Validation of the 3M™ Molecular Detection Assay 2—STEC Gene Screen (stx and eae) for Detection of Shiga Toxin Gene (stx1 and/or stx2) and Intimin Gene (eae) in Dried Cannabis Flower and Dried Hemp Flower: AOAC Performance Tested MethodSM 071902

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Abstract

Background: The 3M™ Molecular Detection Assay 2—STEC Gene Screen (stx and eae) 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) and intimin gene (eae) from Shiga toxin-producing Escherichia coli (STEC) in enriched products. The 3M Molecular Detection Assay 2—STEC Gene Screen (stx and eae) was approved as AOAC Performance Tested MethodSM Certificate No. 071902.

Objective: This matrix extension study evaluated the 3M Molecular Detection Assay 2—STEC Gene Screen (stx and eae) method for detection of STECs in dried cannabis flower (>0.3% delta 9-tetrahydrocannabinol (THC)) and dried hemp flower (≤0.3% THC) at a 10 g test portion size.

Method: Testing followed procedures outlined in 3M Molecular Detection Assay 2—STEC Gene Screen (stx and eae) product instructions and Standard Method Performance Requirements (SMPR®) for Detection of Shiga Toxin-Producing Escherichia coli in Cannabis and Cannabis Products (AOAC SMPR 2020.012). The method was evaluated at low, high, and non-inoculated levels.

Results: Results showed no statistically significant difference between the presumptive positive 3M Molecular Detection Assay 2—STEC Gene Screen (stx and eae) results and the SMPR 2020.012 recommended cultural confirmations.

Conclusions: This study provides data that demonstrate the 3M Molecular Detection Assay 2—STEC Gene Screen (stx and eae) is a reliable method for the rapid and specific detection of STEC organisms in dried cannabis flower and dried hemp flower.

Highlights: The 3M Molecular Detection Assay 2—STEC Gene Screen (stx and eae) method is suitable for the rapid and specific detection of STEC organisms in dried cannabis flower and dried hemp flower.
General Information

*Escherichia coli* (E. coli) is a bacterium that is commonly found in the human gastrointestinal tract. Some strains can be pathogenic to humans. STEC are *E. coli* that produce Shiga toxins encoded by stx genes. STEC are not necessarily associated with human disease. The main modes of transmission of STEC infections to humans is in the consumption of contaminated food such as meat products, milk and dairy products made with pasteurized or unpasteurized cow’s milk or goat’s milk, consumption of raw vegetables contaminated by animal feces, and ingestion of contaminated water and contact with animals (particularly bovines).

Principle

The 3MTM Molecular Detection Assay 2—STEC Gene Screen (stx and eae) is used with the 3M Molecular Detection System for the rapid and specific screening of Shiga toxin-producing *E. coli* genes stx1 and/or stx2 and intimin gene (eae) in enriched food samples. The 3M Molecular Detection Assays use loop-mediated isothermal amplification 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 and eae) is Performance Tested MethodsSM (PTM) certified for fresh raw ground beef and pork, raw beef trim, raw poultry parts, fresh spinach, and sprouts.

Scope of Matrix Extension

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

(b) **Matrices**—Dried cannabis flower (>0.3% delta 9-tetrahydrocannabinol (THC)) and dried hemp flower (<0.3% THC).

(c) **Summary of validated performance claims**—Performance met the acceptance criteria established in the Standard Method Performance Requirements (SMPR) for Detection of Shiga Toxin-Producing *Escherichia coli* in Cannabis and Cannabis Products [AOAC SMPR 2020:012; (1)] for dried cannabis flower (>0.3% THC) and dried hemp flower (<0.3% THC) at a 10 g test portion size.

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.

(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, then 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 and eae).

(b) **Cat no.**—MDA2STXEA48.

(c) **Ordering information**—https://www.3m.com/

Test Kit Components

(a) **STEC Gene Screen (stx) Reagent Tubes**—48 orange tubes (two pouches; containing three strips of eight tubes).

(b) **STEC Gene Screen (eae) Reagent Tubes**—48 red tubes (two pouches; containing three strips of eight tubes).

(c) **Lysis Solution Tubes**—96 clear tubes (12 strips of eight tubes, with each tube containing 580 μL of lysis solution).

(d) **Reagent control (RC)**—16 individual clear flip-top tubes (two pouches of eight individual flip-top tubes).

(e) **Extra reagent tube caps**—96 orange caps, 96 red caps.

(f) **Product instructions**.

Additional Supplies and Reagent

(a) **3M Molecular Detection System Matrix Control**—Cat. No. MDMC96NA.

(b) **3M Buffered Peptone Water (ISO formulation) (3M BPW ISO)**—Cat. No. BPW500 (or equivalent).

(c) **3M Molecular Detection System Instrument**—Cat. No. MDST100.

(d) **Laptop with 3M Molecular Detection System Software**—Version 2.6.0.0.

(e) **3M Molecular Detection Speed Loading Tray**—Cat. No. MDSSLT.

(f) **3M Molecular Detection Chill Block Insert**—Cat. No. MDSCBIN.

(g) **3M Molecular Detection Heat Block Insert**—Cat. No. MDHIBIN.

(h) **3M Molecular Detection Cap/Decap Tool (Reagent)**—Cat. No. MDSGDR.

(i) **3M Molecular Detection Cap/Decap Tool (Lysis)**—Cat. No. MDSGDL.

(j) **Empty lysis tube rack**.

(k) **Empty reagent tube rack**.

Apparatus

(a) **Incubators**—Capable of maintaining 37 ± 1 and 41.5 ± 1°C.

(b) **Filter laboratory blender bags**.

(c) **Serological pipet bulbs (automatic pipet)**—For sampling and delivering of 1–10 mL.

(d) **Serological pipets**—Aerosol resistant.

(e) **Precision pipettors**—For sampling and delivering of 20 μL.

(f) **Sterile pipet tips**—Capable of 20 μL.

(g) **Multi-channel pipet**—Capable of 20 μL.

(h) **Laboratory paddle blender**.

(i) **Sterile collection sponge and swab**—Environmental surface sampling.

(j) **Thermometer**—Calibrated range to include 100 ± 1°C range.

(k) **Dry bath incubator**—Capable of maintaining a temperature of 100 ± 1°C.

(l) **Dry double block heater unit**—Capable of maintaining 100 ± 1°C; or a water bath capable of maintaining 100 ± 1°C.

(m) **Refrigerator**—Capable of maintaining 2–8°C, for storing the 3M MDA2 and lysates.
Safety Precautions

The user should read, understand, and follow all safety information in the instructions for the 3M Molecular Detection System and the 3M Molecular Detection Assay 2—STEC Gene Screen (stx). Retain the safety instructions for future reference.

To reduce the risks associated with exposure to chemicals and biohazards: (1) Perform pathogen testing in a properly equipped laboratory under the control of trained personnel. Incubated enrichment media and equipment or surfaces that have come into contact with 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. (2) Dispose of enriched samples according to current local/regional/national regulatory standards. (3) 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.

To reduce the risks associated with exposure to hot liquids: (1) Do not exceed the recommended temperature setting on 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 (pipets, 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°C. Homogenize by hand-massaging.

Caution: Should you select to use neutralizing buffer that contains aryl sulfonate complex as a hydrating solution for environmental sponge samples, it is required 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.

Sample Enrichment

(a) Pre-warm BPW-ISO enrichment medium to 41.5 ± 1°C.
(b) Aseptically transfer 10 g of sample (dried cannabis flower or dried hemp flower) to a 24 oz filter bag and add 90 mL BPW-ISO.
(c) Homogenize by hand-massaging.
(d) Incubate the bag aerobically at 41.5 ± 1°C for 28–32 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.
   (2) Rinse the 3M Molecular Detection Speed Loader Tray with water.
   (3) Use a disposable towel to wipe the 3M Molecular Detection Speed Loader Tray dry.
   (4) Ensure the 3M Molecular Detection Speed Loader Tray is dry before use.
(b) Place the 3M Molecular Detection Chill Block Insert directly on the laboratory bench; the 3M Molecular Detection Chill Block Tray is not used. Use the block at ambient laboratory temperature (20–25°C).
(c) Place the 3M Molecular Detection Heat 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 (e.g., 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.
(d) 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.
(e) Turn on the 3M Molecular Detection Instrument.
(f) Create or edit a run with data for each sample. Selection of the STXAE-2 icon in the software selects two adjacent wells (such as A1, A2, B1, B2, etc.), one for stx and the other for eae reagent tube, as each sample is run with two assays. The NC is set up for each of the reagent tubes, and one RC is set up for the kit. Refer to the 3M Molecular Detection System 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.

Lysis

(a) 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 Lysis Solution tubes on the laboratory bench for at least 2 h, incubate the 3M Lysis Solution Tubes in a 37 ± 1°C incubator for 1 h, or place them in a dry double block heater for 30 s at 100 ± 1°C.
(b) Invert the capped tubes to mix. Proceed to next step within 4 h after inverting.
(c) Remove the enrichment broth from the incubator.
(d) One 3M Lysis Solution Tube is required for each sample and the negative control (NC) sample (sterile enrichment medium).
(1) 3M Lysis Solution Tubes strips can be cut to the desired tube number. Select the number of individual 3M Lysis Solution Tubes or 8-tube strips needed. Place the 3M Lysis Solution Tubes in an empty rack.

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

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

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

(5) Discard the 3M Lysis Solution Tube cap—if lysate will be retained for retest, place the caps into a clean container for reapplication after lysis.

(6) Transfer 20μL of sample into a 3M Lysis Solution Tube.

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

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

(g) 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 Tubes 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.

(h) 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 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.

(i) Remove the rack of 3M Lysis Solution Tubes from the 3M Molecular Detection Chill Block Insert.

Amplification

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

(1) Tube strips can be cut to the desired tube number. Select the number of individual 3M Molecular Detection Assay 2—STEC Gene Screen (stx) and 3M Molecular Detection Assay 2—STEC Gene Screen (eae) Reagent Tubes or 8-tube strips needed.

(2) Place 3M Molecular Detection Assay 2—STEC Gene Screen (stx) tubes in an empty rack in one column.

(3) Place 3M Molecular Detection Assay 2—STEC Gene Screen (eae) tubes in the adjacent right column.

(4) Avoid disturbing the reagent pellets from the bottom of the tubes.

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

(c) 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 pipet 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.

(1) First, transfer each of the sample lysate to a 3M Molecular Detection Assay 2—STEC Gene Screen (stx) Reagent Tube.

(2) Second, transfer each of the same sample lysate to a 3M Molecular Detection Assay 2—STEC Gene Screen (eae) Reagent Tube in the adjacent right column.

(3) After all sample lysate transfer, add NC lysate to each of the 3M Molecular Detection Assay 2—STEC Gene Screen (stx) Reagent Tubes and 3M Molecular Detection Assay 2—STEC Gene Screen (eae) Reagent Tubes.

(e) 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.

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

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

(h) 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.

(i) 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.

(j) Note: To minimize the risk of false positives due to cross-contamination, never open reagent tubes containing amplified DNA. This includes 3M Molecular Detection Assay 2—STEC Gene Screen (stx and eae) Reagent, 3M Reagent Control, and 3M Matrix Control tubes. Always dispose of sealed reagent tubes by soaking in a 1–5% (v/v in water) household bleach solution for 1 h and away from the assay preparation.

Results and Interpretation

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. Note: Even a negative sample will not give a zero reading as the system and 3M Molecular Detection Assay 2—STEC Gene Screen (stx and eae) amplification reagents have a “background” relative light unit (RLU) reading.

Confirmation

Presumptive positive samples should be confirmed as per the laboratory standard operating procedures or by following the
appropriate reference method confirmation, as relevant to the
matrix, beginning with transfer from the primary enrichment
broth to selective plates, confirmation of isolates using appro-
priate biochemical, microscopic, and serological methods.

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 the appropriate ref-
ence method.

In the event of discordant results [presumptive positive with
the 3M Molecular Detection Assay 2—STEC Gene Screen (stx and
eae), nonconfirmed by the reference method], the laboratory
should follow their established standard operating procedures
to report their results.

**Matrix Extension Study**

This matrix extension study was conducted under the AOAC
PTM program according to the Appendix J: AOAC
INTERNATIONAL Methods Committee Guidelines for Validation of
Microbiological Methods for Food and Environmental Surfaces (2) and
AOAC SMPR 2020.012. The matrix study was conducted inde-
pendently by TEQ Analytical Laboratories, Inc. (Aurora, CO). The
3M Molecular Detection Assay 2—STEC Gene Screen (stx and
eae) is PTM certified in conformance with the TOP 7 STEC inclu-
sivity (E. coli serogroups O26, O111, O121, O45, O103, O145 and
O157: H7) and exclusivity requirements listed in SMPR 2020.012.
The method will not be making a broad STEC claim, so no addi-
tional inclusivity/exclusivity testing was conducted in that
respect.

The 3M Molecular Detection Assay 2—STEC Gene Screen (stx and
eae) allows for detection of STEC and Salmonella spp. from
the same enriched portion. For this study the dried cannabis
flower and the dried hemp flower were co-inoculated with both
a STEC strain and a Salmonella strain. Results for the Salmonella
analysis will be discussed in a separate PTM report. The 3M
Molecular Detection Assay 2—STEC Gene Screen (stx and eae)
study is reported here.

**Matrix Study**

(a) Methodology.—Prior to inoculation, a total aerobic plate
count was performed following U.S. Food and Drug
Administration Bacteriological Analytical Manual Chapter 3
(5) and the matrix was pre-screened for the target organ-
ism as outlined in SMPR 2020.012. No natural contamina-
tion was found, so artificial contamination was required.
Strains from the American Type Culture Collection (ATCC,
Manassas, VA) and the Centers for Disease Control and
Prevention (CDC, Atlanta, GA) were used to artificially con-
taminate the dried cannabis flower and dried hemp flower
materials.

Dried cannabis flower was inoculated with lyophilized cul-
tures of E. coli O157: H7 (ATCC 43895) and S. Typhimurium
(ATCC 14028). Dried hemp flower was inoculated with E. coli
O26 (CDC 03-3014) and S. Enteritidis (ATCC 13076). Both
matrix types were inoculated in the following manner:
Dense buds of dried cannabis flower and dried hemp
flower were obtained by the independent laboratory. The
dried flower materials were broken up by hand, placed into
large plastic stomacher-type bags, and then hand-mas-
saged further to create small particles. The bags were
shaken to mix the particles. A portion of each material was
set aside prior to inoculation to serve as the non-
inoculated level (0 CFU/test portion). The indicated bacte-
rial strains, in the form of lyophilized pellets, were crushed
into nonfat dry milk (NFDM), and then serial dilutions of
the inoculated NFDM were made. A three-level MPN was
conducted to determine the CFU/pellet. A portion of the in-
oculum was added to the dried cannabis flower or dried
hemp in the large bag, and then hand-massaged and agi-
tated within the sterile bag to mix. The spiked materials
were tested in an MPN format, using the same AOAC PTM
certified methods that were used for the screening. After
the STEC and Salmonella levels were determined, the dried
cannabis flower and dried hemp flower materials were ad-
justed by either adding non-inoculated material to create a
lower contamination level, or by adding more inoculum to
create a higher contamination level. Contamination levels
were targeted at approximately 2 CFU/test portion (for the
fractional low level) and approximately 10 CFU/test portion
(for the high level). The bags were hand-massaged and agi-
tated after any adjustments to ensure homogeneity. The
materials were allowed to equilibrate for a minimum of
two weeks at 18–25 °C prior to testing.

After the equilibration period, 10 g portions of each material
were tested at each contamination level using the candidate
method. An MPN analysis was conducted on the low- and
high-level contaminated materials. The test portion prepa-
ration, analysis, and presumptive positive confirmations
were performed according to SMPR 2020.012 for STEC.

(b) 3M Molecular Detection Assay 2—STEC Gene Screen (stx and
eae) method.—The 10 g test portions of dried cannabis
flower or dried hemp flower were aseptically transferred to
a sterile filter bag and combined with 90 mL of pre-warmed
BPW-ISO enrichment media and hand-massaged for 30–
60 s. The portions were incubated at 41.5 ± 1 °C for 28 and
32 h. After incubation, all the portions were homogenized
by hand again, and then analyzed by 3M Molecular
Detection Assay 2—STEC Gene Screen (stx and eae) as de-
scribed in the Analysis section. All portions, regardless of
screening result, were culturally confirmed as recom-
ended in SMPR 2020.012.

(c) Confirmation.—Following incubation, each overnight
enriched portion was serially diluted in Butterfield’s phos-
phate buffer and spread-plated (50 μL of the 10−2 to 10−4
dilutions) in duplicate onto selective agars. For E. coli O157:
H7, tellurite cefixime-sorbitol MacConkey agar and
CHROMagar™ STEC were used. For non-O157 STEC (E. coli
O26), Levine’s eosin-methylene blue agar and CHROMagar
STEC were used. All plates were incubated at 37 ± 1.0 °C for
18–24 h.

To confirm E. coli O157: H7, typical colonies on selective
agar were screened using O157 antigen by latex agglutina-
tion. One positive colony was streaked to tryptic soy agar
with yeast extract (TSA-YE). A CoLiComplete (CC) disc
(MilliporeSigma) was placed onto the heaviest streak area
and the plate was incubated at 37 ± 1 °C for 18–24 h. Plates
were analyzed for a blue color [galactopyranoside (X-gal)
positive] and fluorescence under UV light. A spot indole
test was performed. X-gal positive, glucuronidase (MUG)
negative, and indole positive isolates were confirmed bio-
chemically using API20E (bioMérieux, Hazelwood, MO). The
presence of O157 and H7 antigens was confirmed using
Pro-Lab Diagnostics E. coli O157 Latex Test Reagent Kit
(Round Rock, TX). The presence of stx in the E. coli O157: H7
Table 1. 3M Molecular Detection Assay—STEC Gene Screen (stx and eae) presumptive versus confirmed results in dried cannabis flower (>0.3% THC) and dried hemp flower (<0.3% THC)

| Matrix and inoculum | Enrichment time | MPN*Test portion | N<sup>b</sup> | x<sup>c</sup> | POD<sub>cp</sub><sup>d</sup> | 95% Cl | x | POD<sub>cc</sub><sup>e</sup> | 95% Cl | dPOD<sub>cp</sub><sup>f</sup> | 95% Cl |
|---------------------|----------------|------------------|------------|--------|--------|------|------|--------|------|----------------|------|
| Dried cannabis flower, 10 g | 28 h | NA<sup>h</sup> | 5 | 0 | 0.00 | 0.00, 0.43 | 0 | 0.00 | 0.00, 0.43 | 0.00 | (-0.47, 0.47) |
| E. coli O157: H7 (ATCC 43895)<sup>i</sup> | 28 h | 0.88 (0.40, 2.02) | 20 | 10 | 0.50 | 0.30, 0.70 | 10 | 0.50 | 0.30, 0.70 | 0.00 | (-0.13, 0.13) |
| | 32 h | 2.96 (1.54, 9.78) | 5 | 5 | 1.00 | 0.57, 1.00 | 5 | 1.00 | 0.57, 1.00 | 0.00 | (-0.47, 0.47) |
| | 28 h | 0.88 (0.40, 2.02) | 20 | 11 | 0.55 | 0.34, 0.74 | 10 | 0.50 | 0.30, 0.70 | 0.00 | (-0.47, 0.47) |
| | 32 h | 2.96 (1.54, 9.78) | 5 | 5 | 1.00 | 0.57, 1.00 | 5 | 1.00 | 0.57, 1.00 | 0.00 | (-0.47, 0.47) |
| Dried hemp flower, 10 g | 28 h | NA | 5 | 0 | 0.00 | 0.00, 0.43 | 0 | 0.00 | 0.00, 0.43 | 0.00 | (-0.47, 0.47) |
| E. coli O26 (CDC 03–3014)<sup>j</sup> | 28 h | 0.88 (0.40, 2.02) | 20 | 10 | 0.50 | 0.30, 0.70 | 10 | 0.50 | 0.30, 0.70 | 0.00 | (-0.13, 0.13) |
| | 32 h | 2.96 (1.54, 9.78) | 5 | 5 | 1.00 | 0.57, 1.00 | 5 | 1.00 | 0.57, 1.00 | 0.00 | (-0.47, 0.47) |
| | 28 h | 1.15 (0.61, 2.45) | 20 | 10 | 0.50 | 0.30, 0.70 | 10 | 0.50 | 0.30, 0.70 | 0.00 | (-0.47, 0.47) |
| | 32 h | 1.15 (0.61, 2.45) | 20 | 10 | 0.50 | 0.30, 0.70 | 10 | 0.50 | 0.30, 0.70 | 0.00 | (-0.13, 0.13) |
| | 28 h | 2.96 (1.54, 9.78) | 5 | 5 | 1.00 | 0.57, 1.00 | 5 | 1.00 | 0.57, 1.00 | 0.00 | (-0.47, 0.47) |

<sup>a</sup>MPN – Most probable number is based on the POD of reference method test portions using the Least Cost Formulations MPN calculator, with 95% confidence interval.

<sup>b</sup>N – Number of test portions.

<sup>c</sup>x – Number of positive test portions.

<sup>d</sup>POD<sub>cp</sub> – Candidate method presumptive positive outcomes divided by the total number of trials.

<sup>e</sup>POD<sub>cc</sub> – Candidate method confirmed positive outcomes divided by the total number of trials.

<sup>f</sup>dPOD<sub>cp</sub> – Difference between the candidate method presumptive result and candidate method confirmed result POD values.

<sup>g</sup>95% CI – If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

<sup>h</sup>Not applicable.

<sup>i</sup>American Type Culture Collection, Manassas, VA.

<sup>j</sup>Centers for Disease Control and Prevention, Atlanta, GA.
isolation was confirmed by reprocessing an isolated colony using a PTM certified real-time STEC screening PCR assay. To confirm non-O157 STEC, one typical colony from selective agar was streaked to TSAYE. A CC disc was placed onto the heaviest streak area, and then the plate was incubated at 37 ± 1 °C for 18–24 h. Plates were analyzed for a blue color (X-gal positive) and fluorescence (MUG positive if present). A spot indole test was performed on X-gal positive strains. X-gal positive, MUG positive or negative, and indole positive isolates were confirmed as E. coli using API20E. The presence of stx in the E. coli O157:H7 isolates was confirmed by reprocessing an isolated colony using a PTM approved method.

Results

The POD and dPOD statistical analyses for paired studies were calculated according to Appendix J, Annex C (3). Background aerobic microbial counts for dried cannabis flower and dried hemp flower were 5.0 × 10^3 and 7.9 × 10^2 CFU/g, respectively. Initial gene screens for the presence of indigenous stx in each product were negative.

POD analysis of the presumptive versus confirmed results showed no statistically significant differences (Table 1). There was one unconfirmed positive for the 3M MDA2STEC (stx and eae) screening kit at the 32 h time point when analyzing dried cannabis flower. No discrepant results were obtained with the candidate method for dried hemp flower in the study. Results of the MPN analysis were interpreted using the Least Cost Formulation MPN calculator (Virginia Beach, VA). The dried cannabis flower had a low inoculation level of 0.88 CFU/test portion and a high level of 2.96 CFU/test portion. For the dried hemp, the low inoculation level was 1.15 CFU/test portion, and the high inoculation level was 2.96 CFU/test portion.

Discussion

The 3M Molecular Detection Assay 2—STEC Gene Screen (stx and eae) successfully detected the target STEC species in dried cannabis flower and dried hemp flower at a 10 g sample size. The difference in POD analysis for the presumptive versus confirmed positives showed no statistically significant differences, with all ranges of the 95% confidence intervals containing the zero point. There was one presumptive positive result in the dried cannabis flower after 32 h of enrichment that was not confirmed. It is possible that a very low level of a STEC organism was present in the sample, but no STEC was detected culturally.

The independent laboratory reported that processing samples was very user-friendly with a standard heat-dependent lysis step and transfer into pre-aliquoted lyophilized pellets in reagent tube wells. The assay was able to be run from a single set of lysis tubes. A short run time of roughly 60 min, with presumptive positive results displaying quickly in the run, was a very beneficial aspect.

The 3M Molecular Detection Assay 2—STEC Gene Screen (stx and eae) allows users to obtain presumptive positive results after 28 h of incubation and 1 to 2 h of processing and assay run time. Presumptive results are easily visualized, denoted by a plus or minus sign within the software.

Conclusions

The 3M Molecular Detection Assay 2—STEC Gene Screen (stx and eae) method successfully recovered STEC from dried cannabis flower and dried hemp flower after 28 h of enrichment, using BPW-ISO as the enrichment medium. Using POD analysis, no statistically significant differences were observed between the number of presumptive positive results detected by the candidate method and the confirmed positive results determined by the reference method for any of the samples tested, at any of the time points tested.

The data collected in this matrix study demonstrate that the 3M Molecular Detection Assay 2 - STEC Gene Screen (stx and eae) method is suitable for PTM certification for rapid and specific detection of Shiga toxin-producing E. coli in dried cannabis flower and dried hemp flower.

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Conflict of Interest
All authors declare no conflict of interest.

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