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Research Article

MICROBIOLOGICAL METHODS

Validation of the 3M™ Molecular Detection Assay 2—*Salmonella* for detection of *Salmonella* in Dried Cannabis Flower and Dried Hemp Flower: AOAC *Performance Tested Methods*℠ 091501

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Abstract

Background: The 3M™ Molecular Detection Assay 2—*Salmonella* method is based on real-time loop-mediated isothermal amplification when used with the 3M Molecular Detection System for the rapid and specific detection of *Salmonella* in enriched products. The 3M Molecular Detection Assay 2—*Salmonella* was approved as AOAC INTERNATIONAL (AOAC) *Performance Tested Method*℠ (PTM) Certificate No. 091501 and as AOAC *Official Method of Analysis*℠ **2016.01**.

Objective: This matrix extension study evaluated the 3M Molecular Detection Assay 2—*Salmonella* for detection of *Salmonella* 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: Matrix studies in dried cannabis and hemp flowers followed procedures outlined in 3M Molecular Detection Assay 2—*Salmonella* product instructions and *Standard Method Performance Requirement* (SMPR®) for Detection of *Salmonella* species in Cannabis and Cannabis Products (AOAC SMPR 2020.002). The method was evaluated at low, high, and non-contaminated levels.

Results: Results showed no statistically significant difference between the presumptive positive 3M Molecular Detection Assay 2—*Salmonella* results and the SMPR 2020.002 recommended cultural confirmations.

Conclusions: This study demonstrates that the 3M Molecular Detection Assay 2—*Salmonella* is a reliable method for the rapid and specific detection of *Salmonella* in dried cannabis flower and dried hemp flower.

Highlights: The 3M Molecular Detection Assay 2—*Salmonella* method is suitable for the rapid and specific detection of *Salmonella* in dried cannabis flower and dried hemp flower.

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General Information

Salmonella has been recognized as a primary cause of foodborne illness worldwide. This genus of bacteria is classified into two species: *S. enterica* and *S. bongori*, the former containing the majority of serotypes associated with human disease. *Salmonella* can contaminate a wide range of foods, including poultry, meat, eggs, dairy, fruit, and vegetables as well as pet food.

In most cases, particularly in the United States, *Salmonella* infection is characterized by acute gastroenteritis. Symptoms include diarrhea, fever, abdominal cramps, and vomiting lasting 4–7 days in most people. Worldwide, especially in developing countries, salmonellosis in the form of enteric fever (typhoid) is more common than in the United States. Typhoid is a more serious bacterial invasion of the bloodstream; symptoms include high fever, weakness, abdominal cramps, headache, loss of appetite, and sometimes a rash. According to the World Health Organization, 16 million people contract typhoid annually, leading to 600 000 fatalities worldwide (1, 2).

Principle

The 3M™ Molecular Detection Assay 2—*Salmonella* is used with the 3M Molecular Detection System for the rapid and specific detection of *Salmonella* in enrichments of food, animal feed, and food process environmental 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. Presumptive positive results are reported in real time, while negative results are displayed after the assay is completed. Presumptive positive results should be confirmed using your preferred method or as specified by local regulations.

The 3M Molecular Detection Assay 2—*Salmonella* 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 or beverage. For example, 3M has not documented this product for testing pharmaceutical, cosmetics, clinical, or veterinary samples. The 3M Molecular Detection Assay 2—*Salmonella* has not been evaluated with all possible food products, food processes, testing protocols or with all possible strains of bacteria.

The 3M Molecular Detection Assay 2—*Salmonella* is *Performance Tested Methods*℠ (PTM) certified for a broad range of foods and select environmental surfaces, including raw ground beef and chicken, cooked breaded chicken, dry dog food, black pepper, raw whole shrimp and head-on raw shrimp, spinach, American cheese, nonfat dry milk (NFDM), cocoa powder, whole milk, liquid egg, peanut butter, chicken carcass rinse and sponges, sprout irrigation water, and sealed concrete, stainless steel, and ceramic tile environmental surfaces.

Scope of Matrix Extension

(a) *Target organisms*.—*Salmonella* species.
(b) *Matrixes*.—Dried cannabis flower [>0.3% delta 9-tetrahydrocannabinol (THC)] and dried hemp flower (≤0.3% THC), 10 g.
(c) *Summary of validated performance claims*.—Performance met the acceptance criteria established in the *Standard Method Performance Requirement* (SMPR®) for Detection of *Salmonella* Species in Cannabis and Cannabis Products [AOAC SMPR 2020.002; (3)] 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—*Salmonella*.
(b) *Cat no*.—MDA2SAL96.
(c) *Ordering information*.—https://www.3m.com/.

Test Kit Components

(a) *3M Lysis Solution Tubes*.—96 (12 strips of eight tubes).
(b) *3M Molecular Detection Assay 2—Salmonella Reagent Tubes*.—96 (12 strips of eight tubes).
(c) *Extra caps*.—96 (12 strips of eight caps).
(d) *3M Reagent Control*.—16 (two pouches of eight).
(e) *Quick Start Guide*.

Additional Supplies and Reagents

(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. MDS100.
(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. MDSHBIN.
(h) *3M Molecular Detection Cap/Decap Tool [Reagent]*.—Cat. No. MDSCDR.
(i) *3M Molecular Detection Cap/Decap Tool [Lysis]*.—Cat. No. MDSCDL.
(j) *Empty lysis tube rack*.
(k) *Empty reagent tube rack*.

Apparatus

(a) *Incubators*.—Capable of maintaining 41.5 ± 1°C.
(b) *Filter laboratory blender bags*.
(c) *Serological pipette 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 assay kit.

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—*Salmonella*. 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. *(2)* Always follow standard laboratory safety practices, including wearing appropriate protective apparel and eye protection while handling reagents and contaminated samples. *(3)* Avoid contact with the contents of the enrichment media and reagent tubes after amplification. *(4)* Dispose of enriched samples according to current industry standards. *(5)* 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 environmental contamination, follow current industry standards for disposal of contaminated waste. To reduce the risks associated with exposure to hot liquids: *(1)* Do not exceed the recommended temperature setting on heater. *(2)* Do not exceed the recommended heating time. *(3)* 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 prewarmed to 41.5 ± 1°C before use. For all meat and highly particulate samples, the use of filter bags is recommended.

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. 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 Tube 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) 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.
  (4) Transfer 20 μL of sample into a 3M Lysis Solution Tube.
(e) When all samples have been transferred, transfer 20 μL 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—*Salmonella* 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 reagent tubes or 8-tube strips needed.
  (2) Place reagent tubes in an empty rack.
  (3) 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 Reagent Tube Strips one at a time and use a new pipet tip for each transfer step.
(d) Transfer each sample lysate into an individual 3M Molecular Detection Assay 2—*Salmonella* Reagent Tube 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—*Salmonella* Reagent Tubes—one strip at a time. Discard cap.
  (1) Transfer 20 μL of sample lysate from the upper half of the liquid (avoid precipitate) in the 3M Lysis Solution Tube into the corresponding 3M Molecular Detection Assay 2—*Salmonella* Reagent Tube. Dispense at an angle to avoid disturbing the pellets. Mix by gently pipetting up and down 5 times.
  (2) Repeat until individual sample lysate has been added to a corresponding 3M Molecular Detection Assay 2—*Salmonella* Reagent Tube in the strip.
  (3) Cover the 3M Molecular Detection Assay 2—*Salmonella* Reagent Tubes with the provided extra caps 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 of NC lysate into a 3M Molecular Detection Assay 2—*Salmonella* Reagent Tube.
  (6) Transfer 20 μL of NC lysate into a Reagent Control tube. Dispense at an angle to avoid disturbing the pellets. Mix by gently pipetting up and down 5 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 amplified DNA. This includes 3M Molecular Detection Assay 2—*Salmonella* 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—*Salmonella* 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 appropriate 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 reference method.

In the event of discordant results (presumptive positive with the 3M Molecular Detection Assay 2—*Salmonella,* nonconfirmed by the reference method), the laboratory should follow their established standard operating procedures to report their results.

Validation Study

This matrix extension study was conducted under the AOAC Research Institute PTM program according to the Appendix J: *AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces* (4) and AOAC SMPR 2020.002. This study was conducted independently by TEQ Analytical Laboratories, Inc. (Aurora, CO). The 3M Molecular Detection Assay 2—*Salmonella* certified method is in conformance with the inclusivity and exclusivity requirements listed in SMPR 2020.002, so no additional testing was needed for this study.

The 3M Molecular Detection Assay 2—*Salmonella* assay allows for detection of *Salmonella* and Shiga toxin-producing *Escherichia coli* (STEC) from the same enriched portion. For this study the dried cannabis flower and the dried hemp flower were co-inoculated with both a *Salmonella* strain and a STEC strain. Results for the STEC analysis will be discussed in a separate PTM report. The 3M Molecular Detection Assay 2—*Salmonella* results are 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, Aerobic Plate Count Method (5), and the matrix was pre-screened for the target organisms as outlined in SMPR 2020.002 and 2020.012 (6). No natural *Salmonella* or STEC contamination 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 contaminate the dried cannabis flower and dried hemp flower materials.

Dried cannabis flower was inoculated with lyophilized cultures of *S.* Typhimurium (ATCC 14028) and *E. coli* O157: H7 (ATCC 43895). Dried hemp flower was inoculated with *S.* Enteritidis (ATCC 13076) and *E. coli* O26 (CDC 03-3014). 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-massaged 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 bacterial strains, in the form of lyophilized pellets, were crushed into NFDM, and then serial dilutions of the inoculated NFDM were made. A three-level most probable number (MPN) was conducted to determine the CFU/pellet. A portion of the inoculum was added to the dried cannabis flower or dried hemp in the large bag, and then hand-massaged and agitated 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 *Salmonella* and STEC levels were determined, the dried cannabis flower and dried hemp flower materials were adjusted by either adding non-inoculated material to create a lower contamination level, or by adding more inoculum to create a higher contamination level. The targeted levels of contamination were 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 agitated 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 preparation, analysis, and presumptive positive confirmations were performed according to SMPR 2020.002 for *Salmonella*.

(b) *3M Molecular Detection Assay* 2—Salmonella *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°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—*Salmonella* as described in the *Analysis* section. All portions, regardless of screening result, were culturally confirmed as recommended in SMPR 2020.002.

(c) *Confirmation*.—Following incubation, 0.1 mL of each test portion was transferred to 10 mL Rappaport-Vassiliadis (RV) medium and 1 to 10 mL Tetrathionate (TT) Broth. The RV and TT tubes were incubated at 42 ± 0.2°C for 22–26 h in a circulating water bath. Secondary selective enrichments were streaked to xylose lysine desoxycholate and CHROMagar *Salmonella* plates and incubated at 37 ± 2°C for 21–27 h. One typical colony from one agar plate was stabbed and streaked to triple sugar iron (TSI) agar and lysine iron agar and then incubated at 35 ± 2°C for 22–26 h. Positive reactions from TSI slant were streaked onto a TSA plate and incubated at 35 ± 2°C for 18–24 h. One isolated colony was selected, and spot polyvalent O and polyvalent H serology tests were performed. Positive agglutinating colonies were biochemically confirmed using API20E.

Results

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 screens for the presence of indigenous *Salmonella* in each product were negative.

The POD and dPOD statistical analyses for paired studies were calculated according to Appendix J, Annex C. POD analysis

Table 1. Matrix study: 3M Molecular Detection Assay 2—*Salmonella* presumptive versus confirmed results in dried cannabis flower (>0.3% THC) and dried hemp flower (≤0.3% THC)

					Presumptive			Confirmed			
Matrix and inoculum	Enrichment time	MPN[a]/Test portion	N[b]	x[c]	POD_{cp}[d]	95% CI	x	POD_{cc}[e]	95% CI	$dPOD_{cp}$[f]	95% CI[g]
Dried cannabis flower, 10 g	28 h	NA[h]	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(–0.47, 0.47)
(*S.* Typhimurium ATCC[i] 14028)		1.74 (0.91, 8.08)	20	14	0.70	0.48, 0.86	14	0.70	0.48, 0.86	0.00	(–0.13, 0.13)
		4.90 (2.50, 16.2)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(–0.47, 0.47)
	32 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(–0.47, 0.47)
		1.74 (0.91, 8.08)	20	15	0.75	0.53, 0.88	14	0.70	0.48, 0.86	0.05	(–0.11, 0.21)
		4.90 (2.50, 16.2)	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)
(*S.* Enteritidis ATCC 13076)		1.03 (0.46, 2.53)	20	12	0.60	0.39, 0.78	12	0.60	0.39, 0.78	0.00	(–0.13, 0.13)
		4.03 (2.10, 16.2)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(–0.47, 0.47)
	32 h	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(–0.47, 0.47)
		1.03 (0.46, 2.53)	20	12	0.60	0.39, 0.78	12	0.60	0.39, 0.78	0.00	(–0.13, 0.13)
		4.03 (2.10, 16.2)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(–0.47, 0.47)

[a] MPN = Most probable number is based on the POD of reference method test portions using the Least Cost Formulations MPN calculator, with 95% confidence interval.
[b] N = Number of test portions.
[c] x = Number of positive test portions.
[d] POD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials.
[e] POD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials.
[f] $dPOD_{CP}$ = Difference between the candidate method presumptive result and candidate method confirmed result POD values.
[g] 95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[h] Not applicable.
[i] American Type Culture Collection, Manassas, VA.

of the presumptive versus confirmed results showed no statistically significant differences (Table 1). There was one unconfirmed positive for the 3M Molecular Detection Assay 2—*Salmonella* method 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 1.74 CFU/test portion and a high level of 4.90 CFU/test portion. For the dried hemp, the low inoculation level was 1.03 CFU/test portion, and the high inoculation level was 4.03 CFU/test portion.

Discussion

The 3M Molecular Detection Assay 2—*Salmonella* successfully detected the target *Salmonella* in dried cannabis flower and dried hemp flower at a 10 g sample size. 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 *Salmonella* was present in the sample, but no *Salmonella* 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—*Salmonella* 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—*Salmonella* method successfully recovered *Salmonella* from dried cannabis flower and dried hemp flower after 28 h of enrichment, using BPW-ISO as the enrichment medium. After 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—*Salmonella* method is suitable for PTM certification for rapid and specific detection of *Salmonella* in dried cannabis flower and dried hemp flower.

Acknowlegments

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Conflict of Interest

All authors declare no conflict of interest.

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