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CERTIFICATION

AOAC® *Performance Tested*SM

Certificate No.

082001

The AOAC Research Institute hereby certifies the test kit known as:

Solus *Listeria monocytogenes* ELISA

manufactured by

Solus Scientific Solutions Ltd.
9 Mansfield Networkcentre
Millennium Business Park
Concord Way, Mansfield
Nottinghamshire, NG19 7JZ

This method has been evaluated in the AOAC® *Performance Tested Methods*SM Program and found to perform as stated by the manufacturer contingent to the comments contained in the manuscript. This certificate means that an AOAC® Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC *Performance Tested* SM certification mark along with the statement - "THIS METHOD'S PERFORMANCE WAS REVIEWED BY AOAC RESEARCH INSTITUTE AND WAS FOUND TO PERFORM TO THE MANUFACTURER'S SPECIFICATIONS" - on the above mentioned method for a period of one calendar year from the date of this certificate (November 25, 2020 – December 31, 2021). Renewal may be granted at the end of one year under the rules stated in the licensing agreement.

Scott Coates

Scott Coates, Senior Director
Signature for AOAC Research Institute

December 01, 2020
Date

METHOD AUTHORS
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KIT NAME(S)
Solus *Listeria monocytogenes* ELISA

CATALOG NUMBERS
LISM-0096 (1 x 96 well microplate), LISM-0480 (5 x 96 well microplate)

INDEPENDENT LABORATORY
Q Laboratories
Cincinnati, OH USA

AOAC EXPERTS AND PEER REVIEWERS
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APPLICABILITY OF METHOD
Analyte – *Listeria monocytogenes*

Matrixes – (MLG 8.11) - Beef hot dogs (25 g, 125 g), sliced deli ham (25 g, 125 g), raw ground beef (~70% lean) (25 g)
(BAM CH 10) - bagged Romaine lettuce (25 g, 125 g), Pasteurized Brie cheese (25 g, 125 g), frozen raw shrimp (25 g), stainless steel (18 gauge: 304 food grade with a brushed finish, 4" x 4" sponge) and plastic (polystyrene, 1" x 1" swab) environmental surfaces

Performance claims - No statistical difference was detected between Solus *Listeria monocytogenes* ELISA and the reference culture methods of U.S. Food and Drug Administration *Bacteriological Analytical Manual* (FDA/BAM) Chapter 10, "Detection of Listeria monocytogenes in Foods and Environmental Samples, and Enumeration of *Listeria monocytogenes* in foods"(2017) (2) for pasteurized Brie cheese, bagged Romaine lettuce, frozen raw shrimp, stainless steel and polystyrene. In addition, no statistical difference was detected between Solus *Listeria monocytogenes* ELISA and the U.S. Department of Agriculture Food Safety and Inspection Service *Microbiology Laboratory Guidebook* (USDA-FSIS/MLG) 8.11 "Isolation and Identification of Listeria monocytogenes from Red Meat, Poultry, Ready-to-Eat Siluriformes (fish) and Egg Products, and Environmental Samples" (2019) (3) reference culture method for hot dogs, ready-to-eat sliced deli ham and raw ground beef.

REFERENCE METHOD
Food and Drug Administration Bacteriological Analytical Manual Chapter 10: *Detection of Listeria monocytogenes in Foods and Environmental Samples, and Enumeration of Listeria monocytogenes in Foods*. October 2017. (2)
United States Department of Agriculture Microbiology Laboratory Guidelines 8.11: *Isolation and Identification of Listeria monocytogenes from Red Meat, Poultry, Egg Products, and Environmental Sponges*. January 2nd, 2019. (3)

ORIGINAL CERTIFICATION DATE
August 07, 2020

CERTIFICATION RENEWAL RECORD
New Approval

METHOD MODIFICATION RECORD
1. November 2020 Level 1

SUMMARY OF MODIFICATION
1. Editorial changes.

Under this AOAC® *Performance Tested*SM License Number, 082001 this method is distributed by:
NONE

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NONE

PRINCIPLE OF THE METHOD (1)
Solus *Listeria monocytogenes* is an immunoassay-based test system for the detection of *L. monocytogenes* in food and production environmental samples. The Solus *Listeria monocytogenes* ELISA relies on a highly specific antibody-antigen reaction. The ELISA is paired with a two-step enrichment protocol. *Listeria monocytogenes* specific antibodies are bound to the microplate. Following enrichment, samples are heat treated and an aliquot is added to the coated wells. If *L. monocytogenes* specific antigen is present in the sample, it will bind immunologically to the antibody on the plate. An enzyme-labeled antibody conjugate is added to the well and binds to the *L. monocytogenes* specific antigen if present in the sample. Following incubation, any unbound sample or conjugate is washed away, and an enzyme substrate is added. The substrate reacts in the presence of the enzyme producing a blue color change in the sample well. The substrate reaction is stopped after 30 minutes by the addition of sulfuric acid which changes the blue color to yellow which allows colorimetric analysis at 450nm. Samples with OD_{450} <0.2 are presumptive negative and OD_{450} ≥0.2 are presumptive positive for the presence of *L. monocytogenes*

DISCUSSION OF THE VALIDATION STUDY (1)

The Solus *Listeria monocytogenes* ELISA using the Dynex DS2 automated immunoassay instrument and manual immunoassay method successfully detected 50 strains of *L. monocytogenes* and all 37 exclusivity strains were correctly excluded. The same method also successfully detected *L. monocytogenes* from beef hot dogs (25 and 125 g), sliced deli ham (25 and 125 g), raw ground beef (~70% Lean) (25 g), bagged Romaine lettuce (25 and 125 g), pasteurized Brie cheese (25 and 125 g), frozen raw shrimp (25 g), stainless steel (4" X 4" sponge), and plastic (1" X 1" swab). Using POD analysis, no statistically significant differences were observed between the number of positive samples detected by the reference methods and the Solus *Listeria monocytogenes* ELISA using the Dynex DS2 automated immunoassay instrument and manual immunoassay method.

Product consistency was evaluated using three lots of Solus *Listeria monocytogenes* ELISA kits that were at their expiration date, near the middle of the expiration period and recently manufactured. Using the automated method, undiluted *E. faecalis* non-target cultures were found to be negative. No significant difference at the 5% level between the numbers of positive results could be determined by POD analysis between the lots of Solus *Listeria monocytogenes* test kits when tested against diluted *Listeria monocytogenes 4b* cultures at the LOD_{50} of the method. This study supports the Solus *Listeria monocytogenes* current shelf-life of 12 months from date of manufacture. A three-parameter factorial study, where both high and low parameter values were incorporated, plus a combination of the nominal parameter values was used to assess robustness, where a matrix of Deli ham was tested in the presence or absence of *L. monocytogenes.* The POD analysis of Solus *Listeria monocytogenes* ELISA method indicated that there was no significant difference at the 5% level between nominal and experimental combinations using the automated preparation method.

The method offers the benefit to the user of either a manual preparation or automated preparation to obtain results. Each method is specific, quick, and simple to perform, providing results in 3 hours post incubation of the enrichment. The small footprint of both methods offers the ability to test in various laboratories. The Solus *Listeria monocytogenes* ELISA recovered *Listeria monocytogenes* within 24 hours of primary enrichment in Half Fraser broth and 24 hours of secondary enrichment in Solus Palcam broth (48 total hours). A combination of selective media and the Solus *Listeria monocytogenes* ELISA provides users with a sensitive and specific test for the detection of *Listeria monocytogenes* in food and production environments.

Table 1. Detailed Inclusivity Results of the Solus *Listeria monocytogenes* ELISA (1)

Sample No.	Strain	Source	Serotype	Origin	Result
1	*L. monocytogenes*	UVM[a] CWD 1553	1/2c	Unknown	+
2	*L. monocytogenes*	UVM CWD 1554	1/2a	Carlisle 1981	+
3	*L. monocytogenes*	UVM CWD 1563	4b	Lausanne 1987	+
4	*L. monocytogenes*	UVM CWD 1567	4b	LA outbreak 1985	+
5	*L. monocytogenes*	UVM CWD 1571	4b	Not Available	+
6	*L. monocytogenes*	UVM CWD 1590	4b	San Francisco	+
7	*L. monocytogenes*	UVM CWD 1600	3b	Not Available	+
8	*L. monocytogenes*	UVM CWD 1609	1/2a	Turkey frank factory	+
9	*L. monocytogenes*	UVM CWD 1620	1/2a	Turkey frank factory	+
10	*L. monocytogenes*	UVM CWD 1626	1/2b	Oklahoma turkey franks	+
11	*L. monocytogenes*	UVM CWD 1627	1/2b	Mother/baby CWD1628	+
12	*L. monocytogenes*	ATCC[b] 19117	4d	Sheep, USA	+
13	*L. monocytogenes*	ATCC 51772	1/2a	Not Available	+
14	*L. monocytogenes*	ATCC 51778	4b	Dairy products (Belgium)	+
15	*L. monocytogenes*	ATCC 51780	1/2b	Dairy products (cheese)	+
16	*L. monocytogenes*	ATCC BAA-751	1/2b	Not Available	+
17	*L. monocytogenes*	NCTC[c] 10890	7	Human feces	+
18	*L. monocytogenes*	Cornell[d] FSL-F6- 366	4b	Not Available	+
19	*L. monocytogenes*	Cornell FSL J1-129	4ab	Not Available	+
20	*L. monocytogenes*	Cornell FSL J1-049	3c	Not Available	+
21	*L. monocytogenes*	ATCC 7644	1/2c	Human Isolate	+
22	*L. monocytogenes*	ATCC 13932	4b	Spinal fluid of child with meningitis, Germany	+
23	*L. monocytogenes*	ATCC 15313	1/2a	Rabbit, Cambridge, England	+
24	*L. monocytogenes*	ATCC 19111	1	Poultry, England	+
25	*L. monocytogenes*	ATCC 19112	2	Spinal fluid of man, Scotland	+
26	*L. monocytogenes*	ATCC 19113	N/A	Not Available	+
27	*L. monocytogenes*	ATCC 19114	4a	Tissue, animal (ruminant brain)	+
28	*L. monocytogenes*	ATCC 19115	4b	Human	+
29	*L. monocytogenes*	ATCC 19116	4c	Chicken, England	+
30	*L. monocytogenes*	ATCC 19118	4e	Chicken, England	+
31	*L. monocytogenes*	ATCC 43256	N/A	Not Available	+
32	*L. monocytogenes*	ATCC 49594	1/2a	Not Available	+
33	*L. monocytogenes*	ATCC 51782	3a	Dairy products (cheese)	+
34	*L. monocytogenes*	ATCC BAA- 2658	N/A	Not Available	+
35	*L. monocytogenes*	QL[e] 030911-10	N/A	Shellfish	+
36	*L. monocytogenes*	UVM CWD 1561	4b	Mother/baby TS34,TS28 placenta	+
37	*L. monocytogenes*	UVM CWD 1601	1/2b	LA	+
38	*L. monocytogenes*	UVM CWD 1612	1/2a	Turkey frank factory	+

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39	*L. monocytogenes*	UVM CWD 1613	1/a	Turkey frank factory	+
40	*L. monocytogenes*	UVM CWD 1614	1/2a	Oklahoma	+
41	*L. monocytogenes*	UVM CWD 1618	1/2a	Turkey frank factory	+
42	*L. monocytogenes*	UVM CWD 1629	1/2a	Oklahoma turkey franks	+
43	*L. monocytogenes*	UVM CWD 1630	1/2a	Turkey frank factory	+
44	*L. monocytogenes*	UVM CWD 1574	4b	Halifax 1983	+
45	*L. monocytogenes*	UVM CWD 1584	1/2b	Not Available	+
46	*L. monocytogenes*	UVM CWD 1586	3b	Not Available	+
47	*L. monocytogenes*	UVM CWD 1588	1/2b	Not Available	+
48	*L. monocytogenes*	UVM CWD 1596	4b	Not Available	+
49	*L. monocytogenes*	UVM CWD 1597	1/2b	Not Available	+
50	*L. monocytogenes*	UVM CWD 1611	1/2b	Turkey frank factory	+

[a] UVM = University of Vermont, Burlington, VT, [b] ATCC = American Type Culture Collection, Manassas, VA, [c] NCTC = National Collection of Type Cultures, Porton Down, Salisbury, UK, [d] Cornell = Cornell University, Ithaca, NY, [e] QL = Q Laboratories, Cincinnati, OH.

Table 2. Detailed Exclusivity Results of the Solus *Listeria monocytogenes* ELISA (1)

Sample No.	Strain	Source	Origin	Result
1	*Bacillus cereus*	QL[a] 15166-1	Psyllium	-
2	*Bacillus coagulans*	QL 7050	Dairy Products (Evaporated Milk)	-
3	*Bacillus licheniformis*	QL 12759	Plant	-
4	*Bacillus mycoides*	ATCC[b] 6462	Soil	-
5	*Bacillus subtilis* subsp. *subtilis*	ATCC 6051	Not available	-
6	*Brochothrix thermosphacta*	ATCC 11509	Animal-derived foodstuff	-
7	*Enterococcus durans Collins*	ATCC 19432	Not Available	-
8	*Enterococcus faecalis*	ATCC 29212	Human cerebrospinal fluid	-
9	*Enterococcus faecium*	ATCC 19434	Not available	-
10	*Enterococcus hirae*	ATCC 8043	Not Available	-
11	*Escherichia coli*	ATCC 8739	Feces	-
12	*Klebsiella oxytoca*	ATCC 43165	Clinical Isolate	-
13	*Klebsiella pneumoniae*	ATCC 13883	Not Available	-
14	*Kurthia gibsonii*	ATCC 43195	Meat	-
15	*Kurthia zopfii*	ATCC 10538	Not Available	-
16	*Listeria coloradensis*	ATCC BAA-2414	Not available	-
17	*Listeria cornellensis*	FSL[c] F6-0969	Water	-
18	*Listeria fleischmannii*	FSL S10-1203	Not available	-
19	*Listeria floridensis*	FSL S10-1187	Running Water	-
20	*Listeria grandiensis*	FSL F6-0971	Water	-
21	*Listeria grayi*	ATCC 25400	Standing corn stalks and leaves	-
22	*Listeria innocua*	ATCC 43547	Bovine brain	-
23	*Listeria ivanovii*	ATCC 700402	Not available	-
24	*Listeria marthii*	ATCC BAA-1595	Soil	-
25	*Listeria riparia*	FSL S10-1204	Running water	-
26	*Listeria rocourtiae*	FSL F6-0920	Not available	-
27	*Listeria seeligeri*	QL 030911-6	Not available	-
28	*Listeria weihenstephanensis*	FSL R9-0317	Not available	-
29	*Listeria welshimeri*	ATCC 49591	Not available	-
30	*Rhodococcus fascians*	ATCC 12974	Not available	-
31	*Serratia liquifaciens*	ATCC 27592	Milk, Cork, Ireland	-
32	*Staphylococcus aureus*	ATCC 29247	Not available	-
33	*Staphylococcus epidermidis*	ATCC 12228	Not available	-
34	*Staphylococcus haemolyticus*	ATCC 29970	Human Skin	-
35	*Staphylococcus warneri*	ATCC 29885	Not available	-
36	*Streptococcus pneumoniae*	ATCC 6302	Not available	-
37	*Streptococcus pyogenes*	ATCC 19615	Pharynx of child following episode of sore throat.	-

[a] QL = Q Laboratories, Cincinnati, OH., [b] ATCC = American Type Culture Collection, Manassas, VA., [c] Cornell = Cornell University, Ithaca, NY.

Table 3. Solus *Listeria monocytogenes* ELISA Method Results: Candidate vs. Reference – POD Results, 25 g Test Portions (1)

Matrix	Strain	Method	MPN[a]/ Test Portion	N[b]	x[c]	POD$_C$[d]	95% CI	X	POD$_R$[f]	95% CI	dPOD$_C$[g]	95% CI[h]
						Solus *Listeria monocytogenes*			Reference method[e]			
Beef Hot Dogs (25 g)	*L. monocytogenes* ATCC[i] 7644	Dynex DS2 Automated	N/A[j]	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.70 (0.40, 1.17)	20	8	0.40	0.22, 0.61	10	0.50	0.30, 0.70	-0.10	-0.37, 0.19
			2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Sliced Deli Ham (25 g)	*L. monocytogenes* ATCC 19116	Dynex DS2 Automated	N/A	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, 1.04)	20	7	0.35	0.18, 0.57	9	0.45	0.26, 0.66	-0.10	-0.37, 0.19
			1.97 (0.91, 4.27)	5	5	1.00	0.57, 0.100	5	1.00	0.57,1.00	0.00	-0.43, 0.43
Raw Ground Beef (~70% Lean) (25 g)	*L. monocytogenes* UVM[k] CWD 1609	Dynex DS2 Automated	N/A	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, 0.84)	20	10	0.50	0.30, 0.70	7	0.35	0.18, 0.57	0.15	-0.15, 0.41
			3.70 (1.52, 9.02)	5	5	1.00	0.57, 0.100	5	1.00	0.57,1.00	0.00	-0.43, 0.43
Bagged Romaine Lettuce (25 g)	*L. monocytogenes* ATCC 49594	Dynex DS2 Automated	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.50 (0.25, 0.86)	20	7	0.35	0.18, 0.57	8	0.40	0.22, 0.61	-0.05	-0.32, 0.23
			1.97 (0.91, 4.27)	5	5	1.00	0.57, 0.100	5	1.00	0.57,1.00	0.00	-0.43, 0.43
Pasteurized Brie Cheese (25 g)	*L. monocytogenes* CWD 1554	Dynex DS2 Automated	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.51 (0.27, 0.87)	20	10	0.50	0.30, 0.70	7	0.35	0.18, 0.57	0.15	-0.15,0.41
			2.58 (1.15, 5.78)	5	5	1.00	0.57, 0.100	5	1.00	0.57,1.00	0.00	-0.43, 0.43
Frozen Raw Shrimp (25 g)	*L. monocytogenes* QL[l] 030911-10	Dynex DS2 Automated	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.55 (0.29, 0.93)	20	7	0.35	0.18, 0.57	8	0.40	0.22, 0.61	-0.05	-0.32, 0.23
			1.97 (0.91, 4.27)	5	5	1.00	0.57, 0.100	5	1.00	0.57,1.00	0.00	-0.43, 0.43

[a]MPN = Most Probable Number is calculated using the LCF MPN calculator ver. 1.6 provided by AOAC RI, with 95% confidence interval.

[b]N = Number of test portions.

[c]x = Number of positive test portions.

[d]POD$_C$ = Candidate method presumptive positive outcomes that confirmed positive divided by the total number of trials.

[e]Reference method = USDA/FSIS/MLG 8.11 for hot dogs, deli ham and raw ground beef; BAM Ch. 10 for Romaine lettuce, pasteurized Brie cheese and frozen raw shrimp.

[f]POD$_R$ = Reference method confirmed positive outcomes divided by the total number of trials.

[g]dPOD$_C$= Difference between the confirmed candidate method result and reference method confirmed result POD values.

[h]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

[i]ATCC = American Type Culture Collection, Manassas, VA.

[j]N/A = Not applicable.

[k]UVM = University of Vermont, Burlington, VT.

[l]QL = Q Laboratories, Cincinnati, OH.

Table 4. Solus *Listeria monocytogenes* ELISA Method Results: Candidate vs. Reference – POD Results, 125 g Test Portions (1)

Matrix	Strain	Method	MPN[a]/ Test Portion	N[b]	Solus *Listeria monocytogenes*[c]				Reference method[f]			dPOD_C[h]	95% CI[i]
					X[d]	POD_C[e]	95% CI	X	POD_R[g]	95% CI			
Beef Hot Dogs (125 g)	*L. monocytogenes* ATCC 7644	Dynex DS2 Automated	N/A[k]	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.70 (0.40, 1.17)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.28, 0.28
			2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Sliced Deli Ham (125 g)	*L. monocytogenes* ATCC 19116	Dynex DS2 Automated & Manual Immunoassay	N/A	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, 1.04)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.28, 0.28
			1.97 (0.91, 4.27)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Bagged Romaine Lettuce (125 g)	*L. monocytogenes* ATCC 49594	Dynex DS2 Automated & Manual Immunoassay	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.50 (0.25, 0.86)	20	7	0.35	0.18, 0.57	8	0.40	0.22, 0.61	-0.05	-0.32, 0.23
			1.97 (0.91, 4.27)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Pasteurized Brie Cheese (125 g)	*L. monocytogenes* UVM[l] CWD 1554	Dynex DS2 Automated	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.51 (0.27, 0.87)	20	8	0.40	0.22, 0.61	7	0.35	0.18, 0.57	0.05	-0.23, 0.32
			2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

[a]MPN = Most Probable Number is calculated using the LCF MPN calculator ver. 1.6 provided by AOAC RI, with 95% confidence interval.

[b]N = Number of test portions.

[c]Identical results produced for both analyses conducted with the Dynex DS2 Automated instrument and manual immunoassay method.

[d]x = Number of positive test portions.

[e]POD_C = Candidate method presumptive positive outcomes confirmed positive divided by the total number of trials.

[f]Reference method = USDA/FSIS/MLG 8.11 for beef hot dogs, sliced deli ham; BAM Ch. 10 for bagged Romaine lettuce, pasteurized Brie cheese.

[g]POD_R = Reference method confirmed positive outcomes divided by the total number of trials.

[h]dPOD_C = Difference between the confirmed candidate method result and reference method confirmed result POD values.

[i]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

[j]ATCC = American Type Culture Collection, Manassas, VA.

[k]N/A = Not applicable.

[l]UVM = University of Vermont, Burlington, VT.

Table 5. Solus *Listeria monocytogenes* ELISA Method Results: Candidate vs. Reference – POD Results, Environmental Surfaces (1)

Matrix	Strain	Method	CFU[a]/ Test Area	N[b]	X[d]	POD$_C$[e]	95% CI	X	POD$_R$[f]	95% CI	dPOD$_C$[g]	95% CI[h]
						Solus *Listeria monocytogenes*[c]			FDA/BAM Ch. 10			
Stainless Steel (4" x 4")	*L. monocytogenes* ATCC[i] 19117 & *E. faecalis* ATCC 29212	Dynex DS2 Automated & Manual Immunoassay	N/A[j]	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			48 & 500	20	9	0.45	0.26, 0.66	8	0.40	0.22, 0.61	0.05	-0.24, 0.33
			110 & 1200	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Polystyrene (1" x 1")	*L. monocytogenes* ATCC 51782	Dynex DS2 Automated	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			40	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.28, 0.28
			134	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

[a]CFU/Test Area = Results of the CFU/Test area were determined by plating the inoculum for the matrix in triplicate.

[b]N = Number of test portions.

[c]Identical results produced for both analyses conducted with the Dynex DS2 Automated instrument and manual immunoassay method.

[d]x = Number of positive test portions.

[e]POD$_C$ = Candidate method presumptive outcomes confirmed positive divided by the total number of trials.

[f]POD$_R$ = Reference method confirmed positive outcomes divided by the total number of trials.

[g]dPOD$_C$= Difference between the confirmed candidate method result and reference method confirmed result POD values.

[h]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

[i]ATCC = American Type Culture Collection, Manassas, VA.

[j]N/A = Not applicable.

Table 6. Solus *Listeria monocytogenes* Method Results: Presumptive vs. Confirmed – POD Results, 25 g Test Portions (1)

Matrix	Strain	MPN[a]/Test Portion	N[b]	x[c]	POD$_{CP}$[d]	95% CI	X	POD$_{CC}$[e]	95% CI	dPOD$_{CP}$[f]	95% CI[g]
					Solus *Listeria monocytogenes* Presumptive			Solus *Listeria monocytogenes* Confirmed			
Beef Hot Dogs (25 g)	*L. monocytogenes* ATCC[h] 7644	N/A[i]	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.70 (0.40, 1.17)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.13, 0.13
		2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Sliced Deli Ham (25 g)	*L. monocytogenes* ATCC 19116	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.61 (0.33, 1.04)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.13, 0.13
		1.97 (0.91, 4.27)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Raw Ground Beef (~70% Lean) (25 g)	*L. monocytogenes* UVM[j] CWD 1609	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.49 (0.25, 0.84)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.13, 0.13
		3.70 (1.52, 9.02)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Bagged Romaine Lettuce (25 g)	*L. monocytogenes* ATCC 49594	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.50 (0.25, 0.86)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.13, 0.13
		1.97 (0.91, 4.27)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Pasteurized Brie Cheese (25 g)	*L. monocytogenes* CWD 1554	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.51 (0.27, 0.87)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.13, 0.13
		2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Frozen Raw Shrimp (25 g)	*L. monocytogenes* QL[k] 030911-10	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.55 (0.29, 0.93)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.13, 0.13
		1.97 (0.91, 4.27)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47

[a]MPN = Most Probable Number is calculated using the LCF MPN calculator ver. 1.6 provided by AOAC RI, with 95% confidence interval.

[b]N = Number of test portions.

[c]x = Number of positive test portions.

[d]POD$_{CP}$ = Candidate method presumptive positive outcomes divided by the total number of trials.

[e]POD$_{CC}$ = Candidate method confirmed positive outcomes divided by the total number of trials.

[f]dPOD$_{CP}$= Difference between the candidate method presumptive result and candidate method confirmed result POD values.

[g]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

[h]ATCC = American Type Culture Collection, Manassas, VA.

[i]N/A = Not applicable.

[j]UVM = University of Vermont, Burlington, VT.

[k]QL = Q Laboratories, Cincinnati, OH.

Table 7. Solus *Listeria monocytogenes* Method Results: Presumptive vs. Confirmed – POD Results, 125 g Test Portions (1)

Matrix	Strain	MPN[a]/ Test Portion	N[b]	x[c]	Solus *Listeria monocytogenes* Presumptive POD_CP[d]	95% CI	X	Solus *Listeria monocytogenes* Confirmed POD_CC[e]	95% CI	dPOD_CP[f]	95% CI[g]
Beef Hot Dogs (125 g)	*L. monocytogenes* ATCC[h] 7644	N/A[i]	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.70 (0.40, 1.17)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.13, 0.13
		2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Sliced Deli Ham (125 g)	*L. monocytogenes* ATCC 19116	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.61 (0.33, 1.04)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.13, 0.13
		1.97 (0.91, 4.27)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Bagged Romaine Lettuce (125 g)	*L. monocytogenes* ATCC 49594	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.50 (0.25, 0.86)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.13, 0.13
		1.97 (0.91, 4.27)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Pasteurized Brie Cheese (125 g)	*L. monocytogenes* UVM[j] CWD 1554	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.51 (0.27, 0.87)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.13, 0.13
		2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47

[a]MPN = Most Probable Number is calculated using the LCF MPN calculator ver. 1.6 provided by AOAC RI, with 95% confidence interval.
[b]N = Number of test portions.
[c]x = Number of positive test portions.
[d]POD_CP = Candidate method presumptive positive outcomes divided by the total number of trials.
[e]POD_CC = Candidate method confirmed positive outcomes divided by the total number of trials.
[f]dPOD_CP = Difference between the candidate method presumptive result and candidate method confirmed result POD values.
[g]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[h]ATCC = American Type Culture Collection, Manassas, VA.
[i]N/A = Not applicable.
[j]UVM = University of Vermont, Burlington, VT.

Table 8. Solus *Listeria monocytogenes* Method Results, Presumptive vs. Confirmed- POD Results - Environmental Surfaces (1)

Matrix	Strain	CFU[a]/ Test Area	N[b]	x[c]	Presumptive POD_CP[d]	95% CI	X	Confirmed POD_CC[e]	95% CI	dPOD_CP[f]	95% CI[g]
Stainless Steel (4" x 4")	*L. monocytogenes* ATCC[h] 19117 & *Enterococcus faecalis* ATCC 29212	N/A[i]	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		48 & 500	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.13, 0.13
		110 & 1200	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Polystyrene (1" x 1")	*L. monocytogenes* ATCC 51782	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		40	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.13, 0.13
		134	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47

[a]CFU/Test Area = Results of the CFU/Test area were determined by plating the inoculum for the matrix in triplicate.
[b]N = Number of test portions.
[c]x = Number of positive test portions.
[d]POD_CP = Candidate method presumptive positive outcomes divided by the total number of trials.
[e]POD_CC = Candidate method confirmed positive outcomes divided by the total number of trials.
[f]dPOD_CP = Difference between the candidate method presumptive result and candidate method confirmed result POD values.
[g]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[h]ATCC = American Type Culture Collection, Manassas, VA.
[i]N/A = Not applicable.

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