Evaluation of the 3M™ Molecular Detection System for the Detection of STEC and *Salmonella* in Beef and Poultry Matrices

Background

Escherichia coli (E. coli) is a normal inhabitant of the intestinal tracts of humans and other animals. While most *E. coli* are non-pathogenic, some types of *E. coli* such as Shiga toxin-producing *E. coli* (STEC) are pathogenic. STEC, also known as "VTEC, verocytotoxin-producing *E. coli,*" is a type of pathogenic *E. coli* that produces a potent toxin called Shiga toxin (Stx), also known as verotoxin or verocytotoxin. STEC can produce shiga toxin type 1 (Stx1), type 2 (Stx2), or both, encoded by *stx1* and *stx2* genes, respectively. STEC are further classified based on other virulence factors that they produce. For example, STEC containing virulence genes for *stx1* and/or *stx2* and for *eae* (the intimin gene involved in attaching and effacing phenotype) are designated enterohemorrhagic *E. coli* (EHEC) making EHEC a subset of STEC. The terms "STEC" and "EHEC" are often used interchangeably

The STEC group of *E. coli* are is important etiologic agent of food borne illness worldwide, causing diarrhea, hemorrhagic colitis, and life-threatening hemolytic uremic syndrome (1). The routes of transmission to humans is often through ingestion of contaminated beef, raw milk, and fresh produce. Globally, STEC is estimated to result in more than 1 million illnesses per year (2). According to CDC estimates, 265,000 STEC infections occur each year in the United States (US) with STEC O157 accounting for about 36% of these infections, and the rest by non-O157 STEC (3).

Salmonella is recognized as a major cause of foodborne infection in humans (4, 5). Since 1998, there have been 2,711 outbreaks in US due to food-borne *Salmonella* contamination (6). Annually, *Salmonella* is implicated as the causative agent resulting in 1.2 million illnesses, 23,000 hospitalizations and 450 deaths in US (7).

Ruminants are the primary reservoir of EHEC (8-11), and cattle have been identified as important reservoirs for STEC as well as *Salmonella*. The hides, hooves, and gastrointestinal (GI) tracts of cattle can contain these pathogens and during slaughter operations, raw products can be contaminated. In 2019, more than 12 million pounds of beef products were recalled due to *Salmonella* contamination (12) and 160,000 pounds of ground beef contaminated with STEC O103 was also recalled (13). Beef products have been the prominent cause of *E. coli* outbreaks, accounting for 143 (23%) of the 615 *E. coli* outbreaks reported since 1998. Although *Salmonella* is commonly considered a poultry adulterant, several outbreaks have also been traced to beef products (6).

In 1994, the USDA-FSIS declared *Escherichia coli* O157:H7 in raw ground beef (14), and then in 2012, declared six non-O157 STEC serogroups (O26, O45, O103, O111, O121 and O145), to be adulterants in non-intact raw beef products (zero tolerance policy) (15). Since June 2014, FSIS has been analyzing *Salmonella* in all raw beef samples along with required STEC analysis. FSIS has announced its intentions to develop new ground beef performance standards based on these data, and intends to expand non-O157 STEC and *Salmonella* testing to all raw beef products (16).

In order to ensure safe food, food processors need rapid, accurate and sensitive methods to detect the pathogens faster than traditional cultural methods. The regulatory requirement for zero tolerance policy foods requires detection of 1 colony forming unit (CFU) per sample. The use of DNA amplification has enabled the rapid detection of pathogenic microorganisms in the food industry by targeting and amplifying specific genetic markers. After a sample is enriched, a portion is processed to lyse bacterial cells present in the sample. Bacterial DNA is then amplified by methods such as polymerase chain reaction (PCR) and the amplification products are detected by various means such as fluorescence, bioluminescence, calorimetry, etc. Compared to traditional methods that rely on selective differential agar plating with further biochemical testing, the detection of unique DNA sequences enables very rapid and highly specific screening of a sample to determine if it is contaminated. Recently, USDA FSIS has updated its Microbiology Laboratory Guidebook (MLG) to include rapid molecular screening assays as primary screen for *Salmonella* (17), *Listeria monocytogenes* (18) and the top seven STEC (19) to support its regulatory activities, giving credence to acceptance of these DNA based methods. It is also critical that rapid DNA-based methods should be validated for various matrices to provide food manufacturers and testing laboratories, the confidence that these methods are fit for their purpose.

PCR has been used as the primary method for food-borne pathogens for the last several years. 3M has introduced the 3M™ Molecular Detection System based on Loop-mediated Isothermal Amplification (LAMP) (20) with bioluminescent detection for various food-borne pathogens such as *Salmonella*, *Listeria monocytogenes*, *Campylobacter*, etc. The LAMP-bioluminescent method offers simplicity, ease of use and cost effectiveness, allowing both big and small food processors to adopt the method. LAMP is the result of novel developments in molecular biology and has been widely used for rapid molecular testing (21, 22) with over 800 peer-reviewed publications citing this technology. It is recognized throughout the scientific literature as a highly robust, efficient, sensitive, specific, and simple nucleic acid amplification technique. Studies have shown that when compared to PCR, LAMP has higher or equivalent sensitivity and greater ability to amplify DNA in the presence of interfering substances often found in food and environmental samples (23-26). USDA FSIS has recognized these methods by adopting 3M™ Molecular Detection Assay 2 - *Salmonella* and 3M™ Molecular Detection Assay 2 – *Listeria monocytogenes* as a primary method in its MLG.

In 2019, 3M introduced LAMP-bioluminescent method for detection of STEC using a gene screen assay for *stx* and *eae* genes (27). The 3M™ Molecular Detection Assay 2 - STEC Gene Screen (*stx* and *eae*) method has received AOAC® *Performance Tested Method*^{SM} (PTM) approval for beef matrices (trim and ground beef) (28). The 3M™ Molecular Detection Assay 2 - *Salmonella* (29) method has previously received AOAC® PTM (30), AOAC® *Official Method of Analysis*^{SM} (OMA) (31)

and AFNOR certification by NF VALIDATION (32) approvals for various matrices. USDA FSIS has recently updated the MLG for *Salmonella* to include 3M Molecular Detection Assay 2 - *Salmonella* method as a rapid screen (17).

The purpose of this third-party study, conducted at an ISO/IEC 17025 accredited laboratory, was to compare the 3M Molecular Detection System for the detection of STEC and *Salmonella* (MLG 4.10 method, 17) in beef and poultry matrices. The 3M Molecular Detection Assay 2 - STEC Gene Screen (*stx* and *eae*) method for STEC detection in beef and poultry matrices was compared to the MLG 5C.00 gene screen method (19). In addition, dual enrichments for STEC and *Salmonella* were also evaluated by 3M methods.

Material and Methods

The enrichment conditions for the 3M methods and for the MLG reference method for poultry and beef matrices are shown in Table 1. For STEC, the 3M Molecular Detection Assay 2 - STEC Gene Screen (*stx* and *eae*), and the MLG 5C.00 reference method were used. For *Salmonella, the* 3M Molecular Detection Assay 2 - *Salmonella* method and the MLG 4.10 culture confirmation procedure with modification were used. The flow chart for detection of STEC (paired and unpaired) and *Salmonella* in matrices tested is shown in Figure 1 and 2.

Table 1. Enrichment protocols.

Sample Matrix	Sample Size	Enrichment Broth (pre-warmed)		Enrichment Temperature (± 1°C)	Enrichment Time (hours)
		3M Method (STEC and *Salmonella*)[a]	MLG 5C.00 Method (STEC)[b]		
Raw ground beef, Beef trim	325 g	975 mL BPW-ISO	975 mL mTSB	42	15
Chicken breast, Mechanically separated chicken (MSC), Chicken parts	325 g	975 mL BPW-ISO	975 mL mTSB	42	15

[a]For 3M method, matrices were inoculated with both STEC and *Salmonella*.
[b]For MLG 5C.00 method, matrices were inoculated with only STEC.

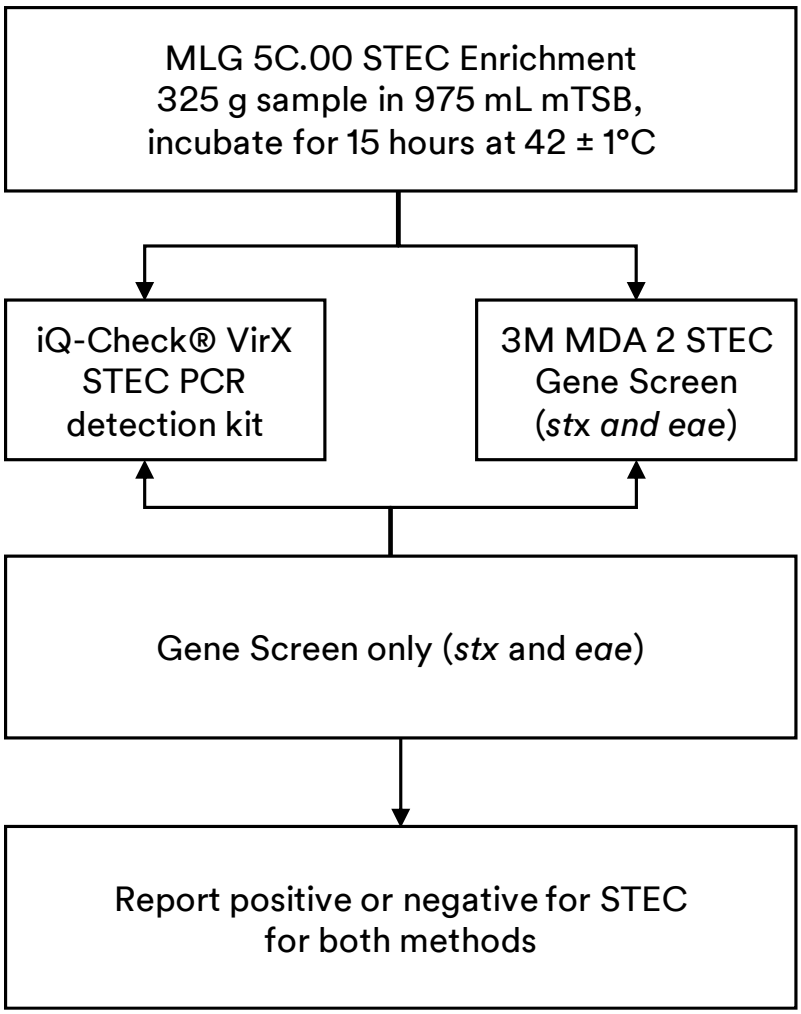

Figure 1. Flow chart for Detection of STEC in beef and poultry matrices (MLG 5C.00 enrichment).

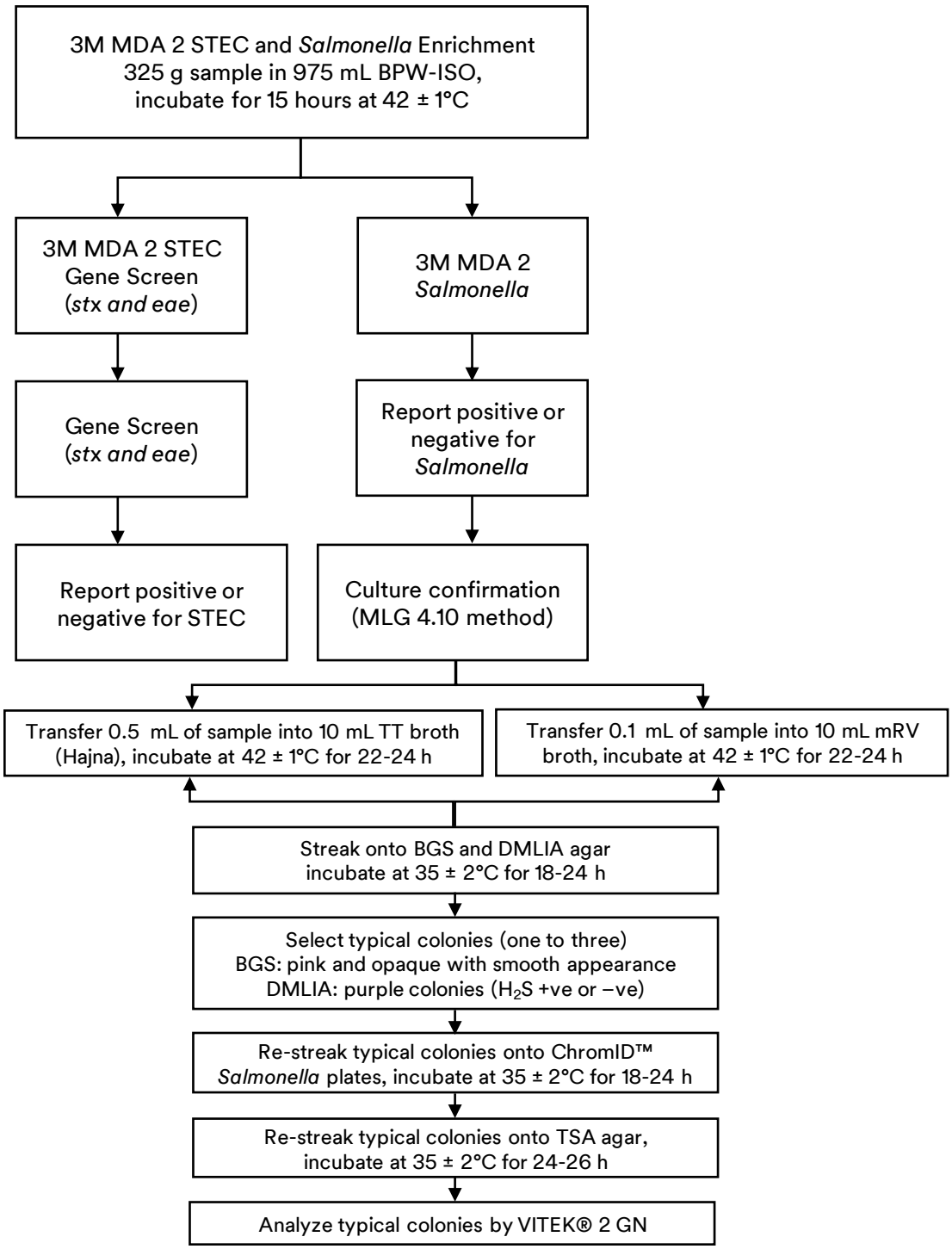

Figure 2. Flow chart for Detection of STEC and *Salmonella* in beef and poultry matrices (3M method enrichment). MLG 4.10 culture confirmation method was modified to use ChromID™ *Salmonella* chromogenic plates for further selective isolation of *Salmonella*.

Preparation of inoculum and inoculation of matrices

Three STEC isolates (*E. coli* O157:H7, ATCC® 35150™, *E. coli* O111:H8, ATCC® BAA-179™, *E. coli* O103:H11, ATCC® BAA-2215™) were obtained from ATCC, Manassas, VA. For each strain, an isolated colony taken from a tryptic soy agar streak plate (TSA, Edge Biologicals, Memphis, TN) was inoculated into 10 mL of Brain Heart Infusion Broth (BHI, Becton Dickinson, Franklin Lakes, NJ) using

a sterile inoculating loop and incubated for 18 to 24 hours at 35°C. After incubation, a 40 mL tube of BHI was inoculated with 0.5 mL of the culture for each of the isolates and incubated for 18 to 24 hours at 35°C. After incubation, the three BHI tubes were combined and centrifuged at 5000 x g for 15 min at 6°C. The pellet was washed twice with 100 mL of sterile 0.85% saline, then resuspended in 100 mL of 0.85% saline. Serial 10-fold dilutions were prepared in 0.85% saline, and 100 µL of each dilution was spread plated on TSA in triplicate and incubated at 35°C for 18 hours. Colonies on TSA plates were counted, and an average count of each dilution was used to determine the appropriate amount of inoculum to add to each sample.

For the preparation of the *Salmonella* cocktail, three isolates, *Salmonella* Senftenberg, ATCC® 43845™, *Salmonella* Enteritidis, ATCC® 49222™, and *Salmonella* Heidelberg, ATCC® 8326™ were used, and the inoculum was prepared as described for STEC isolates.

Prior to inoculation, each product type was screened for natural contamination with Hygiena BAX methods. Naturally occurring STEC or *Salmonella* were not detected in any beef or chicken breast samples. The chicken parts and mechanically separated chicken (MSC) were naturally contaminated with *Salmonella,* but not with STEC. Total aerobic counts in all matrices were also determined by serially diluting the sample homogenates in Butterfield's phosphate buffer (3M Food Safety) and plating on 3M™ Petrifilm™ Aerobic Count Plates (33).

Three hundred twenty-five (325) g samples of raw beef and poultry products were cut from each product type using a pre-heated bead sterilizer and metal cutting utensils. The 325 g products were added to a 4 L sterile Whirl-Pak filter bag (Nasco, Fort Atkinson, WI). The samples were inoculated with 100 µL of STEC (single inoculum) or STEC and *Salmonella* (dual inoculum) suspension, described above, at various CFU levels per 325 g sample, and then stored at 4-8°C for 20 to 30 min. For STEC MLG 5C.00 enrichments, 975 mL of pre-warmed modified Tryptone Soy Broth (mTSB) was added and incubated at 42° C for 15 hours. For STEC and *Salmonella* enrichments (dual inoculum), 975 mL of pre-warmed Buffered Peptone Water (BPW) - ISO was added and incubated at 42°C for 15 hours.

STEC detection

3M Molecular Detection Assay 2 - STEC Gene Screen (*stx* and *eae*) method. After enrichment, 20 µL of sample was collected and processed for STEC detection using the 3M Molecular Detection System following the product instructions (27). Each enriched sample (20 µL) was added to an individual lysis tube, heated for 15 minutes in a heating block at 100 ± 1°C, and cooled for 5 minutes at ambient temperature in a chill block. Afterwards, 20 µL of each lysate was transferred first to a *stx* reagent tube and mixed by gently pipetting up and down 5 times. Then 20 µL of the same lysate was transferred to an *eae* reagent tube and mixed by gently pipetting up and down 5 times. The sample run was configured using the 3M™ Molecular Detection Software and the reagent tubes were then loaded to the 3M™ Molecular Detection Instrument for DNA amplification and detection. Presumptive positive results were reported in real time, while negative results were displayed at the end of the 60 minute run.

MLG 5C.00 gene screen method. After enrichment, samples were analyzed by the iQ-Check® STEC VirX kit (BioRad, Hercules, CA) following manufacturer's instructions (34). Briefly, the lysis

reagent was prepared by mixing reagent F (lysis beads) with reagent A (lysis reagent) and 100 µL of homogenized lysis reagent was added to wells of a deep well plate. One hundred (100) µL of the enriched sample was added to the wells containing lysis reagent and mixed by pipetting up and down until homogenized. The deep well plate was sealed with film and then placed in an agitator-incubator for 15 to 20 min at 95-100°C at a speed of 1300 rpm. The PCR reagent mix was prepared by combining amplification solution (reagent C) and the fluorescent probes (reagent B) for each of the samples, and 20 µL of the PCR mix was added to each well of a PCR plate. Five (5) µL of the lysed sample was added to the corresponding well of the PCR plate containing PCR mix. The plate was sealed with optically clear caps and placed in the real-time PCR system for DNA amplification and detection. The presumptive positive and negative results were displayed at the end of the 2 hour run.

***Salmonella* detection**

MLG 4.10 method (3M Molecular Detection Assay 2 - *Salmonella*). After enrichment, 20 µL of sample was collected and processed for *Salmonella* detection using the 3M Molecular Detection System following the product instructions (29). Each enriched sample (20 µL) was added to an individual lysis tube, heated for 15 minutes in a heating block at 100 ± 1°C, and cooled for 5 minutes at ambient temperature in a chill block. Afterwards, 20 µL of each lysate was transferred to a *Salmonella* reagent tube and mixed by gently pipetting up and down 5 times. The sample run was configured using the 3M Molecular Detection Software and the reagent tubes were then loaded to the 3M Molecular Detection Instrument for DNA amplification and detection. Presumptive positive results were reported in real time, while negative results were displayed at the end of the 60 minute run.

For the 3M method, a randomly selected sample for each product type was also analyzed with the external amplification control, 3M™ Molecular Detection Matrix Control (35) to assess sample matrix interference during the DNA isothermal amplification reaction.

Salmonella culture confirmation. Regardless of the results obtained by the MLG 4.10 rapid detection assay (3M Molecular Detection Assay 2 - *Salmonella*), primary enrichments were confirmed per the MLG 4.10 culture confirmation procedure with modification (17). From the primary enrichment, 0.5 mL was transferred to 10 mL of Tetrathionate (TT) broth Hajna (Edge Biologicals) and 0.1 mL to 10 mL of modified Rappaport Vassiliadis (mRV) broth (Edge Biologicals) and both tubes were incubated at 42 ± 1°C for 22-24 hours. After incubation, the tubes were vortexed and streaked on Brilliant Green Sulfa (BGS) agar and Double Modified Lysine Iron Agar (DMLIA) plates (Edge Biologicals) and incubated at 35 ± 2°C for 22-26 hours. Three typical colonies were selected, and each colony was streaked onto ChromID™ *Salmonella* plates (bioMerieux, Inc., Hazelwood, MO) and incubated at 35 ± 2°C for 18-24 hours. Typical colonies were re-streaked onto TSA to isolate pure colonies and then the colonies were subjected to biochemical analysis using VITEK® 2 Gram-Negative identification card (GN) (bioMerieux, Inc., Durham, NC). The VITEK results were used to determine the colony identification.

For all the samples, Probability of Detection (POD) was computed for the 3M Molecular Detection Assay 2 - STEC Gene Screen (*stx* and *eae*) method (POD alternate, POD_a) and the reference method

(POD reference, POD_r) and used as a statistical model to validate the 3M method (36). For *Salmonella* detection, POD was computed for 3M Molecular Detection Assay 2 - *Salmonella* method (POD reference method presumptive results, POD_p) and the culture confirmation (POD reference method confirmed results, POD_c) and used as a statistical model to validate the 3M method (36). The difference between the two PODs, dPOD, was computed and 95% confidence interval for dPOD was calculated.

Results and Discussion

The aerobic counts for ground beef and trim was about 8 to 8.5 Log CFU/g, for chicken breast and parts it was 1 to 1.5 Log CFU/g and for MSC it was about 5.7 to 7.8 Log CFU/g.

Paired analysis

The results for STEC detection in mTSB enrichments (paired analysis) for the matrices tested by the MLG 5C.00 gene screen method and the 3M Molecular Detection Assay 2 - STEC Gene Screen (*stx* and *eae*) is shown in Table 2. There was complete agreement between the two methods for the ground beef, beef trim and chicken part samples tested. For chicken breast samples, the MLG 5C.00 method had one additional positive compared to the 3M Molecular Detection Assay 2 - STEC Gene Screen (*stx* and *eae*); for MSC, the 3M Molecular Detection Assay 2 - STEC Gene Screen (*stx* and *eae*) had an additional positive compared to the MLG 5C.00 method. Based on POD analysis, there was no significant difference between the two methods (Table 3). Matrix control results were valid for all the matrices tested in mTSB enrichment indicating that there was no sample matrix interference.

Table 2. Paired comparison of STEC detection in beef and poultry matrices with the 3M Molecular Detection Assay 2 (MDA2) - STEC Gene Screen (*stx* and *eae*) assay and the MLG 5C.00 method.

Sample Matrix	STEC Inoculation Level[a]	Number of Samples	USDA FSIS MLG 5C.00 Enrichment mTSB (42°C, 15 hours)	
			3M MDA2 STEC Gene Screen (*stx* and *eae*) Presumptive Positive Result	MLG 5C.00 PCR Gene Screen (*stx* and *eae*) Presumptive Positive Result
Ground beef	Uninoculated	2	0	0
	4 CFU	10	10	10
	9 CFU	3	3	3
Beef trim	Uninoculated	3	0	0
	4 CFU	10	10	10
	9 CFU	2	2	2
Chicken breast	Uninoculated	1	0	0
	1 to 2 CFU	9	2	3
Mechanically separated chicken (MSC)[b]	Uninoculated	3	0	0
	7 CFU	5	5	4
Chicken parts[b]	Uninoculated	2	0	0
	7 to 10 CFU	6	6	6

[a]Sample size:325g.

[b]Mechanically separated chicken (MSC) and chicken parts were naturally contaminated with *Salmonella*.

Table 3. POD analysis of paired comparison of STEC detection in beef and poultry matrices with 3M Molecular Detection Assay 2 (MDA2) - STEC Gene Screen (*stx* and *eae*) and MLG 5C.00 method.

Sample Matrix	STEC Inoculation Level[a]	N[b]	3M MDA2 STEC Gene Screen (*stx* and *eae*)		MLG 5C.00 PCR Gene Screen (*stx* and *eae*)		dPOD$_a$[f]	95% CI[g]	
			X[c]	POD$_a$[d]	X	POD$_r$[e]			
Ground beef	Uninoculated	2	0	0	0	0	0	-0.66	0.66
	4 CFU	10	10	1	10	1	0	-0.28	0.28
	9 CFU	3	3	1	3	1	0	-0.56	0.56
Beef trim	Uninoculated	3	0	0	0	0	0	-0.56	0.56
	4 CFU	10	10	1	10	1	0	-0.28	0.28
	9 CFU	2	2	1	2	1	0	-0.66	0.66
Chicken breast	Uninoculated	1	0	0	0	0	0	-0.79	0.79
	1 to 2 CFU	9	2	0.2	3	0.3	-0.1	-0.46	0.28
MSC[h]	Uninoculated	3	0	0	0	0	0	-0.56	0.56
	7 CFU	5	5	1	4	0.8	0.2	-0.26	0.62
Chicken parts[h]	Uninoculated	2	0	0	0	0	0	-0.66	0.66
	7 to 10 CFU	6	6	1	6	1	0	-0.39	0.39

[a]Sample size: 325g.
[b]N = Number of test portions.
[c]X = Number of positive test portions.
[d]POD$_a$ = alternate method positives divided by N.
[e]POD$_r$ = Reference method positives divided by N.
[f]dPOD$_a$ = Difference between the alternate method and the reference method POD values.
[g]95% CI = If the confidence interval of dPOD includes zero, then the difference between the methods is not significant.
[h]MSC and chicken parts were naturally contaminated with *Salmonella*.

Non-paired analysis

The results for non-paired analysis of STEC detection in BPW-ISO (3M method) and mTSB enrichments (MLG 5C.00) for the matrices tested by 3M Molecular Detection Assay 2 - STEC method and MLG 5C.00 gene screen method is shown in Table 4. There was complete agreement between the two methods for ground beef, beef trim, chicken breast and chicken parts tested. For MSC, the 3M method had one additional positive compared to the MLG 5C.00 method. Based on POD analysis, there was no significant difference between the two methods (Table 5). Matrix control results were valid for all the matrices tested in BPW-ISO enrichment indicating that there was no sample matrix interference.

Table 4. Non-paired comparison of STEC detection in beef and poultry matrices with 3M Molecular Detection Assay 2 (MDA2) - STEC Gene Screen (*stx* and *eae*) and MLG 5C.00 method.

Sample Matrix	STEC Inoculation Level[a]	Number of Samples	3M MDA2 STEC Gene Screen (*stx* and *eae*) Presumptive Positive Result[b]	MLG 5C.00 PCR Gene Screen (*stx* and *eae*) Presumptive Positive Result[c]
Ground beef	Uninoculated	2	0	0
	4 CFU	10	10	10
	7 to 9 CFU	3	3	3
Beef trim	Uninoculated	3	0	0
	4 CFU	10	10	10
	7 to 9 CFU	2	2	2
Chicken breast	Uninoculated	1	0	0
	1 to 2 CFU	9	3	3
MSC[a]	Uninoculated	3	0	0
	7 CFU	5	5	4
Chicken parts[a]	Uninoculated	2	0	0
	7 to 10 CFU	6	6	6

[a]Ground beef, beef trim and chicken breast was also inoculated with *Salmonella* along with STEC for 3M method. MSC and chicken parts were naturally contaminated with *Salmonella* and hence inoculated with only STEC. Sample size: 325g

[b]Enrichment in BPW-ISO for 15 hours at 42°C.

[c]Enrichment in mTSB for 15 hours at 42°C.

Table 5. POD analysis of non-paired comparison of STEC detection in beef and poultry matrices with 3M Molecular Detection Assay 2 (MDA2) - STEC Gene Screen (*stx* and *eae*) and MLG 5C.00 method.

Matrix	STEC Inoculation Level[a]	N[b]	3M MDA2 STEC Gene Screen (*stx* and *eae*)[c]		MLG 5C.00 PCR Gene Screen (*stx* and *eae*)[c]		dPOD$_a$[g]	95% CI[h]	
			X[d]	POD$_a$[e]	X	POD$_r$[f]			
Ground beef	Uninoculated	2	0	0	0	0	0	-0.66	0.66
	4 CFU	10	10	1	10	1	0	-0.28	0.28
	7 to 9 CFU	3	3	1	3	1	0	-0.56	0.56
Beef trim	Uninoculated	3	0	0	0	0	0	-0.56	0.56
	4 CFU	10	10	1	10	1	0	-0.28	0.28
	7 to 9 CFU	2	2	1	2	1	0	-0.66	0.66
Chicken breast	Uninoculated	1	0	0	0	0	0	-0.79	0.79
	1 to 2 CFU	9	3	0.3	3	0.3	0	-0.38	0.38
MSC[a]	Uninoculated	3	0	0	0	0	0	-0.56	0.56
	7 CFU	5	5	1	4	0.8	0.2	-0.26	0.62
Chicken parts[a]	Uninoculated	2	0	0	0	0	0	-0.66	0.66
	7 to 10 CFU	6	6	1	6	1	0	-0.39	0.39

[a]Ground beef, beef trim and chicken breast was also inoculated with *Salmonella* along with STEC for 3M method. MSC and chicken parts were naturally contaminated with *Salmonella* and hence inoculated with only STEC. Sample size: 325g.

[b]N = Number of test portions.

[c]Enrichment was done in BPW-ISO for 3M method and in mTSB for MLG 5C.00 method for 15 hours at 42°C.

[d]X = Number of positive test portions.

[e]POD$_a$ = Alternate method positives divided by N.

[f]POD$_r$ = Reference method positives divided by N.

[g]dPOD$_a$ = Difference between the alternate method and the reference method POD values.

[h]95% CI = If the confidence interval of dPOD includes zero, then the difference between the methods is not significant.

Detection of STEC and *Salmonella* in the same enrichment (dual inoculation)

The beef and chicken samples were inoculated with a cocktail of STEC and *Salmonella* and upon enrichment of samples in BPW-ISO for 15 hours at 42°C, the samples were tested with the 3M methods for STEC Gene Screen (*stx* and *eae*) and for *Salmonella*. The results are shown in Table 6. All the enriched samples were also culturally confirmed for *Salmonella* by MLG 4.10 method (17). *Salmonella* was detected and confirmed in all inoculated and naturally contaminated samples for all the matrices tested. STEC was detected in all inoculated samples, with only the chicken breast giving fractional positives. Chicken breast samples had fractional positives for STEC for both enrichment schemes, 3M method and MLG 5C.00 (Table 2 and 4). Based on POD analysis, there was no significant difference between the presumptive detection by the 3M Molecular Detection Assay 2 - *Salmonella* and the culture confirmation for *Salmonella* (Table 7).

Table 6. STEC and *Salmonella* detection in beef and poultry matrices inoculated with a cocktail of both cultures (dual inoculation) with 3M Molecular Detection Assay 2 (MDA2).

Product	Inoculation Level[a]	Number of Samples	3M MDA2 BPW-ISO Enrichment (15 hour at 42°C)		
			3M MDA2 STEC Gene Screen (*stx* and *eae*) Presumptive Positive Result	*Salmonella*	
				3M MDA2 *Salmonella* Presumptive Positive Result	Culture Confirmation
Ground beef	Uninoculated	2	0	0	0
	4 CFU STEC and 3 CFU *Salmonella*	10	10	10	10
	7 CFU each of STEC and *Salmonella*	3	3	3	3
Beef trim	Uninoculated	3	0	1	0
	4 CFU STEC and 3 CFU *Salmonella*	10	10	10	10
	7 CFU each of STEC and *Salmonella*	2	2	2	2
Chicken breast	Uninoculated	1	0	1	1
	1 to 2 CFU STEC and 2 to 6 CFU *Salmonella*	9	3	9	9
MSC[a]	Uninoculated STEC/Natural *Salmonella*	3	0	3	3
	7 CFU STEC/Natural *Salmonella*	5	5	5	5
Chicken parts[a]	Uninoculated STEC/Natural *Salmonella*	2	0	2	2
	7 to 10 CFU STEC/Natural *Salmonella*	6	6	6	6

[a]MSC and chicken parts were naturally contaminated with *Salmonella*. Sample size: 325g.

Table 7. POD analysis of *Salmonella* detection in beef and poultry matrices inoculated with a cocktail of both cultures (STEC and *Salmonella*).

Matrix	Level[a]	N[b]	MLG 4.10				$dPOD_p$[f]	95% CI[g]	
			3M MDA2 *Salmonella*		Culture Confirmed				
			X[c]	POD_p[d]	X	POD_c[e]			
Ground beef	Uninoculated	2	0	0	0	0	0	-0.66	0.66
	4 CFU STEC and 3 CFU *Salmonella*	10	10	1	10	1	0	-0.28	0.28
	7 CFU each of STEC and *Salmonella*	3	3	1	3	1	0	-0.56	0.56
Beef trim	Uninoculated	3	1	0.3	0	0	0.3	-0.29	0.79
	4 CFU STEC and 3 CFU *Salmonella*	10	10	1	10	1	0	-0.28	0.28
	7 CFU each of STEC and *Salmonella*	2	2	1	2	1	0	-0.66	0.66
Chicken breast	Uninoculated	1	1	1	1	1	0	-0.79	0.79
	1 to 2 CFU STEC and 2 to 6 CFU *Salmonella*	9	9	1	9	1	0	-0.30	0.30
MSC[a]	Uninoculated STEC/Natural *Salmonella*	3	3	1	3	1	0	-0.32	0.32
	7 CFU STEC/Natural *Salmonella*	5	5	1	5	1	0	-0.43	0.43
Chicken parts[a]	Uninoculated STEC/Natural *Salmonella*	3	3	1	3	1	0	-0.56	0.56
	7 to 10 CFU STEC/Natural *Salmonella*	5	5	1	5	1	0	-0.43	0.43

[a]Ground beef, beef trim and chicken breast was inoculated with both *Salmonella* and STEC. MSC and chicken parts were naturally contaminated with *Salmonella* and hence inoculated with only STEC. Samples size: 325g.

[b]N = Number of test portions.

[c]X = Number of positive test portions.

[d]POD_p = Reference method presumptive positives divided by N.

[e]POD_c = Reference method confirmed positives divided by N.

[f]$dPOD_p$ = Difference between the presumptive and the confirmed positives for the reference method POD values.

[g]95% CI = If the confidence interval of dPOD includes zero, then the difference between the methods is not significant.

This study evaluated the performance of a new LAMP-based molecular detection method, the 3M Molecular Detection Assay 2 - STEC Gene Screen (*stx* and *eae*), for detection of STEC in beef and poultry matrices as compared to the MLG 5C.00 reference method (PCR). The results from the 3M method were in agreement with the reference method for both the paired and non-paired analysis. The POD analysis between the two methods did not show any significant difference at a 95% confidence interval. Based on these and other results (28), the 3M Molecular Detection Assay 2 - STEC Gene Screen method is an acceptable alternative method for analyzing beef and poultry samples for STEC. The results of the 3M method were not statistically significantly different from the reference PCR method.

3M Molecular Detection Assay 2 - STEC Gene Screen (*stx* and *eae*) method enrichments were also inoculated with *Salmonella* and analyzed by MLG 4.10 method (3M Molecular Detection Assay 2 - *Salmonella*). *Salmonella* was detected in all the matrices co-inoculated with STEC by the 3M Molecular Detection Assay 2 – *Salmonella* method. Out of 56 samples tested, only one sample was not culturally confirmed, indicating high sensitivity and specificity for the 3M method. This indicates that both STEC and *Salmonella* can be analyzed using the same BPW-ISO enrichment using one workflow.

This study demonstrated that the 3M Molecular Detection Assay 2 - STEC Gene Screen (*stx* and *eae*) and the 3M Molecular Detection Assay 2 - *Salmonella* provided rapid and accurate detection of both STEC and *Salmonella* in beef and poultry matrices. The 3M Molecular Detection System offers a streamlined and easy to use protocol with next day results, with the ability to detect both STEC and *Salmonella* from the same enriched sample. The 3M Molecular Detection System allows food manufacturers and testing laboratories to utilize a single solution to assess prevalence and detection of STEC and *Salmonella* in beef and poultry products.

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