



CERTIFICATION

AOAC[®] Performance TestedSM

Certificate No.

051304

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

VeriflowTM *Listeria monocytogenes* (LM)

manufactured by

Invisible Sentinel, Inc.

3711 Market Street, 8th Floor

Philadelphia, PA 19104

USA

This method has been evaluated in the AOAC[®] *Performance Tested MethodsSM* 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 TestedSM* 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

METHOD AUTHORS

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MODIFICATION JULY 2015: Ken Huang and Adam Joelsson
MODIFICATION AUGUST 2015: Adam Joelsson, Ken Huang, and Nicholas A. Siciliano

SUBMITTING COMPANY

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

KIT NAME(S)

Veriflow™ *Listeria monocytogenes* (LM)

CATALOG NUMBERS

IS 1002

INDEPENDENT LABORATORY

Original Validation
 Q Laboratories, Inc
 1400 Harrison Avenue
 Cincinnati, OH 45214
 USA

AOAC EXPERTS AND PEER REVIEWERS

Yi Chen¹, Michael Brodsky², Wayne Ziemer³
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APPLICABILITY OF METHOD

Target organism – *Listeria monocytogenes*
Matrices – USDA FSIS MLG 8.08: stainless steel, sealed concrete, plastic, ceramic tile, hot dogs, turkey deli meat
AOAC 993.12: 2% milk
August 2015 Matrix Extension: USDA FSIS MLG 10: chocolate chip cookies
Performance claims - The Veriflow™ LM system proved equivalent to the reference methods.

REFERENCE METHODS

U.S. Department of Agriculture, Food Safety and Inspection Service (2012) Microbiology Laboratory Guidebook, Chapter 8.08. (3)
 AOAC Official Method 993.12 (1994) *Listeria monocytogenes* in Milk and Dairy Products J. AOAC Int. 77, 395 (4)
 U.S. Department of Agriculture, Food Safety and Inspection Service (2013) Microbiology Laboratory Guidebook, Chapter 10. (11)

ORIGINAL CERTIFICATION DATE

May 2013

CERTIFICATION RENEWAL RECORD

Renewed Annually Through December 2017

METHOD MODIFICATION RECORD

1. July 2015
2. August 2015

SUMMARY OF MODIFICATION

1. Change in sampling volume from 1 mL to 500 µL
2. Matrix Extension to include chocolate chip cookies

Under this AOAC® *Performance Tested*SM License Number, 051304 this method is distributed by:
 NONE

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 NONE

PRINCIPLE OF THE METHOD (1)

Veriflow™ *Listeria monocytogenes* (LM) (Cat no. IS1002) is a molecular based test that detects the foodborne pathogen *Listeria monocytogenes* in environmental, dairy, and RTE (hot dogs and deli meat) food matrices (6). The method combines polymerase chain reaction (PCR) with a rapid, chromatographic vertical flowthrough system that provides specific and highly sensitive detection of pathogen associated molecular signatures coupled with rapid, easy-to-interpret results. In this study, artificially inoculated environmental surfaces, dairy, and RTE food (hot dogs and deli meat) matrices were sampled, enriched and subjected to PCR amplification leading to the generation of a *Listeria monocytogenes* 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 monocytogenes*; whereas appearance of just the control line indicates a negative sample (see Figure 1).

Summary of Results

To satisfy AOAC-RI *Performance-Tested Method*SM (PTM) unpaired method comparison validation requirements, replicate samples of four environmental surfaces (stainless steel, sealed concrete, plastic, and ceramic tile) a dairy and two RTE food matrices (2% milk, hot dogs and deli turkey meat) were inoculated at a low and high level with an additional un-inoculated control set, in duplicate, and sampled according to directions outlined in either the IS Veriflow™ LM assay insert or the USDA/FSIS MLG 8.08 and AOAC 993.12 reference methods (3,4,5). Probability of detection (POD) analysis indicated that no significant difference existed between the reference methods and the Veriflow™ LM rapid assay. All tested strains of *Listeria monocytogenes* were detected in the inclusivity study, while 35 non-specific organisms went undetected in the exclusivity study. Additionally, robustness testing and lot-to-lot stability results indicate that the Veriflow™ LM system is stable, rugged and is uniformly manufactured.

DISCUSSION OF THE ORIGINAL VALIDATION STUDY (1)

The results of this study demonstrated the specificity, accuracy and reliability of the Veriflow™ LM assay as compared to the traditional USDA/FSIS MLG 8.08 and AOAC 993.12 culture based reference methods (3,4) for the detection of *Listeria monocytogenes* on environmental surfaces, dairy, 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™ LM assay was able to accurately detect *Listeria monocytogenes* isolates while correctly excluding all non-specific bacteria tested. The Veriflow™ LM 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 and AOAC 993.12 reference methods (3,4), 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™ LM protocol provides for a sensitive, reliable and simple to use rapid detection method for *Listeria monocytogenes*.

Table 3. Veriflow™ LM Inclusivity Evaluation (1)

No.	Organism	Strain Reference Number	Serotype	IS Presumptive Result	Reference Confirmed Result
1	<i>Listeria monocytogenes</i>	CWD 1553 ¹	1/2C	+	+
2	<i>Listeria monocytogenes</i>	CWD 1554 ¹	1/2A	+	+
3	<i>Listeria monocytogenes</i>	CWD 1555 ¹	1/2A	+	+
4	<i>Listeria monocytogenes</i>	CWD 1559 ¹	4B	+	+
5	<i>Listeria monocytogenes</i>	CWD 1560 ¹	4B	+	+
6	<i>Listeria monocytogenes</i>	CWD 1561 ¹	4B	+	+
7	<i>Listeria monocytogenes</i>	CWD 1563 ¹	4B	+	+
8	<i>Listeria monocytogenes</i>	CWD 1567 ¹	4B	+	+
9	<i>Listeria monocytogenes</i>	CWD 1571 ¹	4B	+	+
10	<i>Listeria monocytogenes</i>	CWD 1574 ¹	4B	+	+
11	<i>Listeria monocytogenes</i>	CWD 1584 ¹	1/2B	+	+
12	<i>Listeria monocytogenes</i>	CWD 1586 ¹	3B	+	+
13	<i>Listeria monocytogenes</i>	CWD 1588 ¹	1/2B	+	+
14	<i>Listeria monocytogenes</i>	CWD 1589 ¹	1/2B	+	+
15	<i>Listeria monocytogenes</i>	CWD 1590 ¹	4B	+	+
16	<i>Listeria monocytogenes</i>	CWD 1591 ¹	3B	+	+
17	<i>Listeria monocytogenes</i>	CWD 1596 ¹	4B	+	+
18	<i>Listeria monocytogenes</i>	CWD 1597 ¹	1/2B	+	+
19	<i>Listeria monocytogenes</i>	CWD 1599 ¹	4B	+	+
20	<i>Listeria monocytogenes</i>	CWD 1600 ¹	3B	+	+
21	<i>Listeria monocytogenes</i>	CWD 1601 ¹	1/2B	+	+
22	<i>Listeria monocytogenes</i>	CWD 1609 ¹	1/2A	+	+
23	<i>Listeria monocytogenes</i>	CWD 1611 ¹	1/2A	+	+
24	<i>Listeria monocytogenes</i>	CWD 1612 ¹	1/2A	+	+
25	<i>Listeria monocytogenes</i>	CWD 1613 ¹	1/2A	+	+
26	<i>Listeria monocytogenes</i>	CWD 1614 ¹	1/2A	+	+
27	<i>Listeria monocytogenes</i>	CWD 1618 ¹	1/2A	+	+
28	<i>Listeria monocytogenes</i>	CWD 1620 ¹	1/2A	+	+
29	<i>Listeria monocytogenes</i>	CWD 1626 ¹	1/2B	+	+
30	<i>Listeria monocytogenes</i>	CWD 1627 ¹	1/2B	+	+
31	<i>Listeria monocytogenes</i>	CWD 1629 ¹	1/2A	+	+
32	<i>Listeria monocytogenes</i>	CWD 1630 ¹	1/2A	+	+
33	<i>Listeria monocytogenes</i>	FSL J1-049 ²	3C	+	+

Invisible Sentinel Veriflow™ *Listeria monocytogenes* AOAC® Certification Number 051304

34	Listeria monocytogenes	FSL J1-129 ²	4AB	+	+
35	Listeria monocytogenes	NCIMB 13726	4B	+	+
36	Listeria monocytogenes	NCTC 10890	7	+	+
37	Listeria monocytogenes	BAA-751 ⁴	1/2B	+	+
38	Listeria monocytogenes	ATCC 76445	1/2C	+	+
39	Listeria monocytogenes	ATCC 191115	1	+	+
40	Listeria monocytogenes	ATCC 191125	2	+	+
41	Listeria monocytogenes	ATCC 191154	4B	+	+
42	Listeria monocytogenes	ATCC 191175	4D	+	+
43	Listeria monocytogenes	ATCC 191185	4E	+	+
44	Listeria monocytogenes	ATCC 139325	4B	+	+
45	Listeria monocytogenes	ATCC 153135	1/2A	+	+
46	Listeria monocytogenes	ATCC 495945	1/2A	+	+
47	Listeria monocytogenes	ATCC 517785	4B	+	+
48	Listeria monocytogenes	ATCC 517805	1/2B	+	+
49	Listeria monocytogenes	ATCC 517825	3A	+	+
50	Listeria monocytogenes	BEI NR-110 ⁶	4B	+	+

¹ Sourced from University of Vermont

² Sourced from Cornell University

³ Sourced from NCIMB

⁴ Sourced from
NCTC

⁵ Sourced from
ATCC

⁶ Sourced from BEI Resources

Table 4. Veriflow™ LM Exclusivity Evaluation (1)

No.	Organism	Reference Number	Veriflow™ Presumptive Result	Reference Confirmed Result
1	<i>Alcaligenes faecalis</i>	ATCC 8750 ¹	-	-
2	<i>Bacillus cereus</i>	ATCC 14579 ²	-	-
3	<i>Bacillus subtilis</i>	ATCC 6051	-	-
4	<i>Campylobacter jejuni</i>	ATCC 33560 ¹	-	-
5	<i>Campylobacter lari</i>	ATCC BAA-1060 ¹	-	-
6	<i>Candida albicans</i>	ATCC 24433 ¹	-	-
7	<i>Citrobacter freundii</i>	ATCC 8090 ¹	-	-
8	<i>Edwardsiella tarda</i>	ATCC 15947 ²	-	-
9	<i>Enterobacter aerogenes</i>	ATCC 13048 ¹	-	-
10	<i>Enterobacter cloacea</i>	ATCC 23355 ¹	-	-
11	<i>Enterococcus faecium</i>	ATCC 19434 ²	-	-
12	<i>Escherichia coli</i>	ATCC 25922 ¹	-	-
13	<i>Escherichia coli</i>	BEI NR-4356 ¹	-	-
14	<i>Escherichia coli</i>	BEI NR-12 ¹	-	-
15	<i>Hafnia alvei</i>	ATCC 51815 ¹	-	-
16	<i>Klebsiella pneumonia</i>	ATCC 13883 ¹	-	-
17	<i>Kocuria rhizophila</i>	ATCC 9341 ¹	-	-
18	<i>Lactobacillus acidophilus</i>	ATCC 314 ¹	-	-
19	<i>Lactobacillus kefir</i>	ATCC 35411 ²	-	-
20	<i>Lactobacillus lactis</i>	ATCC 4797 ²	-	-
21	<i>Listeria innocua</i>	ATCC 33090 ¹	-	-
22	<i>Listeria maarthi</i>	ATCC BAA-1595 ¹	-	-
23	<i>Listeria welshimeri</i>	ATCC 43548 ¹	-	-
24	<i>Listeria murrayi</i>	ATCC 25401 ¹	-	-
25	<i>Listeria ivanovii</i>	ATCC 19119 ¹	-	-
26	<i>Listeria seeligeri</i>	ATCC 35967 ¹	-	-
27	<i>Listeria grayi</i>	ATCC 19120 ¹	-	-
28	<i>Morganella morganii</i>	ATCC 25829 ²	-	-
29	<i>Proteus mirabilis</i>	ATCC 7002 ²	-	-
30	<i>Proteus vulgaris</i>	ATCC 6380 ¹	-	-
31	<i>Pseudomonas aeruginosa</i>	ATCC 27853 ²	-	-
32	<i>Salmonella enterica ser. SaintPaul</i>	ATCC 9712 ²	-	-
33	<i>Salmonella enterica ser. Abaetuba</i>	ATCC 35640 ²	-	-
34	<i>Salmonella enterica ser. Dublin</i>	STS 27 ²	-	-
35	<i>Shigella sonnei</i>	ATCC 29930 ¹	-	-
36	<i>Staphylococcus aureus</i>	ATCC 10832 ¹	-	-
37	<i>Staphylococcus epidermidis</i>	ATCC 12228 ²	-	-
38	<i>Staphylococcus haemolyticus</i>	ATCC 29970 ²	-	-
39	<i>Staphylococcus hominis</i>	ATCC 27844 ²	-	-

1

¹Isolates obtained from Invisible Sentinel²Isolates obtained from Q Laboratories

Table 5. Veriflow™ LM Presumptive vs. Confirmed for Environmental Surfaces – POD Results (1)

Matrix	Strain	CFU/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	<i>Listeria monocytogenes</i> ATCC# 7644 & <i>E. coli</i> ATCC# 25922	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		36	20	6	0.30	0.15, 0.52	6	0.30	0.15, 0.52	0.00	-0.27, 0.27
		217	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Ceramic Tile	<i>Listeria monocytogenes</i> ATCC# 51782	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		17	20	12	0.60	0.39, 0.78	13	0.65	0.43, 0.82	-0.05	-0.32, 0.23
		189	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Concrete	<i>Listeria monocytogenes</i> ATCC# 19115	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		95	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
		560	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Plastic	<i>Listeria monocytogenes</i> ATCC# 13932	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		33	20	12	0.60	0.39, 0.78	12	0.60	0.39, 0.78	0.00	-0.28, 0.28
		280	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 candidate method confirmed results 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

Table 6: Veriflow LM Environmental Surfaces, Candidate vs. Reference – POD Results (1)

Matrix	Strain	CFU/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	<i>Listeria monocytogenes</i> ATCC# 7644 & <i>E. coli</i> ATCC# 25922	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		36	20	6	0.30	0.15, 0.52	7	0.35	0.18, 0.57	-0.05	-0.32, 0.23
		217	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Ceramic Tile	<i>Listeria monocytogenes</i> ATCC# 51782	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		17	20	12	0.60	0.39, 0.78	10	0.50	0.30, 0.70	0.10	-0.19, 0.37
		189	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Concrete	<i>Listeria monocytogenes</i> ATCC# 19115	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		95	20	7	0.35	0.18, 0.57	9	0.45	0.26, 0.66	-0.10	-0.37, 0.19
		560	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Plastic	<i>Listeria monocytogenes</i> ATCC# 13932	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		33	20	12	0.60	0.39, 0.78	7	0.35	0.18, 0.57	0.25	-0.05, 0.50
		280	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 candidate method confirmed results 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

Table 7. Veriflow™ LM Presumptive vs. Confirmed for dairy and 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	<i>Listeria monocytogenes</i> ATCC# 13932	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.46	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.28, 0.28
		1.88	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Hot Dogs	<i>Listeria monocytogenes</i> ATCC# 7644	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.53	20	5	0.25	0.11, 0.47	5	0.25	0.11, 0.47	0.00	-0.26, 0.26
		4.38	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
2% Pasteurized Milk	<i>Listeria monocytogenes</i> ATCC# 19115	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.48	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0.00	-0.28, 0.28
		4.38	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 based on the POD of reference method test portions across labs using the AOAC MPN calculator, 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 candidate method confirmed results 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

Table 8: Veriflow LM Dairy and RTE Matrices, Candidate vs. Reference – POD Results (1)

Matrix	Strain	MPN ^a /25g	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	<i>Listeria monocytogenes</i> ATCC# 13932	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.46	20	9	0.45	0.26, 0.66	7	0.35	0.18, 0.57	0.10	-0.19, 0.37
		1.88	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Hot Dogs	<i>Listeria monocytogenes</i> ATCC# 7644	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.53	20	5	0.25	0.11, 0.47	8	0.40	0.22, 0.61	-0.15	-0.40, 0.13
		4.38	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
2% Pasteurized Milk	<i>Listeria monocytogenes</i> ATCC# 19115	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.48	20	11	0.55	0.34, 0.74	9	0.45	0.26, 0.66	0.10	-0.19, 0.37
		4.38	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 based on the POD of reference method test portions across labs using the AOAC MPN calculator, 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 candidate method confirmed results 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

Discussion of Modification Validation Approved July 2015 (9)

The results of this study demonstrated the specificity, accuracy and reliability of the modified Veriflow® *LM* assay as compared to the traditional USDA/FSIS MLG 8.08 and AOAC 993.12 culture based reference methods (3,4) for the detection of *Listeria monocytogenes* on environmental surfaces, dairy, and in RTE (deli meat) foods. POD statistical analysis of all three 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® *LM* 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 and AOAC 993.12 reference methods (3,4), 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. Thus the results of this study demonstrated that the easy to follow Veriflow® *LM* protocol provides for a sensitive, reliable and simple to use rapid detection method for *Listeria monocytogenes*.

Table 1. Veriflow® *LM* Presumptive vs. Confirmed for Environmental Surfaces – POD Results (9)

Matrix	Strain	CFU/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	<i>Listeria monocytogenes</i> ATCC# 7644 & <i>E. faecalis</i> ATCC# 29212	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	-0.27, 0.27
		110	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 candidate method confirmed results 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

Table 2: Veriflow® *LM* Environmental Surfaces, Candidate vs. Reference – POD Results (9)

Matrix	Strain	CFU/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	<i>Listeria monocytogenes</i> ATCC# 7644 & <i>E. faecalis</i> ATCC# 29212	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	-0.09, 0.45
		110	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 candidate method confirmed results 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

Table 3. Veriflow™ LM Presumptive vs. Confirmed for dairy and RTE Food matrices – 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		
Deli Turkey	<i>Listeria monocytogenes</i> ATCC# 7644	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.74	20	10	0.50	0.29, 0.70	10	0.50	0.29, 0.70	0.00	-0.29, 0.29
		1.76	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
2% Pasteurized Milk	<i>Listeria monocytogenes</i> ATCC# 7644	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.38	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.28, 0.28
		3.20	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 based on the POD of reference method test portions across labs using the AOAC MPN calculator, 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 candidate method confirmed results 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

Table 4: Veriflow® LM Dairy and RTE Matrices, Candidate vs. Reference – POD Results (9)

Matrix	Strain	MPN ^a /25g	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	<i>Listeria monocytogenes</i> ATCC# 7644	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.74	20	10	0.50	0.29, 0.70	13	0.65	0.43, 0.82	-0.15	-0.42, 0.15
		1.76	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
2% Pasteurized Milk	<i>Listeria monocytogenes</i> ATCC# 7644	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.38	20	8	0.40	0.22, 0.61	5	0.25	0.11, 0.47	0.15	-0.13, 0.40
		3.20	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 based on the POD of reference method test portions across labs using the AOAC MPN calculator, 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 candidate method confirmed results 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

Discussion of the Matrix Extension August 2015 (10)

Analysis of chocolate chip cookie samples was performed on three inoculation levels of *L. monocytogenes* (ATCC 7644): 0, .59 and 1.36 CFU/25g for the Veriflow® LM assay and the FDA BAM chapter 10 reference method. For the low level of contamination, there were 9 presumptive positive results and 11 confirmed positives for the Veriflow® LM samples after 26 hours of enrichment. The unpaired FDA BAM method detected 8 positive test portions. All un-inoculated control test portions were negative for both methods and the Veriflow® method detected 3 out of the 5 high level test portions, and the reference method detected 4 out of the 5 test portions. There were no significant differences between the Veriflow® LM assay results and the FDA BAM reference method results based on the POD analysis

Table 1. Veriflow® LM Presumptive vs. Confirmed for confectionery food matrix – POD Results (10)

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		
Chocolate Chip Cookies	<i>Listeria monocytogenes</i> ATCC# 7644	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43,0.43
		0.59	20	9	0.45	0.26, 0.66	11	0.55	0.34, 0.74	-0.10	-0.37, 0.19
		1.38	5	3	0.60	0.23, 0.88	3	0.60	0.23, 0.88	0.00	-0.46, 0.46

^aMPN = Most Probable Number is based on the POD of reference method test portions across labs using the AOAC MPN calculator, 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 candidate method confirmed results 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

Table 2: Veriflow LM Dairy , RTE and confectionery Matrices, Candidate vs. Reference – POD Results (10)

Matrix	Strain	MPN ^a /25g	N ^b	Candidate			Reference			dPOD _C ^f	95% CI ^g
				x ^c	POD _C ^d	95% CI	x	POD _R ^e	95% CI		
Chocolate Chip Cookies	<i>Listeria monocytogenes</i> ATCC# 7644	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43,0.43
		0.59	20	9	0.45	0.26, 0.66	8	0.40	0.22, 0.61	0.05	-0.24, 0.33
		1.38	5	3	0.60	0.23, 0.88	4	0.80	0.38, 0.96	-0.20	-0.20, 0.71

^aMPN = Most Probable Number is based on the POD of reference method test portions across labs using the AOAC MPN calculator, 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 candidate method confirmed results 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

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