Independent Validation for the Polyskope 1.0 Multiplex Pathogen Detection Assay for the Detection of Shiga-Toxin Producing *Escherichia coli* non-O157 STEC, *Escherichia coli* O157, *Listeria monocytogenes*, and *Salmonella* species

*AOAC Performance Tested MethodSM XXXXX*

**Abstract**

The Polyskope 1.0 Multiplex Assay is a test based on gene amplification and detection by real-time PCR. This multiplex pathogen detection test is intended for simultaneous qualitative detection of *Escherichia coli* O157, non-O157 Shiga-Toxin Producing *E. coli* (STEC), *Listeria monocytogenes*, and *Salmonella* species. This assay was evaluated in an unpaired independent validation study according to AOAC validation guidelines. Polyskope 1.0 evaluated fresh raw ground beef (25 g), deli turkey (25 g), fresh baby spinach (25 g), and stainless steel environmental surface sponges (4” x 4” test area) after inoculation with a suspension of 3 microorganisms (STEC, *Listeria monocytogenes*, *Salmonella* species). All matrices were compared to appropriate reference methods from the FDA-BAM, USDA/FSIS-MLG or ISO reference standards. Polyskope 1.0 demonstrated no statistically significant differences between candidate and reference method results (dPOD<sub>C</sub>) or between presumptive and confirmed results (dPOD<sub>CP</sub>) for all three food matrices and one environmental surface analyzed. Results from all 4 inclusivity and exclusivity evaluations indicated the test method can accurately detect the target analytes and correctly excluded all non-target organisms. No differences were observed with the stability (both real-time and accelerated) or lot-to-lot evaluations. During the robustness study, three operational parameters of the method were evaluated: sample enrichment time and two separate lysis step times. POD values and 95% Confidence Intervals were calculated for each target analyte and treatment combination. Polyskope 1.0 demonstrated robustness by remaining unaffected by small variations in method parameters, which had no statistically significant effect on the results for all eight variations.

**Study Report Authors**

Benjamin Bastin, Leo Horine, Patrick Bird, M. Joseph Benzinger, Jr., James Agin, and David Goins

Q Laboratories, Inc., Cincinnati, OH, USA 45214
Scope of Methods

(a) **Target Organisms**
   1) STEC (E. coli O157:H7 & Non-O157 STEC Big 6 (E. coli O26, O45, O103, O111, O121, O145)
   2) *Listeria monocytogenes*
   3) *Salmonella* species

(b) **Matrices**
   1) Fresh Raw Ground Beef (73% lean) (25 g)
   2) Deli Turkey (25 g)
   3) Fresh Baby Spinach (25 g)
   4) Stainless Steel Environmental Surface (4” x 4”)

(c) **Summary of Validated Performance Claims**

The Polyskope 1.0 Multiplex Pathogen Detection Assay is considered equivalent to the US Department of Agriculture (USDA)/Food Safety and
Definitions

(a) Probability of Detection

Probability of Detection (POD) is the proportion of positive analytical outcomes for a qualitative method for a given matrix at a given analyte level or concentration. POD is concentration dependent. There are several POD measures that can be calculated, e.g., POD\(_R\) (reference method POD), POD\(_C\) (confirmed candidate method POD), POD\(_{CP}\) (candidate method presumptive result POD), POD\(_{CC}\) (candidate method confirmation result POD), and dPOD, the difference between any two POD values.

(b) PCR

Polymerase Chain Reaction

(c) Multiplex

Use of PCR to amplify several different DNA sequences simultaneously (as performing many separate PCR reactions all together in one reaction).

(d) STEC

Shiga-Toxin Producing *Escherichia coli*

(e) stx 1

Shiga-like toxin 1, a toxin produced by pathogenic *E. coli* that is closely related to Shiga toxin which is produced by *Shigella dysenteriae*.

(f) stx 2
Shiga-like toxin 2, a toxin produced by pathogenic *E. coli* that is closely related to Shiga toxin which is produced by STEC

\((g)\) *eaе*

Gene associated with *E. coli* attaching and effacing

\((h)\) *Intimin*

A virulence factor of Enteropathogenic *E. coli* (EPEC) and Enterohemorrhagic *E. coli* (EHEC) *E. coli* strains. It is an attaching and effacing protein, which with other virulence factors is responsible for Enteropathogenic and Enterohemorrhagic diarrhea.

**General Information**

The Centers of Disease Control and Prevention (CDC) estimates that roughly 1 in 6 Americans (48 million people) get sick, 128,000 are hospitalized, and 3,000 die from foodborne diseases each year. Among the most prevalent foodborne pathogens causing issues in food safety in the United States are *Listeria monocytogenes*, *Salmonella* species, non-O157 STECs, and *E. coli* O157. These estimates show that there is still a lot of work that remains to be done, specifically in focusing efforts on the top known pathogens and identifying the additional causes of foodborne illness and death.

Listeriosis is a serious infection that is usually caused by eating food contaminated with the bacterium *Listeria monocytogenes*. Outbreaks are primarily linked to deli meats, hot dogs, dairy products and produce. It has also been traced to cheeses, celery, sprouts, cantaloupe, and ice cream. The infection is most likely to sicken pregnant women and their newborns, adults aged 65 or older, and people with weakened immune systems

Salmonellosis is identified in the stool or blood from an infected person. Most of the outbreaks are from foods that are of animal origin. Therefore, people should not eat raw or undercooked eggs, poultry, or meat. Cross contamination of foods should be avoided. Uncooked meats should never come into contact with produce. When handling animals, hands should be washed thoroughly to avoid the transfer of *Salmonella*.

Symptoms of Shiga-Toxin Producing *E. coli* (STEC) vary from person to person, but often include severe stomach cramps, diarrhea, and vomiting. Some people may have a mild fever. *E. coli* is found in the environment, foods (mostly meat, pork, raw milk, unpasteurized dairy products, and unpasteurized juices), and intestines of people and animals. Most *E. coli* are harmless and are actually an important part of the human intestinal tract. (CDC) [8].

**Principle of the Method**
The Polyskope 1.0 Multiplex Pathogen Detection Assay is a test based on gene amplification and detection by real-time PCR. Ready-to-use PCR reagents contain oligonucleotides (primers and probes) specific to *E. coli* O157, non-O157 STEC, *Listeria monocytogenes*, and *Salmonella* species as well as DNA polymerase and nucleotides. PCR is a well-established technique used to rapidly generate profuse copies of target DNA. During the PCR reaction, cycles of heating and cooling promote DNA denaturation, followed by primers binding to specific target regions. The DNA polymerase then recognizes these primers and utilizes deoxynucleotide triphosphates (dNTPs) to extend the DNA, creating copies of the target DNA, called amplicons. Next, specific probes are used to detect the DNA during the amplification, by hybridizing to the amplicons. These probes are bound to a fluorophore which fluoresces only when hybridized to the correct target sequence. In the absence of target DNA, no fluorescence will be detected. As the amplicons increase with each round of amplification, fluorescence intensity also increases. At the annealing step of each PCR cycle, the detector measures this fluorescence and the associated software plots the fluorescence intensity versus number of cycles. This method allows a simple determination of the presence, or absence, of up to five targets in a single reaction. An unrelated DNA "internal control" is included in the reaction mix. This control is amplified with a specific probe at the same time as the other probe target DNA sequences and detected by a specific fluorophore. It allows for the validation of any negative result. Polyskope 1.0 Multiplex Pathogen Detection assay is specifically designed to detect pathogenic bacteria capable of human infection. The oligonucleotides are targeted to specific pathogen-related genes that are present in these bacteria and distinguish them from closely related non-pathogenic bacteria. The PolySkope 1.0 Multiplex Pathogen Detection method allows the simultaneous detection of *E. coli* O157 STEC, *E. coli* non-O157 STEC, *Salmonella* spp. and *Listeria monocytogenes* in select environmental samples and select food products enriched with Polyskope Multiplex Enrichment Media (PMEM). [9]

**Materials and Methods**

*Test Kit Information*

(a) Polyskope 1.0 Multiplex Pathogen Detection Assay

*Test Kit Components*

(a) Amplification Mix (Reagent A): 1 Tube (0.66 mL)
(b) Probes (Reagent B): 2 Tubes (2 x 0.75 mL)
(c) Lysis Component 1 (Reagent C): 1 Bottle (7.5 mL)
(d) Lysis Component 2 (Reagent D): 1 Bottle (7.5 mL)
(e) Lysis Component 3, Beads (Reagent E): 1 Bottle (8.8 g)
(f) PCR Positive Control (Reagent F): 1 Tube (0.25 mL)
(g) PCR Negative Control (Reagent G): 1 Tube (0.25 mL)

Ordering Information

PolySkope Labs
755 Research Parkway, Suite 465
Oklahoma City, OK. 73104
405-633-0540
info@polyskopelabs.com

Additional Supplies and Reagents

(a) Polyskope Multiplex Enrichment Media (PMEM): Dehydrated Powder
(b) Applied Biosystems® QuantStudio™ 5 Real-Time PCR System
(c) Computer with QuantStudio™ 5 Design and Analysis software, version 1.4.2.
(d) Environmental Sampling Sponges – Nasco CAT# B01422WA or equivalent
(e) Sterile Laboratory Filtered Stomacher Bags, or equivalent
(f) Neutralizing Buffer
(g) Wide mouth micropipette tips – capable of sampling and delivering 150 µL
(h) Aerosol resistant micropipette tips – capable of sampling and delivering 1 µL - 1000 µL
(i) Agitator-thermomixer for deepwell plates, capable of 65 ± 5°C and 95 ± 5°C and shaking at 1,400 RPM – Eppendorf Thermomixer or equivalent
(j) Deepwell Plates
(k) PCR Plate
(l) Pre-pierced Film
(m) PCR plate Sealing Film

Apparatus

(a) Incubators – capable of maintaining 37 ± 1°C
(b) Micropipettors (1 - 1000 µL)
(c) Freezer – capable of maintaining -20 ± 2°C
(d) Vortex Mixer
(e) Laboratory paddle blender – Seward 400 or equivalent: for sample homogenization
(f) Balance, 2,000 g capacity, sensitivity of 0.1 g
(g) Serological Pipette Bulbs (Automatic Pipette) – For sampling and delivering 1 mL - 10 mL.
(h) Serological Pipettes – Aerosol resistant

Reference Materials

Organisms used in the method comparison study were obtained from the following sources:

(a) American Type Culture Collection (ATCC; Manassas, VA)
(b) National Collection of Type Cultures (NCTC; Salisbury, UK)
(c) Michigan State University STEC Center (MSU; East Lansing, MI)
(d) University of Pennsylvania Culture Collection (UPENN; Philadelphia, PA)
(e) Pennsylvania State University Culture Collection (PSU; State College, PA)
(f) Cornell University Culture Collection (FSL; Ithaca, New York)
(g) University of Vermont Culture Collection (CWD; Burlington, VT)
(h) US Food and Drug Administration Culture Collection (FDA; Silver Spring, MD)
(i) Q Laboratories Inc. Culture Collection (QL; Cincinnati, OH)

Safety Precautions

Polyskope 1.0 Multiplex Pathogen Detection Assay

The Polyskope 1.0 Multiplex Pathogen Detection Assay should be disposed of following procedures for infections or potentially infectious products. User should wear appropriate personal protective equipment, including (but not limited to) protective disposable gloves, laboratory coats, and eye protection when handling samples and kit reagents. Wash hands thoroughly after handling specimens and reagents. It is the responsibility of each laboratory to handle waste and effluents produced according to their type and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with local, state, and federal regulations. Strict compliance with biosafety level (BSL)-2 practices should be followed.

Enrichment

*E. coli* O157:H7, non-O157 STEC, *Listeria monocytogenes*, and *Salmonella* are BSL-2 organisms. *Listeria monocytogenes* is of particular concern for pregnant women, newborns, the elderly, and the immunocompromised. Biological samples such as enrichments have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations on disposal of biological wastes. Wear appropriate protective equipment, which includes but is not limited to: protective
eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities utilizing the appropriate safety equipment (for example, physical containment devices). Individuals should be trained in accordance with applicable regulatory and company/institution requirements before working with potentially infectious materials. All enrichment broths should be sterilized following any culture based confirmatory steps.

**General Preparation**

(a) Use aseptic techniques.

(b) Use filter laboratory bags during enrichment to minimize particulates.

(c) Separate work areas for the following: media preparation, sample preparation, and pathogen detection.

(d) Clean the work stations and lab equipment with a disinfectant of choice before and after use. (Sodium hypochlorite solution, phenol solution, Quaternary ammonium solution, etc.)

(e) Do not reuse kit disposables.

(f) Change pipette tips in between samples.

(g) Wear personal protective equipment (PPE).

**DNA Lysis**

(a) Change pipette tips in between samples.

(b) Prewarm Thermomixer heat block before initiating extraction.

**DNA Amplification**

(a) Use aseptic technique.

(b) Change pipette tips between samples.

(c) Use gloves and protective laboratory wear.

(d) Do not touch any PCR equipment and supplies without wearing gloves.

(e) Avoid the transfer of Lysis Beads to the PCR plate.

(f) Avoid bubble formation.
Sample Preparation

(a) Performing Pre-Enrichment

1) Fresh Raw Ground Beef, Deli Turkey, and Fresh Baby Spinach (25 g test portions)

A 25 g test portion is added to 225 mL of pre-warmed (37 ± 1°C) Polyskope Multiple Enrichment Media (PMEM), at 130 RPM for 30 seconds, and incubate at 37 ± 1°C for 23 ± 1 hour.

2) Stainless Steel Environmental Sponges (4” x 4”)

A sponge (pre-wetted with neutralizing buffer) is added to 100 mL of pre-warmed (37 ± 1°C) PMEM, homogenized for 30 seconds, and incubated at 37 ± 1°C for 23 ± 1 hour.

Polyskope 1.0 Multiplex Pathogen Detection Assay

(a) Lysis Procedure

1) Aliquot 150 µL, using the wide mouth pipette tips, of homogenized lysis reagent (reagents C+D+E) into the wells of a deepwell plate. Note: The lysis reagent has a shelf life of 1 month when stored at 2-8°C. Before every use, gently agitate the lysis reagent by hand to resuspend the resin. Then repeat pipette rapidly, to keep the lysis buffer in suspension while pipetting from the lysis bottle into the deepwell plate.

2) After removing from the incubator, resuspend the food matrix by repeatedly squeezing/agitating the filter bag by hand (or in the stomacher) for at least 10 seconds. Add 50 µL of decanted, enriched sample. Mix by repeat pipetting and seal the deepwell plate with pre-pierced sealing film.

3) Incubate the deepwell plate on the heat block at 65 ± 5°C for 15±2 minutes, shaking at 1,400 RPM. Note: Secure the deepwell plate with laboratory tape if necessary.

4) Remove the block from the Thermomixer and adjust temperature to 95 ± 5°C. After Thermomixer has achieved proper temperature, reinsert
the deepwell plate and shake for 1,400 RPM for an additional 10±2 minutes.
5) After 10 minutes, remove the deepwell plate and allow samples to cool to ambient temperature (20-25ºC).

(b) Prepare PCR Reaction Mix

1) Prepare PCR mixture containing the amplification solution (reagent A) and the fluorescent probes (reagent B) depending on the number of samples and controls to analyze (reference kit insert for table to prepare PCR master mix). **Note:** A positive and negative control must be analyzed for every run.
2) After preparation, the PCR mix (reagent A+B) must be used immediately. Is stable for only one hour at 2-8 ºC.
3) Pipette 19 µL of the PCR mix into each well of the PCR plate according to the plate setup.
4) Add 1 µL of sample, negative control (reagent F), and positive control (reagent E). Hermetically seal the wells of the PCR plate by lightly applying pressure after the plate film is in place. **Note:** Avoid bubbles at the bottom of the wells by pipetting carefully. If necessary, to eliminate bubbles, centrifuge the sealed PCR plate.
5) Place the plate in the QuantStudio 5 Real-Time PCR System. Be sure to place the PCR plate correctly: A1 well at the upper left corner.
6) Close the Real-Time PCR System and initiate the run.

(c) Data Analysis and Interpreting Results

1) Data can be analyzed directly after the end of the PCR run or at a later time by opening the stored data file.
2) Once the data analysis parameters have been set, results are interpreted by analyzing the Cq (the cycle at which the amplification curve crosses the threshold, also known as Ct) values of each sample. In addition, each amplification curve should be analyzed in conjunction with the Cq values. Table A, B, and C shows the interpretation of the results.

<table>
<thead>
<tr>
<th>Fluorophore</th>
<th>Color</th>
<th>Target</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAM</td>
<td>Red</td>
<td>stx1 and stx2</td>
</tr>
<tr>
<td>ABY</td>
<td>Blue</td>
<td>eae</td>
</tr>
<tr>
<td>VIC</td>
<td>Green</td>
<td>Listeria monocytogenes</td>
</tr>
<tr>
<td>ALEXA 647</td>
<td>Purple</td>
<td>Salmonella enterica</td>
</tr>
<tr>
<td>JUN</td>
<td>Yellow</td>
<td>Internal Control</td>
</tr>
</tbody>
</table>
Table B: Control Interpretation

<table>
<thead>
<tr>
<th>Target</th>
<th>Target Probe Detection</th>
<th>Internal Control Detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Control</td>
<td>14 &lt; Cq &lt; 40</td>
<td>14 &lt; Cq &lt; 40</td>
</tr>
<tr>
<td>Negative Control</td>
<td>Cq = N/A</td>
<td>14 &lt; Cq &lt; 40</td>
</tr>
</tbody>
</table>

Table C: Interpretation of Sample Test Outcome

<table>
<thead>
<tr>
<th>Target Probe Detection (Target Fluorophore)</th>
<th>Internal Control Detection</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cq ≥ 10</td>
<td>Cq ≥ 14</td>
<td>Positive</td>
</tr>
<tr>
<td>Cq = N/A</td>
<td>Cq ≥ 14</td>
<td>Negative</td>
</tr>
<tr>
<td>Cq = N/A</td>
<td>Cq = N/A</td>
<td>Inhibition</td>
</tr>
</tbody>
</table>

Confirmation

All samples are confirmed according to the appropriate reference methods specified for the matrix and analyte:

(a) USDA/FSIS-MLG 5.09: Detection, Isolation and Identification of Escherichia coli O157:H7 from Meat Products and Carcass and Environmental Sponges

(b) USDA/FSIS-MLG 5B.05: Detection and Isolation of non-O157 Shiga Toxin-Producing Escherichia coli (STEC) from Meat Products and Carcass and Environmental Sponges

(c) USDA/FSIS-MLG 4.09: Isolation and Identification of Salmonella from Meat, Poultry, Pasteurized Egg, and Siluriformes (Fish) Products and Carcass and Environmental Sponges.

(d) USDA/FSIS-MLG 8.10: Isolation and Identification of Listeria monocytogenes from Read Meat, Poultry, RTE Siluriformes (Fish) and Egg Products, and Environmental Samples

(e) FDA/BAM Chapter 5: Salmonella

(f) FDA/BAM Chapter 10: Detection of Listeria monocytogenes in Foods and Environmental Samples, and Enumeration of Listeria monocytogenes in Foods

(g) ISO/TS 13136:2012: Microbiology of food and animal feed – Real-time polymerase chain reaction (PCR)-based method for the detection of food-borne pathogens – Horizontal method for the detection of Shiga toxin-
producing Escherichia coli (STEC) and the determination of O157, O111, O26, O103 and O145 serogroups.

Independent Validation Study

The study was conducted according to the procedures outlined in the AOAC Research Institute: Performance Tested Methods Program – Comparative Evaluation of the PolySkope 1 Test Kit for the Detection of E. coli O157, E. coli non-O157 STEC, Listeria monocytogenes and Salmonella species (Version 14: January 2018) [10]. The Polyskope 1.0 Multiplex Pathogen Detection Assay was compared to the USDA/FSIS-MLG 5.09, 5B.05, 4.09, 8.10 and to the FDA/BAM Chapters 5 and 10, and to the ISO/TS 13136: 2012 reference methods. Each target pathogen was evaluated after 23 ± 1 hour of enrichment. The study outline consisted of an unpaired method comparison study, inclusivity and exclusivity evaluation for all target organisms, robustness, and product stability and lot to lot variation. Regardless of the presumptive results for the method comparison, all samples were culturally confirmed following the appropriate reference method.

Inclusivity and Exclusivity

For the inclusivity and exclusivity evaluation of the PolySkope 1.0 Pathogen Detection Assay: 50 pathogenic non-O157 STEC, 50 pathogenic E. coli O157, 50 Listeria monocytogenes, and 100 Salmonella species were cultured in PMEM for 23 ± 1 hour at 37 ± 1ºC. The cultures tubes were diluted to 100x the Limit of Detection (LOD50).

For the exclusivity portion of the non-O157 STEC and E. coli O157 evaluation, 30 species/strains (including non-pathogenic E. coli) closely related to STEC’s were grown in Brain Heart Infusion (BHI) broth for 23 ± 1 hour at 37 ± 1ºC.

For the exclusivity portion of the Listeria monocytogenes evaluation, 30 non-Listeria monocytogenes (including Listeria innocua, Listeria ivanovii, Listeria seeligeri, and Listeria welshimeri) were grown in BHI broth for 23 ± 1 hour at 37 ± 1ºC.

For the exclusivity portion of the Salmonella evaluation, 50 closely related Gram-negative organisms were grown in BHI for 23 ± 1 hour at 37 ± 1ºC.

All exclusivity organisms were analyzed undiluted. The inclusivity and exclusivity cultures were randomized, blind-coded and then analyzed by the PolySkope 1.0 Pathogen Detection Assay.

Method Comparison Study

Raw ground beef (73% lean), deli turkey, and fresh baby spinach was purchased from a local distributor, prescreened for natural contamination of the target analyte and analyzed for total aerobic count by the FDA/BAM Chapter 3 method [11]. Following the screening, no natural contamination was present. Therefore, each food matrix and
environmental surface was inoculated with a cocktail of pathogenic \textit{E. coli}, \textit{Listeria monocytogenes}, and \textit{Salmonella} as indicated in Table D. The method comparison study consisted of evaluating a total of 30 un-paired sample replicates. Within each food matrix sample set, there were 5 uninoculated samples (0 CFU/test portion), 20 low-level inoculated samples (0.2-2 CFU/test portion), and 5 high-level inoculated samples (2-10 CFU/test portion). Within the stainless steel environmental surface sample set, there were 5 uninoculated samples (0 CFU/test area), 20 low-level inoculated samples (50 CFU/test area), and 5 high-level inoculated samples (~500 CFU/test area). All samples analyzed by the PolySkope 1.0 Multiplex Pathogen Detection Assay, regardless of presumptive results, were culturally confirmed by the appropriate reference method.

### Table D: Matrices and Inoculating Organisms

<table>
<thead>
<tr>
<th>Matrix/Test Portion Size</th>
<th>Inoculating Organism</th>
<th>Target Inoculum Level</th>
<th># of Replicates</th>
<th>PolySkope Testing Time Point</th>
<th>Reference Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh Raw Ground Beef (73% lean) (25 g)</td>
<td>\textit{E. coli} O157, ATCC\textsuperscript{1} 39895, \textit{Listeria monocytogenes} ATCC 7644, \textit{Salmonella} Typhimurium ATCC 14028</td>
<td>0 CFU/ Test Portion</td>
<td>5</td>
<td>23 ± 1 hrs.</td>
<td>USDA/FSIS-MLG 5.09, USDA/FSIS-MLG 8.10, USDA/FSIS-MLG 4.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.2-2 CFU/ Test Portion</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2-10 CFU/ Test Portion</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deli Turkey (25 g)</td>
<td>\textit{E. coli} O26, MSU TW00971, \textit{Listeria monocytogenes} ATCC 19115, \textit{Salmonella} Dublin ATCC 15480</td>
<td>0 CFU/ Test Portion</td>
<td>5</td>
<td>23 ± 1 hrs.</td>
<td>USDA/FSIS-MLG 5B.05, USDA/FSIS-MLG 8.10, USDA/FSIS-MLG 4.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.2-2 CFU/ Test Portion</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2-10 CFU/ Test Portion</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh Baby Spinach (25 g)</td>
<td>\textit{E. coli} O145, MSU TW09153, \textit{Listeria monocytogenes} ATCC BAA-2658, \textit{Salmonella} Enteritidis ATCC 13076</td>
<td>0 CFU/ Test Portion</td>
<td>5</td>
<td>23 ± 1 hrs.</td>
<td>ISO/TS 13136: 2012, FDA/BAM Chapter 10, FDA/BAM Chapter 5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.2-2 CFU/ Test Portion</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2-10 CFU/ Test Portion</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stainless Steel (4” x 4”)</td>
<td>\textit{E. coli} O103, MSU TW08101, \textit{Listeria monocytogenes} ATCC 51780, \textit{Salmonella} Kentucky ATCC 9263</td>
<td>0 CFU/ Test Area</td>
<td>5</td>
<td>23 ± 1 hrs.</td>
<td>USDA FSIS-MLG 5B.05, FDA/BAM Chapter 10, FDA/BAM Chapter 5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>~50 CFU/ Test Area</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>~500 CFU/ Test Area</td>
<td>5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{1}ATCC - American Type Culture Collection  
\textsuperscript{2}MSU – Michigan State University Culture Collection

Each matrix was artificially contaminated with a cocktail of the target strains. Each inoculum was prepared by transferring a single colony from trypticase soy agar with 5% sheep blood (SBA) into BHI broth and incubating the culture at 35 ± 2°C for 24 ± 2 hours. Using BHI broth as the diluent, the culture was diluted to a low-level expected to yield fractional positive results (5-15 positive results) and a high-level expected to yield all positive results. Following inoculation, a bulk lot of the matrix was homogenized by hand and held for 48-72 hours at refrigerated temperature (2-8°C) prior to analysis to allow time for the organism to equilibrate within the sample.

Prior to inoculation of deli turkey, the broth culture inoculum was heat stressed for 10 ± 1 minute at 50 ± 1°C in a water-bath. The degree of injury of the culture was estimated by plating an aliquot of diluted culture onto Modified Rainbow Agar (RBA),
Modified Oxford Agar (MOX), Xylose Lysine Tergitol 4 (XLT4) and Tryptic Soy agar (TSA). The agars were incubated at 35 ± 1°C for 24 ± 2 hours and the colonies enumerated. The degree of injury was estimated as

\[ (1 - \frac{n_{select}}{n_{nonselect}}) \times 100 \]

Where \( n_{select} \) = number of colonies on selective agar and \( n_{nonselect} \) = number of colonies on non-selective agar.

For stainless steel, 4” x 4” areas were inoculated with 0.25 mL of the diluted cocktail and sampled using sampling sponges with neutralizing buffer (Nasco Part#: B01422WA). For the uninoculated test portions, sterile BHI broth was applied to the test area. The surface was dried for 16-24 hours at ambient temperature (24 ± 2 °C) prior to sampling.

The level of target analyte in the low-level inoculum for all 25 g test portions was determined by Most Probable Number (MPN) on the day of analysis by evaluating 5 x 50 g, 20 x 25 g (reference method test portions), and 5 x 10 g inoculated test samples. The level of the target analyte in the high-level inoculum for all 25 g test portions was determined by MPN on the day of analysis by evaluating 5 x 50 g, 5 x 25 g (reference method test portions), and 5 x 10 g inoculated test samples.

The test portion size for the MPN of each matrix is presented below in Table E. Each test portion was enriched with the reference method enrichment and analyzed by the reference method procedure. The number of positives from the 3 test levels was used to calculate the MPN using the LCF MPN calculator (version 1.6) provided by AOAC RI. [12] (http://www.lcfltd.com/customer/LCFMPNCalculator.exe)

<table>
<thead>
<tr>
<th>Reference Method Test Portion</th>
<th>Inoculation Level</th>
<th>MPN Test Portions</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 g</td>
<td>Low</td>
<td>5 x 50 g</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 x 25 g*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 x 10 g</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>5 x 50 g</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 x 25 g*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 x 10 g</td>
</tr>
</tbody>
</table>

*Test portions from reference method

**USDA/FSIS-MLG 5.09: Detection, Isolation and Identification of Escherichia coli O157:H7 from Meat Products and Carcass and Environmental Sponges**

The fresh raw ground beef, test portions consisting of 25 ± 2.5 g were enriched with 75 ± 1.5 mL of pre-warmed (42 ± 1°C) modified tryptic soy broth (mTSB), homogenized by homogenizing for 2 minutes, and incubated for 15-24 hours at 42 ± 1°C. After incubation, the primary enrichment from each sample was screened using a USDA/FSIS-MLG 5.09 validated lateral flow device test system (PTM# 070801). Regardless of the screening result, all samples were subjected to isolation by
immunomagnetic separation (IMS) by transferring 1.0 mL aliquots of the primary enrichment to a microcentrifuge tube containing a 50 µL suspension of *E. coli* O157 immunomagnetic (paramagnetic) beads. The solution was placed onto a Labquake agitator and rotated for 10 to 15 minutes at 18-30°C. After rotation, the bead and sample solution were transferred to a MACS® large cell separation (ferromagnetic) column and was washed four times with E buffer (pre-warmed to 18-35 °C) before the final elute was collected with 1 mL of E buffer into a sterile tube. Following the IMS procedure, a 1:10 dilution and a 1:100 dilution of each IMS suspension in E Buffer, were spread plated onto modified Rainbow® agar (mRBA).

A 450 µL aliquot of each remaining IMS suspension was transferred into a microcentrifuge tube and mixed with 25 µL of a 1 N hydrochloric acid (HCl) solution. The microcentrifuge tubes were vortexed briefly and placed onto a Labquake agitator and rotated for 1 hour at 18 - 30 °C. After rotating, 475 µL of E buffer was added to each sample tube. The acid treated IMS suspension and a 1:10 dilution of this suspension in E Buffer were plated onto mRBA. All mRBA plates were incubated for 20 - 24 hours at 35 ± 2°C. After incubation, plates were observed for typical colonies (purple-blue colonies). The mRBA plates containing typical colonies were tested for O157 latex agglutination and up to 5 isolated colonies were streaked to SBA. The SBA plates were incubated for 16 - 24 hours at 35 ± 2°C. After incubation, SBA plates were observed for purity. Isolated colonies from SBA were confirmed positive by conducting a H7 latex agglutination test and for the presence of Shiga-toxins using the USDA approved Real-Time PCR assay (PTM# 091301). Final biochemical confirmations were obtained by VITEK 2 GN Biochemical Identification following AOAC OMA 2011.17. [13].

**USDA/FSIS-MLG 5B.05: Detection and Isolation of non-O157 Shiga Toxin-Producing Escherichia coli (STEC) from Meat Products and Carcass and Environmental Sponges**

For the deli turkey, 25 ± 2.5 g were enriched with 75 ± 1.5 mL of pre-warmed mTSB, homogenized by homogenizing for 2 minutes, and incubated for 15-24 hours at 42 ± 1°C.

The environmental sponges, with the addition of Dey-Engley (D/E) neutralizing broth, were combined with 50 ± 5 mL of mTSB. Each sponge was homogenized by hand until well mixed.

Following incubation, 30 µL of the primary enrichment from each sample was screened using a USDA/FSIS-MLG 5B.05 approved commercially available Real-Time PCR assay (DuPont Nutrition and Health, BAX System: PTM# 091301)). Each sample was screened for the presence of STEC virulence factors Shiga-like toxin 1 and/or Shiga-like toxin 2 and intimin (stx1/stx2 and eae). If a sample produced positive results for stx1 or stx2 and eae an additional screen for the targeted 6 serogroups (O26, O45, O103, O111, O121, and O145) was conducted. Regardless of the screening result, all samples were subjected to isolation by IMS in a ferromagnetic column with paramagnetic beads by transferring a 1.0 mL aliquot of the primary enrichment to microcentrifuge tubes containing a 50 µL suspension of *E. coli* O26 or *E. coli* O103 immunomagnetic beads.
The solution was placed onto a Labquake agitator and rotated for 10-15 minutes at 18 - 30°C. After rotation, the bead and sample solution were transferred to a MACS large cell separation column and was washed four times with 1 mL of E buffer before the final elute was collected with 1 mL of E buffer into a sterile tube.

Following the IMS procedure, a 1:10 dilution and a 1:100 dilution of each IMS suspension in E Buffer were spread plated onto mRBA. A 450 µL aliquot of each remaining sample was transferred into a microcentrifuge tube and mixed with 25 µL of 1 N HCl solution. The microcentrifuge tubes were gently mixed and placed onto a Labquake agitator and rotated for 1 hour ± 10 mins at 18 - 30°C. After rotating, 475 µL of E buffer was added to each sample tube. The acid washed IMS suspension and a 1:10 dilution of the acid washed IMS suspension in E Buffer were plated onto mRBA. All mRBA plates were incubated for 20-24 hours at 35 ± 1°C. After incubation, plates were observed for typical colonies (purple-magenta colonies) and any mRBA plates containing typical colonies were tested by serogroup specific latex agglutination. Up to 5 isolated colonies were streaked to SBA and incubated for 16-24 hours at 35 ± 1°C. After incubation, SBA plates were observed for purity. Isolated colonies from SBA were confirmed positive for the presence of stx1 or stx2 and eae by Real-Time PCR. The serogroup was confirmed by latex agglutination and by Real-Time PCR. Final biochemical confirmations were obtained by VITEK 2 GN Biochemical Identification following AOAC OMA 2011.17.

ISO/TS 13136: 2012: Microbiology of food and animal feed – Real-time polymerase chain reaction (PCR)-based method for the detection of food-borne pathogens – Horizontal method for the detection of Shiga toxin-producing Escherichia coli (STEC) and the determination of O157, O111, O26, O103 and O145 serogroups

For fresh baby spinach, test portions consisting of 25 g of product were enriched with 225 mL of mTSB with the addition of novobiocin (16 mg/L). All test portions were homogenized for 2 minutes by stomaching and incubated at 37 ± 1°C for 18-24 hours.

Following incubation, 30 µL of the primary enrichment from each sample was screened using a Real-Time PCR Assay (DuPont Nutrition and Health, BAX System STEC Assays). Each sample was screened for the presence of STEC virulence factors Shiga-like toxin 1 and/or Shiga-like toxin 2 and intimin (stx1/stx2 and eae). If a sample produced positive results for stx1 or stx2 and eae, an additional screen for the targeted 4 serogroups (O26, O103, O111 and O145) and by the BAX System E. coli O157 Assay was conducted. Regardless of the screening result, all samples were subjected to isolation by immunomagnetic separation (IMS) in a ferromagnetic column with paramagnetic beads by transferring a 1.0 mL aliquot of the primary enrichment to microcentrifuge tubes containing a 50 µL suspension of E. coli O145 immunomagnetic beads. The solution was placed onto a Labquake agitator and rotated for 10-15 minutes at 18 - 30°C. After rotation, the bead and sample solution were transferred to a MACS large cell separation column and was washed four times with 1 mL of ISO IMS Wash Buffer before the final elute was collected with 1 mL of ISO IMS Wash Buffer into a sterile tube.
Following the IMS procedure, a 1:10 dilution and a 1:100 dilution of each IMS suspension in ISO IMS Wash Buffer were spread plated onto Tryptone Bile X-Glucuronide Agar (TBX) and mRBA. All plates were incubated for 20-24 hours at 37 ± 1°C. After incubation, plates were observed for typical colonies and up to 50 colonies from each sample were struck to Nutrient Agar (NA) and incubated for 18-24 hours at 37 ±1°C. Following isolation of the typical colonies, isolated colonies from NA were confirmed for the presence of stx1 or stx2 and eae by Real-Time PCR (BAX STEC Screening Kit). The serogroup was confirmed by Real-Time PCR (BAX STEC Panel 1, BAX STEC Panel 2 and BAX E. coli O157). Final biochemical confirmations were obtained by VITEK 2 GN Biochemical Identification following AOAC OMA 2011.17.

**USDA/FSIS-MLG Method 8.10 Isolation and Identification of Listeria from Red Meat, Poultry and Egg Products, and Environmental Samples**

For the USDA/FSIS MLG 8.10 reference method, 25 g test portions were enriched with 225 mL ± 5 mL of modified University of Vermont Medium broth (UVM). All test portions were mechanically stomached for two minutes. The test portions were incubated at 30 ± 2 °C for 20-26 hours.

After incubation of all test portions, 0.1 ± 0.02 mL of the sample enrichment was transferred to 10 ± 0.5 mL of Fraser Broth (FB) containing 0.1 mL of 5% ferric ammonium citrate and incubated at 35 ± 2°C for 26 ± 2 hours. A loopful of the sample enrichment was also streaked to MOX and incubated at 35 ± 2°C for 26 ± 2 hours.

After 26 ± 2 hours, FB was examined for any degree of darkening due to esculin hydrolysis. Any FB that displayed darkening was streaked to a MOX plate. If no darkening occurred, FB was re-incubated at 35 ± 2°C for a total of 48 ± 2 hours and re-examined for evidence of darkening. If darkening occurred, the FB was streaked to a MOX plate, if no darkening occurred, samples were considered negative. All FB streaked MOX plates were incubated at 35°C for 26 ± 2 hours.

MOX agar plates streaked from the primary enrichment or the FB secondary enrichment were examined after 26 ± 2 hours and if no suspect colonies were present, the MOX agar plate was re-incubated for additional 26 ± 2 hours at 35°C ± 2°C for a total of 48 ± 2 hours. If suspect colonies were present on the MOX agar plates, these suspect colonies were streaked to Horse Blood Overlay agar (HBO) and incubated at 35 ± 2°C for 22 ± 4 hours. HBO plates were examined for hemolysis reactions and well-isolated colonies were transferred to BHI broth and incubated at 25°C for 16-18 hours. Sample isolates from BHI broth were analyzed for tumbling motility by preparing a wet mount, analyzed by a catalase test and examined for morphology by preparing a Gram stain. Additionally, purified HBO isolates were identified using the VITEK® 2 GP Biochemical Identification following AOAC OMA 2012.02. [14].

**FDA/BAM Chapter 10 Detection and Enumeration of Listeria monocytogenes in Foods**
All test portions and sponges were enriched in 225 mL ± 5 mL of Buffered Listeria Enrichment Broth (BLEB) homogenized for 2 minutes and incubated at 30 ± 1°C for 4 hours. After an hour at room temperature, the test portions were incubated at 30 ± 1°C for 4 hours ± 30 mins. Following 4 hours of incubation, selective supplements acriflavine (10mg/L), sodium nalidixate (40mg/L) and cycloheximide (50mg/L) were added to each test portion and incubated for an additional 20 hours ± 30 mins. After 24 hours of total incubation, the enriched samples were streaked to MOX agar and Agar Listeria Ottavani and Agosti (ALOA) plates and incubated at 35 ±1 °C for 24-48 hours. The enriched samples were re-incubated for an additional 24 hours at 30 ± 1°C and then streaked to a second MOX agar and ALOA plate which was incubated for 24-48 hours at 35 ± 1°C. All agar plates were examined for suspect colonies (MOX: approximately 1 mm in diameter, brown-black in color, black zone, ALOA: blue-green colonies with a halo), and if present, at least 5 colonies were streaked to Tryptic Soy Agar containing 0.6% yeast extract (TSA/YE). The TSA/YE plates were incubated at 35 ± 1°C for 24-48 hours and then examined for purity. Pure colonies were tested for catalase reactivity and a Gram Stain was conducted. A pure Listeria colony was transferred to Trypticase Soy Broth containing 0.6% yeast extract (TSB/YE). The TSB/YE cultures were incubated at 25 ± 1 °C overnight, or until the broth was turbid, indicating sufficient growth. Catalase-positive organisms were stabbed into plates of 5% SBA and incubated at 35 ± 1°C for 24-48 hours. The TSB/YE tubes incubated at 25 ±1°C were used to prepare a wet mount slide to determine motility pattern. After incubation, the SBA plates were examined for Beta-hemolysis. Final confirmation was conducted using the VITEK® 2 GP Biochemical Identification card following AOAC OMA 2012.02.

USDA/FSIS-MLG Method 4.09 Isolation and Identification of Salmonella from Meat, Poultry, Pasteurized Egg, Siluriformes (Fish) Products and Carcass and Environmental Sponges

The fresh raw ground beef test portions, consisting of 25 ± 2.5 g, were enriched with 75 ± 1.5 mL of pre-warmed (42 ± 1°C) modified Tryptic Soy Broth (mTSB), homogenized by stomaching for 2 minutes, and incubated for 15-24 hours at 42 ± 1°C.

The deli turkey test portions, consisting of 25 ± 2.5 g, were enriched with 225 ± 4.5 mL of Buffered Peptone Water (BPW), homogenized by stomaching for 2 minutes, and incubated for 18-24 hours at 35 ± 1°C.

After incubation, 0.1 ± 0.02 mL of each sample was transferred to 10 mL of modified RV broth (mRV) and 0.5 ± 0.05 mL into 10 mL of TT Hajna broth. The broths were incubated in a circulating water bath at 42 ± 0.5°C for 18-24 hours. Following incubation, a loopful from each broth replicate was streaked to Xylose-Lysine-Tergitol 4 (XLT4) and Brilliant Green Sulfa agar (BGSA). Both selective agars were incubated at 35 ± 2°C for 18-24 hours. In no growth was present, plates were incubated for an additional 24 hours. Presumptive positive Salmonella colonies (XLT4: yellow-red colonies with black center and BGSA: pink-white colonies surrounded by a brilliant red-
zone) from each selective agar were picked and transferred to TSI and LIA slants and incubated at 35 ± 2°C for 24 ± 2 hours. Growth from samples producing typical biochemical reactions in TSI and LIA were streaked to TSA slants and incubated at 35 ± 2°C for 18-24 hours. Following incubation, isolates were serologically tested for both somatic O and flagellar H agglutination. Additionally, purified TSA isolates were identified using the VITEK® 2 GN Biochemical Identification card following AOAC Official Method 2011.17.

**FDA/BAM Chapter 5 Salmonella**

All 25 g fresh baby spinach test portions were enriched with 225 mL of Lactose Broth (LB), homogenized by swirling 25 times clockwise and 25 times counter-clockwise. Following homogenization, test portions were allowed to stand at room temperature (24 ± 2°C) for 60 ± 5 minutes. If necessary, the pH of the enrichments for all matrixes was adjusted to 6.8 ± 0.2. Subsequently, all matrix enrichments were incubated at 35 ± 2°C for 24 ± 2 hours.

Environmental sponges were enriched with 225 mL of Lactose Broth (LB). Environmental test portions were homogenized by hand massaging and were allowed to stand at room temperature (24 ± 2°C) for 60 ± 5 minutes. If necessary, the pH of the enrichments were adjusted to 6.8 ± 0.2. Subsequently, all enrichments were incubated at 35 ± 2°C for 24 ± 2 hours.

Following incubation, 0.1 mL of primary enrichment was transferred into 10 mL of RV and 1.0 mL into 10 mL of TT broth. RV tubes were incubated at 42 ± 0.2°C for 24 ± 2 hours. For the environmental sponges, the TT tubes were incubated at 35 ± 2°C for 24 ± 2 hours. The fresh baby spinach contained a high microbial background (>10^4), therefore the TT tubes were incubated at 43 ± 0.2°C for 24 ± 2 hours. Following incubation, a loopful of the secondary enrichments were streaked to Bismuth Sulfite agar (BS), Hektoen Enteric agar (HE) and Xylose Lysine Deoxycholate agar (XLD) and incubated at 35 ± 2°C for 24 ± 2 hours. If no visible colonies were present after 24 hours of incubation on the BS plates, they were re-incubated for additional 24 ± 2 hours at 35 ± 2°C. A minimum of two suspect colonies from each selective agar were transferred to Triple Sugar Iron agar (TSI) and Lysine Iron agar (LIA) slants and incubated at 35 ± 2°C for 24 ± 2 hours. Following incubation, TSI and LIA slants were examined for typical reactions. Slants producing typical reactions were streaked to TSA and incubated for 35 ± 2°C for 18-24 hours. Following incubation, isolates were serologically tested for both somatic O and flagellar H agglutination. Additionally, purified TSA isolates were identified using the VITEK® 2 GN Biochemical Identification card following AOAC Official Method 2011.17.

**PolySkope 1.0 Multiplex Pathogen Detection Assay**

All test portions were enriched and incubated according to the AOAC protocol as described previously in “General Preparation”. After incubation, all test portions were processed by the PolySkope 1.0 Multiplex Pathogen Detection Assay.
**Analysis**

The reaction plate containing master mix and samples were loaded into the QuantStudio 5 and prompts were followed by the QuantStudio 5 software to identify samples and controls. The PolySkope 1.0 Multiplex Pathogen Detection Assay was initiated and results were obtained within 90 minutes.

**Interpretation of Results**

The presence or absence of the pathogens is determined by manually interpreting the amplification curves in conjunction with the analysis of the CT values provided by the software. Both sets of data have to be compared to make a final determination of the results.

**Confirmation**

All samples analyzed by the PolySkope 1.0 Multiplex Pathogen Detection Assay, regardless of presumptive result, were culturally confirmed by procedures outlined in the reference methods specified for matrix or environmental surface. Final confirmation was achieved by VITEK® 2 GN Biochemical Identification, AOAC OMA 2011.17 or by VITEK® 2 GP Biochemical Identification, AOAC OMA 2012.02.

**Product Stability and Lot to Lot**

The product stability and lot to lot design consisted of combining reagents within three separate lot of the PolySkope 1.0 Multiplex Pathogen Detection Assay and testing pure cultures at multiple storage time points accelerated and real-time, see Table F below.

<table>
<thead>
<tr>
<th>Storage Type</th>
<th>Storage Temperature</th>
<th>Time Points (From the Date of Production)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Real Time</td>
<td>2-8°C</td>
<td>1 month, 2.5 months, 5 months, 6 months</td>
</tr>
<tr>
<td>Accelerated</td>
<td>25 ± 2°C</td>
<td>4 days, 9 days, 17 days, 20 days</td>
</tr>
</tbody>
</table>

Each of the storage time points were analyzed with pure cultures: *E. coli* O45 MSU TW09183, *Listeria monocytogenes* ATCC 13932, and *Salmonella Choleraesuis* ATCC 10708 cultured in PMEM. Each culture was diluted to a level that yielded fractional positive results (2-8 positives) and analyzed for 10 replicates per strain. A non-pathogenic *E. coli* strain was cultured in BHI and analyzed undiluted. In addition, three uninoculated lysis buffer controls were
analyzed. All samples were randomized and blind-coded and analyzed at each storage time point in Table F.

Robustness Study

The study was conducted according to the procedures outlined in the AOAC approved protocol. The parameters of enrichment time and two different lysis times were varied. Using a factorial design, 8 treatment combinations were evaluated and compared to a nominal treatment combination. Ten (10) individual 25 g test portions of fresh raw ground beef were inoculated with a low-level of target strains and 10 individual 25 g test portions of fresh raw ground beef were inoculated with Enterococcus faecalis and non-pathogenic E. coli at a high inoculation level. The samples were enriched using PMEM, incubated at 37 ± 1°C, and assayed using the PolySkope 1.0 Pathogen Detection Assay following the treatment combinations listed in Table G.

Table G: PolySkope 1.0 Pathogen Detection Assay Robustness Parameters

<table>
<thead>
<tr>
<th>Treatment Combination</th>
<th>Enrichment Time</th>
<th>First Lysis Time</th>
<th>Second Lysis Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20 Hours</td>
<td>10 Minutes</td>
<td>5 Minutes</td>
</tr>
<tr>
<td>2</td>
<td>20 Hours</td>
<td>10 Minutes</td>
<td>15 Minutes</td>
</tr>
<tr>
<td>3</td>
<td>20 Hours</td>
<td>20 Minutes</td>
<td>5 Minutes</td>
</tr>
<tr>
<td>4</td>
<td>20 Hours</td>
<td>20 Minutes</td>
<td>15 Minutes</td>
</tr>
<tr>
<td>5</td>
<td>26 Hours</td>
<td>10 Minutes</td>
<td>5 Minutes</td>
</tr>
<tr>
<td>6</td>
<td>26 Hours</td>
<td>10 Minutes</td>
<td>15 Minutes</td>
</tr>
<tr>
<td>7</td>
<td>26 Hours</td>
<td>20 Minutes</td>
<td>5 Minutes</td>
</tr>
<tr>
<td>8</td>
<td>26 Hours</td>
<td>20 Minutes</td>
<td>15 Minutes</td>
</tr>
<tr>
<td>9 (Normal)</td>
<td>22-24 Hours</td>
<td>15 Minutes</td>
<td>10 Minutes</td>
</tr>
</tbody>
</table>

1 Michigan State University Culture Collection
2 American Type Culture Collection

Results

Inclusivity and Exclusivity Study

Of the 50 inclusivity for the non-O157 STEC, E. coli O157, and Listeria monocytogenes, all 50 inclusivity organisms were correctly identified. Of the 100
inclusivity organisms for *Salmonella* species, all 100 organisms were correctly identified. All of the exclusivity organisms were correctly excluded.

Detailed results for the inclusivity and exclusivity evaluations are presented in Tables 1-6 of the Appendix.

**Method Comparison**

As per criteria outlined in Appendix J of the Official Methods of Analysis Manual, fractional positive results were obtained [15]. A summary of the method comparison results is presented in Table H.

The pre-evaluation pathogen screen results and APC results are presented in Table 7 of the Appendix. The heat stress data for the deli turkey is presented in Table 8 of the Appendix. An inoculum summary for stainless steel environmental surface is presented in Table 9 of the Appendix. A summary of the MPN results is presented in Tables 10A-10C, 11A-11C, and 12A-12C of the Appendix. A detailed summary of results for each target analyte and each matrix is presented in Tables 13-16 of the Appendix.

The POD was calculated as the number of positive outcomes divided by the total number of trials [16]. The POD was calculated for the candidate presumptive results, $POD_{CP}$, the candidate confirmatory results, $POD_{CC}$, the difference in the candidate presumptive and confirmatory results, $dPOD_{CP}$, presumptive candidate results that confirmed positive, $POD_{C}$, the reference method, $POD_{R}$, and the difference in the confirmed candidate and reference methods, $dPOD_{C}$. The POD analysis between the PolySkope 1.0 Pathogen Detection Assay and the reference methods for all matrices indicated that there was no significant difference at the 5% level between the number of positive results by the two methods. The POD analysis between the PolySkope 1.0 Pathogen Detection Assay presumptive and confirmed results for all matrices and 1 environmental surface (for all target analytes) indicated that there was no significant difference at the 5% level. A summary of POD analyses [17] are presented in Tables 17-24 of the Appendix.

**Table H: Summary of Results**

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Fresh Raw Ground Beef</th>
</tr>
</thead>
<tbody>
<tr>
<td>Result</td>
<td>PolySkope 1.0 Pathogen Detection Assay</td>
</tr>
<tr>
<td>Target</td>
<td></td>
</tr>
<tr>
<td>Presumptive</td>
<td></td>
</tr>
<tr>
<td>Confirmed</td>
<td></td>
</tr>
<tr>
<td>Big 6 STEC &amp; E. coli/O157</td>
<td></td>
</tr>
<tr>
<td><em>L. monocytogenes</em></td>
<td></td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td></td>
</tr>
<tr>
<td>Big 6 STEC &amp; E. coli/O157</td>
<td></td>
</tr>
<tr>
<td><em>L. monocytogenes</em></td>
<td></td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td></td>
</tr>
<tr>
<td>Matrix</td>
<td>Deli Turkey</td>
</tr>
<tr>
<td>----------</td>
<td>-------------</td>
</tr>
<tr>
<td>Uninoculated</td>
<td>0/5 0/5 0/5 0/5 0/5 0/5 0/5 0/5</td>
</tr>
<tr>
<td>High</td>
<td>5/5 5/5 5/5 5/5 5/5 5/5 5/5 5/5</td>
</tr>
</tbody>
</table>

**Table H: Summary of Results (Continued)**

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Fresh Baby Spinach (25g)</th>
<th>Method</th>
<th>PolySkope 1.0 Pathogen Detection Assay</th>
<th>FDA/BAM Chapter 4A</th>
<th>FDA/BAM Chapter 10</th>
<th>FDA/BAM Chapter 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninoculated</td>
<td>0/5 0/5 0/5 0/5 0/5 0/5 0/5 0/5</td>
<td>0/5 0/5 0/5 0/5 0/5 0/5 0/5 0/5</td>
<td>0/5 0/5 0/5 0/5 0/5 0/5 0/5 0/5</td>
<td>0/5 0/5 0/5 0/5 0/5 0/5 0/5 0/5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>5/5 5/5 5/5 5/5 5/5 5/5 5/5 5/5</td>
<td>5/5 5/5 5/5 5/5 5/5 5/5 5/5 5/5</td>
<td>5/5 5/5 5/5 5/5 5/5 5/5 5/5 5/5</td>
<td>5/5 5/5 5/5 5/5 5/5 5/5 5/5 5/5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Target</td>
<td>Big 6 STEC &amp; E. coli O157</td>
<td>L. monocytogenes</td>
<td>Salmonella</td>
<td>Big 6 STEC &amp; E. coli O157</td>
<td>L. monocytogenes</td>
<td>Salmonella</td>
</tr>
<tr>
<td>-----------------</td>
<td>---------------------------</td>
<td>------------------</td>
<td>------------</td>
<td>---------------------------</td>
<td>------------------</td>
<td>------------</td>
</tr>
<tr>
<td>Uninoculated</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>High</td>
<td>5/5</td>
<td>5/5</td>
<td>5/5</td>
<td>5/5</td>
<td>5/5</td>
<td>5/5</td>
</tr>
</tbody>
</table>

**Fresh Raw Ground Beef – STEC (Inoculating Organism - E. coli O157)**

For the low inoculation level of the PolySkope 1.0 Pathogen Detection Assay, there were 7 presumptive positives and 7 confirmed positives for the PolySkope method. There were 6 confirmed positives for the reference method. For the high inoculation level, there were 5 presumptive positives and 5 confirmed positives for the PolySkope 1.0 Pathogen Detection Assay and reference method.

For the low inoculation level, a dPOD$_C$ value of 0.05 was obtained with a 95% confidence interval of (-0.23, 0.32), indicating no statistically significant difference between the candidate and reference method. A dPOD$_{CP}$ value of 0.00 was obtained with a 95% confidence interval of (-0.13, 0.13), indicating no statistically significant difference between the candidate presumptive and confirmed results.

For the high inoculation level, a dPOD$_C$ value of 0.00 was obtained with a 95% confidence interval of (-0.43, 0.43), indicating no statistically significant difference between the candidate and reference method. A dPOD$_{CP}$ value of 0.00 was obtained with a 95% confidence interval of (-0.47, 0.47), indicating no statistically significant difference between the candidate presumptive and confirmed results. Detailed results of the POD analyses are presented in Tables 17 and 21 of the Appendix.

**Fresh Raw Ground Beef – Listeria monocytogenes**

For the low inoculation level of the PolySkope 1.0 Pathogen Detection Assay, there were 7 presumptive positives and 7 confirmed positives for the PolySkope method. There were 8 confirmed positives for the reference method. For the high inoculation level, there were 5 presumptive positives and 5
confirmed positives for the PolySkope 1.0 Pathogen Detection Assay and reference method.

For the low inoculation level, a dPOD_C value of -0.05 was obtained with a 95% confidence interval of (-0.32, 0.23), indicating no statistically significant difference between the candidate and reference method. A dPOD_CP value of 0.00 was obtained with a 95% confidence interval of (-0.13, 0.13), indicating no statistically significant difference between the candidate presumptive and confirmed results.

For the high inoculation level, a dPOD_C value of 0.00 was obtained with a 95% confidence interval of (-0.43, 0.43), indicating no statistically significant difference between the candidate and reference method. A dPOD_CP value of 0.00 was obtained with a 95% confidence interval of (-0.47, 0.47), indicating no statistically significant difference between the candidate presumptive and confirmed results. Detailed results of the POD analyses are presented in Tables 17 and 21 of the Appendix.

Fresh Raw Ground Beef – Salmonella

For the low inoculation level of the PolySkope 1.0 Pathogen Detection Assay, there were 9 presumptive positives and 9 confirmed positives for the PolySkope method. There were 5 confirmed positives for the reference method. For the high inoculation level, there were 5 presumptive positives and 5 confirmed positives for the PolySkope 1.0 Pathogen Detection Assay and reference method.

For the low inoculation level, a dPOD_C value of 0.20 was obtained with a 95% confidence interval of (-0.09, 0.45), indicating no statistically significant difference between the candidate and reference method. A dPOD_CP value of 0.00 was obtained with a 95% confidence interval of (-0.13, 0.13), indicating no statistically significant difference between the candidate presumptive and confirmed results.

For the high inoculation level, a dPOD_C value of 0.00 was obtained with a 95% confidence interval of (-0.43, 0.43), indicating no statistically significant difference between the candidate and reference method. A dPOD_CP value of 0.00 was obtained with a 95% confidence interval of (-0.47, 0.47), indicating no statistically significant difference between the candidate presumptive and confirmed results. Detailed results
of the POD analyses are presented in Tables 17 and 21 of the Appendix.

**Deli Turkey – STEC (Inoculating Organism E. coli O26)**

For the low inoculation level of the PolySkope 1.0 Pathogen Detection Assay, there were 7 presumptive positives and 7 confirmed positives for the PolySkope method. There were 5 confirmed positives for the reference method. For the high inoculation level, there were 5 presumptive positives and 5 confirmed positives for the PolySkope 1.0 Pathogen Detection Assay and reference method.

For the low inoculation level, a dPOD<sub>C</sub> value of 0.10 was obtained with a 95% confidence interval of (-0.18, 0.36), indicating no statistically significant difference between the candidate and reference method. A dPOD<sub>CP</sub> value of 0.00 was obtained with a 95% confidence interval of (-0.13, 0.13), indicating no statistically significant difference between the candidate presumptive and confirmed results.

For the high inoculation level, a dPOD<sub>C</sub> value of 0.00 was obtained with a 95% confidence interval of (-0.43, 0.43), indicating no statistically significant difference between the candidate and reference method. A dPOD<sub>CP</sub> value of 0.00 was obtained with a 95% confidence interval of (-0.47, 0.47), indicating no statistically significant difference between the candidate presumptive and confirmed results. Detailed results of the POD analyses are presented in Tables 18 and 22 of the Appendix.

**Deli Turkey – Listeria monocytogenes**

For the low inoculation level of the PolySkope 1.0 Pathogen Detection Assay, there were 11 presumptive positives and 11 confirmed positives for the PolySkope method. There were 8 confirmed positives for the reference method. For the high inoculation level, there were 5 presumptive positives and 5 confirmed positives for the PolySkope 1.0 Pathogen Detection Assay and reference method.

For the low inoculation level, a dPOD<sub>C</sub> value of 0.15 was obtained with a 95% confidence interval of (-0.15, 0.41), indicating no statistically significant difference between the
candidate and reference method. A dPOD<sub>CP</sub> value of 0.00 was obtained with a 95% confidence interval of (-0.13, 0.13), indicating no statistically significant difference between the candidate presumptive and confirmed results.

For the high inoculation level, a dPOD<sub>C</sub> value of 0.00 was obtained with a 95% confidence interval of (-0.43, 0.43), indicating no statistically significant difference between the candidate and reference method. A dPOD<sub>CP</sub> value of 0.00 was obtained with a 95% confidence interval of (-0.47, 0.47), indicating no statistically significant difference between the candidate presumptive and confirmed results. Detailed results of the POD analyses are presented in Tables 18 and 22 of the Appendix.

**Deli Turkey – Salmonella**

For the low inoculation level of the PolySkope 1.0 Pathogen Detection Assay, there were 14 presumptive positives and 13 confirmed positives for the PolySkope method. There were 10 confirmed positives for the reference method. For the high inoculation level, there were 5 presumptive positives and 5 confirmed positives for the PolySkope 1.0 Pathogen Detection Assay and reference method.

For the low inoculation level, a dPOD<sub>C</sub> value of 0.15 was obtained with a 95% confidence interval of (-0.15, 0.41), indicating no statistically significant difference between the candidate and reference method. A dPOD<sub>CP</sub> value of 0.05 was obtained with a 95% confidence interval of (-0.11, 0.21), indicating no statistically significant difference between the candidate presumptive and confirmed results.

For the high inoculation level, a dPOD<sub>C</sub> value of 0.00 was obtained with a 95% confidence interval of (-0.43, 0.43), indicating no statistically significant difference between the candidate and reference method. A dPOD<sub>CP</sub> value of 0.00 was obtained with a 95% confidence interval of (-0.47, 0.47), indicating no statistically significant difference between the candidate presumptive and confirmed results. Detailed results of the POD analyses are presented in Tables 18 and 22 of the Appendix.

**Fresh Baby Spinach – STEC (Inoculating Organism - E. coli O145)**

For the low inoculation level of the PolySkope 1.0 Pathogen Detection Assay, there were 9 presumptive positives and 9
confirmed positives for the PolySkope method. There were 7 confirmed positives for the reference method. For the high inoculation level, there were 5 presumptive positives and 5 confirmed positives for the PolySkope 1.0 Pathogen Detection Assay and reference method.

For the low inoculation level, a dPOD$_c$ value of 0.10 was obtained with a 95% confidence interval of (-0.19, 0.37), indicating no statistically significant difference between the candidate and reference method. A dPOD$_{cp}$ value of 0.00 was obtained with a 95% confidence interval of (-0.13, 0.13), indicating no statistically significant difference between the candidate presumptive and confirmed results.

For the high inoculation level, a dPOD$_c$ value of 0.00 was obtained with a 95% confidence interval of (-0.43, 0.43), indicating no statistically significant difference between the candidate and reference method. A dPOD$_{cp}$ value of 0.00 was obtained with a 95% confidence interval of (-0.47, 0.47), indicating no statistically significant difference between the candidate presumptive and confirmed results. Detailed results of the POD analyses are presented in Tables 19 and 23 of the Appendix.

*Fresh Baby Spinach – Listeria monocytogenes*

For the low inoculation level of the PolySkope 1.0 Pathogen Detection Assay, there were 7 presumptive positives and 7 confirmed positives for the PolySkope method. There were 5 confirmed positives for the reference method. For the high inoculation level, there were 5 presumptive positives and 5 confirmed positives for the PolySkope 1.0 Pathogen Detection Assay and reference method.

For the low inoculation level, a dPOD$_c$ value of 0.10 was obtained with a 95% confidence interval of (-0.18, 0.36), indicating no statistically significant difference between the candidate and reference method. A dPOD$_{cp}$ value of 0.00 was obtained with a 95% confidence interval of (-0.13, 0.13), indicating no statistically significant difference between the candidate presumptive and confirmed results.

For the high inoculation level, a dPOD$_c$ value of 0.00 was obtained with a 95% confidence interval of (-0.43, 0.43), indicating no statistically significant difference between the candidate and reference method. A dPOD$_{cp}$ value of 0.00 was obtained with a 95% confidence interval of (-0.47, 0.47),
indicating no statistically significant difference between the candidate presumptive and confirmed results. Detailed results of the POD analyses are presented in Tables 19 and 23 of the Appendix.

**Fresh Baby Spinach – Salmonella**

For the low inoculation level of the PolySkope 1.0 Pathogen Detection Assay, there were 7 presumptive positives and 7 confirmed positives for the PolySkope method. There were 6 confirmed positives for the reference method. For the high inoculation level, there were 5 presumptive positives and 5 confirmed positives for the PolySkope 1.0 Pathogen Detection Assay and reference method.

For the low inoculation level, a dPOD\textsubscript{C} value of 0.05 was obtained with a 95% confidence interval of (-0.23, 0.32), indicating no statistically significant difference between the candidate and reference method. A dPOD\textsubscript{CP} value of 0.00 was obtained with a 95% confidence interval of (-0.13, 0.13), indicating no statistically significant difference between the candidate presumptive and confirmed results.

For the high inoculation level, a dPOD\textsubscript{C} value of 0.00 was obtained with a 95% confidence interval of (-0.43, 0.43), indicating no statistically significant difference between the candidate and reference method. A dPOD\textsubscript{CP} value of 0.00 was obtained with a 95% confidence interval of (-0.47, 0.47), indicating no statistically significant difference between the candidate presumptive and confirmed results. Detailed results of the POD analyses are presented in Tables 19 and 23 of the Appendix.

**Stainless Steel – STEC (Inoculating Organism - E. coli O103)**

For the low inoculation level of the PolySkope 1.0 Pathogen Detection Assay, there were 7 presumptive positives and 7 confirmed positives for the PolySkope method. There were 6 confirmed positives for the reference method. For the high inoculation level, there were 5 presumptive positives and 5 confirmed positives for the PolySkope 1.0 Pathogen Detection Assay and reference method.

For the low inoculation level, a dPOD\textsubscript{C} value of 0.05 was obtained with a 95% confidence interval of (-0.23, 0.32), indicating no statistically significant difference between the
candidate and reference method. A dPOD<sub>CP</sub> value of 0.00 was obtained with a 95% confidence interval of (-0.13, 0.13), indicating no statistically significant difference between the candidate presumptive and confirmed results.

For the high inoculation level, a dPOD<sub>C</sub> value of 0.00 was obtained with a 95% confidence interval of (-0.43, 0.43), indicating no statistically significant difference between the candidate and reference method. A dPOD<sub>CP</sub> value of 0.00 was obtained with a 95% confidence interval of (-0.47, 0.47), indicating no statistically significant difference between the candidate presumptive and confirmed results. Detailed results of the POD analyses are presented in Tables 20 and 24 of the Appendix.

**Stainless Steel – Listeria monocytogenes**

For the low inoculation level of the PolySkope 1.0 Pathogen Detection Assay, there were 4 presumptive positives and 4 confirmed positives for the PolySkope method. There were 7 confirmed positives for the reference method. For the high inoculation level, there were 5 presumptive positives and 5 confirmed positives for the PolySkope 1.0 Pathogen Detection Assay and reference method.

For the low inoculation level, a dPOD<sub>C</sub> value of -0.15 was obtained with a 95% confidence interval of (-0.40, 0.12), indicating no statistically significant difference between the candidate and reference method. A dPOD<sub>CP</sub> value of 0.00 was obtained with a 95% confidence interval of (-0.13, 0.13), indicating no statistically significant difference between the candidate presumptive and confirmed results.

For the high inoculation level, a dPOD<sub>C</sub> value of 0.00 was obtained with a 95% confidence interval of (-0.43, 0.43), indicating no statistically significant difference between the candidate and reference method. A dPOD<sub>CP</sub> value of 0.00 was obtained with a 95% confidence interval of (-0.47, 0.47), indicating no statistically significant difference between the candidate presumptive and confirmed results. Detailed results of the POD analyses are presented in Tables 20 and 24 of the Appendix.

**Stainless Steel – Salmonella**

For the low inoculation level of the PolySkope 1.0 Pathogen Detection Assay, there were 5 presumptive positives and 5
confirmed positives for the PolySkope method. There were 7 confirmed positives for the reference method. For the high inoculation level, there were 5 presumptive positives and 5 confirmed positives for the PolySkope 1.0 Pathogen Detection Assay and reference method.

For the low inoculation level, a dPOD\(_C\) value of -0.10 was obtained with a 95% confidence interval of (-0.36, 0.18), indicating no statistically significant difference between the candidate and reference method. A dPOD\(_{CP}\) value of 0.00 was obtained with a 95% confidence interval of (-0.13, 0.13), indicating no statistically significant difference between the candidate presumptive and confirmed results.

For the high inoculation level, a dPOD\(_C\) value of 0.00 was obtained with a 95% confidence interval of (-0.43, 0.43), indicating no statistically significant difference between the candidate and reference method. A dPOD\(_{CP}\) value of 0.00 was obtained with a 95% confidence interval of (-0.47, 0.47), indicating no statistically significant difference between the candidate presumptive and confirmed results. Detailed results of the POD analyses are presented in Tables 20 and 24 of the Appendix.

**Product Stability and Lot-to-Lot**

**Accelerated Stability**

For the accelerated product stability and lot-to-lot consistency, the PolySkope 1.0 Multiplex Pathogen Detection Assay detected the target analytes at all four (4) time points while the assay was held at 25 ± 2ºC, with no observed effect on the results. A summary of results is displayed below in Table I for the stability evaluation. A detailed summary of results and POD results is displayed in Tables 26-29 and 34 of the Appendix.

**Table I: Accelerated Product Stability and Lot-to-Lot**

<table>
<thead>
<tr>
<th>Time Point</th>
<th>Sample</th>
<th>Result</th>
<th>Sample</th>
<th>Result</th>
<th>Sample</th>
<th>Result</th>
<th>Sample</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 Days</td>
<td>Low Level</td>
<td>4/10</td>
<td>Low Level</td>
<td>6/10</td>
<td>Low Level</td>
<td>4/10</td>
<td>Low Level</td>
<td>5/10</td>
</tr>
<tr>
<td></td>
<td>Non-Target Organism</td>
<td>0/10</td>
<td>Non-Target Organism</td>
<td>0/10</td>
<td>Non-Target Organism</td>
<td>0/10</td>
<td>Non-Target Organism</td>
<td>0/10</td>
</tr>
<tr>
<td></td>
<td>Lysis Blank</td>
<td>0/3</td>
<td>Lysis Blank</td>
<td>0/3</td>
<td>Lysis Blank</td>
<td>0/3</td>
<td>Lysis Blank</td>
<td>0/3</td>
</tr>
</tbody>
</table>

Page 31 of 95
<table>
<thead>
<tr>
<th>Time Point</th>
<th>Sample</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 Days</td>
<td>Low Level</td>
<td>4/10</td>
</tr>
<tr>
<td></td>
<td>Non-Target Organism</td>
<td>0/10</td>
</tr>
<tr>
<td></td>
<td>Lysis Blank</td>
<td>0/3</td>
</tr>
<tr>
<td>9 Days</td>
<td>Low Level</td>
<td>4/10</td>
</tr>
<tr>
<td></td>
<td>Non-Target Organism</td>
<td>0/10</td>
</tr>
<tr>
<td></td>
<td>Lysis Blank</td>
<td>0/3</td>
</tr>
<tr>
<td>17 Days</td>
<td>Low Level</td>
<td>5/10</td>
</tr>
<tr>
<td></td>
<td>Non-Target Organism</td>
<td>0/10</td>
</tr>
<tr>
<td></td>
<td>Lysis Blank</td>
<td>0/3</td>
</tr>
<tr>
<td>20 Days</td>
<td>Low Level</td>
<td>3/10</td>
</tr>
<tr>
<td></td>
<td>Non-Target Organism</td>
<td>0/10</td>
</tr>
<tr>
<td></td>
<td>Lysis Blank</td>
<td>0/3</td>
</tr>
</tbody>
</table>

---

### Accelerated: Day 4

There were 4 presumptive positives out of 10 replicates for *E. coli* O45, 4 presumptive positives out of 10 replicates for *Listeria monocytogenes*, and 5 presumptive positives out of 10 replicates for *Salmonella* spp. at the low inoculation level. For the 10 non-target organism test portions, there were 0 presumptive positives out of 5 replicates and all 3 lysis blank controls there were 0 presumptive positives out of 3 replicates.

For the low inoculation level, a POD value of 0.40 was obtained with a 95% confidence interval of (0.17, 0.69) for *E. coli* O45 and *Listeria monocytogenes*. A POD value of 0.50 was obtained with a 95% confidence interval of (0.24, 0.76) for *Salmonella* spp. All 10 non-target organism test portions were negative with a POD value of 0.00 with a 95% confidence interval of (0.00, 0.28). All 3 lysis blanks were negative with a POD value of 0.00 with a 95% confidence interval of (0.00, 0.56).

### Accelerated: Day 9

There were 6 presumptive positives out of 10 replicates for *E. coli* O45, 4 presumptive positives out of 10 replicates for *Listeria monocytogenes*, and 6 presumptive positives out of 10 replicates for *Salmonella* spp. at the low inoculation level. For the 10 non-target organism test portions, there were 0 presumptive positives out of 5 replicates and all 3 lysis blank controls there were 0 presumptive positives out of 3 replicates.

For the low inoculation level, a POD value of 0.60 was obtained with a 95% confidence interval of (0.31, 0.83) for *E. coli* O45 and *Salmonella* spp. A POD value of 0.40 was obtained with a 95% confidence interval of...
(0.17, 0.69) for *Listeria monocytogenes*. All 10 non-target organism test portions were negative with a POD value of 0.00 with a 95% confidence interval of (0.00, 0.28). All 3 lysis blanks were negative with a POD value of 0.00 with a 95% confidence interval of (0.00, 0.56).

**Accelerated: Day 17**

There were 4 presumptive positives out of 10 replicates for *E. coli* O45, 5 presumptive positives out of 10 replicates for *Listeria monocytogenes*, and 5 presumptive positives out of 10 replicates for *Salmonella* spp. at the low inoculation level. For the 10 non-target organism test portions, there were 0 presumptive positives out of 5 replicates and all 3 lysis blank controls there were 0 presumptive positives out of 3 replicates.

For the low inoculation level, a POD value of 0.40 was obtained with a 95% confidence interval of (0.17, 0.69) for *E. coli* O45. A POD value of 0.50 was obtained with a 95% confidence interval of (0.24, 0.76) for *Salmonella* spp and *Listeria monocytogenes*. All 10 non-target organism test portions were negative with a POD value of 0.00 with a 95% confidence interval of (0.00, 0.28). All 3 lysis blanks were negative with a POD value of 0.00 with a 95% confidence interval of (0.00, 0.56).

**Accelerated: Day 20**

There were 5 presumptive positives out of 10 replicates for *E. coli* O45, 3 presumptive positives out of 10 replicates for *Listeria monocytogenes*, and 7 presumptive positives out of 10 replicates for *Salmonella* spp. at the low inoculation level. For the 10 non-target organism test portions, there were 0 presumptive positives out of 5 replicates and all 3 lysis blank controls there were 0 presumptive positives out of 3 replicates.

For the low inoculation level, a POD value of 0.50 was obtained with a 95% confidence interval of (0.24, 0.76) for *E. coli* O45 and a POD value of 0.30 with a 95% confidence interval of (0.11, 0.60) for *Listeria monocytogenes*. A POD value of 0.70 was obtained with a 95% confidence interval of (0.40, 0.89) for *Salmonella* spp. All 10 non-target organism test portions were negative with a POD value of 0.00 with a 95% confidence interval of (0.00, 0.28). All 3 lysis blanks were negative with a POD value of 0.00 with a 95% confidence interval of (0.00, 0.56).

**Real Time Stability**

For the real-time product stability and lot-to-lot consistency, the PolySkope 1.0 Multiplex Pathogen Detection Assay detected the target analytes at all four (4) time points while the assay was held at 2-8°C, with no observed effect on the results. A summary of results is displayed below in Table J.
for the stability evaluation. A detailed summary of results and the POD results is displayed in Tables 30-34 of the Appendix.

**Table J: Real Time Product Stability and Lot-to-Lot**

<table>
<thead>
<tr>
<th>Time Point 1 Month</th>
<th>Time Point 2.5 Months</th>
<th>Time Point 5 Months</th>
<th>Time Point 6 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>Result</td>
<td>Sample</td>
<td>Result</td>
</tr>
<tr>
<td>Low Level</td>
<td>6/10</td>
<td>Low Level</td>
<td>6/10</td>
</tr>
<tr>
<td>Non-Target Organism</td>
<td>0/10</td>
<td>Non-Target Organism</td>
<td>0/10</td>
</tr>
<tr>
<td>Lysis Blank</td>
<td>0/3</td>
<td>Lysis Blank</td>
<td>0/3</td>
</tr>
</tbody>
</table>

**Listeria monocytogenes**

<table>
<thead>
<tr>
<th>Time Point 1 Month</th>
<th>Time Point 2.5 Months</th>
<th>Time Point 5 Months</th>
<th>Time Point 6 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>Result</td>
<td>Sample</td>
<td>Result</td>
</tr>
<tr>
<td>Low Level</td>
<td>3/10</td>
<td>Low Level</td>
<td>3/10</td>
</tr>
<tr>
<td>Non-Target Organism</td>
<td>0/10</td>
<td>Non-Target Organism</td>
<td>0/10</td>
</tr>
<tr>
<td>Lysis Blank</td>
<td>0/3</td>
<td>Lysis Blank</td>
<td>0/3</td>
</tr>
</tbody>
</table>

**Salmonella**

<table>
<thead>
<tr>
<th>Time Point 1 Month</th>
<th>Time Point 2.5 Months</th>
<th>Time Point 5 Months</th>
<th>Time Point 6 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>Result</td>
<td>Sample</td>
<td>Result</td>
</tr>
<tr>
<td>Low Level</td>
<td>5/10</td>
<td>Low Level</td>
<td>5/10</td>
</tr>
<tr>
<td>Non-Target Organism</td>
<td>0/10</td>
<td>Non-Target Organism</td>
<td>0/10</td>
</tr>
<tr>
<td>Lysis Blank</td>
<td>0/3</td>
<td>Lysis Blank</td>
<td>0/3</td>
</tr>
</tbody>
</table>

**Real Time: Month 1**

There were 6 presumptive positives out of 10 replicates for *E. coli* O45, 3 presumptive positives out of 10 replicates for *Listeria monocytogenes*, and 5 presumptive positives out of 10 replicates for *Salmonella* spp. at the low inoculation level. For the 10 non-target organism test portions, there were 0 presumptive positives out of 5 replicates and all 3 lysis blank controls there were 0 presumptive positives out of 3 replicates.

For the low inoculation level, a POD value of 0.60 was obtained with a 95% confidence interval of (0.31, 0.83) for *E. coli* O45 and a POD value of 0.30 was obtained with a 95% confidence interval of (0.11, 0.60) for *Listeria monocytogenes*. A POD value of 0.50 was obtained with a 95% confidence interval of (0.24, 0.76) for *Salmonella* spp. All 10 non-target organism test portions were negative with a POD value of 0.00 with a 95%
confidence interval of (0.00, 0.28). All 3 lysis blanks were negative with a POD value of 0.00 with a 95% confidence interval of (0.00, 0.56).

**Real Time: Month 2.5**

There were 6 presumptive positives out of 10 replicates for *E. coli* O45, 3 presumptive positives out of 10 replicates for *Listeria monocytogenes*, and 5 presumptive positives out of 10 replicates for *Salmonella* spp. at the low inoculation level. For the 10 non-target organism test portions, there were 0 presumptive positives out of 5 replicates and all 3 lysis blank controls there were 0 presumptive positives out of 3 replicates.

For the low inoculation level, a POD value of 0.60 was obtained with a 95% confidence interval of (0.31, 0.83) for *E. coli* O45 and a POD value of 0.30 was obtained with a 95% confidence interval of (0.11, 0.60) for *Listeria monocytogenes*. A POD value of 0.50 was obtained with a 95% confidence interval of (0.24, 0.76) for *Salmonella* spp. All 10 non-target organism test portions were negative with a POD value of 0.00 with a 95% confidence interval of (0.00, 0.28). All 3 lysis blanks were negative with a POD value of 0.00 with a 95% confidence interval of (0.00, 0.56).

**Real Time: Month 5**

There were 6 presumptive positives out of 10 replicates for *E. coli* O45, 3 presumptive positives out of 10 replicates for *Listeria monocytogenes*, and 5 presumptive positives out of 10 replicates for *Salmonella* spp. at the low inoculation level. For the 10 non-target organism test portions, there were 0 presumptive positives out of 5 replicates and all 3 lysis blank controls there were 0 presumptive positives out of 3 replicates.

For the low inoculation level, a POD value of 0.60 was obtained with a 95% confidence interval of (0.31, 0.83) for *E. coli* O45 and a POD value of 0.30 was obtained with a 95% confidence interval of (0.11, 0.60) for *Listeria monocytogenes*. A POD value of 0.50 was obtained with a 95% confidence interval of (0.24, 0.76) for *Salmonella* spp. All 10 non-target organism test portions were negative with a POD value of 0.00 with a 95% confidence interval of (0.00, 0.28). All 3 lysis blanks were negative with a POD value of 0.00 with a 95% confidence interval of (0.00, 0.56).

**Real Time: Month 6**

There were 6 presumptive positives out of 10 replicates for *E. coli* O45, 3 presumptive positives out of 10 replicates for *Listeria monocytogenes*, and 5 presumptive positives out of 10 replicates for *Salmonella* spp. at the low inoculation level. For the 10 non-target organism test portions, there were
0 presumptive positives out of 5 replicates and all 3 lysis blank controls there were 0 presumptive positives out of 3 replicates.

For the low inoculation level, a POD value of 0.60 was obtained with a 95% confidence interval of (0.31, 0.83) for *E. coli* O45 and a POD value of 0.30 was obtained with a 95% confidence interval of (0.11, 0.60) for *Listeria monocytogenes*. A POD value of 0.50 was obtained with a 95% confidence interval of (0.24, 0.76) for *Salmonella* spp. All 10 non-target organism test portions were negative with a POD value of 0.00 with a 95% confidence interval of (0.00, 0.28). All 3 lysis blanks were negative with a POD value of 0.00 with a 95% confidence interval of (0.00, 0.56).

**Robustness Study**

For the robustness evaluation, there were no observed discrepant results observed for all 8 treatment combinations when changing the operational parameters of the enrichment lysis time and the two heat lysis durations of the sample.

**STEC (Inoculating Organism - *E. coli* O121 MSU TW07931)**

For these 8 treatment combinations, there were 6 presumptive positives out of 10 replicates for each variation evaluated. For the 10 non-target test portions, there were 0 presumptive positives out of 10 replicates.

For the low inoculation level, a POD value of 0.60 was obtained with a 95% confidence interval of (0.31, 0.83). All 10 non-target test portions were negative with a POD value of 0.00 with a 95% confidence interval of (0.00, 0.28).

**Listeria monocytogenes ATCC 19118**

For these 8 treatment combinations, there were 3 presumptive positives out of 10 replicates for each variation evaluated. For the 10 non-target test portions, there were 0 presumptive positives out of 10 replicates.

For the low inoculation level, a POD value of 0.30 was obtained with a 95% confidence interval of (0.11, 0.60). All 10 non-target test portions were negative with a POD value of 0.00 with a 95% confidence interval of (0.00, 0.28).

**Salmonella Hadar ATCC 51956**
For these 8 treatment combinations, there were 4 presumptive positives out of 10 replicates for each variation evaluated. For the 10 non-target test portions, there were 0 presumptive positives out of 10 replicates.

For the low inoculation level, a POD value of 0.40 was obtained with a 95% confidence interval of (0.17, 0.69). All 10 non-target test portions were negative with a POD value of 0.00 with a 95% confidence interval of (0.00, 0.28).

For the robustness evaluation, a summary of results is displayed below in Table K. A detailed summary of results is displayed in Tables 36-44 of the Appendix.

**Table K: Robustness Results**

<table>
<thead>
<tr>
<th>Treatment Combination</th>
<th>1</th>
<th>2</th>
<th>Treatment Combination</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Level</td>
<td>6/10</td>
<td>6/10</td>
<td>Low Level</td>
<td>6/10</td>
<td>5/10</td>
</tr>
<tr>
<td>Non-Target Organisms&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0/10</td>
<td>0/10</td>
<td>Non-Target Organisms&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0/10</td>
<td>0/10</td>
</tr>
<tr>
<td>Treatment Combination</td>
<td>5</td>
<td>6</td>
<td>Treatment Combination</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Low Level</td>
<td>6/10</td>
<td>6/10</td>
<td>Low Level</td>
<td>6/10</td>
<td>6/10</td>
</tr>
<tr>
<td>Non-Target Organisms&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0/10</td>
<td>0/10</td>
<td>Non-Target Organisms&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0/10</td>
<td>0/10</td>
</tr>
<tr>
<td>Treatment Combination</td>
<td>9 (Nominal)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low Level</td>
<td>6/10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Target Organisms&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0/10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table K: Robustness Results (Continued)**

<table>
<thead>
<tr>
<th>Treatment Combination</th>
<th>1</th>
<th>2</th>
<th>Treatment Combination</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Level</td>
<td>3/10</td>
<td>3/10</td>
<td>Low Level</td>
<td>3/10</td>
<td>3/10</td>
</tr>
<tr>
<td>Non-Target Organisms&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0/10</td>
<td>0/10</td>
<td>Non-Target Organisms&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0/10</td>
<td>0/10</td>
</tr>
<tr>
<td>Treatment Combination</td>
<td>5</td>
<td>6</td>
<td>Treatment Combination</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Low Level</td>
<td>3/10</td>
<td>3/10</td>
<td>Low Level</td>
<td>3/10</td>
<td>3/10</td>
</tr>
</tbody>
</table>
Non-Target Organisms² | 0/10 | 0/10 | Non-Target Organisms² | 0/10 | 0/10

**Treatment Combination 9 (Nominal)**

| Low Level | 3/10 |
| Non-Target Organisms² | 0/10 |

**Robustness¹**

*Salmonella Hadar ATCC 51956*

<table>
<thead>
<tr>
<th>Treatment Combination</th>
<th>1</th>
<th>2</th>
<th>Treatment Combination</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Level</td>
<td>4/10</td>
<td>4/10</td>
<td>Low Level</td>
<td>4/10</td>
<td>4/10</td>
</tr>
<tr>
<td>Non-Target Organisms²</td>
<td>0/10</td>
<td>0/10</td>
<td>Non-Target Organisms²</td>
<td>0/10</td>
<td>0/10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment Combination</th>
<th>5</th>
<th>6</th>
<th>Treatment Combination</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Level</td>
<td>4/10</td>
<td>4/10</td>
<td>Low Level</td>
<td>4/10</td>
<td>4/10</td>
</tr>
<tr>
<td>Non-Target Organisms²</td>
<td>0/10</td>
<td>0/10</td>
<td>Non-Target Organisms²</td>
<td>0/10</td>
<td>0/10</td>
</tr>
</tbody>
</table>

**Treatment Combination 9 (Nominal)**

| Low Level | 4/10 |
| Non-Target Organisms² | 0/10 |

¹- All samples were analyzed from a common sample
²-Non-target organisms: *Enterococcus faecalis* ATCC 29212 and *E. coli* ATCC 8739
³-MSU: Michigan State University Culture Collection
⁴-ATCC: American Type Culture Collection

For the Robustness study, a summary of the parameters, experimental design, and a list of the strains used in the evaluation are presented in Tables 34 and 35. Overall, the method demonstrated that small changes in testing parameters did not impact the performance of the assay. Tables 36-44 present a detailed summary of the results. The POD results and 95% Confidence Intervals for each target analyte and treatment combination are presented in Table 45.

**Discussion**

The PolySkope 1.0 Multiplex Pathogen Detection Assay provides qualitative detection of virulence factors (*stx1, stx 2 and eae*) for *E. coli* O157 and non-*E. coli* O157 STEC (O26, O45, O103, O111, O121 and O145), *Listeria monocytogenes* and *Salmonella* spp. Because the PolySkope method utilizes a multiplex reaction, it has the ability to detect multiple common pathogens within a single reaction. This enables the user to save time and cost per test by only having to prepare a single enrichment, conduct a single lysis sample, and run a single PCR reaction. The software is simple and easy to navigate and allows the user to view Real-Time results. Each individual reaction taking place within a single sample can be interpreted throughout the entire run, including the final analysis. The software does not present the typical stop light result (Green -
positive, red - negative), but requires interpretation of the results. An analysis of the curves and the Cq values by a trained analyst are required to obtain a final result.

In the inclusivity and exclusivity evaluations, all inclusivity organisms were correctly included and all exclusivity organisms were correctly excluded. In the method comparison study, the PolySkope 1.0 Multiplex Pathogen Detection Assay demonstrated no statistically significant differences between candidate and reference method results (dPOD$_C$), or between presumptive and confirmed results (dPOD$_{CP}$) for all target pathogens. During the robustness evaluation, the change to the operational parameters of the method proved the method is robust and had not negative impact on the testing. For the product stability evaluation, the test kit proved to be unaffected by the storage conditions and lot to lot variations.

Conclusion

The data from the study, within their statistical uncertainty, support the product claims of the PolySkope 1.0 Multiplex Pathogen Detection Assay for detection of E. coli O157:H7, non-O157 STEC (O26, O45, O103, O111, O121 and O145), Listeria monocytogenes and Salmonella in fresh raw ground beef (25 g), deli turkey (25 g), fresh baby spinach (25 g) and stainless steel environmental surface (4” x 4”).

References


APPENDIX
<table>
<thead>
<tr>
<th>No.</th>
<th>Species</th>
<th>Serotype</th>
<th>Source</th>
<th>Origin</th>
<th>Result</th>
<th>No.</th>
<th>Species</th>
<th>Serotype</th>
<th>Source</th>
<th>Origin</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>E. coli</td>
<td>O26</td>
<td>ATCC BAA-1653</td>
<td>Stool</td>
<td>+</td>
<td>26</td>
<td>E. coli</td>
<td>O103</td>
<td>QL 15071-2</td>
<td>Meat Powder</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>E. coli</td>
<td>O26</td>
<td>MSU TW 07862</td>
<td>Calf, Cow</td>
<td>+</td>
<td>27</td>
<td>E. coli</td>
<td>O111:H12</td>
<td>MSU DEC 6A</td>
<td>Infant</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>E. coli</td>
<td>O26</td>
<td>MSU TW02295</td>
<td>Infant</td>
<td>+</td>
<td>28</td>
<td>E. coli</td>
<td>O111:H8</td>
<td>MSU DEC 6C</td>
<td>Human</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>E. coli</td>
<td>O26</td>
<td>MSU DEC 9F</td>
<td>Human</td>
<td>+</td>
<td>29</td>
<td>E. coli</td>
<td>O111</td>
<td>MSU DEC 8D</td>
<td>Infant</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>E. coli</td>
<td>O26</td>
<td>MSU TW04270</td>
<td>Human</td>
<td>+</td>
<td>30</td>
<td>E. coli</td>
<td>O111</td>
<td>MSU TW07926</td>
<td>Human</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>E. coli</td>
<td>O26</td>
<td>MSU TW04284</td>
<td>Child</td>
<td>+</td>
<td>31</td>
<td>E. coli</td>
<td>O111</td>
<td>MSU TW14960</td>
<td>Human</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>E. coli</td>
<td>O26</td>
<td>MSU TW08031</td>
<td>Human</td>
<td>+</td>
<td>32</td>
<td>E. coli</td>
<td>O111</td>
<td>MSU TW06296</td>
<td>Child</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>E. coli</td>
<td>O26</td>
<td>MSU TW07814</td>
<td>Human</td>
<td>+</td>
<td>33</td>
<td>E. coli</td>
<td>O111</td>
<td>MSU TW05614</td>
<td>Human</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>E. coli</td>
<td>O26</td>
<td>MSU TW00971</td>
<td>Human Feces</td>
<td>+</td>
<td>34</td>
<td>E. coli</td>
<td>O111</td>
<td>MSU TW00186</td>
<td>Human</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>E. coli</td>
<td>O26</td>
<td>MSU TW05992</td>
<td>Human</td>
<td>+</td>
<td>35</td>
<td>E. coli</td>
<td>O111</td>
<td>MSU TW01387</td>
<td>Human</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>E. coli</td>
<td>O26</td>
<td>MSU TW10121</td>
<td>Human</td>
<td>+</td>
<td>36</td>
<td>E. coli</td>
<td>O121</td>
<td>PSU 10.0709</td>
<td>Not Available</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>E. coli</td>
<td>O45</td>
<td>MSU TW14003</td>
<td>Human</td>
<td>+</td>
<td>37</td>
<td>E. coli</td>
<td>O121</td>
<td>PSU 5.0959</td>
<td>Not Available</td>
<td>+</td>
</tr>
<tr>
<td>13</td>
<td>E. coli</td>
<td>O45</td>
<td>MSU TW07947</td>
<td>Human</td>
<td>+</td>
<td>38</td>
<td>E. coli</td>
<td>O121</td>
<td>PSU 7.1686</td>
<td>Not Available</td>
<td>+</td>
</tr>
<tr>
<td>14</td>
<td>E. coli</td>
<td>O45</td>
<td>MSU DEC 11C</td>
<td>Human</td>
<td>+</td>
<td>39</td>
<td>E. coli</td>
<td>O121</td>
<td>PSU 7.1709</td>
<td>Not Available</td>
<td>+</td>
</tr>
<tr>
<td>15</td>
<td>E. coli</td>
<td>O45</td>
<td>PSU 1.2622</td>
<td>Not Available</td>
<td>+</td>
<td>40</td>
<td>E. coli</td>
<td>O121</td>
<td>PSU 7.1732</td>
<td>Not Available</td>
<td>+</td>
</tr>
<tr>
<td>16</td>
<td>E. coli</td>
<td>O45</td>
<td>PSU 1.2635</td>
<td>Not Available</td>
<td>+</td>
<td>41</td>
<td>E. coli</td>
<td>O121</td>
<td>MSU TW07931</td>
<td>Human</td>
<td>+</td>
</tr>
<tr>
<td>17</td>
<td>E. coli</td>
<td>O45</td>
<td>PSU 2.0164</td>
<td>Not Available</td>
<td>+</td>
<td>42</td>
<td>E. coli</td>
<td>O121</td>
<td>MSU TW07614</td>
<td>Human</td>
<td>+</td>
</tr>
<tr>
<td>18</td>
<td>E. coli</td>
<td>O45</td>
<td>PSU 11.1079</td>
<td>Not Available</td>
<td>+</td>
<td>43</td>
<td>E. coli</td>
<td>O121</td>
<td>MSU TW08023</td>
<td>Human</td>
<td>+</td>
</tr>
<tr>
<td>19</td>
<td>E. coli</td>
<td>O103</td>
<td>MSU TW09101</td>
<td>Human</td>
<td>+</td>
<td>44</td>
<td>E. coli</td>
<td>O145</td>
<td>QL 15071-1</td>
<td>Meat Powder</td>
<td>+</td>
</tr>
<tr>
<td>20</td>
<td>E. coli</td>
<td>O103</td>
<td>MSU TW07971</td>
<td>Human</td>
<td>+</td>
<td>45</td>
<td>E. coli</td>
<td>O145</td>
<td>PSU 7.1711</td>
<td>Not Available</td>
<td>+</td>
</tr>
<tr>
<td>21</td>
<td>E. coli</td>
<td>O103</td>
<td>MSU TW11239</td>
<td>Child</td>
<td>+</td>
<td>46</td>
<td>E. coli</td>
<td>O145</td>
<td>PSU 10.0707</td>
<td>Not Available</td>
<td>+</td>
</tr>
<tr>
<td>22</td>
<td>E. coli</td>
<td>O103</td>
<td>MSU TW07697</td>
<td>Human</td>
<td>+</td>
<td>47</td>
<td>E. coli</td>
<td>O145</td>
<td>MSU TW09153</td>
<td>Human</td>
<td>+</td>
</tr>
<tr>
<td>23</td>
<td>E. coli</td>
<td>O103</td>
<td>PSU 5.1658</td>
<td>Not Available</td>
<td>+</td>
<td>48</td>
<td>E. coli</td>
<td>O145</td>
<td>MSU TW07596</td>
<td>Human</td>
<td>+</td>
</tr>
<tr>
<td>24</td>
<td>E. coli</td>
<td>O103</td>
<td>PSU 7.1691</td>
<td>Not Available</td>
<td>+</td>
<td>49</td>
<td>E. coli</td>
<td>O145</td>
<td>MSU TW09356</td>
<td>Human</td>
<td>+</td>
</tr>
<tr>
<td>25</td>
<td>E. coli</td>
<td>O103</td>
<td>PSU 9.0036</td>
<td>Not Available</td>
<td>+</td>
<td>50</td>
<td>E. coli</td>
<td>O145</td>
<td>MSU TW01664</td>
<td>Human</td>
<td>+</td>
</tr>
<tr>
<td>No.</td>
<td>Species</td>
<td>Serotype</td>
<td>Source</td>
<td>Origin</td>
<td>Result</td>
<td>No.</td>
<td>Species</td>
<td>Serotype</td>
<td>Source</td>
<td>Origin</td>
<td>Result</td>
</tr>
<tr>
<td>-----</td>
<td>---------</td>
<td>----------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td>-----</td>
<td>---------</td>
<td>----------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td>1</td>
<td>E. coli</td>
<td>O157</td>
<td>MSU TW00116</td>
<td>Human</td>
<td>+</td>
<td>26</td>
<td>E. coli</td>
<td>O157</td>
<td>MSU DEC3B</td>
<td>Human</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>E. coli</td>
<td>O157</td>
<td>MSU TW00975</td>
<td>Human</td>
<td>+</td>
<td>27</td>
<td>E. coli</td>
<td>O157</td>
<td>MSU DEC3C</td>
<td>Human</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>E. coli</td>
<td>O157</td>
<td>MSU TW02302</td>
<td>Hamburger</td>
<td>+</td>
<td>28</td>
<td>E. coli</td>
<td>O157</td>
<td>MSU DEC3D</td>
<td>Human</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>E. coli</td>
<td>O157</td>
<td>MSU TW04863</td>
<td>Human</td>
<td>+</td>
<td>29</td>
<td>E. coli</td>
<td>O157</td>
<td>MSU DEC3E</td>
<td>Human</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>E. coli</td>
<td>O157</td>
<td>MSU TW05356</td>
<td>Human</td>
<td>+</td>
<td>30</td>
<td>E. coli</td>
<td>O157</td>
<td>MSU DEC4A</td>
<td>Human</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>E. coli</td>
<td>O157</td>
<td>MSU TW07587</td>
<td>Human</td>
<td>+</td>
<td>31</td>
<td>E. coli</td>
<td>O157</td>
<td>MSU DEC4B</td>
<td>Human</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>E. coli</td>
<td>O157</td>
<td>ATCC BAA-460</td>
<td>Human Feces</td>
<td>+</td>
<td>32</td>
<td>E. coli</td>
<td>O157</td>
<td>MSU DEC4C</td>
<td>Buffalo</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>E. coli</td>
<td>O157</td>
<td>NCTC 12900</td>
<td>Not Available</td>
<td>+</td>
<td>33</td>
<td>E. coli</td>
<td>O157</td>
<td>MSU DEC4D</td>
<td>Cow, Calf</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>E. coli</td>
<td>O157</td>
<td>NCTC 13125</td>
<td>Human Stool</td>
<td>+</td>
<td>34</td>
<td>E. coli</td>
<td>O157</td>
<td>MSU DEC4E</td>
<td>Human</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>E. coli</td>
<td>O157</td>
<td>NCTC 13126</td>
<td>Not Available</td>
<td>+</td>
<td>35</td>
<td>E. coli</td>
<td>O157</td>
<td>QL 4 164673</td>
<td>Beef Trim</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>E. coli</td>
<td>O157</td>
<td>NCTC 13127</td>
<td>Not Available</td>
<td>+</td>
<td>36</td>
<td>E. coli</td>
<td>O157</td>
<td>QL 2-202</td>
<td>Meat</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>E. coli</td>
<td>O157</td>
<td>ATCC 35150</td>
<td>Human Feces</td>
<td>+</td>
<td>37</td>
<td>E. coli</td>
<td>O157</td>
<td>QL 2-203</td>
<td>Meat</td>
<td>+</td>
</tr>
<tr>
<td>13</td>
<td>E. coli</td>
<td>O157</td>
<td>ATCC 43888</td>
<td>Human Feces</td>
<td>+</td>
<td>38</td>
<td>E. coli</td>
<td>O157</td>
<td>QL 2-204</td>
<td>Meat</td>
<td>+</td>
</tr>
<tr>
<td>14</td>
<td>E. coli</td>
<td>O157</td>
<td>ATCC 43890</td>
<td>Human Feces</td>
<td>+</td>
<td>39</td>
<td>E. coli</td>
<td>O157</td>
<td>QL 2-205</td>
<td>Meat</td>
<td>+</td>
</tr>
<tr>
<td>15</td>
<td>E. coli</td>
<td>O157</td>
<td>ATCC 43895</td>
<td>Human Feces</td>
<td>+</td>
<td>40</td>
<td>E. coli</td>
<td>O157</td>
<td>QL 2-206</td>
<td>Meat</td>
<td>+</td>
</tr>
<tr>
<td>16</td>
<td>E. coli</td>
<td>O157</td>
<td>ATCC 43895</td>
<td>Human Feces</td>
<td>+</td>
<td>41</td>
<td>E. coli</td>
<td>O157</td>
<td>QL 2-207</td>
<td>Meat</td>
<td>+</td>
</tr>
<tr>
<td>17</td>
<td>E. coli</td>
<td>O157</td>
<td>ATCC 43894</td>
<td>Human Feces</td>
<td>+</td>
<td>42</td>
<td>E. coli</td>
<td>O157</td>
<td>QL 2-214</td>
<td>Meat</td>
<td>+</td>
</tr>
<tr>
<td>18</td>
<td>E. coli</td>
<td>O157</td>
<td>ATCC 51657</td>
<td>Clinical Isolate</td>
<td>+</td>
<td>43</td>
<td>E. coli</td>
<td>O157</td>
<td>QL 2-701</td>
<td>Beef</td>
<td>+</td>
</tr>
<tr>
<td>19</td>
<td>E. coli</td>
<td>O157</td>
<td>ATCC 51658</td>
<td>Clinical Isolate</td>
<td>+</td>
<td>44</td>
<td>E. coli</td>
<td>O157</td>
<td>QL 2-704</td>
<td>Beef</td>
<td>+</td>
</tr>
<tr>
<td>20</td>
<td>E. coli</td>
<td>O157</td>
<td>ATCC 51659</td>
<td>Clinical Isolate</td>
<td>+</td>
<td>45</td>
<td>E. coli</td>
<td>O157</td>
<td>QL 2-705</td>
<td>Beef</td>
<td>+</td>
</tr>
<tr>
<td>21</td>
<td>E. coli</td>
<td>O157</td>
<td>ATCC 700531</td>
<td>Clinical Isolate</td>
<td>+</td>
<td>46</td>
<td>E. coli</td>
<td>O157</td>
<td>QL 2-706</td>
<td>Beef</td>
<td>+</td>
</tr>
<tr>
<td>22</td>
<td>E. coli</td>
<td>O157</td>
<td>ATCC 700599</td>
<td>Salami</td>
<td>+</td>
<td>47</td>
<td>E. coli</td>
<td>O157</td>
<td>QL 2-707</td>
<td>Beef</td>
<td>+</td>
</tr>
<tr>
<td>23</td>
<td>E. coli</td>
<td>O157</td>
<td>ATCC 700927</td>
<td>Not Available</td>
<td>+</td>
<td>48</td>
<td>E. coli</td>
<td>O157</td>
<td>QL 2-708</td>
<td>Beef</td>
<td>+</td>
</tr>
<tr>
<td>24</td>
<td>E. coli</td>
<td>O157</td>
<td>ATCC 700927</td>
<td>Human</td>
<td>+</td>
<td>49</td>
<td>E. coli</td>
<td>O157</td>
<td>QL 2-710</td>
<td>Beef</td>
<td>+</td>
</tr>
<tr>
<td>25</td>
<td>E. coli</td>
<td>O157</td>
<td>MSU DEC3A</td>
<td>Human</td>
<td>+</td>
<td>50</td>
<td>E. coli</td>
<td>O157</td>
<td>QL 14077.1</td>
<td>Meat</td>
<td>+</td>
</tr>
</tbody>
</table>

1. MSU – Michigan State University Culture Collection, 2. ATCC – American Type Culture Collection, 3. NCTC – National Culture Type Collection, 4. QL – Q Laboratories Culture Collection
### Table 3: Inclusivity Results for *Salmonella*

<table>
<thead>
<tr>
<th>No.</th>
<th>Organism</th>
<th>Source</th>
<th>Origin</th>
<th>Result</th>
<th>No.</th>
<th>Organism</th>
<th>Source</th>
<th>Origin</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Salmonella bongori</em></td>
<td>NCTC(^1) 12419</td>
<td>Not Available</td>
<td>+</td>
<td>26</td>
<td><em>Salmonella Berta</em></td>
<td>UPENN STS 13</td>
<td>Not Available</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td><em>Salmonella bongori</em></td>
<td>ATCC(^2) 43975</td>
<td>Not Available</td>
<td>+</td>
<td>27</td>
<td><em>Salmonella Bina</em></td>
<td>UPENN STS 14</td>
<td>Not Available</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td><em>Salmonella bongori</em></td>
<td>NCTC 10946</td>
<td>Amphibian, Frog</td>
<td>+</td>
<td>28</td>
<td><em>Salmonella Bovis-Morbificans</em></td>
<td>UPENN STS 16</td>
<td>Not Available</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td><em>Salmonella Artes</em></td>
<td>ATCC 700149</td>
<td>Not Available</td>
<td>+</td>
<td>29</td>
<td><em>Salmonella Brandenburg</em></td>
<td>UPENN STS 18</td>
<td>Not Available</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td><em>Salmonella Salamae</em></td>
<td>QL(^3) 02415</td>
<td>Clinical Isolate</td>
<td>+</td>
<td>30</td>
<td><em>Salmonella Bredeney</em></td>
<td>NCTC 5731</td>
<td>Not Available</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td><em>Salmonella Basel</em></td>
<td>ATCC 700151</td>
<td>Not Available</td>
<td>+</td>
<td>31</td>
<td><em>Salmonella California</em></td>
<td>NCTC 6018</td>
<td>Not Available</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td><em>Salmonella Arizonae</em></td>
<td>ATCC 13314</td>
<td>Not Available</td>
<td>+</td>
<td>32</td>
<td><em>Salmonella Cerro</em></td>
<td>UPENN STS 22</td>
<td>Not Available</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td><em>Salmonella Arizonae</em></td>
<td>ATCC BAA-1577</td>
<td>Not Available</td>
<td>+</td>
<td>33</td>
<td><em>Salmonella Choleraeuis</em></td>
<td>ATCC 10708</td>
<td>Not Available</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td><em>Salmonella Arizonae</em></td>
<td>QL 11007-4</td>
<td>Veterinary</td>
<td>+</td>
<td>34</td>
<td><em>Salmonella Choleraeuis var Kunzendorf</em></td>
<td>ATCC 12011</td>
<td>Not Available</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td><em>Salmonella Diarizonae</em></td>
<td>ATCC BAA-1579</td>
<td>Not Available</td>
<td>+</td>
<td>35</td>
<td><em>Salmonella Cubana</em></td>
<td>UPENN STS 24</td>
<td>Not Available</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td><em>Salmonella Diarizonae</em></td>
<td>ATCC BAA-216</td>
<td>Human Blood</td>
<td>+</td>
<td>36</td>
<td><em>Salmonella Derby</em></td>
<td>NCTC 5721</td>
<td>Not Available</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td><em>Salmonella Diarizonae</em></td>
<td>ATCC BAA-639</td>
<td>Human Feces</td>
<td>+</td>
<td>37</td>
<td><em>Salmonella Drypool</em></td>
<td>UPENN STS 26</td>
<td>Not Available</td>
<td>+</td>
</tr>
<tr>
<td>13</td>
<td><em>Salmonella Abaetetuba</em></td>
<td>ATCC 35640</td>
<td>Creek Water</td>
<td>+</td>
<td>38</td>
<td><em>Salmonella Dublin</em></td>
<td>UPENN STS 27</td>
<td>Not Available</td>
<td>+</td>
</tr>
<tr>
<td>14</td>
<td><em>Salmonella Abortusequi</em></td>
<td>FDA(^4) 9842</td>
<td>Not Available</td>
<td>+</td>
<td>39</td>
<td><em>Salmonella Eastbourne</em></td>
<td>FDA 4017H</td>
<td>Not Available</td>
<td>+</td>
</tr>
<tr>
<td>15</td>
<td><em>Salmonella Abortusovis</em></td>
<td>NCTC10241</td>
<td>Not Available</td>
<td>+</td>
<td>40</td>
<td><em>Salmonella Enteritidis</em></td>
<td>ATCC 13076</td>
<td>Not Available</td>
<td>+</td>
</tr>
<tr>
<td>16</td>
<td><em>Salmonella Abony</em></td>
<td>NCTC 6017</td>
<td>Not Available</td>
<td>+</td>
<td>41</td>
<td><em>Salmonella Galiema</em></td>
<td>QL 024.2</td>
<td>Clinical Isolate</td>
<td>+</td>
</tr>
<tr>
<td>17</td>
<td><em>Salmonella Adelaide</em></td>
<td>UPENN(^5) STS 2</td>
<td>Not Available</td>
<td>+</td>
<td>42</td>
<td><em>Salmonella Give</em></td>
<td>UPENN STS 42</td>
<td>Not Available</td>
<td>+</td>
</tr>
<tr>
<td>18</td>
<td><em>Salmonella Agona</em></td>
<td>ATCC 51957</td>
<td>Not Available</td>
<td>+</td>
<td>43</td>
<td><em>Salmonella Haardt</em></td>
<td>UPENN STS 44</td>
<td>Not Available</td>
<td>+</td>
</tr>
<tr>
<td>19</td>
<td><em>Salmonella Agama</em></td>
<td>UPENN STS 3</td>
<td>Not Available</td>
<td>+</td>
<td>44</td>
<td><em>Salmonella Hadar</em></td>
<td>ATCC 51956</td>
<td>Not Available</td>
<td>+</td>
</tr>
<tr>
<td>20</td>
<td><em>Salmonella Agouye</em></td>
<td>UPENN STS 5</td>
<td>Not Available</td>
<td>+</td>
<td>45</td>
<td><em>Salmonella Havana</em></td>
<td>UPENN STS 47</td>
<td>Not Available</td>
<td>+</td>
</tr>
<tr>
<td>21</td>
<td><em>Salmonella Alachua</em></td>
<td>UPENN STS 6</td>
<td>Not Available</td>
<td>+</td>
<td>46</td>
<td><em>Salmonella Heidelberg</em></td>
<td>ATCC 8326</td>
<td>Not Available</td>
<td>+</td>
</tr>
<tr>
<td>22</td>
<td><em>Salmonella Albany</em></td>
<td>UPENN STS 7</td>
<td>Not Available</td>
<td>+</td>
<td>47</td>
<td><em>Salmonella Illinois</em></td>
<td>ATCC 11646</td>
<td>Not Available</td>
<td>+</td>
</tr>
<tr>
<td>23</td>
<td><em>Salmonella Anatum</em></td>
<td>ATCC 9270</td>
<td>Pork Liver</td>
<td>+</td>
<td>48</td>
<td><em>Salmonella Indiana</em></td>
<td>NCTC 11304</td>
<td>Turkey</td>
<td>+</td>
</tr>
<tr>
<td>24</td>
<td><em>Salmonella Arkansas</em></td>
<td>UPENN STS 11</td>
<td>Not Available</td>
<td>+</td>
<td>49</td>
<td><em>Salmonella Infantis</em></td>
<td>ATCC 51741</td>
<td>Pasta</td>
<td>+</td>
</tr>
<tr>
<td>25</td>
<td><em>Salmonella Bareilly</em></td>
<td>FDA 1206H</td>
<td>Not Available</td>
<td>+</td>
<td>50</td>
<td><em>Salmonella Javiana</em></td>
<td>ATCC 10721</td>
<td>Not Available</td>
<td>+</td>
</tr>
</tbody>
</table>

1. NCTC – National Culture Type Collection, 2. ATCC – American Type Culture Collection, 3. QL – Q Laboratories Culture Collection, 4. FDA – US Food and Drug Administration Culture Collection, 5. UPENN – University of Pennsylvania Culture Collection
### Table 3: Inclusivity Results for *Salmonella* (continued)

<table>
<thead>
<tr>
<th>No.</th>
<th>Organism</th>
<th>Source</th>
<th>Origin</th>
<th>Result</th>
<th>No.</th>
<th>Organism</th>
<th>Source</th>
<th>Origin</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>51</td>
<td><em>Salmonella Jerusalem</em></td>
<td>QL^1^ 024.12</td>
<td>Dry Dog Food</td>
<td>+</td>
<td>76</td>
<td><em>Salmonella Paratyphi A</em></td>
<td>ATCC 9150</td>
<td>Not Available</td>
<td>+</td>
</tr>
<tr>
<td>52</td>
<td><em>Salmonella Johannesburg</em></td>
<td>UPENN^2^ STS 56</td>
<td>Not Available</td>
<td>+</td>
<td>77</td>
<td><em>Salmonella Paratyphi B</em></td>
<td>ATCC 10719</td>
<td>Not Available</td>
<td>+</td>
</tr>
<tr>
<td>53</td>
<td><em>Salmonella Kahla</em></td>
<td>ATCC^3^ 17980</td>
<td>Human Feces</td>
<td>+</td>
<td>78</td>
<td><em>Salmonella Paratyphi C</em></td>
<td>ATCC 13428</td>
<td>Not Available</td>
<td>+</td>
</tr>
<tr>
<td>54</td>
<td><em>Salmonella Kaitaan</em></td>
<td>QL 024.7</td>
<td>Clinical Isolate</td>
<td>+</td>
<td>79</td>
<td><em>Salmonella Pomona</em></td>
<td>ATCC 10729</td>
<td>Clinical Isolate</td>
<td>+</td>
</tr>
<tr>
<td>55</td>
<td><em>Salmonella Kentucky</em></td>
<td>ATCC 9263</td>
<td>Not Available</td>
<td>+</td>
<td>80</td>
<td><em>Salmonella Poona</em></td>
<td>NCTC 4840</td>
<td>Infant</td>
<td>+</td>
</tr>
<tr>
<td>56</td>
<td><em>Salmonella Krefeld</em></td>
<td>UPENN STS 58</td>
<td>Not Available</td>
<td>+</td>
<td>81</td>
<td><em>Salmonella Preston</em></td>
<td>QL 024.16</td>
<td>Clinical Isolate</td>
<td>+</td>
</tr>
<tr>
<td>57</td>
<td><em>Salmonella Indica</em></td>
<td>ATCC BAA-1578</td>
<td>Unknown, India</td>
<td>+</td>
<td>82</td>
<td><em>Salmonella Pullorum</em></td>
<td>ATCC 13036</td>
<td>Egg</td>
<td>+</td>
</tr>
<tr>
<td>58</td>
<td><em>Salmonella Ferlac</em></td>
<td>ATCC 43976</td>
<td>Not Available</td>
<td>+</td>
<td>83</td>
<td><em>Salmonella Rubislaw</em></td>
<td>UPENN STS 92</td>
<td>Not Available</td>
<td>+</td>
</tr>
<tr>
<td>59</td>
<td><em>Salmonella Ferlac</em></td>
<td>NCTC^4^ 10458</td>
<td>Desiccated Coconut</td>
<td>+</td>
<td>84</td>
<td><em>Salmonella Saintpaul</em></td>
<td>ATCC 9712</td>
<td>Cystitis</td>
<td>+</td>
</tr>
<tr>
<td>60</td>
<td><em>Salmonella Lille</em></td>
<td>UPENN STS 59</td>
<td>Not Available</td>
<td>+</td>
<td>85</td>
<td><em>Salmonella San-Diego</em></td>
<td>UPENN STS 94</td>
<td>Not Available</td>
<td>+</td>
</tr>
<tr>
<td>61</td>
<td><em>Salmonella Livingstone</em></td>
<td>UPENN STS 63</td>
<td>Not Available</td>
<td>+</td>
<td>86</td>
<td><em>Salmonella Schalkwijk</em></td>
<td>QL 024.10</td>
<td>Clinical Isolate</td>
<td>+</td>
</tr>
<tr>
<td>62</td>
<td><em>Salmonella London</em></td>
<td>UPENN STS 64</td>
<td>Not Available</td>
<td>+</td>
<td>87</td>
<td><em>Salmonella Schwarzengrund</em></td>
<td>UPENN STS 95</td>
<td>Not Available</td>
<td>+</td>
</tr>
<tr>
<td>63</td>
<td><em>Salmonella Manhattan</em></td>
<td>UPENN STS 65</td>
<td>Not Available</td>
<td>+</td>
<td>88</td>
<td><em>Salmonella Serftenberg</em></td>
<td>ATCC 43845</td>
<td>Not Available</td>
<td>+</td>
</tr>
<tr>
<td>64</td>
<td><em>Salmonella Mbankaka</em></td>
<td>FDA^5^ 37N</td>
<td>Not Available</td>
<td>+</td>
<td>89</td>
<td><em>Salmonella Stanley</em></td>
<td>ATCC 7308</td>
<td>Not Available</td>
<td>+</td>
</tr>
<tr>
<td>65</td>
<td><em>Salmonella Menden</em></td>
<td>ATCC 15992</td>
<td>Human Feces</td>
<td>+</td>
<td>90</td>
<td><em>Salmonella Tallahasee</em></td>
<td>ATCC 12002</td>
<td>Not Available</td>
<td>+</td>
</tr>
<tr>
<td>66</td>
<td><em>Salmonella Meleagridis</em></td>
<td>QL 12074-1</td>
<td>Not Available</td>
<td>+</td>
<td>91</td>
<td><em>Salmonella Tennessee</em></td>
<td>QL 024.6</td>
<td>Clinical Isolate</td>
<td>+</td>
</tr>
<tr>
<td>67</td>
<td><em>Salmonella Menhaden</em></td>
<td>QL 024.20</td>
<td>Clinical Isolate</td>
<td>+</td>
<td>92</td>
<td><em>Salmonella Thompson</em></td>
<td>FDA 2051H</td>
<td>Not Available</td>
<td>+</td>
</tr>
<tr>
<td>68</td>
<td><em>Salmonella Montevideo</em></td>
<td>ATCC 8387</td>
<td>Not Available</td>
<td>+</td>
<td>93</td>
<td><em>Salmonella Typhi</em></td>
<td>ATCC 6539</td>
<td>Not Available</td>
<td>+</td>
</tr>
<tr>
<td>69</td>
<td><em>Salmonella Muenchen</em></td>
<td>ATCC BAA-1594</td>
<td>Roma Tomatoes</td>
<td>+</td>
<td>94</td>
<td><em>Salmonella Typhimurium</em></td>
<td>ATCC 14028</td>
<td>Animal Tissue</td>
<td>+</td>
</tr>
<tr>
<td>70</td>
<td><em>Salmonella Neasden</em></td>
<td>QL 024.4</td>
<td>Clinical Isolate</td>
<td>+</td>
<td>95</td>
<td><em>Salmonella Utrecht</em></td>
<td>NCTC 10077</td>
<td>Not Available</td>
<td>+</td>
</tr>
<tr>
<td>71</td>
<td><em>Salmonella Newington</em></td>
<td>QL 0248</td>
<td>Clinical Isolate</td>
<td>+</td>
<td>96</td>
<td><em>Salmonella Urbana</em></td>
<td>UPENN STS 110</td>
<td>Not Available</td>
<td>+</td>
</tr>
<tr>
<td>72</td>
<td><em>Salmonella Newport</em></td>
<td>ATCC 6962</td>
<td>Unknown, England</td>
<td>+</td>
<td>97</td>
<td><em>Salmonella Vellore</em></td>
<td>ATCC 15611</td>
<td>Rectal Swab</td>
<td>+</td>
</tr>
<tr>
<td>73</td>
<td><em>Salmonella Ohio</em></td>
<td>UPENN STS 81</td>
<td>Unknown, Illinois Hospital</td>
<td>+</td>
<td>98</td>
<td><em>Salmonella Virchow</em></td>
<td>ATCC 51955</td>
<td>Not Available</td>
<td>+</td>
</tr>
<tr>
<td>74</td>
<td><em>Salmonella Oranienburg</em></td>
<td>ATCC 9239</td>
<td>Not Available</td>
<td>+</td>
<td>99</td>
<td><em>Salmonella Volta</em></td>
<td>QL 024.9</td>
<td>Clinical Isolate</td>
<td>+</td>
</tr>
<tr>
<td>75</td>
<td><em>Salmonella Orthmarshen</em></td>
<td>QL 024.13</td>
<td>Clinical Isolate</td>
<td>+</td>
<td>100</td>
<td><em>Salmonella Westhampton</em></td>
<td>QL 024.14</td>
<td>Clinical Isolate</td>
<td>+</td>
</tr>
</tbody>
</table>

1. QL – Q Laboratories Culture Collection, 2. UPENN – University of Pennsylvania Culture Collection, 3. ATCC – American Type Culture Collection, 4. NCTC – National Culture Type Collection, 5. FDA – US Food and Drug Administration Culture Collection
### Table 4: Exclusivity Results for Gram Negative Organisms

<table>
<thead>
<tr>
<th>No</th>
<th>Organism</th>
<th>Source</th>
<th>Origin</th>
<th>Result</th>
<th>No</th>
<th>Organism</th>
<th>Source</th>
<th>Origin</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Alcaligenes faecalis</em></td>
<td>ATCC(^1) 8750</td>
<td>Not Available</td>
<td>-</td>
<td>16</td>
<td><em>Escherichia hermanii</em></td>
<td>ATCC 33650</td>
<td>Mouse Brain</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td><em>Aeromonas hydrophila</em></td>
<td>ATCC 49140</td>
<td>Clinical Isolate</td>
<td>-</td>
<td>17</td>
<td><em>Escherichia vulneris</em></td>
<td>ATCC 29943</td>
<td>Human Wound</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td><em>Citrobacter braakii</em></td>
<td>ATCC 43162</td>
<td>Clinical Isolate</td>
<td>-</td>
<td>18</td>
<td><em>Haemophilus influenzae</em></td>
<td>ATCC 51815</td>
<td>Milk</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td><em>Citrobacter farmeri</em></td>
<td>ATCC 51633</td>
<td>Human Feces</td>
<td>-</td>
<td>19</td>
<td><em>Haemophilus influenzae</em></td>
<td>ATCC 19418</td>
<td>Not Available</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td><em>Cronobacter sakazakii</em></td>
<td>QL(^2) 17031.4</td>
<td>Infant Formula</td>
<td>-</td>
<td>20</td>
<td><em>Klebsiella pneumoniae</em></td>
<td>ATCC 4352</td>
<td>Cow Milk</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td><em>Edwardsiella tarda</em></td>
<td>ATCC 15947</td>
<td>Human Feces</td>
<td>-</td>
<td>21</td>
<td><em>Moranella morganii</em></td>
<td>ATCC 25829</td>
<td>Human</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td><em>Enterobacter aerogenes</em></td>
<td>ATCC 13048</td>
<td>Sputum</td>
<td>-</td>
<td>22</td>
<td><em>Mycobacterium smegmatis</em></td>
<td>ATCC 19420</td>
<td>Not Available</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td><em>Escherichia blattae</em></td>
<td>ATCC 29907</td>
<td>Insect</td>
<td>-</td>
<td>23</td>
<td><em>Pantoea agglomerans</em></td>
<td>ATCC 19552</td>
<td>Sewage</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td><em>Escherichia coli</em> O55</td>
<td>MSU(^3) DEC1A</td>
<td>Human Feces</td>
<td>-</td>
<td>24</td>
<td><em>Proteus mirabilis</em></td>
<td>ATCC 7002</td>
<td>Urine</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td><em>Escherichia coli</em> O113</td>
<td>NCTC(^4) 9113</td>
<td>Not Available</td>
<td>-</td>
<td>25</td>
<td><em>Providencia rettgeri</em></td>
<td>ATCC 14505</td>
<td>Not Available</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td><em>Escherichia coli</em> O115</td>
<td>NCTC10444</td>
<td>Calf</td>
<td>-</td>
<td>26</td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>ATCC 9027</td>
<td>Ear Infection</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td><em>Escherichia coli</em> O117</td>
<td>NCTC 9117</td>
<td>Not Available</td>
<td>-</td>
<td>27</td>
<td><em>Rahnella aquatilis</em></td>
<td>ATCC 55046</td>
<td>Soil</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td><em>Escherichia coli</em> O118</td>
<td>NCTC 9118</td>
<td>Not Available</td>
<td>-</td>
<td>28</td>
<td><em>Serratia marcescens</em></td>
<td>ATCC 13880</td>
<td>Human</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td><em>Escherichia coli</em> O163</td>
<td>NCTC 11021</td>
<td>Human Feces</td>
<td>-</td>
<td>29</td>
<td><em>Shigella boydii</em></td>
<td>ATCC 9290</td>
<td>Pork Liver</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td><em>Escherichia fergusonii</em></td>
<td>ATCC 35469</td>
<td>Human Feces</td>
<td>-</td>
<td>30</td>
<td><em>Vibrio vulnificus</em></td>
<td>QL 02111-1A</td>
<td>Seafood Product</td>
<td>-</td>
</tr>
</tbody>
</table>

1. ATCC – American Type Culture Collection, 2. QL – Q Laboratories Culture Collection, 3. MSU – Michigan State University Culture Collection, 4. NCTC – National Culture Type Collection
Table 5: Inclusivity Results for *Listeria monocytogenes*

<table>
<thead>
<tr>
<th>No.</th>
<th>Organism</th>
<th>Source</th>
<th>Origin</th>
<th>Result</th>
<th>No.</th>
<th>Organism</th>
<th>Source</th>
<th>Origin</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>L. monocytogenes (1/2C)</td>
<td>CWD 1 553</td>
<td>Not Available</td>
<td>+</td>
<td>26</td>
<td>L. monocytogenes (N/A)</td>
<td>ATCC 19113</td>
<td>Not Available</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>L. monocytogenes (1/2A)</td>
<td>CWD 1554</td>
<td>Unknown, Carlisle, 1981</td>
<td>+</td>
<td>27</td>
<td>L. monocytogenes (4A)</td>
<td>ATCC 19114</td>
<td>Animal Tissue</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>L. monocytogenes (4B)</td>
<td>CWD 1563</td>
<td>Unknown, Lausanne, 1987</td>
<td>+</td>
<td>28</td>
<td>L. monocytogenes (4B)</td>
<td>ATCC 19115</td>
<td>Human</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>L. monocytogenes (4B)</td>
<td>CWD 1567</td>
<td>Unknown, Los Angeles, 1985</td>
<td>+</td>
<td>29</td>
<td>L. monocytogenes (4C)</td>
<td>ATCC 19116</td>
<td>Chicken</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>L. monocytogenes (4B)</td>
<td>CWD 1571</td>
<td>Not Available</td>
<td>+</td>
<td>30</td>
<td>L. monocytogenes (4E)</td>
<td>ATCC 19118</td>
<td>Chicken</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>L. monocytogenes (4B)</td>
<td>CWD 1590</td>
<td>Unknown, San Francisco</td>
<td>+</td>
<td>31</td>
<td>L. monocytogenes (N/A)</td>
<td>ATCC 49953</td>
<td>Goat, Belgium</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>L. monocytogenes (3B)</td>
<td>CWD 1600</td>
<td>Not Available</td>
<td>+</td>
<td>32</td>
<td>L. monocytogenes (1/2A)</td>
<td>ATCC 49594</td>
<td>Food, France</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>L. monocytogenes (1/2A)</td>
<td>CWD 1609</td>
<td>Turkey Factory</td>
<td>+</td>
<td>33</td>
<td>L. monocytogenes (3A)</td>
<td>ATCC 51782</td>
<td>Cheese</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>L. monocytogenes (1/2A)</td>
<td>CWD 1620</td>
<td>Turkey Factory</td>
<td>+</td>
<td>34</td>
<td>L. monocytogenes (N/A)</td>
<td>ATCC BAA- 2658</td>
<td>Not Available</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>L. monocytogenes (1/2B)</td>
<td>CWD 1626</td>
<td>Turkey Franks</td>
<td>+</td>
<td>35</td>
<td>L. monocytogenes (N/A)</td>
<td>QL5 030911-10</td>
<td>Clinical</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>L. monocytogenes (1/2B)</td>
<td>CWD 1627</td>
<td>Mother/Baby</td>
<td>+</td>
<td>36</td>
<td>L. monocytogenes (4B)</td>
<td>CWD 1561</td>
<td>Placenta</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>L. monocytogenes (4D)</td>
<td>ATCC 19117</td>
<td>Sheep</td>
<td>+</td>
<td>37</td>
<td>L. monocytogenes (1/2B)</td>
<td>CWD 1601</td>
<td>Unknown, Los Angeles</td>
<td>+</td>
</tr>
<tr>
<td>13</td>
<td>L. monocytogenes (1/2A)</td>
<td>ATCC 51772</td>
<td>Not Available</td>
<td>+</td>
<td>38</td>
<td>L. monocytogenes (1/2A)</td>
<td>CWD 1612</td>
<td>Turkey Factory</td>
<td>+</td>
</tr>
<tr>
<td>14</td>
<td>L. monocytogenes (4B)</td>
<td>ATCC 51778</td>
<td>Dairy Products</td>
<td>+</td>
<td>39</td>
<td>L. monocytogenes (1/A)</td>
<td>CWD 1613</td>
<td>Turkey Factory</td>
<td>+</td>
</tr>
<tr>
<td>15</td>
<td>L. monocytogenes (1/2B)</td>
<td>ATCC 51780</td>
<td>Cheese</td>
<td>+</td>
<td>40</td>
<td>L. monocytogenes (1/2A)</td>
<td>CWD 1614</td>
<td>Unknown, Oklahoma</td>
<td>+</td>
</tr>
<tr>
<td>16</td>
<td>L. monocytogenes (1/2B)</td>
<td>ATCC BAA-751</td>
<td>Not Available</td>
<td>+</td>
<td>41</td>
<td>L. monocytogenes (1/2A)</td>
<td>CWD 1618</td>
<td>Turkey Factory</td>
<td>+</td>
</tr>
<tr>
<td>17</td>
<td>L. monocytogenes (7)</td>
<td>NCTC 10890</td>
<td>Human Feces</td>
<td>+</td>
<td>42</td>
<td>L. monocytogenes (1/2A)</td>
<td>CWD 1629</td>
<td>Turkey Franks</td>
<td>+</td>
</tr>
<tr>
<td>18</td>
<td>L. monocytogenes (4B)</td>
<td>FSL 4-F6-367</td>
<td>Not Available</td>
<td>+</td>
<td>43</td>
<td>L. monocytogenes (1/2A)</td>
<td>CWD 1630</td>
<td>Turkey Factory</td>
<td>+</td>
</tr>
<tr>
<td>19</td>
<td>L. monocytogenes (4AB)</td>
<td>FSL J1-129</td>
<td>Not Available</td>
<td>+</td>
<td>44</td>
<td>L. monocytogenes (4B)</td>
<td>CWD 1574</td>
<td>Unknown, Halifax, 1983</td>
<td>+</td>
</tr>
<tr>
<td>20</td>
<td>L. monocytogenes (3C)</td>
<td>FSL J1-049</td>
<td>Not Available</td>
<td>+</td>
<td>45</td>
<td>L. monocytogenes (1/2B)</td>
<td>CWD 1584</td>
<td>Not Available</td>
<td>+</td>
</tr>
<tr>
<td>21</td>
<td>L. monocytogenes (1/2C)</td>
<td>ATCC 7644</td>
<td>Human</td>
<td>+</td>
<td>46</td>
<td>L. monocytogenes (3B)</td>
<td>CWD 1586</td>
<td>Not Available</td>
<td>+</td>
</tr>
<tr>
<td>22</td>
<td>L. monocytogenes (4B)</td>
<td>ATCC 13932</td>
<td>Child with Meningitis</td>
<td>+</td>
<td>47</td>
<td>L. monocytogenes (1/2B)</td>
<td>CWD 1588</td>
<td>Not Available</td>
<td>+</td>
</tr>
<tr>
<td>23</td>
<td>L. monocytogenes (1/2A)</td>
<td>ATCC 15313</td>
<td>Rabbit</td>
<td>+</td>
<td>48</td>
<td>L. monocytogenes (4B)</td>
<td>CWD 1596</td>
<td>Not Available</td>
<td>+</td>
</tr>
</tbody>
</table>
Table 6: Exclusivity Results for *Listeria monocytogenes*

<table>
<thead>
<tr>
<th>No</th>
<th>Organism</th>
<th>Source</th>
<th>Origin</th>
<th>Result</th>
<th>No</th>
<th>Organism</th>
<th>Source</th>
<th>Origin</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>L. grayi</em></td>
<td>ATCC 19120</td>
<td>Animal Feces</td>
<td>-</td>
<td>16</td>
<td><em>Enterococcus faecalis</em></td>
<td>ATCC 19433</td>
<td>Not Available</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td><em>L. innocua</em></td>
<td>ATCC 33090</td>
<td>Cow Brain</td>
<td>-</td>
<td>17</td>
<td><em>Kurthia gibsonii</em></td>
<td>ATCC 43195</td>
<td>Meat</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td><em>L. ivanovii</em></td>
<td>ATCC 19119</td>
<td>Sheep</td>
<td>-</td>
<td>18</td>
<td><em>Lactobacillus fermentum</em></td>
<td>ATCC 9338</td>
<td>Not Available</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td><em>L. marthii</em></td>
<td>ATCC BAA-1595</td>
<td>Soil</td>
<td>-</td>
<td>19</td>
<td><em>Lactobacillus acidophilus</em></td>
<td>ATCC 314</td>
<td>Not Available</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td><em>L. rocourtiae</em></td>
<td>FSL^2^ F6-0920</td>
<td>Not Available</td>
<td>-</td>
<td>20</td>
<td><em>Lactobacillus plantarum</em></td>
<td>ATCC 8014</td>
<td>Not Available</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td><em>L. welshimeri</em></td>
<td>ATCC 35897</td>
<td>Not Available</td>
<td>-</td>
<td>21</td>
<td><em>Lactococcus lactis</em></td>
<td>ATCC 4797</td>
<td>Not Available</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td><em>L. seeligeri</em></td>
<td>ATCC 35967</td>
<td>Soil</td>
<td>-</td>
<td>22</td>
<td><em>Rhodococcus equi</em></td>
<td>ATCC 6939</td>
<td>Not Available</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td><em>Aeromonas hydrophila</em></td>
<td>ATCC 49140</td>
<td>Clinical Isolate</td>
<td>-</td>
<td>23</td>
<td><em>Staphylococcus aureus</em></td>
<td>ATCC 29213</td>
<td>Wound</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td><em>Bacillus cereus</em></td>
<td>ATCC 6464</td>
<td>Soil</td>
<td>-</td>
<td>24</td>
<td><em>Staphylococcus saprophyticus</em></td>
<td>ATCC 15305</td>
<td>Urine</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td><em>Bacillus mycoides</em></td>
<td>ATCC 6462</td>
<td>Soil</td>
<td>-</td>
<td>25</td>
<td><em>Staphylococcus epidermidis</em></td>
<td>ATCC 12228</td>
<td>Not Available</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td><em>Bacillus subtilis</em></td>
<td>ATCC 27370</td>
<td>Not Available</td>
<td>-</td>
<td>26</td>
<td><em>Staphylococcus haemolyticus</em></td>
<td>ATCC 29970</td>
<td>Human Skin</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td><em>Bacillus licheniformis</em></td>
<td>ATCC 12759</td>
<td>Plant</td>
<td>-</td>
<td>27</td>
<td><em>Staphylococcus hominis</em></td>
<td>ATCC 27844</td>
<td>Human Skin</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td><em>Brochothrix thermosphacta</em></td>
<td>ATCC 11509</td>
<td>Animal Derived Foodstuff</td>
<td>-</td>
<td>28</td>
<td><em>Staphylococcus warneri</em></td>
<td>ATCC 29885</td>
<td>Not Available</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td><em>Enterobacter cloacae</em></td>
<td>ATCC 23355</td>
<td>Not Available</td>
<td>-</td>
<td>29</td>
<td><em>Streptococcus mutans</em></td>
<td>ATCC 25175</td>
<td>Not Available</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td><em>Enterococcus durans</em></td>
<td>ATCC 19432</td>
<td>Not Available</td>
<td>-</td>
<td>30</td>
<td><em>Streptococcus pyogenes</em></td>
<td>ATCC 19615</td>
<td>Pharynx of Child</td>
<td>-</td>
</tr>
</tbody>
</table>

1. ATCC – American Type Culture Collection, 2. FSL – Cornell University Culture Collection
Table 7: Aerobic Plate Count and Background Results (Prior to Inoculation)

| Matrix                | APC\(^1\) (CFU/g) | E. coli O157 Pathogen Screen\(^2\)  
(325 g test portions) | Listeria monocytogenes Pathogen Screen\(^3\)  
(25 g test portions) | Salmonella Pathogen Screen\(^4\)  
(25 g test portions) |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Deli Turkey</td>
<td>1.8 x 10(^5)</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>Ground Beef</td>
<td>1.8 x 10(^5)</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>Fresh Baby Spinach</td>
<td>1.8 x 10(^5)</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
</tbody>
</table>

\(^1\) APC conducted in accordance with FDA/BAM Chapter 3  
\(^2\) E. coli O157:H7 screen conducted following the USDA/FSIS MLG 5.09 reference method  
\(^3\) Listeria monocytogenes screen conducted following the USDA/FSIS MLG 8.10 reference method  
\(^4\) Salmonella screen conducted following the USDA/FSIS MLG 4.09 reference method  
\(^5\) Non-O157 STEC screen conducted following the USDA/FSIS MLG 5B.05 reference method  
\(^6\) STEC screen conducted following the ISO/TS STEC 13136: 2012 reference method  
\(^7\) Listeria monocytogenes screen conducted following the FDA/BAM Chapter 10 reference method  
\(^8\) Salmonella screen conducted following the FDA/BAM Chapter 5 reference method

Table 8: Inoculum Heat Stress Results

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Inoculating Organism</th>
<th>Agar</th>
<th>CFU/ g</th>
<th>Percent Injury</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deli Turkey</td>
<td>E. coli O26</td>
<td>TSA</td>
<td>6.0 x 10(^9)</td>
<td>58.33 %</td>
</tr>
<tr>
<td></td>
<td>MSU TW00971</td>
<td>mRBA</td>
<td>2.5 x 10(^8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Listeria monocytogenes</td>
<td>TSA</td>
<td>2.0 x 10(^9)</td>
<td>72.50 %</td>
</tr>
<tr>
<td></td>
<td>ATCC 19115</td>
<td>MOX</td>
<td>5.5 x 10(^7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Salmonella Dublin</td>
<td>TSA</td>
<td>4.2 x 10(^9)</td>
<td>64.29 %</td>
</tr>
<tr>
<td></td>
<td>ATCC 15480</td>
<td>XLT4</td>
<td>1.5 x 10(^8)</td>
<td></td>
</tr>
</tbody>
</table>

TSA: Trypticase Soy Agar  
mRBA: Modified Rainbow Agar  
MOX: Modified Oxford Agar  
XLT4: Xylose Lysine Tergitol 4 agar
### Table 9: Inoculum Summary Table for Stainless Steel Environmental Surface

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Stainless Steel</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculating Organism</td>
<td>*, O103</td>
<td>Listeria monocytogenes</td>
<td>Salmonella Kentucky</td>
<td></td>
</tr>
<tr>
<td>Low-Inoculum Level CFU(^a)/Test Area(^b)</td>
<td>51</td>
<td>64</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>High-Inoculum Level CFU(^a)/Test Area(^b)</td>
<td>440</td>
<td>650</td>
<td>580</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) CFU: aliquots of the inocula were plated in triplicate onto TSA and averaged

\(^b\) Test Area: 4" x 4" Surface Area

### Table 10A: MPN Summary Table for Fresh Raw Ground Beef

#### Escherichia coli O157:H7 ATCC 43895

<table>
<thead>
<tr>
<th>Low Level Inoculum (0.2-2 MPN/Test Portion)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
<td>E</td>
</tr>
<tr>
<td>5 x 50 g</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>20 x 25 g (Reference Samples)</td>
<td></td>
<td></td>
<td></td>
<td>6/20</td>
</tr>
<tr>
<td>5 x 10 g</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MPN/Test portion</td>
<td></td>
<td></td>
<td></td>
<td>0.44</td>
</tr>
<tr>
<td>Low Conf. Limit MPN/Test Portion</td>
<td></td>
<td></td>
<td></td>
<td>0.21</td>
</tr>
<tr>
<td>High Conf. Limit MPN/Test Portion</td>
<td></td>
<td></td>
<td></td>
<td>0.76</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>High Level Inoculum (2-5 MPN/Test Portion)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
<td>E</td>
</tr>
<tr>
<td>5 x 50 g</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>20 x 25 g (Reference Samples)</td>
<td></td>
<td></td>
<td></td>
<td>5/5</td>
</tr>
<tr>
<td>5 x 10 g</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>MPN/Test portion</td>
<td></td>
<td></td>
<td></td>
<td>3.01</td>
</tr>
<tr>
<td>Low Conf. Limit MPN/Test Portion</td>
<td></td>
<td></td>
<td></td>
<td>1.31</td>
</tr>
<tr>
<td>High Conf. Limit MPN/Test Portion</td>
<td></td>
<td></td>
<td></td>
<td>6.89</td>
</tr>
</tbody>
</table>

\(^1\) MPN was calculated for the low level inoculation for fresh raw ground beef using five 50 g, five 10 g, and the 20 low level reference method samples (25 g) using the LCF MPN Calculator version 1.6 provided by AOAC-RI [http://www.lclfld.com/customer/LCFMPNCalculator](http://www.lclfld.com/customer/LCFMPNCalculator)

\(^2\) MPN was calculated for the high level inoculation for fresh raw ground beef using five 50 g, five 10 g and the 5 high level reference method samples (25 g) using the LCF MPN Calculator version 1.6 provided by AOAC-RI [http://www.lclfld.com/customer/LCFMPNCalculator](http://www.lclfld.com/customer/LCFMPNCalculator)
Table 10B: MPN Summary Table for Fresh Raw Ground Beef

<table>
<thead>
<tr>
<th>Listeria monocytogenes ATCC 7644</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Low Level Inoculum (0.2-2 MPN/Test Portion)</strong></td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
<td>E</td>
</tr>
<tr>
<td>5 x 50 g</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>20 x 25 g (Reference Samples)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8/20</td>
</tr>
<tr>
<td>5 x 10 g</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>MPN/Test portion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.55</td>
</tr>
<tr>
<td>Low Conf. Limit MPN/Test Portion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.29</td>
</tr>
<tr>
<td>High Conf. Limit MPN/Test Portion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.94</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>High Level Inoculum (2-5 MPN/Test Portion)</strong></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 x 50 g</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>20 x 25 g (Reference Samples)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5/5</td>
</tr>
<tr>
<td>5 x 10 g</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>MPN/Test portion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.01</td>
</tr>
<tr>
<td>Low Conf. Limit MPN/Test Portion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.31</td>
</tr>
<tr>
<td>High Conf. Limit MPN/Test Portion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6.89</td>
</tr>
</tbody>
</table>

1 MPN was calculated for the low level inoculation for fresh raw ground beef using five 50 g, five 10 g, and the 20 low level reference method samples (25 g) using the LCF MPN Calculator version 1.6 provided by AOAC-RI [http://www.lcfltd.com/customer/LCFMPNCalculator](http://www.lcfltd.com/customer/LCFMPNCalculator)

2 MPN was calculated for the high level inoculation for fresh raw ground beef using five 50 g, five 10 g and the 5 high level reference method samples (25 g) using the LCF MPN Calculator version 1.6 provided by AOAC-RI [http://www.lcfltd.com/customer/LCFMPNCalculator](http://www.lcfltd.com/customer/LCFMPNCalculator)
Table 10C: MPN Summary Table for Fresh Raw Ground Beef

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Low Level Inoculum (0.2-2 MPN/Test Portion)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 x 50 g</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>20 x 25 g (Reference Samples)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 x 10 g</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MPN/Test portion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low Conf. Limit MPN/Test Portion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High Conf. Limit MPN/Test Portion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>High Level Inoculum (2-5 MPN/Test Portion)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 x 50 g</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>20 x 25 g (Reference Samples)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 x 10 g</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MPN/Test portion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low Conf. Limit MPN/Test Portion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High Conf. Limit MPN/Test Portion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1MPN was calculated for the low level inoculation for fresh raw ground beef using five 50 g, five 10 g, and the 20 low level reference method samples (25 g) using the LCF MPN Calculator version 1.6 provided by AOAC-RI [http://www.lcfltd.com/customer/LCFMPNCalculator](http://www.lcfltd.com/customer/LCFMPNCalculator)

2MPN was calculated for the high level inoculation for fresh raw ground beef using five 50 g, five 10 g and the 5 high level reference method samples (25 g) using the LCF MPN Calculator version 1.6 provided by AOAC-RI [http://www.lcfltd.com/customer/LCFMPNCalculator](http://www.lcfltd.com/customer/LCFMPNCalculator)
# Table 11A: MPN Summary Table for Deli Turkey

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Escherichia coli</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O26 MSU TW00971</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Low Level Inoculum (0.2-2 MPN/Test Portion)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 x 50 g</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>20 x 25 g (Reference Samples)</td>
<td>5/20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 x 10 g</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>MPN/Test portion</td>
<td>0.35</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low Conf. Limit MPN/Test Portion</td>
<td>0.14</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High Conf. Limit MPN/Test Portion</td>
<td>0.62</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>High Level Inoculum (2-5 MPN/Test Portion)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 x 50 g</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>20 x 25 g (Reference Samples)</td>
<td>5/5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 x 10 g</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>MPN/Test portion</td>
<td>3.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low Conf. Limit MPN/Test Portion</td>
<td>1.31</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High Conf. Limit MPN/Test Portion</td>
<td>6.89</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1MPN was calculated for the low level inoculation for deli turkey using five 50 g, five 10 g, and the 20 low level reference method samples (25 g) using the LCF MPN Calculator version 1.6 provided by AOAC-RI [http://www.lcfltd.com/customer/LCFMPNCalculator](http://www.lcfltd.com/customer/LCFMPNCalculator)

2MPN was calculated for the high level inoculation for deli turkey using five 50 g, five 10 g and the 5 high level reference method samples (25 g) using the LCF MPN Calculator version 1.6 provided by AOAC-RI [http://www.lcfltd.com/customer/LCFMPNCalculator](http://www.lcfltd.com/customer/LCFMPNCalculator)
Table 11B: MPN Summary Table for Deli Turkey

Listeria monocytogenes ATCC 19115

<table>
<thead>
<tr>
<th>Low Level Inoculum (0.2-2 MPN/Test Portion)</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 x 50 g</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>20 x 25 g (Reference Samples)</td>
<td>8/20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 x 10 g</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>MPN/Test portion</td>
<td>0.55</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low Conf. Limit MPN/Test Portion</td>
<td>0.29</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High Conf. Limit MPN/Test Portion</td>
<td>0.93</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>High Level Inoculum (2-5 MPN/Test Portion)</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 x 50 g</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>20 x 25 g (Reference Samples)</td>
<td>5/5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 x 10 g</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>MPN/Test portion</td>
<td>3.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low Conf. Limit MPN/Test Portion</td>
<td>1.31</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High Conf. Limit MPN/Test Portion</td>
<td>6.89</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 MPN was calculated for the low level inoculation for deli turkey using five 50 g, five 10 g, and the 20 low level reference method samples (25 g) using the LCF MPN Calculator version 1.6 provided by AOAC-RI [http://www.lcfltd.com/customer/LCFMPNCalculator](http://www.lcfltd.com/customer/LCFMPNCalculator)

2 MPN was calculated for the high level inoculation for deli turkey using five 50 g, five 10 g and the 5 high level reference method samples (25 g) using the LCF MPN Calculator version 1.6 provided by AOAC-RI [http://www.lcfltd.com/customer/LCFMPNCalculator](http://www.lcfltd.com/customer/LCFMPNCalculator)
Table 11C: MPN Summary Table for Deli Turkey

<table>
<thead>
<tr>
<th>Salmonella Dublin ATCC 15480</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Low Level Inoculum (0.2-2 MPN/Test Portion)</strong></td>
</tr>
<tr>
<td>5 x 50 g</td>
</tr>
<tr>
<td>20 x 25 g (Reference Samples)</td>
</tr>
<tr>
<td>5 x 10 g</td>
</tr>
<tr>
<td>MPN/Test portion</td>
</tr>
<tr>
<td>Low Conf. Limit MPN/Test Portion</td>
</tr>
<tr>
<td>High Conf. Limit MPN/Test Portion</td>
</tr>
</tbody>
</table>

| **High Level Inoculum (2-5 MPN/Test Portion)** | A | B | C | D | E |
|-------------------------------------------------|
| 5 x 50 g | + | + | + | + | + |
| 20 x 25 g (Reference Samples) | 5/5 |
| 5 x 10 g | - | + | + | + | + |
| MPN/Test portion | 4.38 |
| Low Conf. Limit MPN/Test Portion | 1.72 |
| High Conf. Limit MPN/Test Portion | 11.15 |

1 MPN was calculated for the low level inoculation for deli turkey using five 50 g, five 10 g, and the 20 low level reference method samples (25 g) using the LCF MPN Calculator version 1.6 provided by AOAC-RI http://www.lcfltd.com/customer/LCFMPNCalculator

2 MPN was calculated for the high level inoculation for deli turkey using five 50 g, five 10 g and the 5 high level reference method samples (25 g) using the LCF MPN Calculator version 1.6 provided by AOAC-RI http://www.lcfltd.com/customer/LCFMPNCalculator
Table 12A: MPN Summary Table for Fresh Baby Spinach

<table>
<thead>
<tr>
<th>Escherichia coli O145 MSU TW09153</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Low Level Inoculum (0.2-2 MPN/Test Portion)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 x 50 g</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>20 x 25 g (Reference Samples)</td>
<td>7/20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 x 10 g</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>MPN/Test portion</td>
<td>0.54</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low Conf. Limit MPN/Test Portion</td>
<td>0.29</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High Conf. Limit MPN/Test Portion</td>
<td>0.90</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>High Level Inoculum (2-5 MPN/Test Portion)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 x 50 g</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>20 x 25 g (Reference Samples)</td>
<td>5/5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 x 10 g</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>MPN/Test portion</td>
<td>3.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low Conf. Limit MPN/Test Portion</td>
<td>1.31</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High Conf. Limit MPN/Test Portion</td>
<td>6.89</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 MPN was calculated for the low level inoculation for fresh baby spinach using five 50 g, five 10 g, and the 20 low level reference method samples (25 g) using the LCF MPN Calculator version 1.6 provided by AOAC-RI [http://www.lcfttd.com/customer/LCFMPNCalculator](http://www.lcfttd.com/customer/LCFMPNCalculator)

2 MPN was calculated for the high level inoculation for fresh baby spinach using five 50 g, five 10 g and the 5 high level reference method samples (25 g) using the LCF MPN Calculator version 1.6 provided by AOAC-RI [http://www.lcfttd.com/customer/LCFMPNCalculator](http://www.lcfttd.com/customer/LCFMPNCalculator)
Table 12B: MPN Summary Table for Fresh Baby Spinach

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Low Level Inoculum (0.2-2 MPN/Test Portion)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 x 50 g</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>20 x 25 g (Reference Samples)</td>
<td></td>
<td></td>
<td></td>
<td>5/20</td>
<td></td>
</tr>
<tr>
<td>5 x 10 g</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MPN/Test portion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.35</td>
</tr>
<tr>
<td>Low Conf. Limit MPN/Test Portion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.14</td>
</tr>
<tr>
<td>High Conf. Limit MPN/Test Portion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.63</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>High Level Inoculum (2-5 MPN/Test Portion)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 x 50 g</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>20 x 25 g (Reference Samples)</td>
<td></td>
<td></td>
<td></td>
<td>5/5</td>
<td></td>
</tr>
<tr>
<td>5 x 10 g</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>MPN/Test portion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.01</td>
</tr>
<tr>
<td>Low Conf. Limit MPN/Test Portion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.31</td>
</tr>
<tr>
<td>High Conf. Limit MPN/Test Portion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6.89</td>
</tr>
</tbody>
</table>

1 MPN was calculated for the low level inoculation for fresh baby spinach using five 50 g, five 10 g, and the 20 low level reference method samples (25 g) using the LCF MPN Calculator version 1.6 provided by AOAC-RI [http://www.lcfltd.com/customer/LCFMPNCalculator](http://www.lcfltd.com/customer/LCFMPNCalculator)

2 MPN was calculated for the high level inoculation for fresh baby spinach using five 50 g, five 10 g and the 5 high level reference method samples (25 g) using the LCF MPN Calculator version 1.6 provided by AOAC-RI [http://www.lcfltd.com/customer/LCFMPNCalculator](http://www.lcfltd.com/customer/LCFMPNCalculator)
### Table 12C: MPN Summary Table for Fresh Baby Spinach

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Salmonella Enteritidis ATCC 13076</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>1 Low Level Inoculum (0.2-2 MPN/Test Portion)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 x 50 g</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>20 x 25 g (Reference Samples)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 x 10 g</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>MPN/Test portion</td>
<td>0.34</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low Conf. Limit MPN/Test Portion</td>
<td>0.14</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High Conf. Limit MPN/Test Portion</td>
<td>0.61</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>2 High Level Inoculum (2-5 MPN/Test Portion)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 x 50 g</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>20 x 25 g (Reference Samples)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 x 10 g</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>MPN/Test portion</td>
<td>2.29</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low Conf. Limit MPN/Test Portion</td>
<td>1.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High Conf. Limit MPN/Test Portion</td>
<td>5.02</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 MPN was calculated for the low level inoculation for fresh baby spinach using five 50 g, five 10 g, and the 20 low level reference method samples (25 g) using the LCF MPN Calculator version 1.6 provided by AOAC-RI [http://www.lcf ltd.com/customer/LCFMPNCalculator](http://www.lcf ltd.com/customer/LCFMPNCalculator)

2 MPN was calculated for the high level inoculation for fresh baby spinach using five 50 g, five 10 g and the 5 high level reference method samples (25 g) using the LCF MPN Calculator version 1.6 provided by AOAC-RI [http://www.lcf ltd.com/customer/LCFMPNCalculator](http://www.lcf ltd.com/customer/LCFMPNCalculator)
### Table 13: Detailed Results for the PolySkope 1.0 Multiplex Pathogen Detection Assay for Fresh Raw Ground Beef

<table>
<thead>
<tr>
<th>Sample #</th>
<th>PolySkope 1.0 Multiplex Pathogen Detection Assay</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Presumptive</td>
<td>Confirmed</td>
<td>USDA/FSIS MLG 5.09</td>
<td>USDA/FSIS MLG 8.10</td>
<td>USDA/FSIS MLG 5B.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>FAM stx1/stx2</td>
<td>ABY eae</td>
<td>VIC L. monocytogenes ALEXA Salmonella JUN Internal Control Big 6 STEC, including O157 L. monocytogenes Salmonella</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>7/20</td>
<td>7/20</td>
<td>7/20</td>
<td>9/20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### High Level

<p>| | | | | | | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>5/5</td>
<td>5/5</td>
<td>5/5</td>
<td>5/5</td>
<td>5/5</td>
<td>5/5</td>
<td>5/5</td>
<td>5/5</td>
<td>5/5</td>
<td>5/5</td>
</tr>
</tbody>
</table>

#### Uninoculated

<p>| | | | | | | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
</tbody>
</table>
Table 14: Detailed Results for the PolySkope 1.0 Multiplex Pathogen Detection Assay for Deli Turkey

<table>
<thead>
<tr>
<th>Sample #</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>16</th>
<th>17</th>
<th>18</th>
<th>19</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAM stx1/stx2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>ABY eae</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>VIC</td>
<td></td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>L. monocytogenes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>ALEXA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Salmonella</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>JUN Internal Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Big 6 STEC, Including O157</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>L. monocytogenes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Salmonella</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>USDA/FSIS MLG 5B.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>USDA/FSIS MLG 8.10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>USDA/FSIS MLG 5B.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

**Low Level**

**Total**


**High Level**

**Total**

5/5 5/5 5/5 5/5 5/5 5/5 5/5 5/5 5/5 5/5

**Uninoculated**

1 2 3 4
Table 15: Detailed Results for the PolySkope 1.0 Multiplex Pathogen Detection Assay for Fresh Baby Spinach

<table>
<thead>
<tr>
<th>Sample #</th>
<th>PolySkope 1.0 Multiplex Pathogen Detection Assay</th>
<th>Low Level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Presumptive</td>
<td>Confirmed</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>15</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>16</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>17</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>18</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>19</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>20</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample #</th>
<th>PolySkope 1.0 Multiplex Pathogen Detection Assay</th>
<th>High Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Total</td>
<td>5/5</td>
<td>5/5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample #</th>
<th>PolySkope 1.0 Multiplex Pathogen Detection Assay</th>
<th>Uninoculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Page 63 of 95
Table 16: Detailed Results for the PolySkope 1.0 Multiplex Pathogen Detection Assay for Stainless Steel

<table>
<thead>
<tr>
<th>Sample #</th>
<th>E. coli O103 MSU TW08101, Listeria monocytogenes ATCC 51780, and Salmonella Kentucky ATCC 9263</th>
<th>PolySkope 1.0 Multiplex Pathogen Detection Assay</th>
<th>Presumptive</th>
<th>Confirmed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>USDA/FSIS MLG 5B.05</td>
<td>FDA/BAM Chapter 10</td>
<td>FDA/BAM Chapter 5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FAM sstI/stx2</td>
<td>ABY eae</td>
<td>VIC L. monocytogenes</td>
<td>ALEXA Salmonella</td>
</tr>
<tr>
<td>1</td>
<td>+ + - - +</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>+ + - - +</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>- - - - +</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>- - - - +</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>- - - - +</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>- - - - +</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>+ + - - +</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>- - - - +</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>- - - - +</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>+ + - - +</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>- - - - +</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>- - - - +</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>+ + - - +</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>- - - - +</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>+ + - - +</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>16</td>
<td>- - - - +</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>17</td>
<td>- - - - +</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>18</td>
<td>+ + - - +</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>19</td>
<td>+ + - - +</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>20</td>
<td>- - + - +</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

High Level

<table>
<thead>
<tr>
<th></th>
<th>USDA/FSIS MLG 5B.05</th>
<th>FDA/BAM Chapter 10</th>
<th>FDA/BAM Chapter 5</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FAM sstI/stx2</td>
<td>ABY eae</td>
<td>VIC L. monocytogenes</td>
<td>ALEXA Salmo nella</td>
</tr>
<tr>
<td>1</td>
<td>+ + + + +</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>+ + + + +</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>+ + + + +</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>+ + + + +</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>+ + + + +</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Total</td>
<td>5/5</td>
<td>5/5</td>
<td>5/5</td>
<td>5/5</td>
</tr>
</tbody>
</table>

Uninoculated

Page 64 of 95
<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0/5</td>
</tr>
</tbody>
</table>
## Table 17: PolySkope 1.0 Multiplex Pathogen Detection Assay, Candidate vs. Reference – POD Results

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Strain</th>
<th>MPN(^a)/ Test Portion</th>
<th>N(^b)</th>
<th>Candidate</th>
<th>Reference</th>
<th>dPOD(^c)</th>
<th>95% Cl(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X (^x)</td>
<td>POD(^c)(^e) 95% CI</td>
<td>X</td>
<td>POD(^e)(^f) 95% CI</td>
</tr>
<tr>
<td></td>
<td>E. coli O157 ATCC 43895</td>
<td></td>
<td></td>
<td>0</td>
<td>0.00, 0.43</td>
<td>0</td>
<td>0.00, 0.43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.44 (0.21, 0.76)</td>
<td>20</td>
<td>3.5</td>
<td>0.18, 0.57</td>
<td>6</td>
<td>0.30, 0.52</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.01 (1.31, 6.89)</td>
<td>5</td>
<td>1.00</td>
<td>0.57, 1.00</td>
<td>5</td>
<td>1.00, 0.57</td>
</tr>
<tr>
<td>Fresh Raw Ground Beef</td>
<td>L. monocytogenes ATCC 7644</td>
<td></td>
<td></td>
<td>0</td>
<td>0.00, 0.43</td>
<td>0</td>
<td>0.00, 0.43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.55 (0.29, 0.94)</td>
<td>20</td>
<td>3.5</td>
<td>0.18, 0.57</td>
<td>8</td>
<td>0.40, 0.61</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.01 (1.31, 6.89)</td>
<td>5</td>
<td>1.00</td>
<td>0.57, 1.00</td>
<td>5</td>
<td>1.00, 0.57</td>
</tr>
<tr>
<td></td>
<td>Salmonella Typhimurium ATCC 14028</td>
<td></td>
<td></td>
<td>0</td>
<td>0.00, 0.43</td>
<td>0</td>
<td>0.00, 0.43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.35 (0.17, 0.62)</td>
<td>20</td>
<td>4.5</td>
<td>0.26, 0.66</td>
<td>5</td>
<td>0.25, 0.47</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.29 (1.05, 5.02)</td>
<td>5</td>
<td>1.00</td>
<td>0.57, 1.00</td>
<td>5</td>
<td>1.00, 0.57</td>
</tr>
</tbody>
</table>

\(^a\)MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval

\(^b\)N = Number of test portions

\(^c\)X = Number of positive test portions

\(^d\)POD\(^c\) = Candidate method confirmed positive outcomes divided by the total number of trials

\(^e\)POD\(^R\) = Reference method confirmed positive outcomes divided by the total number of trials

\(^f\)dPOD\(^c\) = Difference between the confirmed candidate method result and reference method confirmed result POD values

\(^g\)95% Cl = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level
Table 18: PolySkope 1.0 Multiplex Pathogen Detection Assay, Candidate vs. Reference – POD Results

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Strain</th>
<th>MPN&lt;sup&gt;a&lt;/sup&gt;/&lt;br&gt;Test Portion</th>
<th>N&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Candidate</th>
<th>Reference</th>
<th>dPOD&lt;sup&gt;c&lt;/sup&gt;</th>
<th>95% CI&lt;sup&gt;d&lt;/sup&gt;</th>
<th>95% CI&lt;sup&gt;e&lt;/sup&gt;</th>
<th>95% CI&lt;sup&gt;f&lt;/sup&gt;</th>
<th>95% CI&lt;sup&gt;g&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deli Turkey</td>
<td>E. coli O26 MSU TW00971</td>
<td>-</td>
<td>5</td>
<td>0</td>
<td>0.00</td>
<td>0.00, 0.43</td>
<td>0</td>
<td>0.00</td>
<td>0.00, 0.43</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.35</td>
<td>20</td>
<td>7</td>
<td>0.35</td>
<td>0.18, 0.57</td>
<td>5</td>
<td>0.25</td>
<td>0.11, 0.47</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.01</td>
<td>5</td>
<td>5</td>
<td>1.00</td>
<td>0.57, 1.00</td>
<td>5</td>
<td>1.00</td>
<td>0.57, 1.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Deli Turkey</td>
<td>L. monocytogenes ATCC 19115</td>
<td>-</td>
<td>5</td>
<td>0</td>
<td>0.00</td>
<td>0.00, 0.43</td>
<td>0</td>
<td>0.00</td>
<td>0.00, 0.43</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.55</td>
<td>20</td>
<td>11</td>
<td>0.55</td>
<td>0.34, 0.74</td>
<td>8</td>
<td>0.40</td>
<td>0.22, 0.61</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.01</td>
<td>5</td>
<td>5</td>
<td>1.00</td>
<td>0.57, 1.00</td>
<td>5</td>
<td>1.00</td>
<td>0.57, 1.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Deli Turkey</td>
<td>Salmonella Dublin ATCC 15480</td>
<td>-</td>
<td>5</td>
<td>0</td>
<td>0.00</td>
<td>0.00, 0.43</td>
<td>0</td>
<td>0.00</td>
<td>0.00, 0.43</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.76</td>
<td>20</td>
<td>13</td>
<td>0.65</td>
<td>0.43, 0.82</td>
<td>10</td>
<td>0.50</td>
<td>0.30, 0.70</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.38</td>
<td>5</td>
<td>5</td>
<td>1.00</td>
<td>0.57, 1.00</td>
<td>5</td>
<td>1.00</td>
<td>0.57, 1.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

<sup>a</sup>MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval

<sup>b</sup>N = Number of test portions

<sup>c</sup>x = Number of positive test portions

<sup>d</sup>POD<sub>C</sub> = Candidate method confirmed positive outcomes divided by the total number of trials

<sup>e</sup>POD<sub>R</sub> = Reference method confirmed positive outcomes divided by the total number of trials

<sup>f</sup>dPOD<sub>C</sub> = Difference between the confirmed candidate method result and reference method confirmed result POD values

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level
Table 19: PolySkope 1.0 Multiplex Pathogen Detection Assay, Candidate vs. Reference – POD Results

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Strain</th>
<th>MPN(^a)/Test Portion</th>
<th>N(^b)</th>
<th>Candidate</th>
<th>Reference</th>
<th>dPOD(^f)</th>
<th>95% CI(^g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh Baby Spinach</td>
<td>E. coli O145 Strain MSU TW09153</td>
<td>-</td>
<td>5</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>-0.43, 0.43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.54 (0.29, 0.90)</td>
<td>20</td>
<td>9.45</td>
<td>0.26, 0.66</td>
<td>7.35</td>
<td>0.18, 0.57</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.01 (1.31, 6.89)</td>
<td>5</td>
<td>1.00</td>
<td>0.57, 1.00</td>
<td>5.00</td>
<td>0.00, -0.43</td>
</tr>
<tr>
<td>Fresh Baby Spinach</td>
<td>L. monocytogenes Strain ATCC BAA-2658</td>
<td>-</td>
<td>5</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>-0.43, 0.43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.35 (0.14, 0.63)</td>
<td>20</td>
<td>0.35</td>
<td>0.18, 0.57</td>
<td>5.00</td>
<td>0.11, 0.47</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.01 (1.31, 6.89)</td>
<td>5</td>
<td>1.00</td>
<td>0.57, 1.00</td>
<td>5.00</td>
<td>0.00, -0.43</td>
</tr>
<tr>
<td>Fresh Baby Spinach</td>
<td>Salmonella Enteritidis Strain ATCC 13076</td>
<td>-</td>
<td>5</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>-0.43, 0.43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.34 (0.14, 0.61)</td>
<td>20</td>
<td>0.35</td>
<td>0.18, 0.57</td>
<td>6.00</td>
<td>0.15, 0.52</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.29 (1.05, 5.02)</td>
<td>5</td>
<td>1.00</td>
<td>0.57, 1.00</td>
<td>5.00</td>
<td>0.00, -0.43</td>
</tr>
</tbody>
</table>

\(^a\)MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval
\(^b\)N = Number of test portions
\(^c\)x = Number of positive test portions
\(^d\)POD\(_C\) = Candidate method confirmed positive outcomes divided by the total number of trials
\(^e\)POD\(_R\) = Reference method confirmed positive outcomes divided by the total number of trials
\(^f\)dPOD\(_C\) = Difference between the confirmed candidate method result and reference method confirmed result POD values
\(^g\)95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level
Table 20: PolySkope 1.0 Multiplex Pathogen Detection Assay, Candidate vs. Reference – POD Results

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Strain</th>
<th>CFU(^a)/Test Area</th>
<th>N(^b)</th>
<th>Candidate</th>
<th>Reference</th>
<th>dPOD(^c)</th>
<th>95% CI</th>
<th>95% CI(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stainless Steel</td>
<td><em>E. coli</em> O103 MSU TW08101</td>
<td>-</td>
<td>5</td>
<td>0</td>
<td>0.00</td>
<td>0.00, 0.43</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>51</td>
<td>20</td>
<td>7</td>
<td>0.35</td>
<td>0.18, 0.57</td>
<td>6</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>440</td>
<td>5</td>
<td>5</td>
<td>1.00</td>
<td>0.57, 1.00</td>
<td>5</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>L. monocytogenes ATCC 51780</td>
<td>-</td>
<td>5</td>
<td>0</td>
<td>0.00</td>
<td>0.00, 0.43</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>64</td>
<td>20</td>
<td>4</td>
<td>0.20</td>
<td>0.08, 0.42</td>
<td>7</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>650</td>
<td>5</td>
<td>5</td>
<td>1.00</td>
<td>0.57, 1.00</td>
<td>5</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td><em>Salmonella</em> Kentucky ATCC 9263</td>
<td>-</td>
<td>5</td>
<td>0</td>
<td>0.00</td>
<td>0.00, 0.43</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>45</td>
<td>20</td>
<td>5</td>
<td>0.25</td>
<td>0.11, 0.47</td>
<td>7</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>580</td>
<td>5</td>
<td>5</td>
<td>1.00</td>
<td>0.57, 1.00</td>
<td>5</td>
<td>1.00</td>
</tr>
</tbody>
</table>

\(^a\)CFU/Test Area = Results of the CFU/Test area were determined by plating the inoculum for each matrix in triplicate

\(^b\)N = Number of test portions

\(^c\)x = Number of positive test portions

\(^d\)POD\(_C\) = Candidate method confirmed positive outcomes divided by the total number of trials

\(^e\)POD\(_R\) = Reference method confirmed positive outcomes divided by the total number of trials

\(^f\)dPOD\(_C\) = Difference between the confirmed candidate method result and reference method confirmed result POD values

\(^g\)95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level
Table 21: PolySkope 1.0 Multiplex Pathogen Detection Assay, Presumptive vs. Confirmed – POD Results

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Strain</th>
<th>MPN&lt;sup&gt;a&lt;/sup&gt; / Test Portion</th>
<th>N&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Presumptive</th>
<th>Confirmed</th>
<th>dPOD&lt;sub&gt;CP&lt;/sub&gt;&lt;sup&gt;f&lt;/sup&gt;</th>
<th>95% CI&lt;sup&gt;g&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh Raw</td>
<td>E. coli O157</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ground Beef</td>
<td>ATCC 43895</td>
<td>0.44 (0.21, 0.76)</td>
<td>20</td>
<td>7</td>
<td>0.35</td>
<td>0.18, 0.57</td>
<td>0.00, 0.43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.01 (1.31, 6.89)</td>
<td>5</td>
<td>5</td>
<td>1.00</td>
<td>0.57, 1.00</td>
<td>0.00, 0.43</td>
</tr>
<tr>
<td>L. monocytogenes</td>
<td>ATCC 7644</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.55 (0.29, 0.94)</td>
<td>20</td>
<td>7</td>
<td>0.35</td>
<td>0.18, 0.57</td>
<td>0.00, 0.43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.01 (1.31, 6.89)</td>
<td>5</td>
<td>5</td>
<td>1.00</td>
<td>0.57, 1.00</td>
<td>0.00, 0.43</td>
</tr>
<tr>
<td>Salmonella</td>
<td>Typhimurium</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATCC 14028</td>
<td></td>
<td>0.35 (0.17, 0.62)</td>
<td>20</td>
<td>9</td>
<td>0.45</td>
<td>0.26, 0.66</td>
<td>0.00, 0.43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.29 (1.05, 5.02)</td>
<td>5</td>
<td>5</td>
<td>1.00</td>
<td>0.57, 1.00</td>
<td>0.00, 0.43</td>
</tr>
</tbody>
</table>

<sup>a</sup>MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval

<sup>b</sup>N = Number of test portions

<sup>c</sup>x = Number of positive test portions

<sup>d</sup>POD<sub>CP</sub> = Candidate method presumptive positive outcomes divided by the total number of trials

<sup>e</sup>POD<sub>CC</sub> = Candidate method confirmed positive outcomes divided by the total number of trials

<sup>f</sup>dPOD<sub>CP</sub> = Difference between the candidate method presumptive result and candidate method confirmed result POD values

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level
Table 22: PolySkope 1.0 Multiplex Pathogen Detection Assay, Presumptive vs. Confirmed – POD Results

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Strain</th>
<th>MPN&lt;sup&gt;a&lt;/sup&gt;/ Test Portion</th>
<th>N&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Presumptive</th>
<th>Confirmed</th>
<th>dPOD&lt;sub&gt;CP&lt;/sub&gt;&lt;sup&gt;f&lt;/sup&gt;</th>
<th>95% CI&lt;sup&gt;g&lt;/sup&gt;</th>
<th>95% CI&lt;sup&gt;h&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E. coli O26</td>
<td>-</td>
<td>5</td>
<td>0.00</td>
<td>0.00, 0.43</td>
<td>0.00</td>
<td>0.00, 0.43</td>
<td>0.00, -0.47, 0.47</td>
</tr>
<tr>
<td></td>
<td>MSU TW00971</td>
<td>0.35</td>
<td>20</td>
<td>0.35</td>
<td>0.18, 0.57</td>
<td>0.35</td>
<td>0.18, 0.57</td>
<td>0.00, -0.13, 0.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.01</td>
<td>5</td>
<td>1.00</td>
<td>0.57, 1.00</td>
<td>1.00</td>
<td>0.57, 1.00</td>
<td>0.00, -0.47, 0.47</td>
</tr>
<tr>
<td>Deli</td>
<td>Turkey</td>
<td>-</td>
<td>5</td>
<td>0.00</td>
<td>0.00, 0.43</td>
<td>0.00</td>
<td>0.00, 0.43</td>
<td>0.00, -0.47, 0.47</td>
</tr>
<tr>
<td></td>
<td>L. monocytogenes</td>
<td>0.55</td>
<td>20</td>
<td>0.55</td>
<td>0.34, 0.74</td>
<td>0.55</td>
<td>0.34, 0.74</td>
<td>0.00, -0.13, 0.13</td>
</tr>
<tr>
<td></td>
<td>ATCC 19115</td>
<td>3.01</td>
<td>5</td>
<td>1.00</td>
<td>0.57, 1.00</td>
<td>1.00</td>
<td>0.57, 1.00</td>
<td>0.00, -0.47, 0.47</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-</td>
<td>5</td>
<td>0.00</td>
<td>0.00, 0.43</td>
<td>0.00</td>
<td>0.00, 0.43</td>
<td>0.00, -0.47, 0.47</td>
</tr>
<tr>
<td></td>
<td>Salmonella</td>
<td>0.76</td>
<td>20</td>
<td>0.70</td>
<td>0.48, 0.85</td>
<td>0.65</td>
<td>0.43, 0.82</td>
<td>0.05, -0.11, 0.21</td>
</tr>
<tr>
<td></td>
<td>Dublin</td>
<td>4.38</td>
<td>5</td>
<td>1.00</td>
<td>0.57, 1.00</td>
<td>1.00</td>
<td>0.57, 1.00</td>
<td>0.00, -0.47, 0.47</td>
</tr>
<tr>
<td></td>
<td>ATCC 15480</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval

<sup>b</sup>N = Number of test portions

<sup>c</sup>x = Number of positive test portions

<sup>d</sup>POD<sub>CP</sub> = Candidate method presumptive positive outcomes divided by the total number of trials

<sup>e</sup>POD<sub>CC</sub> = Candidate method confirmed positive outcomes divided by the total number of trials

<sup>f</sup>dPOD<sub>CP</sub> = Difference between the candidate method presumptive result and candidate method confirmed result POD values

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level
Table 23: PolySkope 1.0 Multiplex Pathogen Detection Assay, Presumptive vs. Confirmed – POD Results

| Matrix                | Strain                        | MPN<sup>a</sup> | N<sup>b</sup> | Presumptive | Confirmed | dPOD<sub>CP</sub><sup>f</sup> | 95% CI    | 95% CI<sup>g</sup> |
|-----------------------|-------------------------------|----------------|-------------|-------------|-----------|-----------------|-----------|
| Fresh Baby Spinach    | E. coli O145 MSU TW09153      | -             | 5           | 0           | 0.00      | 0.00, 0.43      | 0.00      | -0.47, 0.47        |
|                       |                               | 0.54 (0.29, 0.90) | 20          | 9           | 0.45      | 0.26, 0.66      | 9.00      | -0.13, 0.13        |
|                       |                               | 3.01 (1.31, 6.89) | 5           | 5           | 1.00      | 0.57, 1.00      | 5.00      | -0.47, 0.47        |
| L. monocytogenes      | ATCC BAA-2658                 | -             | 5           | 0           | 0.00      | 0.00, 0.43      | 0.00      | -0.47, 0.47        |
|                       |                               | 0.35 (0.14, 0.63) | 20          | 7           | 0.35      | 0.18, 0.57      | 7.00      | -0.13, 0.13        |
|                       |                               | 3.01 (1.31, 6.89) | 5           | 5           | 1.00      | 0.57, 1.00      | 5.00      | -0.47, 0.47        |
| Salmonella Enteritidis | ATCC 13076                   | -             | 5           | 0           | 0.00      | 0.00, 0.43      | 0.00      | -0.47, 0.47        |
|                       |                               | 0.34 (0.14, 0.61) | 20          | 7           | 0.35      | 0.18, 0.57      | 7.00      | -0.13, 0.13        |
|                       |                               | 2.29 (1.05, 5.02) | 5           | 5           | 1.00      | 0.57, 1.00      | 5.00      | -0.47, 0.47        |

<sup>a</sup>MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval
<br><sup>b</sup>N = Number of test portions
<br><sup>c</sup>x = Number of positive test portions
<br><sup>d</sup>POD<sub>CP</sub> = Candidate method presumptive positive outcomes divided by the total number of trials
<br><sup>e</sup>POD<sub>CC</sub> = Candidate method confirmed positive outcomes divided by the total number of trials
<br><sup>f</sup>dPOD<sub>CP</sub> = Difference between the candidate method presumptive result and candidate method confirmed result POD values
<br><sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level
Table 24: PolySkope 1.0 Multiplex Pathogen Detection Assay, Presumptive vs. Confirmed – POD Results

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Strain</th>
<th>CFU&lt;sup&gt;a&lt;/sup&gt;/Test Area</th>
<th>N&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Presumptive</th>
<th>Confirmed</th>
<th>dPOD&lt;sub&gt;CP&lt;/sub&gt;&lt;sup&gt;f&lt;/sup&gt;</th>
<th>95% CI</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>X&lt;sup&gt;c&lt;/sup&gt;</td>
<td>POD&lt;sub&gt;CP&lt;/sub&gt;&lt;sup&gt;a&lt;/sup&gt;</td>
<td>95% CI</td>
<td>X</td>
<td>POD&lt;sub&gt;CC&lt;/sub&gt;&lt;sup&gt;e&lt;/sup&gt;</td>
<td>95% CI</td>
<td>95% CI</td>
</tr>
<tr>
<td>Stainless Steel</td>
<td>E. coli O103</td>
<td>-</td>
<td>5</td>
<td>0</td>
<td>0.00</td>
<td>0.00, 0.43</td>
<td>0</td>
<td>0.00, 0.43</td>
</tr>
<tr>
<td></td>
<td>MSU TW08101</td>
<td>51</td>
<td>20</td>
<td>7</td>
<td>0.35</td>
<td>0.18, 0.57</td>
<td>7</td>
<td>0.35, 0.57</td>
</tr>
<tr>
<td></td>
<td></td>
<td>440</td>
<td>5</td>
<td>5</td>
<td>1.00</td>
<td>0.57, 1.00</td>
<td>5</td>
<td>1.00, 0.57</td>
</tr>
<tr>
<td></td>
<td>L. monocytogenes</td>
<td>-</td>
<td>5</td>
<td>0</td>
<td>0.00</td>
<td>0.00, 0.43</td>
<td>0</td>
<td>0.00, 0.43</td>
</tr>
<tr>
<td></td>
<td>ATCC 51780</td>
<td>64</td>
<td>20</td>
<td>4</td>
<td>0.20</td>
<td>0.08, 0.42</td>
<td>4</td>
<td>0.20, 0.42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>650</td>
<td>5</td>
<td>5</td>
<td>1.00</td>
<td>0.57, 1.00</td>
<td>5</td>
<td>1.00, 0.57</td>
</tr>
<tr>
<td></td>
<td>Salmonella Kentucky</td>
<td>-</td>
<td>5</td>
<td>0</td>
<td>0.00</td>
<td>0.00, 0.43</td>
<td>0</td>
<td>0.00, 0.43</td>
</tr>
<tr>
<td></td>
<td>ATCC 9263</td>
<td>45</td>
<td>20</td>
<td>5</td>
<td>0.25</td>
<td>0.11, 0.47</td>
<td>5</td>
<td>0.25, 0.47</td>
</tr>
<tr>
<td></td>
<td></td>
<td>580</td>
<td>5</td>
<td>5</td>
<td>1.00</td>
<td>0.57, 1.00</td>
<td>5</td>
<td>1.00, 0.57</td>
</tr>
</tbody>
</table>

<sup>a</sup>CFU/Test Area = Results of the CFU/Test area were determined by plating the inoculum for each matrix in triplicate

<sup>b</sup>N = Number of test portions

<sup>c</sup>X = Number of positive test portions

<sup>d</sup>POD<sub>CP</sub> = Candidate method presumptive positive outcomes divided by the total number of trials

<sup>e</sup>POD<sub>CC</sub> = Candidate method confirmed positive outcomes divided by the total number of trials

<sup>f</sup>dPOD<sub>CP</sub> = Difference between the candidate method presumptive result and candidate method confirmed result POD values

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level
Table 25: Product Stability and Lot to Lot Outline and Information

<table>
<thead>
<tr>
<th>Storage Type</th>
<th>Storage Temperature</th>
<th>Time Points (From the Date of Production)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accelerated</td>
<td>25 ± 2°C</td>
<td>4 days, 9 days, 17 days, 20 days</td>
</tr>
<tr>
<td>Real Time</td>
<td>2-8°C</td>
<td>1 month, 2.5 months, 5 months, 6 months</td>
</tr>
</tbody>
</table>

**Lot Information**

<table>
<thead>
<tr>
<th>Lot</th>
<th>Time Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lot 1</td>
<td>052717</td>
</tr>
<tr>
<td>Lot 2</td>
<td>081217</td>
</tr>
<tr>
<td>Lot 3</td>
<td>102417</td>
</tr>
<tr>
<td>Lot 4</td>
<td>121717</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Strain</th>
<th>Source</th>
<th>Identification</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Target</strong></td>
<td>E. coli O45</td>
<td>MSU² TW09183</td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>ATCC³</td>
<td>13932</td>
</tr>
<tr>
<td>Salmonella Choleraesuis</td>
<td>ATCC</td>
<td>10708</td>
</tr>
<tr>
<td><strong>non-Target</strong></td>
<td>Escherichia coli</td>
<td>ATCC 8739</td>
</tr>
</tbody>
</table>

¹All three lots were combined to make a single lot and the product stability and lot to lot was analyzed at once.
²Michigan State Shiga toxin-producing Escherichia coli Center
³American Type Culture Collection
Table 26: Accelerated Product Stability and Lot to Lot Detailed Results – 4 Days

<table>
<thead>
<tr>
<th>Sample</th>
<th>Low-Level 1 Target</th>
<th>Non-Target 2 Target</th>
<th>Lysis Buffer Blank Target</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>stx1/stx2</td>
<td>eae</td>
<td>L. monocytogenes</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>- +</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>4/10</td>
<td>4/10</td>
<td>4/10</td>
</tr>
</tbody>
</table>

1 Target pathogens listed in Table 25 were diluted to a level that were expected to yield fractional positive results.

2 Uninoculated samples were inoculated with the non-target pathogens listed in Table 25 a high level of 2-10 CFU/ test portion.
Table 27: Accelerated Product Stability and Lot to Lot Detailed Results – 9 Days

<table>
<thead>
<tr>
<th>Sample</th>
<th>Low-Level&lt;sup&gt;1&lt;/sup&gt;</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>stx1/stx2</td>
<td>eae</td>
<td>L. monocytogenes</td>
<td>Salmonella</td>
<td>Internal Control</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>6/10</td>
<td>6/10</td>
<td>4/10</td>
<td>6/10</td>
<td>10/10</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>Non-Target&lt;sup&gt;2&lt;/sup&gt;</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>stx1/stx2</td>
<td>eae</td>
<td>L. monocytogenes</td>
<td>Salmonella</td>
<td>Internal Control</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>10/10</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>Lysis Buffer Blank</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>stx1/stx2</td>
<td>eae</td>
<td>L. monocytogenes</td>
<td>Salmonella</td>
<td>Internal Control</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>10/10</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup> Target pathogens listed in Table 25 were diluted to a level that were expected to yield fractional positive results

<sup>2</sup> Uninoculated samples were inoculated with the non-target pathogens listed in Table 25 a high level of 2-10 CFU/test portion
### Table 28: Accelerated Product Stability and Lot to Lot Detailed Results – 17 Days

<table>
<thead>
<tr>
<th>Sample</th>
<th>Low-Level[^1]</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>stx1/stx2</td>
<td>eae</td>
<td><em>L. monocytogenes</em></td>
<td><em>Salmonella</em></td>
<td>Internal Control</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Total</td>
<td>4/10</td>
<td>4/10</td>
<td>5/10</td>
<td>5/10</td>
<td>10/10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>Non-Target[^2]</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>stx1/stx2</td>
<td>eae</td>
<td><em>L. monocytogenes</em></td>
<td><em>Salmonella</em></td>
<td>Internal Control</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Total</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>10/10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>Lysis Buffer Blank</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>stx1/stx2</td>
<td>eae</td>
<td><em>L. monocytogenes</em></td>
<td><em>Salmonella</em></td>
<td>Internal Control</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Total</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>10/10</td>
</tr>
</tbody>
</table>
Target pathogens listed in Table 25 were diluted to a level that were expected to yield fractional positive results.

Uninoculated samples were inoculated with the non-target pathogens listed in Table 25 at a high level of 2-10 CFU/test portion.

Table 29: Accelerated Product Stability and Lot to Lot Detailed Results – 20 Days

<table>
<thead>
<tr>
<th>Sample</th>
<th>Low-Level¹</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>stx1/stx2</td>
<td>eae</td>
<td>L. monocytogenes</td>
<td>Salmonella</td>
<td>Internal Control</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Total</td>
<td>5/10</td>
<td>5/10</td>
<td>3/10</td>
<td>7/10</td>
<td>10/10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>Non-Target²</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>stx1/stx2</td>
<td>eae</td>
<td>L. monocytogenes</td>
<td>Salmonella</td>
<td>Internal Control</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Total</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>10/10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>Lysis Buffer Blank</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>stx1/stx2</td>
<td>eae</td>
<td>L. monocytogenes</td>
<td>Salmonella</td>
<td>Internal Control</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>
Target pathogens listed in Table 25 were diluted to a level that were expected to yield fractional positive results.

Uninoculated samples were inoculated with the non-target pathogens listed in Table 25 a high level of 2-10 CFU/ test portion.

Table 30: Real Time Product Stability and Lot to Lot Detailed Results – 1 Month

<table>
<thead>
<tr>
<th>Sample</th>
<th>Low-Level&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Non-Target&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Lysis Buffer Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>stx1/stx2</td>
<td>eae</td>
<td>L. monocytogenes</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>6/10</td>
<td>6/10</td>
<td>3/10</td>
</tr>
<tr>
<td>Sample</td>
<td>stx1/stx2</td>
<td>eae</td>
<td>L. monocytogenes</td>
</tr>
<tr>
<td>--------</td>
<td>------------</td>
<td>-----</td>
<td>-----------------</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
</tr>
</tbody>
</table>

1. Target pathogens listed in Table 25 were diluted to a level that were expected to yield fractional positive results.
2. Uninoculated samples were inoculated with the non-target pathogens listed in Table 25 a high level of 2-10 CFU/ test portion.

Table 31: Real Time Product Stability and Lot to Lot Detailed Results – 2.5 Months

<table>
<thead>
<tr>
<th>Sample</th>
<th>Target</th>
<th>Non-Target</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>stx1/stx2</td>
<td>eae</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>6/10</td>
<td>6/10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Sample pathogen results:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Lysis Buffer Blank</th>
<th>Target</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>stx1/stx2</td>
<td>eae</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>0/10</td>
<td>0/10</td>
</tr>
</tbody>
</table>

* Target pathogens listed in Table 25 were diluted to a level that were expected to yield fractional positive results.

* Uninoculated samples were inoculated with the non-target pathogens listed in Table 25 at a high level of 2-10 CFU/test portion.

### Table 32: Real Time Product Stability and Lot to Lot Detailed Results – 5 Months

| Sample | Low-Level<sup>1</sup> |
|---|---|---|---|---|---|
|  | stx1/stx2 | eae | L. monocytogenes | Salmonella | Internal Control |
| 1 | + | + | - | + | + |
| 2 | - | - | - | + | + |
| 3 | - | - | - | - | + |
| 4 | + | + | + | - | + |
| 5 | - | - | + | - | + |
| 6 | + | + | - | - | + |
| 7 | - | - | - | + | + |
| 8 | + | + | - | - | + |
| 9 | + | + | + | + | + |
| 10 | + | + | - | + | + |
| **Total** | 6/10 | 6/10 | 3/10 | 5/10 | 10/10 |

<p>| Sample | Non-Target&lt;sup&gt;2&lt;/sup&gt; |
|---|---|---|---|---|---|
|  | stx1/stx2 | eae | L. monocytogenes | Salmonella | Internal Control |
| 1 | - | - | - | - | + |
| 2 | - | - | - | - | + |
| 3 | - | - | - | - | + |
| 4 | - | - | - | - | + |
| 5 | - | - | - | - | + |
| 6 | - | - | - | - | + |
| 7 | - | - | - | - | + |</p>
<table>
<thead>
<tr>
<th>Sample</th>
<th>Lysis Buffer Blank</th>
<th>Target</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>stx1/stx2</td>
<td>eae</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>0/10</td>
<td>0/10</td>
</tr>
</tbody>
</table>

1. Target pathogens listed in Table 25 were diluted to a level that were expected to yield fractional positive results.

2. Uninoculated samples were inoculated with the non-target pathogens listed in Table 25 at a high level of 2-10 CFU/test portion.

**Table 33: Real Time Product Stability and Lot to Lot Detailed Results – 6 Months**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Low-Level(^1)</th>
<th>Non-Target(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>stx1/stx2</td>
<td>eae</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Total</td>
<td>6/10</td>
<td>6/10</td>
</tr>
</tbody>
</table>
Table 34: PolySkope 1.0 Multiplex Pathogen Detection Assay – Stability and Lot to Lot Inoculated Test Portions – POD Results

<table>
<thead>
<tr>
<th>Stability</th>
<th>Time Point</th>
<th>Target</th>
<th>N^a</th>
<th>x^b</th>
<th>POD_t^c</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>E. coli O45</td>
<td>10</td>
<td>4</td>
<td>0.40</td>
<td>0.17, 0.69</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Listeria monocytogenes</td>
<td>10</td>
<td>4</td>
<td>0.40</td>
<td>0.17, 0.69</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Salmonella Choleraesuis</td>
<td>10</td>
<td>5</td>
<td>0.50</td>
<td>0.24, 0.76</td>
</tr>
<tr>
<td>Accelerated</td>
<td>4 Days</td>
<td>E. coli O45</td>
<td>10</td>
<td>6</td>
<td>0.60</td>
<td>0.31, 0.83</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Listeria monocytogenes</td>
<td>10</td>
<td>4</td>
<td>0.40</td>
<td>0.17, 0.69</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Salmonella spp.</td>
<td>10</td>
<td>6</td>
<td>0.60</td>
<td>0.31, 0.83</td>
</tr>
<tr>
<td></td>
<td>9 Days</td>
<td>E. coli O45</td>
<td>10</td>
<td>5</td>
<td>0.50</td>
<td>0.24, 0.76</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Listeria monocytogenes</td>
<td>10</td>
<td>4</td>
<td>0.40</td>
<td>0.17, 0.69</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Salmonella Choleraesuis</td>
<td>10</td>
<td>5</td>
<td>0.50</td>
<td>0.24, 0.76</td>
</tr>
<tr>
<td></td>
<td>17 Days</td>
<td>E. coli O45</td>
<td>10</td>
<td>6</td>
<td>0.60</td>
<td>0.31, 0.83</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Listeria monocytogenes</td>
<td>10</td>
<td>5</td>
<td>0.50</td>
<td>0.24, 0.76</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Salmonella spp.</td>
<td>10</td>
<td>5</td>
<td>0.50</td>
<td>0.24, 0.76</td>
</tr>
<tr>
<td></td>
<td>20 Days</td>
<td>E. coli O45</td>
<td>10</td>
<td>5</td>
<td>0.50</td>
<td>0.24, 0.76</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Listeria monocytogenes</td>
<td>10</td>
<td>3</td>
<td>0.30</td>
<td>0.11, 0.60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Salmonella spp.</td>
<td>10</td>
<td>7</td>
<td>0.70</td>
<td>0.40, 0.89</td>
</tr>
<tr>
<td>Real Time</td>
<td>1 Month</td>
<td>E. coli O45</td>
<td>10</td>
<td>6</td>
<td>0.60</td>
<td>0.31, 0.83</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Listeria monocytogenes</td>
<td>10</td>
<td>3</td>
<td>0.30</td>
<td>0.11, 0.60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Salmonella spp.</td>
<td>10</td>
<td>5</td>
<td>0.50</td>
<td>0.24, 0.76</td>
</tr>
<tr>
<td></td>
<td>2.5 Months</td>
<td>E. coli O45</td>
<td>10</td>
<td>6</td>
<td>0.60</td>
<td>0.31, 0.83</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Listeria monocytogenes</td>
<td>10</td>
<td>3</td>
<td>0.30</td>
<td>0.11, 0.60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Salmonella spp.</td>
<td>10</td>
<td>5</td>
<td>0.50</td>
<td>0.24, 0.76</td>
</tr>
</tbody>
</table>

^a Target pathogens listed in Table 25 were diluted to a level that were expected to yield fractional positive results.

^b Uninoculated samples were inoculated with the non-target pathogens listed in Table 25 a high level of 2-10 CFU/test portion.
<table>
<thead>
<tr>
<th>Treatment Combination</th>
<th>Enrichment Time</th>
<th>Initial Lysis Time</th>
<th>Second Lysis Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20 Hours</td>
<td>10 Minutes</td>
<td>5 Minutes</td>
</tr>
<tr>
<td>2</td>
<td>20 Hours</td>
<td>10 Minutes</td>
<td>15 Minutes</td>
</tr>
<tr>
<td>3</td>
<td>20 Hours</td>
<td>20 Minutes</td>
<td>5 Minutes</td>
</tr>
<tr>
<td>4</td>
<td>20 Hours</td>
<td>20 Minutes</td>
<td>15 Minutes</td>
</tr>
<tr>
<td>5</td>
<td>26 Hours</td>
<td>10 Minutes</td>
<td>5 Minutes</td>
</tr>
<tr>
<td>6</td>
<td>26 Hours</td>
<td>10 Minutes</td>
<td>15 Minutes</td>
</tr>
<tr>
<td>7</td>
<td>26 Hours</td>
<td>20 Minutes</td>
<td>5 Minutes</td>
</tr>
<tr>
<td>8</td>
<td>26 Hours</td>
<td>20 Minutes</td>
<td>15 Minutes</td>
</tr>
<tr>
<td>9 (Normal)</td>
<td>22-24 Hours</td>
<td>15 Minutes</td>
<td>10 Minutes</td>
</tr>
</tbody>
</table>

**Table 36: Strain List for Robustness Study**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Source</th>
<th>Identification</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Target</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em> O121</td>
<td>MSU(^1)</td>
<td>TW07931</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>ATCC(^2)</td>
<td>19118</td>
</tr>
<tr>
<td>Sample</td>
<td>Target</td>
<td>Low-Level¹</td>
</tr>
<tr>
<td>--------</td>
<td>--------</td>
<td>------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td>stx1/stx2</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Total</td>
<td>6/10</td>
<td>6/10</td>
</tr>
</tbody>
</table>

1 Michigan State Shiga toxin-producing *Escherichia coli* Center
2 American Type Culture Collection

Table 37: PolySkope 1.0 Multiplex Pathogen Detection Assay Robustness
Treatment Combination 1
Low-Level samples were inoculated with the target pathogens listed in Table 36 at a low level of 0.2-2 CFU/test portion.

Uninoculated samples were inoculated with the non-target pathogens listed in Table 36 at a high level of 2-10 CFU/test portion.

Table 38: PolySkope 1.0 Multiplex Pathogen Detection Assay Robustness
Treatment Combination 2

<table>
<thead>
<tr>
<th>Sample</th>
<th>Low-Level&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>stx1/stx2</td>
</tr>
<tr>
<td></td>
<td>Target</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>+</td>
</tr>
<tr>
<td>Total</td>
<td>6/10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>Non-Target&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>stx1/stx2</td>
</tr>
<tr>
<td></td>
<td>Target</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 39: PolySkope 1.0 Multiplex Pathogen Detection Assay Robustness
Treatment Combination 3

<table>
<thead>
<tr>
<th>Sample</th>
<th>stx1/stx2</th>
<th>eae</th>
<th>L. monocytogenes</th>
<th>Salmonella</th>
<th>Internal Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Total</td>
<td>6/10</td>
<td>6/10</td>
<td>3/10</td>
<td>4/10</td>
<td>10/10</td>
</tr>
</tbody>
</table>

1 Low-Level samples were inoculated with the target pathogens listed in Table 36 at a low level of 0.2-2 CFU/test portion
2 Uninoculated samples were inoculated with the non-target pathogens listed in Table 36 a high level of 2-10 CFU/test portion
Low-Level samples were inoculated with the target pathogens listed in Table 36 at a low level of 0.2-2 CFU/test portion.

Uninoculated samples were inoculated with the non-target pathogens listed in Table 36 at a high level of 2-10 CFU/test portion.

Table 40: PolySkope 1.0 Multiplex Pathogen Detection Assay Robustness
Treatment Combination 4

<table>
<thead>
<tr>
<th>Sample</th>
<th>stx1/stx2</th>
<th>eae</th>
<th>L. monocytogenes</th>
<th>Salmonella</th>
<th>Internal Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Total</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>10/10</td>
</tr>
<tr>
<td>Sample</td>
<td>Low-Level</td>
<td>Target</td>
<td>Internal Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>-----------</td>
<td>--------</td>
<td>------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>stx1/stx2</td>
<td>eae</td>
<td>L. monocytogenes</td>
<td>Salmonella</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Total</td>
<td>6/10</td>
<td>6/10</td>
<td>3/10</td>
<td>4/10</td>
<td>10/10</td>
</tr>
</tbody>
</table>

1Low-Level samples were inoculated with the target pathogens listed in Table 36 at a low level of 0.2-2 CFU/test portion
2Uninoculated samples were inoculated with the non-target pathogens listed in Table 36 a high level of 2-10 CFU/test portion
| Sample | Non-Target<sup>2</sup> | Target |
| --- | --- | --- | --- | --- | --- |
|  | stx1/stx2 | eae | *L*. monocytogenes | Salmonella | Internal Control |
| 1 | - | - | - | - | + |
| 2 | - | - | - | - | + |
| 3 | - | - | - | - | + |
| 4 | - | - | - | - | + |
| 5 | - | - | - | - | + |
| 6 | - | - | - | - | + |
| 7 | - | - | - | - | + |
| 8 | - | - | - | - | + |
| 9 | - | - | - | - | + |
| 10 | - | - | - | - | + |
| Total | 0/10 | 0/10 | 0/10 | 0/10 | 10/10 |

<sup>1</sup>Low-Level samples were inoculated with the target pathogens listed in Table 36 at a low-level of 0.2-2 CFU/test portion.

<sup>2</sup>Uninoculated samples were inoculated with the non-target pathogens listed in Table 36 at a high level of 2-10 CFU/test portion.

---

**Table 42:** PolySkope 1.0 Multiplex Pathogen Detection Assay Robustness

**Treatment Combination 6**

| Sample | Low-Level<sup>1</sup> | Target |
| --- | --- | --- | --- | --- | --- |
|  | stx1/stx2 | eae | *L*. monocytogenes | Salmonella | Internal Control |
| 1 | + | + | + | - | + |
| 2 | - | - | - | - | + |
| 3 | + | + | + | - | + |
| 4 | - | - | + | - | + |
| 5 | + | + | + | - | + |
| 6 | + | + | + | - | + |
| 7 | + | + | - | + | + |
| 8 | - | - | - | - | + |
| 9 | - | - | + | + | + |
| 10 | + | + | - | - | + |
**Table 43: PolySkope 1.0 Multiplex Pathogen Detection Assay Robustness Treatment Combination 7**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Low-Level¹ Target</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>stx1/stx2</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
</tr>
</tbody>
</table>

¹Low-Level samples were inoculated with the target pathogens listed in Table 36 at a low level of 0.2-2 CFU/test portion
²Uninoculated samples were inoculated with the non-target pathogens listed in Table 36 at a high level of 2-10 CFU/test portion
### Table 44: PolySkope 1.0 Multiplex Pathogen Detection Assay Robustness

<table>
<thead>
<tr>
<th>Sample</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Non-Target</strong></td>
<td>6/10</td>
<td>6/10</td>
<td>3/10</td>
<td>4/10</td>
<td>10/10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Target</strong></td>
<td>stx1/stx2</td>
<td>eae</td>
<td>L. monocytogenes</td>
<td>Salmonella</td>
<td>Internal Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>10/10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. Low-Level samples were inoculated with the target pathogens listed in Table 36 at a low level of 0.2-2 CFU/test portion.
2. Uninoculated samples were inoculated with the non-target pathogens listed in Table 36 at a high level of 2-10 CFU/test portion.

---

Page 92 of 95
<table>
<thead>
<tr>
<th>Sample</th>
<th>9</th>
<th>10</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Total</td>
<td>6/10</td>
<td>6/10</td>
<td>3/10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>Non-Target(^*)</th>
<th>Target</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>stx1/stx2</td>
<td>eae</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>0/10</td>
<td>0/10</td>
</tr>
</tbody>
</table>

\(^1\)Low-Level samples were inoculated with the target pathogens listed in Table 36 at a low level of 0.2-2 CFU/test portion

\(^2\)Uninoculated samples were inoculated with the non-target pathogens listed in Table 36 at a high level of 2-10 CFU/test portion

---

Table 45: PolySkope 1.0 Multiplex Pathogen Detection Assay Robustness Treatment Combination 9

<table>
<thead>
<tr>
<th>Sample</th>
<th>Low-Level(^1) Target</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>stx1/stx2</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>+</td>
</tr>
<tr>
<td>Sample</td>
<td>Non-Target²</td>
</tr>
<tr>
<td>--------</td>
<td>-------------</td>
</tr>
<tr>
<td></td>
<td>stx1/stx2</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>0/10</td>
</tr>
</tbody>
</table>

¹Low-Level samples were inoculated with the target pathogens listed in Table 36 at a low level of 0.2-2 CFU/test portion
²Uninoculated samples were inoculated with the non-target pathogens listed in Table 36 at a high level of 2-10 CFU/test portion
<table>
<thead>
<tr>
<th>Matrix</th>
<th>Strain</th>
<th>Treatment Combination</th>
<th>N(^a)</th>
<th>Treatment Combination</th>
<th>(x)^(b)</th>
<th>POD(^c)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh Raw Ground Beef</td>
<td>(E. coli) O121 MSU TW07931</td>
<td>1</td>
<td>10 6</td>
<td>2</td>
<td>0.60</td>
<td>0.31, 0.83</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>10 6</td>
<td>3</td>
<td>0.60</td>
<td>0.31, 0.83</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>10 6</td>
<td>5</td>
<td>0.60</td>
<td>0.31, 0.83</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>10 6</td>
<td>7</td>
<td>0.60</td>
<td>0.31, 0.83</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>10 6</td>
<td>9</td>
<td>0.60</td>
<td>0.31, 0.83</td>
<td></td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>ATCC 19118</td>
<td>1</td>
<td>10 3</td>
<td>2</td>
<td>0.30</td>
<td>0.11, 0.60</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>10 3</td>
<td>4</td>
<td>0.30</td>
<td>0.11, 0.60</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>10 3</td>
<td>6</td>
<td>0.30</td>
<td>0.11, 0.60</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>7</td>
<td>10 3</td>
<td>8</td>
<td>0.30</td>
<td>0.11, 0.60</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>9</td>
<td>10 3</td>
<td>10</td>
<td>0.30</td>
<td>0.11, 0.60</td>
<td></td>
</tr>
<tr>
<td>Salmonella Hadar</td>
<td>ATCC 51956</td>
<td>1</td>
<td>10 4</td>
<td>2</td>
<td>0.40</td>
<td>0.17, 0.69</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>10 4</td>
<td>4</td>
<td>0.40</td>
<td>0.17, 0.69</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>10 4</td>
<td>6</td>
<td>0.40</td>
<td>0.17, 0.69</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>7</td>
<td>10 4</td>
<td>8</td>
<td>0.40</td>
<td>0.17, 0.69</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>9</td>
<td>10 4</td>
<td>10</td>
<td>0.40</td>
<td>0.17, 0.69</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) N = Number of test portions  
\(^b\) \(x\) = Number of positive test portions  
\(^c\) POD\(_t\) = Treatment combination confirmed positive outcomes divided by the total number of trials