CERTIFICATION

AOAC® *Performance Tested*SM

Certificate No.

041601

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

Solus Scientific *Listeria* ELISA

manufactured by

Solus Scientific Ltd.
Unit 9 Mansfield Networkcentre
Millennium Business Park
Concorde Way, Mansfield
Nottinghamshire, NG9 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 (December 05, 2018 – December 31, 2019). 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 05, 2018
Date

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SUBMITTING COMPANY
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United Kingdom

KIT NAME(S)
Solus Scientific *Listeria* ELISA

CATALOG NUMBERS
LIS 0480S, LIS 0096S

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APPLICABILITY OF METHOD
Target organism – *Listeria* species (*L. grayi, L. innocua, L. ivanovii, L. monocytogenes, L. seeligeri, L. welshimeri*)

Matrixes – (25g) – bagged romaine lettuce, hot dogs, frozen raw shrimp, smoked salmon, soft cheese (brie), stainless steel (swab, 1 x 1 in), polystyrene (sponge, 4 x 4 in)

Performance claims - The Solus Scientific *Listeria* ELISA method is considered equivalent to the reference methods.

REFERENCE METHODS
United States Department of Agriculture Microbiological Laboratory Guidelines 8.09: *Isolation and Identification of Listeria monocytogenes from Red Meat, Poultry, Egg Products, and Environmental Sponges*. May 1st, 2013. (Accessed October 2015)
http://www.fsis.usda.gov/wps/wcm/connect/1710bee8-76b9-4e6c-92fc-fdc290dbfa92/MLG-8.pdf?MOD=AJPERES (2)
Food and Drug Administration Bacteriological Analytical Manual Chapter 10: *Detection and Enumeration of Listeria monocytogenes in Foods*. February, 2013. (Accessed October 2015)
http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm071400.htm (3)

ORIGINAL CERTIFICATION DATE
April 04, 2016

CERTIFICATION RENEWAL RECORD
Renewed annually through December 2019

METHOD MODIFICATION RECORD
1. **January 2018 Level 1**
2. **December 2018 Level 1**

SUMMARY OF MODIFICATION
1. **Reformatting and editorial changes**
2. **Editorial/clerical changes to text to bring the language and style in line with more recently approved products.**

Under this AOAC® *Performance Tested*SM License Number, 041601 this method is distributed by:

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PRINCIPLE OF THE METHOD (1)
The Solus Scientific *Listeria* ELISA method is used for the rapid and specific detection of *Listeria* species in enriched food and environmental samples. The Solus Scientific *Listeria* ELISA method relies on antibodies attached to the wells of the microplate strips that are specific to *Listeria* antigens. Samples are heat treated and an aliquot is added to the antibody coated wells.
Listeria specific antigens present in the samples will bind immunologically to the antibody. After washing to remove unbound material, an enzyme-labeled antibody will bind to the proteins and thus to the well. After a second wash step to remove any unbound enzyme-antibody, the 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 with the addition of dilute sulfuric acid changing any blue color present in the wells to yellow. [4]

DISCUSSION OF THE VALIDATION STUDY (1)
The Solus Scientific *Listeria* ELISA methods successfully recovered *Listeria* species from all of the food matrixes and environmental surfaces analyzed. Using POD analysis, no statistically significant differences were observed between the number of positive samples detected by the candidate method and the reference methods for all matrixes and environmental surfaces tested.
The results of the inclusivity and exclusivity evaluation demonstrated 100% agreement with expected results for the test panels, and confirmed the high specificity and selectivity of the method to *Listeria* species
The method offers the benefit of the use of the manual immunoassay method and the automated immunoassay method to obtain results. Each method is quick and simple to perform, providing results in less than 2 hours post incubation of the selective enrichment. The small footprint of both methods offers the ability to test in various laboratories. The Dynex DS2 software is user friendly with the ability to track lot information and sample identification quickly and with ease. The Dynex DS2 software and instrument also offer the ability to run multiple assays at one time and has an open platform.

Table 1: Inclusivity Results (1)

Organism	Source	Origin	Result	Organism	Source	Origin	Result
Listeria grayi	NCTC[1] 19120	Animal Feces	+	*Listeria monocytogenes*	CWD[5] 1552 1/2C	Not Available	+
Listeria grayi	ATCC[2] 25401	Corn Stalks	+	*Listeria monocytogenes*	CWD[5] 1553 1/2C	Not Available	+
Listeria grayi	ATCC[2] 700545	Not Available	+	*Listeria monocytogenes*	CWD[5] 1554 1/2A	Carlisle 1981e	+
Listeria innocua	QL[3] 030911-12	Environmental	+	*Listeria monocytogenes*	CWD[5] 1555 1/2A	Carlisle 1981	+
Listeria innocua	QL[3] 051111-1	Environmental	+	*Listeria monocytogenes*	CWD[5] 1561 4B	Human Placenta	+
Listeria innocua	QL[3] 32811.2	Seasoning Powder	+	*Listeria monocytogenes*	CWD[5] 1563 4B	Lausanne	+
Listeria innocua	ATCC[2] 33091	Human Feces	+	*Listeria monocytogenes*	CWD[5] 1590 4B	San Francisco	+
Listeria innocua	QL[3] 32911.1	Environmental	+	*Listeria monocytogenes*	CWD[5] 1611 1/2A	Turkey	+
Listeria innocua	CSU[4] W1-301	Not Available	+	*Listeria monocytogenes*	CWD[5] 1613 1/2A	Turkey	+
Listeria innocua	CSU[4] W1-305	Not Available	+	*Listeria monocytogenes*	CWD[5] 1614 1/2A	Oklahoma	+
Listeria ivanovii	ATCC[2] 49954	Food, France	+	*Listeria monocytogenes*	CWD[5] 1626 1/2B	Oklahoma	+
Listeria ivanovii	ATCC[2] BAA-678	Sheep Fetus	+	*Listeria monocytogenes*	CWD[5] 1627 1/2B	Mother/Baby	+
Listeria ivanovii	ATCC[2] Liv004	Not Available	+	*Listeria monocytogenes*	CWD[5] 1629 1/2A	Oklahoma	+
Listeria ivanovii	ATCC[2] Liv005	Not Available	+	*Listeria monocytogenes*	CWD[5] 1630 1/2A	Turkey	+
Listeria ivanovii	QL[3] 030911-9	Clinical Isolate	+	*Listeria monocytogenes*	QL[3] 030911-10	Shellfish	+
Listeria monocytogenes	ATCC[2] 7644 1/2C	Human Isolate	+	*Listeria seeligeri*	ATCC[2] 11289 6B	Human Feces	+
Listeria monocytogenes	ATCC[2] 13932 4B	Spinal Fluid	+	*Listeria seeligeri*	ATCC[2] 11856	Not Available	+
Listeria monocytogenes	ATCC[2] 15313 1/2A	Rabbit	+	*Listeria seeligeri*	ATCC[2] 35967 1/2B	Soil	+
Listeria monocytogenes	ATCC[2] 19114 4A	Animal Tissue	+	*Listeria seeligeri*	FSL[6]-S4-035	Not Available	+
Listeria monocytogenes	ATCC[2] 19115 4B	Human Isolate	+	*Listeria seeligeri*	QL[3] 030911-2	Creamer	+
Listeria monocytogenes	ATCC[2] 19117 4D	Sheep	+	*Listeria welshimeri*	ATCC[2] 35897	Not Available	+
Listeria monocytogenes	ATCC[2] 49594 1/2A	Not Available	+	*Listeria welshimeri*	ATCC[2] 43548 6A	Not Available	+
Listeria monocytogenes	ATCC[2] 51778 4B	Dairy Products	+	*Listeria welshimeri*	ATCC[2] 43549 6B	Soil	+
Listeria monocytogenes	ATCC[2] 51780 1/2B	Dairy Products	+	*Listeria welshimeri*	ATCC[2] 43550 1/2B	Human Feces	+
Listeria monocytogenes	ATCC[2] Li2 4B	Human Isolate	+	*Listeria welshimeri*	LW[7] 003	Not Available	+

+ = Indicates detection of the target analyte.
[1]NCTC-National Culture Type Collection
[2]ATCC-American Type Culture Collection
[3]QL-Q Laboratories Inc. Culture Collection
[4]CSU-Colorado State Culture Collection
[5]CWD-University of Vermont Culture Collection
[6]FSL-Cornell University Culture Collection
[7]LW-University of Vermont Culture Collection

Table 2: Exclusivity Results (1)

Organism	Source	Origin	Result	Organism	Source	Origin	Result
Bacillus mycoides	ATCC[1] 6462	Soil	-	*Lactobacillus fermentum*	ATCC[1] 9338	Not Available	-
Brochothrix thermosphacta	ATCC[1] 11509	Pork Sausage	-	*Lactobacillus lactis*	ATCC[1] 4797	Not Available	-
Bacillus cereus	ATCC[1] 14579	Not Available	-	*Lactobacillus plantarum*	ATCC[1] 8014	Not Available	-
Geobacillus stearothermophilus	ATCC[1] 12980	Not Available	-	*Micrococcus luteus*	ATCC[1] 7468	Not Available	-
Rhodococcus fascians	ATCC[1] 12974	Not Available	-	*Proteus mirabilis*	ATCC[1] 7002	Urine	-
Enterococcus hirae	ATCC[1] 8043	Not Available	-	*Streptococcus mutans*	ATCC[1] 25715	Not Available	-
Enterococcus faecium	ATCC[1] 19434	Not Available	-	*Rhodococcus equi*	ATCC[1] 6939	Lung Abscess	-
Enterococcus durans	ATCC[1] 19432	Not Available	-	*Salmonella* Typhimurium	ATCC[1] 14028	Chicken Hearts and Livers	-
Enterococcus faecalis	ATCC[1] 29212	Urine	-	*Bacillus subtilis*	ATCC[1] 6051	Not Available	-
Kurthia gibsonii	ATCC[1] 43195	Not Available	-	*Staphylococcus aureus*	ATCC[1] 29247	Not Available	-
Escherichia coli	ATCC[1] 8739	Feces	-	*Staphylococcus epidermidis*	ATCC[1] 12228	Not Available	-
Klebsiella oxytoca	ATCC[1] 43165	Clinical Isolate	-	*Staphylococcus haemolyticus*	ATCC[1] 29970	Human Skin	-
Klebsiella pneumoniae	ATCC[1] 13883	Not Available	-	*Staphylococcus warneri*	ATCC[1] 29885	Not Available	-
Kurthia zopfii	ATCC[1] 10538	Not Available	-	*Streptococcus pneumoniae*	ATCC[1] 6302	Not Available	-
Lactobacillus casei	ATCC[1] 11578	Oral Cavity	-	*Streptococcus pyogenes*	ATCC[1] 19615	Pharynx of Child	-

- = Indicates no detection of the target analyte.
[1]ATCC-American Type Culture Collection

Table 17: Solus Scientific *Listeria* ELISA, Candidate vs. Reference – POD Results (1)

Matrix/Test Portion	Strain	ELISA Method	MPN[a]/ Test Portion	N[b]	Candidate			Reference			dPOD$_C$[f]	95% CI[g]
					x[c]	POD$_C$[d]	95% CI	X	POD$_R$[e]	95% CI		
Hot Dogs (All Beef) (25 g)	*Listeria monocytogenes* CWD 1618	Manual	-	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, 0.94)	20	6	0.30	0.15, 0.52	8	0.40	0.22, 0.61	-0.10	-0.36, 0.18
			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/Test Portion	**Strain**	**ELISA Method**	**MPN[a]/ Test Portion**	**N[b]**	**Candidate**			**Reference**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
Hot Dogs (All Beef) (25 g)	*Listeria monocytogenes* CWD 1618	Automated	-	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, 0.94)	20	6	0.30	0.15, 0.52	8	0.40	0.22, 0.61	-0.10	-0.36, 0.18
			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/Test Portion	**Strain**	**ELISA Method**	**MPN[a]/ Test Portion**	**N[b]**	**Candidate**			**Reference**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
Frozen Raw Shrimps (25 g)	*Listeria innocua* ATCC 33091	Manual	-	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, 0.94)	20	6	0.30	0.15, 0.52	8	0.40	0.22, 0.61	-0.10	-0.36, 0.18
			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/Test Portion	**Strain**	**ELISA Method**	**MPN[a]/ Test Portion**	**N[b]**	**Candidate**			**Reference**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
Smoked Salmon (25 g)	*Listeria welshimeri* ATCC 35897	Manual	-	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	9	0.45	0.26, 0.66	7	0.35	0.18, 0.57	0.10	-0.19, 0.37
			2.29 (1.04, 5.02)	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 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 confirmed positive outcomes divided by the total number of trials
[e]POD$_R$ = Reference method confirmed positive outcomes divided by the total number of trials
[f]dPOD$_C$= Difference between the confirmed candidate method result and reference 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

Table 18: Solus Scientific *Listeria* ELISA, Candidate vs. Reference – POD Results (1)

Matrix/Test Portion	Strain	ELISA Method	MPN[a]/ Test Portion	N[b]	Candidate			Reference			dPOD$_C$[f]	95% CI[g]
					x[c]	POD$_C$[d]	95% CI	X	POD$_R$[e]	95% CI		
Bagged Romaine Lettuce (25 g)	*Listeria seeligeri* ATCC 35967	Manual	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.34 (0.14, 0.62)	20	3	0.15	0.05, 0.36	6	0.30	0.14, 0.52	-0.15	-0.39, 0.11
			2.29 (1.04, 5.02)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix/Test Portion	**Strain**	**ELISA Method**	**MPN[a]/ Test Portion**	**N[b]**	**Candidate**			**Reference**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
Soft Cheese (Brie) (25 g)	*Listeria monocytogenes* ATCC 51782	Manual	-	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/Test Portion	**Strain**	**ELISA Method**	**MPN[a]/ Test Portion**	**N[b]**	**Candidate**			**Reference**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
Soft Cheese (Brie) (25 g)	*Listeria monocytogenes* ATCC 51782	Automated	-	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

[a]MPN = Most Probable Number is calculated using the LCF MPN calculator 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 confirmed positive outcomes divided by the total number of trials

[e]POD_R = Reference method confirmed positive outcomes divided by the total number of trials
[f]$dPOD_C$= Difference between the confirmed candidate method result and reference 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

Table 19: Solus Scientific *Listeria* ELISA, Candidate vs. Reference – POD Results (1)

Matrix	Strain	ELISA Method	CFU[a]/ Test Area	N[b]	Candidate			Reference			dPOD$_C$[f]	95% CI[g]
					x[c]	POD$_C$[d]	95% CI	X	POD$_R$[e]	95% CI		
Stainless Steel	*Listeria monocytogenes* ATCC 19115 & *Enterococcus faecalis* ATCC 29212	Manual	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			41 & 170	20	8	0.40	0.22, 0.61	6	0.30	0.15, 0.52	0.10	-0.18, 0.36
			550 & 1300	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	**Strain**	**ELISA Method**	**CFU[a]/ Test Area**	**N[b]**	**Candidate**			**Reference**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
Stainless Steel	*Listeria monocytogenes* ATCC 19115 & *Enterococcus faecalis* ATCC 29212	Automated	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			41 & 170	20	8	0.40	0.22, 0.61	6	0.30	0.15, 0.52	0.10	-0.18, 0.36
			550 & 1300	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	**Strain**	**ELISA Method**	**CFU[a]/ Test Area**	**N[b]**	**Candidate**			**Reference**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
Polystyrene	*Listeria innocua* ATCC BAA-680 & *Enterococcus faecalis* ATCC 51299	Manual	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			35 & 200	20	7	0.35	0.18, 0.57	8	0.40	0.22, 0.61	-0.05	-0.32, 0.23
			790 & 5300	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 each matrix in triplicate
[b]N = Number of test portions
[c]x = Number of positive test portions
[d]POD_C = Candidate method confirmed positive outcomes divided by the total number of trials
[e]POD_R = Reference method confirmed positive outcomes divided by the total number of trials
[f]$dPOD_C$= Difference between the confirmed candidate method result and reference 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

Table 20: Solus Scientific *Listeria* ELISA, Presumptive vs. Alternative Confirmed – POD Results (1)

Matrix/Test Portion	Strain	ELISA Method	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		
Hot Dogs (All Beef) (25 g)	*Listeria monocytogenes* CWD 1618	Manual	-	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, 0.94)	20	6	0.30	0.15, 0.52	6	0.30	0.15, 0.52	0.00	-0.27, 0.27
			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/Test Portion	**Strain**	**ELISA Method**	**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**		
Hot Dogs (All Beef) (25 g)	*Listeria monocytogenes* CWD 1618	Automated	-	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, 0.94)	20	6	0.30	0.15, 0.52	6	0.30	0.15, 0.52	0.00	-0.27, 0.27
			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/Test Portion	**Strain**	**ELISA Method**	**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**		
Frozen Raw Shrimps (25 g)	*Listeria innocua* ATCC 33091	Manual	-	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, 0.94)	20	6	0.30	0.15, 0.52	6	0.30	0.15, 0.52	0.00	-0.27, 0.27
			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/Test Portion	**Strain**	**ELISA Method**	**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**		
Smoked Salmon (25 g)	*Listeria welshimeri* ATCC 35897	Manual	-	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	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.28, 0.28
			2.29 (1.04, 5.02)	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 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

Table 21: Solus Scientific *Listeria* ELISA, Presumptive vs. Alternative Confirmed – POD Results (1)

Matrix/Test Portion	Strain	ELISA Method	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		
Bagged Romaine Lettuce (25 g)	*Listeria seeligeri* ATCC 35967	Manual	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.34 (0.14, 0.62)	20	3	0.15	0.05, 0.36	3	0.15	0.05, 0.36	0.00	-0.23, 0.23
			2.29 (1.04, 5.02)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix/Test Portion	**Strain**	**ELISA Method**	**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**		
Soft Cheese (Brie) (25 g)	*Listeria monocytogenes* ATCC 51782	Manual	-	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/Test Portion	**Strain**	**ELISA Method**	**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**		
Soft Cheese (Brie) (25 g)	*Listeria monocytogenes* ATCC 51782	Automated	-	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

[a]MPN = Most Probable Number is calculated using the LCF MPN calculator 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

Table 22: Solus Scientific *Listeria* ELISA, Presumptive vs. Alternative Confirmed – POD Results (1)

Matrix	Strain	ELISA Method	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	*Listeria monocytogenes* ATCC 19115 & *Enterococcus faecalis* ATCC 29212	Automated	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			41 & 170	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.28, 0.28
			550 & 1300	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	**Strain**	**ELISA Method**	**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	*Listeria monocytogenes* ATCC 19115 & *Enterococcus faecalis* ATCC 29212	Manual	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			41 & 170	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.25, 0.25
			550 & 1300	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	**Strain**	**ELISA Method**	**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**		
Polystyrene	*Listeria innocua* ATCC BAA-680 & *Enterococcus faecalis* ATCC 51299	Manual	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			35 & 200	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.27, 0.27
			790 & 5300	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 each 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

Table 23: Solus Scientific *Listeria* ELISA, Presumptive vs. Traditional Confirmed – POD Results (1)

Matrix/Test Portion	Strain	ELISA Method	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		
Hot Dogs (All Beef) (25 g)	*Listeria monocytogenes* CWD 1618	Manual	-	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, 0.94)	20	6	0.30	0.15, 0.52	6	0.30	0.15, 0.52	0.00	-0.27, 0.27
			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/Test Portion	**Strain**	**ELISA Method**	**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**		
Hot Dogs (All Beef) (25 g)	*Listeria monocytogenes* CWD 1618	Automated	-	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, 0.94)	20	6	0.30	0.15, 0.52	6	0.30	0.15, 0.52	0.00	-0.27, 0.27
			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/Test Portion	**Strain**	**ELISA Method**	**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**		
Frozen Raw Shrimps (25 g)	*Listeria innocua* ATCC 33091	Manual	-	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, 0.94)	20	6	0.30	0.15, 0.52	6	0.30	0.15, 0.52	0.00	-0.27, 0.27
			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/Test Portion	**Strain**	**ELISA Method**	**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**		
Smoked Salmon (25 g)	*Listeria welshimeri* ATCC 35897	Manual	-	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	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.28, 0.28
			2.29 (1.04, 5.02)	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 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

Table 24: Solus Scientific *Listeria* ELISA, Presumptive vs. Traditional Confirmed – POD Results (1)

Matrix/Test Portion	Strain	ELISA Method	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		
Bagged Romaine Lettuce (25 g)	*Listeria seeligeri* ATCC 35967	Manual	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.34 (0.14, 0.62)	20	3	0.15	0.05, 0.36	3	0.15	0.05, 0.36	0.00	-0.23, 0.23
			2.29 (1.04, 5.02)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix/Test Portion	**Strain**	**ELISA Method**	**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**		
Soft Cheese (Brie) (25 g)	*Listeria monocytogenes* ATCC 51782	Manual	-	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/Test Portion	**Strain**	**ELISA Method**	**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**		
Soft Cheese (Brie) (25 g)	*Listeria monocytogenes* ATCC 51782	Automated	-	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

[a] MPN = Most Probable Number is calculated using the LCF MPN calculator 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

Table 25: Solus Scientific *Listeria* ELISA, Presumptive vs. Traditional Confirmed – POD Results (1)

Matrix	Strain	ELISA Method	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	*Listeria monocytogenes* ATCC 19115 & *Enterococcus faecalis* ATCC 29212	Automated	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			41 & 170	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.28, 0.28
			550 & 1300	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	**Strain**	**ELISA Method**	**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	*Listeria monocytogenes* ATCC 19115 & *Enterococcus faecalis* ATCC 29212	Manual	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			41 & 170	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.28, 0.28
			550 & 1300	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	**Strain**	**ELISA Method**	**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**		
Polystyrene	*Listeria innocua* ATCC BAA-680 & *Enterococcus faecalis* ATCC 51299	Manual	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			35 & 200	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
			790 & 5300	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 each 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

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