



CERTIFICATION

AOAC Research Institute *Performance Tested Methods*SM

Certificate No.
111501

The AOAC Research Institute hereby certifies the method known as:

Molecular Detection Assay 2 – *Listeria* species (MDA2 – LIS)

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*SM 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*SM 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
Expiration Date

December 09, 2024
December 31, 2026

AUTHORS ORIGINAL VALIDATION: Lisa Monteroso and DeAnn Benesh MODIFICATION FEBRUARY 2024: April Schumacher and Micki Rosauer	SUBMITTING COMPANY 3M Company 3M Center, Bldg. 275-5W-05 St. Paul, MN 55144-1000	CURRENT SPONSOR Neogen Food Safety Corporation 620 Leshar Place Lansing, MI 48912
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METHOD NAME Neogen® Molecular Detection Assay 2 – <i>Listeria</i> species (MDA2 – LIS) Formerly 3M™ Molecular Detection Assay 2 – <i>Listeria</i> species (MDA2 – LIS)	CATALOG NUMBER MDA2LIS96
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INDEPENDENT LABORATORY Q Laboratories, Inc. 1400 Harrison Ave. Cincinnati, OH 45214 USA

APPLICABILITY OF METHOD Target organism – <i>Listeria</i> species (<i>L. monocytogenes</i> , <i>L. seeligeri</i> , <i>L. ivanovii</i> , <i>L. innocua</i> , <i>L. grayi</i> , <i>L. welshimeri</i> , <i>L. fleishmanii</i> supsp. <i>coloradensis</i> , <i>L. cornellensis</i> , <i>L. grandensis</i> , <i>L. marthii</i> , and <i>L. riparia</i>). Matrixes – USDA/FSIS MLG 8.09 – beef hot dogs (25 g), raw chicken (leg pieces and fillets) (25 g), deli turkey (125 g), stainless steel (4" x 4", sponge enriched in 225 mL), sealed concrete (4" x 4", sponge enriched in 225 mL), plastic (1" x 1", Tecra™ Enviroswab [Australia Pty Ltd] enriched in 10 mL) FDA-BAM Ch 10 – whole melon, cold smoked salmon (25 g), romaine lettuce AOAC 993.12 – vanilla ice cream (25 g), queso fresco (25 g), 4% milk fat cottage cheese (25 g) ISO 11290-1/A1 – bagged raw spinach (25 g), cold smoked salmon (25 g). Performance claims – Performance equivalent to that of the U.S. Food and Drug Administration <i>Bacteriological Analytical Manual</i> (FDA/BAM) Chapter 10 [1] for whole melon, cold smoked salmon, bagged raw spinach; the U.S. Department of Agriculture Food Safety and Inspection Service <i>Microbiology Laboratory Guidebook</i> (USDA-FSIS/MLG) 8.09 [2] for beef hot dogs, deli turkey, raw chicken, sealed concrete, plastic and stainless steel and AOAC 993.12 [3] for queso fresco, vanilla ice cream, 4% milk fat cottage cheese and for ISO 11290-1/A1 [4] for cold smoked salmon and bagged raw spinach.	REFERENCE METHODS Food and Drug Administration Bacteriological Analytical Manual Chapter 10: <i>Detection and Enumeration of Listeria monocytogenes in Foods</i> . February 2013. (Accessed January 2015) (3) United States Department of Agriculture Microbiological Laboratory Guidelines 8.09: <i>Isolation and Identification of Listeria monocytogenes from Red Meat, Poultry and Egg Products, and Environmental Samples</i> . May 2013. (4) Official Methods of Analysis of AOAC INTERNATIONAL (2012) 19th Ed., AOAC Official Method 993.12 <i>Listeria monocytogenes</i> in Milk and Dairy Products: Selective Enrichment and Isolation Method, First Action 1993, Final Action 1996 (5) ISO 11290-1/A1 (2004) - Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of <i>Listeria monocytogenes</i> - Part 1: detection of <i>Listeria monocytogenes</i> in foods (6)
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ORIGINAL CERTIFICATION DATE November 03, 2015	CERTIFICATION RENEWAL RECORD Renewed through December 2026.
METHOD MODIFICATION RECORD 1. December 2018 Level 1 2. February 2020 Level 1 3. January 2024 Level 1 4. February 2024 Level 2	SUMMARY OF MODIFICATION 1. Editorial and formatting changes to insert. 2. Editorial changes for clarity. 3. Editorial/clerical changes to rebrand method from 3M to Neogen Corporation. 4. Manufacturing location change from Columbia, Missouri to Lansing, Michigan.
Under this AOAC <i>Performance Tested Methods</i> SM License Number, 111501 this method is distributed by: NONE	Under this AOAC <i>Performance Tested Methods</i> SM License Number, 111501 this method is distributed as: NONE

PRINCIPLE OF THE METHOD (1)

Neogen® Molecular Detection Assay 2 – *Listeria* species (MDA2 – LIS) is used with the Neogen Molecular Detection System for the rapid and specific detection of *Listeria* species 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)

All matrixes evaluated in this validation study, including the raw chicken, resulted in no statistical differences when compared to the reference methods. Three different brands of Demi Fraser with FAC were used in this study, referred to as X, Y, and Z.

There were no differences between the 24 and 26 hour primary enrichment time points for the environmental surfaces tested, therefore a 24 hour minimum enrichment time will be recommended for these matrixes: stainless steel, sealed concrete and plastic.

The five false negative results with raw chicken (leg pieces) evaluated in the internal study resulted in an investigation of protocol. It was determined that the contract laboratory conducting the study used Demi Fraser with FAC brand X, which demonstrated low productivity. Brand X had also been used to analyze the inclusive list, spinach, and cold smoked salmon.

When the protocol for raw chicken was performed at the independent laboratory, using a different brand Y, two lots of naturally contaminated raw chicken (leg pieces) analyzed showed no statistical difference when compared to the reference method. Lot 1 of the naturally contaminated raw chicken (leg pieces) had zero false negatives, while Lot 2 had two false negatives.

Table 2a. Inclusivity Study Results (1)

Genus	Species	Reference	Origin	Inoculation level (cfu/225ml)	Molecular Detection Assay 2 Half Fraser broth 24h at 37°C			
					MDA 2 - <i>Listeria</i> spp	Confirmation 100µl		
						ALOA	PALCAM	
1	<i>Listeria</i>	<i>monocytogenes</i>	1011/1410	Frozen broccoli	28	+	H+	+
2	<i>Listeria</i>	<i>monocytogenes</i>	153	Soft cheese (Munster)	39	+	H+	+
3	<i>Listeria</i>	<i>monocytogenes</i>	1973/2400	Egg and ham pastry (Quiche Lorraine)	38	+	H+	+
4	<i>Listeria</i>	<i>monocytogenes</i>	38/181	Toulouse sausages	33	+	H+	+
5	<i>Listeria</i>	<i>monocytogenes</i>	7111/7516	Pâté (Rillettes)	64	+	H+	+
6	<i>Listeria</i>	<i>monocytogenes</i>	913/1048	Black pudding	39	+	H+	+
7	<i>Listeria</i>	<i>monocytogenes</i>	A00C036	Poultry (guinea)	41	+	H+	+
8	<i>Listeria</i>	<i>monocytogenes</i>	A00C041	Sausage	38	+	H+	+
9	<i>Listeria</i>	<i>monocytogenes</i>	A00C044	Poultry (Duck)	34	+	H+	+
10	<i>Listeria</i>	<i>monocytogenes</i>	A00L097	Milk	60	+	H+	+
11	<i>Listeria</i>	<i>monocytogenes</i>	A00M009	Smoked salmon	40	+	H+	+
12	<i>Listeria</i>	<i>monocytogenes</i>	Ad 253	Semi-hard cheese	58	+	H+	+
13	<i>Listeria</i>	<i>monocytogenes</i>	Ad 266	Poultry	26	+	H+	+
14	<i>Listeria</i>	<i>monocytogenes</i>	Ad 270	Fermented sausage	42	+	H+	+
15	<i>Listeria</i>	<i>monocytogenes</i>	Ad 273	Cured delicatessen	27	+	H+	+
16	<i>Listeria</i>	<i>monocytogenes</i>	Ad 274	Ready-to-eat food (Asiatic meal)	40	+	H+	+
17	<i>Listeria</i>	<i>monocytogenes</i>	Ad 534	Fruits	54	+	H+	+
18	<i>Listeria</i>	<i>monocytogenes</i>	Ad 548	Environment (Seafood)	37	+	H+	+
19	<i>Listeria</i>	<i>monocytogenes</i>	Ad 623	Bread crumbs	50	+	H+	+
20	<i>Listeria</i>	<i>monocytogenes</i>	Ad 665	Raw milk	33	+	H+	+
21	<i>Listeria</i>	<i>grayi</i>	Ad 1198	Smoked salmon	158 (+25ml UHT milk)	+	H-	st
					1300	+	H-(2)	-
					1300 (+25ml UHT milk)	+	H-	-
22	<i>Listeria</i>	<i>grayi</i>	Ad 1443	Pork meat sausages	12(+25ml UHT milk)	+	H-	-
23	<i>Listeria</i>	<i>innocua</i>	1	Smoked salmon	40	+	H-	+
24	<i>Listeria</i>	<i>innocua</i>	Ad 658	Gorgonzola	28	+	H-	+
25	<i>Listeria</i>	<i>innocua</i>	Ad 655	Brine	19	+	H-	+
26	<i>Listeria</i>	<i>innocua</i>	Ad 660	Bread crumbs	35	+	H-	+
27	<i>Listeria</i>	<i>innocua</i>	Ad 663	Environment (dairy industry)	25	+	H-	+
28	<i>Listeria</i>	<i>innocua</i>	Ad 671	Smoked bacon	10	+	H-	+
29	<i>Listeria</i>	<i>innocua</i>	Ad 661	Soft cheese (Pont L'Evêque)	15	+	H-	+
30	<i>Listeria</i>	<i>innocua</i>	Ad 659	Environment (dairy industry)	14	+	H-	+
31	<i>Listeria</i>	<i>ivanovii</i>	Ad 466	Raw veal meat	11	+	H+	+
32	<i>Listeria</i>	<i>ivanovii</i>	Ad 662	Environment (dairy industry)	20	+	H+	+
33	<i>Listeria</i>	<i>ivanovii</i>	BR11	Environment (fish)	29	+	H+	+
34	<i>Listeria</i>	<i>ivanovii</i>	Ad 1289	Raw milk cheese	30	+	H+	+
35	<i>Listeria</i>	<i>ivanovii</i>	Ad 1290	Milk powder	17	+	H+	+
36	<i>Listeria</i>	<i>ivanovii</i>	Ad 1291	Poultry	18	+	H+	+
37	<i>Listeria</i>	<i>ivanovii</i>	Ad 1288	Sheep milk	80	+	H+	+
38	<i>Listeria</i>	<i>ivanovii</i> subsp. <i>londoniensis</i>	CIP103466	Unknown	14	+	H-	+
39	<i>Listeria</i>	<i>seeligeri</i>	Ad 649	Cheese	29	+	H-	+
40	<i>Listeria</i>	<i>seeligeri</i>	Ad 651	Environment	36	+	H-	+
41	<i>Listeria</i>	<i>seeligeri</i>	Ad 652	Environment (dairy industry)	28	+	H-	+
42	<i>Listeria</i>	<i>seeligeri</i>	Ad 674	Soft cheese (Munster)	240	+	H-	H-
43	<i>Listeria</i>	<i>seeligeri</i>	BR1	Trout	28	+	H-	+
44	<i>Listeria</i>	<i>seeligeri</i>	BR18	Environment (fish)	36	+	H-	+
45	<i>Listeria</i>	<i>seeligeri</i>	CIP100100	Unknown	8	+	st	1col
46	<i>Listeria</i>	<i>welshimeri</i>	Ad1276	Environment (Slaughterhouse)	44	+	H-	+
47	<i>Listeria</i>	<i>welshimeri</i>	Ad1235	Beef meat	26	+	H-	+
48	<i>Listeria</i>	<i>welshimeri</i>	191424	Poultry	24	+	H-	+
49	<i>Listeria</i>	<i>welshimeri</i>	Ad 1175	Ready-to-eat-food	46	+	H-	+
50	<i>Listeria</i>	<i>welshimeri</i>	Ad 650	Poultry	33	+	H-	+

All the strains are wild strains isolated in Adria Developpement, Quimper, France.

Ad = Adria

A= Adria

BR = ANSES Lab strain

CIP = collection of Pasteur Institute

H+/- = Presence/absence Phospho-Lipolysis halo

St = plate without colonies

ANSES = French Agency for Food, Environmental, and Occupational Health and Safety, Cedex, France

Listeria strains #21 and #22 utilized UHT to assist with growth as ISO 16140 directs.

Table 2b. Inclusivity Study Results (1)

#	<i>Listeria</i> species	Reference	Origin	Level tested	MDA2LIS
51	<i>fleischmannii</i> subsp. <i>coloradensis</i>	FSL S10-1203	Running water, FL	1.8e6 CFU/mL	+
52	<i>marthii</i>	FSL S4-120	Pristine environment, forest, NY	2.65e5 CFU/mL	+
53	<i>cornellensis</i>	FSL F6-969	Water, CO	3.5e7 CFU/mL	+
54	<i>grandensis</i>	FSL F6-971	Water, CO	1.95e7 CFU/mL	+
55	<i>riparia</i>	FSL S10-1204	Running water, FL	McFarland 1 *	+

Table 2b are *Listeria* strains analyzed at St. Paul, MN laboratory

Cultures 51-54 were obtained from the laboratory of [Dr. Martin Wiedmann](#) at Cornell University, NY. Frozen suspensions were thawed and streaked to sheep blood agar. Single isolated colonies were picked and sub-cultured in 10 mL Demi Fraser broth with FAC overnight at 37 ± 1°C. The cultures were diluted with fresh Demi Fraser broth with FAC before testing using the MDA2LIS kit.

Culture 55 was also obtained from Cornell. The frozen suspension was thawed and streaked to sheep blood agar. This organism did not grow in broth at 37°C or at lower temperatures, therefore a McFarland 1* suspension (approximately 3e8 CFU/mL) was prepared from a colonial isolate prior to MDA2LIS testing.

FSL = Food Safety Laboratory Cornell University, NY

Table 3. Exclusivity Study Results (1)

	Genus	Species	Reference	Origin	Inoculation level (cfu/ml)	Molecular Detection Assay 2 – <i>Listeria</i>
1	<i>Bacillus</i>	<i>cereus</i>	Ad 465	Salmon Terrine	7,2x10 ⁴	-
2	<i>Bacillus</i>	<i>circulans</i>	Ad 760	Vegetables	1,0 x 10 ⁴ (opacity +)	-
3	<i>Bacillus</i>	<i>coagulans</i>	Ad 731	Dairy product	<2,0x10 ³ (opacity +)	-
4	<i>Bacillus</i>	<i>licheniformis</i>	Ad 978	Dairy product	<2,0x10 ³ (opacity+)	-
5	<i>Bacillus</i>	<i>mycoïdes</i>	Ad 762	Milk	5,6x10 ⁴	-
6	<i>Bacillus</i>	<i>pseudomycoïdes</i>	Ad 765	Vegetables	2,0x10 ⁴	-
7	<i>Bacillus</i>	<i>pumilus</i>	Ad 284	Ready-to-eat	1,7x10 ⁵	-
8	<i>Bacillus</i>	<i>weihenstephanensis</i>	Ad 726	Egg product	6,8x10 ⁴	-
9	<i>Brochothrix</i>	<i>thermosphacta</i>	EN 15129	Trout	8,5x10 ⁵	-
10	<i>Brochrotrix</i>	<i>campestris</i>	CIP 102920T	Environment	<2,0x10 ³ (opacity +)	-
11	<i>Carnobacterium</i>	<i>divergens</i>	CIP 101029T	Unknown	>2,0x10 ³ (opacity +)	-
12	<i>Carnobacterium</i>	<i>piscicola</i>	Ad 369	Raw milk	<2,0x10 ³ (opacity +)	-
13	<i>Enterococcus</i>	<i>durans</i>	Ad 149	Ham	<2,0x10 ³ (opacity +)	-
14	<i>Enterococcus</i>	<i>faecalis</i>	89L326	Soft cheese (Vacherin)	1,6x10 ⁵	-
15	<i>Lactobacillus</i>	<i>brevis</i>	86L126	Ham	1,4x10 ⁵	-
16	<i>Lactobacillus</i>	<i>curvatus</i>	Ad 380	Delicatessen	1,7x10 ⁵	-
17	<i>Lactobacillus</i>	<i>fermentum</i>	Ad 482	Tomatoes juice	1,2x10 ⁵	-
18	<i>Lactobacillus</i>	<i>sakei</i>	Ad 473	Ham	4,9x10 ⁵	-
19	<i>Lactococcus</i>	<i>lactis</i> subsp <i>cremoris</i>	Ad 137	Dairy product	>2,0x10 ³ (opacity +)	-
20	<i>Leuconostoc</i>	<i>carnosum</i>	Ad 411	Ham	6,8x10 ⁵	-
21	<i>Leuconostoc</i>	<i>citreum</i>	Ad 396	Ham	4,9x10 ⁵	-
22	<i>Micrococcus</i>	<i>luteus</i>	Ad 432	Cocktail	<2,0x10 ³ (opacity+)	-
23	<i>Pediococcus</i>	<i>pentosaceus</i>	ATCC 33316	Unknown	1,0x10 ⁵	-
24	<i>Propionibacterium</i>	<i>freundenreichii</i>	CNRZ 725	Dairy product	<2,0x10 ³ (opacity +)	-
25	<i>Staphylococcus</i>	<i>aureus</i>	Ad 165	Smoked delicatessen	1,3x10 ⁵	-
26	<i>Staphylococcus</i>	<i>aureus</i>	Ad 902	Nems	8,2x10 ⁴	-
27	<i>Staphylococcus</i>	<i>epidermidis</i>	Ad 931	Fruits	2,0x10 ³	-
28	<i>Staphylococcus</i>	<i>haemolyticus</i>	Ad 989	Dairy product	2,8x10 ⁴	-
29	<i>Streptococcus</i>	<i>bovis</i>	92L622	Dairy product	2,0x10 ³	-
30	<i>Streptococcus</i>	<i>salivarius</i>	Ad 441	Dairy product	<2,0x10 ³ (opacity +)	-

All the strains are wild strains isolated in Adria Developpement, Quimper, France.

Ad = Adria

A= Adria

BR = ANSES Lab strain

CIP = collection of Pasteur Institute

ANSES = French Agency for Food, Environmental, and Occupational Health and Safety, Cedex, France

CNRZ = National Centre for Zootechnical Research, Jouy-en-Josas, France

ATCC = American Type Culture Collection

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

Matrix	Strain	Analysis Time Point	MPN ^a / Test Portion	N ^b	MDA2			USDA/FSIS MLG			dPOD _c ^f	95% CI ^g
					x ^c	POD _c ^d	95% CI	X	POD _R ^e	95% CI		
Raw Chicken Leg Pieces ^h (25 g)	<i>L. ivanovii</i> Ad 1291	28 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.31 (0.14, 0.57)	20	4	0.20	0.08, 0.42	5	0.25	0.11, 0.47	-0.05	-0.30, 0.21
			0.5	5	3	0.60	0.23, 0.88	1	0.20	0.04, 0.62	0.40	-0.16, 0.73
Raw Chicken Fillet ⁱ (25 g)	<i>L. ivanovii</i> Ad 1291	28Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.3 (0.10, 0.5)	20	4	0.20	0.08, 0.42	5	0.25	0.11, 0.47	-0.05	-0.30, 0.21
			0.5	5	2	0.40	0.12, 0.77	4	0.80	0.38, 0.96	-0.40	-0.73, 0.16
Bagged Raw Spinach ^h (25 g)	<i>L. seeligeri</i> Ad 1754	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.26 (0.10, 0.47)	20	6	0.30	0.15, 0.52	3	0.15	0.05, 0.36	0.15	-0.11, 0.39
			1.7	5	3	0.60	0.23, 0.88	4	0.80	0.38, 0.96	-0.20	-0.60, 0.31
Bagged Raw Spinach ^h (25 g)	<i>L. seeligeri</i> Ad 1754	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.26 (0.10, 0.47)	20	6	0.30	0.15, 0.52	9	0.45	0.26, 0.66	-0.15	-0.41, 0.14
			1.7	5	3	0.60	0.23, 0.88	4	0.80	0.38, 0.96	-0.20	-0.60, 0.31
Cold Smoked Salmon ^h (25 g)	<i>L. innocua</i> Ad 1674	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.27 (0.10, 0.49)	20	10	0.50	0.30, 0.70	7	0.35	0.18, 0.57	0.15	-0.15, 0.41
			1.7	5	5	1.00	0.57, 1.00	4	0.80	0.38, 0.96	0.20	-0.26, 0.62
Cold Smoked Salmon ^h (25 g)	<i>L. innocua</i> Ad 1674	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.27 (0.10, 0.49)	20	10	0.50	0.30, 0.70	8	0.40	0.22, 0.61	0.10	-0.19, 0.37
			1.7	5	5	1.00	0.57, 1.00	4	0.80	0.38, 0.96	0.20	-0.26, 0.62

^aMPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval

^bN = Number of test portions

^cx = Number of positive test portions

^dPOD_c = Candidate method confirmed positive outcomes divided by the total number of trials

^ePOD_R = Reference method confirmed positive outcomes divided by the total number of trials

^fdPOD_c = Difference between the confirmed candidate method result and reference method confirmed result POD values

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

^hTested using Demi Fraser (with FAC) brand X

ⁱTested using Demi Fraser (with FAC) brand Z

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

Matrix	Strain	Analysis Time Point	MPN ^a / Test Portion	N ^b	MDA2			Reference			dPOD _c ^f	95% CI ^g
					x ^c	POD _c ^d	95% CI	X	POD _R ^e	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
Matrix	Strain	Analysis Time Point	MPN ^a / Test Portion	N ^b	MDA2			Reference			dPOD _c ^f	95% CI ^g
x ^c	POD _c ^d	95% CI	X	POD _R ^e	95% CI							
Raw Chicken Leg Pieces (25 g): Lot 1	Naturally Contaminated	28 Hour	1.91 (1.23, 3.80)	20	18	0.90	0.70, 0.97	16	0.80	0.58, 0.92	0.10	-0.13, 0.33
Matrix	Strain	Analysis Time Point	MPN ^a / Test Portion	N ^b	MDA2			Reference			dPOD _c ^f	95% CI ^g
x ^c	POD _c ^d	95% CI	X	POD _R ^e	95% CI							
Raw Chicken Leg Pieces (25 g): Lot 2	Naturally Contaminated	28 Hour	1.12 (0.68, 1.91)	20	12	0.60	0.39, 0.78	14	0.70	0.48, 0.85	-0.10	-0.36, 0.18
Matrix	Strain	Analysis Time Point	MPN ^a / Test Portion	N ^b	MDA2			Reference			dPOD _c ^f	95% CI ^g
x ^c	POD _c ^d	95% CI	X	POD _R ^e	95% CI							
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
Matrix	Strain	Analysis Time Point	MPN ^a / Test Portion	N ^b	MDA2			Reference			dPOD _c ^f	95% CI ^g
x ^c	POD _c ^d	95% CI	X	POD _R ^e	95% CI							
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

^aMPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval

^bN = Number of test portions

^cx = Number of positive test portions

^dPOD_c = Candidate method confirmed positive outcomes divided by the total number of trials

^ePOD_R = Reference method confirmed positive outcomes divided by the total number of trials

^fdPOD_c = Difference between the confirmed candidate method result and reference method confirmed result POD values

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

All matrixes were in Table 9 were tested with Demi Fraser brand Y.

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

Matrix	Strain	Analysis Time Point	MPN ^a / Test Portion	N ^b	MDA2			Reference			dPOD _c ^f	95% CI ^g
					x ^c	POD _c ^d	95% CI	X	POD _R ^e	95% CI		
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. welshimeri</i> ATCC 35897	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.69 (0.40, 1.14)	20	12	0.60	0.39, 0.78	9	0.45	0.26, 0.66	0.15	-0.15, 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
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

^aMPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval

^bN = Number of test portions

^cx = Number of positive test portions

^dPOD_c = Candidate method confirmed positive outcomes divided by the total number of trials

^ePOD_R = Reference method confirmed positive outcomes divided by the total number of trials

^fdPOD_c = Difference between the confirmed candidate method result and reference method confirmed result POD values

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

All matrixes tested in Table 10 used Demi Fraser brand Y.

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

Matrix	Strain	Analysis Time Point	CFU ^a / Test Area	N ^b	MDA2			Reference			dPOD _c ^f	95% CI ^g
					x ^c	POD _c ^d	95% CI	X	POD _R ^e	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 ^a / Test Area	N ^b	MDA2			Reference			dPOD _c ^f	95% CI ^g
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 ^a / Test Area	N ^b	MDA2			Reference			dPOD _c ^f	95% CI ^g
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
			64 & 540	20	15	0.75	0.53, 0.89	11	0.55	0.34, 0.74	0.20	-0.09, 0.45
			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
			64 & 540	20	15	0.75	0.53, 0.89	11	0.55	0.22, 0.74	0.20	-0.09, 0.45
			590 & 3600	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

^aCFU/Test Area = Results of the CFU/Test area were determined by plating the inoculum for each matrix in triplicate

^bN = Number of test portions

^cx = Number of positive test portions

^dPOD_c = Candidate method confirmed positive outcomes divided by the total number of trials

^ePOD_R = Reference method confirmed positive outcomes divided by the total number of trials

^fdPOD_c = Difference between the confirmed candidate method result and reference method confirmed result POD values

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

All matrixes tested in Table 11 used Demi Fraser brand Y.

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

Matrix	Strain	Analysis Time Point	MPN ^a / Test Portion	N ^b	Presumptive			Confirmed			dPOD _{CP} ^f	95% CI ^g
					x ^c	POD _{CP} ^d	95% CI	X	POD _{CC} ^e	95% CI		
Raw Chicken Leg Pieces ^h (25 g)	<i>L. ivanovii</i> Ad 1291	28 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.31 (0.14, 0.57)	20	4	0.20	0.08, 0.42	9	0.45	0.26, 0.66	-0.25	-0.49, 0.05
			0.5	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Raw Chicken Fillet ⁱ (25 g)	<i>L. ivanovii</i> Ad 1291	28 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.3 (0.1, 0.5)	20	4	0.80	0.38, 0.96	4	0.80	0.38, 0.96	0.00	-0.25, 0.25
			0.5	5	2	0.40	0.12, 0.77	2	0.40	0.12, 0.77	0.00	-0.46, 0.46
Bagged Raw Spinach ^h (25 g)	<i>L. seeligeri</i> Ad 1754	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.26 (0.10, 0.47)	20	6	0.30	0.15, 0.52	6	0.30	0.15, 0.52	0.00	-0.27, 0.31
			1.7	5	3	1.00	0.57, 1.00	3	1.00	0.57, 1.00	0.00	-0.43, 0.43
Cold Smoked Salmon ^h (25 g)	<i>L. innocua</i> Ad 1674	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.27 (0.1, 0.49)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.20	-0.26, 0.62
			1.7	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

^aMPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval

^bN = Number of test portions

^cx = Number of positive test portions

^dPOD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials

^ePOD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials

^fdPOD_{CP} = Difference between the candidate method presumptive result and candidate method confirmed result POD values

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

^hTested using Demi Fraser brand (with FAC) brand X.

ⁱTested using Demi Fraser brand (with FAC) brand Z.

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

Matrix	Strain	Analysis Time Point	MPN ^a / Test Portion	N ^b	Presumptive			Confirmed			dPOD _{CP} ^f	95% CI ^g
					x ^c	POD _{CP} ^d	95% CI	X	POD _{CC} ^e	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
Raw Chicken Leg Pieces (25 g): Lot 1	Naturally Contaminated	28 Hours	1.91 (1.23, 3.80)	20	18	0.90	0.70, 0.97	18	0.90	0.70, 0.97	0.00	-0.21, 0.21
			1.12 (0.68, 1.91)	20	12	0.60	0.39, 0.78	12	0.60	0.39, 0.78	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	12	0.60	0.39, 0.78	10	0.50	0.30, 0.70	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

^aMPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval

^bN = Number of test portions

^cx = Number of positive test portions

^dPOD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials

^ePOD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials

^fdPOD_{CP} = Difference between the candidate method presumptive result and candidate method confirmed result POD values

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

All matrixes tested in Table 13 used Demi Fraser (with FAC) brand Y.

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

Matrix	Strain	Analysis Time Point	MPN ^a / Test Portion	N ^b	Presumptive			Confirmed			dPOD _{CP} ^f	95% CI ^g
					x ^c	POD _{CP} ^d	95% CI	X	POD _{CC} ^e	95% CI		
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. welshimeri</i> ATCC 35897	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.69 (0.40, 01.14)	20	12	0.60	0.39, 0.78	12	0.60	0.39, 0.78	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

^aMPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval

^bN = Number of test portions

^cx = Number of positive test portions

^dPOD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials

^ePOD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials

^fdPOD_{CP} = Difference between the candidate method presumptive result and candidate method confirmed result POD values

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

All matrixes tested in Table 13 used Demi Fraser (with FAC) brand Y.

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

Matrix	Strain	Analysis Time Point	CFU ^a / Test Area	N ^b	Presumptive			Confirmed			dPOD _{CP} ^f	95% CI ^g
					x ^c	POD _{CP} ^d	95% CI	X	POD _{CC} ^e	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 ^a / Test Area	N ^b	Presumptive			Confirmed			dPOD _{CP} ^f	95% CI ^g
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	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
Matrix	Strain	Analysis Time Point	CFU ^a / Test Area	N ^b	x ^c	POD _{CP} ^d	95% CI	X	POD _{CC} ^e	95% CI	dPOD _{CP} ^f	95% CI ^g
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
			64 & 540	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0.00	-0.26, 0.26
			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
			64 & 540	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0.00	-0.26, 0.26
			590 & 3600	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

^aCFU/Test Area = Results of the CFU/Test area were determined by plating the inoculum for each matrix in triplicate

^bN = Number of test portions

^cx = Number of positive test portions

^dPOD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials

^ePOD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials

^fdPOD_{CP} = Difference between the candidate method presumptive result and candidate method confirmed result POD values

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the statistically significant at the 5% level

All matrixes tested in Table 13 used Demi Fraser (with FAC) brand Y.

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