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Dr. George W Latimer, Jr. (ed.)

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CHAPTER

17.4.09 AOAC Official Method 2017.01 *Escherichia coli* O157:H7 in Selected Foods: Neogen® Molecular Detection Assay (MDA) 2–*E. coli* O157 (Including H7) Method

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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. *See* Table 2017.01B for detailed results of the interlaboratory study.

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Table 2017.01A. Summary of results for detection of *E. coli* O157:H7 in raw ground beef (325 g)

Method[a]	Neogen MDA 2–*E. coli* O157 (including H7)		
Inoculation level	Uninoculated	Low	High
Candidate presumptive positive/total No. of samples analyzed	0/120	32/120	97/120
Candidate presumptive POD (CP)	0.00	0.27	0.81
	(0.00, 0.03)	(0.19, 0.35)	(0.67, 0.93)
s_r [b]	0.00	0.45	0.37
	(0.00, 0.17)	(0.40, 0.52)	(0.33, 0.42)
s_L [c]	0.00	0.00	0.15
	(0.00, 0.17)	(0.00, 0.16)	(0.07, 0.32)
s_R [d]	0.00	0.45	0.40
	(0.00, 0.24)	(0.40, 0.52)	(0.35, 0.50)
P value[e]	1.00	0.760	0.005
Candidate confirmed positive/total No. of samples analyzed	3/120[f]	31/120	96/120
Candidate confirmed POD (CC)[g]	0.03	0.26	0.80
	(0.01, 0.07)	(0.18, 0.34)	(0.69, 0.91)
s_r	0.15	0.45	0.38
	(0.14, 0.18)	(0.40, 0.52)	(0.34, 0.44)
s_L	0.03	0.00	0.13
	(0.00, 0.09)	(0.00, 0.13)	(0.04, 0.29)
s_R	0.16	0.45	0.40
	(0.14, 0.19)	(0.40, 0.51)	(0.36, 0.49)
P value	0.12	0.920	0.018
Candidate confirmed positive/total No. of samples analyzed	0/120	29/120	92/120
Candidate presumptive positive that confirmed POD (C)[h]	0.00	0.24	0.77
	(0.00, 0.03)	(0.16, 0.32)	(0.63, 0.91)
s_r	0.00	0.44	0.39
	(0.00, 0.17)	(0.39, 0.51)	(0.34, 0.45)
s_L	0.00	0.00	0.18
	(0.00, 0.17)	(0.00, 0.13)	(0.09, 0.37)
s_R	0.00	0.44	0.43

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	(0.00, 0.24)	(0.39, 0.50)	(0.38, 0.52)
P value	1.00	0.908	0.002
Positive reference samples/total No. of samples analyzed	3/120[i]	42/120	95/120
Reference POD	0.03	0.35	0.79
	(0.01, 0.07)	(0.26, 0.44)	(0.67, 0.92)
s_r	0.14	0.48	0.38
	(0.00, 0.17)	(0.42, 0.52)	(0.34, 0.44)
s_L	0.07	0.05	0.15
	(0.03, 0.14)	(0.00, 0.23)	(0.07, 0.33)
s_R	0.16	0.48	0.41
	(0.00, 0.20)	(0.43, 0.52)	(0.37, 0.51)
P Value	0.001	0.342	0.01
dLPOD (candidate vs. reference)[j]	−0.025	−0.11	−0.025
dLPOD (candidate presumptive vs. candidate confirmed)[j]	(−0.071, 0.010)	(−0.22, 0.01)	(−0.129, 0.080)
	−0.025	0.01	0.008
	(−0.071, 0.010)	(−0.10, 0.12)	(−0.092, 0.109)

a Results include 95% confidence intervals.

b r = Repeatability standard deviation.

c Among-laboratory standard deviation.

d Reproducibility standard deviation.

e *P* value = Homogeneity test of laboratory PODs.

f 16s rRNA identified one of the reported positives as non-*E. coli.*

g Confirmed positive (including false negatives).

h Presumptive positives that confirmed (excluding false negatives).

i 16s rRNA identified all three isolates as non-*E. coli.*

j Confidence interval for dLPOD that does not contain the value 0 indicates statistical significant difference between the two methods.

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Table 2017.01B. Comparative results for detection of *E. coli* O157:H7 in raw ground beef (73% lean) by Neogen MDA 2–*E. coli* O157 (including H7) method vs USDA/FSIS MLG Chapter 5.09 in collaborative study

			Uninoculated control													
			Candidate presumptive (CP)			Candidate confirmed (CC)[a]			Candidate result (C)[b]			Reference method (R)			C vs R	
Statistic	Matrix/inoculation level	Lab	*N*[c]	*X*[d]	POD (CP)	*N*	*X*	POD (CC)	*N*	*X*	POD (C)	*N*	*X*	POD (R)	dLPOD (C,R)	dLPOD (CP,CC)
	Raw ground beef (73% lean)/uninoculated	1	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		2	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		3[e]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		4	12	0	0.00	NA	0	0.00	NA	0	0.00	12	0	0.00	0.00	0.00
		5	12	0	0.00	12	2	0.167	12	0	0.00	12	0	0.00	0.00	0.00
		6	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		7[e]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		8	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		9	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		10[e]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		11	12	0	0.00	12	1[f]	0.08	12	0	0.00	12	0	0.00	0.00	-0.08

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	12	12	0	0.00	12	0	0.00	12	0	0.00	12	3[f]	0.25	-0.25	0.00
	13[e]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	14	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
	15[e]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Estimate	All	120	0	0.00	120	3	0.03	120	0	0.00	120	3	0.03	−0.025	−0.025
LCL[g]				0.00			0.01			0.00			0.01	−0.071	−0.071
UCL[h]				0.03			0.07			0.03			0.07	0.010	0.010
s_r [i]				0.00			0.15			0.00			0.14		
LCL				0.00			0.14			0.00			0.13		
UCL				0.17			0.18			0.17			0.17		
s_L [j]				0.00			0.03			0.00			0.07		
LCL				0.00			0.00			0.00			0.03		
UCL				0.17			0.09			0.17			0.14		
s_R [k]				0.00			0.16			0.00			0.16		
UCL				0.00			0.14			0.00			0.14		
LCL				0.24			0.19			0.24			0.20		
P_T [l]				1.00			0.122			1.00			0.001		

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		(Low inoculum level)														
			Candidate presumptive (CP)			Candidate confirmed (CC)[a]			Candidate result (C)[b]			Reference method (R)			C vs R	
Statistic	Matrix/inoculation level	Lab	*N*[c]	*X*[d]	POD (CP)	*N*	*X*	POD (CC)	*N*	*X*	POD (C)	*N*	*X*	POD (R)	dLPOD (C,R)	dLPOD (CP,CC)
	Raw ground beef (73% lean)/low	1	12	2	0.167	12	2	0.167	12	2	0.167	12	2	0.167	0.00	0.00
		2	12	5	0.417	12	3	0.250	12	3	0.250	12	5	0.417	−0.167	0.167
		3[a]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		4	12	2	0.167	NA	2	0.167	NA	2	0.167	12	4	0.333	−0.167	0.00
		5	12	2	0.167	NA	2	0.167	NA	2	0.167	NA	4	0.333	−0.167	0.00
		6	12	5	0.417	12	5	0.417	12	5	0.417	12	7	0.583	−0.167	0.00
		7[e]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		8	12	3	0.250	12	3	0.250	12	3	0.250	12	1	0.083	0.167	0.00
		9	12	3	0.250	12	3	0.250	12	3	0.250	12	4	0.333	−0.083	0.00
		10[e]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	MPN/test portion	11	12	4	0.333	12	3	0.250	12	3	0.250	12	4	0.333	−0.083	0.083
		12	12	4	0.333	12	4	0.333	12	4	0.333	12	5	0.417	−0.083	0.00

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		13[e]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		14	12	2	0.167	12	4	0.333	12	2	0.167	12	6	0.500	−0.333	−0.167
		15[e]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Estimate		All	120	32	0.27	120	31	0.26	120	29	0.24	120	42	0.35	−0.11	0.01
LCL	0.29				0.19			0.18			0.16			0.26	−0.22	−0.10
UCL	−0.09				0.35			0.34			0.32			0.44	0.01	0.12
s_r	0.55				0.45			0.45			0.44			0.48		
LCL					0.40			0.40			0.39			0.42		
UCL					0.52			0.52			0.51			0.52		
s_L					0.00			0.00			0.00			0.05		
LCL					0.00			0.00			0.00			0.00		
UCL					0.16			0.13			0.13			0.23		
s_R					0.45			0.45			0.44			0.48		
UCL					0.40			0.40			0.39			0.43		
LCL					0.52			0.51			0.50			0.52		
P_T					0.760			0.920			0.908			0.342		
			High inoculum level													

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			Candidate presumptive (CP)			Candidate confirmed (CC)[a]			Candidate result (C)[b]			Reference method (R)			C vs R	
Statistic	Matrix/inoculation level	Lab	N [c]	X [d]	POD (CP)	N	X	POD (CC)	N	X	POD (C)	N	X	POD (R)	dLPOD (C,R)	dLPOD (CP,CC)
	Raw ground beef (73% lean)/high	1	12	6	0.500	12	6	0.500	12	6	0.500	12	8	0.667	−0.167	0.00
		2	12	12	0.917	12	11	0.917	11	11	0.917	12	11	0.917	0.00	0.083
		3 [e]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		4	12	12	1.000	12	12	1.000	12	12	1.000	12	9	0.750	0.250	0.00
		5	12	8	0.667	12	8	0.667	12	6	0.500	12	10	0.833	−0.333	0.00
		6	12	12	1.000	12	12	1.000	12	12	1.000	12	11	0.917	0.083	0.00
		7 [e]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		8	12	11	0.917	12	11	0.917	12	11	0.917	12	12	1.000	−0.083	0.00
		9	12	11	0.917	12	11	0.917	12	11	0.917	12	11	0.917	0.00	0.00
		10[e]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	MPN/test portion	11	12	7	0.583	12	8	0.667	12	6	0.500	12	10	0.833	−0.333	−0.083
		12	12	9	0.750	12	9	0.750	12	9	0.750	12	4	0.333	0.417	0.00
		13[e]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

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		14	12	9	0.750	12	8	0.667	12	8	0.667	12	9	0.750	−0.083	0.083
		15[e]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Estimate		All	120	97	0.81	120	96	0.80	120	92	0.77	120	95	0.79	−0.025	0.008
LCL	1.92				0.67			0.69			0.63			0.67	−0.129	−0.092
UCL	−1.00				0.93			0.91			0.91			0.92	0.080	0.109
s_r	3.42				0.37			0.38			0.39			0.38		
LCL					0.33			0.34			0.34			0.34		
UCL					0.42			0.44			0.45			0.44		
s_L					0.15			0.13			0.18			0.15		
LCL					0.07			0.04			0.09			0.07		
UCL					0.32			0.29			0.37			0.33		
s_R					0.40			0.40			0.43			0.41		
UCL					0.35			0.36			0.38			0.37		
LCL					0.50			0.49			0.52			0.51		
P_T					0.005			0.018			0.002			0.01		

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a CC = Number of confirmed positives (including false negatives).

b C = Presumptive positives that confirmed positive (excluding false negatives).

c *N* = Samples analyzed.

d *X* = Number of samples that obtained a positive.

e NA = Laboratory did not submit data for this matrix because of laboratory error.

f 16S rRNA results identified isolates as non-*E. coli*.

g LCL = Lower confidence limit.

h UCL = Upper confidence limit.

i Repeatability standard deviation.

j Among-laboratory standard deviation.

k Reproducibility standard deviation.

l *P* value = Homogeneity test of laboratory PODs.

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A Principle

The Molecular Detection Assay (MDA) 2–*E. coli* O157 (including H7) method is used with the Neogen Molecular Detection System (MDS) for the rapid and specific detection of *E. coli* O157 (including H7) in enriched food samples. The MDA 2–*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 min. Samples are enriched in Neogen Buffered Peptone Water (BPW; ISO formulation).

B Apparatus and Reagents

Items (**a**)–(**m**) are available from Neogen Corp. (Lansing, MI, USA; www.neogen.com).

Items **(b)–(e)** are available as the MDA 2–*E. coli* O157 (including H7) kit from Neogen Corp. (Cat. No. MDA2ECO96; Product SKU 700002229).

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(a) *MDS.*—Model MDS100; Product SKU 700002195.

(b) *MDA 2–E. coli O157 (including H7) reagent tubes.*—Twelve strips of eight tubes.

(c) *Lysis solution (LS) tubes.*—Twelve strips of eight tubes.

(d) *Extra caps.*—Twelve strips of eight caps.

(e) *Reagent control.*—Eight reagent tubes.

(f) *Molecular Detection Speed loader tray* .

(g) *Molecular Detection Chill Block insert* .

(h) *Molecular Detection Heat Block insert* .

(i) *Molecular Detection cap/ decap tool for reagent tubes* .

(j) *Molecular Detection cap/ decap tool for lysis tubes* .

(k) *Empty lysis tube rack* .

(l) *Empty reagent tube rack* .

(m) *BPW (ISO formulation).*—Formulation equivalent to ISO 6579:2002 Annex B or Neogen equivalent.

(n) *Disposable pipet.*—Capable of 20 µL.

(o) *Multichannel (8-channel) pipet.*—Capable of 20 µL.

(p) *Pipet tips.*—Sterile filter tip, capable of 20 µL.

(q) *Filter Stomacher® bags.*—Seward (Islandia, NY, USA) or equivalent.

(r) *Smasher® XL bags.*—Available from bioMérieux Industry (Hazelwood, MO, USA).

(s) *Stomacher.*—Seward or equivalent.

(t) *Partial immersion thermometer.*—Calibrated range to include 100 ± 1°C.

(u) *Dry block heater unit.*—Capable of maintaining 100 ± 1°C.

(v) *Incubators.*—Capable of maintaining 37 ± 1°C or 41.5 ± 1°C.

(w) *Refrigerator.*—Capable of maintaining 2–8°C, for storing MDA components.

(x) *Computer.*—Compatible with Molecular Detection Instrument.

C General Instructions

(a) Store MDA 2–*E. coli* O157 (including H7) at 2–8°C. Do not freeze. Keep kit away from light during storage. After opening kit, check that foil pouch is undamaged. If pouch is damaged, do not use. After opening, unused reagent tubes should always be stored in the resealable 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 MDA 2–*E. coli* O157 (including H7) past the expiration date.

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

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p. C17-98 The MDA 2–*E. coli* O157 (including H7) is intended for use in a laboratory environment by professionals trained in laboratory techniques. Neogen has not documented the use of this product in industries other than the food and beverage industries. For example, Neogen has not documented this product for testing drinking water, pharmaceutical, cosmetics, clinical, or veterinary samples. The 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 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 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 MDS instrument.

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

Periodically decontaminate laboratory benches and equipment (pipets, cap/decap tools, etc.) with 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 Safety Data Sheet for additional information and local regulations for disposal.

To reduce risks associated with exposure to chemicals and biohazards, perform pathogen testing in 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 contents of enrichment media and reagent tubes after amplification. Dispose of enriched samples according to current industry standards.

To reduce 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 prewarm to 41.5 ± 1°C. *See* Table 2017.01C for matrix-specific enrichment protocols.

(b) Aseptically combine 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 min. Incubate matrices according to instructions provided in Table 2017.01C.

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Table 2017.01C. Neogen MDA 2–*E. coli* O157 (including H7) enrichment protocols using prewarmed BPW ISO at 41.5 ± 1°C according to 2017.01

Sample matrix	Sample size, g	Enrichment broth volume, mL	Enrichment time, h	Homogenized
Raw ground beef (73% lean)	325	975	10–18	Manually by hand or by stomaching
Raw bagged spinach[a]	200	450	18–24	Gently agitated by hand for 5 min, do not stomach
Fresh bean sprouts	25	225	18–24	Gently agitated by hand for 5 min, do not stomach
Frozen blueberries[a, b]	25	225	18–24	Gently agitated by hand for 5 min, do not stomach

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

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

E Preparation of Molecular Detection Speed Loader Tray

(a) Wet a cloth or paper towel with 1–5% (v/v in water) household bleach solution, and wipe Molecular Detection Speed Loader Tray.

(b) Rinse Molecular Detection Speed Loader Tray with water.

(c) Use disposable towel to wipe Molecular Detection Speed Loader Tray dry.

(d) Ensure Molecular Detection Speed Loader Tray is dry before use.

F Preparation of the Molecular Detection Chill Block Insert

Place Molecular Detection Chill Block Insert directly on laboratory bench: The Molecular Detection Chill Block Tray is not used. Use the block at ambient laboratory temperature (20–25°C).

G Preparation of Molecular Detection Heat Block Insert

Place Molecular Detection Heat Block Insert in dry double block heater unit. Turn on dry block heater unit and set temperature to allow Molecular Detection Heat Block Insert to reach and maintain temperature of 100 ± 1°C.

Note: Depending on heater unit, allow approximately 30 min for Molecular Detection Heat Block Insert to reach temperature. Using appropriate, calibrated thermometer (e.g., partial immersion thermometer, digital thermocouple thermometer, not total immersion thermometer) placed in designated location, verify that Molecular Detection Heat Block Insert is at 100 ± 1°C.

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H Preparation of Molecular Detection Instrument

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

(b) Turn on Molecular Detection Instrument.

(c) Create or edit a run with data for each sample. Refer to MDS User Manual for details.

Note: The Molecular Detection Instrument must reach and maintain temperature of 60°C before inserting 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 instrument is ready to start a run, the status bar will turn GREEN.

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I Lysis

(a) Allow LS tubes to warm up by setting rack at room temperature (20–25°C) overnight (16–18 h). Alternatives to equilibrate LS tubes to room temperature are to set LS tubes on laboratory bench for at least 2 h, incubate LS tubes in 37 ± 1°C incubator for 1 h, or place them in dry double block heater for 30 s at 100°C.

(b) Invert capped tubes to mix. Proceed to next step within 4 h.

(c) Remove enrichment broth from incubator.

(d) One LS tube is required for each sample and negative control (NC) sample (sterile enrichment medium).

(1) LS tube strips can be cut to desired LS tube number. Select number of individual LS tubes or 8-tube strips needed. Place LS tubes in an empty rack.

(2) To avoid cross-contamination, decap one LS tubes strip at a time and use a new pipet tip for each transfer step.

(3) Transfer enriched sample to LS tubes as follows: Transfer each enriched sample into individual LS tube first. Transfer the NC last.

(4) Use Molecular Detection Cap/Decap Tool-Lysis to decap one LS tube strip, one strip at a time.

(5) Discard LS tube cap. If lysate will be retained for retest, place caps into clean container for reapplication after lysis.

(6) Transfer 20 µL sample into LS tube unless otherwise indicated in the protocol table.

(e) Repeat step **I(d)***(2)* until each individual sample has been added to a corresponding LS tube in the strip (Figure 2017.01A).

(f) Repeat steps **I(d)***(1)*–*(6)* as needed, for number of samples to be tested.

(g) When all samples have been transferred, transfer 20 µL NC (sterile enrichment medium, e.g., BPW) into LS tube. Do not use water as NC.

(h) Verify that temperature of Molecular Detection Heat Block Insert is at 100 ± 1°C.

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

(j) Remove uncovered rack of LS tubes from heating block and allow to cool in Molecular Detection Chill Block. Insert at least 5 min and maximum of 10 min. The Molecular Chill Block Insert, used at ambient temperature without Molecular Detection Chill Block Tray, should sit directly on the laboratory bench. When cool, lysis solution will revert to a pink color.

(k) Remove rack of LS tubes from Molecular Detection Chill Block Insert (Figure 2017.01B).

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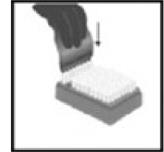
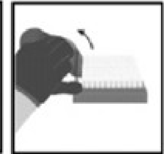

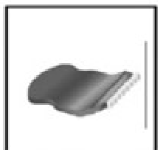
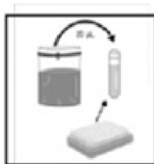

Figure 2017.01A

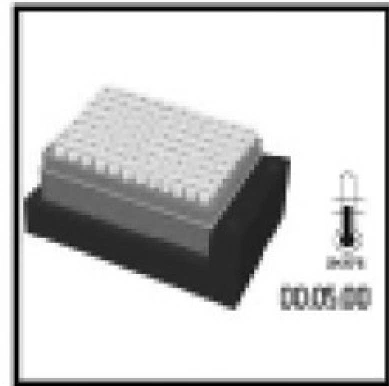

Figure 2017.01B

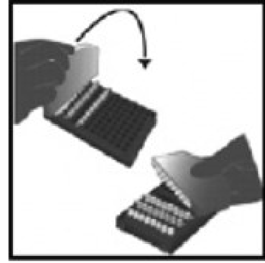
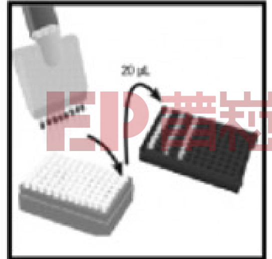

Figure 2017.01C

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J Amplification

(a) One reagent tube is required for each sample and the NC.

(1) Reagent tubes strips can be cut to desired tube number. Select number of individual reagent tubes or 8-tube strips needed.

(2) Place reagent tubes in empty rack.

(3) Avoid disturbing reagent pellets from bottom of tubes.

(b) Select one reagent control (RC) tube and place in rack.

(c) To avoid cross-contamination, decap one reagent tube strip at a time and use new pipet tip for each transfer step.

(d) Transfer lysate to reagent tubes and RC tube as follows: Transfer each sample lysate into individual reagent tubes first followed by the NC. Hydrate RC tube last.

(1) Use Molecular Detection Cap/Decap Tool-Reagent to decap reagent tubes, one reagent tubes strip at a time. Discard cap.

(2) Transfer 20 µL of sample lysate from upper half of liquid (avoid precipitate) in LS tube into corresponding reagent tube. Dispense at angle to avoid disturbing pellets. Mix by gently pipetting up and down five times.

(3) Repeat step **J(d)** *(2)* until individual sample lysate has been added to corresponding reagent tube in strip.

(4) Cover reagent tubes with the provided extra cap and use rounded side of Molecular Detection Cap/Decap Tool-Reagent to apply pressure in back and forth motion ensuring that cap is tightly applied.

(5) Repeat steps **J(d)** *(1)–(4)* as needed, for number of samples to be tested.

(6) When all sample lysates have been transferred, repeat **J(d)** *(1)–(4)* to transfer 20 µL NC lysate into reagent tube.

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

(e) Load capped tubes into clean and decontaminated Molecular Detection Speed Loader Tray. Close and latch Molecular Detection Speed Loader Tray lid (Figure 2017.01C).

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

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

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

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

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Note: To minimize risk of false positives due to cross-contamination, never open reagent tubes containing amplified DNA. This includes RC, reagent, and matrix control tubes. Always dispose of sealed reagent tubes by soaking in 1–5% (v/v in water) household bleach solution for 1 h and away from assay preparation area.

K Results and Interpretation

An algorithm interprets light output curve resulting from detection of nucleic acid amplification. Results are analyzed automatically by software and are color-coded based on result. 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 laboratory standard operating procedures or by following the appropriate reference method confirmation beginning with transfer from 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 negative sample will not give zero reading as system and MDA 2–*E. coli* O157 (including H7) amplification reagents have "background" relative light unit (RLU) reading.

In the rare event of any unusual light output, the algorithm labels this as "Inspect." Neogen 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.

For questions about specific applications or procedures, visit www.neogen.com or contact your local Neogen representative or distributor.

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