AOAC Official Method 2017.01
***Escherichia coli* O157:H7 in Selected Foods**
3M™ Molecular Detection Assay (MDA) 2–*E. coli* O157 (Including H7) Method
First Action 2017

[Applicable to detection of *Escherichia coli* O157 (including H7) in raw ground beef (73% lean), frozen blueberries, fresh bean sprouts, and fresh baby spinach.]

See Table **2017.01A** for a summary of results of the interlaboratory study supporting acceptance of the method.

A. Principle

The 3M™ Molecular Detection Assay (MDA) 2 – *E. coli* O157 (including H7) method is used with the 3M™ Molecular Detection System (MDS) for the rapid and specific detection of *E. coli* O157 (including H7) in enriched food samples. The 3M MDA2 –*E. coli* O157 (including H7) uses loop-mediated isothermal amplification of unique DNA target 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, 60 minutes. Samples are enriched in 3M™ Buffered Peptone Water (BPW) ISO formulation.

B. Apparatus and Reagents

Items (**a**)-(**g**) are available as the 3M™ Molecular Detection Assay (MDA) 2 – *E. coli* O157 (including H7) kit from 3M Food Safety (St. Paul, MN 55144-1000, USA).

(a) *3M Molecular Detection System* (MDS100) – Available from 3M Food Safety (St. Paul, MN 55144-1000, USA).
(b) *3M Molecular Detection Assay 2 – E. coli* O157 (including H7) *Catalog number:* MDA2ECO96, *reagent tubes* - 12 strips of 8 tubes. Available from 3M Food Safety (St. Paul, MN 55144-1000, USA).
(c) *Lysis Solution (LS) tubes* – 12 strips of 8 tubes.
(d) *Extra caps* – 12 strips of 8 caps
(e) *Reagent Control* – 8 reagent tubes
(f) *Quick Start Guide*
(g) *3M™ Molecular Detection Speed Loader Tray* - Available from 3M Food Safety (St. Paul, MN 55144-1000, USA).
(h) *3M™ Molecular Detection Chill Block Insert* - Available from 3M™ Food Safety (St. Paul, MN 55144-1000, USA).
(i) *3M™ Molecular Detection Heat Block Insert* - Available from 3M Food Safety (St. Paul, MN 55144-1000, USA).
(j) *3M™ Molecular Detection Cap/Decap Tool for Reagent tubes* - Available from 3M Food Safety (St. Paul, MN 55144-1000, USA).
(k) *3M™ Molecular Detection Cap/Decap Tool for Lysis tubes* – Available from 3M Food Safety (St. Paul, MN 55144-1000, USA).
(l) *Empty Lysis Tube Rack* - Available from 3M Food Safety (St. Paul, MN 55144-1000, USA).
(m) *Empty Reagent Tube Rack* - Available from 3M Food Safety (St. Paul, MN 55144-1000, USA).
(n) *Buffered Peptone Water (ISO Formulation)* – Available from 3M Food Safety (St. Paul, MN 55144-1000, USA). (Formulation equivalent to ISO 6579:2002 Annex B or 3M equivalent)
(o) *Disposable pipette* – capable of 20 µL
(p) *Multi-channel (8-channel) pipette* - capable of 20 µL
(q) *Sterile filter tip pipette tips* - capable of 20 µL
(r) *Filter Stomacher® bags* – Seward (Islandia, NY 11749, USA) or equivalent.
(s) *Smasher® XL bags* – Available from bioMerieux Industry (Hazelwood, MO USA)
(t) *Stomacher®* – Seward (Islandia, NY 11749, USA) or equivalent.
(u) *Partial immersion thermometer* – calibrated range to include 100 ± 1°C
(v) *Dry block heater unit* – capable of maintaining 100 ± 1°C
(w) *Incubators* – Capable of maintaining 37 ± 1°C or 41.5 ± 1°C.

(x) *Refrigerator* – capable of maintaining 2-8°C, for storing the 3M Molecular Detection Assay components
(y) *Computer* – compatible with the 3M™ Molecular Detection Instrument
(z) *Modified TSB*– Available from Mediabox™ Actero™ (Calgary, Alberta, Canada T2R 1J6)
(aa) *OctoMACS™* (Milteny Biotec, Gaithersburg, MD, 20878, USA)
(bb) *Multistand to support OctoMACS*(Milteny Biotec, Gaithersburg, MD, 20878, USA)
(cc) *MACS® Large Cell Separation Columns*-((Milteny Biotec,Gaithersburg, MD, 20878, USA)
(dd) *Dynabeads® anti-E. coli O157* (Dynal Inc., Lake Success, NY 11042, USA)
(ee) *Tryptic Soy Agar with 5% sheep blood (SBA)*- (TS-BD, Franklin Lakes, New Jersey 07417, USA; Sheep blood-Quad Five, Ryegate, Montana 59074, ,USA)
(ff) *Rainbow® Agar O157, Dehydrated* (Biolog Inc., Hayward California, 94545, USA)
(gg) *Novobiocin (5.0 mg/L)* (Alfa Aesar, Tewksbury, MA 01876, USA)
(hh) *Cefixime (0.05 mg/L)*(Sigma Aldrich, St. Louis, MO 63103, USA)
(ii) *Potassium Tellurite (*0.15 mg/L*)* (Oxoid, Hampshire RG24 8PW, UK)
(jj) *1N HCL* (Acros, New Jersey, USA)
(kk) *E. coli* O157:H7 latex agglutination test kit (RIM® *E. coli* O157:H7 Latex Test Kit, REMEL, 12076 Santa Fe Drive, Lenexa, KS 66215)
(ll) *3M™ Petrifilm™ Rapid Aerobic Count* – Available from 3M Food Safety (St. Paul, MN, 55144-1000, USA)
(mm) *3M™ Rapid Plate Spreader* – Available from 3M Food Safety (St. Paul, MN, 55144-1000, USA)

C. General Instructions

(a) Store the 3M Molecular Detection Assay 2 – *E. coli* O157 (including H7) at 2-8°C. Do not freeze. Keep kit away from light during storage. After opening the kit, check that the foil pouch is undamaged. If the pouch is damaged, do not use. After opening, unused reagent tubes should always be stored in the re-sealable pouch with the desiccant inside to maintain stability of the lyophilized reagents. Store resealed pouches at 2-8°C for no longer than 60 days. Do not use 3M Molecular Detection Assay 2 - *E. coli* O157 (including H7) past the expiration date.

(b) Follow all instructions carefully. Failure to do so may lead to inaccurate results.

Safety Precautions

The 3M MDA 2 – *E. coli* O157 (including H7) 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 the food and beverage industries. For example, 3M has not documented this product for testing drinking water, pharmaceutical, cosmetics, clinical or veterinary samples. The 3M MDA 2 – *E. coli* O157 (including H7) has not been evaluated with all possible food products, food processes, testing protocols or with all possible strains of bacteria.

As with all test methods, the source of enrichment medium can influence the results. The 3M MDA 2 – *E. coli* O157 (including H7) has only been evaluated for use with the enrichment media specified in the Instructions for Use section.

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 MDS instrument.

The user should read, understand and follow all safety information in the instructions for the 3M MDS and the 3M MDA 2 – *E. coli* O157 (including H7). Retain the safety instructions for future reference.

Periodically decontaminate laboratory benches and equipment (pipettes, cap/decap tools, etc.) with a 1- 5% (v:v in water) household bleach solution or DNA removal solution. When testing is complete, follow current industry standards for the disposal of contaminated waste. Consult the Safety Data Sheet for additional information and local regulations for disposal.

To reduce the risks associated with exposure to chemicals and biohazards: 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. Dispose of enriched samples according to current industry standards.

To reduce the risks associated with environmental contamination: Follow current industry standards for disposal of contaminated waste.

D. Sample Enrichment

Foods

(a) Allow BPW ISO enrichment medium to pre-warm to 41.5±1°C. *See* Table **2017.01B** for matrix-specific enrichment protocols.
(b) Aseptically combine the enrichment medium and sample. For all meat and highly particulate samples, the use of filter bags is recommended.
(c) Homogenize by stomacher, hand, or gentle agitation thoroughly for 2 ± 0.2 minutes. Incubate matrices according to the instructions provided in Table **2017.01B**.

E. Preparation of the 3M™ Molecular Detection Speed Loader Tray

(a) Wet a cloth or paper towel with a 1-5% (v:v in water) household bleach solution and wipe the 3M™ Molecular Detection Speed Loader Tray.
(b) Rinse the 3M Molecular Detection Speed Loader Tray with water.
(c) Use a disposable towel to wipe the 3M Molecular Detection Speed Loader Tray dry.
(d) Ensure the 3M Molecular Detection Speed Loader Tray is dry before use

F. Preparation of the 3M™ Molecular Detection Chill Block Insert

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).

G. Preparation of the 3M™ Molecular Detection Heat Block Insert

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 30minutes for the 3M Molecular Detection Heat Block Insert to reach temperature. Using an appropriate, calibrated thermometer (e.g., a partial immersion thermometer, 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.

H. Preparation of the 3M Molecular Detection Instrument

1. 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.
2. Turn on the 3M Molecular Detection Instrument.
3. Create or edit a run with data for each sample. Refer to the 3M Molecular Detection System User Manual for details.

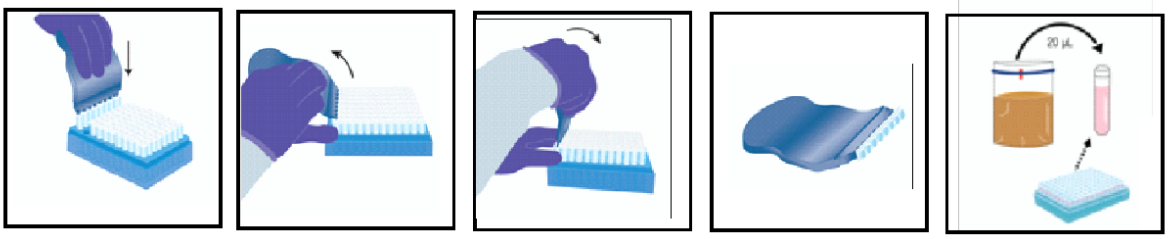
NOTE: The 3M Molecular Detection Instrument must reach and maintain temperature of 60°C before inserting the 3M Molecular Detection Speed Loader Tray with reaction tubes. This heating step takes approximately 20 minutes and is indicated by an ORANGE light on the instrument's status bar. When the instrument is ready to start a run, the status bar will turn GREEN.

I. Lysis

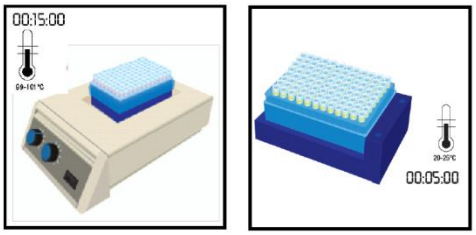
1. Allow the lysis solution (LS) tubes to warm up by setting the rack at room temperature (20-25°C) overnight (16-18 hours). Alternatives to equilibrate the LS tubes to room temperature are to set the LS tubes on the laboratory bench for at least 2 hours, incubate the LS tubes in a 37 ±1°C incubator for 1 hour or place them in a dry double block heater for 30 seconds at 100°C.
2. Invert the capped tubes to mix. Proceed to next step within 4 hours.
3. Remove the enrichment broth from the incubator.
4. One LS tube is required for each sample and the Negative Control (NC) sample (sterile enrichment medium).
   - 4.1 LS tube strips can be cut to desired LS tube number. Select the number of individual LS tubes or 8-tube strips needed. Place the LS tubes in an empty rack.
   - 4.2 To avoid cross-contamination, decap one LS tubes strip at a time and use a new pipette tip for each transfer step.
   - 4.3 Transfer enriched sample to LS tubes as described below:

Transfer each enriched sample into individual LS tube **first**. Transfer the NC **last**.

   - 4.4 Use the 3M™ Molecular Detection Cap/Decap Tool-Lysis to decap one LS tube strip -one strip at a time.
   - 4.5 Discard the LS tube cap – if lysate will be retained for retest, place the caps into a clean container for re-application after lysis
   - 4.6 Transfer 20 µL of sample into a LS tube unless otherwise indicated in the protocol table.
5. Repeat step 4.2 until each individual sample has been added to a corresponding LS tube in the strip



6. Repeat steps 4.1 to 4.6 as needed, for the number of samples to be tested.
7. When all samples have been transferred, transfer 20 µL of NC (sterile enrichment medium e.g. BPW) into a LS tube. Do not use water as a NC
8. Verify that the temperature of the 3M Molecular Detection Heat Block Insert is at 100 ±1°C.
9. Place the uncovered rack of LS tubes in the 3M Molecular Detection Heat Block Insert and heat for 15 ±1 minutes. During heating, the LS 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.
10. Remove the uncovered rack of LS tubes from the heating block and allow to cool in the 3M Molecular Detection Chill Block Insert at least 5 minutes and a maximum of 10 minutes. The 3M Molecular Chill Block Insert, used at ambient temperature 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.
11. Remove the rack of LS tubes from the 3M Molecular Detection Chill Block Insert.



J. AMPLIFICATION

1. One Reagent tube is required for each sample and the NC.
   1.1 Reagent tubes strips can be cut to desired tube number. Select the number of individual Reagent tubes or 8-tube strips needed.
   1.2 Place Reagent tubes in an empty rack.
   1.3 Avoid disturbing the reagent pellets from the bottom of the tubes.
2. Select 1 Reagent Control (RC) tube and place in rack.
3. To avoid cross-contamination, decap one Reagent tubes strip at a time and use a new pipette tip for each transfer step.
4. Transfer lysate to Reagent tubes and RC tube as described below:

Transfer each sample lysate into individual Reagent tubes **first** followed by the NC. Hydrate the RC tube **last**.

   4.1 Use the 3M™ Molecular Detection Cap/Decap Tool-Reagent to decap the Reagent tubes –one Reagent tubes strip at a time. Discard cap.
   4.2 Transfer 20 µL of Sample lysate from the upper ½ of the liquid (avoid precipitate) in the LS tube into corresponding Reagent tube. Dispense at an angle to avoid disturbing the pellets. Mix by gently pipetting up and down 5 times.
   4.3 Repeat step 4.2 until individual Sample lysate has been added to a corresponding Reagent tube in the strip.
   4.4 Cover the Reagent tubes with the provided extra cap 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.
   4.5 Repeat steps 4.1 to 4.4 as needed, for the number of samples to be tested.
   4.6 When all sample lysates have been transferred, repeat 4.1 to 4.4 to transfer 20 µL of NC lysate into a Reagent tube.
   4.7 Transfer **20 µL of <u>NC lysate</u> into a RC tube**. Dispense at an angle to avoid disturbing the pellets. Mix by gently pipetting up and down 5 times.
5. 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.

6. Review and confirm the configured run in the 3M Molecular Detection Software.
7. Click the Start button in the software and select instrument for use. The selected instrument's lid automatically opens.
8. Place the 3M Molecular Detection Speed Loader Tray into the 3M MDS Instrument and close the lid to start the assay. Results are provided within 60 minutes, although positives may be detected sooner.
9. 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 hour and away from the assay preparation area.

NOTICE: To minimize the risk of false positives due to cross-contamination, never open reagent tubes containing amplified DNA. This includes Reagent Control, Reagent and Matrix Control tubes. Always dispose of sealed reagent tubes by soaking in a 1-5% (v:v in water) household bleach solution for 1 hour and away from the assay preparation area.

K. 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 and Inspect results will be displayed after the run is completed.

Presumptive positive samples should be confirmed as per the laboratory standard operating procedures or by following the appropriate reference method confirmation beginning with transfer from the primary BPW ISO enrichment to secondary enrichment broth(s), followed by subsequent plating and confirmation of isolates using appropriate biochemical and serological methods.

NOTE: Even a negative sample will not give a zero reading as the system and 3M Molecular Detection Assay 2 - *E. coli* O157 (including H7) amplification reagents have a "background" relative light unit (RLU) reading.

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 your preferred method or as specified by local regulations.

If you have questions about specific applications or procedures, please visit our website at www.3M.com/foodsafety or contact your local 3M representative or distributor.

Reference: *J. AOAC Int*. (future issue)

Posted: June 2017

Table 2017.01A. Summary of Results for the Detection of *E. coli* O157:H7 in Raw Ground Beef (325g)

Method[a]	**3M™ MDA 2 *E. coli* O157 (including H7)**		
Inoculation Level	**Un-inoculated**	**Low**	**High**
Candidate Presumptive Positive/ Total # of Samples Analyzed	0/120	32/120	97/120
Candidate Presumptive POD (CP)	0.00 (0.00, 0.03)	0.27 (0.19, 0.35)	0.81 (0.67, 0.93)
s_r[b]	0.00 (0.00, 0.17)	0.45 (0.40, 0.52)	0.37 (0.33, 0.42)
s_L[c]	0.00 (0.00, 0.17)	0.00 (0.00, 0.16)	0.15 (0.07, 0.32)
s_R[d]	0.00 (0.00, 0.24)	0.45 (0.40, 0.52)	0.40 (0.35, 0.50)
P Value[e]	1.00	0.760	0.005
Candidate Confirmed Positive/ Total # of Samples Analyzed	3/120[i]	31/120	96/120
Candidate Confirmed POD (CC)[f]	0.03 (0.01, 0.07)	0.26 (0.18, 0.34)	0.80 (0.69, 0.91)
s_r	0.15 (0.14, 0.18)	0.45 (0.40, 0.52)	0.38 (0.34, 0.44)
s_L	0.03 (0.00, 0.09)	0.00 (0.00, 0.13)	0.13 (0.04, 0.29)
s_R	0.16 (0.14, 0.19)	0.45 (0.40, 0.51)	0.40 (0.36, 0.49)
P Value	0.12	0.920	0.018
Candidate Confirmed Positive/ Total # of Samples Analyzed	0/120	29/120	92/120
Candidate Presumptive Positive that Confirmed POD (C)[g]	0.00 (0.00, 0.03)	0.24 (0.16, 0.32)	0.77 (0.63, 0.91)
s_r	0.00 (0.00, 0.17)	0.44 (0.39, 0.51)	0.39 (0.34, 0.45)
s_L	0.00 (0.00, 0.17)	0.00 (0.00, 0.13)	0.18 (0.09, 0.37)
s_R	0.00 (0.00, 0.24)	0.44 (0.39, 0.50)	0.43 (0.38, 0.52)
P Value	1.00	0.908	0.002
Positive Reference Samples/ Total # of Samples Analyzed	3/120[J]	42/120	95/120
Reference POD	0.03 (0.01, 0.07)	0.35 (0.26, 0.44)	0.79 (0.67, 0.92)

s_r	0.14 (0.00, 0.17)	0.48 (0.42, 0.52)	0.38 (0.34, 0.44)
s_L	0.07 (0.03, 0.14)	0.05 (0.00, 0.23)	0.15 (0.07, 0.33)
s_R	0.16 (0.00, 0.20)	0.48 (0.43, 0.52)	0.41 (0.37, 0.51)
P Value	0.001	0.342	0.01
dLPOD (Candidate vs. M Reference)[h]	-0.025 (-0.071, 0.010)	-0.11 (-0.22, 0.01)	-0.025 (-0.129, 0.080)
dLPOD (Candidate Presumptive vs. Candidate Confirmed)[h]	-0.025 (-0.071, 0.010)	0.01 (-0.10, 0.12)	0.008 (-0.092, 0.109)

[a] Results include 95% Confidence Intervals

[b] Repeatability Standard Deviation

[c] Among-Laboratory Standard Deviation

[d] Reproducibility Standard Deviation

[e] P Value = Homogeneity test of laboratory PODs

[f] Confirmed positive (including false negatives)

[g] Presumptive positives that confirmed (excluding false negatives)

[h] A confidence interval for dLPOD that does not contain the value 0 indicates a statistical significant difference between the two methods

[i] 16s rRNA identified one of the reported positives as a non-*E. coli*

[J] 16s rRNA identified all three isolates as non-*E. coli*

Table 2017.01B. 3M MDA 2 – *E. coli* O157 (including H7) enrichment protocols using pre-warmed BPW ISO at 41.5 ± 1°C

Sample Matrix	Sample Size	Enrichment Broth Volume	Enrichment Time (hour)	Homogenized
Raw ground beef (73% lean)	325 g	975 mL	10-18	Manually by hand or by Stomaching
Raw bagged spinach(a)	200 g	450 mL	18-24	Gently agitated by hand for 5 minutes, Do not stomach.
Fresh bean sprouts	25 g	225 mL	18-24	Gently agitated by hand for 5 minutes, Do not stomach.
Frozen blueberries(a)(b)	25 g	225 mL	18-24	Gently agitated by hand for 5 minutes, Do not stomach.

(a) Leafy produce and fruit samples should be gently agitated by hand for 5 minutes. Do not blend or stomach.

(b) Frozen samples should be equilibrated to 4-8°C before addition to enrichment broth.