Validation of the 3M™ Molecular Detection Assay 2 - STEC Gene Screen (stx) for the Detection of Shiga Toxin Gene (stx1 and/or stx2) in Fresh Raw Ground Beef and Fresh Spinach: AOAC Performance Tested Method™ 071903

Micki L. Rosauer 1,*, Christina Barnes 1, Karen M. Silbernagel 1, and Kateland M. Koch 2

13M Company, Food Safety Department, St. Paul, MN 55144-1000, USA, 2Research and Development, Q Laboratories, Cincinnati, OH 45204, USA

*Corresponding author’s e-mail: mirosauer@mmm.com

Abstract

Background: The 3M™ Molecular Detection Assay 2 - STEC Gene Screen (stx) method is based on gene amplification by the use of real time loop-mediated isothermal amplification when used with the 3M Molecular Detection System for the rapid and specific detection of Shiga toxin gene (stx1 and/or stx2) from Shiga toxin-producing Escherichia coli (STEC) in enriched foods. The stx assay does not differentiate between stx1 and stx2 but detects the presence of stx1 and/or stx2.

Objective: The 3M Molecular Detection Assay 2 - STEC Gene Screen (stx) method was evaluated for AOAC® Performance Tested Methods™ certification.

Methods: Matrix studies, inclusivity/exclusivity, robustness testing, product stability, and lot-to-lot variability testing were conducted to assess the method’s performance.

Results: The 3M Molecular Detection Assay 2 - STEC Gene Screen (stx) demonstrated equivalent results to the United States Department of Agriculture/Food Safety and Inspection Service Microbiology Laboratory Guidebook Chapter 5C.00 reference method for fresh raw ground beef, and the U.S. Food and Drug Administration Bacteriological Analytical Manual Chapter 4A reference method for fresh spinach. The 3M Molecular Detection Assay 2 - STEC Gene Screen (stx) detected all STEC E. coli strains (E. coli strains with stx1 and/or stx2 genes) and did not detect any of the 45 strains from the exclusivity panel. Robustness testing indicated that small variations in critical test parameters did not adversely affect the assay’s performance. Product consistency and stability testing demonstrated no differences between the lots evaluated.

Conclusion: The data collected in these studies demonstrate that the 3M Molecular Detection Assay 2 - STEC Gene Screen (stx) is a reliable method for the rapid and specific detection of Shiga toxin-producing E. coli in raw ground beef and spinach.
**Highlights:** The 3M Molecular Detection Assay 2 - STEC Gene Screen (stx) method is suitable for the rapid and specific detection of Shiga toxin-producing E. coli in fresh raw ground beef, and spinach.

### General Information

*Escherichia coli* (E. coli) is found in the environment, in foods (notably in meat, pork, raw milk, unpasteurized dairy products, and unpasteurized juices), and in the intestines of people and animals. Most *E. coli* strains are harmless and are important commensals in the human intestinal tract; however, some strains can cause diarrhea or other illnesses.

Infection with *E. coli* which produce a toxin known as Shiga toxin can cause illness; these *E. coli* strains are often transmitted through water and food contaminated from contact with animals or animal waste. These strains are collectively called Shiga toxin-producing *E. coli* (STEC). Symptoms of illnesses caused by STEC can include abdominal cramps, diarrhea (which may progress to bloody diarrhea), fever, and vomiting. In 5 to 15% of patients, it can lead to hemolytic uremic syndrome (HUS), characterized by thrombocytopenia (low blood platelet levels), hemolytic anemia, and acute renal failure (1). In fact, STEC-related HUS is the leading cause of acute renal failure in young children (2).

As with many foodborne illnesses, health ministries and other health-related organizations estimate that STEC-related illnesses are significantly underestimated. In the United States, for instance, there were 8672 cases of STEC reported to the National Notifiable Diseases Surveillance System (NNDSS) for the United States and US Territories in 2017 (3). However, the estimate for STEC illness each year in the United States is more than 265,000 (4). The global incidence of STEC-related illness has been estimated to be 2,800,000 acute illnesses annually (5).

The major sources of STEC infections are related to the consumption of undercooked or raw meat, raw milk, and unpasteurized dairy products, and increasingly, ready-to-eat foods such as fresh fruits and vegetables (1, 6).

There are several actions that can be taken to avoid STEC infection, including washing of hands after handling raw meat, cooking meats like ground beef thoroughly to an internal temperature of 71°C, and separating food preparation areas to prevent cross contamination (7).

### Principle

The 3M™ Molecular Detection Assay 2 - STEC Gene Screen (stx) is used with the 3M Molecular Detection System for the rapid and specific screening of *E. coli* genes *stx1* and/or *stx2* in enriched food samples. The 3M Molecular Detection Assays use loop-mediated isothermal amplification (LAMP) to rapidly amplify nucleic acid sequences with high specificity and sensitivity, combined with bioluminescence to detect the amplification.

An algorithm interprets the light output curve resulting from the detection of the nucleic acid amplification. Results are analyzed automatically by the software and are color-coded based on the result. A positive or negative result is determined by analysis of a number of unique curve parameters. Presumptive positive results are reported in real-time while negative results will be displayed after the run is completed.

The 3M Molecular Detection Assay 2 - STEC Gene Screen (stx) is intended for use in a laboratory environment by professionals trained in laboratory techniques. 3M has not documented the use of this product in industries other than food. For example, 3M has not documented this product for testing pharmaceutical, cosmetic, clinical, or veterinary samples. The 3M Molecular Detection Assay 2 - STEC Gene Screen (stx) has not been evaluated with all possible food products, food processes, testing protocols, or with all possible strains of bacteria. The 3M Molecular Detection Instrument is intended for use with samples that have undergone heat treatment during the assay lysis step, which is designed to destroy organisms present in the sample. Samples that have not been properly heat treated during the assay lysis step may be considered a potential biohazard and should not be inserted into the 3M Molecular Detection Instrument.

As with all test methods, the source of enrichment medium can influence the results. The STEC Molecular Detection Assay 2 - STEC Gene Screen (stx) has been evaluated for use with the 3M Buffered Peptone Water (ISO Formulation; 3M BPW-ISO) enrichment broth.

### Scope of Method

(a) **Target organisms.**—Shiga toxin-producing *E. coli* (strains containing *E. coli* gene *stx1*, coding for Shiga toxin type 1, and/or *stx2*, coding for Shiga toxin type 2).

(b) **Matrixes.**—Fresh raw ground beef (375 g, approximately 73% lean), fresh raw spinach (200 g).

(c) **Summary of validated performance claims.**—Performance equivalent to that of U.S. Department of Agriculture-Food Safety and Inspection Service Microbiology Laboratory Guidebook (USDA/FSIS MLG), Chapter 5C.00, Detection, isolation and identification of top seven shiga toxin-producing *Escherichia coli* (STECs) from meat products and carcass and environmental sponges for fresh raw ground beef (8), and to the U.S. Food and Safety Administration Diarrheagenic *Escherichia coli* for spinach (9).

### Definitions

(a) **Probability of detection (POD).**—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. Several POD measures can be calculated: POD$_A$ (reference method POD), POD$_C$ (confirmed candidate method POD), POD$_{CP}$ (candidate method presumptive result POD), and POD$_{CC}$ (candidate method confirmation result POD).

(b) **Difference of probabilities of detection (dPOD).**—Difference of probabilities of detection is the difference between any two POD values. If the confidence interval of a dPOD does not contain zero, the difference is statistically significant at the 5% level.

### Materials and Methods

**Test Kit Information**

(a) **Kit name.**—3M Molecular Detection Assay 2 - STEC Gene Screen (stx).
Dry bath incubator.—

3M Molecular Detection Cap/Decap Tool—Reagent.—

Extra reagent tube caps.—

Serological pipettes.—

IEC 17025 (10), or ISO 7218 (11). Incubated enrichment media testing techniques: for example, good laboratory practices, ISO/IEC 17025 (10), or ISO 7218 (11). Incubated enrichment media may contain pathogens at levels sufficient to cause risk to human health. Always follow standard laboratory safety practices, including wearing appropriate protective apparel and eye protection while handling reagents and contaminated samples. Avoid contact with the contents of the enrichment media and reagent tubes after amplification. Dispose of enriched samples according to current local/regional/national regulations and industry standards. Samples that have not been properly heat treated during the assay lysis step may be considered a potential biohazard and should not be inserted into the 3M Molecular Detection Instrument. Do not exceed the recommended temperature setting on the heater. Do not exceed the recommended heating time. Use an appropriate, calibrated thermometer to verify the 3M Molecular Detection Heat Block Insert temperature (e.g., a partial immersion thermometer or digital thermocouple thermometer, not a total immersion thermometer). The thermometer must be placed in the designated location in the 3M Molecular Detection Heat Block Insert.

General Preparation
Follow all instructions carefully. Failure to do so may lead to inaccurate results. Decontaminate laboratory benches and equipment (pipettes, cap/decap tools, etc.) periodically with a 1–5% (v/v in water) household bleach solution or DNA removal solution (pipettes, cap/decap tools, etc.) periodically with a 1–5% (v/v in water) household bleach solution or DNA removal solution. Prepare 3M BPW-ISO as per product instructions. Store prepared broth at 2–8°C if it will not be immediately used after preparation. Ensure enrichment media is pre-warmed to 41.5 ± 1°C before use. For all meat and highly particulate samples, the use of filter bags is recommended.

Sample Preparation
Note: Sample preparation instructions below are excerpted from the full product Instructions For Use, and so include references to full categories of raw ground beef, pieces and trim, and leafy produce. For this matrix study, fresh raw ground beef and fresh spinach were tested.

(a) Raw ground beef.—Aseptically transfer 375 g sample to a sterile bag and add 1125 mL 3M BPW-ISO, pre-warmed to 41.5 ± 1°C. Hand massage the samples for 30–60 s to disperse and break apart clumps after adding BPW-ISO.

(b) Leafy produce.—Aseptically transfer 200 g sample to a sterile bag and add 450 mL 3M BPW-ISO, pre-warmed to 41.5 ± 1°C. Rinse enrichment broth over leaves and agitate gently for 30–60 s. Do not massage or homogenize leaves.

Sample Enrichment
(a) Incubate the bag aerobically at 41.5 ± 1°C.
(b) Incubate raw ground beef for 10–18 h. Incubate leafy produce for 18–24 h.

Analysis
(a) Preparation of the 3M Molecular Detection Speed Loader Tray:
   (1) Wet a cloth or disposable towel with a 1–5% (v/v in water) household bleach solution and wipe the 3M Molecular Detection Speed Loader Tray.
Launch the 3M Molecular Detection Software and log in.

One 3M Lysis Solution tube is required for each sample and allow the 3M Lysis Solution Tubes to warm up by setting the 3M Molecular Detection Heat Block Insert to reach temperature of 100 °C.

Turn on the 3M Molecular Detection Instrument.

Place the 3M Molecular Detection Chill Block Insert in a dry double block heater unit. Turn on the dry block heater unit and set the temperature to allow the 3M Molecular Detection Heat Block Insert to reach and maintain a temperature of 100 ± 1 °C. Note: Depending on the heater unit, allow approximately 30 min for the 3M Molecular Detection Heat Block Insert to reach temperature. Using an appropriate, calibrated thermometer (i.e., a partial immersion thermometer or digital thermocouple thermometer, not a total immersion thermometer) placed in the designated location, verify that the 3M Molecular Detection Heat Block Insert is at 100 ± 1 °C.

Launch the 3M Molecular Detection Software and log in. Contact your 3M Food Safety representative to ensure you have the most updated version of the software.

Turn on the 3M Molecular Detection Instrument.

Create or edit a run with data for each sample. Refer to the 3M Molecular Detection User Manual for details. Note: The 3M Molecular Detection Instrument must reach and maintain “Ready” state before inserting the 3M Molecular Detection Speed Loader Tray with reaction tubes. This heating step takes approximately 20 min and is indicated by an orange light on the instrument’s status bar. When the instrument is ready to start a run, the status bar will turn green.

Allow the 3M Lysis Solution Tubes to warm up by setting the rack at ambient temperature (20–25 °C) overnight (16–18 h). Alternatives to equilibrate the 3M Lysis Solution Tubes to ambient temperature are to set the 3M Molecular Detection Heat Block Insert to reach and maintain 37 ± 1 °C incubator for 1 h, or place them in a dry double block heater for 30 s at 100 ± 1 °C.

Invert the capped tubes to mix. Proceed to the next step within 4 h after inverting.

Remove the enrichment broth from the incubator.

Gently massage the bottom of the enrichment bag before transferring the sample to the 3M Lysis Solution tube.

Additional sample may be required for re-testing or confirmatory steps. After collecting the sample, roll down bag to minimize headspace and reduce exposure of the enrichment to air. If confirmation of presumptive results is required, proceed to confirmatory steps as soon as presumptive result is obtained.

One 3M Lysis Solution tube is required for each sample and the negative control (NC) sample (sterile enrichment medium).

3M Lysis Solution Tube strips can be cut to desired tube number. Select the number of individual 3M Lysis Solution or eight-tube strips needed. Place the 3M Lysis Solution Tubes in an empty rack.

To avoid cross-contamination, decap one 3M Lysis Solution Tube strip at a time and use a new pipette tip for each transfer step.

Transfer enriched sample to 3M Lysis Solution Tube. Transfer each enriched sample into an individual 3M Lysis Solution Tube first. Transfer the NC last.

Use the 3M Molecular Detection Cap/Decap Tool—Lysis to decap one Lysis Solution tube strip—one strip at a time.

Discard the 3M Lysis Solution Tube cap—if lysate will be retained for retest, place the caps into a clean container for re-application after lysis.

Transfer 20 μL sample into a 3M Lysis Solution Tube. Warning: Should you choose to use neutralizing buffer that contains aryl sulfonate complex as a hydrating solution for environmental sponge samples, it is necessary to perform a 1:2 dilution (1 part sample into 1 part sterile enrichment broth) of the enriched environmental sample before testing in order to reduce the risks associated with a false-negative result leading to the release of contaminated product. Another option is to transfer 10 μL of the neutralizing buffer enrichment into the 3M Lysis Solution tubes.

When all samples have been transferred, transfer 20 μL NC (sterile enrichment medium) into a 3M Lysis Solution Tube. Do not use water as a NC.

Verify that the temperature of the 3M Molecular Detection Heat Block Insert is at 100 ± 1 °C.

Place the uncovered rack of 3M Lysis Solution Tubes in the 3M Molecular Detection Heat Block Insert and heat for 15 ± 1 min. During heating, the 3M Lysis Solution will change from pink (cool) to yellow (hot). Samples that have not been properly heat treated during the assay lysis step may be considered a potential biohazard and should not be inserted into the 3M Molecular Detection Instrument.

Remove the uncovered rack of 3M Lysis Solution Tubes from the heating block and allow to cool in the 3M Molecular Detection Chill Block Insert for at least 5 min and a maximum of 10 min. The 3M Molecular Chill Block Insert, used at ambient temperature (20–25 °C) without the 3M Molecular Detection Chill Block Tray, should sit directly on the laboratory bench. When cool, the lysis solution will revert to a pink color.

Remove the rack of 3M Lysis Solution tubes from the 3M Molecular Detection Chill Block Insert.

Amplification

One 3M Molecular Detection Assay 2 - STEC Gene Screen (stx) Reagent Tube is required for each sample and the NC.

Select the number of individual Reagent tubes or eight-tube strips needed.

Place Reagent tubes in an empty rack.

Avoid disturbing the reagent pellets from the bottom of the tubes.

Select one 3M Reagent Control Tube and place in rack.

To avoid cross-contamination, decap one 3M Molecular Detection Assay 2 - STEC Gene Screen (stx) Reagent Tube.
strip at a time and use a new pipette tip for each transfer step.

(d) Transfer each sample lysate into individual 3M Molecular Detection Assay 2 - STEC Gene Screen (stx) Reagent tubes first followed by the NC. Hydrate the 3M Reagent Control Tube last.

(e) Use the 3M Molecular Detection Cap/Decap Tool—Reagent to decap one 3M Molecular Detection Assay 2 - STEC Gene Screen (stx) Reagent Tube strip at a time. Discard cap.

(i) Transfer 20 μL sample lysate from the upper half of the liquid (avoid precipitate) in the 3M Lysis Solution Tube into a corresponding 3M Molecular Detection Assay 2 - STEC Gene Screen (stx) Reagent Tube. Dispense at an angle to avoid disturbing the pellets. Mix by gently pipetting up and down five times.

(2) Repeat until all individual sample lysates have been added to a corresponding 3M Molecular Detection Assay 2 - STEC Gene Screen (stx) Reagent tube in the strip.

(3) Cover the 3M Molecular Detection Assay 2 - STEC Gene Screen (stx) Reagent tubes with the extra caps provided and use the rounded side of the 3M Molecular Detection Cap/Decap Tool—Reagent to apply pressure in a back and forth motion ensuring that the cap is tightly applied.

(4) Repeat steps as needed, for the number of samples to be tested.

(5) When all sample lysates have been transferred, transfer 20 μL NC lysate into a 3M Molecular Detection Assay 2 - STEC Gene Screen (stx) Reagent Tube.

(6) Transfer 20 μL NC lysate into a RC tube. Dispense at an angle to avoid disturbing the pellets. Mix by gently pipetting up and down five times.

(f) Load capped tubes into a clean and decontaminated 3M Molecular Detection Speed Loader Tray. Close and latch the 3M Molecular Detection Speed Loader Tray lid.

(g) Review and confirm the configured run in the 3M Molecular Detection Software.

(h) Click the “Start” button in the software and select instrument for use. The selected instrument’s lid automatically opens.

(i) Place the 3M Molecular Detection Speed Loader Tray into the 3M Molecular Detection Instrument and close the lid to start the assay. Results are provided within 60 min, although positives may be detected sooner.

(j) After the assay is complete, remove the 3M Molecular Detection Speed Loader Tray from the 3M Molecular Detection Instrument and dispose of the tubes by soaking in a 1–5% (v/v in water) household bleach solution for 1 h and away from the assay preparation area.

(k) Note: To minimize the risk of false positives due to cross-contamination, never open reagent tubes containing amplification reagents have a “background” relative light unit (RLU) reading.

Confidential
Presumptive positive samples should be confirmed as per the laboratory standard operating procedures or by following the appropriate reference method confirmation, USDA/FSIS MLG Chapter 5C.00 or FDA BAM Chapter 4A as relevant to the matrix, beginning with transfer from the primary enrichment broth to selective plates, to confirmation of isolates using appropriate biochemical, microscopic, and serological methods. For matrices specified by USDA/FSIS MLG Chapter 5C, immunomagnetic separation (IMS) should be done prior to plating on selective medium.

In the rare event of any unusual light output, the algorithm labels this as “Inspect”. 3M recommends the user to repeat the assay for any Inspect samples. If the result continues to be Inspect, proceed to confirmation test using USDA/FSIS MLG Chapter 5C.00 or FDA BAM Chapter 4A.

In the event of discordant results (presumptive positive with the 3M Molecular Detection Assay 2 - STEC Gene Screen (stx), non-confirmed by USDA/FSIS MLG Chapter 5C.00 or FDA BAM Chapter 4A), the laboratory should follow their established standard operating procedures to report their results.

Validation Study
This validation study was conducted under the AOAC Research Institute Performance Tested Method™ (PTM) program according to the AOAC Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces (12). The inclusivity/exclusivity, robustness, and stability testing were performed by 3M. Matrix studies were conducted independently by Q Laboratories (Cincinnati, OH, USA), and SGS Vanguard Sciences, Inc. (North Sioux City, SD, USA); both American Association for Laboratory Accreditation (A2LA) accredited and by following the AOAC Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces (12).

Presumptive positive samples should be confirmed as per the laboratory standard operating procedures or by following the appropriate reference method confirmation, USDA/FSIS MLG Chapter 5C.00 or FDA BAM Chapter 4A as relevant to the matrix, beginning with transfer from the primary enrichment broth to selective plates, to confirmation of isolates using appropriate biochemical, microscopic, and serological methods. For matrices specified by USDA/FSIS MLG Chapter 5C, immunomagnetic separation (IMS) should be done prior to plating on selective medium.

In the rare event of any unusual light output, the algorithm labels this as “Inspect”. 3M recommends the user to repeat the assay for any Inspect samples. If the result continues to be Inspect, proceed to confirmation test using USDA/FSIS MLG Chapter 5C.00 or FDA BAM Chapter 4A.

In the event of discordant results (presumptive positive with the 3M Molecular Detection Assay 2 - STEC Gene Screen (stx), non-confirmed by USDA/FSIS MLG Chapter 5C.00 or FDA BAM Chapter 4A), the laboratory should follow their established standard operating procedures to report their results.

Inclusivity/Exclusivity
(a) Methodology.—Inclusivity and exclusivity testing were conducted to ensure that the 3M Molecular Detection Assay 2 - STEC Gene Screen (stx) specifically detects Shiga toxin-producing E. coli strains, while not reacting with non-target strains and species. For inclusivity, 50 E. coli strains containing the stx1 and/or stx2 genes were tested (Table 1); these strains were previously characterized in external collections as STEC strains. Additionally, strains were screened by PCR using primer sequences for stx1 and stx2 outlined in Appendix 4.00 of USDA/FSIS MLG Chapter 5C.00 (13). Each strain was cultured in 3M BPW-ISO for 10 h at 41.5 ± 1°C and diluted 1000-fold before testing. Fifty exclusivity strains, consisting of closely related non-E. coli species and E. coli non-STEC strains, were grown in BPW-ISO and incubated at 37 ± 2°C for 24 h or until turbid (Table 2). Exclusive cultures were diluted one log when visually turbid and the average CFU/mL tested on the Molecular
| Number | Strain source | Strain ID | Genus           | Species | Serogroup         | stx1<sup>a</sup> | stx2<sup>b</sup> | Isolation source       | 3M MDA<sup>c</sup> - STEC (stx) results |
|--------|---------------|-----------|----------------|---------|------------------|------------------|------------------|------------------------|-------------------------------------------|
| 1      | E. coli Reference Center (PSU)<sup>b</sup> | 10.2360   | Escherichia    | coli    | O45: H2          | +                | −                | Unknown                | Positive                                |
| 2      | MSU STEC Center<sup>c</sup> | TW04257   | Escherichia    | coli    | O111:−           | +                | +                | Washington—BD<sup>d</sup> fever/vomit | Positive                                |
| 3      | MSU STEC Center | TW09991   | Escherichia    | coli    | O103: NM         | +                | −                | Ohio—unknown           | Positive                                |
| 4      | Minnesota Department of Health<sup>e</sup> | 201024930-1 | Escherichia  | coli    | O103: H2         | +                | −                | Minnesota—stool/Diarrhea | Positive                                |
| 5      | MSU STEC Center | TW08101   | Escherichia    | coli    | O103: H2         | +                | −                | Denmark—Feces          | Positive                                |
| 6      | Minnesota Department of Health | 2011027007-3 | Escherichia  | coli    | O103: H2         | +                | −                | Minnesota—Stool/Diarrhea | Positive                                |
| 7      | Minnesota Department of Health | 2011032087-1 | Escherichia  | coli    | O26: H11         | +                | −                | Minnesota—Stool/Diarrhea | Positive                                |
| 8      | MSU STEC Center | TW08039   | Escherichia    | coli    | O121             | −                | +                | Montana—Unknown        | Positive                                |
| 9      | E. coli Reference Center (PSU) | 99.0723   | Escherichia    | coli    | O26              | −                | −                | Unknown                | Positive                                |
| 10     | E. coli Reference Center (PSU) | 99.0704   | Escherichia    | coli    | O26              | +                | −                | Unknown                | Positive                                |
| 11     | MSU STEC Center | TW07814   | Escherichia    | coli    | O26: H11         | +                | +                | Idaho—HUS<sup>f</sup>   | Positive                                |
| 12     | MSU STEC Center | TW07705   | Escherichia    | coli    | O26: H46         | +                | +                | Utah—Watery Stool      | Positive                                |
| 13     | USDA ARS<sup>g</sup> | 96-3285   | Escherichia    | coli    | O45: H2          | +                | −                | CDC<sup>h</sup>-Human Stool | Positive                                |
| 14     | MSU STEC Center | TW14003   | Escherichia    | coli    | O45: H2          | +                | −                | Michigan—Unknown       | Positive                                |
| 15     | MSU STEC Center | TW11239   | Escherichia    | coli    | O103: H25        | +                | −                | Washington—Unknown     | Positive                                |
| 16     | MSU STEC Center | TW05997   | Escherichia    | coli    | O103: N          | +                | −                | Idaho—Unknown          | Positive                                |
| 17     | MSU STEC Center | TW07990   | Escherichia    | coli    | O103: NM         | +                | −                | Washington—Feces       | Positive                                |
| 18     | ATCC<sup>i</sup> | BAA179    | Escherichia    | coli    | O111: H8         | +                | +                | Alabama—HUS            | Positive                                |
| 19     | ATCC | BAA181    | Escherichia    | coli    | O111: H8         | +                | +                | South Dakota—HUS       | Positive                                |
| 20     | MSU STEC Center | TW07931   | Escherichia    | coli    | O121: H19        | −                | +                | Massachusetts—Bloody Diarrhea | Positive                                |
| 21     | USDA ARS | 08023     | Escherichia    | coli    | O121: H19        | −                | +                | FDA—Human              | Positive                                |
| 22     | ATCC | BAA-2129 | BAA-5544      | Escherichia  | coli    | O145: H11        | +                | −                | Germany—Diarrhea        | Positive                                |
| 23     | USDA ARS | 05-6544  | Escherichia    | coli    | O26: H11         | +                | −                | PHAC<sup>k</sup>-Human | Positive                                |
| 24     | USDA ARS | TB285     | Escherichia    | coli    | O26: H2          | +                | −                | University of Washington—Human | Positive                                |
| 25     | USDA ARS | 93-3118  | Escherichia    | coli    | O26: H11         | +                | −                | PHAC—Human             | Positive                                |
| 26     | USDA ARS | 96-1415  | Escherichia    | coli    | O26: H11         | +                | −                | PHAC—Human             | Positive                                |
| 27     | USDA ARS | 96-001   | Escherichia    | coli    | O26: H11         | +                | −                | PHAC—Human             | Positive                                |
| 28     | USDA ARS | b8026 C1 | Escherichia    | coli    | O45: H2          | +                | −                | CDC—Calf               | Positive                                |
| 29     | USDA ARS | 05-6545  | Escherichia    | coli    | O45: H2          | +                | −                | PHAC—Human             | Positive                                |
| 30     | USDA ARS | SJ7      | Escherichia    | coli    | O45: H2          | +                | −                | CDC—Human              | Positive                                |
| 31     | USDA ARS | SJ8      | Escherichia    | coli    | O45: H2          | +                | −                | CDC—Human              | Positive                                |
| 32     | USDA ARS | SJ9      | Escherichia    | coli    | O45: H2          | +                | −                | CDC—Human              | Positive                                |
| 33     | USDA ARS | B8227 C8 | Escherichia    | coli    | O45              | +                | −                | CDC—Calf               | Positive                                |
| 34     | USDA ARS | 97-3112  | Escherichia    | coli    | O103: H25        | +                | −                | CDC—Human              | Positive                                |
| 35     | USDA ARS | tb154    | Escherichia    | coli    | O103: H6         | +                | −                | University of Washington—Human | Positive                                |
| 36     | USDA ARS | 03-2444  | Escherichia    | coli    | O103: H25        | +                | −                | PHAC—Human             | Positive                                |
| 37     | USDA ARS | 04162    | Escherichia    | coli    | O103: H6         | +                | −                | FDA—Human              | Positive                                |
| 38     | USDA ARS | 96-3166  | Escherichia    | coli    | O111: NM         | +                | −                | CDC—Human              | Positive                                |
| 39     | USDA ARS | TB226    | Escherichia    | coli    | O111: HN         | +                | −                | University of Washington—Human | Positive                                |
| 40     | USDA ARS | 01387    | Escherichia    | coli    | O111: H8         | +                | −                | FDA—Human              | Positive                                |
| 41     | USDA ARS | 96-1585  | Escherichia    | coli    | O121: H19        | +                | −                | PHAC—Human             | Positive                                |
| 42     | USDA ARS | 97-3068  | Escherichia    | coli    | O121: H19        | +                | −                | CDC—Human              | Positive                                |
| 43     | USDA ARS | 03-4064  | Escherichia    | coli    | O121: NM         | +                | −                | PHAC—Human             | Positive                                |
| 44     | USDA ARS | DA-1     | Escherichia    | coli    | O121             | +                | −                | STEC Center MSU—Human | Positive                                |

(continued)
Table 1. (continued)

| Number | Strain source | Strain ID | Genus       | Species        | Serogroup | Isolation source |
|--------|---------------|-----------|-------------|----------------|-----------|-----------------|
| 45     | USDA ARS      | 0145: H28 | Escherichia | coli           | O157: H7  | FDA—Cow feces   |
| 46     | USDA ARS      | 01357: H7 | Escherichia | coli           | O145: H28 | Beef Patty Outbreak |
| 47     | USDA ARS      | 01357: H7 | Escherichia | coli           | O157: H7  | Beef Brisket    |
| 48     | USDA ARS      | 01357: H7 | Escherichia | coli           | O157: H7  | Minnesota—Stool/Diarrhea |
| 49     | USDA ARS      | 01357: H7 | Escherichia | coli           | O157: H7  | Minnesota—Stool/Diarrhea |
| 50     | Minnesota Department of Health | 2011020423-1 | Escherichia | coli           | O111: NM  | Minnesota—Stool/Diarrhea |

a PCR gene characterization (internally screened with PCR using primers from Appendix 4C of USDA/FSIS MLG Chapter 5C.00).

Matrix Study

(a) Methodology.—Bulk samples of the matrices studied (fresh raw ground beef, approximately 73% lean, and fresh spinach) were purchased from local supplier(s). The fresh raw ground beef was prescreened for natural contamination with STEC organisms following the USDA/FSIS MLG Chapter 5C.00 reference method, and the spinach was prescreened following FDA BAM Chapter 4A: no natural contamination by the target organism was detected during product screening. Each matrix was artificially contaminated with an STEC strain at low and high contamination levels, while a sample of each matrix was set aside to run as an uncontrolled test. Thirty test portions of each matrix were analyzed in the unpaired comparison; 20 portions at a low contamination level, five portions at a high contamination level, and five portions uncontaminated. The low contamination level was targeted at a level to achieve a fractional response (5–15 positive results/20 replicates portions tested), while the high contamination level was targeted 10 times higher to produce 5 positive results/5 replicate portions tested. A 15-tube most probable number (MPN) was performed for both the high and low load samples to determine the final concentration of target per sample and determined using the Least Cost Formulation MPN Calculator v2.0, Least Cost Formulations, Ltd, Virginia Beach, VA.

E. coli strains from the American Type Culture Collection (ATCC; Manassas, VA, USA) were used to artificially contaminate the matrices. Fresh raw ground beef samples were inoculated with E. coli O26 BAA 1653. The culture was grown in brain heart infusion (BHI) broth at 35 ± 1°C for 24 ± 2h. Following incubation, the culture was diluted to a target level using BHI as the diluent and added to the matrix at an appropriate amount where the low-level inoculated samples would yield 0.2–2 CFU/25 g and the high-level inoculated samples would yield 2–10 CFU/25 g. Inoculated matrix was mixed to ensure homogeneous distribution of the organisms within the matrix and was held for 48–72h at 2–8°C to allow for equilibration of the organisms. For the 375 g test portions analyzed by the 3M Molecular Detection Assay 2 - STEC Gene Screen (stx) method, 25 g from each contamination level was combined with 350 g uninoculated matrix on the day of analysis. For 25 g test portions analyzed by the USDA/FSIS MLG Chapter 5C.00 method, 25 g replicates for each level of contamination were transferred to sterile filter laboratory blender bags on the day of analysis.

Fresh spinach was inoculated with E. coli O111 strain C4-61-1. The culture was grown in BHI overnight at 35–37°C, then serial dilutions were prepared in tubes of sterile distilled water to achieve an inoculation level of 0.2–2 CFU/200 g for the low level, and 2–10 CFU/200 g for the high level. The raw spinach Detection System was 9.32 ± 10^7 CFU/mL. Inclusivity and exclusivity cultures were randomized and blind-coded for testing on the 3M Molecular Detection Assay 2 - STEC Gene Screen (stx).

(b) Results.—The results of the inclusivity and exclusivity testing are presented in Tables 1 and 2. Fifty out of 50 inclusivity strains tested were detected by the 3M Molecular Detection Assay 2 - STEC Gene Screen (stx). Zero out of 45 exclusivity strains tested were detected by the 3M Molecular Detection Assay 2 - STEC Gene Screen (stx).
| Number | Strain ID | Genus     | Species          | Isolation source | Serogroup<sup>a</sup> | 3M MDA<sup>2</sup> - STEC (stx) results |
|--------|-----------|-----------|------------------|------------------|-----------------------|---------------------------------------|
| 1      | ATCC<sup>b</sup> 9372 | Bacillus atrophaeus | Unknown           | —                | Negative              |
| 2      | ATCC 8090 | Citrobacter freundii | Unknown           | —                | Negative              |
| 3      | ATCC 43162 | Citrobacter braakii | Clinical isolate, California | —                | Negative              |
| 4      | ATCC 27156 | Citrobacter laceri | Type strain of Citrobacter diversus | —                | Negative              |
| 5      | ATCC 25944 | Cronobacter sakazakii | Child's throat    | —                | Negative              |
| 6      | ATCC 51816 | Enterobacter amnigenus | Milk, Minnesota | —                | Negative              |
| 7      | ATCC 49464 | Enterococcus raffinosus | Clinical isolate | —                | Negative              |
| 8      | ATCC 11303 | Escherichia coli | Unknown           | —                | Negative              |
| 9      | ATCC 10536 | Escherichia coli | Unknown           | —                | Negative              |
| 10     | ATCC 8739 | Escherichia coli | Clinical isolate | —                | Negative              |
| 11     | ATCC 12345 | Escherichia coli | Feces            | —                | Negative              |
| 12     | ATCC 13567 | Escherichia coli | Unknown           | —                | Negative              |
| 13     | BEI<sup>c</sup> 53169 | Escherichia coli | Human isolate    | O25: H4          | Negative              |
| 14     | BEI 85.1284 | Escherichia coli | Human isolate, 1985 | O6: H31         | Negative              |
| 15     | BEI U9-41 | Escherichia coli | Human urine isolate | O2: K1: H4      | Negative              |
| 16     | BEI F11119-41 | Escherichia coli | Unknown           | O16: K1: H-      | Negative              |
| 17     | BEI E3b | Escherichia coli | Human peritoneum  | O75: K95: H5     | Negative              |
| 18     | ATCC 51815 | Hafnia abei | Milk, Minnesota | —                | Negative              |
| 19     | ATCC 51817 | Klebsiella oxytox | Milk, Minnesota | —                | Negative              |
| 20     | ATCC 13438 | Klebsiella pneumoniae | Water           | —                | Negative              |
| 21     | ATCC 13882 | Klebsiella pneumoniae | Moto, starter of sake | —                | Negative              |
| 22     | ATCC 9595 | Lactobacillus rhamnosus | Unknown       | —                | Negative              |
| 23     | ATCC 8014 | Lactobacillus plantarum | Unknown       | —                | Negative              |
| 24     | ATCC 19111 | Listeria monocytogenes | Poultry, England | —                | Negative              |
| 25     | ATCC 19119 | Listeria ivanovii | Sheep, Bulgaria | —                | Negative              |
| 26     | ATCC 4698 | Micrococcus luteus | Unknown           | —                | Negative              |
| 27     | ATCC 49143 | Moraxella catarrhalis | Clinical isolate | —                | Negative              |
| 28     | ATCC 43071 | Proteus mirabilis | Rectum, Georgia  | —                | Negative              |
| 29     | ATCC 13515 | Proteus vulgaris | Unknown           | —                | Negative              |
| 30     | ATCC 27853 | Pseudomonas aeruginosa | Blood culture    | —                | Negative              |
| 31     | ATCC 13525 | Pseudomonas fluorescens | Pre-filter tanks, England | —                | Negative              |
| 32     | ATCC 51812 | Salmonella Typhimurium | Human blood, Minnesota | —                | Negative              |
| 33     | ATCC 6962 | Salmonella Newport | Food poisoning fatality, England | —                | Negative              |
| 34     | ATCC 51741 | Salmonella Infantis | Pasta             | —                | Negative              |
| 35     | ATCC 12022 | Shigella flexneri | Unknown           | —                | Negative              |
| 36     | ATCC 8700 | Shigella boydii | Unknown           | —                | Negative              |
| 37     | ATCC 25931 | Shigella sonnei | Feces, human     | —                | Negative              |
| 38     | ATCC 6538 | Staphylococcus aureus | Human lesion     | —                | Negative              |
| 39     | ATCC 33317 | Streptococcus bovis | Cow dung         | —                | Negative              |
| 40     | ATCC 23715 | Yersinia enterocolitica | Human blood, petechiae, from anterior chamber of the eye, 47-year-old female, Missouri, 1968 | —                | Negative              |

<sup>a</sup> Serogroup information is not provided for all strains.

(continued)
was inoculated in bulk, thoroughly mixed, and then separated into 200 g samples. The product was stored at 2–8°C for 72–96 h. A portion remained uninoculated to serve as an uninoculated negative control.

3M Molecular Detection Assay 2 - STEC Gene Screen (Stx)
The 375 g test portions of raw ground beef were prepared by mixing the 25 g pre-inoculated sample with 350 g uncontaminated material. Each 375 g test portion was aseptically transferred to a sterile bag and combined with 1125 mL pre-warmed 41.5 ± 1°C BPW-ISO enrichment media and hand massaged for 30–60 s to disperse and break apart clumps. For fresh spinach, 450 mL pre-warmed 41.5 ± 1°C BPW-ISO was added to each 200 g test portion, and the liquid was gently rinsed over the leaves for 30–60 s without massaging or homogenizing. The fresh raw ground beef samples were incubated at 41.5 ± 1°C for 10–18 h, and the fresh spinach samples were incubated at 41.5 ± 1°C for 18 h. After incubation, all the samples were analyzed by 3M Molecular Detection Assay 2 - STEC Gene Screen (stx) as described in Analysis. All samples, regardless of screening result, were confirmed using the corresponding reference method.

USDA/FSIS MLG Chapter 5C.00 and FDA BAM Chapter 4A
All media for the reference method and cultural confirmations were prepared according to USDA/FSIS MLG Chapter 5C.00 or FDA BAM Chapter 4A methods. For raw ground beef samples, 25 g sample test portions were aseptically added to sterile bags, then 225 ± 4.5 mL modified tryptic soy broth (mTSB) was added to each bag. Samples were massaged by hand for 30–60 s to disperse clumps and then were incubated for 15–24 h at 42 ± 1°C. For spinach samples, 200 g sample test portions were aseptically added to sterile bags, then 450 mL of 1x modified Buffered Petone Water with pyruvate was added to each 200 g test portion. Bags were gently pressed to facilitate the flow of enrichment broth over the leaves. Bags were incubated at 37 ± 1°C for 5 h, then 1 mL each of the Acriflavin, Cefsulodin, and Vancomycin supplements was added per 225 mL mBPWp. Samples were further incubated at 42 ± 1°C for an additional 18–24 h.

Following the 18 h enrichment time point, all matrix samples, regardless of presumptive results, were culturally confirmed. For fresh raw ground beef, confirmation was completed per the USDA/FSIS ISMLG Chapter 5C.00 method; final confirmation was determined by VITEK2 GN Official Method 2011.17. For fresh spinach, confirmation was completed by plating on Levine’s eosin-methylene blue, MacConkey Agar with Sorbitol, Cefixime, and Tellurite and Rainbow® V Agar, and subsequent analysis of biochemical and serological characteristics of suspect colonies according to the FDA BAM Chapter 4A methods.

(a) Results.—The POD and dPOD statistical analyses for paired studies and unpaired studies were calculated according to Appendix J, Annex C (10). Background aerobic microbial counts for fresh raw ground beef, conducted per USDA/FSIS MLG Chapter 3.02 Quantitative analysis of bacteria in foods as sanitary indicators reference method, were 6.51 CFU/g. For fresh spinach, the background aerobic microbial counts were 6.14 CFU/g per FDA BAM Chapter 3 Aerobic plate count (14, 15).

Paired dPOD analyses were used to compare the 3M Molecular Detection Assay 2 - STEC Gene Screen (stx) presumptive

| Number | Strain ID | Genus | Species | Isolation source | Serogroup | Reference source |
|--------|-----------|-------|---------|------------------|-----------|------------------|
| 41     | BEI       | Escherichia | coli     | Infant diarrhea outbreak, Taunton, England. | O127: H6  | Negative |
| 42     | BEI       | Escherichia | coli     | Infant diarrhea, Seattle, Washington | O55: H7   | Negative |
| 43     | BEI       | Escherichia | coli     | Rabbit diarrhea | O15: H2   | Negative |
| 44     | RDEC-1    | Escherichia | coli     | Unknown | O95: H2 | Negative |
| 45     | BEI       | Escherichia | coli     | Unknown | O54: H2 | Negative |

* If applicable; ** ATCC = American Type Culture Collection, Manassas, VA, USA. ** BEI = BEI Resources, NIAID, NIH, Manassas, VA, USA. ** BRL = BEI Resource Center (PSU) – The E. coli Reference Center at Pennsylvania State University, University Park, PA, USA. ** USDA ARS = U.S. Department of Agriculture Research Service, Washington, DC, USA. ** Molecular Detection Assay 2. ** Not available.
results from the 3M BPW-ISO enrichment broth to the 3M method confirmed results as described above. Data are shown in Table 3.

For the low contamination level of fresh raw ground beef, the eight presumptive positive results at both 10 and 18 h confirmed as positive, with a dPOD of 0.00 and confidence interval (CI) of (0.13, 0.13). For the low contamination level of fresh spinach, there were 16 presumptive positives at 18 h, with all 16 confirming as positive, with a dPOD of 0.00 and CI of (0.13, 0.13). All other portions for each matrix lot and contamination level that were presumptive positive with the 3M Molecular Detection Assay 2 - STEC Gene Screen (stx) confirmed as positive. There were no 3M method negative results that confirmed positive. All dPOD comparisons showed no significant statistical difference.

Unpaired dPOD analyses were used to compare the 3M Molecular Detection Assay 2 - STEC Gene Screen (stx) confirmed results from the 3M BPW-ISO broth to the reference method enriched cultural results. Data are shown in Table 4. There were no significant statistical differences seen between the 3M Molecular Detection Assay 2 - STEC Gene Screen (stx) confirmed results from the 3M BPW-ISO enrichment broth and the USDA/FSIS MLG Chapter 5C.00 reference method confirmed results for fresh raw ground beef, or between the 3M Molecular Detection Assay 2 - STEC Gene Screen (stx) results from the 3M BPW-ISO broth and the USDA/FSIS MLG Chapter 5C.00 reference method confirmed results for fresh spinach.
Table 5. Robustness: 3M Molecular Detection Assay 2 - STEC Gene Screen (stx) results: test conditions versus nominal condition

| Test condition | n\(^a\) | x\(^b\) | POD\(_{TC}\)^c | 95% CI | Test condition results | Nominal condition results^d | dPOD\(^e\) | 95% CI^f |
|----------------|--------|--------|----------------|-------|------------------------|-----------------------------|----------|----------|
| Ground beef samples inoculated with Escherichia coli O157 culture (FSIS 258-93)h | | | | | | | | |
| 9.5 h, 13 min, 18 μL | 10 | 6 | 0.60 (0.31, 0.83) | 6 | 0.60 (0.31, 0.83) | 0.00 (–0.25, 0.25) |
| 9.5 h, 13 min, 22 μL | 10 | 6 | 0.60 (0.31, 0.83) | 6 | 0.60 (0.31, 0.83) | 0.00 (–0.25, 0.25) |
| 9.5 h, 17 min, 18 μL | 10 | 6 | 0.60 (0.31, 0.83) | 6 | 0.60 (0.31, 0.83) | 0.00 (–0.25, 0.25) |
| 9.5 h, 17 min, 22 μL | 10 | 6 | 0.60 (0.31, 0.83) | 6 | 0.60 (0.31, 0.83) | 0.00 (–0.25, 0.25) |
| 26 h, 13 min, 18 μL | 10 | 6 | 0.60 (0.31, 0.83) | 6 | 0.60 (0.31, 0.83) | 0.00 (–0.25, 0.25) |
| 26 h, 13 min, 22 μL | 10 | 6 | 0.60 (0.31, 0.83) | 6 | 0.60 (0.31, 0.83) | 0.00 (–0.25, 0.25) |
| 26 h, 17 min, 18 μL | 10 | 6 | 0.60 (0.31, 0.83) | 6 | 0.60 (0.31, 0.83) | 0.00 (–0.25, 0.25) |
| 26 h, 17 min, 22 μL | 10 | 6 | 0.60 (0.31, 0.83) | 6 | 0.60 (0.31, 0.83) | 0.00 (–0.25, 0.25) |

| Uninoculated ground beef samples | | | | | | | | |
| 9.5 h, 13 min, 18 μL | 10 | 0 | 0.00 (0.28) | 6 | 0.00 (0.28) | 0.00 (–0.25, 0.25) |
| 9.5 h, 13 min, 22 μL | 10 | 0 | 0.00 (0.28) | 6 | 0.00 (0.28) | 0.00 (–0.25, 0.25) |
| 9.5 h, 17 min, 18 μL | 10 | 0 | 0.00 (0.28) | 6 | 0.00 (0.28) | 0.00 (–0.25, 0.25) |
| 9.5 h, 17 min, 22 μL | 10 | 0 | 0.00 (0.28) | 6 | 0.00 (0.28) | 0.00 (–0.25, 0.25) |
| 26 h, 13 min, 18 μL | 10 | 0 | 0.00 (0.28) | 6 | 0.00 (0.28) | 0.00 (–0.25, 0.25) |
| 26 h, 13 min, 22 μL | 10 | 0 | 0.00 (0.28) | 6 | 0.00 (0.28) | 0.00 (–0.25, 0.25) |
| 26 h, 17 min, 18 μL | 10 | 0 | 0.00 (0.28) | 6 | 0.00 (0.28) | 0.00 (–0.25, 0.25) |
| 26 h, 17 min, 22 μL | 10 | 0 | 0.00 (0.28) | 6 | 0.00 (0.28) | 0.00 (–0.25, 0.25) |

^n = Number of test portions.

x = Number of positive test portions.

POD\(_{TC}\) = Test condition parameter positive outcomes divided by the total number of trials.

Nominal condition results – 22 h enrichment, 15 min lysis time, and 20 μL lysate into reaction tube.

POD\(_{AV}\) = Nominal value positive outcomes divided by the total number of trials.

dPOD = Difference between the test condition and the nominal condition POD values.

95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

United States Department of Agriculture, Food Safety and Inspection Service, Agricultural Research Service, Washington, DC, USA.

Enrichment time.

Lysis heating time.

Volume of lysate into reaction tube.

Robustness Study

(a) Methodology.—The robustness study was conducted to determine whether variations to the method parameters, specifically those that may occur when performed by an end user, affect the 3M Molecular Detection Assay 2 - STEC Gene Screen (stx) method performance. Upper and lower limits of three method parameters were evaluated; enrichment time (9.5 and 26 h), lysis heating time (13 and 17 min), and volume of lysate to reaction tube (18 and 22 μL). The nominal conditions for the assay are 10.5 h enrichment time, 15 min lysis time, and 20 μL lysate to reaction tube, and were run alongside the test conditions in a factorial design. Ground beef samples were used in the robustness evaluation. Samples were prepared by distributing 375 g 85% ground beef into 96 oz filter enrichment bags and adding 1125 mL pre-warmed BPW-ISO to each sample. Samples were homogenized by hand. Ten of the rinse samples were inoculated at a fractional level with Shiga toxin-producing E. coli O157 strain FSIS 258-93. This culture was obtained from the USDA Agriculture Research Service (ARS, Washington, DC, USA). Ten additional samples were left non-inoculated. Samples were incubated aerobically at 41.5 °C for 9.5, 10.5, and 26 h. At each assessment time, samples were subjected to 13 and 17 min of heat treatment in lysis at 100 °C. The third robustness parameter, volume of lysate to reaction tube, was evaluated by analyzing replicates by transferring 18 or 22 μL lysate into each 3M Molecular Detection Assay 2 - STEC Gene Screen (stx) reagent tube.

(b) Results.—Results of the robustness study are presented in Table 5. Six of the 10 replicate fractional-level inoculated portions were positive by the nominal parameters, and by each test condition. All non-inoculated portions were negative. The dPOD values comparing the test conditions to the nominal parameters were zero in all cases, indicating that small variations in these test parameters (enrichment time, lysis time, and lysed sample reaction volume) do not adversely affect the 3M Molecular Detection Assay 2 - STEC Gene Screen (stx) performance.

Product Stability and Lot-to-Lot Variability Study

(a) Methodology.—The stability and lot-to-lot variability of three different test kits were evaluated. Two lots were at eight months of real-time shelf life, which were the oldest available at the time of the product stability study. These lots were prepared in the same timeframe but varied by lots of raw materials used in production. The third lot was at one month of real-time shelf life. The three lots were evaluated by testing with pure culture. Ten replicate samples of each lot were tested with a fractional level preparation of target organism, Shiga toxin-producing E. coli O157: H7 (FSIS 258-93), and ten replicate samples of a non-diluted, related organism, Salmonella Typhimurium ATCC 14028.

The target STEC E. coli strain was grown for 10 h in BPW-ISO at 41.5 ± 1 °C and diluted with sterile BPW-ISO before testing. The non-target S. Typhimurium strain was grown in...
Table 6. Product consistency and stability study: 3M Molecular Detection Assay 2 - STEC Gene Screen (stx) results

| Lot No. | Age of lot at time of testing, months | n* | Pure culture; Shiga toxin-producing Escherichia coli O157 (FSIS 258–93)*; fractional (low) level | Pure culture; Salmonella Typhimurium (ATCC 14028)*; undiluted |
|---------|-------------------------------------|----|------------------------------------------------|----------------------------------------------------------|
| 100418–1 | 8 | 10 | 7 | 0.70 | (0.40, 0.89) | 10 | 0 | 0.00 | (0.00, 0.28) |
| 100418–2 | 8 | 10 | 7 | 0.70 | (0.40, 0.89) | 10 | 0 | 0.00 | (0.00, 0.28) |
| 040319 | 1 | 10 | 7 | 0.70 | (0.40, 0.89) | 10 | 0 | 0.00 | (0.00, 0.28) |

* n – Number of test portions.
* United States Department of Agriculture, Food Safety and Inspection Service, Agricultural Research Service, Washington, DC, USA.
* x – Number of positive test portions.
* PODT – Target strain positive outcomes divided by the total number of trials.
* American Type Culture Collection, Manassas, VA, USA.
* PODNT – Non-target strain positive outcomes divided by the total number of trials.

Results.—Results of the product stability and lot-to-lot variability study are presented in Table 6. Seven of the 10 replicate fractional-level inoculated portions were positive by each of the three lots of the 3M Molecular Detection Assay 2 - STEC Gene Screen (stx). All non-inoculated portions were negative. Results indicate that the age and lot-to-lot variability of 3M Molecular Detection Assay 2 - STEC Gene Screen (stx) product tested do not adversely affect assay performance.

Discussion

The results of the inclusivity and exclusivity testing show that all 50 out of 50 inclusivity strains tested were detected by the 3M Molecular Detection Assay 2 - STEC Gene Screen (stx). Zero out of the 45 exclusivity strains tested were detected by the 3M Molecular Detection Assay 2 - STEC Gene Screen (stx).

As per criteria outlined in Appendix J of the Official Methods of Analysis Manual for evaluations in matrices, fractional positive results were obtained for both 10 and 18 h enrichment time points for fresh raw ground beef, and at 18 h enrichment time point for fresh spinach. The probability of detection (POD) was calculated as the number of positive outcomes divided by the total number of trials. The POD was calculated for the candidate presumptive results, PODCP, the candidate confirmatory results, PODCC, the difference in the candidate presumptive and confirmatory results, dPODCP, presumptive candidate results that confirmed positive, PODC, the reference method, PODR, and the difference in the confirmed candidate and reference methods, dPODC. The POD analysis between the 3M Molecular Detection Assay 2 - STEC Gene Screen (stx) and the appropriate reference method for each matrix indicated that there was no significant difference at the 5% level between the number of positive results by the two methods at any time points tested (10 and 18 h of enrichment for fresh raw ground beef, and 18 h of enrichment for fresh spinach). A summary of POD analyses is presented in Tables 4 and 5.

Results from the robustness study and product stability and lot-to-lot variability study results both indicate the rigor of the method, since small variations in test parameters (enrichment time, lysis time, and lysed sample reaction volume), age of the product, and variations between product lots do not adversely affect the 3M Molecular Detection Assay 2 - STEC Gene Screen (stx) performance.

Feedback from laboratory analysts from independent matrix studies highlighted additional strengths of the 3M Molecular Detection Assay 2 - STEC Gene Screen (stx) method. Analysts found the method quick and simple to perform, providing results in less than 2 h post incubation for up to 90 sample replicates. Another benefit mentioned was the simplicity of the method; with only two sample transfers, risks of possible contamination are minimized. The small footprint and ability to link multiple Molecular Detection Systems to a single laptop computer, offering high throughput, was noted. Analysts also found the 3M Molecular Detection System software to be user friendly, with the ability to track assay lot information and sample identification quickly and with ease, with real-time curves allowing for improvement of any troubleshooting issues that may arise.

Conclusions

The 3M Molecular Detection Assay 2 - STEC Gene Screen (stx) method successfully recovered STEC from fresh raw ground beef after 10 and 18 h of enrichment, and from fresh spinach after 18 h of enrichment, using 3M BPW-ISO as the enrichment medium. Using POD analysis, no statistically significant differences were observed between the number of positive samples detected by the candidate method and the appropriate reference method for any of the samples tested, at any of the time points tested.

Matrix studies, inclusivity/exclusivity, robustness testing, product stability, and lot-to-lot variability testing were conducted to assess the performance of the 3M Molecular Detection Assay 2 - STEC Gene Screen (stx) method. The data collected in these studies demonstrate that the 3M Molecular Detection Assay 2 - STEC Gene Screen (stx) method is suitable for Performance Tested MethodSM certification for rapid and specific detection of Shiga toxin-producing E. coli in fresh raw ground beef, and spinach.

Acknowledgments

Submitting Company

3M Company
Food Safety Department
2501 Hudson Road
References

1. World Health Organization (2018) E. coli, https://www.who.int/news-room/fact-sheets/detail/e-coli (accessed June 5, 2019)
2. Tarr, P., Gordon, C., & Chandler, W. (2005) Lancet. 365, 1073–1086. doi:10.1016/S0140-6736(05)71144-2
3. CDC Division of Health Informatics and Surveillance (2018) National Notifiable Diseases Surveillance System, 2017 Annual Tables of Infectious Disease Data, TABLE 2m. Reported Cases of Notifiable Diseases, by Region and Reporting Area, https://wonder.cdc.gov/ndss/static/2017/annual/2017-table2m.html (accessed June 5, 2019)
4. National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention (2006) Estimates of Foodborne Illness in the United States, Reference CS228412, https://www.cdc.gov/foodborneburden/pdfs/scallan-estimated-illnesses-foodborne-pathogens.pdf (accessed June 5, 2019)
5. Majowicz, S., Scallan, E., Jones-Bitton, A., Sargeant, J., Stapleton, J., Angulo, F., Yeung, D., & Kirk, M. (2014) Foodborne Pathog. Dis. 11, 447–455. doi:10.1089/ftp.2013.1704
6. Kintz, E., Brainard, J., Hooper, L., & Hunter, P. (2017) Int. J. Hyg. Environ. Health 220, 57–67. doi:10.1016/j.ijheh.2016.10.011
7. Stop Foodborne Illness (2019) What is foodpoisoning?, http://stopfoodborneillness.org/awareness/what-is-foodborne-illness/ (accessed June 5, 2019)
8. U.S. Department of Agriculture Food Safety and Inspection Service (2019) Microbiology Laboratory Guidebook, Ch. SC.00, Detection, Isolation and Identification of Top Seven Shiga Toxin-Producing Escherichia coli (STEC) from Meat Products and Carcass and Environmental Sponges, https://www.fsis.usda.gov/sites/default/files/media_file/2021-08/MLG-SC.02.pdf (accessed version SC.00 May 29, 2019)
9. U.S. Food and Safety Administration (2018) Bacteriological Analytical Manual, Ch. 4A, Diarrheagenic Escherichia coli, https://www.fda.gov/food/laboratory-methods-food/bam-chapter-4a-diarrheagenic-escherichia-coli (accessed May 29, 2019)
10. ISO/IEC 17025:2017: General Requirements for the Competence of Testing and Calibration Laboratories, Geneva, Switzerland.
11. ISO 7218:2007: Microbiology of Food and Animal Feeding Stuffs – General Rules for Microbiological Examination, Geneva, Switzerland.
12. Official Methods of Analysis (2019) 21st Ed., AOAC INTERNATIONAL, Rockville, MD, Appendix J, http://www.eoma.aoac.org/app_j.pdf (accessed June 1, 2019)
13. U.S. Department of Agriculture Food Safety and Inspection Service (2019) Microbiology Laboratory Guidebook, Ch. SC Appendix 4.00: Primer and Probe Sequences and Reagent Concentrations for non-O157 Shiga Toxin-Producing Escherichia coli (STEC) Real-Time PCR Assay, https://www.fsis.usda.gov/sites/default/files/media_file/2021-03/mlg-4-appendix-4.pdf (accessed June 7, 2019)
14. U.S. Department of Agriculture Food Safety and Inspection Service (2019) Microbiology Laboratory Guidebook, Ch. 3.02 Appendix 3. Quantitative Analysis of Bacteria in Foods as Sanitary Indicators, https://www.fsis.usda.gov/sites/default/files/media_file/2021-03/MLG-3.pdf (accessed June 2, 2019)
15. U.S. Food and Drug Administration (2001) Bacteriological Analytical Manual, Ch. 3 Aerobic Plate Count, https://www.fda.gov/food/laboratory-methods-food/bam-chapter-3-aerobic-plate-count (accessed July 2018)