AOAC
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CERTIFICATION

AOAC® *Performance Tested*^{SM}

Certificate No.

121302

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

Veriflow^{TM} *Listeria* spp.

manufactured by

Invisible Sentinel, Inc.
3711 Market Street, 8th Floor
Philadelphia, PA 19104
USA

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 (January 15, 2017 – December 31, 2017). Renewal may be granted at the end of one year under the rules stated in the licensing agreement.

Deborah McKenzie

Deborah McKenzie, Senior Director
Signature for AOAC Research Institute

January 15, 2017

Date

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MODIFICATION FEBRUARY 2016: Ken Huang and Adam C. Joelsson

SUBMITTING COMPANY
Invisible Sentinel, Inc.
3711 Market Street, 8th Floor
Philadelphia, PA 19104

KIT NAME(S)
VeriflowTM *Listeria* spp.

INDEPENDENT LABORATORY
Q Laboratories, Inc.
1400 Harrison Ave.
Cincinnati, OH 45214
USA

AOAC EXPERTS AND PEER REVIEWERS
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[4] January 2016 and February 2016 modification

APPLICABILITY OF METHOD
Target organism – *Listeria* species
Matrices – Deli turkey (125 g), beef hot dogs (25 g), whey protein isolate (25 g)
(1 x 1 in swab, 4 x 4 in sponge): stainless steel, ceramic tile, sealed concrete, plastic
(4 x 4 sponge pre-moistened with neutralizing broth) - stainless steel
Performance claims - The Veriflow® *LS* system allows for the rapid presumptive detection of *Listeria* species with equivalent performance as compared to the reference methods.

REFERENCE METHODS
U.S. Department of Agriculture, Food Safety and Inspection Service (2012)
Microbiology Laboratory Guidebook, Chapter 8.08, *Isolation and Identification of Listeria monocytogenes from Red Meat, Poultry and Egg Products, and Environmental Samples* . (3)
Food and Drug Administration Bacterial Analytical Manual Chapter 10 (2011)
"Detection and Enumeration of *Listeria monocytogenes* in Foods".(10)

ORIGINAL CERTIFICATION DATE
December 31, 2013

CERTIFICATION RENEWAL RECORD
Renewed Annually through December 2017

METHOD MODIFICATION RECORD
1. **January 2016**
2. **February 2016**

SUMMARY OF MODIFICATION
1. **Modification to use pre-moistened (neutralizing broth) sponges**
2. **Modification to include whey protein isolate**

Under this AOAC® *Performance Tested*SM License Number, 121302 this method is distributed by:
NONE

Under this AOAC® *Performance Tested*SM License Number, 121302 this method is distributed as:
NONE

PRINCIPLE OF THE METHOD (1)
Veriflow® *Listeria* species *(LS)* (Cat no. IS1004) is a molecular based test that detects *Listeria* species including the foodborne pathogen *Listeria monocytogenes,* in environmental (stainless steel, sealed concrete, plastic, ceramic tile) and RTE (hot dogs and deli meat) food matrices (3).   The method combines polymerase chain reaction (PCR) with a rapid, chromatographic vertical flowthrough system that provides specific and highly sensitive detection of target associated molecular signatures coupled with rapid, easy-to-interpret results.

In this study, artificially inoculated environmental surfaces (stainless steel, sealed concrete, plastic, ceramic tile) and RTE food (hot dogs and deli meat) matrices were sampled, enriched and subjected to PCR amplification leading to the generation of a *Listeria* species-*specific* analyte. For final analysis, the PCR generated analyte is applied directly to the sample window of the assay cassette, and the signal is allowed to develop for a total of 3 minutes, after which the cassette switch is retracted to remove the conjugate pad and reveal the underlying test membrane and results. In the event of a positive sample, the target analyte is captured and immobilized on the nitrocellulose test membrane and detected by a colloidal gold-protein conjugate, which generates a visual signal at the test line. The aggregation of the colloidal gold results in a distinct red line in the area indicated as "T" on the test cassette. A control line will also develop, indicated as "C" on the test cassette, and reacts only with the colloidal gold conjugate providing the user an indication that the test was run properly. The appearance of two distinct red lines is indicative of a positive sample for *Listeria* species; whereas appearance of just the control line indicates a negative sample.

DISCUSSION OF THE VALIDATION STUDY (1)
The results of this study demonstrated the specificity, accuracy and reliability of the Veriflow® *LS* assay as compared to the traditional USDA/FSISI MLG 8.08 culture based reference method (1) for the detection of *Listeria* species on environmental surfaces (stainless steel, sealed concrete, plastic, ceramic tile) and in RTE (hot dogs and deli meat) foods. POD statistical analysis of all seven matrices tested indicate that there is no significant difference in performance between the methods at specific time points as assayed in this study. The successful validation of the assay is further supported by the results of the inclusivity and exclusivity testing, indicating that the Veriflow® *LS* assay was able to accurately detect *Listeria* species isolates while correctly excluding all non-specific bacteria tested.

The Veriflow® *LS* assay provides flexibility and ease of use for the end user by providing accurate results across multiple surfaces with sampling by either swabs or sponges, and across multiple food matrices, without complex sample preparation after enrichment. The Veriflow® system also offers significant savings in time compared to the USDA/FSIS MLG 8.08 reference method (1), by producing accurate presumptive results after an enrichment time of only 24 hours, as compared to the reference methods that require 3-4 days to reach presumptive results. The robustness and lot-to-lot stability data also indicated that the assay is reproducible and rugged and that it can be manufactured

uniformly and consistently. Thus the results of this study demonstrated that the easy to follow Veriflow® *LS* protocol provides for a sensitive, reliable and simple to use rapid detection method for *Listeria monocytogenes*.

	Table 3. Veriflow® LS Inclusivity Evaluation (1)					
No.	Organism	Strain Reference Number	Serotype	Source	IS Presumptive Result	Reference Confirmed Result
1	*Listeria grayi*[1]	ATCC 19120	NA[7]	Feces, Chincilla, Denmark	+	+
2	*Listeria grayi*[1]	ATCC 25401	NA	Standing Corn Stalks	+	+
3	*Listeria grayi*[1]	ATCC 25402	NA	Standing Corn Stalks	+	+
4	*Listeria grayi*[1]	ATCC 25403	NA	Standing Corn Stalks	+	+
5	*Listeria innocua*[2]	NCTC 10528	4AB	Standing Corn Stalks	+	+
6	*Listeria innocua*[1]	ATCC 33090	6A	Unknown	+	+
7	*Listeria innocua*[1]	ATCC 33091	6B	Cow Brain	+	+
8	*Listeria innocua*[1]	ATCC 51742	4B	Feces, pregnant woman	+	+
9	*Listeria innocua*[1]	ATCC BAA-680	6A	Cabbage	+	+
10	*Listeria ivanovii*[1]	ATCC 19119	NA	Cheese, Mexico	+	+
11	*Listeria ivanovii*[1]	ATCC 49953	NA	Sheep, Bulgaria	+	+
12	*Listeria ivanovii*[1]	ATCC 49954	NA	Goat, Belgium	+	+
13	*Listeria ivanovii*[1]	ATCC BAA-139	NA	Food, France	+	+
14	*Listeria ivanovii*[1]	ATCC BAA-678	5	Washing Water, Switzerland	+	+
15	*Listeria seeligeri*[1]	ATCC 35967	1/2B	Sheep fetus	+	+
16	*Listeria seeligeri*[1]	ATCC 51334	NA	Soil, Germany	+	+
17	*Listeria seeligeri*[1]	ATCC 51335	4A	Intestinal contents of Clethrionomys glareolus, Bratislava, Slovakia	+	+
18	*Listeria seeligeri*[2]	NCTC 11889	NA	Unknown	+	+
19	*Listeria seeligeri*[1]	ATCC 11289	6B	Unknown	+	+
20	*Listeria welshimeri*[2]	NCTC 11857	NA	Feces, pregnant woman	+	+
21	*Listeria welshimeri*[1]	ATCC 35897	6B	Plant	+	+
22	*Listeria welshimeri*[3]	ATCC 43549	NA	Unknown	+	+
23	*Listeria welshimeri*[1]	ATCC 43550	6B	Unknown	+	+
24	*Listeria welshimeri*[1]	ATCC 43551	1/2B	Soil	+	+
25	*Listeria grayi sbsp Murrayi*[2]	NCTC 10812	NA	Feces, man	+	+
26	*Listeria monocytogenes*[4]	CWD 1563	4B	Unknown	+	+
27	*Listeria monocytogenes*[4]	CWD 1567	4B	Lausanna, 1987	+	+
28	*Listeria monocytogenes*[4]	CWD 1571	4B	LA outbreak 1985	+	+
29	*Listeria monocytogenes*[4]	CWD 1574	4B	Unknown	+	+
30	*Listeria monocytogenes*[4]	CWD 1584	1/2B	Halifax, 1983	+	+

31	*Listeria monocytogenes*[4]	CWD 1586	3B	Unknown	+	+
32	*Listeria monocytogenes*[4]	CWD 1588	1/2B	Unknown	+	+
33	*Listeria monocytogenes*[4]	FSL J1-049[2]	NA	Unknown	+	+
34	*Listeria monocytogenes*[4]	CWD 1589	1/2B	LA, USA	+	+
35	*Listeria monocytogenes*[5]	NCIMB# 13726	4B	Unknown	+	+
36	*Listeria monocytogenes*[2]	NCTC# 10890	7	Human feces	+	+
37	*Listeria monocytogenes*[1]	BAA-751	1/2B	Unknown	+	+
38	*Listeria monocytogenes*[1]	ATCC 19111	NA	Poultry, England	+	+
39	*Listeria monocytogenes*[1]	ATCC 19112	NA	Spinal fluid of man, Scotland	+	+
40	*Listeria monocytogenes*[1]	ATCC# 19114	4A	Ruminant Brain	+	+
41	*Listeria monocytogenes*[1]	ATCC# 19115	4B	Human Isolate	+	+
42	*Listeria monocytogenes*[1]	ATCC# 19116	4C	Chicken, England	+	+
43	*Listeria monocytogenes*[1]	ATCC# 19117	4D	Sheep, USA	+	+
44	*Listeria monocytogenes*[1]	ATCC# 19118	4E	Chicken, England	+	+
45	*Listeria monocytogenes*[1]	ATCC# 13932	4B	Spinal fluid of child	+	+
46	*Listeria monocytogenes*[1]	ATCC# 15313	1/2A	Rabbit, England	+	+
47	*Listeria monocytogenes*[1]	ATCC# 49594	1/2A	Unknown	+	+
48	*Listeria monocytogenes*[1]	ATCC# 51780	1/2B	Cheese	+	+
49	*Listeria monocytogenes*[1]	ATCC# 51782	3A	Cheese	+	+
50	*Listeria monocytogenes*[1]	ATCC 7644	1/2C	Human Isolate	+	+
51	*Listeria maarthi*[6]	ATCC BAA-1595	NA	Soil, NY, USA	+	+
	[1]	ATCC: American Type Culture Collection				
	[2]	NCTC: HPA Culture Collection				
	[3]	Culture obtained from Invisible Sentinel Culture Collection				
	[4]	University of Vermont				
	[5]	National collection of Industrial Marine and Food Bacteria				
	[6]	Assayed at Invisible Sentinel Laboratory				
	[7]	NA = Not available				

Table 4. Veriflow® LS Exclusivity Evaluation (1)

No.	Organism	Reference Number	Source	Veriflow® Presumptive Result	Reference Confirmed Result
1	*Alcaligenes faecalis*	ATCC 8750[1]	**Unknown**	-	-
2	*Bacillus coagulans*	ATCC 7050	**Dairy Products**	–	–
3	*Campylobacter jejuni*	ATCC 33560	**Herring Gull cloacal swab**	-	-
4	*Campylobacter lari*	ATCC BAA-1060	**Bovine Feces**	-	-
5	*Candida albicans*	ATCC 24433	**Nail Infection**	-	-
6	*Carnobacterium maltaromaticum*	ATCC 43224	**Vacuum Packed Beef**	-	-
7	*Citrobacter freundii*	ATCC 8090	**Unknown**	-	-
8	*Edwardsiella tarda*	ATCC 15947	**Feces, Human**	-	-
9	*Enterobacter aerogenes*	ATCC 13048	**Sputum**	-	-
10	*Enterobacter cloacea*	ATCC 23355	**Unknown**	-	-
11	*Enterococcus faecalis*	ATCC 29212	**Urine**	-	-
12	*Enterococcus faecium*	ATCC 19434	**Unknown**	+/-[4]	-
13	*Escherichia coli*	ATCC 25922	**Clinical Isolate**	-	-
14	*Escherichia coli*	BEI NR-4356[2]	**Unknown**	-	-
15	*Escherichia coli*	BEI NR-12[2]	**Unknown**	-	-
16	*Hafnia alvei*	ATCC 51815	**Unknown**	-	-
17	*Klebsiella pneumonia*	ATCC 13883	**Unknown**	-	-
18	*Kocuria rhizophila*	ATCC 9341	**Soil**	-	-
19	*Lactobacillus acidophilus*	ATCC 314	**Unknown**	-	-
20	*Lactobacillus kefiri*	ATCC 35411	**Kefir**	-	-
21	*Lactobacillus lactis*	ATCC 4797	**Unknown**	-	-
22	*Lactobacillus plantarum*	ATCC 8014	**Unknown**	-	-
23	*Morganella morganii*	ATCC 25829	**Human**	-	-
24	*Proteus mirabilis*	ATCC 7002	**Urine**	-	-
25	*Proteus vulgaris*	ATCC 6380	**Clinical Isolate**	-	-
26	*Pseudomonas aeruginosa*	ATCC 27853	**Clinical Isolate**	-	-
27	*Salmonella enterica ser. Abaetuba*	ATCC 35640[2]	**Creek Water, Argentina**	-	-
28	*Salmonella enterica ser. Dublin*[3]	STS 27	**Dublin**	-	-
29	*Salmonella enterica ser. SaintPaul*	ATCC 9712	**Unknown**	-	-
30	*Shigella sonnei*	ATCC 29930	**Unknown**	-	-
31	*Staphylococcus aureus*	ATCC 10832	**Unknown**	-	-
32	*Staphylococcus epidermidis*	ATCC 12228	**Unknown**	-	-
33	*Staphylococcus haemolyticus*	ATCC 29970	**Human Skin**	-	-
34	*Staphylococcus hominis*	ATCC 27844	**Human Skin**	-	-
35	*Streptococcus pneumoniae*	ATCC 6302	Unknown	-	-

[1]ATCC: American Type Culture Collection
[2]Isolates obtained from IS culture collection
[3] STS- Culture obtained from the University of Pennsylvania Culture Collection
[4] Sample produced a presumptive positive result from non-selective enrichment and a negative result from the selective enrichment used by the assay

Table 5. Veriflow® *LS* Presumptive vs. Confirmed Results for Environmental Surfaces – POD Results (1)											
Matrix	Strain	CFU/Test Area[a]	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# 7644	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		21	20	12	0.60	0.39, 0.78	12	0.60	0.39, 0.78	0.00	-0.28, 0.28
		110	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Sealed Concrete	*Listeria innocua* ATCC# 33090	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		60	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	-0.05	-0.26, 0.26
		170	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Ceramic Tile	*Listeria welshimeri* ATCC# 43548	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		36	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.28, 0.28
		75	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Plastic	*Listeria ivanovii* ATCC# 19119	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		140	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0.00	-0.26, 0.26
		311	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 potions
[c]x = Number of positive test potions
[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 6: Veriflow® *LS* **Environmental Surfaces, Candidate vs. Reference – POD Results (1)**

Matrix	Strain	CFU/Test Area[a]	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# 7644	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		21	20	12	0.60	0.39, 0.78	8	0.40	0.22, 0.61	0.20	-0.10, 0.46
		110	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Sealed Concrete	*Listeria innocua* ATCC# 33090	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		60	20	15	0.75	0.53, 0.89	14	0.70	0.48, 0.85	0.05	-0.22, 0.31
		170	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Ceramic Tile	*Listeria welshimeri* ATCC# 43548	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		36	20	8	0.40	0.22, 0.61	11	0.55	0.34, 0.74	-0.15	-0.41, 0.15
		75	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Plastic	*Listeria ivanovii* ATCC# 19119	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		140	20	15	0.75	0.53, 0.89	13	0.65	0.43, 0.82	0.10	-0.18, 0.36
		311	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 potions
[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 candidate method confirmed results 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 7. Veriflow® *LS* Presumptive vs. Confirmed for RTE Food matrices – POD Results (1)

Matrix	Strain	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	*Listeria innocua* ATCC# 33090	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.22 (0.11, 0.47)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.28, 0.28
		0.98 (0.46, 2.07)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Hot Dogs	*Listeria monocytogenes* ATCC# 7644	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.64 (0.36, 0.1.07)	20	13	0.65	0.43, 0.82	13	0.65	0.43, 0.82	0.00	-0.28, 0.28
		1.19 (0.62, 2.25)	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 based on the POD of reference method test portions using the AOAC MPN calculator, with 95% confidence interval
[b]N = Number of test potions
[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 candidate method confirmed results 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 8: Veriflow® *LS* RTE Matrices, Candidate vs. Reference – POD Results (1)

Matrix	Strain	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		
Deli Turkey	*Listeria innocua* ATCC# 33090	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.22 (0.11, 0.47)	20	8	0.40	0.22, 0.61	7	0.35	0.18, 0.57	0.05	-0.23, 0.32
		0.98 (0.46, 2.07)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Hot Dogs	*Listeria monocytogenes* ATCC# 7644	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.64 (0.36, 0.1.07)	20	13	0.65	0.43, 0.82	10	0.50	0.30, 0.70	0.15	-0.15, 0.41
		1.19 (0.62, 2.25)	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 based on the POD of reference method test portions using the AOAC MPN calculator, with 95% confidence interval
[b]N = Number of test potions
[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 candidate method confirmed results 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

DISCUSSION OF MODIFICATION STUDY APPROVED JANUARY 2016 (8)

The results of this study demonstrated the specificity, accuracy and reliability of the Veriflow® *LS* assay as compared to the traditional USDA/FSIS MLG 8.08 culture based reference methods (1) for the detection of *Listeria* species from environmental surfaces (stainless steel). POD statistical analysis of all eight matrices tested indicate that there is no significant difference in performance between the methods at specific time points as assayed in this study.

The Veriflow® *LS* assay provides flexibility and ease of use for the end user by providing accurate results across multiple surfaces with sampling by either swabs or sponges, and across multiple food matrices, without complex sample preparation after enrichment. The Veriflow® system also offers significant savings in time compared to the USDA/FSIS reference methods (1), by producing accurate presumptive results after an enrichment time of only 24-28 hours, as compared to the reference methods that require 3-4 days to reach presumptive results. Thus the results of this study demonstrated that the easy to follow Veriflow® *LS* protocol provides for a sensitive, reliable and simple to use rapid detection method for *Listeria* species.

Table 1. Veriflow® *LS* Presumptive vs. Confirmed for Stainless Steel Surface– POD Results (8)

Matrix	Strain	CFU per 4x4" Surface	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# 7644	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		41	20	12	0.60	0.39, 0.78	12	0.60	0.39, 0.78	0.00	-0.28, 0.28
		120	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 based on the POD of reference method test portions across labs using the AOAC MPN calculator, with 95% confidence interval
[b]N = Number of test potions
[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 candidate method confirmed results 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 2: Veriflow *LS* Stainless Steel Surface Candidate vs. Reference – POD Results (8)

Matrix	Strain	CFU per 4x4" Surface	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# 7644	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		41	20	12	0.60	0.39, 0.78	12	0.60	0.39, 0.78	0.00	-0.28, 0.28
		120	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 based on the POD of reference method test portions across labs using the AOAC MPN calculator, with 95% confidence interval
[b]N = Number of test potions
[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 candidate method confirmed results 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

DISCUSSION OF MODIFICATION STUDY APPROVED FEBRUARY 2016 (9)

The results of this study demonstrated the specificity, accuracy and reliability of the Veriflow® LS assay as compared to the traditional FDA/BAM Chapter 10 culture based reference method (1) for the detection of *Listeria* species in whey protein isolate. POD statistical analysis of the results indicate that there is no significant difference in performance between the methods.

The Veriflow® LS assay provides flexibility and ease of use for the end user by providing accurate results across multiple surfaces with sampling by either swabs or sponges, and across multiple food matrices, without complex sample preparation after enrichment. The Veriflow® system also offers significant savings in time compared to the FDA BAM reference method (1), by producing accurate presumptive results after an enrichment time of only 26 hours, as compared to the reference methods that require multiple days to reach presumptive results. Thus the results of this study demonstrated that the easy to follow Veriflow® LS protocol provides for a sensitive, reliable and simple to use rapid detection method for *Listeria* species from whey protein isolate.

Table 1. Veriflow® LS Presumptive vs. Confirmed for Whey Protein Isolate– POD Results (9)

Matrix	Strain	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		
Whey Protein Isolate	*Listeria innocua* ATCC# 33090	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.50	20	12	0.60	0.39, 0.78	11	0.55	0.34, 0.74	0.05	-0.23, 0.32
		2.03	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 based on the POD of reference method test portions using the AOAC MPN calculator, with 95% confidence interval
[b]N = Number of test potions
[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 candidate method confirmed results 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 2: Veriflow® LS, Candidate vs. Reference for Whey Protein Isolate – POD Results (9)

Matrix	Strain	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		
Whey Protein Isolate	*Listeria innocua* ATCC# 33090	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.50	20	12	0.60	0.39, 0.78	6	0.30	0.18, 0.57	0.30	-0.04, 0.51
		2.03	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 based on the POD of reference method test portions using the AOAC MPN calculator, with 95% confidence interval
[b]N = Number of test potions
[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 candidate method confirmed results 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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