



# CERTIFICATION

## AOAC Research Institute *Performance Tested Methods*<sup>SM</sup>

Certificate No.

**081501**

The AOAC Research Institute hereby certifies the method known as:

### **Molecular Detection Assay 2 - *Listeria monocytogenes* (MDA2 – LMO)**

manufactured by

**Neogen Corporation**

**620 Leshar Place**

**Lansing, Michigan 48912**

**USA**

This method has been evaluated and certified according to the policies and procedures of the AOAC *Performance Tested Methods*<sup>SM</sup> Program. This certificate indicates an AOAC Research Institute Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC Research Institute *Performance Tested Methods*<sup>SM</sup> certification mark on the above-mentioned method for the period below. Renewal may be granted by the Expiration Date under the rules stated in the licensing agreement.

A handwritten signature in black ink, appearing to read "Bradley A. Stawick".

Bradley A. Stawick, Senior Director  
Signature for AOAC Research Institute

Issue Date

December 05, 2024

Expiration Date

December 31, 2026

<b>AUTHORS</b> ORIGINAL VALIDATION: Lisa Monteroso and DeAnn Benesh MODIFICATION FEBRUARY 2024: April Schumacher and Micki Rosauer	<b>SUBMITTING COMPANY</b> 3M Company 3M Center, Bldg. 275-5W-05 St. Paul, MN 55144	<b>CURRENT SPONSOR</b> Neogen Food Safety Corporation 620 Leshler Place Lansing, MI 48912
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<b>METHOD NAME</b> Neogen® Molecular Detection Assay 2 - <i>Listeria monocytogenes</i> (MDA2 – LMO) Formerly 3M™ Molecular Detection Assay 2 - <i>Listeria monocytogenes</i> (MDA2 – LMO)	<b>CATALOG NUMBER</b> MDA2LM96
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<b>INDEPENDENT LABORATORY</b> Q Laboratories, Inc. 1400 Harrison Ave Cincinnati, OH 45214 USA	<b>APPLICABILITY OF METHOD</b> Target organism – <i>Listeria monocytogenes</i> .  Matrixes – USDA MLG 8.09: beef hot dogs (25 g), deli turkey (125 g), raw chicken (25 g), sealed concrete (sponge enriched in 100 mL, 4” x 4”), plastic (Enviroswab enriched in 10 mL, 1” x 1”), stainless steel (sponge enriched in 225 mL, 4” x 4”), FDA BAM Ch 10: whole melon (25 g), cold smoked salmon (25 g), romaine lettuce (25 g) AOAC 993.12: queso fresco (25 g), vanilla ice cream (25 g), 4% milk fat cottage cheese (25 g), 3% chocolate whole milk (25 mL) ISO 11290-1/A1: cold smoked salmon (25 g), bagged raw spinach (25 g)  Performance claim – Performance equivalent to that of the U.S. Food and Drug Administration <i>Bacteriological Analytical Manual</i> (FDA/BAM) Chapter 10 (2) for whole melon, cold smoked salmon, romaine lettuce; the U.S. Department of Agriculture Food Safety and Inspection Service <i>Microbiology Laboratory Guidebook</i> (USDA-FSIS/MLG) 8.09 (3) for beef hot dogs, deli turkey, raw chicken leg pieces, sealed concrete, plastic and stainless steel and AOAC 993.12 (4) for queso fresco, vanilla ice cream, 4% milk fat cottage cheese, 3% chocolate whole milk and the ISO 11290-1/A1 (5) for cold smoked salmon and bagged raw spinach.
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<b>ORIGINAL CERTIFICATION DATE</b> August 18, 2015	<b>CERTIFICATION RENEWAL RECORD</b> Renewed through December 2026.
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<b>METHOD MODIFICATION RECORD</b>	<b>SUMMARY OF MODIFICATION</b>
1. December 2018 Level 1	1. Editorial changes to insert to include safety information and testing information.
2. February 2020 Level 1	2. Editorial changes.
3. January 2024 Level 1	3. Editorial changes to rebrand method from 3M to Neogen Corporation.
4. February 2024 Level 2	4. Manufacturing location change from Columbia, Missouri to Lansing, Michigan.

<b>Under this AOAC Performance Tested Methods<sup>SM</sup> License Number, 081501 this method is distributed by:</b> NONE	<b>Under this AOAC Performance Tested Methods<sup>SM</sup> License Number, 081501 this method is distributed as:</b> NONE
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#### PRINCIPLE OF THE METHOD (1)

Neogen® Molecular Detection Assay 2 – *Listeria monocytogenes* is used with the Neogen Molecular Detection System for the rapid and specific detection of *Listeria monocytogenes* in enriched food and environmental samples.

The 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 the laboratory’s preferred method or as specified by local regulations.

#### DISCUSSION OF THE VALIDATION STUDY (1)

There were no differences between the 24- and 26-hour enrichment time points for the environmental surfaces tested, therefore a 24-hour minimum enrichment will be recommended for these matrixes.

The MDA2 - *Listeria monocytogenes* AOAC PTM validation study included an inclusivity/exclusivity, lot-to-lot/stability, robustness and matrix studies. The MDA2 – *Listeria monocytogenes* was compared to the USDA/FSIS-MLG 8.09, the FDA-BAM Chapter 10, the AOAC 993.12 or the ISO 11290-1/A1 reference methods for the detection of *Listeria monocytogenes*. There were no significant differences between the Neogen MDA2 - *Listeria monocytogenes* method and the corresponding reference method for any of the matrixes evaluated. The MDA2 - *Listeria monocytogenes* demonstrated reliability as a rapid and sensitive method for the detection of *Listeria monocytogenes* for the following matrixes: beef hot dogs (25 g), deli turkey (125 g), queso fresco (25 g), vanilla ice cream (25 g), 4% milk fat cottage cheese (25 g), 3% chocolate whole milk (25 mL), whole melon, romaine lettuce (25 g), bagged raw spinach (25 g), cold smoked salmon (25 g), raw chicken leg pieces (25 g), sealed concrete (sponge enriched in 100 mL), plastic (Enviroswab enriched in 10 mL), stainless steel (sponge enriched in 225 mL).

Table 2. Inclusivity Study Results (1)

INCLUSIVITY								
Strain	Species	Reference <sup>a</sup>	Origin	Inoculation level (cfu/225ml)	MDA 2			
					Demi Fraser broth 24h at 37°C			
					MDA2 - <i>Listeria monocytogenes</i>	Confirmation 100µl		
					ALOA <sup>b</sup>	PALCAM		
1	<i>Listeria</i>	<i>monocytogenes</i>	153	Soft cheese (Munster)	39	+	H+	+
2	<i>Listeria</i>	<i>monocytogenes</i>	1011/1410	Frozen broccoli	28	+	H+	+
3	<i>Listeria</i>	<i>monocytogenes</i>	1972/2399	Puff pastry with mushrooms	38	+	H+	+
4	<i>Listeria</i>	<i>monocytogenes</i>	1973/2400	Puff pastry egg and ham (Quiche-lorraine)	36	+	H+	+
5	<i>Listeria</i>	<i>monocytogenes</i>	2407/3139	Tripes with tomatoes	31	+	H+	+
6	<i>Listeria</i>	<i>monocytogenes</i>	2760/3145	Raw bacon	44	+	H+	+
7	<i>Listeria</i>	<i>monocytogenes</i>	32.183	Croque-Monsieur	31	+	H+	+
8	<i>Listeria</i>	<i>monocytogenes</i>	38/181	Toulouse sausages	33	+	H+	+
9	<i>Listeria</i>	<i>monocytogenes</i>	5721/6179	Smoked bacon	49	+	H+	+
10	<i>Listeria</i>	<i>monocytogenes</i>	7111/7516	Pâté (Rillettes)	64	+	H+	+
11	<i>Listeria</i>	<i>monocytogenes</i>	850/109	Ready-To-Eat (RTE) food (deli salad with seafood)	34	+	H+	+
12	<i>Listeria</i>	<i>monocytogenes</i>	877/113	Environmental sample (pastry)	28	+	H+	+
13	<i>Listeria</i>	<i>monocytogenes</i>	913/1048	Black pudding	39	+	H+	+
14	<i>Listeria</i>	<i>monocytogenes</i>	A00C014	Sausage	37	+	H+	+
15	<i>Listeria</i>	<i>monocytogenes</i>	A00C022	Merguez	33	+	H+	+
16	<i>Listeria</i>	<i>monocytogenes</i>	A00C024	Sausage	21	+	H+	+
17	<i>Listeria</i>	<i>monocytogenes</i>	A00C036	Poultry (guinea)	41	+	H+	+
18	<i>Listeria</i>	<i>monocytogenes</i>	A00C039	Sausages	20	+	H+	+
19	<i>Listeria</i>	<i>monocytogenes</i>	A00C040	Cooked delicatessen (Museau)	36	+	H+	+
20	<i>Listeria</i>	<i>monocytogenes</i>	A00C041	Sausage	38	+	H+	+
21	<i>Listeria</i>	<i>monocytogenes</i>	A00C042	Raw sausage	32	+	H+	+
22	<i>Listeria</i>	<i>monocytogenes</i>	A00C043	Smoked Bacon	50	+	H+	+
23	<i>Listeria</i>	<i>monocytogenes</i>	A00C044	Poultry (duck)	34	+	H+	+
24	<i>Listeria</i>	<i>monocytogenes</i>	A00C052	RTE food (Osso bucco with turkey)	34	+	H+	+
25	<i>Listeria</i>	<i>monocytogenes</i>	A00C053	Gizzards	30	+	H+	+
26	<i>Listeria</i>	<i>monocytogenes</i>	A00C054	Beef heart	30	+	H+	+
27	<i>Listeria</i>	<i>monocytogenes</i>	A00C055	Raw sausages	43	+	H+	+
28	<i>Listeria</i>	<i>monocytogenes</i>	A00E008	Environmental sample	27	+	H+	+
29	<i>Listeria</i>	<i>monocytogenes</i>	A00E049	Environmental sample (smoked salmon)	41	+	H+	+
30	<i>Listeria</i>	<i>monocytogenes</i>	A00E082	Environmental sample (smoked salmon)	33	+	H+	+
31	<i>Listeria</i>	<i>monocytogenes</i>	A00L097	Milk	60	+	H+	+
32	<i>Listeria</i>	<i>monocytogenes</i>	A00M009	Smoked salmon	40	+	H+	+
33	<i>Listeria</i>	<i>monocytogenes</i>	A00M032	Smoked salmon	11	+	H+	+
34	<i>Listeria</i>	<i>monocytogenes</i>	A00M045	Smoked salmon	62	+	H+	+
35	<i>Listeria</i>	<i>monocytogenes</i>	A00M088	Smoked salmon	79	+	H+	+
36	<i>Listeria</i>	<i>monocytogenes</i>	Ad235	Poultry	45	+	H+	+
37	<i>Listeria</i>	<i>monocytogenes</i>	Ad253	Hard cheese	58	+	H+	+
38	<i>Listeria</i>	<i>monocytogenes</i>	Ad260	Semi hard cheese	27	+	H+	+
39	<i>Listeria</i>	<i>monocytogenes</i>	Ad265	Tongue	62	+	H+	+
40	<i>Listeria</i>	<i>monocytogenes</i>	Ad266	Poultry	26	+	H+	+
41	<i>Listeria</i>	<i>monocytogenes</i>	Ad267	Dry sausage	26	+	H+	+
42	<i>Listeria</i>	<i>monocytogenes</i>	Ad268	Cured ham	35	+	H+	+
43	<i>Listeria</i>	<i>monocytogenes</i>	Ad270	Fermented sausage	42	+	H+	+
44	<i>Listeria</i>	<i>monocytogenes</i>	Ad272	Fermented sausage	56	+	H+	+
45	<i>Listeria</i>	<i>monocytogenes</i>	Ad273	Cured delicatessen	27	+	H+	+
46	<i>Listeria</i>	<i>monocytogenes</i>	Ad274	Ready-to-eat food (Asiatic meal)	40	+	H+	+
47	<i>Listeria</i>	<i>monocytogenes</i>	Ad534	Fruits	54	+	H+	+
48	<i>Listeria</i>	<i>monocytogenes</i>	Ad544	Onion	43	+	H+	+
49	<i>Listeria</i>	<i>monocytogenes</i>	Ad546	Flour	83	+	H+	+
50	<i>Listeria</i>	<i>monocytogenes</i>	Ad623	Bread crumbs	50	+	H+	+

<sup>a</sup>All the strains are wild strains isolated in Adria Developpement, Quimper, France., Ad = Adria, A= Adria, C= Origin from Meat and Meat products, M = Origin from Fish and Seafood, L = Origin from Milk and Dairy products, E = Origin from Production environment

<sup>b</sup>H+ = Phospho-Lipolysis halo

**Table 3. Exclusivity Study Results (1)**

EXCLUSIVITY						
Strain	Species	Reference <sup>a</sup>	Origin	Inoculation level (cfu/ml)	MDA2 – <i>Listeria monocytogenes</i>	
1	<i>Listeria</i>	<i>grayi</i>	Ad 1198	Smoked salmon	3.2 x 10 <sup>5</sup>	-
2	<i>Listeria</i>	<i>grayi</i>	Ad 1443	Pork meat sausages	4.9 x 10 <sup>5</sup>	-
3	<i>Listeria</i>	<i>innocua</i>	1	Smoked salmon	4.9 x 10 <sup>5</sup>	-
4	<i>Listeria</i>	<i>innocua</i>	Ad 658	Gorgonzola	5.9 x 10 <sup>5</sup>	-
5	<i>Listeria</i>	<i>ivanovii</i>	Ad 466	Raw veal meat	3.3 x 10 <sup>5</sup>	-
6	<i>Listeria</i>	<i>ivanovii</i>	Ad 662	Environment (dairy industry)	3.6 x 10 <sup>5</sup>	-
7	<i>Listeria</i>	<i>seeligeri</i>	Ad 649	Cheese	5.4 x 10 <sup>5</sup>	-
8	<i>Listeria</i>	<i>seeligeri</i>	BR1	Trout	5.1 x 10 <sup>5</sup>	-
9	<i>Listeria</i>	<i>welshimeri</i>	Ad1276	Environment (Slaughterhouse)	5.2 x 10 <sup>5</sup>	-
10	<i>Listeria</i>	<i>welshimeri</i>	Ad1175	Ready-to-eat-food	6.4 x 10 <sup>5</sup>	-
11	<i>Bacillus</i>	<i>cereus</i>	Ad 465	Salmon Terrine	7.2 x 10 <sup>4</sup>	-
12	<i>Bacillus</i>	<i>circulans</i>	Ad 760	Vegetables	2.0 x 10 <sup>4</sup>	-
13	<i>Bacillus</i>	<i>coagulans</i>	Ad 731	Dairy product	<2.0 x 10 <sup>3</sup> (opacity +)	-
14	<i>Bacillus</i>	<i>licheniformis</i>	Ad 978	Dairy product	1.6 x 10 <sup>4</sup>	-
15	<i>Bacillus</i>	<i>pumilus</i>	Ad 284	Ready-to-eat	1.7 x 10 <sup>5</sup>	-
16	<i>Brochotrix</i>	<i>campestris</i>	CIP 102920T	Environment	<2.0 x 10 <sup>3</sup> (opacity +)	-
17	<i>Carnobacterium</i>	<i>piscicola</i>	Ad 369	Raw milk	<2.0 x 10 <sup>3</sup> (opacity +)	-
18	<i>Enterococcus</i>	<i>durans</i>	Ad 149	Ham	<2.0 x 10 <sup>3</sup> (opacity +)	-
19	<i>Enterococcus</i>	<i>faecalis</i>	89L326	Soft cheese (Vacherin)	1.6 x 10 <sup>5</sup>	-
20	<i>Lactobacillus</i>	<i>brevis</i>	86L126	Ham	1.4 x 10 <sup>5</sup>	-
21	<i>Lactobacillus</i>	<i>curvatus</i>	Ad 380	Delicatessen	<2.0 x 10 <sup>3</sup> (opacity +)	-
22	<i>Lactobacillus</i>	<i>sakei</i>	Ad 473	Ham	<2.0 x 10 <sup>3</sup> (opacity +)	-
23	<i>Leuconostoc</i>	<i>carnosum</i>	Ad 411	Ham	<2.0 x 10 <sup>3</sup> (opacity +)	-
24	<i>Leuconostoc</i>	<i>citreum</i>	Ad 396	Ham	4.9 x 10 <sup>5</sup>	-
25	<i>Micrococcus</i>	<i>luteus</i>	Ad 432	Cocktail	<2.0 x 10 <sup>3</sup> (opacity +)	-
26	<i>Staphylococcus</i>	<i>aureus</i>	Ad 165	Smoked delicatessen	<2.0 x 10 <sup>3</sup> (opacity +)	-
27	<i>Staphylococcus</i>	<i>epidermidis</i>	Ad 931	Fruits	2.0 x 10 <sup>3</sup>	-
28	<i>Staphylococcus</i>	<i>haemolyticus</i>	Ad 989	Dairy product	2.8 x 10 <sup>4</sup>	-
29	<i>Streptococcus</i>	<i>bovis</i>	92L622	Cheese	2.0 x 10 <sup>3</sup>	-
30	<i>Streptococcus</i>	<i>salivarius</i>	Ad 441	Dairy product	<2.0 x 10 <sup>3</sup> (opacity +)	-

<sup>a</sup>All the strains are wild strains isolated in Adria Developpement, Quimper, France, Ad = Adria, A= Adria, C= Origin from Meat and Meat products, M = Origin from Fish and Seafood, L = Origin from Milk and Dairy products, E = Origin from Production environment

**Table 8: MDA 2 *Listeria monocytogenes* Assay, Candidate vs. Reference – POD Results (INTERNAL STUDY) (1)**

Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	MDA2			USDA/FSIS MLG			dPOD <sup>c</sup>	95% CI <sup>e</sup>
					x <sup>c</sup>	POD <sup>c</sup>	95% CI	X	POD <sup>R</sup> <sup>e</sup>	95% CI		
Raw Chicken Leg Pieces (25 g)	<i>L. monocytogenes</i> Ad 668	28 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.91 (0.6, 1.39)	20	10	0.50	0.30, 0.70	12	0.60	0.39, 0.78	-0.10	-0.37, 0.19
			2.2	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Bagged Raw Spinach (25 g)	<i>L. monocytogenes</i> Ad 543	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			1.17 (0.74, 1.84)	20	9	0.45	0.26, 0.66	10	0.50	0.30, 0.70	-0.05	-0.34, 0.25
			2.9	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Cold Smoked Salmon (25 g)	<i>L. monocytogenes</i> Ad 670	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.4 (0.18, 0.72)	20	10	0.50	0.30, 0.70	7	0.35	0.15, 0.60	0.15	-0.20, 0.47
			2.7	5	3	0.60	0.23, 0.88	5	1.00	0.57, 1.00	-0.40	-0.77, 0.12
Cold Smoked Salmon (25 g)	<i>L. monocytogenes</i> Ad 670	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.4 (0.18, 0.72)	20	10	0.50	0.30, 0.70	9	0.45	0.26, 0.66	0.05	-0.27, 0.36
			2.7	5	3	0.60	0.23, 0.88	4	0.80	0.38, 0.96	-0.20	-0.60, 0.31

<sup>a</sup>MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval

<sup>b</sup>N = Number of test portions

<sup>c</sup>x = Number of positive test portions

<sup>a</sup>POD<sub>c</sub> = Candidate method confirmed positive outcomes divided by the total number of trials  
<sup>e</sup>POD<sub>r</sub> = Reference method confirmed positive outcomes divided by the total number of trials  
<sup>f</sup>dPOD<sub>c</sub> = Difference between the confirmed candidate method result and reference 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

**Table 9: MDA 2 *Listeria monocytogenes* Assay, Candidate vs. Reference – POD Results (INDEPENDENT STUDY) (1)**

Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	MDA2			Reference			dPOD <sub>c</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>c</sub> <sup>d</sup>	95% CI	X	POD <sub>r</sub> <sup>e</sup>	95% CI		
Deli Turkey (125 g)	<i>L. monocytogenes</i> ATCC 19116	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.49 (0.25, 0.85)	20	8	0.40	0.22, 0.61	7	0.35	0.18, 0.57	0.05	-0.23, 0.32
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Raw Chicken Leg Pieces (25 g): Lot 1	Naturally Contaminated	28 Hour	1.91 (1.23, 3.80)	20	17	0.85	0.64, 0.95	16	0.80	0.58, 0.92	0.05	-0.19, 0.29
Raw Chicken Leg Pieces (25 g): Lot 2	Naturally Contaminated	28 Hour	1.12 (0.68, 1.91)	20	10	0.50	0.30, 0.70	14	0.70	0.48, 0.85	-0.20	-0.45, 0.10
Whole Melon	<i>L. monocytogenes</i> FSL J1-049	26 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
7			20	10	0.50	0.30, 0.70	8	0.40	0.22, 0.61	0.10	-0.19, 0.37	
55			5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43	
Vanilla Ice Cream (25 g)	<i>L. monocytogenes</i> ATCC 19114	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
0.59 (0.33, 0.97)			20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28	
4.38 (1.72, 11.15)			5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43	

<sup>a</sup>MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, 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>c</sub> = Candidate method confirmed positive outcomes divided by the total number of trials

<sup>e</sup>POD<sub>r</sub> = Reference method confirmed positive outcomes divided by the total number of trials

<sup>f</sup>dPOD<sub>c</sub> = Difference between the confirmed candidate method result and reference 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

**Table 10: MDA 2 *Listeria monocytogenes* Assay, Candidate vs. Reference – POD Results (INDEPENDENT STUDY) (1)**

Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	MDA2			Reference			dPOD <sub>c</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>c</sub> <sup>d</sup>	95% CI	X	POD <sub>r</sub> <sup>e</sup>	95% CI		
Romaine Lettuce (25 g)	<i>L. monocytogenes</i> ATCC 49594	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.40 (0.18, 0.70)	20	7	0.35	0.18, 0.57	6	0.30	0.15, 0.52	0.05	-0.23, 0.32
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Queso Fresco (25 g)	<i>L. monocytogenes</i> CWD 1554	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
0.61 (0.33, 1.02)			20	11	0.55	0.34, 0.74	9	0.45	0.26, 0.66	0.10	-0.19, 0.37	
4.38 (1.72, 11.15)			5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43	
4% Milk Fat Cottage Cheese (25 g)	<i>L. monocytogenes</i> ATCC 19112	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
0.55 (0.40, 0.92)			20	10	0.50	0.30, 0.70	8	0.40	0.22, 0.61	0.10	-0.19, 0.37	
1.64 (0.84, 3.20)			5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43	

3% Chocolate Whole Milk (25 g)	<i>L. monocytogenes</i> ATCC BAA-751	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.44 (0.21, 0.76)	20	9	0.45	0.26, 0.66	6	0.30	0.15, 0.52	0.15	-0.14, 0.41
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	MDA2			Reference			dPOD <sub>c</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>c</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI		
Beef Hot Dogs (25 g)	<i>L. monocytogenes</i> ATCC 7644	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.44 (0.21, 0.76)	20	5	0.25	0.11, 0.47	6	0.30	0.15, 0.52	-0.05	-0.31, 0.22
			4.38 (1.72, 11.15)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

<sup>a</sup>MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, 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>c</sub> = Candidate method confirmed positive outcomes divided by the total number of trials

<sup>e</sup>POD<sub>R</sub> = Reference method confirmed positive outcomes divided by the total number of trials

<sup>f</sup>dPOD<sub>c</sub> = Difference between the confirmed candidate method result and reference 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

**Table 11: MDA 2 *Listeria monocytogenes* Assay, Candidate vs. Reference – POD Results (INDEPENDENT STUDY) (1)**

Matrix	Strain	Analysis Time Point	CFU <sup>a</sup> / Test Area	N <sup>b</sup>	MDA2			Reference			dPOD <sub>c</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>c</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI		
Stainless Steel (225 mL)	<i>L. monocytogenes</i> ATCC 19118 & <i>Enterococcus faecium</i> ATCC 19434	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			42 & 530	20	5	0.25	0.11, 0.47	8	0.40	0.22, 0.61	-0.10	-0.40, 0.13
			400 & 4000	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
		26 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			42 & 530	20	5	0.25	0.11, 0.47	8	0.40	0.22, 0.61	-0.10	-0.40, 0.13
			400 & 4000	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	Analysis Time Point	CFU <sup>a</sup> / Test Area	N <sup>b</sup>	MDA2			Reference			dPOD <sub>c</sub> <sup>f</sup>	95% CI <sup>g</sup>
x <sup>c</sup>	POD <sub>c</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI							
Sealed Concrete (100 mL)	<i>L. monocytogenes</i> ATCC 19117	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			35	20	15	0.75	0.53, 0.89	11	0.55	0.34, 0.74	0.20	-0.09, 0.45
			360	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
		26 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			35	20	15	0.75	0.53, 0.89	11	0.55	0.34, 0.74	0.20	-0.09, 0.45
			360	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	Analysis Time Point	CFU <sup>a</sup> / Test Area	N <sup>b</sup>	MDA2			Reference			dPOD <sub>c</sub> <sup>f</sup>	95% CI <sup>g</sup>
x <sup>c</sup>	POD <sub>c</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI							
Plastic (10 mL)	<i>L. monocytogenes</i> ATCC 51782 & <i>Enterococcus faecalis</i> ATCC 29212	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			42 & 540	20	12	0.60	0.39, 0.78	10	0.50	0.30, 0.70	0.10	-0.19, 0.37
			590 & 3600	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
		26 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			42 & 540	20	12	0.60	0.39, 0.78	10	0.50	0.30, 0.70	0.10	-0.19, 0.37
			590 & 3600	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

<sup>a</sup>CFU/Test Area = Results of the CFU/Test area were determined by plating the inoculum for each matrix in triplicate

<sup>b</sup>N = Number of test portions

<sup>c</sup>x = Number of positive test portions

<sup>d</sup>POD<sub>c</sub> = Candidate method confirmed positive outcomes divided by the total number of trials

<sup>e</sup>POD<sub>R</sub> = Reference method confirmed positive outcomes divided by the total number of trials

<sup>f</sup>dPOD<sub>c</sub> = Difference between the confirmed candidate method result and reference 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

**Table 12: MDA 2 *Listeria monocytogenes* Assay, Presumptive vs. Confirmed – POD Results (INTERNAL STUDY) (1)**

Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	Presumptive			Confirmed			dPOD <sub>cp</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>cp</sub> <sup>d</sup>	95% CI	X	POD <sub>cc</sub> <sup>e</sup>	95% CI		
Raw Chicken Leg Pieces (25 g)	<i>L. monocytogenes</i> Ad 668	28 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.91 (0.6, 1.39)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.28, 0.28
			2.2	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	x <sup>c</sup>	POD <sub>cp</sub> <sup>d</sup>	95% CI	X	POD <sub>cc</sub> <sup>e</sup>	95% CI	dPOD <sub>cp</sub> <sup>f</sup>	95% CI <sup>g</sup>
Bagged Raw Spinach (25 g)	<i>L. monocytogenes</i> Ad 543	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			1.17 (0.74, 1.84)	20	9	0.45	0.26, 0.66	10	0.50	0.30, 0.70	-0.05	-0.33, 0.24
			2.9	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Cold Smoked Salmon (25 g)	<i>L. monocytogenes</i> Ad 670	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.4 (0.18, 0.72)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.28, 0.28
			2.7	5	3	0.60	0.23, 0.88	3	0.60	0.23, 0.88	0.00	-0.46, 0.46

<sup>a</sup>MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, 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

**Table 13: MDA 2 *Listeria monocytogenes* Assay, Presumptive vs. Confirmed – POD Results (INDEPENDENT STUDY) (1)**

Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	Presumptive			Confirmed			dPOD <sub>cp</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>cp</sub> <sup>d</sup>	95% CI	X	POD <sub>cc</sub> <sup>e</sup>	95% CI		
Deli Turkey (125 g)	<i>L. monocytogenes</i> ATCC 19116	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.49 (0.25, 0.85)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.28, 0.28
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	x <sup>c</sup>	POD <sub>cp</sub> <sup>d</sup>	95% CI	X	POD <sub>cc</sub> <sup>e</sup>	95% CI	dPOD <sub>cp</sub> <sup>f</sup>	95% CI <sup>g</sup>
Raw Chicken Leg Pieces (25 g): Lot 1	Naturally Contaminated	28 Hours	1.91 (1.23, 3.80)	20	17	0.85	0.64, 0.95	17	0.85	0.64, 0.95	0.00	-0.23, 0.23
Raw Chicken Leg Pieces (25 g): Lot 2	Naturally Contaminated	28 Hours	1.12 (0.68, 1.91)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.28, 0.28
Whole Melon	<i>L. monocytogenes</i> FSL J1-049	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			7	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.28, 0.28
			55	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

Vanilla Ice Cream (25 g)	<i>L. monocytogenes</i> ATCC 19114	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.59 (0.33, 0.97)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
			4.38 (1.72, 11.15)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

<sup>a</sup>MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, 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

**Table 14: MDA 2 *Listeria monocytogenes* Assay, Presumptive vs. Confirmed – POD Results (INDEPENDENT STUDY) (1)**

Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	Presumptive			Confirmed			dPOD <sub>CP</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	X	POD <sub>CC</sub> <sup>e</sup>	95% CI		
Romaine Lettuce (25 g)	<i>L. monocytogenes</i> ATCC 49594	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.40 (0.18, 0.70)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Queso Fresco (25 g)	<i>L. monocytogenes</i> CWD 1554	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.61 (0.33, 1.02)	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0.00	-0.28, 0.28
			4.38 (1.72, 11.15)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
4% Milk Fat Cottage Cheese (25 g)	<i>L. monocytogenes</i> ATCC 19112	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.55 (0.40, 0.92)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.28, 0.28
			1.64 (0.84, 3.20)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
3% Chocolate Whole Milk (25 g)	<i>L. monocytogenes</i> ATCC BAA-751	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.44 (0.21, 0.76)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.28, 0.28
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Beef Hot Dogs (25 g)	<i>L. monocytogenes</i> ATCC 7644	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.44 (0.21, 0.76)	20	4	0.20	0.08, 0.42	5	0.25	0.11, 0.47	-0.05	-0.30, 0.21
			4.38 (1.72, 11.15)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

<sup>a</sup>MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, 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

**Table 15: MDA 2 *Listeria monocytogenes* Assay, Presumptive vs. Confirmed – POD Results (INDEPENDENT STUDY) (1)**

Matrix	Strain	Analysis Time Point	CFU <sup>a</sup> / Test Area	N <sup>b</sup>	Presumptive			Confirmed			dPOD <sub>CP</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	X	POD <sub>CC</sub> <sup>e</sup>	95% CI		
Stainless Steel (225 mL)	<i>L. monocytogenes</i> ATCC 19118 & <i>Enterococcus faecium</i> ATCC 19434	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			42 & 530	20	5	0.25	0.11, 0.47	5	0.25	0.11, 0.47	0.00	-0.26, 0.26
			400 & 4000	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
		26 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			42 & 530	20	5	0.25	0.11, 0.47	5	0.25	0.11, 0.47	0.00	-0.26, 0.26
			400 & 4000	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	Analysis Time Point	CFU <sup>a</sup> / Test Area	N <sup>b</sup>	Presumptive			Confirmed			dPOD <sub>CP</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	X	POD <sub>CC</sub> <sup>e</sup>	95% CI		
Sealed Concrete (100 mL)	<i>L. monocytogenes</i> ATCC 19117	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			35	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0.00	-0.26, 0.26
			360	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
		26 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			35	20	16	0.80	0.58, 0.92	15	0.75	0.53, 0.89	0.05	-0.21, 0.30
			360	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	Analysis Time Point	CFU <sup>a</sup> / Test Area	N <sup>b</sup>	Presumptive			Confirmed			dPOD <sub>CP</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	X	POD <sub>CC</sub> <sup>e</sup>	95% CI		
Plastic (10 mL)	<i>L. monocytogenes</i> ATCC 51782 & <i>Enterococcus faecalis</i> ATCC 29212	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			42 & 540	20	12	0.60	0.39, 0.78	12	0.60	0.39, 0.78	0.00	-0.28, 0.28
			590 & 3600	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
		26 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			42 & 540	20	12	0.60	0.39, 0.78	12	0.60	0.39, 0.78	0.00	-0.28, 0.28
			590 & 3600	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

<sup>a</sup>CFU/Test Area = Results of the CFU/Test area were determined by plating the inoculum for each matrix in triplicate

<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 statistically significant at the 5% level

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