



CERTIFICATION

AOAC[®] Performance TestedSM

Certificate No.

062001

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

Simultaneous Multiplex Real Time PCR (SIMUL-qPCR) *Listeria* species and *monocytogenes* Assay

manufactured by

Applied Food Diagnostics, Inc.

387 Hazle Street

Nuremberg, PA

USA

This method has been evaluated as a single-site method 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.

Scott Coates

Scott Coates, Senior Director
Signature for AOAC Research Institute

January 28, 2021

Date

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KIT NAME(S) Simultaneous Multiplex Real Time PCR (SIMUL-qPCR) <i>Listeria</i> species and <i>monocytogenes</i> Assay (Single source service-based method)	CATALOG NUMBER SMRT-LSLM-096
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 Consultant, Loganville, GA, USA
APPLICABILITY OF METHOD Analyte – <i>Listeria</i> species and <i>Listeria monocytogenes</i> Matrixes – (MLG 8.10): Frankfurters (125 g), ready to eat (RTE) sliced turkey (125 g), cooked eggs (25 g) (BAM Ch 10): soft fresh raw cheese (25 g), chicken salad (25 g), ice cream (25 g), pasteurized milk (25 g), frozen/cooked shrimp (25 g), stainless steel (4" x 4", 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 comparable to that of the reference methods.	REFERENCE METHODS U. S Department of Agriculture Food Safety and Inspection Service <i>Microbiology Laboratory Manual</i>, Isolation and Identification of <i>Listeria monocytogenes</i> from Red Meat, Poultry and Egg Products, and Environmental Samples, 8.10 (2017) (2) Food and Drug Administration <i>Bacteriological Analytical Manual</i>, Detection and Enumeration of <i>Listeria monocytogenes</i> in Foods, Chapter 10 (2017) (3)
ORIGINAL CERTIFICATION DATE June 04, 2020	CERTIFICATION RENEWAL RECORD New Approval
METHOD MODIFICATION RECORD NONE	SUMMARY OF MODIFICATION NONE
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PRINCIPLE OF THE METHOD (1)

This protocol is a multifaceted approach to the detection of *Listeria* species in a variety of food products and environmental samples. Specifically formulated media are utilized for enriching samples followed by cultural (detection plate) and rapid (qPCR) detection procedures. *Listeria* Recovery and Enrichment Broth (LREB) combines nutritional components with additional ingredients that are necessary to selectively improve recovery and growth of *Listeria*. The selective agents present in LREB have been optimized to efficiently inhibit competing normal bacterial flora without affecting the growth of *Listeria* species. LREB is formulated for buffering capacity to ensure growth in a variety of matrixes.

The sample is enriched at a specific temperature. Detection procedures can occur after a specified minimal enrichment time.

During PCR amplification, forward and reverse primers hybridize to unique sequences of *Listeria* species and *monocytogenes* genomic DNA. A fluorogenic probe is included in the same reaction mixture which consists of a DNA probe labeled with a 5'-dye and a 3'-quencher. 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. Two unique and specific primers and probe mixtures are present in this assay.

DISCUSSION OF THE VALIDATION STUDY (1)

Inclusivity and Exclusivity

Of the 75 inclusivity strains analyzed by the SIMUL-qPCR *Listeria* species and *monocytogenes* Assay, all 75 inclusivity strains were correctly detected by the SIMUL-qPCR *Listeria* assay kit. The kit also properly identified all 50 *L. monocytogenes* strains.

Of the 30 exclusivity strains, none were detected by the SIMUL-qPCR method. All the samples were negative for both the *Listeria* species channel and the *L. monocytogenes* channel.

Matrix Study

Results from both the method developer and independent studies of the SIMUL-qPCR *Listeria* species and *monocytogenes* Assay for the food and environmental samples are outlined in Tables 4 and 5. 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 samples 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.

During confirmation testing for the raw products, the method developer laboratory had issues with competing flora growing on plates. Colonies had to be re-streak for further isolation to obtain the intended target. Method developer results are reported here.

For frankfurters, there were nine presumptive positive results and nine confirmed positives by the SIMUL-qPCR method in the low contamination level. The FSIS MLG 8.10 reference method had 13 positive portions. POD analysis showed that the difference in results were not statistically significant. All portions in the high level were positive (presumptive and confirmed) for both methods.

For the ready to eat turkey, there were 10 presumptive positive results in the low contamination level for the SIMUL-qPCR method and eight portions confirmed positive. The background bacteria made it difficult to culturally confirm some of the positives. For the FSIS MLG 8.10 reference method, 12 were positive. POD analysis showed no statistically significant differences between the candidate and reference methods. All portions in the high level were positive (presumptive and confirmed) for both methods.

For the cooked eggs, there were no differences in results between the candidate individual and FSIS MLG 8.10 reference method in the low contamination level. Fifteen portions were presumptive positive and confirmed positive. At the high contamination level, four portions were presumptive positive and four confirmed positive. For the reference method, five samples were positive. POD analysis showed no significant difference in the statistical results.

For the cheese matrix, all 10 of the presumptive positive portions in the low level confirmed positive. For the reference method, 8 portions confirmed positive, compared to the 10 the confirmed by the SIMUL-qPCR method. All portions in the high level were positive (presumptive and confirmed) for both methods. There were no statistical differences shown by POD analysis.

For the frozen/cooked shrimp, 16 portions were presumptive positive and 17 confirmed positive for the individual samples. For the FDA BAM Chapter 10 reference method, 13 samples were positive. All portions in the high level were positive (presumptive and confirmed) for individual and reference methods. No statistical differences between methods were indicated by POD analysis.

For chicken salad, there were five presumptive positive portions in the low contamination level, but only four samples confirmed positive. This matrix had a high APC count relative to the target contamination level, which made confirmation difficult. There were 7 positive portions for reference method. For the high contamination level, there were 4 positive results (presumptive and confirmed) individual and reference methods. POD analysis showed no statistically significant difference between the candidate and reference methods.

For ice cream, seven portions were positive (presumptive and confirmed) for the SIMUL-qPCR method and for the reference method in the low contamination level. For the high contamination level, there were 5 positive results (presumptive and confirmed) individual and reference methods. POD analysis showed no statistically significant difference between the candidate and reference methods.

The pasteurized milk had 16 presumptive positive results and all confirmed positive. The FDA BAM Chapter 10 reference method had 12 confirmed positives. For the high contamination level, there were 5 positive results (presumptive and confirmed) individual and reference methods. POD analysis showed no statistically significant difference between the candidate and reference methods.

For stainless steel, a 1" x 1" area was tested using a swab, and a 4" x 4" was tested using a sponge via the candidate method. For the low contamination level, the swab detected 15 presumptive positive results in the 1" x 1" area, and all 15 confirmed positive. The sponge detected 12 presumptive positive results, but 15 confirmed positive. The low amount of target cells led to some portions not to be detected by the candidate method. For the high contamination level, the swab detected all five high portions, and all confirmed positive. The sponge also detected five of the five high portions, in which all five confirmed positive. The FDA BAM Chapter 10 method had four of the five high contamination level positive portions as well as 7 positive results for the low set. For the 1"x 1" swabs, the low level showed a statistical difference in results favoring the SIMUL-qPCR method, otherwise there were no statistically significant differences between the methods.

The plastic was swabbed in a 1" x 1" area. Eleven portions in the low contamination level were presumptive positive, and nine portions were confirmed positive. It is possible that the PCR method detected dead cells from the swab that were not able to be confirmed culturally. All five at the high contamination level confirmed positive. Seven portions at the low contamination level and 4 portions at the high contamination level were positive for the FDA BAM method, leading to no statistically significant differences between the two methods.

The rubber was also swabbed in a 1" x 1" area. For the low contamination level, 12 portions were presumptive positive by the SIMUL-qPCR, and all 12 portions confirmed positive. For the FDA BAM Chapter 10 method, 14 were positive. For the high contamination level, there were 5 positive results (presumptive and confirmed) individual and reference methods. POD analysis showed no statistically significant difference between the candidate and reference methods.

For ceramic, a 1" x 1" area was swabbed. For the low contamination level, the candidate method had 9 presumptive positive results, and all 9 confirmed positive. The FDA BAM Chapter 10 method had 9 positive results. For the high contamination level, both the candidate and reference methods had 4 positive results (presumptive and confirmed for the candidate method). POD analysis showed no statistical difference.

For concrete, a 1" x 1" surface area was also swabbed. For the low contamination level, the candidate method had six presumptive positive results, and 8 portions confirmed positive. The reference method had 10 positive results. For the high contamination level, the candidate method had 4 presumptive positive results, and all 4 confirmed positive. The reference method had 5 positive results. POD analysis did not show any statistically significant differences in the methods.

Independent Laboratory Studies

For the SIMUL-qPCR method of frankfurters, at the 30 h enrichment time point for the individual set, 11 out of 20 portions were presumptive positive, and 13 confirmed positive. For the reference method, 6 portions were positive. For the high contamination level of the individual set, 5 out of 5 samples were presumptive positive and confirmed positive for the candidate method. The reference method had 4 out of 5 positive portions.

For fresh raw soft cheese, 13 out of 20 test portions were presumptive positive. Fourteen samples culturally confirmed positive. For the reference method, 14 out of 20 test portions were positive. All five portions at the high contamination level were for both the candidate and reference methods.

For the stainless steel sponges, 7 out of 20 test portions were presumptive positive, and all seven confirmed positive. For the reference method, 6 out of 20 samples were positive. For both the candidate and reference methods, all five portions at the high contamination level were positive.

There were no statistically significant differences found between the candidate presumptive versus confirmed results at any time point, and no significant differences found between the candidate and reference methods for any of the matrixes.

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

No.	Genus	Species	Serotype	Source	Origin	SIMUL-qPCR Result
1	<i>Listeria</i>	<i>grayi</i>		ATCC ^a 25401	Standing corn stalks and leaves	+
2	<i>Listeria</i>	<i>welshimeri</i>		USDA ^b ERRC B-33266	Florida, USA	+
3	<i>Listeria</i>	<i>grayi</i>		ATCC 19120	Animal feces	+
4	<i>Listeria</i>	<i>grayi</i>		USDA ERRC B-33214	Wheat processing plant	+
5	<i>Listeria</i>	<i>seeligeri</i>		USDA ERRC B-57212	Unknown	+
6	<i>Listeria</i>	<i>innocua</i>		USDA ERRC B-33314	Unknown	+
7	<i>Listeria</i>	<i>ivanovii</i> subsp. <i>ivanovii</i>		USDA ERRC B-33165	Bovine	+
8	<i>Listeria</i>	<i>marthii</i>		BEI ^c NR-9581	Run-off water	+
9	<i>Listeria</i>	<i>marthii</i>		BEI NR-9582	Stream water	+
10	<i>Listeria</i>	<i>marthii</i>		BEI NR-9579	Soil	+
11	<i>Listeria</i>	<i>marthii</i>		BEI NR-9580	Standing water puddle	+
12	<i>Listeria</i>	<i>seeligeri</i>		USDA ERRC B-33019	Soil	+
13	<i>Listeria</i>	<i>ivanovii</i>		USDA ERRC B-33017	Sheep	+
14	<i>Listeria</i>	<i>welshimeri</i>		USDA ERRC B-33020	Decaying vegetation	+
15	<i>Listeria</i>	<i>innocua</i>		USDA ERRC B-33003	California, USA	+
16	<i>Listeria</i>	<i>innocua</i>		ATCC 33091	Human feces	+
17	<i>Listeria</i>	<i>ivanovii</i> subsp. <i>londoniensis</i>		ATCC BAA-139	Washing water	+
18	<i>Listeria</i>	<i>welshimeri</i>		ATCC 35897	Decaying plant material	+
19	<i>Listeria</i>	<i>Innocua</i>	6a	ATCC 33090	Cow brain	+
20	<i>Listeria</i>	<i>grayi</i>		USDA ERRC B-33023	Chinchilla feces	+
21	<i>Listeria</i>	<i>seeligeri</i>		ATCC 35967	Soil	+
22	<i>Listeria</i>	<i>aquatica</i>		USDA ERRC B-57629	Running water	+
23	<i>Listeria</i>	<i>riparia</i>		USDA ERRC B-57632	Running water	+
24	<i>Listeria</i>	<i>welshimeri</i>		USDA ERRC B-33194	Wheat processing plant	+
25	<i>Listeria</i>	<i>grayi</i>		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>		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>		USDA ERRC B-33259	Chicken	+
53	<i>Listeria</i>	<i>monocytogenes</i>		USDA ERRC B-33260	Beef sausage links	+

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54	<i>Listeria</i>	<i>monocytogenes</i>		USDA ERRC B-33261	Beef jerky	+
55	<i>Listeria</i>	<i>monocytogenes</i>		USDA ERRC B-33264	Sliced cooked beef	+
56	<i>Listeria</i>	<i>monocytogenes</i>		USDA ERRC B-33274	Florida, USA	+
57	<i>Listeria</i>	<i>monocytogenes</i>		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>		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>		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>		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>		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>		USDA ERRC B-33246	White chicken salad	+
70	<i>Listeria</i>	<i>monocytogenes</i>		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>		USDA ERRC B-33253	Cooked ham	+
74	<i>Listeria</i>	<i>monocytogenes</i>		BEI HM-1048	Human	+
75	<i>Listeria</i>	<i>monocytogenes</i>	4c	USDA ERRC B-33115	Arabian oryx	+

^aAmerican Type Culture Collection, Manassas, VA.

^bAmerican Genetic Stock Centre, University of Calgary, Canada.

^cBEI Resources, Manassas, VA.

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

No.	Genus	Species	Source	Origin	SIMUL-qPCR Result
1	<i>Alcaligenes</i>	<i>faecalis</i> subsp. <i>faecalis</i>	USDA ERRC ^a 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 4. SIMUL-qPCR *Listeria* Presumptive vs. Confirmed Results (1)

Matrix	Strain	MPN ^a /test portion	N ^b	SIMUL-qPCR <i>Listeria</i> species and <i>monocytogenes</i> presumptive			SIMUL-qPCR <i>Listeria</i> species and <i>monocytogenes</i> confirmed			dPOD _{CF} ^f	95% CI ^g
				x ^c	POD _{CF} ^d	95% CI	x	POD _{CC} ^e	95% CI		
Frankfurters 125 g	<i>L. monocytogenes</i> 1/2b (USDA ERRC B-33258)	N/A ^h	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.91 (0.54, 1.50)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	0.28, 0.28
		5.01 (2.46, 10.2)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Frankfurters ⁱ 125 g	<i>L. monocytogenes</i> 1/2b (ATCC BAA839)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.25 (0.10, 0.48)	20	11	0.55	0.34, 0.74	13	0.65	0.43, 0.82	-0.10	-0.37, 0.19
		1.13 (0.49, 2.59)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
RTE Sliced Turkey 125 g	<i>L. ivanovii</i> (USDA ERRC B-33017)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.19 (0.77, 2.08)	20	10	0.50	0.30, 0.70	8	0.40	0.22, 0.61	0.10	-0.19, 0.37
		4.79 (2.51, 9.17)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Cooked Eggs 25 g	<i>L. seeligeri</i> (USDA ERRC B-33019)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.72 (0.47, 1.07)	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0.00	-0.26, 0.26
		9.37 (5.07, 1.00E+12) ^j	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
Fresh Raw Soft Cheese 25 g	<i>L. monocytogenes</i> 4b (USDA ERRC B-33000)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.57 (0.31, 0.96)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.28, 0.28
		9.37 (5.07, 1.00E+12) ^j	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Fresh Raw Soft Cheese ^k 25 g	<i>L. monocytogenes</i> 4b (ATCC 19115)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.21 (0.79, 1.93)	20	13	0.65	0.43, 0.82	14	0.70	0.48, 0.85	-0.05	-0.32, 0.23
		4.92 (2.27, 10.7)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Frozen/Cooked Shrimp 25 g	<i>L. monocytogenes</i> 1/2a (BEI NR-13229)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.27 (0.81, 2.08)	20	16	0.80	0.58, 0.92	17	0.85	0.64, 0.95	-0.05	-0.29, 0.19
		6.45 (2.88, 14.5)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Chicken Salad 25 g	<i>L. monocytogenes</i> 4e (USDA ERRC B-33120)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.78 (0.47, 1.23)	20	5	0.25	0.11, 0.47	4	0.20	0.08, 0.42	0.05	-0.21, 0.30
		4.79 (2.51, 9.17)	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
Ice Cream 25 g	<i>L. welshimeri</i> (USDA ERRC B-33194)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.56 (0.30, 0.96)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
		9.37 (5.07, 1.00E+12) ^j	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Pasteurized Milk 25 g	<i>L. monocytogenes</i> 4c (BEI NR-111)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.86 (0.51, 1.40)	20	16	0.80	0.58, 0.92	16	0.80	0.58, 0.92	0.00	-0.25, 0.25
		3.63 (1.74, 7.57)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Stainless steel	<i>L. monocytogenes</i> 4a	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43

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1"x 1" AFD swab	(BEI NR-109)/10X <i>E. faecalis</i> (NR-31884)	N/A	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0.00	-0.26, 0.26
		N/A	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Stainless steel	<i>L. monocytogenes</i> 4a	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
4"x 4" Sponge	(BEI NR-109)/10X <i>E. faecalis</i> (NR-31884)	N/A	20	12	0.60	0.39, 0.78	15	0.75	0.53, 0.89	-0.15	-0.40, 0.13
		N/A	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Stainless steel ^l	<i>L. monocytogenes</i> 4a	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
4"x 4" Sponge	(ATCC 19114)/10X <i>E. faecalis</i> (ATCC 29212)	N/A	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.13, 0.13
		N/A	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Plastic	<i>L. innocua</i> (USDA ERRC 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
1"x 1" AFD swab		N/A	20	11	0.55	0.34, 0.74	9	0.45	0.26, 0.66	0.10	-0.19, 0.43
		N/A	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Rubber	<i>L. 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
1"x 1" AFD swab		N/A	20	12	0.60	0.39, 0.78	12	0.60	0.39, 0.78	0.00	-0.28, 0.28
		N/A	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Ceramic tile	<i>L. 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
1"x 1" AFD swab		N/A	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.28, 0.28
		N/A	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
Sealed concrete	<i>L. 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
1"x 1" AFD swab		N/A	20	6	0.30	0.15, 0.52	8	0.40	0.22, 0.61	-0.10	-0.36, 0.18
		N/A	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47

^aMPN = Most Probable Number is based on the POD of reference method test portions using the LCF MPN calculator, with 95% confidence interval.

^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 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.

^hNot applicable.

ⁱAll test portions in the MPN were positive, leading to the high value.

^jMatrix tested by the independent laboratory.

Table 5. SIMUL-qPCR *Listeria* species and *monocytogenes* Assay vs. MLG 4.09 and BAM Ch. 5 (1)

Matrix	Strain	MPN ^a /test portion	N ^b	SIMUL-qPCR <i>Listeria</i> species and <i>monocytogenes</i> results			Reference method results ^c				
				x ^c	POD _C ^d	95% CI	x	POD _R ^f	95% CI	dPOD _C ^g	95% CI ^h
Frankfurters 125 g	<i>L. monocytogenes</i> 1/2b (USDA ERRC B-33258)	N/A ^a	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.91 (0.54, 1.50)	20	9	0.45	0.26, 0.66	13	0.65	0.43, 0.82	-0.20	-0.46, 0.10
		5.01 (2.46, 10.2)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Frankfurters ⁱ 125 g	<i>L. monocytogenes</i> 1/2b (ATCC BAA839)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.25 (0.10, 0.48)	20	11	0.55	0.34, 0.74	6	0.30	0.15, 0.52	0.25	-0.05, 0.50
		1.13 (0.49, 2.59)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
RTE Sliced Turkey 125 g	<i>L. ivanovii</i> (USDA ERRC B-33017)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.19 (0.77, 2.08)	20	8	0.40	0.22, 0.61	12	0.60	0.39, 0.78	-0.20	-0.46, 0.10
		4.79 (2.51, 9.17)	5	5	1.00	0.57, 1.00	4	0.80	0.38, 1.00	0.20	-0.28, 0.62
Cooked Eggs 25 g	<i>L. seeligeri</i> (USDA ERRC B-33019)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.72 (0.47, 1.07)	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0.00	-0.26, 0.26
		9.37 (5.07, 1.00E+12) ^y	5	4	0.80	0.38, 1.00	5	1.00	0.57, 1.00	-0.20	-0.62, 0.28
Fresh Raw Soft Cheese 25 g	<i>L. monocytogenes</i> 4b (USDA ERRC B-33000)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.57 (0.31, 0.96)	20	10	0.50	0.30, 0.70	8	0.40	0.22, 0.61	0.10	-0.19, 0.37
		9.37 (5.07, 1.00E+12) ^y	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Fresh Raw Soft Cheese ⁱ 25 g	<i>L. monocytogenes</i> 4b (ATCC 19115)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.21 (0.79, 1.93)	20	13	0.65	0.43, 0.82	14	0.70	0.48, 0.85	-0.05	-0.32, 0.23
		4.92 (2.27, 10.7)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Frozen/Cooked Shrimp 25 g	<i>L. monocytogenes</i> 1/2a (BEI NR-13229)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.27 (0.81, 2.08)	20	16	0.80	0.58, 0.92	13	0.65	0.43, 0.82	0.15	-0.12, 0.40
		6.45 (2.88, 14.5)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Chicken Salad 25 g	<i>L. monocytogenes</i> 4e (USDA ERRC B-33120)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.78 (0.47, 1.23)	20	4	0.20	0.08, 0.42	7	0.35	0.18, 0.57	-0.15	-0.40, 0.12
		4.79 (2.51, 9.17)	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
Ice Cream 25 g	<i>L. welshimeri</i> (USDA ERRC B-33194)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.56 (0.30, 0.96)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
		9.37 (5.07, 1.00E+12) ^y	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Pasteurized Milk 25 g	<i>L. monocytogenes</i> 4c (BEI NR-111)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.86 (0.51, 1.40)	20	16	0.80	0.58, 0.92	12	0.60	0.39, 0.78	0.20	-0.08, 0.44
		3.63 (1.74, 7.57)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Stainless steel 1"x 1" AFD swab	<i>L. monocytogenes</i> 4a (BEI NR-109)/10X <i>E.</i> <i>faecalis</i> (NR-31884)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		N/A	20	15	0.75	0.53, 0.89	7	0.35	0.18, 0.57	0.40	0.09, 0.62

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		N/A	5	5	1.00	0.57, 1.00	4	0.80	0.38, 1.00	0.20	-0.28, 0.62
Stainless steel 4" x 4" Sponge	<i>L. monocytogenes</i> 4a (BEI NR-109)/10X <i>E. faecalis</i> (NR-31884)	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		N/A	20	12	0.60	0.39, 0.78	7	0.35	0.18, 0.57	0.25	-0.05, 0.50
		N/A	5	5	1.00	0.57, 1.00	4	0.80	0.38, 1.00	0.20	-0.28, 0.62
Stainless steel ^l 4" x 4" Sponge	<i>L. monocytogenes</i> 4a (ATCC 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
		N/A	20	7	0.35	0.18, 0.57	6	0.30	0.15, 0.52	0.10	-0.23, 0.32
		N/A	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" AFD swab	<i>L. innocua</i> (USDA ERRC 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
		N/A	20	9	0.45	0.26, 0.66	7	0.35	0.18, 0.57	0.10	-0.19, 0.43
		N/A	5	5	1.00	0.57, 1.00	4	0.80	0.38, 1.00	0.20	-0.28, 0.62
Rubber 1" x 1" AFD swab	<i>L. 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
		N/A	20	12	0.60	0.39, 0.78	14	0.70	0.48, 0.85	-0.10	-0.36, 0.18
		N/A	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" AFD swab	<i>L. 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
		N/A	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.28, 0.28
		N/A	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
Sealed concrete 1" x 1" AFD swab	<i>L. 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
		N/A	20	6	0.30	0.15, 0.52	10	0.50	0.30, 0.70	-0.20	-0.45, 0.10
		N/A	5	4	0.80	0.38, 1.00	5	1.00	0.57, 1.00	-0.20	-0.62, 0.28

^aMPN = Most Probable Number is based on the POD of reference method test portions using the LCF MPN calculator, with 95% confidence interval.

^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.

^eReference method = MLG 8.10 for frankfurters, RTE sliced turkey, cooked eggs; FDA BAM Ch. 5 for fresh raw soft cheese, frozen/cooked shrimp, chicken salad, ice cream, pasteurized milk, environmental surfaces.

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

^gdPOD_c = Difference between the candidate method result and reference method result POD values.

^h95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

ⁱNot applicable.

^jAll test portions in the MPN were positive, leading to the high value.

^kMatrix tested by the independent laboratory.

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