Evaluation of the 3M™ Molecular Detection Assay 2 – STEC Gene Screen for the Detection of *Escherichia coli* O157:H7 in Poultry Matrices

Introduction

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 *E. coli* O157:H7 are highly pathogenic. *E. coli* O157:H7 produces a potent toxin called Shiga toxin (Stx) type 1 (Stx1), type 2 (Stx2), or both, encoded by *stx*1 and *stx*2 genes, respectively (1). In addition, *E. coli* O157:H7 produces *eae* (the intimin gene involved in attaching and effacing phenotype). *E. coli* O157:H7 infection causes diarrhea, hemorrhagic colitis, and life-threatening hemolytic uremic syndrome (1). Shiga toxin production, especially Stx2, has been implicated as an important factor in causing severe disease and hemolytic uremic syndrome (HUS) (1).

E. coli O157:H7 is an important etiologic agent of foodborne illness worldwide. Transmission to humans often occurs through ingestion of contaminated beef, raw milk, and fresh produce. Globally, *E. coli* O157:H7 is estimated to result in more than 1 million illnesses per year (2). According to CDC estimates, *E. coli* O157:H7 causes about 95,000 infections each year in the United States (US) accounting for about 36% of all Shiga toxin-producing *E. coli* (STEC) infections (3).

Ruminants, and particularly cattle, are the primary reservoir (4-8) for STEC including *E. coli* O157:H7 (4-8). The hides, hooves, and gastrointestinal (GI) tracts of cattle can contain these pathogens and during slaughter operations, raw beef products can be contaminated. Beef has been one of the prominent causes of *E. coli* outbreaks in US, accounting for 143 (23%) of the 615 *E. coli* outbreaks reported since 1998 (9). In 1994, the USDA-FSIS declared *E. coli* O157:H7 as an adulterant in raw ground beef (10). Although poultry products are not typically associated with *E. coli* O157:H7, several publications indicate that it is not a rare contaminant in poultry products (11-15).

In order to ensure safe food, food processors need rapid, accurate and sensitive methods to detect the pathogens faster than traditional cultural methods. Regulatory zero tolerance policies for certain pathogens in food requires detection of 1 colony forming unit (CFU) per food sample. The use of DNA amplification for food pathogen testing has enabled the rapid detection of pathogenic microorganisms 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 the traditional methods that rely on plating to selective and/or differential agar followed by further biochemical

testing, the detection of unique DNA sequences enables very rapid and highly specific screening of a sample.

Recently, the United States Department of Agriculture Food Safety and Inspection Service (USDA FSIS) has updated its Microbiology Laboratory Guidebook (MLG) to include rapid molecular screening assays for *Salmonella* (16), *Listeria monocytogenes* (17) and the top seven STEC (18) as the primary screen to support its regulatory activities, giving credence to these DNA-based methods. In addition to regulatory recognition, it is also critical that rapid DNA-based methods are validated for various matrices to provide food manufacturers and testing laboratories, confidence that these methods are fit for their purpose.

PCR has been used as the primary method for foodborne pathogens for the last several years. 3M has introduced the 3M™ Molecular Detection System based on Loop-mediated Isothermal Amplification (LAMP) (19) with bioluminescent detection for various foodborne pathogens such as *Salmonella*, *Listeria monocytogenes*, *Campylobacter*, and others. 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 (20, 21) with over 1000 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 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 (22-25). USDA FSIS has recognized LAMP methods by adopting 3M™ Molecular Detection Assay 2 - *Salmonella* and 3M™ Molecular Detection Assay 2 – *Listeria monocytogenes* as the primary screening methods for those pathogens in the MLG (16, 17).

E. coli O157:H7 is typically detected by culturing in special media, serological and molecular methods. Molecular methods based on the somatic antigen gene *rfb* O157 and/or the flagella gene *fliC* H7 are commonly used for detection of *E. coli* O157:H7. However, these methods may not accurately reflect the virulence of isolates. O157 detection based *rfb* gene needs further confirmation of the O157:H7 genotype. H7 detection based on *fliC* gene does not detect non-motile variants. In addition, non-toxigenic *E. coli* O157:H7 have been isolated which may not fully reflect the pathogenicity of the isolates (1-3). The virulence genes *stx*1 and *stx*2 are the most commonly assayed virulence factors of STEC and provide a better screening tool for detecting toxigenic *E. coli* O157:H7 and other STECs. The presumptive positive results from the gene screen can be followed with specific assays for O157 and/or H7 detection (18) and culture based assays (26).

In 2019, 3M introduced LAMP-bioluminescent method for detection of STEC using gene screen for *stx* and *eae* genes (27) or *stx* gene alone (28). These kits have received AOAC *Performance Tested Methods*℠ certificates for detection of STEC in a variety of matrices, including fresh raw poultry parts (325 g samples) (29, 30). The purpose of this third-party study, conducted at an ISO/IEC 17025 accredited laboratory, was to compare the 3M Molecular Detection Assay 2 - STEC Gene Screen with an *E. coli* O157:H7 PCR method (31) for the detection of *E. coli* O157:H7 in poultry matrices.

Material and Methods

The enrichment conditions for poultry matrices are shown in Table 1. The samples (25 g) were enriched in buffered peptone water (BPW) (ISO)) for 18 hours at 41.5°C. The flow chart for detection of *E. coli* O157:H7 in poultry matrices tested is shown in Figure 1.

Table 1. Enrichment protocols.

Sample Matrix[a]	Sample Size	Enrichment Broth (pre-warmed)	Enrichment Temperature (± 1°C)	Enrichment Time (hours)
Mechanically separated chicken (MSC)	25 g	225 mL BPW (ISO)	41.5	18
Ground chicken	25 g	225 mL BPW (ISO)	41.5	18
Chicken parts	25 g	225 mL BPW (ISO)	41.5	18

[a]Samples were hand massaged for 30-60 seconds to disperse and break apart clumps after adding pre-warmed BPW (ISO).

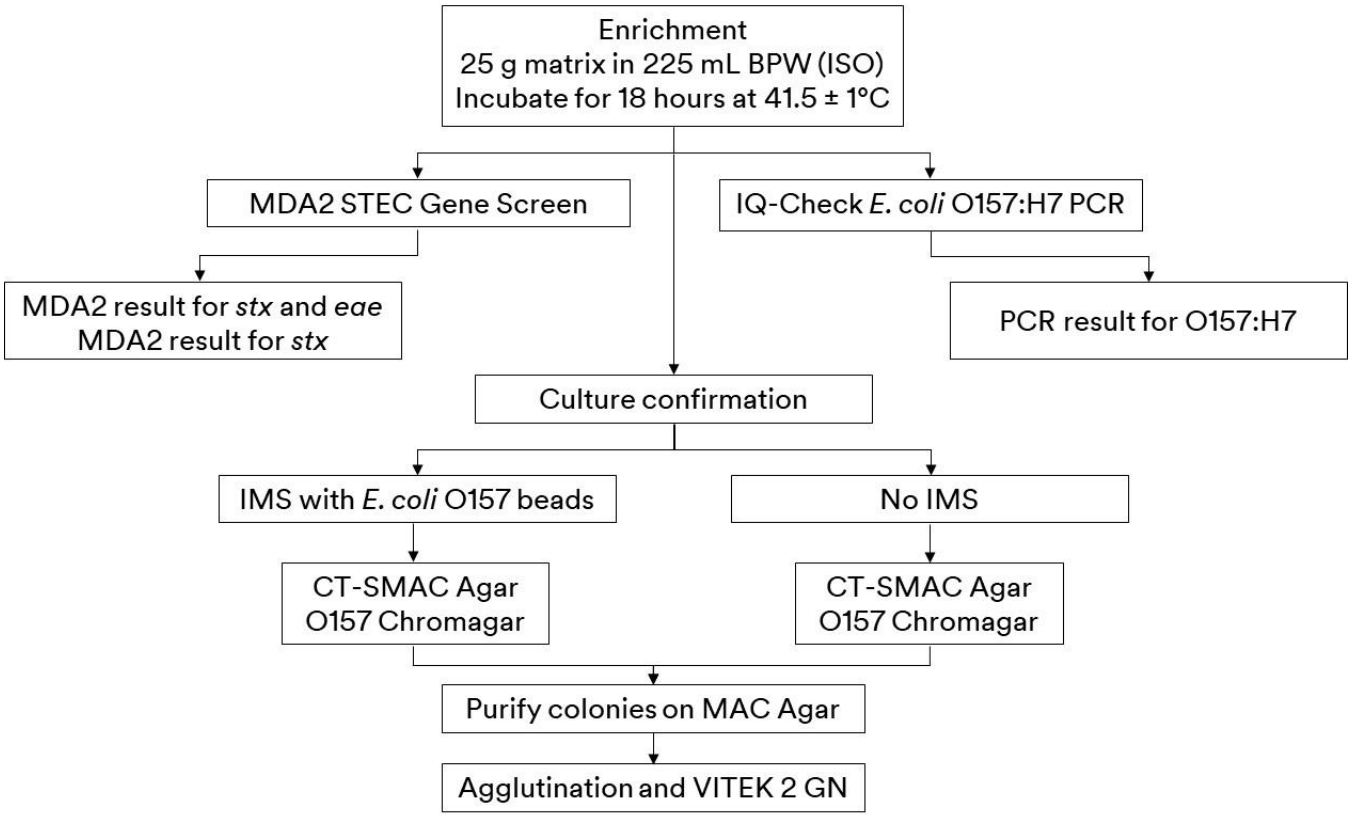

Figure 1. Flow chart for Detection of *E. coli* O157:H7 in poultry matrices.

Preparation of inoculum and inoculation of matrices

A toxigenic strain of *E. coli* 0157:H7 (ATCC 35150) was obtained from ATCC (Manassas, VA). An isolated colony 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 20 hours at 37°C. After incubation, a 40 mL tube of BHI broth was inoculated with 0.5 mL of the culture and incubated for 20 hours at 37°C. After incubation,

the 40 mL of culture was centrifuged at 5000 x g for 15 min at 4°C. The pellet was washed twice with 40 mL of sterile 0.85% saline and then resuspended in 40 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. The inoculum was stored at 4°C till the counts were obtained. 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. Based on the counts obtained, an average of approximately 2 CFU for low inoculum and approximately 5 CFU for high inoculum was used. The inoculation levels were also verified by plating the inoculum volume (100 µL) in triplicates onto TSA and counts obtained after the incubation of TSA plates.

Prior to inoculation, 25 g of each product type was screened for natural contamination of non-O157 Shiga toxin-producing *E. coli* and *E. coli* O157:H7 with Hygiena BAX® PCR testing methods (Hygiena, Camarillo, CA). Naturally occurring non-O157 STEC or *E. coli* O157:H7 were not detected in any of the samples. Total aerobic counts in all matrices were also determined by serially diluting the sample homogenates in Butterfield's Buffer and plating on 3M™ Petrifilm™ Aerobic Count Plates (both 3M Food Safety) (32).

Twenty-five (25) gram samples of mechanically separated chicken (MSC), ground chicken or poultry parts were cut from each product type using metal cutting utensils sterilized before each use with a pre-heated bead sterilizer. The 25 g products were added to a 1 L sterile Whirl-Pak filter bag (Nasco, Fort Atkinson, WI). The samples were inoculated with 100 µL of inoculum suspension, described above, at various CFU levels per 25 g sample. For MSC and ground chicken, five samples were used as control (uninoculated), twenty samples were inoculated at a low level (approximately 2 CFU), and five samples were inoculated at a high level (approximately 5 CFU). Five different types of poultry parts (drums, wings, breasts, thighs, and tenders) were used. For each part one sample was used as control, one sample for high inoculum and four parts for low inoculum. After inoculation, all samples were blinded and stored at 4°C for 2 hours. After 2 hours, 225 mL of pre-warmed BPW (ISO) was added to each of the sample, homogenized for 30 sec and incubated at 41.5 ± 1°C for 18 hours.

***E. coli* O57:H7 detection**

3M Molecular Detection Assay 2 - STEC Gene Screen method. After enrichment, 20 µL of sample was collected and processed for STEC detection using the 3M Molecular Detection System following the product instructions (21, 22). 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 loaded to the 3M™ Molecular Detection Instrument for DNA amplification and detection. For *stx* assay, only the sample results from the *stx* assay were used to assess 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 (33) to assess sample matrix interference during the DNA isothermal amplification reaction.

E. coli O157:H7 PCR method. After enrichment, samples were analyzed by the iQ-Check® *E. coli* O157:H7 PCR Detection Kit (BioRad, Hercules, CA) following manufacturer's instructions (31). 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 45 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.

Culture confirmation. All samples were confirmed per the culture confirmation procedure with modification (Fig. 1; 34). From each of the enrichment, immunomagnetic separation (IMS) was done using *E. coli* 0157:H7 immunomagnetic bead (Dynabeads™ anti-*E. coli* O157, catalog number 71004. Thermo Fisher Scientific) following MLG 5C (Fig. 2). Fifty µL of IMS beads were plated onto Cefixime tellurite sorbitol MacConkey (CT-SMAC) agar (Hardy Diagnostics, Santa Maria, CA) and CHROMagar™ O157 agar (Microbiology International, Frederick, MD). Each enrichment sample was also streaked to both CT-SMAC agar and CHROMagar™ O157 agar using a sterile 10 µL loop for isolation of colonies without IMS. The plates were incubated at 37°C for 18-24 hours. Typical colonies from either agar were re-streaked onto MacConkey (MAC) agar (Edge Biologicals) and incubated at 37°C for 18-24 hours. Typical colonies from MAC agar were biochemically identified using VITEK® 2 Gram-Negative identification card (GN) (bioMerieux, Inc., Durham, NC). MAC colonies were also screened using Remel™ *E. coli* 0157 Latex kit (Thermo Fisher Scientific). Both the VITEK results and agglutination results were used to confirm the colony identification.

For all samples, probability of detection (POD) analysis was done to compare the 3M Molecular Detection Assay 2 - STEC Gene Screen method (POD LAMP method, POD_1) and the *E. coli* O157:H7 PCR method (POD PCR method, POD_2) (35). POD analysis was also done to compare the 3M Molecular Detection Assay 2 – STEC Gene Screen method presumptive results (POD_p) and the culture confirmation (POD confirmed results, POD_{cr}) (35). POD analysis was also done to compare the H7 PCR method presumptive results (POD_p) and the culture confirmation (POD confirmed results, POD_{cr}). The difference between the two PODs, dPOD, was computed and the 95% confidence interval for dPOD was calculated.

Results and Discussion

The aerobic count was approximately 5.2 log CFU/g for MSC, approximately 3.2 log CFU/g for ground chicken, approximately 2 log CFU/g for poultry parts (drums, wings, thighs, tenders) and approximately 2.6 logs CFU/g for breasts. The low inoculum samples met the criteria for fractional recovery.

STEC gene screen using *stx* and *eae*

The results for *E. coli* O157:H7 detection using the 3M Molecular Detection Assay 2 - STEC Gene Screen (*stx* and *eae*) and H7 PCR is shown in Table 2. The STEC gene screen method detected 8 positive portions out of 20 low inoculum MSC and ground chicken samples, while H7 PCR detected 2 additional positives (Table 2). For poultry parts there was no difference between the two methods for the detection of *E. coli* O157:H7. For high inoculum samples (5 CFU), the results were identical for the STEC gene screen method and H7 PCR method for all the three matrices. Based on POD analysis, there was no significant difference between the STEC gene screen (*stx* and *eae*) method and H7 PCR method (Table 3). Matrix control results were valid for all the matrices indicating that there was no sample matrix interference. For low inoculum MSC samples, 12 samples were positive for *eae* with the 3M Molecular Detection Assay 2 - STEC Gene Screen (*stx* and *eae*). These samples were considered STEC negative for the 3M method as the *stx* results were negative. Two samples out of these 12 samples were positive for H7 PCR.

Table 2. Paired comparison of STEC detection in poultry matrices with the 3M Molecular Detection Assay 2 (MDA2) - STEC Gene Screen (*stx* and *eae*) assay and the *E. coli* O157:H7 PCR method.

Sample Matrix	STEC Inoculation Level[a]	Number of Samples	BPW (ISO) Enrichment (41.5 ± 1°C, 18 hours)	
			3M MDA2 STEC Gene Screen (*stx* and *eae*) Presumptive Positive Result[b]	*E. coli* O157:H7 PCR Presumptive Positive Result
Mechanically separated chicken (MSC)	Uninoculated	5	0	0
	2 CFU	20	8	10
	5 CFU	5	5	5
Ground chicken	Uninoculated	5	0	0
	2 CFU	20	8	10
	5 CFU	5	5	5
Poultry parts-drums	Uninoculated	1	0	0
	2 CFU	4	2	2
	5 CFU	1	1	1
Poultry parts-wings	Uninoculated	1	0	0
	2 CFU	4	2	2
	5 CFU	1	1	1
Poultry parts-thighs	Uninoculated	1	0	0
	2 CFU	4	3	3
	5 CFU	1	1	1
Poultry parts-breasts	Uninoculated	1	0	0
	2 CFU	4	3	3
	5 CFU	1	1	1

Poultry parts-tenders	Uninoculated	1	0	0
	2 CFU	4	1	1
	5 CFU	1	1	1
Parts cumulative	Uninoculated	5	0	0
	2 CFU	20	11	11
	5 CFU	5	5	5

[a]Sample size: 25 g. *E. coli* 0157:H7 (ATCC 35150) was used for inoculated samples.
[b]Sample positive for both *stx* and *eae* for the 3M method.

Table 3. POD analysis of paired comparison of STEC detection in poultry matrices with 3M Molecular Detection Assay 2 (MDA2) - STEC Gene Screen (*stx* and *eae*) and the *E. coli* O157:H7 PCR method.

Sample Matrix	STEC Inoculation Level[a]	N[b]	3M MDA2 STEC Gene Screen (*stx* and *eae*)		*E. coli* O157:H7 PCR		dPOD$_1$[f]	95% CI[g]	
			X[c]	POD$_1$[d]	X[c]	POD$_2$[e]			
Mechanically separated chicken (MSC)	Uninoculated	5	0	0	0	0	0	-0.43	0.43
	2 CFU	20	8	0.4	10	0.5	-0.1	-0.31	0.25
	5 CFU	5	5	1	5	1	0	-0.43	0.43
Ground chicken	Uninoculated	5	0	0	0	0	0	-0.43	0.43
	2 CFU	20	8	0.4	10	0.5	-0.1	-0.31	0.25
	5 CFU	5	5	1	2	1	0	-0.43	0.43
Poultry parts	Uninoculated	5	0	0	0	0	0	-0.43	0.43
	2 CFU	20	11	0.55	11	0.55	0	-0.28	0.28
	5 CFU	5	5	1	5	1	0	-0.43	0.43

[a]Sample size: 25 g. *E. coli* 0157:H7 (ATCC 35150) was used for inoculated samples.
[b]N = Number of test portions.
[c]X = Number of positive test portions. Samples positive for both *stx* and *eae* for the 3M method.
[d]POD$_1$ = Alternative method 1 positives divided by N.
[e]POD$_2$ = Alternative method 2 positives divided by N.
[f]dPOD$_1$ = Difference between the alternative method 1 and the alternative method 2 POD values.
[g]95% CI = If the confidence interval of dPOD includes zero, then the difference between the methods is not significant.

STEC gene screen using *stx*

The results for *E. coli* O157:H7 detection using the 3M Molecular Detection Assay 2 - STEC Gene Screen (*stx*) and H7 PCR is shown in Table 4. The STEC gene screen method detected 8 positive portions out of 20 low inoculum MSC and ground chicken samples, while H7 PCR detected 2 additional positives (Table 4). For poultry parts there was no difference between the two methods for the detection of *E. coli* O157:H7. For high inoculum samples (5 CFU), the results were identical for the STEC gene screen (*stx*) method and H7 PCR method for all the three matrices. The results using *stx* and *eae* were identical to those using *stx* alone. Based on POD analysis, there was no significant difference between the STEC gene screen method and H7 PCR method (Table 5). Matrix control results were valid for all the matrices indicating that there was no sample matrix interference.

Table 4. Paired comparison of STEC detection in poultry matrices with the 3M Molecular Detection Assay 2 (MDA2) - STEC Gene Screen (*stx*) assay and the *E. coli* O157:H7 PCR method.

Sample Matrix	STEC Inoculation Level[a]	Number of Samples	BPW (ISO) Enrichment (41.5 ± 1°C, 18 hours)	
			3M MDA2 STEC Gene Screen (*stx*) Presumptive Positive Result	*E. coli* O157:H7 PCR Presumptive Positive Result
Mechanically separated chicken (MSC)	Uninoculated	5	0	0
	2 CFU	20	8	10
	5 CFU	5	5	5
Ground chicken	Uninoculated	5	0	0
	2 CFU	20	8	10
	5 CFU	5	5	5
Poultry parts-drums	Uninoculated	1	0	0
	2 CFU	4	2	2
	5 CFU	1	1	1
Poultry parts-wings	Uninoculated	1	0	0
	2 CFU	4	2	2
	5 CFU	1	1	1
Poultry parts-thighs	Uninoculated	1	0	0
	2 CFU	4	3	3
	5 CFU	1	1	1
Poultry parts-breasts	Uninoculated	1	0	0
	2 CFU	4	3	3
	5 CFU	1	1	1
Poultry parts-tenders	Uninoculated	1	0	0
	2 CFU	4	1	1
	5 CFU	1	1	1
Parts cumulative	Uninoculated	5	0	0
	2 CFU	20	11	11
	5 CFU	5	5	5

[a]Sample size: 25 g. *E. coli* 0157:H7 (ATCC 35150) was used for inoculated samples.

Table 5. POD analysis of paired comparison of STEC detection in poultry matrices with 3M Molecular Detection Assay 2 (MDA2) - STEC Gene Screen (*stx*) and the *E. coli* O157:H7 PCR method.

Sample Matrix	STEC Inoculation Level[a]	N[b]	3M MDA2 STEC Gene Screen (*stx*)		*E. coli* O157:H7 PCR		$dPOD_1$[f]	95% CI[g]	
			X[c]	POD_1[d]	X[c]	POD_2[e]			
Mechanically separated chicken (MSC)	Uninoculated	5	0	0	0	0	0	-0.43	0.43
	2 CFU	20	8	0.4	10	0.5	-0.1	-0.31	0.25
	5 CFU	5	5	1	5	1	0	-0.43	0.43
Ground chicken	Uninoculated	5	0	0	0	0	0	-0.43	0.43
	2 CFU	20	8	0.4	10	0.5	-0.1	-0.31	0.25
	5 CFU	5	5	1	5	1	0	-0.43	0.43
Poultry parts	Uninoculated	5	0	0	0	0	0	-0.43	0.43
	2 CFU	20	11	0.55	11	0.55	0	-0.28	0.28
	5 CFU	5	5	1	5	1	0	-0.43	0.43

[a]Sample size: 25 g. *E. coli* 0157:H7 (ATCC 35150) was used for inoculated samples.

[b]N = Number of test portions.

[c]X = Number of positive test portions.
[d]POD_1 = Alternative method 1 positives divided by N.
[e]POD_2 = Alternative method 2 positives divided by N.
[f]$dPOD_1$ = Difference between the alternative method 1 and the alternative method 2 POD values.
[g]95% CI = If the confidence interval of dPOD includes zero, then the difference between the methods is not significant.

The PCR method involved multiple steps including preparation of lysis buffer, addition of lysis buffer to deep well plate followed by addition of enriched sample, lysis step, preparation of PCR reagent mix, addition of PCR reagent mix to PCR plate followed by addition of lysed sample to PCR plate. The 3M method involved only two transfers post enrichment, as the assay kits contain ready-to-use lysis buffer and ready-to-use reagent tubes. For the 3M method, the enriched samples were transferred to lysis solution tubes, heated for 15 minutes to lyse the samples and cooled for 5 minutes followed by addition of lysed samples to ready-to-use reagent tubes. The 3M method offered an easy to use protocol relative to the H7 PCR method used in the study.

Confirmation of *E. coli* O157:H7

All samples were culturally confirmed by the ISO method (28). All presumptive positive results by the molecular method (STEC gene screen and H7 PCR) for poultry parts were culturally confirmed (Table 6). For ground chicken, STEC gene screen method had two false positives and H7 PCR had four false positives (Table 6). For MSC, only two samples were culturally confirmed. As an alternative to cultural confirmation, the IMS beads from MSC samples were tested with STEC gene screen and the presumptive results for the IMS bead samples were identical to the non-IMS samples. The *E. coli* O157:H7 PCR had two additional positives for MSC and ground chicken than the STEC gene screen method and these samples were not confirmed by culture (Table 6).

The POD analysis for molecular method results and cultural confirmation results for MSC was not done as there were only two culturally confirmed results. POD analysis was done for the STEC gene screen method and culture confirmation results and the H7 PCR method and culture confirmation results for ground chicken and poultry parts. Based on the POD analysis, there was no significant difference between the STEC gene screen method presumptive results and the culture confirmation results (Table 7) or the H7 PCR method presumptive results and the culture confirmation results (Table 8) for ground chicken and poultry parts.

Table 6. *E. coli* O157:H7 detection in poultry matrices with 3M Molecular Detection Assay 2 – STEC Gene Screen and H7 PCR.

Product	Inoculation Level[a]	Number of Samples	BPW (ISO) Enrichment (18 hour at 41.5°C)		
			Molecular Method		Culture Confirmation
			3M MDA2 STEC Gene Screen[b]	H7 PCR	
Ground chicken	Uninoculated	5	0	0	0
	2 CFU	20	8	10	7
	5 CFU	5	5	5	4
Poultry parts	Uninoculated	5	0	0	0
	2 CFU	20	11	11	11
	5 CFU	5	5	5	5

[a]Sample size: 25 g. *E. coli* 0157:H7 (ATCC 35150) was used for inoculated samples.
[b]Results were identical for STEC gene screen method using either *stx* and *eae* or *stx* alone.

Table 7. POD analysis of *E. coli* O157:H7 detection in poultry matrices using 3M Molecular Detection Assay 2 – STEC Gene Screen.

Matrix	Level[a]	N[b]	3M MDA2 STEC Gene Screen		Culture Confirmed		$dPOD_p$[f]	95% CI[g]	
			X[c]	POD_p[d]	X[c]	POD_{cr}[e]			
Ground chicken	Uninoculated	5	0	0	0	0	0	-0.43	0.43
	2 CFU	20	8	0.4	7	0.35	0.05	-0.27	0.30
	5 CFU	5	5	1	4	0.8	0.2	-0.28	0.43
Poultry parts	Uninoculated	5	0	0	0	0	0	-0.43	0.43
	2 CFU	20	11	1	11	1	0	-0.28	0.28
	5 CFU	5	5	1	5	1	0	-0.43	0.43

[a]Sample size: 25 g. *E. coli* 0157:H7 (ATCC 35150) was used for inoculated samples. POD analysis was not done for MSC samples.
[b]N = Number of test portions.
[c]X = Number of positive test portions. The number of positive test portions were identical for STEC gene screen method using either *stx* and *eae* or *stx* alone.
[d]POD_p = Alternative method presumptive positives divided by N.
[e]POD_{cr} = Alternative method confirmed positives divided by N.
[f]$dPOD_p$ = Difference between the presumptive and the confirmed positives for the alternative method POD values.
[g]95% CI = If the confidence interval of dPOD includes zero, then the difference between the methods is not significant.

Table 8. POD analysis of *E. coli* O157:H7 detection in poultry matrices using H7 PCR.

Matrix	Level[a]	N[b]	H7 PCR		Culture Confirmed		$dPOD_p$[f]	95% CI[g]	
			X[c]	POD_p[d]	X[c]	POD_{cr}[e]			
Ground chicken	Uninoculated	5	0	0	0	0	0	-0.43	0.43
	2 CFU	20	10	0.5	7	0.35	0.15	-0.25	0.36
	5 CFU	5	5	1	4	0.8	0.2	-0.28	0.43
Poultry parts	Uninoculated	5	0	0	0	0	0	-0.43	0.43
	2 CFU	20	11	1	11	1	0	-0.28	0.28
	5 CFU	5	5	1	5	1	0	-0.43	0.43

[a]Sample size: 25 g. *E. coli* 0157:H7 (ATCC 35150) was used for inoculated samples. POD analysis was not done for MSC samples.
[b]N = Number of test portions.
[c]X = Number of positive test portions. The number of positive test portions were identical for STEC gene screen method using either *stx* and *eae* or *stx* alone.
[d]POD_p = Alternative method presumptive positives divided by N.
[e]POD_{cr} = Alternative method confirmed positives divided by N.
[f]$dPOD_p$ = Difference between the presumptive and the confirmed positives for the alternative 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 LAMP-based molecular detection methods, the 3M Molecular Detection Assay 2 - STEC Gene Screen using the virulence genes *stx* and *eae* or *stx* alone, for detection of *E. coli* O157:H7 in poultry matrices as compared to the *E. coli* O157:H7 PCR method. 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, 29), the 3M Molecular Detection Assay 2 - STEC Gene Screen method using both *stx* and *eae* or *stx* alone is an acceptable alternative method for analyzing poultry samples for detection of toxigenic *E. coli* O157:H7 and other STECs. The positive screening results need to be followed up with a specific O157:H7 assay if further confirmation is needed.

The results of the 3M method or the H7 PCR method were not statistically significantly different from the cultural confirmation method for ground chicken and poultry parts. For MSC only two samples were culturally confirmed. Several different agars were used and none of the agars were able to isolate *E. coli* O157 colonies. The IMS beads from MSC enrichments were tested with STEC gene

screen method and the results were identical to results with no beads. The cultures probably failed to grow due to high background in MSC samples.

The virulence gene screen methods offer the potential to assess the presence of toxigenic *E. coli* O157:H7 than the use of O157 gene and/or flagella gene screen (36-40). In addition, the 3M Molecular Detection STEC Gene Screen method offers a streamlined and easy to use protocol relative to the H7 PCR method. The positives from the gene screen method can be confirmed further using specific *E. coli* O157:H7 methods.

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