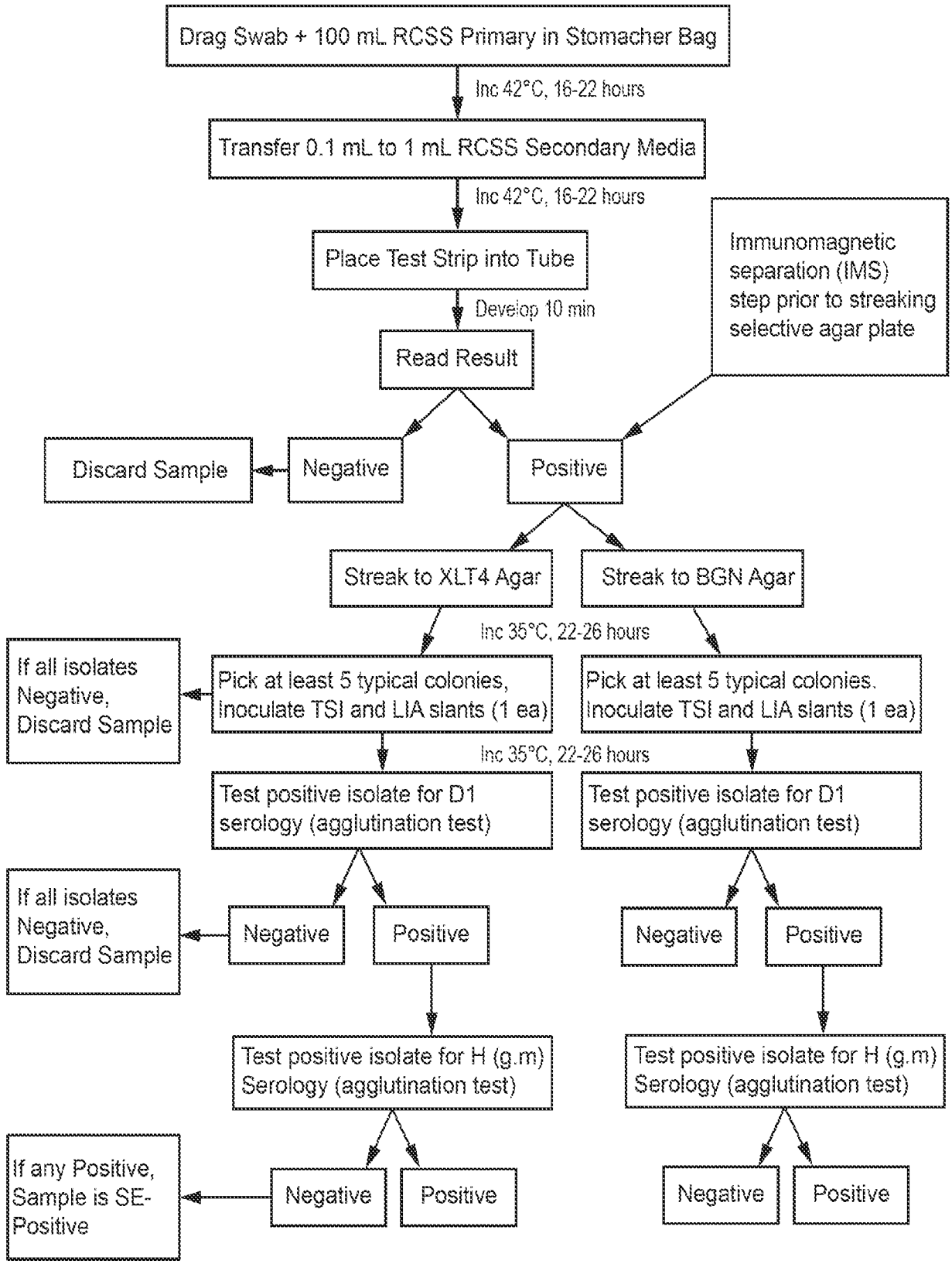

Drag Swab + 100 mL RCSS Primary in Stomacher Bag
Inc 42°C, 16-22 hours
Transfer 0.1 mL to 1 mL RCSS Secondary Media
Inc 42°C, 16-22 hours
Place Test Strip into Tube
Develop 10 min
Read Result
Immunomagnetic separation (IMS) step prior to streaking selective agar plate
Discard Sample
Negative
Positive
Streak to XLT4 Agar
Streak to BGN Agar
Inc 35°C, 22-26 hours
If all isolates Negative, Discard Sample
Pick at least 5 typical colonies, Inoculate TSI and LIA slants (1 ea)
Pick at least 5 typical colonies. Inoculate TSI and LIA slants (1 ea)
Inc 35°C, 22-26 hours
Test positive isolate for D1 serology (agglutination test)
Test positive isolate for D1 serology (agglutination test)
If all isolates Negative, Discard Sample
Negative
Positive
Negative
Positive
Test positive isolate for H (g.m) Serology (agglutination test)
Test positive isolate for H (g.m) Serology (agglutination test)
If any Positive, Sample is SE-Positive
Negative
Positive
Negative
Positive

Fig. 5

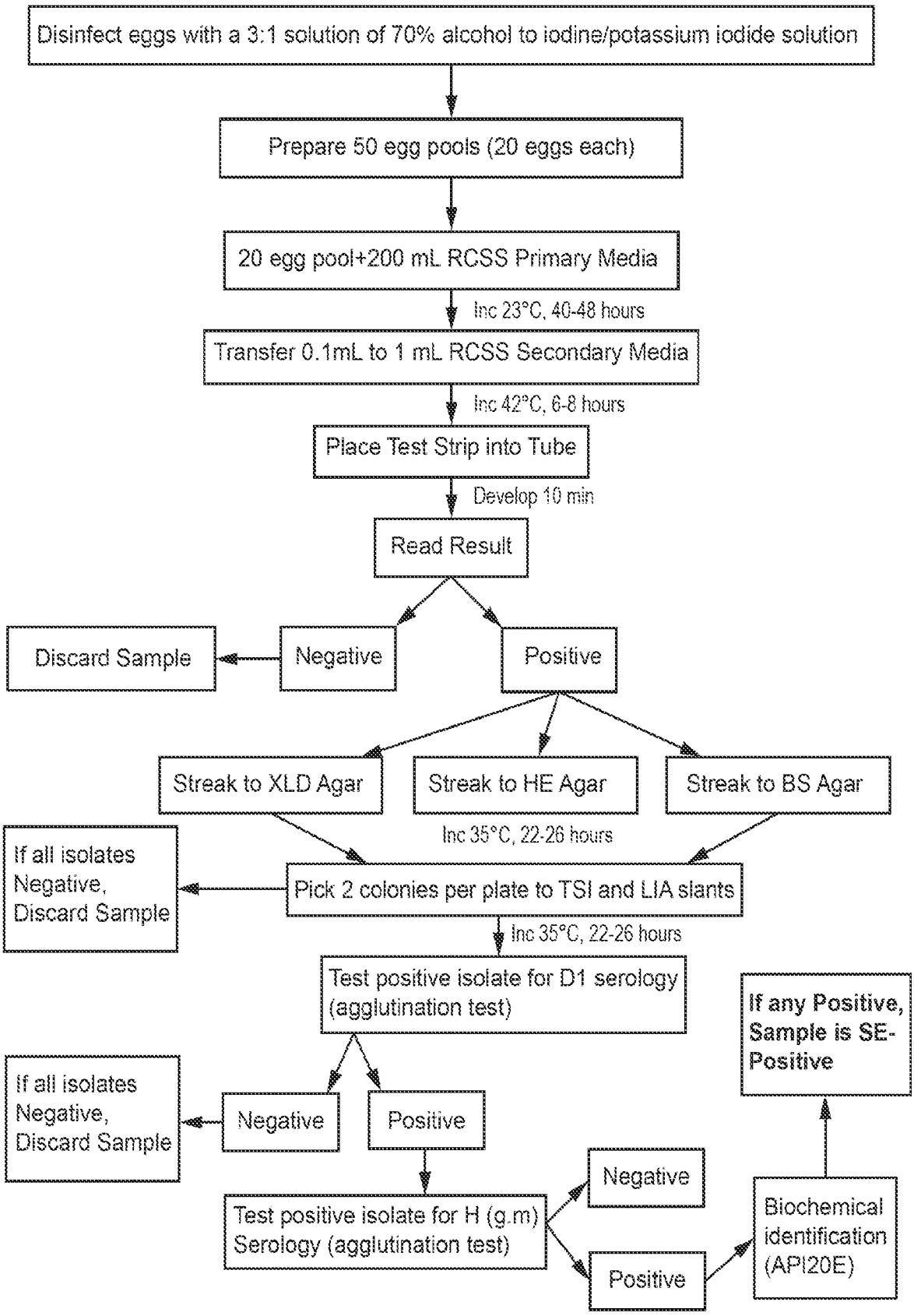

Disinfect eggs with a 3:1 solution of 70% alcohol to iodine/potassium iodide solution
Prepare 50 egg pools (20 eggs each)
20 egg pool+200 mL RCSS Primary Media
Inc 23°C, 40-48 hours
Transfer 0.1mL to 1 mL RCSS Secondary Media
Inc 42°C, 6-8 hours
Place Test Strip into Tube
Develop 10 min
Read Result
Discard Sample
Negative
Positive
Streak to XLD Agar
Streak to HE Agar
Streak to BS Agar
Inc 35°C, 22-26 hours
If all isolates Negative, Discard Sample
Pick 2 colonies per plate to TSI and LIA slants
Inc 35°C, 22-26 hours
Test positive isolate for D1 serology (agglutination test)
If any Positive, Sample is SE-Positive
If all isolates Negative, Discard Sample
Negative
Positive
Test positive isolate for H (g.m) Serology (agglutination test)
Negative
Positive
Biochemical identification (API20E)

Fig. 6

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2011/045828

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/569 C07K16/12 G01N33/543
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, INSPEC, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	"SDIX to Help Egg Producers Meet New FDA Regulatory Challenge", SDIX Press Release , 12 July 2010 (2010-07-12), XP002663198, Retrieved from the Internet: URL:http://www.sdix.com/About-SDIX/Press--Media-Relations/Press-Releases/2010/SDIX-to-Help-Egg-Producers-Meet-New-FDA-Regulatory-Challenge.aspx [retrieved on 2011-11-10] the whole document -----	1-37
X	CA 2 078 162 A1 (TSANG RAYMOND [CA]; NG MUM H [HK]) 15 March 1994 (1994-03-15) page 9 page 11 claims 1-10 -----	1-37
	-/--	

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 November 2011	28/11/2011

Name and mailing address of the ISA/	Authorized officer
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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2011/045828

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 2 141 176 A1 (PROFOS AG [DE] BIOMERIEUX SA [FR]) 6 January 2010 (2010-01-06) example 7 table 5 claim 14 -----	1-37

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2011/045828

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
CA 2078162 A1	15-03-1994	NONE	
EP 2141176 A1	06-01-2010	AT 520706 T	15-09-2011
		AU 2009265666 A1	07-01-2010
		CN 102099371 A	15-06-2011
		EP 2141176 A1	06-01-2010
		EP 2310403 A2	20-04-2011
		WO 2010000854 A2	07-01-2010