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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 Method^{SM} 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 ($dPOD_C$) or between presumptive and confirmed results ($dPOD_{CP}$) 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

Method Authors

Paul Smith, Michael Centola

Submitting Company

PolySkope Labs
755 Research Pkwy.
Oklahoma City, OK 73104
United States
www.polyskopelabs.com

Independent Laboratory

Q Laboratories, Inc.
1400 Harrison Ave
Cincinnati, OH 45214
United States

Reviewers

Michael Brodsky, Brodsky Consultants, Thornhill, Ontario, Canada

Yvonne Salfinger, Independent Consultant, Florida, USA

Brad Stawick, SGS North America, New Jersey, USA

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

Inspection Service (FSIS)-Microbiology Laboratory Guidebook (MLG) 5.09 [1] and 5B.05 [2], USDA/FSIS-MLG 8.10 [3], and the USDA/FSIS-MLG 4.09 [4] for fresh raw ground beef. The method is considered equivalent to the USDA/FSIS MLG 5.09 and 5B.05, USDA/FSIS MLG 8.10, and the USDA/FSIS MLG 4.09 for deli turkey. The method is also considered equivalent to the ISO/TS 13136: 2012 [5], US Food and Drug Administration (FDA)/Bacteriological Analytical Manual (BAM) Chapter 10 [6], and the FDA/BAM Chapter 5 [7] for fresh baby spinach. The method is also considered equivalent to the USDA/FSIS MLG 5B.05, FDA/BAM Chapter 10, and FDA/BAM Chapter 5 for stainless steel environmental surface.

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) eae

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 ± 1ºC) Polyskope Multiplex Enrichment Media (PMEM), at 130 RPM for 30 seconds, and incubate at 37 ± 1ºC 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 ± 1ºC) PMEM, homogenized for 30 seconds, and incubated at 37 ± 1ºC 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 2-8ºC. 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 ± 5ºC 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 ± 5ºC. 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 A: Identification of the Fluorophores

Fluorophore	Color	Target
FAM	Red	*stx1* and *stx2*
ABY	Blue	*eae*
VIC	Green	*Listeria monocytogenes*
ALEXA 647	Purple	*Salmonella enterica*
JUN	Yellow	Internal Control

Table B: Control Interpretation

Target	Target Probe Detection	Internal Control Detection
Positive Control	14 < Cq < 40	14 < Cq < 40
Negative Control	Cq = N/A	14 < Cq < 40

Table C: Interpretation of Sample Test Outcome

Target Probe Detection (Target Fluorophore)	Internal Control Detection	Interpretation
Cq ≥ 10	Cq ≥ 14	Positive
Cq = N/A	Cq ≥ 14	Negative
Cq = N/A	Cq = N/A	Inhibition

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) UDSA/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 (LOD_{50}).

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 *E. coli, Listeria monocytogenes,* and *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

Matrix/Test Portion Size	Inoculating Organism	Target Inoculum Level	# of Replicates	PolySkope Testing Time Point	Reference Method
Fresh Raw Ground Beef (73% lean) (25 g)	*E. coli* O157 ATCC[1] 43895, *Listeria monocytogenes* ATCC 7644, *Salmonella* Typhimurium ATCC 14028	0 CFU/ Test Portion	5	23 ± 1 hrs.	USDA/FSIS-MLG 5.09, USDA/FSIS-MLG 8.10, USDA/FSIS-MLG 4.09
		0.2-2 CFU/ Test Portion	20		
		2-10 CFU/ Test Portion	5		
Deli Turkey (25 g)	*E. coli* O26 MSU[2] TW00971, *Listeria monocytogenes* ATCC 19115, *Salmonella* Dublin ATCC 15480	0 CFU/ Test Portion	5	23 ± 1 hrs.	USDA/FSIS-MLG 5B.05, USDA/FSIS-MLG 8.10, USDA/FSIS-MLG 4.09
		0.2-2 CFU/ Test Portion	20		
		2-10 CFU/ Test Portion	5		
Fresh Baby Spinach (25 g)	*E. coli* O145 MSU TW09153, *Listeria monocytogenes* ATCC BAA-2658, *Salmonella* Enteritidis ATCC 13076	0 CFU/ Test Portion	5	23 ± 1 hrs.	ISO/TS 13136: 2012, FDA/BAM Chapter 10, FDA/BAM Chapter 5
		0.2-2 CFU/ Test Portion	20		
		2-10 CFU/ Test Portion	5		
Stainless Steel (4" x 4")	*E. coli* O103 MSU TW08101, *Listeria monocytogenes* ATCC 51780, *Salmonella* Kentucky ATCC 9263	0 CFU/ Test Area	5	23 ± 1 hrs.	USDA FSIS-MLG 5B.05, FDA/BAM Chapter 10, FDA/BAM Chapter 5
		~50 CFU/ Test Area	20		
		~500 CFU/ Test Area	5		

[1] ATCC - American Type Culture Collection
[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

$$(1-\frac{n_{select}}{n_{nonselect}})x100$$

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 ^{o}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 E: MPN Test Portion Sizes

Reference Method Test Portion	Inoculation Level	MPN Test Portions		
25 g	Low	5 x 50 g	20 x 25 g*	5 x 10 g
	High	5 x 50 g	20 x 25 g*	5 x 10 g

***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 (*stx*1/*stx*2 and *eae*). If a sample produced positive results for *stx*1 or *stx*2 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 *stx*1 or *stx*2 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 ^{o}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 (*stx*1/*stx*2 and *eae*). If a sample produced positive results for *stx*1 or *stx*2 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 ±1ºC. Following isolation of the typical colonies, isolated colonies from NA were confirmed for the presence of *stx*1 or *stx*2 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^{o}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^{o}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^{o}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^{o}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 F: Product Stability Conditions

Storage Type	Storage Temperature	Time Points (From the Date of Production)
Real Time	2-8ºC	1 month, 2.5 months, 5 months, 6 months
Accelerated	25 ± 2ºC	4 days, 9 days, 17 days, 20 days

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

Strain		Source	Identification
Target	*Escherichia coli* O121	MSU[1]	TW07931
	Listeria monocytogenes	ATCC[2]	19118
	Salmonella Hadar	ATCC	51956
non-Target	*Escherichia coli*	ATCC	8739
	Enterococcus faecalis	ATCC	29212
Treatment Combination	**Enrichment Time**	**First Lysis Time**	**Second Lysis Time**
1	20 Hours	10 Minutes	5 Minutes
2	20 Hours	10 Minutes	15 Minutes
3	20 Hours	20 Minutes	5 Minutes
4	20 Hours	20 Minutes	15 Minutes
5	26 Hours	10 Minutes	5 Minutes
6	26 Hours	10 Minutes	15 Minutes
7	26 Hours	20 Minutes	5 Minutes
8	26 Hours	20 Minutes	15 Minutes
9 (Normal)	22-24 Hours	15 Minutes	10 Minutes

[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

Matrix	**Fresh Raw Ground Beef**								
Method	**PolySkope 1.0 Pathogen Detection Assay**						**USDA/FSIS-MLG 5.09**	**USDA/FSIS-MLG8.10**	**USDA/FSIS-MLG 4.09**
Result	**Presumptive**			**Confirmed**					
Target	Big 6 STEC & *E. coli* O157	*L. monocytogenes*	*Salmonella*	Big 6 STEC & *E. coli* O157	*L. monocytogenes*	*Salmonella*			

Uninoculated	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Low	7/20	7/20	9/20	7/20	7/20	9/20	6/20	8/20	5/20
High	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Matrix	**Deli Turkey**								
Method	**PolySkope 1.0 Pathogen Detection Assay**						**USDA/ FSIS-MLG 5B.05**	**USDA/ FSIS-MLG8.10**	**USDA /FSIS-MLG 4.09**
Result	**Presumptive**			**Confirmed**					
Target	Big 6 STEC & *E. coli* O157	*L. monocytogenes*	*Salmonella*	Big 6 STEC & *E. coli* O157	*L. monocytogenes*	*Salmonella*			
Uninoculated	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Low	7/20	11/20	14/20	7/20	11/20	13/20	5/20	8/20	10/20
High	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5

Table H: Summary of Results (Continued)

Matrix	**Fresh Baby Spinach (25g)**								
Method	**PolySkope 1.0 Pathogen Detection Assay**						**FDA/BAM Chapter 4A**	**FDA/BAM Chapter 10**	**FDA/BAM Chapter 5**
Result	**Presumptive**			**Confirmed**					
Target	Big 6 STEC & *E. coli* O157	*L. monocytogenes*	*Salmonella*	Big 6 STEC & *E. coli* O157	*L. monocytogenes*	*Salmonella*			
Uninoculated	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Low	9/20	7/20	7/20	9/20	7/20	7/20	7/20	5/20	6/20
High	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Matrix	**Stainless Steel (4"x4")**								
Method	**PolySkope 1.0 Pathogen Detection Assay**						**FSIS-MLB**	**AMCh**	**AMCh**
Result	**Presumptive**			**Confirmed**					

Target	Big 6 STEC & *E. coli* O157	*L. monocytogenes*	*Salmonella*	Big 6 STEC & *E. coli* O157	*L. monocytogenes*	*Salmonella*			
Uninoculated	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Low	7/20	4/20	5/20	7/20	5/20	4/20	6/20	7/20	7/20
High	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5

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 $dPOD_C$ 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 18 and 22 of the Appendix.

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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 $dPOD_C$ 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 $dPOD_{CP}$ 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 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 $dPOD_C$ 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 $dPOD_{CP}$ 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 $dPOD_C$ 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 18 and 22 of the Appendix.

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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 $dPOD_C$ 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 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 $dPOD_C$ 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 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 $dPOD_{C}$ 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 $dPOD_{CP}$ 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.

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

Time Point		**Time Point**		**Time Point**		**Time Point**	
4 Days		**9 Days**		**17 Days**		**20 Days**	
Sample	**Result**	**Sample**	**Result**	**Sample**	**Result**	**Sample**	**Result**
STEC (Inoculating Organism - *E. coli* O45)							
Low Level	**4/10**	**Low Level**	6/10	Low Level	4/10	Low Level	5/10
Non-Target Organism	0/10	Non-Target Organism	0/10	Non-Target Organism	0/10	Non-Target Organism	0/10
Lysis Blank	0/3	Lysis Blank	0/3	Lysis Blank	0/3	Lysis Blank	0/3
Time Point		**Time Point**		**Time Point**		**Time Point**	

4 Days		9 Days		17 Days		20 Days	
Sample	**Result**	**Sample**	**Result**	**Sample**	**Result**	**Sample**	**Result**
Listeria monocytogenes							
Low Level	4/10	Low Level	4/10	Low Level	5/10	Low Level	3/10
Non-Target Organism	0/10	Non-Target Organism	0/10	Non-Target Organism	0/10	Non-Target Organism	0/10
Lysis Blank	0/3	Lysis Blank	0/3	Lysis Blank	0/3	Lysis Blank	0/3
Time Point		**Time Point**		**Time Point**		**Time Point**	
4 Days		**9 Days**		**17 Days**		**20 Days**	
Sample	**Result**	**Sample**	**Result**	**Sample**	**Result**	**Sample**	**Result**
Salmonella spp.							
Low Level	5/10	Low Level	6/10	Low Level	5/10	Low Level	7/10
Non-Target Organism	0/10	Non-Target Organism	0/10	Non-Target Organism	0/10	Non-Target Organism	0/10
Lysis Blank	0/3	Lysis Blank	0/3	Lysis Blank	0/3	Lysis Blank	0/3

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 2-8ºC, 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

Time Point		Time Point		Time Point		Time Point	
1 Month		2.5 Months		5 Months		6 Months	
Sample	Result	Sample	Result	Sample	Result	Sample	Result
E. coli O45							
Low Level	6/10	Low Level	6/10	Low Level	6/10	Low Level	6/10
Non-Target Organism	0/10	Non-Target Organism	0/10	Non-Target Organism	0/10	Non-Target Organism	0/10
Lysis Blank	0/3	Lysis Blank	0/3	Lysis Blank	0/3	Lysis Blank	0/3
Time Point		**Time Point**		**Time Point**		**Time Point**	
1 Month		**2.5 Months**		**5 Months**		**6 Months**	
Sample	**Result**	**Sample**	**Result**	**Sample**	**Result**	**Sample**	**Result**
Listeria monocytogenes							
Low Level	3/10	Low Level	3/10	Low Level	3/10	Low Level	3/10
Non-Target Organism	0/10	Non-Target Organism	0/10	Non-Target Organism	0/10	Non-Target Organism	0/10
Lysis Blank	0/3	Lysis Blank	0/3	Lysis Blank	0/3	Lysis Blank	0/3
Time Point		**Time Point**		**Time Point**		**Time Point**	
1 Month		**2.5 Months**		**5 Months**		**6 Months**	
Sample	**Result**	**Sample**	**Result**	**Sample**	**Result**	**Sample**	**Result**
Salmonella							
Low Level	5/10	Low Level	5/10	Low Level	5/10	Low Level	5/10
Non-Target Organism	0/10	Non-Target Organism	0/10	Non-Target Organism	0/10	Non-Target Organism	0/10
Lysis Blank	0/3	Lysis Blank	0/3	Lysis Blank	0/3	Lysis Blank	0/3

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

Robustness[1]					
STEC (Inoculating Organism *E. coli* O121 MSU TW07931)					
Treatment Combination	**1**	**2**	**Treatment Combination**	**3**	**4**
Low Level	6/10	6/10	Low Level	6/10	5/10
Non-Target Organisms[2]	0/10	0/10	Non-Target Organisms[2]	0/10	0/10
Treatment Combination	**5**	**6**	**Treatment Combination**	**7**	**8**
Low Level	6/10	6/10	Low Level	6/10	6/10
Non-Target Organisms[2]	0/10	0/10	Non-Target Organisms[2]	0/10	0/10
Treatment Combination 9 (Nominal)					
Low Level			6/10		
Non-Target Organisms[2]			0/10		

Table K: Robustness Results (Continued)

Robustness[1]					
***Listeria monocytogenes* ATCC 19118**					
Treatment Combination	**1**	**2**	**Treatment Combination**	**3**	**4**
Low Level	3/10	3/10	Low Level	3/10	3/10
Non-Target Organisms[2]	0/10	0/10	Non-Target Organisms[2]	0/10	0/10
Treatment Combination	**5**	**6**	**Treatment Combination**	**7**	**8**
Low Level	3/10	3/10	Low Level	3/10	3/10

Non-Target Organisms[2]	0/10	0/10	Non-Target Organisms[2]	0/10	0/10
Treatment Combination 9 (Nominal)					
Low Level			3/10		
Non-Target Organisms[2]			0/10		
Robustness[1]					
***Salmonella* Hadar ATCC 51956**					
Treatment Combination	**1**	**2**	**Treatment Combination**	**3**	**4**
Low Level	4/10	4/10	Low Level	4/10	4/10
Non-Target Organisms[2]	0/10	0/10	Non-Target Organisms[2]	0/10	0/10
Treatment Combination	**5**	**6**	**Treatment Combination**	**7**	**8**
Low Level	4/10	4/10	Low Level	4/10	4/10
Non-Target Organisms[2]	0/10	0/10	Non-Target Organisms[2]	0/10	0/10
Treatment Combination 9 (Nominal)					
Low Level			4/10		
Non-Target Organisms[2]			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”).

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APPENDIX

Table 1: Inclusivity Results for Non-O157 STEC

No.	Species	Serotype	Source	Origin	Result	No.	Species	Serotype	Source	Origin	Result
1	*E. coli*	O26	ATCC BAA-1653	Stool	+	26	*E. coli*	O103	QL 15071-2	Meat Powder	+
2	*E. coli*	O26	MSU TW 07862	Calf, Cow	+	27	*E. coli*	O111:H12	MSU DEC 6A	Infant	+
3	*E. coli*	O26	MSU TW02295	Infant	+	28	*E. coli*	O111:H8	MSU DEC 6C	Human	+
4	*E. coli*	O26	MSU DEC 9F	Human	+	29	*E. coli*	O111	MSU DEC 8D	Infant	+
5	*E. coli*	O26	MSU TW04270	Human	+	30	*E. coli*	O111	MSU TW07926	Human	+
6	*E. coli*	O26	MSU TW04284	Child	+	31	*E. coli*	O111	MSU TW14960	Human	+
7	*E. coli*	O26	MSU TW08031	Human	+	32	*E. coli*	O111	MSU TW06296	Child	+
8	*E. coli*	O26	MSU TW07814	Human	+	33	*E. coli*	O111	MSU TW05614	Human	+
9	*E. coli*	O26	MSU TW00971	Human Feces	+	34	*E. coli*	O111	MSU TW00186	Human	+
10	*E. coli*	O26	MSU TW05992	Human	+	35	*E. coli*	O111	MSU TW01387	Human	+
11	*E. coli*	O45	MSU TW10121	Human	+	36	*E. coli*	O121	PSU 10.0709	Not Available	+
12	*E. coli*	O45	MSU TW14003	Human	+	37	*E. coli*	O121	PSU 5.0959	Not Available	+
13	*E. coli*	O45	MSU TW07947	Human	+	38	*E. coli*	O121	PSU 7.1686	Not Available	+
14	*E. coli*	O45	MSU DEC 11C	Human	+	39	*E. coli*	O121	PSU 7.1709	Not Available	+
15	*E. coli*	O45	PSU 1.2622	Not Available	+	40	*E. coli*	O121	PSU 7.1732	Not Available	+
16	*E. coli*	O45	PSU 1.2635	Not Available	+	41	*E. coli*	O121	MSU TW07931	Human	+
17	*E. coli*	O45	PSU 2.0164	Not Available	+	42	*E. coli*	O121	MSU TW07614	Human	+
18	*E. coli*	O45	PSU 11.1079	Not Available	+	43	*E. coli*	O121	MSU TW08023	Human	+
19	*E. coli*	O103	MSU TW09101	Human	+	44	*E. coli*	O145	QL 15071-1	Meat Powder	+
20	*E. coli*	O103	MSU TW07971	Human	+	45	*E. coli*	O145	PSU 7.1711	Not Available	+
21	*E. coli*	O103	MSU TW11239	Child	+	46	*E. coli*	O145	PSU 10.0707	Not Available	+
22	*E. coli*	O103	MSU TW07697	Human	+	47	*E. coli*	O145	MSU TW09153	Human	+
23	*E. coli*	O103	PSU 5.1658	Not Available	+	48	*E. coli*	O145	MSU TW07596	Human	+
24	*E. coli*	O103	PSU 7.1691	Not Available	+	49	*E. coli*	O145	MSU TW09356	Human	+
25	*E. coli*	O103	PSU 9.0036	Not Available	+	50	*E. coli*	O145	MSU TW01664	Human	+

Table 2: Inclusivity Results for *E. coli* O157

No.	Species	Serotype	Source	Origin	Result	No.	Species	Serotype	Source	Origin	Result
1	*E. coli*	O157	MSU[1] TW00116	Human	+	26	*E. coli*	O157	MSU DEC3B	Human	+
2	*E. coli*	O157	MSU TW00975	Human	+	27	*E. coli*	O157	MSU DEC3C	Human	+
3	*E. coli*	O157	MSU TW02302	Hamburger	+	28	*E. coli*	O157	MSU DEC3D	Human	+
4	*E. coli*	O157	MSU TW04863	Human	+	29	*E. coli*	O157	MSU DEC3E	Human	+
5	*E. coli*	O157	MSU TW05356	Human	+	30	*E. coli*	O157	MSU DEC4A	Human	+
6	*E. coli*	O157	MSU TW07587	Human	+	31	*E. coli*	O157	MSU DEC4B	Human	+
7	*E. coli*	O157	ATCC[2] BAA-460	Human Feces	+	32	*E. coli*	O157	MSU DEC4C	Buffalo	+
8	*E. coli*	O157	NCTC[3] 12900	Not Available	+	33	*E. coli*	O157	MSU DEC4D	Cow, Calf	+
9	*E. coli*	O157	NCTC 13125	Human Stool	+	34	*E. coli*	O157	MSU DEC4E	Human	+
10	*E. coli*	O157	NCTC 13126	Not Available	+	35	*E. coli*	O157	QL[4] 164673	Beef Trim	+
11	*E. coli*	O157	NCTC 13127	Not Available	+	36	*E. coli*	O157	QL 2-202	Meat	+
12	*E. coli*	O157	NCTC 13128	Not Available	+	37	*E. coli*	O157	QL 2-203	Meat	+
13	*E. coli*	O157	ATCC 35150	Human Feces	+	38	*E. coli*	O157	QL 2-204	Meat	+
14	*E. coli*	O157	ATCC 43888	Human Feces	+	39	*E. coli*	O157	QL 2-205	Meat	+
15	*E. coli*	O157	ATCC 43889	Human Feces	+	40	*E. coli*	O157	QL 2-206	Meat	+
16	*E. coli*	O157	ATCC 43890	Human Feces	+	41	*E. coli*	O157	QL 2-207	Meat	+
17	*E. coli*	O157	ATCC 43894	Human Feces	+	42	*E. coli*	O157	QL 2-214	Meat	+
18	*E. coli*	O157	ATCC 43895	Raw Hamburger	+	43	*E. coli*	O157	QL 2-701	Beef	+
19	*E. coli*	O157	ATCC 51657	Clinical Isolate	+	44	*E. coli*	O157	QL 2-704	Beef	+
20	*E. coli*	O157	ATCC 51658	Clinical Isolate	+	45	*E. coli*	O157	QL 2-705	Beef	+
21	*E. coli*	O157	ATCC 51659	Clinical Isolate	+	46	*E. coli*	O157	QL 2-706	Beef	+
22	*E. coli*	O157	ATCC 700531	Clinical Isolate	+	47	*E. coli*	O157	QL 2-707	Beef	+
23	*E. coli*	O157	ATCC 700599	Salami	+	48	*E. coli*	O157	QL 2-708	Beef	+
24	*E. coli*	O157	ATCC 700927	Not Available	+	49	*E. coli*	O157	QL 2-710	Beef	+
25	*E. coli*	O157	MSU DEC3A	Human	+	50	*E. coli*	O157	QL 14077.1	Meat	+

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*

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

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)

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

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

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

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*

No.	Organism	Source	Origin	Result	No.	Organism	Source	Origin	Result
1	*L. monocytogenes* (1/2C)	CWD[1] 1553	Not Available	+	26	*L. monocytogenes* (N/A)	ATCC 19113	Not Available	+
2	*L. monocytogenes* (1/2A)	CWD 1554	Unknown, Carlisle, 1981	+	27	*L. monocytogenes* (4A)	ATCC 19114	Animal Tissue	+
3	*L. monocytogenes* (4B)	CWD 1563	Unknown, Lausanne ,1987	+	28	*L. monocytogenes* (4B)	ATCC 19115	Human	+
4	*L. monocytogenes* (4B)	CWD 1567	Unknown, Los Angeles, 1985	+	29	*L. monocytogenes* (4C)	ATCC 19116	Chicken	+
5	*L. monocytogenes* (4B)	CWD 1571	Not Available	+	30	*L. monocytogenes* (4E)	ATCC 19118	Chicken	+
6	*L. monocytogenes* (4B)	CWD 1590	Unknown, San Francisco	+	31	*L. monocytogenes* (N/A)	ATCC 49953	Goat, Belgium	+
7	*L. monocytogenes* (3B)	CWD 1600	Not Available	+	32	*L. monocytogenes* (1/2A)	ATCC 49594	Food, France	+
8	*L. monocytogenes* (1/2A)	CWD 1609	Turkey Factory	+	33	*L. monocytogenes* (3A)	ATCC 51782	Cheese	+
9	*L. monocytogenes* (1/2A)	CWD 1620	Turkey Factory	+	34	*L. monocytogenes* (N/A)	ATCC BAA- 2658	Not Available	+
10	*L. monocytogenes* (1/2B)	CWD 1626	Turkey Franks	+	35	*L. monocytogenes* (N/A)	QL[5] 030911-10	Clinical	+
11	*L. monocytogenes* (1/2B)	CWD 1627	Mother/Baby	+	36	*L. monocytogenes* (4B)	CWD 1561	Placenta	+
12	*L. monocytogenes* (4D)	ATCC[2] 19117	Sheep	+	37	*L. monocytogenes* (1/2B)	CWD 1601	Unknown, Los Angeles	+
13	*L. monocytogenes* (1/2A)	ATCC 51772	Not Available	+	38	*L. monocytogenes* (1/2A)	CWD 1612	Turkey Factory	+
14	*L. monocytogenes* (4B)	ATCC 51778	Dairy Products	+	39	*L. monocytogenes* (1/A)	CWD 1613	Turkey Factory	+
15	*L. monocytogenes* (1/2B)	ATCC 51780	Cheese	+	40	*L. monocytogenes* (1/2A)	CWD 1614	Unknown, Oklahoma	+
16	*L. monocytogenes* (1/2B)	ATCC BAA-751	Not Available	+	41	*L. monocytogenes* (1/2A)	CWD 1618	Turkey Factory	+
17	*L. monocytogenes* (7)	NCTC[3] 10890	Human Feces	+	42	*L. monocytogenes* (1/2A)	CWD 1629	Turkey Franks	+
18	*L. monocytogenes* (4B)	FSL[4]-F6-367	Not Available	+	43	*L. monocytogenes* (1/2A)	CWD 1630	Turkey Factory	+
19	*L. monocytogenes* (4AB)	FSL J1-129	Not Available	+	44	*L. monocytogenes* (4B)	CWD 1574	Unknown, Halifax, 1983	+
20	*L. monocytogenes* (3C)	FSL J1-049	Not Available	+	45	*L. monocytogenes* (1/2B)	CWD 1584	Not Available	+
21	*L. monocytogenes* (1/2C)	ATCC 7644	Human	+	46	*L. monocytogenes* (3B)	CWD 1586	Not Available	+
22	*L. monocytogenes* (4B)	ATCC 13932	Child with Meningitis	+	47	*L. monocytogenes* (1/2B)	CWD 1588	Not Available	+
23	*L. monocytogenes* (1/2A)	ATCC 15313	Rabbit	+	48	*L. monocytogenes* (4B)	CWD 1596	Not Available	+

24	*L. monocytogenes* (1)	ATCC 19111	Poultry	+	49	*L. monocytogenes* (1/2B)	CWD 1597	Not Available	+
25	*L. monocytogenes* (2)	ATCC 19112	Spinal Fluid	+	50	*L. monocytogenes* (1/2A)	CWD 1611	Turkey Factory	+

1. CWD – University of Vermont Culture Collection, 2. ATCC – American Type Culture Collection, 3. NCTC – National Culture Type Collection, 4. FSL – Cornell University Culture Collection, 5. QL – Q Laboratories Culture Collection

Table 6: Exclusivity Results for *Listeria monocytogenes*

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

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)
Deli Turkey	1.8×10^5	0/5	0/5	0/5
Matrix	**APC1 (CFU/g)**	**Non-O157 STEC Pathogen Screen[5] (325 g test portions)**	***Listeria monocytogenes* Pathogen Screen[3] (25 g test portions)**	***Salmonella* Pathogen Screen[4] (25 g test portions)**
Ground Beef	1.8×10^5	0/5	0/5	0/5
Matrix	**APC1 (CFU/g)**	**Non-O157 STEC Pathogen Screen[6] (325 g test portions)**	***Listeria monocytogenes* Pathogen Screen[7] (25 g test portions)**	***Salmonella* Pathogen Screen[8] (25 g test portions)**
Fresh Baby Spinach	1.8×10^5	0/5	0/5	0/5

[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

Matrix	Inoculating Organism	Agar	CFU/ g	Percent Injury
Deli Turkey	***E. coli* O26 MSU TW00971**	**TSA**	**6.0×10^9**	**58.33 %**
		mRBA	**2.5×10^8**	
	***Listeria monocytogenes* ATCC 19115**	**TSA**	**2.0×10^9**	**72.50 %**
		MOX	**5.5×10^7**	
	***Salmonella* Dublin ATCC 15480**	**TSA**	**4.2×10^9**	**64.29 %**
		XLT4	**1.5×10^8**	

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

Matrix	Stainless Steel		
Inoculating Organism	*E. coli* O103 MSU TW08101	*Listeria monocytogenes* ATCC 51780	*Salmonella* Kentucky ATCC 9263
Low-Inoculum Level CFU[a]/Test Area[b]	51	64	45
High-Inoculum Level CFU[a]/Test Area[b]	440	650	580

[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**					
[1]Low Level Inoculum (0.2-2 MPN/Test Portion)					
	A	**B**	**C**	**D**	**E**
5 x 50 g	**+**	**+**	**-**	**+**	**-**
20 x 25 g (Reference Samples)	**6/20**				
5 x 10 g	**-**	**+**	**-**	**-**	**+**
MPN/Test portion	**0.44**				
Low Conf. Limit MPN/Test Portion	**0.21**				
High Conf. Limit MPN/Test Portion	**0.76**				
[2]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	**3.01**				
Low Conf. Limit MPN/Test Portion	**1.31**				
High Conf. Limit MPN/Test Portion	**6.89**				

[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
[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

Table 10B: MPN Summary Table for Fresh Raw Ground Beef

Listeria monocytogenes ATCC 7644					
[1]Low Level Inoculum (0.2-2 MPN/Test Portion)					
	A	B	C	D	E
5 x 50 g	-	+	-	+	+
20 x 25 g (Reference Samples)	8/20				
5 x 10 g	-	+	-	-	+
MPN/Test portion	0.55				
Low Conf. Limit MPN/Test Portion	0.29				
High Conf. Limit MPN/Test Portion	0.94				
[2]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	3.01				
Low Conf. Limit MPN/Test Portion	1.31				
High Conf. Limit MPN/Test Portion	6.89				

[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

[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

Table 10C: MPN Summary Table for Fresh Raw Ground Beef

Salmonella Typhimurium ATCC 14028					
[1]Low Level Inoculum (0.2-2 MPN/Test Portion)					
	A	B	C	D	E
5 x 50 g	-	+	-	+	+
20 x 25 g (Reference Samples)	5/20				
5 x 10 g	-	+	-	-	-
MPN/Test portion	0.35				
Low Conf. Limit MPN/Test Portion	0.17				
High Conf. Limit MPN/Test Portion	0.62				
[2]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	2.29				
Low Conf. Limit MPN/Test Portion	1.05				
High Conf. Limit MPN/Test Portion	5.02				

[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

[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

Table 11A: MPN Summary Table for Deli Turkey

Escherichia coli O26 MSU TW00971					
[1]Low Level Inoculum (0.2-2 MPN/Test Portion)					
	A	B	C	D	E
5 x 50 g	+	+	-	+	-
20 x 25 g (Reference Samples)	5/20				
5 x 10 g	-	-	-	+	-
MPN/Test portion	0.35				
Low Conf. Limit MPN/Test Portion	0.14				
High Conf. Limit MPN/Test Portion	0.62				
[2]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	3.01				
Low Conf. Limit MPN/Test Portion	1.31				
High Conf. Limit MPN/Test Portion	6.89				

[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 11B: MPN Summary Table for Deli Turkey

Listeria monocytogenes ATCC 19115					
[1]Low Level Inoculum (0.2-2 MPN/Test Portion)					
	A	B	C	D	E
5 x 50 g	+	-	+	+	-
20 x 25 g (Reference Samples)	8/20				
5 x 10 g	-	-	-	+	+
MPN/Test portion	0.55				
Low Conf. Limit MPN/Test Portion	0.29				
High Conf. Limit MPN/Test Portion	0.93				
[2]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	3.01				
Low Conf. Limit MPN/Test Portion	1.31				
High Conf. Limit MPN/Test Portion	6.89				

[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 11C: MPN Summary Table for Deli Turkey

Salmonella Dublin ATCC 15480					
[1]Low Level Inoculum (0.2-2 MPN/Test Portion)					
	A	B	C	D	E
5 x 50 g	+	-	+	+	+
20 x 25 g (Reference Samples)	10/20				
5 x 10 g	-	-	+	-	+
MPN/Test portion	0.76				
Low Conf. Limit MPN/Test Portion	0.41				
High Conf. Limit MPN/Test Portion	1.27				
[2]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

Escherichia coli O145 MSU TW09153					
[1]Low Level Inoculum (0.2-2 MPN/Test Portion)					
	A	B	C	D	E
5 x 50 g	-	+	-	+	+
20 x 25 g (Reference Samples)	7/20				
5 x 10 g	-	-	+	+	+
MPN/Test portion	0.54				
Low Conf. Limit MPN/Test Portion	0.29				
High Conf. Limit MPN/Test Portion	0.90				
[2]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	3.01				
Low Conf. Limit MPN/Test Portion	1.31				
High Conf. Limit MPN/Test Portion	6.89				

[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

[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

Table 12B: MPN Summary Table for Fresh Baby Spinach

Listeria monocytogenes ATCC BAA-2658					
[1]Low Level Inoculum (0.2-2 MPN/Test Portion)					
	A	B	C	D	E
5 x 50 g	+	-	-	+	+
20 x 25 g (Reference Samples)	5/20				
5 x 10 g	-	-	+	-	-
MPN/Test portion	0.35				
Low Conf. Limit MPN/Test Portion	0.14				
High Conf. Limit MPN/Test Portion	0.63				
[2]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	3.01				
Low Conf. Limit MPN/Test Portion	1.31				
High Conf. Limit MPN/Test Portion	6.89				

[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

[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

Table 12C: MPN Summary Table for Fresh Baby Spinach

Salmonella Enteritidis ATCC 13076					
[1]Low Level Inoculum (0.2-2 MPN/Test Portion)					
	A	B	C	D	E
5 x 50 g	-	+	-	-	+
20 x 25 g (Reference Samples)	6/20				
5 x 10 g	-	-	-	-	+
MPN/Test portion	0.34				
Low Conf. Limit MPN/Test Portion	0.14				
High Conf. Limit MPN/Test Portion	0.61				
[2]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	2.29				
Low Conf. Limit MPN/Test Portion	1.05				
High Conf. Limit MPN/Test Portion	5.02				

[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

[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

Table 13: Detailed Results for the PolySkope 1.0 Multiplex Pathogen Detection Assay for Fresh Raw Ground Beef

***E. coli* O157 ATCC 43895, *Listeria monocytogenes* ATCC 7644, and *Salmonella* Typhimurium ATCC 14028**											
Low Level											
Sample #	**PolySkope 1.0 Multiplex Pathogen Detection Assay**								**USDA/FSIS MLG 5.09**	**USDA/FSIS MLG 8.10**	**USDA/FSIS MLG 5B.05**
	Presumptive					**Confirmed**					
	FAM *stx1/stx2*	**ABY eae**	**VIC *L. monocytogenes***	**ALEXA *Salmonella***	**JUN Internal Control**	**Big 6 STEC, Including O157**	***L. monocytogenes***	***Salmonella***			
1	-	-	+	+	+	-	+	+	-	-	-
2	-	-	-	+	+	-	-	+	+	+	-
3	-	-	+	-	+	-	+	-	+	-	-
4	+	+	+	-	+	+	+	-	-	-	-
5	+	+	-	-	+	+	-	-	-	+	-
6	+	+	+	+	+	+	+	+	-	-	+
7	-	-	-	-	+	-	-	-	-	-	-
8	-	-	-	+	+	-	-	+	+	-	-
9	-	-	+	-	+	-	+	-	-	+	-
10	-	-	-	-	+	-	-	-	-	+	-
11	+	+	-	-	+	+	-	-	-	-	+
12	-	-	+	-	+	-	+	-	-	-	-
13	-	-	-	-	+	-	-	-	+	+	-
14	-	-	-	+	+	-	-	+	-	-	+
15	+	+	-	-	+	+	-	-	-	-	-
16	-	-	-	+	+	-	-	+	-	+	-
17	-	-	-	-	+	-	-	-	+	-	-
18	+	+	-	+	+	+	-	+	+	-	+
19	-	-	+	+	+	-	+	+	-	+	-
20	+	+	-	+	+	+	-	+	-	+	+
Total	**7/20**	**7/20**	**7/20**	**9/20**	**20/20**	**7/20**	**7/20**	**9/20**	**6/20**	**8/20**	**5/20**
High Level											
1	+	+	+	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+	+	+	+
Total	**5/5**	**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	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-
Total	**0/5**	**0/5**	**0/5**	**0/5**	**0/5**	**0/5**	**0/5**	**0/5**	**0/5**	**0/5**	**0/5**

Table 14: Detailed Results for the PolySkope 1.0 Multiplex Pathogen Detection Assay for Deli Turkey

***E. coli* O26 MSU TW00971, *Listeria monocytogenes* ATCC 19115, and *Salmonella* Dublin ATCC 15480**											
Low Level											
Sample #	**PolySkope 1.0 Multiplex Pathogen Detection Assay**								**USDA/FSIS MLG 5B.05**	**USDA/FSIS MLG 8.10**	**USDA/FSIS MLG 5B.05**
	Presumptive					**Confirmed**					
	FAM *stx1/stx2*	**ABY *eae***	**VIC *L. monocytogenes***	**ALEXA *Salmonella***	**JUN Internal Control**	**Big 6 STEC, Including O157**	***L. monocyogenes***	***Salmonella***			
1	-	-	+	+	+	-	+	+	+	-	+
2	+	+	-	+	+	+	-	+	-	+	-
3	-	-	-	-	+	-	-	-	+	-	+
4	-	-	-	+	+	-	-	+	-	-	-
5	+	+	-	-	+	+	-	-	-	-	+
6	-	-	+	+	+	-	+	+	-	+	-
7	-	-	+	+	+	-	+	+	-	-	-
8	+	+	+	+	+	+	+	-	-	-	+
9	+	+	-	-	+	+	-	-	-	-	+
10	-	-	+	+	+	-	+	+	+	-	-
11	-	-	+	+	+	-	+	+	-	+	-
12	-	-	-	+	+	-	-	+	-	-	-
13	-	-	-	-	+	-	-	-	-	+	-
14	-	-	+	+	+	-	+	+	-	-	+
15	+	+	+	+	+	+	+	+	-	+	-
16	-	-	-	-	+	-	-	-	+	-	+
17	-	-	-	-	+	-	-	-	+	-	+
18	+	+	+	+	+	+	+	+	-	+	-
19	-	-	+	+	+	-	+	+	-	+	+
20	+	+	+	+	+	+	+	+	-	+	+
Total	**7/20**	**7/20**	**11/20**	**14/20**	**20/20**	**7/20**	**11/20**	**13/20**	**5/20**	**8/20**	**10/20**
High Level											
1	+	+	+	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+	+	+	+
Total	**5/5**	**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	-	-	-	-	-	-	-	-	-	-	-

5	-	-	-	-	-	-	-	-	-	-	-
Total	**0/5**	**0/5**	**0/5**	**0/5**	**0/5**	**0/5**	**0/5**	**0/5**	**0/5**	**0/5**	**0/5**

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

***E. coli* O145 MSU TW09153, *Listeria monocytogenes* ATCC BAA-2658, and *Salmonella* Enteritidis ATCC 13076**											
Low Level											
Sample #	**PolySkope 1.0 Multiplex Pathogen Detection Assay**								**ISO/TS 13136: 2012**	**FDA/BAM Chapter 10**	**FDA/BAM Chapter 5**
	Presumptive					**Confirmed**					
	FAM *stx1/stx2*	**ABY *eae***	**VIC *L. monocytogenes***	**ALEXA *Salmonella***	**JUN Internal control**	**Big 6 STEC, Including O157**	***L. monocytogenes***	***Salmonella***			
1	-	-	-	-	+	-	-	-	+	-	+
2	+	+	+	+	+	+	+	+	-	+	-
3	+	+	-	-	+	+	-	-	+	-	-
4	-	-	-	-	+	-	-	-	+	-	-
5	+	+	-	-	+	+	-	-	-	-	-
6	+	+	-	+	+	+	-	+	-	+	-
7	+	+	-	-	+	+	-	-	-	-	+
8	-	-	-	-	+	-	-	-	-	-	+
9	-	-	-	-	+	-	-	-	+	-	-
10	+	+	+	+	+	+	+	+	-	-	-
11	-	-	-	-	+	-	-	-	-	+	-
12	-	-	+	-	+	-	+	-	-	-	-
13	-	-	-	-	+	-	-	-	-	-	+
14	+	+	+	+	+	+	+	+	-	-	+
15	-	-	+	+	+	-	+	+	+	+	-
16	-	-	-	-	+	-	-	-	-	-	-
17	+	+	+	+	+	+	+	+	-	-	-
18	-	-	-	-	+	-	-	-	+	+	-
19	+	+	+	-	+	+	+	-	+	-	-
20	-	-	-	+	+	-	-	+	-	-	+
Total	**9/20**	**9/20**	**7/20**	**7/20**	**20/20**	**9/20**	**7/20**	**7/20**	**7/20**	**5/20**	**6/20**
High Level											
1	+	+	+	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+	+	+	+
Total	**5/5**	**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	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-
Total	**0/5**	**0/5**	**0/5**	**0/5**	**0/5**	**0/5**	**0/5**	**0/5**	**0/5**	**0/5**	**0/5**

Table 16: Detailed Results for the PolySkope 1.0 Multiplex Pathogen Detection Assay for Stainless Steel

***E. coli* O103 MSU TW08101, *Listeria monocytogenes* ATCC 51780, and *Salmonella* Kentucky ATCC 9263**											
Low Level											
Sample #	**PolySkope 1.0 Multiplex Pathogen Detection Assay**								**USDA/FSIS MLG 5B.05**	**FDA/BAM Chapter 10**	**FDA/BAM Chapter 5**
	Presumptive					**Confirmed**					
	FAM *stx1/stx2*	**ABY *eae***	**VIC *L. monocytogenes***	**ALEXA *Salmonella***	**JUN Internal Control**	**Big 6 STEC, Including O157**	***L. monocytogenes***	***Salmonella***			
1	+	+	-	+	+	+	-	+	-	-	+
2	-	-	-	-	+	-	-	-	+	+	-
3	-	-	+	-	+	-	+	-	-	+	-
4	-	-	-	-	+	-	-	-	+	-	+
5	-	-	-	+	+	-	-	+	-	-	-
6	-	-	-	-	+	-	-	-	-	+	-
7	+	+	-	-	+	+	-	-	-	-	-
8	-	-	-	-	+	-	-	-	-	-	+
9	-	-	-	+	+	-	-	+	-	-	-
10	+	+	-	-	+	+	-	-	-	-	-
11	-	-	+	-	+	-	+	-	+	+	-
12	-	-	-	-	+	-	-	-	-	-	+
13	+	+	-	-	+	+	-	-	-	-	-
14	-	-	-	-	+	-	-	-	-	-	-
15	+	+	-	-	+	+	-	-	+	+	-
16	-	-	+	+	+	-	+	+	-	+	+
17	-	-	-	-	+	-	-	-	-	-	-
18	+	+	-	+	+	+	-	+	+	+	+
19	+	+	-	-	+	+	-	-	+	-	+
20	-	-	+	-	+	-	+	-	-	-	-
Total	**7/20**	**7/20**	**4/20**	**5/20**	**20/20**	**7/20**	**4/20**	**5/20**	**6/20**	**7/20**	**7/20**
High Level											
1	+	+	+	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+	+	+	+
Total	**5/5**	**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	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-
Total	**0/5**	**0/5**	**0/5**	**0/5**	**0/5**	**0/5**	**0/5**	**0/5**	**0/5**	**0/5**	**0/5**

Table 17: PolySkope 1.0 Multiplex Pathogen Detection Assay, Candidate vs. Reference – POD Results

Matrix	Strain	MPN[a]/ Test Portion	N[b]	Candidate			Reference			dPOD$_C$[f]	95% CI[g]
				x[c]	POD$_C$[d]	95% CI	X	POD$_R$[e]	95% CI		
Fresh Raw Ground Beef	*E. coli* O157 ATCC 43895	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.44 (0.21, 0.76)	20	7	0.35	0.18, 0.57	6	0.30	0.15, 0.52	0.05	-0.23, 0.32
		3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	L. monocytogenes ATCC 7644	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.55 (0.29, 0.94)	20	7	0.35	0.18, 0.57	8	0.40	0.22, 0.61	-0.05	-0.32, 0.23
		3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	Salmonella Typhimurium ATCC 14028	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.35 (0.17, 0.62)	20	9	0.45	0.26, 0.66	5	0.25	0.11, 0.47	0.20	-0.09, 0.45
		2.29 (1.05, 5.02)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

[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 18: PolySkope 1.0 Multiplex Pathogen Detection Assay, Candidate vs. Reference – POD Results

Matrix	Strain	MPN[a]/ Test Portion	N[b]	Candidate			Reference			dPOD$_C$[f]	95% CI[g]
				x[c]	POD$_C$[d]	95% CI	X	POD$_R$[e]	95% CI		
Deli Turkey	*E. coli* O26 MSU TW00971	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.35 (0.14, 0.62)	20	7	0.35	0.18, 0.57	5	0.25	0.11, 0.47	0.10	-0.18, 0.36
		3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	L. monocytogenes ATCC 19115	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.55 (0.29, 0.93)	20	11	0.55	0.34, 0.74	8	0.40	0.22, 0.61	0.15	-0.15, 0.41
		3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	Salmonella Dublin ATCC 15480	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.76 (0.41, 1.27)	20	13	0.65	0.43, 0.82	10	0.50	0.30, 0.70	0.15	-0.15, 0.41
		4.38 (1.72, 11.15)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

[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 19: PolySkope 1.0 Multiplex Pathogen Detection Assay, Candidate vs. Reference – POD Results

Matrix	Strain	MPN[a]/ Test Portion	N[b]	Candidate			Reference			dPOD$_C$[f]	95% CI[g]
				x[c]	POD$_C$[d]	95% CI	X	POD$_R$[e]	95% CI		
Fresh Baby Spinach	*E. coli* O145 MSU TW09153	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.54 (0.29, 0.90)	20	9	0.45	0.26, 0.66	7	0.35	0.18, 0.57	0.10	-0.19, 0.37
		3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	L. monocytogenes ATCC BAA-2658	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.35 (0.14, 0.63)	20	7	0.35	0.18, 0.57	5	0.25	0.11, 0.47	0.10	-0.18, 0.36
		3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	Salmonella Enteritidis ATCC 13076	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.34 (0.14, 0.61)	20	7	0.35	0.18, 0.57	6	0.30	0.15, 0.52	0.05	-0.23, 0.32
		2.29 (1.05, 5.02)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

[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

Matrix	Strain	CFU[a]/ Test Area	N[b]	Candidate			Reference			dPOD$_C$[f]	95% CI[g]
				x[c]	POD$_C$[d]	95% CI	X	POD$_R$[e]	95% CI		
Stainless Steel	*E. coli* O103 MSU TW08101	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		51	20	7	0.35	0.18, 0.57	6	0.30	0.15, 0.52	0.05	-0.23, 0.32
		440	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	L. monocytogenes ATCC 51780	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		64	20	4	0.20	0.08, 0.42	7	0.35	0.18, 0.57	-0.15	-0.40, 0.12
		650	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	Salmonella Kentucky ATCC 9263	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		45	20	5	0.25	0.11, 0.47	7	0.35	0.18, 0.57	-0.10	-0.36, 0.18
		580	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

[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

Matrix	Strain	MPN[a]/ Test Portion	N[b]	Presumptive			Confirmed			dPOD$_{CP}$[f]	95% CI[g]
				x[c]	POD$_{CP}$[d]	95% CI	X	POD$_{CC}$[e]	95% CI		
Fresh Raw Ground Beef	*E. coli* O157 ATCC 43895	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.44 (0.21, 0.76)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.13, 0.13
		3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
	L. monocytogenes ATCC 7644	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.55 (0.29, 0.94)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.13, 0.13
		3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
	Salmonella Typhimurium ATCC 14028	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.35 (0.17, 0.62)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.13, 0.13
		2.29 (1.05, 5.02)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47

[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$_{CP}$ = Candidate method presumptive positive outcomes divided by the total number of trials
[e]POD$_{CC}$ = Candidate method confirmed positive outcomes divided by the total number of trials
[f]dPOD$_{CP}$= Difference between the candidate method presumptive result and candidate 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 22: PolySkope 1.0 Multiplex Pathogen Detection Assay, Presumptive vs. Confirmed – POD Results

Matrix	Strain	MPN[a]/ Test Portion	N[b]	Presumptive			Confirmed			dPOD$_{CP}$[f]	95% CI[g]
				x[c]	POD$_{CP}$[d]	95% CI	X	POD$_{CC}$[e]	95% CI		
Deli Turkey	*E. coli* O26 MSU TW00971	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.35 (0.14, 0.62)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.13, 0.13
		3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
	L. monocytogenes ATCC 19115	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.55 (0.29, 0.93)	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0.00	-0.13, 0.13
		3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
	Salmonella Dublin ATCC 15480	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.76 (0.41, 1.27)	20	14	0.70	0.48, 0.85	13	0.65	0.43, 0.82	0.05	-0.11, 0.21
		4.38 (1.72, 11.15)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47

[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$_{CP}$ = Candidate method presumptive positive outcomes divided by the total number of trials
[e]POD$_{CC}$ = Candidate method confirmed positive outcomes divided by the total number of trials
[f]dPOD$_{CP}$= Difference between the candidate method presumptive result and candidate 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 23: PolySkope 1.0 Multiplex Pathogen Detection Assay, Presumptive vs. Confirmed – POD Results

Matrix	Strain	MPN[a]/ Test Portion	N[b]	Presumptive			Confirmed			dPOD$_{CP}$[f]	95% CI[g]
				x[c]	POD$_{CP}$[d]	95% CI	X	POD$_{CC}$[e]	95% CI		
Fresh Baby Spinach	*E. coli* O145 MSU TW09153	-	5	0	0.00	0.00, 0.43	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	0.45	0.26, 0.66	0.00	-0.13, 0.13
		3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
	L. monocytogenes ATCC BAA-2658	-	5	0	0.00	0.00, 0.43	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	0.35	0.18, 0.57	0.00	-0.13, 0.13
		3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
	Salmonella Enteritidis ATCC 13076	-	5	0	0.00	0.00, 0.43	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	0.35	0.18, 0.57	0.00	-0.13, 0.13
		2.29 (1.05, 5.02)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47

[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$_{CP}$ = Candidate method presumptive positive outcomes divided by the total number of trials
[e]POD$_{CC}$ = Candidate method confirmed positive outcomes divided by the total number of trials
[f]dPOD$_{CP}$= Difference between the candidate method presumptive result and candidate 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 24: PolySkope 1.0 Multiplex Pathogen Detection Assay, Presumptive vs. Confirmed – POD Results

Matrix	Strain	CFU[a]/ Test Area	N[b]	Presumptive			Confirmed			dPOD$_{CP}$[f]	95% CI[g]
				x[c]	POD$_{CP}$[d]	95% CI	X	POD$_{CC}$[e]	95% CI		
Stainless Steel	*E. coli* O103 MSU TW08101	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		51	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.13, 0.13
		440	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
	L. monocytogenes ATCC 51780	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		64	20	4	0.20	0.08, 0.42	4	0.20	0.08, 0.42	0.00	-0.13, 0.13
		650	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
	Salmonella Kentucky ATCC 9263	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		45	20	5	0.25	0.11, 0.47	5	0.25	0.11, 0.47	0.00	-0.13, 0.13
		580	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47

[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$_{CP}$ = Candidate method presumptive positive outcomes divided by the total number of trials
[e]POD$_{CC}$ = Candidate method confirmed positive outcomes divided by the total number of trials
[f]dPOD$_{CP}$= Difference between the candidate method presumptive result and candidate 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 25: Product Stability and Lot to Lot Outline and Information

Storage Type	Storage Temperature	Time Points (From the Date of Production)	
Accelerated	25 ± 2ºC	4 days, 9 days, 17 days, 20 days	
Real Time	2-8ºC	1 month, 2.5 months, 5 months, 6 months	
Lot Information[1]			
Lot 1		052717	
Lot 2		081217	
Lot 3		102417	
Lot 4		121717	
Strain		**Source**	**Identification**
Target	*E. coli* O45	MSU[2]	TW09183
	Listeria monocytogenes	ATCC[3]	13932
	Salmonella Choleraesuis	ATCC	10708
non-Target	*Escherichia coli*	ATCC	8739

[1]All three lots were combined to make a single lot and the product stability and lot to lot was analyzed at once.
[2]Michigan State Shiga toxin-producing *Escherichia coli* Center
[3]American Type Culture Collection

Table 26: Accelerated Product Stability and Lot to Lot Detailed Results – 4 Days

Sample	Low-Level[1]				
	Target				
	stx1/stx2	*eae*	*L. monocytogenes*	*Salmonella*	Internal Control
1	+	+	-	+	+
2	-	-	-	-	+
3	+	+	+	-	+
4	-	-	-	+	+
5	-	-	+	+	+
6	-	-	-	+	+
7	-	-	+	-	+
8	+	+	-	-	+
9	+	+	+	-	+
10	-	-	-	+	+
Total	4/10	4/10	4/10	5/10	10/10
Sample	**Non-Target[2]**				
	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
Sample	**Lysis Buffer Blank**				
	Target				
	stx1/stx2	***eae***	***L. monocytogenes***	***Salmonella***	**Internal Control**
1	-	-	-	-	+
2	-	-	-	-	+
3	-	-	-	-	+
Total	0/10	0/10	0/10	0/10	10/10

[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

Sample	Low-Level[1]				
	Target				
	stx1/stx2	*eae*	*L. monocytogenes*	*Salmonella*	Internal Control
1	-	-	+	-	+
2	-	-	-	+	+
3	+	+	-	+	+
4	+	+	-	-	+
5	-	-	+	+	+
6	+	+	-	+	+
7	+	+	-	+	+
8	+	+	+	-	+
9	+	+	-	+	+
10	-	-	+	-	+
Total	6/10	6/10	4/10	6/10	10/10
Sample	Non-Target[2]				
	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
Sample	Lysis Buffer Blank				
	Target				
	stx1/stx2	*eae*	*L. monocytogenes*	*Salmonella*	Internal Control
1	-	-	-	-	+
2	-	-	-	-	+
3	-	-	-	-	+
Total	0/10	0/10	0/10	0/10	10/10

[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 28: Accelerated Product Stability and Lot to Lot Detailed Results – 17 Days

Sample	Low-Level[1]				
	Target				
	stx1/stx2	*eae*	*L. monocytogenes*	*Salmonella*	Internal Control
1	-	-	+	-	+
2	+	+	-	-	+
3	-	-	-	+	+
4	-	-	+	+	+
5	+	+	-	+	+
6	-	-	-	+	+
7	+	+	+	-	+
8	+	+	-	+	+
9	-	-	+	-	+
10	-	-	+	-	+
Total	4/10	4/10	5/10	5/10	10/10
Sample	**Non-Target[2]**				
	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
Sample	**Lysis Buffer Blank**				
	Target				
	stx1/stx2	*eae*	*L. monocytogenes*	*Salmonella*	Internal Control
1	-	-	-	-	+
2	-	-	-	-	+
3	-	-	-	-	+
Total	0/10	0/10	0/10	0/10	10/10

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

Sample	Low-Level[1]				
	Target				
	stx1/stx2	*eae*	*L. monocytogenes*	*Salmonella*	Internal Control
1	-	-	-	+	+
2	+	+	+	-	+
3	-	-	-	+	+
4	+	+	-	+	+
5	+	+	+	+	+
6	+	+	-	-	+
7	-	-	-	+	+
8	-	-	-	+	+
9	+	+	-	+	+
10	-	-	+	-	+
Total	5/10	5/10	3/10	7/10	10/10
Sample	Non-Target[2]				
	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
Sample	Lysis Buffer Blank				
	Target				
	stx1/stx2	*eae*	*L. monocytogenes*	*Salmonella*	Internal Control
1	-	-	-	-	+

2	-	-	-	-	+
3	-	-	-	-	+
Total	0/10	0/10	0/10	0/10	10/10

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

Sample	Low-Level[1]				
	Target				
	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
Sample	**Non-Target[2]**				
	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
Sample	**Lysis Buffer Blank**				
	Target				

	stx1/stx2	*eae*	*L. monocytogenes*	*Salmonella*	Internal Control
1	-	-	-	-	+
2	-	-	-	-	+
3	-	-	-	-	+
Total	0/10	0/10	0/10	0/10	10/10

[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

Sample	Low-Level[1]				
	Target				
	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
Sample	**Non-Target[2]**				
	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
Sample	**Lysis Buffer Blank**				
	Target				
	stx1/stx2	***eae***	***L. monocytogenes***	***Salmonella***	**Internal Control**
1	-	-	-	-	+
2	-	-	-	-	+
3	-	-	-	-	+
Total	0/10	0/10	0/10	0/10	10/10

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

Sample	**Low-Level[1]**				
	Target				
	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
Sample	**Non-Target[2]**				
	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
Sample	**Lysis Buffer Blank**				
	Target				
	stx1/stx2	***eae***	***L. monocytogenes***	***Salmonella***	**Internal Control**
1	-	-	-	-	+
2	-	-	-	-	+
3	-	-	-	-	+
Total	0/10	0/10	0/10	0/10	10/10

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

Sample	**Low-Level[1]**				
	Target				
	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
Sample	**Non-Target[2]**				
	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
Sample	**Lysis Buffer Blank**				
	Target				
	stx1/stx2	***eae***	***L. monocytogenes***	***Salmonella***	**Internal Control**
1	-	-	-	-	+
2	-	-	-	-	+
3	-	-	-	-	+
Total	0/10	0/10	0/10	0/10	10/10

[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 34: PolySkope 1.0 Multiplex Pathogen Detection Assay – Stability and Lot to Lot Inoculated Test Portions– POD Results

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

	5 Months	*E. coli* O45	10	6	0.60	0.31, 0.83
		Listeria monocytogenes	10	3	0.30	0.11, 0.60
		Salmonella spp.	10	5	0.50	0.24, 0.76
	6 Months	*E. coli* O45	10	6	0.60	0.31, 0.83
		Listeria monocytogenes	10	3	0.30	0.11, 0.60
		Salmonella spp.	10	5	0.50	0.24, 0.76

Table 35: PolySkope 1.0 Multiplex Pathogen Detection Assay Robustness Experimental Design

Treatment Combination	Enrichment Time	Initial Lysis Time	Second Lysis Time
1	20 Hours	10 Minutes	5 Minutes
2	20 Hours	10 Minutes	15 Minutes
3	20 Hours	20 Minutes	5 Minutes
4	20 Hours	20 Minutes	15 Minutes
5	26 Hours	10 Minutes	5 Minutes
6	26 Hours	10 Minutes	15 Minutes
7	26 Hours	20 Minutes	5 Minutes
8	26 Hours	20 Minutes	15 Minutes
9 (Normal)	22-24 Hours	15 Minutes	10 Minutes

Table 36: Strain List for Robustness Study

Strain		Source	Identification
Target	*E. coli* O121	MSU[1]	TW07931
	Listeria monocytogenes	ATCC[2]	19118

	Salmonella Hadar	ATCC	51956
non-Target	*Escherichia coli*	ATCC	8739
	Enterococcus faecalis	ATCC	29212

[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

Sample	Low-Level[1]				
	Target				
	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	4/10	10/10
Sample	**Non-Target[2]**				
	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

[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

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

Sample	Low-Level[1]				
	Target				
	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	4/10	10/10
Sample	**Non-Target[2]**				
	Target				
	stx1/stx2	***eae***	***L.***	***Salmonella***	**Internal Control**

			monocytogenes		
1	-	-	-	-	+
2	-	-	-	-	+
3	-	-	-	-	+
4	-	-	-	-	+
5	-	-	-	-	+
6	-	-	-	-	+
7	-	-	-	-	+
8	-	-	-	-	+
9	-	-	-	-	+
10	-	-	-	-	+
Total	0/10	0/10	0/10	0/10	10/10

[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

Table 39: PolySkope 1.0 Multiplex Pathogen Detection Assay Robustness Treatment Combination 3

Sample	**Low-Level[1]**				
	Target				
	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	4/10	10/10
Sample	**Non-Target[2]**				
	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

[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

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

Sample	Low-Level[1]				
	Target				
	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	4/10	10/10
Sample	Non-Target[2]				

	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

[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

Table 41: PolySkope 1.0 Multiplex Pathogen Detection Assay Robustness Treatment Combination 5

Sample	**Low-Level[1]**				
	Target				
	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	4/10	10/10

Sample	Non-Target[2]				
	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

[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

Table 42: PolySkope 1.0 Multiplex Pathogen Detection Assay Robustness Treatment Combination 6

Sample	Low-Level[1]				
	Target				
	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	4/10	10/10
Sample	**Non-Target[2]**				
	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

[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

Table 43: PolySkope 1.0 Multiplex Pathogen Detection Assay Robustness Treatment Combination 7

Sample	Low-Level[1]				
	Target				
	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	4/10	10/10
Sample	**Non-Target[2]**				
	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

[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

Table 44: PolySkope 1.0 Multiplex Pathogen Detection Assay Robustness Treatment Combination 8

Sample	**Low-Level[1]**				
	Target				
	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	4/10	10/10
Sample	**Non-Target[2]**				
	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

[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

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

Sample	**Low-Level[1]**				
	Target				
	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	4/10	10/10
Sample	**Non-Target[2]**				
	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

[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

Table 46: PolySkope 1.0 Multiplex Pathogen Detection Assay - Robustness Inoculated Test Portions– POD Results

Matrix	Strain	Treatment Combination	N[a]	Treatment Combination		
				x[b]	POD_t[c]	95% CI
Fresh Raw Ground Beef	*E. coli* O121 MSU TW07931	1	10	6	0.60	0.31, 0.83
		2	10	6	0.60	0.31, 0.83
		3	10	6	0.60	0.31, 0.83
		4	10	6	0.60	0.31, 0.83
		5	10	6	0.60	0.31, 0.83
		6	10	6	0.60	0.31, 0.83
		7	10	6	0.60	0.31, 0.83
		8	10	6	0.60	0.31, 0.83
		9	10	6	0.60	0.31, 0.83
	Listeria monocytogenes ATCC 19118	1	10	3	0.30	0.11, 0.60
		2	10	3	0.30	0.11, 0.60
		3	10	3	0.30	0.11, 0.60
		4	10	3	0.30	0.11, 0.60
		5	10	3	0.30	0.11, 0.60
		6	10	3	0.30	0.11, 0.60
		7	10	3	0.30	0.11, 0.60
		8	10	3	0.30	0.11, 0.60
		9	10	3	0.30	0.11, 0.60
	Salmonella Hadar ATCC 51956	1	10	4	0.40	0.17, 0.69
		2	10	4	0.40	0.17, 0.69
		3	10	4	0.40	0.17, 0.69
		4	10	4	0.40	0.17, 0.69
		5	10	4	0.40	0.17, 0.69
		6	10	4	0.40	0.17, 0.69
		7	10	4	0.40	0.17, 0.69
		8	10	4	0.40	0.17, 0.69
		9	10	4	0.40	0.17, 0.69

[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