



CERTIFICATION

AOAC[®] Performance TestedSM

Certificate No.

052003

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

Molecular Environmental Monitoring Program (MEMP) *Listeria* Assay

manufactured by

Applied Food Diagnostics, Inc.

387 Hazle Street

Nuremberg, PA

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 01, 2021 – December 31, 2021). Renewal may be granted at the end of one year under the rules stated in the licensing agreement.

A handwritten signature in black ink that reads "Scott Coates".

Scott Coates, Senior Director
Signature for AOAC Research Institute

January 28, 2021

Date

METHOD AUTHORS Thomas Lonczynski and Laura Cowin	SUBMITTING COMPANY Applied Food Diagnostics, Inc. 387 Hazle Street Nuremberg, PA USA
KIT NAME(S) Molecular Environmental Monitoring Program (MEMP) <i>Listeria</i> Assay	CATALOG NUMBERS MEMP-LIS-032- MEMP <i>Listeria</i> Assay Kit MEMP-SWB-032 – AFD MEMP Swab Kit
INDEPENDENT LABORATORY Q Laboratories 1930 Radcliff Drive Cincinnati, OH 45204 USA	AOAC EXPERTS AND PEER REVIEWERS Yi Chen ¹ , Michael Brodsky ² , Wayne Ziemer ³ ¹ US FDA CFSAN, College Park, MD, USA ² Brodsky Consultants, Ontario, CANADA ³ Independent Consultant, Loganville, MD, USA
APPLICABILITY OF METHOD Analyte – <i>Listeria</i> species Matrixes – Stainless steel (1"x 1"), plastic (1"x 1"), rubber (1"x 1"), ceramic tile (1"x 1") and sealed concrete (1"x 1") Performance claims - Performance equivalent to that of the U.S. Food and Drug Administration (FDA) <i>Bacteriological Analytical Manual</i> (BAM) Chapter 10 (2) for environmental surface swabs.	REFERENCE METHOD Food and Drug Administration <i>Bacteriological Analytical Manual, Detection and Enumeration of Listeria monocytogenes in Foods, Chapter 10 (2017) (2)</i>
ORIGINAL CERTIFICATION DATE May 15, 2020	CERTIFICATION RENEWAL RECORD Renewed annually through December 2021
METHOD MODIFICATION RECORD NONE	SUMMARY OF MODIFICATION NONE
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PRINCIPLE OF THE METHOD (1)

For qPCR amplification and detection, forward and reverse primers hybridize to a unique sequence *Listeria* genomic DNA. A fluorogenic probe consisting of a DNA probe labeled with a 5'-dye and a 3'-quencher is included in the same reaction mixture. During PCR amplification, the probe is cleaved, and the reporter dye and quencher are separated. The resulting increase in fluorescence can be detected on the real-time PCR instrument. Unique primer and probe mixtures specific for *Listeria* are present in this assay.

DISCUSSION OF THE VALIDATION STUDY (1)

Inclusivity and Exclusivity

Of the 75 inclusivity strains analyzed by the MEMP *Listeria* Assay, all 75 inclusivity strains were correctly detected by the *Listeria* assay kit. Of the 30 exclusivity strains, none were detected by the *Listeria* MEMP method. See Tables 1 and 2.

Matrix Study

Results from both the method developer and independent studies of the MEMP *Listeria* Assay for the environmental samples are outlined in Tables 3 and 4. Throughout the study, the method developer had difficulty achieving fractional results, and repeat attempts had to be made to spike at the correct inoculation levels. Environmental surfaces proved challenging to recover cells off the surface at low levels due to the humidity in the environment. Multiple trials were run for some of the surfaces to achieve fractional levels.

For stainless steel, a 1" x 1" area was tested using a swab. For the low contamination level, the swab detected 17 presumptive positive results, 15 of which confirmed positive. The MEMP swabs are able to collect all cell types, including live and dead cells. For the presumptive positive test portions that did not confirm, it is likely that dead cells were present, leading to a positive PCR reaction, but these cells were not enriched. For the high contamination level, the swab detected all five portions, which all confirmed positive. The FDA BAM Chapter 10 method had 5 positive results at the high contamination level and 13 positive results at the low contamination level, which showed no statistically significant difference with the candidate method when analyzed using POD analysis.

For plastic, a 1" x 1" area was tested using a swab. Twelve portions were presumptive positive, and nine confirmed positive. Again, it is likely that dead cells were present, leading to a positive PCR reaction, but these cells were not enriched. The FDA BAM Chapter 10 method had seven positive portions. Both methods had 5 positive portions in the high contamination level, with all candidate method portions confirming positive. There were no statistically significant differences between the two methods.

The rubber was also swabbed in a 1" x 1" area. For the low contamination level, 16 portions were presumptive on the PCR, 14 of which confirmed positive. Again, it is likely that MEMP assay is detecting dead cells from the surface. For the FDA BAM Chapter 10 method, 14 portions were positive. Both methods had 5 positive portions in the high contamination level, with all candidate method portions confirming positive. When performing the POD analysis on the two methods, no statistically significant differences were observed.

For ceramic, a 1" x 1" area was swabbed. For the low contamination level, the candidate method had nine presumptive positive results, and all nine confirmed positive. The FDA BAM Chapter 10 method also had nine positive results. Both methods had 5 positive portions in the high contamination level, with all candidate method portions confirming positive. There were no statistically significant differences between the two methods.

For concrete, a 1" x 1" surface was also swabbed. For the low contamination level, the candidate method had twelve presumptive positive results, all of which confirmed positive. The FDA BAM Chapter 10 reference method had ten positive results. Both methods had 5 positive portions in the high contamination level, with all candidate method portions confirming positive. There were no statistically significant differences between the two methods.

Independent Laboratory Study

In the independent study, there were 8 presumptive positive results at the low contamination level, and 8 confirmed results. For the reference method, there were 7 positive results at the low level. Both methods had 5 positive portions in the high contamination level, with all candidate method portions confirming positive. The POD analysis between the MEMP Assay and the reference method indicated that there was no significant difference, with 95% confidence. A summary of POD analyses is presented in Tables 3 and 4.

Table 1. Inclusivity List – *Listeria* Strains (1)

No.	Genus	Species	Serotype ^a	Source	Origin	MEMP Result
1	<i>Listeria</i>	<i>grayi</i>	N/A ^b	ATCC ^c 25401	Standing corn stalks and leaves	+
2	<i>Listeria</i>	<i>welshimeri</i>	N/A	USDA ^d ERRC B-33266	Florida, USA	+
3	<i>Listeria</i>	<i>grayi</i>	N/A	ATCC 19120	Animal feces	+
4	<i>Listeria</i>	<i>grayi</i>	N/A	USDA ERRC B-33214	Wheat processing plant	+
5	<i>Listeria</i>	<i>seeligeri</i>	N/A	USDA ERRC B-57212	Unknown	+
6	<i>Listeria</i>	<i>innocua</i>	N/A	USDA ERRC B-33314	Unknown	+
7	<i>Listeria</i>	<i>ivanovii</i> subsp. <i>Ivanovii</i>	N/A	USDA ERRC B-33165	Bovine	+
8	<i>Listeria</i>	<i>marthii</i>	N/A	BEI ^e NR-9581	Run-off water	+
9	<i>Listeria</i>	<i>marthii</i>	N/A	BEI NR-9582	Stream water	+
10	<i>Listeria</i>	<i>marthii</i>	N/A	BEI NR-9579	Soil	+
11	<i>Listeria</i>	<i>marthii</i>	N/A	BEI NR-9580	Standing water puddle	+
12	<i>Listeria</i>	<i>seeligeri</i>	N/A	USDA ERRC B-33019	Soil	+
13	<i>Listeria</i>	<i>ivanovii</i>	N/A	USDA ERRC B-33017	Sheep	+
14	<i>Listeria</i>	<i>welshimeri</i>	N/A	USDA ERRC B-33020	Decaying vegetation	+
15	<i>Listeria</i>	<i>innocua</i>	N/A	USDA ERRC B-33003	California, USA	+
16	<i>Listeria</i>	<i>innocua</i>	N/A	ATCC 33091	Human feces	+
17	<i>Listeria</i>	<i>ivanovii</i> subsp. <i>londoniensis</i>	N/A	ATCC BAA-139	Washing water	+
18	<i>Listeria</i>	<i>welshimeri</i>	N/A	ATCC 35897	Decaying plant material	+
19	<i>Listeria</i>	<i>Innocua</i>	6a	ATCC 33090	Cow brain	+
20	<i>Listeria</i>	<i>grayi</i>	N/A	USDA ERRC B-33023	Chinchilla feces	+
21	<i>Listeria</i>	<i>seeligeri</i>	N/A	ATCC 35967	Soil	+
22	<i>Listeria</i>	<i>aquatica</i>	N/A	USDA ERRC B-57629	Running water	+
23	<i>Listeria</i>	<i>riparia</i>	N/A	USDA ERRC B-57632	Running water	+
24	<i>Listeria</i>	<i>welshimeri</i>	N/A	USDA ERRC B-33194	Wheat processing plant	+
25	<i>Listeria</i>	<i>grayi</i>	N/A	ATCC 700545	Unknown	+

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26	<i>Listeria</i>	<i>monocytogenes</i>	4b	USDA ERRC B-33000	Cheese	+
27	<i>Listeria</i>	<i>monocytogenes</i>	1/2b complex	USDA ERRC B-33045	Turkey, Pork, Beef hot dogs	+
28	<i>Listeria</i>	<i>monocytogenes</i>	1/2b	USDA ERRC B-33258	Smoked boneless ham	+
29	<i>Listeria</i>	<i>monocytogenes</i>	1/2b	USDA ERRC B-33272	Environmental isolates	+
30	<i>Listeria</i>	<i>monocytogenes</i>	1/2b	USDA ERRC B-33273	Environmental isolates	+
31	<i>Listeria</i>	<i>monocytogenes</i>	1/2b	USDA ERRC B-33254	Roast beef	+
32	<i>Listeria</i>	<i>monocytogenes</i>	1/2b	USDA ERRC B-33046	Chicken	+
33	<i>Listeria</i>	<i>monocytogenes</i>	1/2b	USDA ERRC B-33073	Bovine	+
34	<i>Listeria</i>	<i>monocytogenes</i>	1/2a	USDA ERRC B-33106	Raw milk	+
35	<i>Listeria</i>	<i>monocytogenes</i>	4d	USDA ERRC B-33116	Sheep	+
36	<i>Listeria</i>	<i>monocytogenes</i>	4e	USDA ERRC B-33120	Chicken	+
37	<i>Listeria</i>	<i>monocytogenes</i>	1/2b	USDA ERRC B-33130	Bovine milk	+
38	<i>Listeria</i>	<i>monocytogenes</i>	1/2b	USDA ERRC B-33162	Bovine	+
39	<i>Listeria</i>	<i>monocytogenes</i>	1/2b complex	BEI NR-108	Human	+
40	<i>Listeria</i>	<i>monocytogenes</i>	3a	BEI NR-110	Human cerebrospinal fluid	+
41	<i>Listeria</i>	<i>monocytogenes</i>	4b	BEI NR-111	Chicken	+
42	<i>Listeria</i>	<i>monocytogenes</i>	4c	BEI NR-112	Sheep	+
43	<i>Listeria</i>	<i>monocytogenes</i>	4d	BEI NR-113	Chicken	+
44	<i>Listeria</i>	<i>monocytogenes</i>	1/2a	BEI NR-13233	Soil	+
45	<i>Listeria</i>	<i>monocytogenes</i>	1/2a	BEI NR-13229	Human	+
46	<i>Listeria</i>	<i>monocytogenes</i>	1/2b	BEI NR-13237	Bovine abortion	+
47	<i>Listeria</i>	<i>monocytogenes</i>	4c	BEI NR-13232	Bovine	+
48	<i>Listeria</i>	<i>monocytogenes</i>	4b	BEI NR-13231	Trout	+
49	<i>Listeria</i>	<i>monocytogenes</i>	1/2b	BEI NR-13230	Human	+
50	<i>Listeria</i>	<i>monocytogenes</i>	N/A	BEI NR-4098	Human meningitis	+
51	<i>Listeria</i>	<i>monocytogenes</i>	4a	BEI NR-109	Ruminant tissue	+
52	<i>Listeria</i>	<i>monocytogenes</i>	N/A	USDA ERRC B-33259	Chicken	+
53	<i>Listeria</i>	<i>monocytogenes</i>	N/A	USDA ERRC B-33260	Beef sausage links	+

54	<i>Listeria</i>	<i>monocytogenes</i>	N/A	USDA ERRC B-33261	Beef jerky	+
55	<i>Listeria</i>	<i>monocytogenes</i>	N/A	USDA ERRC B-33264	Sliced cooked beef	+
56	<i>Listeria</i>	<i>monocytogenes</i>	N/A	USDA ERRC B-33274	Florida, USA	+
57	<i>Listeria</i>	<i>monocytogenes</i>	N/A	USDA ERRC B-33276	Chicken	+
58	<i>Listeria</i>	<i>monocytogenes</i>	1/2a	USDA ERRC B-33814	Clinical Isolate	+
59	<i>Listeria</i>	<i>monocytogenes</i>	3a	USDA ERRC B-33225	Unknown	+
60	<i>Listeria</i>	<i>monocytogenes</i>	N/A	USDA ERRC B-33282	Duck Breast	+
61	<i>Listeria</i>	<i>monocytogenes</i>	3c	USDA ERRC B-33226	Unknown	+
62	<i>Listeria</i>	<i>monocytogenes</i>	N/A	USDA ERRC B-33238	Beef jerky	+
63	<i>Listeria</i>	<i>monocytogenes</i>	1/2b complex	USDA ERRC B-33239	Beef/pork franks	+
64	<i>Listeria</i>	<i>monocytogenes</i>	1/2b complex	USDA ERRC B-33240	Beef/pork franks	+
65	<i>Listeria</i>	<i>monocytogenes</i>	N/A	USDA ERRC B-33241	Cooked apple sausage	+
66	<i>Listeria</i>	<i>monocytogenes</i>	1/2b complex	USDA ERRC B-33242	Roast beef	+
67	<i>Listeria</i>	<i>monocytogenes</i>	N/A	USDA ERRC B-33243	Cooked beef	+
68	<i>Listeria</i>	<i>monocytogenes</i>	1/2b complex	USDA ERRC B-33245	Environmental isolates	+
69	<i>Listeria</i>	<i>monocytogenes</i>	N/A	USDA ERRC B-33246	White chicken salad	+
70	<i>Listeria</i>	<i>monocytogenes</i>	N/A	USDA ERRC B-33247	Roast beef	+
71	<i>Listeria</i>	<i>monocytogenes</i>	1/2b complex	USDA ERRC B-33248	BBQ Chicken	+
72	<i>Listeria</i>	<i>monocytogenes</i>	1/2b complex	USDA ERRC B-33250	Boneless smoked ham steak	+
73	<i>Listeria</i>	<i>monocytogenes</i>	N/A	USDA ERRC B-33253	Cooked ham	+
74	<i>Listeria</i>	<i>monocytogenes</i>	N/A	BEI HM-1048	Human	+
75	<i>Listeria</i>	<i>monocytogenes</i>	4c	USDA ERRC B-33115	Arabian oryx	+

^aSerotype listed if applicable.

^bNot applicable.

^cAmerican Type Culture Collection, Manassas, VA.

^dUnited States Department of Agriculture Eastern Regional Research Center, Wyndmoor, PA.

^eBEI Resources, Manassas, VA.

Table 2. Exclusivity List – Non-Listeria Strains (1)

No.	Genus	Species	Source	Origin	MEMP Result
1	<i>Alcaligenes</i>	<i>faecalis</i> subsp. <i>faecalis</i>	USDA ^a ERRC B-170	USDA, Beltsville, MD	-
2	<i>Citrobacter</i>	<i>koseri</i>	SGSC ^b 5610	Unknown	-
3	<i>Bacillus</i>	<i>subtilis</i>	BEI ^c NR-607	Unknown	-
4	<i>Bacillus</i>	<i>cereus</i>	BEI NR-608	Laboratory isolate	-
5	<i>Citrobacter</i>	<i>freundii</i>	ATCC ^d 43864	Unknown	-
6	<i>Cronobacter</i>	<i>sakazakii</i>	ATCC BAA-894	Human clinical specimen	-
7	<i>Klebsiella</i>	<i>ozaenae</i>	SGSC 2810	Unknown	-
8	<i>Escherichia</i>	<i>fergusonii</i>	SGSC 5718	Human feces	-
9	<i>Escherichia</i>	<i>coli</i> O75:K95:H5	BEI NR-17715	Human	-
10	<i>Enterobacter</i>	<i>taylorae</i>	SGSC 5283	Unknown	-
11	<i>Providencia</i>	<i>stuartii</i>	SGSC 5639	Unknown	-
12	<i>Ewingella</i>	<i>americana</i>	SGSC 5640	Human feces	-
13	<i>Hafnia</i>	<i>alvei</i>	SGSC 5583	Unknown	-
14	<i>Klebsiella</i>	<i>oxytoca</i>	SGSC 5366	Unknown	-
15	<i>Klebsiella</i>	<i>pneumoniae</i> subsp. <i>pneumoniae</i>	SGSC 5926	Unknown	-
16	<i>Lactobacillus</i>	<i>lactis</i>	ATCC 19257	Unknown	-
17	<i>Serratia</i>	<i>marcescens</i>	SGSC 5354	Unknown	-
18	<i>Serratia</i>	<i>odorifera</i>	SGSC 5576	Unknown	-
19	<i>Shigella</i>	<i>sonnei</i>	SGSC 5576	Unknown	-
20	<i>Shigella</i>	<i>flexneri</i>	SGSC 5577	Unknown	-
21	<i>Staphylococcus</i>	<i>aureus</i>	ATCC 29213	Wound	-
22	<i>Pseudomonas</i>	<i>aeruginosa</i>	BEI NR-48982	Human	-
23	<i>Yersinia</i>	<i>enterocolitica</i>	USDA ERRC B-41479	Ground Beef	-
24	<i>Morganella</i>	<i>morganii</i>	SGSC 5435	Unknown	-
25	<i>Proteus</i>	<i>mirabilis</i>	SGSC 5445	Unknown	-
26	<i>Escherichia</i>	<i>coli</i> O157:H7	ATCC 43888	Human feces	-
27	<i>Escherichia</i>	<i>coli</i> O121	MSU ^e TW08004	Human	-
28	<i>Escherichia</i>	<i>coli</i> O111	MSU TW05150	Cow	-
29	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i> Enteritidis	SGSC 2475	Unknown, Connecticut	-
30	<i>Salmonella</i>	<i>enterica</i> subsp. <i>enterica</i> Typhimurium	SGSC 2522	Human, Mexico	-

^aUnited States Department of Agriculture Eastern Regional Research Center, Windsor, PA.

^b*Salmonella* Genetic Stock Centre, University of Calgary, Canada

^cBEI Resources, Manassas, VA.

^dAmerican Type Culture Collection, Manassas, VA.

^eMichigan State University STEC Center, East Lansing, MI.

Table 3. MEMP *Listeria* Assay Presumptive vs. Confirmed Results (1)

Matrix	Strain	CFU ^a /test area	N ^b	MEMP <i>Listeria</i> presumptive results			MEMP <i>Listeria</i> confirmed results			dPOD _{CP} ^f	95% CI ^g
				x ^c	POD _{CP} ^d	95% CI	x	POD _{CC} ^e	95% CI		
Stainless steel (1"x 1")	<i>Listeria monocytogenes</i> 4a (BEI ^h NR-109)/10X <i>E. faecalis</i> (BEI NR-31884)	N/A ^t	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		80 & 1000	20	17	0.85	0.64, 0.95	15	0.75	0.53, 0.89	0.10	-0.15, 0.34
		150 & 3500	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Stainless steel ^l (1"x 1")	<i>Listeria monocytogenes</i> 4a (ATCC ⁱ 19114)/10X <i>E. faecalis</i> (ATCC 29212)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		77 & 800	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.13, 0.13
		190 & 2600	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Plastic (1"x 1")	<i>Listeria innocua</i> (USDA ERRC ^j B-33003)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		200	20	12	0.60	0.39, 0.78	9	0.45	0.26, 0.66	0.15	-0.15, 0.41
		1000	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Rubber (1"x 1")	<i>Listeria grayi</i> (USDA ERRC B-33214)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		200	20	16	0.80	0.58, 0.92	14	0.70	0.48, 0.85	0.10	-0.17, 0.35
		600	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Ceramic tile (1"x 1")	<i>Listeria ivanovii</i> (USDA ERRC B-33165)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		170	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.28, 0.28
		500	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Sealed concrete (1"x 1")	<i>Listeria marthii</i> (BEI NR-9580)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		110	20	12	0.60	0.39, 0.78	12	0.60	0.39, 0.78	0.00	-0.28, 0.28
		500	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

^aCFU = Colony forming units per test 1"x 1" test area

^bN = Number of test portions.

^cx = Number of positive test portions.

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

^ePOD_{CC} = Candidate method confirmed positive outcomes (using the FDA BAM Chapter 10 reference method) divided by the total number of trials.

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

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

^hBEI Resources, Manassas, VA.

ⁱUnited States Department of Agriculture Eastern Regional Research Center, Windsor, PA.

^tN/A = Not applicable.

^lMatrix tested in the independent laboratory.

Table 4. MEMP *Listeria* Assay vs. Reference Method BAM Ch. 5 Results (1)

Matrix	Strain	CFU ^a /test area	MEMP <i>Listeria</i> results				Reference method results				
			N ^b	x ^c	POD _c ^d	95% CI	x	POD _R ^e	95% CI	dPOD _c ^f	95% CI ^g
Stainless steel (1"x 1")	<i>Listeria monocytogenes</i> 4a (BEI ^h NR-109)/10X <i>E. faecalis</i> (BEI NR-31884)	N/A ⁱ	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		80 & 1000	20	15	0.75	0.53, 0.89	13	0.65	0.43, 0.82	0.10	-0.18, 0.36
		150 & 3500	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Stainless steel ^k (1"x 1")	<i>Listeria monocytogenes</i> 4a (ATCC ⁱ 19114)/10X <i>E. faecalis</i> (ATCC 29212)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		77 & 800	20	8	0.40	0.22, 0.61	7	0.35	0.18, 0.57	0.05	-0.23, 0.32
		190 & 2600	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Plastic (1"x 1")	<i>Listeria innocua</i> (USDA ERRC ⁱ B-33003)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		200	20	9	0.45	0.26, 0.66	7	0.35	0.18, 0.57	0.10	-0.19, 0.37
		800	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Rubber (1"x 1")	<i>Listeria grayi</i> (USDA ERRC B-33214)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		200	20	14	0.70	0.48, 0.85	14	0.70	0.48, 0.85	0.00	-0.27, 0.27
		600	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Ceramic tile (1"x 1")	<i>Listeria ivanovii</i> (USDA ERRC B-33165)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		170	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.28, 0.28
		500	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Sealed concrete (1"x 1")	<i>Listeria marthii</i> (BEI NR-9580)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		110	20	12	0.60	0.39, 0.78	10	0.50	0.30, 0.70	0.10	-0.19, 0.37
		500	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

^aCFU = Colony forming units per test 1"x 1" test area.

^bN = Number of test portions.

^cx = Number of positive test portions.

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

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

^fdPOD_c = Difference between the candidate method result and reference method 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.

^hBEI Resources, Manassas, VA.

ⁱUnited States Department of Agriculture Eastern Regional Research Center, Windsor, PA.

^kN/A = Not applicable.

^lMatrix tested in the independent laboratory.

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