



CERTIFICATION

AOAC[®] Performance TestedSM

Certificate No.

081802

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

Listeria Right NowTM

manufactured by

Neogen Corporation

620 Leshar Place

Lansing, Michigan 48912

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 (November 24, 2019 – December 31, 2020). 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

November 24, 2020

Date

METHOD AUTHORS ORIGINAL VALIDATION: Quynh Nhi-Le, Susan Alles, Brooke Roman, Eric Tovar, Edan Hosking, Lei Zhang, Preetha Biswas, Benjamin Bastin ¹ , Patrick M. Bird ¹ , Mark Mozola, and Robert Donofrio November 2018 MODIFICATION: Brooke Roman, Mark Mozola, Robert Donofrio, Benjamin Bastin, Nicole Klass, Patrick Bird		SUBMITTING COMPANY Neogen Corporation 620 Leshar Place Lansing, Michigan 48912 USA	
KIT NAME(S) <i>Listeria</i> Right Now™		CATALOG NUMBERS 9873	
INDEPENDENT LABORATORY Q Laboratories 1400 Harrison Ave. Cincinnati, OH USA		AOAC EXPERTS AND PEER REVIEWERS Yi Chen ^{1,4} , Michael Brodsky ² , Wayne Ziemer ³ ¹ US FDA CFSAN, College Park, MD, USA ² Brodsky Consultants, Thornhill, Ontario, Canada ³ Consultant, Loganville, GA, USA ⁴ Modifications: November 2018	
APPLICABILITY OF METHOD Target organism – <i>Listeria</i> spp. (<i>Listeria</i> spp. rRNA) Matrices – (swab, 1 x 1 in) - stainless steel, sealed concrete November 2018 Modification: (swab, 1 x 1 in) ceramic tile, plastic, rubber Performance claims - As determined by probability of detection analysis, LRN method performance is equivalent to that of the U.S. Food and Drug Administration <i>Bacteriological Analytical Manual</i> (FDA/BAM) reference culture method (7). In an experiment measuring recovery from a stainless steel surface inoculated with a pure culture of <i>L. monocytogenes</i> , the probability of detection was 0.65 at 2 CFU/surface and 1.0 at 6 CFU/surface.		REFERENCE METHOD U.S. FDA (2017) Detection and enumeration of <i>Listeria monocytogenes</i> in foods. <i>Bacteriological Analytical Manual</i> , chapter 10 https://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm071400.htm (7)	
ORIGINAL CERTIFICATION DATE August 14, 2018		CERTIFICATION RENEWAL RECORD Renewed annually through December 2020	
METHOD MODIFICATION RECORD <ol style="list-style-type: none"> November 2018 Level 1 November 2018 Level 2 November 2019 Level 1 		SUMMARY OF MODIFICATION <ol style="list-style-type: none"> Editorial/Clerical changes to inserts. Matrix Extension Editorial changes 	
Under this AOAC® <i>Performance Tested</i> SM License Number, 081802 this method is distributed by: NONE		Under this AOAC® <i>Performance Tested</i> SM License Number, 081802 this method is distributed as: NONE	

PRINCIPLE OF THE METHOD (1)

After sample collection from an environmental surface, the entire sampling swab is subject to sample processing. If present, *Listeria* spp. rRNA is liberated in amounts as high as 1,000–10,000 copies per cell. A portion of the lysate is then tested using the ANSR for *Listeria* isothermal nucleic acid amplification assay. Lysates are incubated, first at 37 ± 2°C for 10 min, then at 80 ± 2°C for 20 min. Next, a portion of the lysate is transferred to a strip tube containing lyophilized ANSR reagents. The tubes are sealed and incubated at 56 ± 2°C on the ANSR reader. Reverse transcriptase produces cDNA from the rRNA target, which is then replicated to produce double-stranded DNA. A specific endonuclease creates nicks in double-stranded DNA, and the nicked DNA is amplified using specific templates and DNA polymerase. Amplified target sequences are detected in real time using fluorescent molecular beacon probes. Results are generated by the reader and displayed in the ANSR software within 18 min as positive, negative or invalid. Invalid assay results must be repeated. Each tube of ANSR reagents contains an internal positive control to ensure that the reagents are functioning properly.

DISCUSSION OF THE VALIDATION STUDY (1)

Results of the study reported here show that the LRN method is an effective and accurate procedure for detection of *Listeria* spp. from selected environmental surfaces, with sensitivity comparable to that of the U.S. FDA reference culture method. The most remarkable feature of the LRN test is that this is accomplished without enrichment of the test sample. A highly sensitive isothermal nucleic acid amplification assay targeting a high copy-number ribosomal RNA target, coupled with processing of the entire collected swab sample, allows detection of as few as two *Listeria* cells. The practical significance of this development is profound. Food industry operations, quality control, and food service personnel can now conduct *Listeria* environmental testing in real time, with results available in less than one hour. Results obtained with the LRN test can inform strategies for additional testing and implementation of corrective actions, without the usual delay of 1-2 days while waiting for results of enrichment-based *Listeria* spp. detection methods.

In theory, the LRN test can detect *Listeria* rRNA from both viable and nonviable cells. This is consistent with the intended use of the test as an “early warning system” or indicator of the current or recent presence of *Listeria* spp. in the environment. In the matrix testing experiments reported here, it seems likely that all detections were due to the presence of viable cells, as there was 100% agreement between LRN assay results, confirmation using the in-house reverse transcriptase PCR method, and conventional culture confirmation.

Table 1. Results of inclusivity testing for the *Listeria* Right Now test (1)

Organism	Serotype	Strain	Source	Origin (if known)	Result
<i>L. aquatica</i>		FSL S10-1188	Cornell Univ. ^a	-	Positive
<i>L. booriae</i>		FSL A5-0281	Cornell Univ.	-	Positive
<i>L. cornellensis</i>		FSL F6-0969	Cornell Univ.	-	Positive ^b
<i>L. fleischmannii</i>		FSL F6-1016	Cornell Univ.	-	Positive
<i>L. floridensis</i>		FSL S10-1187	Cornell Univ.	-	Positive
<i>L. grandensis</i>		FSL F6-0971	Cornell Univ.	-	Positive
<i>L. grayi</i> ^c	-	GT4800	Neogen	Environmental	Positive
<i>L. grayi</i> ^c	-	A203	ATCC ^d 19120	Chinchilla feces	Positive
<i>L. grayi</i> subsp. <i>Murrayi</i> ^c	-	A198	Neogen	-	Positive
<i>L. innocua</i>	6a	GT3627	H. Seeliger ^e	Cheese	Positive
<i>L. innocua</i>	6a	A102	ATCC 33090	Cow brain	Positive
<i>L. innocua</i>	6b	GT1026	H. Seeliger	Cheese	Positive
<i>L. innocua</i>	6b	GT1042	H. Seeliger	Cheese	Positive
<i>L. innocua</i>	6b	GT1044	H. Seeliger	Cheese	Positive
<i>L. innocua</i>	6b	GT1050	H. Seeliger	Cheese	Positive
<i>L. innocua</i>	-	GT3785	CDC ^f	-	Positive
<i>L. innocua</i>	-	GT1052	J. Farber ^g	Raw milk	Positive
<i>L. ivanovii</i>	5	GT1028	H. Seeliger	Mouse	Positive
<i>L. ivanovii</i>	5	GT1040	H. Seeliger	Human	Positive
<i>L. ivanovii</i>	5	GT3699	H. Seeliger	Watercress	Positive
<i>L. ivanovii</i>	-	A140	ATCC 19119	Sheep	Positive
<i>L. marthii</i>	-	S4-696	Cornell Univ.	-	Positive

<i>L. monocytogenes</i>	1/2a	GT3727	H. Seeliger	Human blood	Positive
<i>L. monocytogenes</i>	1/2a	GT4340	CDC	Fish	Positive
<i>L. monocytogenes</i>	1/2a	GT1038	H. Seeliger	Human blood	Positive
<i>L. monocytogenes</i>	1/2b	GT3728	H. Seeliger	Cheese	Positive
<i>L. monocytogenes</i>	1/2b	GT3856	H. Seeliger	Cheese	Positive
<i>L. monocytogenes</i>	1/2c	GT3677	H. Seeliger	Cheese	Positive
<i>L. monocytogenes</i>	1/2c	GT2400	H. Seeliger	Human blood	Positive
<i>L. monocytogenes</i>	1/2c	GT3730	H. Seeliger	-	Positive
<i>L. monocytogenes</i>	1/2c	GT3636	H. Seeliger	Human blood	Positive
<i>L. monocytogenes</i>	1/2c	GT3741	H. Seeliger	-	Positive
<i>L. monocytogenes</i>	1a	GT3829	C. Donnelly ^h	Raw milk	Positive
<i>L. monocytogenes</i>	1a	GT1072	C. Donnelly	Raw milk	Positive
<i>L. monocytogenes</i>	1a	GT1880	J. Lovett ⁱ	Brie cheese	Positive
<i>L. monocytogenes</i>	1a	GT3812	J. Lovett	Chocolate milk	Positive
<i>L. monocytogenes</i>	2	A169	ATCC 19112	Human CSF	Positive
<i>L. monocytogenes</i>	3a	GT3720	H. Seeliger	Cheese	Positive
<i>L. monocytogenes</i>	3a	GT1035	H. Seeliger	-	Positive
<i>L. monocytogenes</i>	3b	GT1057	J. Lovett	Brie cheese	Positive
<i>L. monocytogenes</i>	3b	GT3715	H. Seeliger	Human blood	Positive
<i>L. monocytogenes</i>	3b	GT3817	H. Seeliger	Cheese	Positive
<i>L. monocytogenes</i>	3b	GT3857	J. Lovett	Brie cheese	Positive
<i>L. monocytogenes</i>	4a	A170	ATCC 19114	Ruminant brain	Positive
<i>L. monocytogenes</i>	4b	A207	ATCC 13932	Human CSF	Positive
<i>L. monocytogenes</i>	4b	GT1019	Neogen	-	Positive

<i>L. monocytogenes</i>	4b	GT1081	CDC	-	Positive
<i>L. monocytogenes</i>	4c	GT3819	H. Seeliger	Human	Positive
<i>L. newyorkensis</i>		FSL M6-0635	Cornell Univ.	-	Positive
<i>L. riparia</i>		FSL S10-1204	Cornell Univ.	-	Positive
<i>L. rocourtiae</i>		FSL-F6-0972	Cornell Univ.	-	Positive
<i>L. seeligeri</i>	1/2b	GT3693	H. Seeliger	Sewage	Positive
<i>L. seeligeri</i>	4a	GT289	H. Seeliger	Cheese	Positive
<i>L. seeligeri</i>	-	A201	ATCC 51334	Vole	Positive
<i>L. seeligeri</i>	6b	GT3708	H. Seeliger	Cheese	Positive
<i>L. welshimeri</i>	6a	GT293	H. Seeliger	Cheese	Positive
<i>L. welshimeri</i>	6a	GT3742	H. Seeliger	Environmental isolate	Positive
<i>L. welshimeri</i>	-	A199	ATCC 35897	Plant material	Positive
<i>L. welshimeri</i>	-	A200	ATCC 43550	Soil	Positive
<i>L. welshimeri</i>	-	GT1773	Neogen	Environmental isolate	Positive

^aDepartment of Food Science, Cornell University, Stocking Hall, Ithaca, NY 14853.

^bPositive when grown in TSB at 30°C, negative when grown at 36°C.

^cGrown in TSB-YE (as opposed to TSB) prior to inoculation into LESS Plus broth.

^dAmerican Type Culture Collection, 10801 University Blvd., Manassas, VA 20110.

^eInstitute of Hygiene and Molecular Microbiology, University of Würzburg, D8700 Würzburg, Germany.

^fCenters for Disease Control and Prevention, 1600 Clifton Rd., Atlanta, GA 30333.

^gFood Directorate, Health Canada, Banting Research Centre, Tunney's Pasture, Postal Locator 2203G3, Ottawa, Ontario K1A 0L2, Canada.

^hDepartment of Nutrition and Food Sciences, University of Vermont, Nutrition and Food Sciences, Room 254, Burlington, VT 05405.

ⁱU.S. Food and Drug Administration, 6751 Steger Dr., Cincinnati, OH 45237.

Table 2. Results of exclusivity testing for the *Listeria* Right Now test (1)

Organism	Strain #	Source (ATCC #)	Origin (if known)	Culture Conditions ^a	Result
<i>Bacillus cereus</i>	A208	25621	Cow dung	BHI broth ^b , 5% CO ₂ , 48 h, 25°C	Negative
<i>Bacillus megaterium</i>	GT2128	14581	-		Negative
<i>Bacillus subtilis</i>	GT4402	21556	-		Negative
<i>Brevibacillus parabrevis</i>	GT803	8186	Dairy product		Negative
<i>Brochothrix thermosphacta</i>	GT664	11509	Pork sausage		Negative
<i>Enterococcus durans</i>	GT407	6056	Human feces		Negative
<i>Enterococcus faecalis</i>	GT3242	27275	-		Negative
<i>Enterococcus faecium</i>	GT919	6057	Cheese		Negative
<i>Enterococcus hirae</i>	GT923	35220	Cow dung		Negative
<i>Geobacillus stearothermophilus</i>	GT4373	12980	-		Negative
<i>Gordonia sputi</i>	GT3474	29627	Human	Nutrient broth, 5% CO ₂ , 48 h, 37°C	Negative
<i>Kocuria rosea</i>	GT1944	185	-	BHI broth, 48 h, 26°C	Negative
<i>Kocuria varians</i>	GT4404	15306	Milk		Negative
<i>Kurthia gibsonii</i>	GT2129	43195	Meat	MRS broth ^c , 48 h, 30°C	Negative
<i>Kurthia zopfii</i>	GT1941	33403	Turkey cecum		Negative
<i>Lactobacillus acidophilus</i>	GT256	4356	Human		Negative
<i>Lactobacillus buchneri</i>	GT4082	11307	Beer		Negative
<i>Lactobacillus casei</i>	GT805	393	Cheese		Negative
<i>Lactobacillus fermentum</i>	GT4063	9338	-		Negative
<i>Lactococcus lactis</i>	GT3516	11454	-		Negative
<i>Micrococcus luteus</i>	GT1943	381	Water		Negative
<i>Rhodococcus equi</i>	GT665	6939	Horse		Negative
<i>Rhodococcus fascians</i>	GT3524	12974	-	BHI broth, 48 h, 26°C	Negative
<i>Staphylococcus aureus</i>	A179	12600	Human pleural fluid		Negative
<i>Staphylococcus epidermidis</i>	A183	14990	Human		Negative
<i>Staphylococcus saprophyticus</i>	A185	15305	Human urine		Negative
<i>Streptococcus equi</i>	GT3596	33398	-		Negative
<i>Streptococcus agalactiae</i>	GT405	13813	-		Negative
<i>Streptococcus mutans</i>	GT412	25175	Human mouth		Negative
<i>Streptococcus pneumoniae</i>	GT408	6303	-		Negative
<i>Streptococcus sanguinis</i>	GT411	10556	Human		Negative

^aIf other than TSB, 24 h, 36°C.

^bBrain heart infusion broth.

^cDeMan, Rogosa & Sharpe broth

Table 3. *Listeria* Right Now Results: Presumptive vs. Confirmed per BAM Ch. 10 cultural confirmation procedure (1)

Listeria Right Now Results Presumptive or Confirmed per 2011 CM 26 cultural confirmation procedure (7)											
Matrix	Strain	Inoculation level ^a	Listeria Right Now presumptive				Listeria Right Now confirmed by culture			dPOD _{CP} ^f	95% CI ^g
			N ^b	x ^c	POD _{CP} ^d	95% CI	x	POD _{CC} ^e	95% CI		
Stainless steel, 1" x 1" (swab)	<i>L. monocytogenes</i> 1/2a	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.47, 0.47
	UVM ^h CWD1620/10X <i>E. faecalis</i>	86/1,200	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0	-0.13, 0.13
	ATCC ⁱ 29212	160/10,000	5	5	1	0.57, 1	5	1	0.57, 1	0	-0.47, 0.47
Sealed concrete, 1" x 1" (swab)		0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.47, 0.47
	<i>L. seeligeri</i> ATCC 35967	74	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0	-0.13, 0.13
		230	5	5	1	0.57, 1	5	1	0.57, 1	0	-0.47, 0.47

^aInoculation level = CFU applied to each 1" x 1" surface area (test portion).

^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 (per BAM Ch. 10 cultural confirmation procedure) 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.

^hUniversity of Vermont collection, Burlington, VT.

ⁱAmerican Type Culture Collection, Manassas, VA.

Table 4. Method Comparison Results: *Listeria* Right Now (Culture Confirmation) vs. BAM Ch. 10 (1)

Matrix	Strain	Inoculation level ^a	<i>Listeria</i> Right Now results				BAM Ch. 10 results			dPOD _{CP} ^f	95% CI ^g
			N ^b	x ^c	POD _{CP} ^d	95% CI	x	POD _{CC} ^e	95% CI		
Stainless steel, 1" x 1" (swab)	<i>L. monocytogenes</i> 1/2a	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
	UVM ^h CWD1620/10X <i>E. faecalis</i> ATCC ⁱ 29212	86/1,200	20	9	0.45	0.26, 0.66	7	0.35	0.18, 0.57	0.10	-0.19, 0.37
		160/10,000	5	5	1	0.57, 1	5	1	0.57, 1	0	-0.43, 0.43
Sealed concrete, 1" x 1" (swab)		0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
	<i>L. seeligeri</i> ATCC 35967	74	20	8	0.40	0.22, 0.61	6	0.30	0.15, 0.52	0.10	-0.18, 0.36
		230	5	5	1	0.57, 1	5	1	0.57, 1	0	-0.43, 0.43

^aInoculation level = CFU applied to each 1" x 1" surface area (test portion).

^bN = Number of test portions.

^cx = Number of positive test portions.

^dPOD_C = Candidate method presumptive positive outcomes confirmed positive per BAM Ch. 10 cultural confirmation procedure.

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

^fdPOD_C = Difference between the candidate method and reference method 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.

^hUniversity of Vermont collection, Burlington, VT.

ⁱAmerican Type Culture Collection, Manassas, VA

Table 5. *Listeria* Right Now Results: Presumptive vs. Confirmed by PCR (1)

Matrix	Strain	Inoculation level ^a	<i>Listeria</i> Right Now presumptive				<i>Listeria</i> Right Now confirmed by PCR			dPOD _{CP} ^f	95% CI ^g
			N ^b	x ^c	POD _{CP} ^d	95% CI	x	POD _{CC} ^e	95% CI		
Stainless steel, 1" x 1" (swab)	<i>L. monocytogenes</i> 1/2a	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.47, 0.47
	UVM ^h CWD1620/10X <i>E. faecalis</i> ATCC ⁱ 29212	86/1,200	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0	-0.13, 0.13
		160/10,000	5	5	1	0.57, 1	5	1	0.57, 1	0	-0.47, 0.47
Sealed concrete, 1" x 1" (swab)		0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.47, 0.47
	<i>L. seeligeri</i> ATCC 35967	74	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0	-0.13, 0.13
		230	5	5	1	0.57, 1	5	1	0.57, 1	0	-0.47, 0.47

^aInoculation level = CFU applied to each 1" x 1" surface area (test portion).

^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 (per PCR) 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.

^hUniversity of Vermont collection, Burlington, VT.

ⁱAmerican Type Culture Collection, Manassas, VA

Table 6. Method Comparison Results: Listeria Right Now (PCR Confirmation) vs. BAM Ch. 10 (1)

Matrix	Strain	Inoculation level ^a	N ^b	Listeria Right Now results			BAM Ch. 10 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" (swab)	<i>L. monocytogenes</i> 1/2a	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
	UVM ^h CWD1620/10X <i>E. faecalis</i> ATCC ⁱ 29212	86/1,200	20	9	0.45	0.26, 0.66	7	0.35	0.18, 0.57	0.10	-0.19, 0.37
		160/10,000	5	5	1	0.57, 1	5	1	0.57, 1	0	-0.43, 0.43
Sealed concrete, 1" x 1" (swab)		0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
	<i>L. seeligeri</i> ATCC 35967	74	20	8	0.40	0.22, 0.61	6	0.30	0.15, 0.52	0.10	-0.18, 0.36
		230	5	5	1	0.57, 1	5	1	0.57, 1	0	-0.43, 0.43

^aInoculation level = CFU applied to each 1" x 1" surface area (test portion).

^bN = Number of test portions.

^cx = Number of positive test portions.

^dPOD_C = Candidate method presumptive positive outcomes confirmed positive per PCR.

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

^fdPOD_C = Difference between the candidate method and reference method 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.

^hUniversity of Vermont collection, Burlington, VT.

ⁱAmerican Type Culture Collection, Manassas, VA

DISCUSSION OF THE MODIFICATION APPROVED NOVEMBER 2018 (9)

Results of this study show that the LRN method is an effective procedure for detection of *Listeria* spp. in swab samples from ceramic tile, plastic, and rubber surfaces. There were no significant differences in performance between the LRN and reference culture methods for any of the three matrixes tested as determined by POD analysis.

There were no false-negative results by the LRN method. Traditional culture confirmation and the in-house reverse transcriptase PCR method were in complete agreement. There were a total of five unconfirmed positive results by the LRN (ANSR) assay for the three matrixes combined. All of these occurred on low-level inoculated test portions. It is possible, even likely, that these results represent detection of residual nucleic acid from non-viable cells in these test portions. In this case, one would conclude that the ANSR assay is more sensitive than the confirmatory PCR assay.

The data provide support for extension of the original claims for stainless steel and sealed concrete. The enrichment-free LRN test provides food industry personnel with a powerful tool for monitoring of environmental surfaces for *Listeria* contamination in real time.

Table 2. Method Comparison Results: Listeria Right Now (Culture Confirmation) vs. BAM Ch. 10 (9)

Matrix	Strain	Inoculation level ^a	N ^b	Listeria Right Now results			BAM Ch. 10 results			dPOD _{CP} ^f	95% CI ^g
				x ^c	POD _{CP} ^d	95% CI	x	POD _{CC} ^e	95% CI		
Ceramic tile, 1" x 1" (swab)	<i>L. monocytogenes</i> 4b	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		56	20	10	0.50	0.30, 0.70	7	0.35	0.18, 0.57	0.15	-0.15, 0.41
	ATCC ^h 19115	140	5	5	1	0.57, 1	5	1	0.57, 1	0	-0.43, 0.43
Plastic, 1" x 1" (swab)		0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
	<i>L. innocua</i> ATCC 33091	44	20	9	0.45	0.26, 0.66	8	0.40	0.22, 0.61	0.05	-0.24, 0.33
		110	5	5	1	0.57, 1	5	1	0.57, 1	0	-0.43, 0.43
Rubber, 1" x 1" (swab)	<i>L. monocytogenes</i> 1/2b	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		72	20	14	0.70	0.48, 0.85	12	0.60	0.39, 0.78	0.10	-0.18, 0.36
	ATCC BAA-751	210	5	5	1	0.57, 1	5	1	0.57, 1	0	-0.43, 0.43

^aInoculation level = CFU applied to each 1" x 1" surface area (test portion).

^bN = Number of test portions.

^cx = Number of positive test portions.

^dPOD_C = Candidate method presumptive positive outcomes confirmed positive per BAM Ch. 10 cultural confirmation procedure.

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

^fdPOD_C = Difference between the candidate method and reference method 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.

^hAmerican Type Culture Collection, Manassas, VA.

Table 3. *Listeria* Right Now Results: Presumptive vs. Confirmed by PCR (9)

Matrix	Strain	Inoculation level ^a	N ^b	<i>Listeria</i> Right Now presumptive			<i>Listeria</i> Right Now confirmed by PCR			dPOD _{CP} ^f	95% CI ^g
				x ^c	POD _{CP} ^d	95% CI	x	POD _{CC} ^e	95% CI		
Ceramic tile, 1" x 1" (swab)	<i>L. monocytogenes</i> 4b ATCC ^h 19115	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.47, 0.47
		56	20	13	0.65	0.43, 0.82	10	0.50	0.30, 0.70	0.15	-0.05, 0.35
		140	5	5	1	0.57, 1	5	1	0.57, 1	0	-0.47, 0.47
Plastic, 1" x 1" (swab)	<i>L. innocua</i> ATCC 33091	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.47, 0.47
		44	20	10	0.50	0.30, 0.70	9	0.45	0.26, 0.66	0.05	-0.11, 0.21
		110	5	5	1	0.57, 1	5	1	0.57, 1	0	-0.47, 0.47
Rubber, 1" x 1" (swab)	<i>L. monocytogenes</i> 1/2b ATCC BAA-751	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.47, 0.47
		72	20	15	0.75	0.53, 0.89	14	0.70	0.48, 0.85	0.05	-0.11, 0.21
		210	5	5	1	0.57, 1	5	1	0.57, 1	0	-0.47, 0.47

^aInoculation level = CFU applied to each 1" x 1" surface area (test portion).

^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 (per PCR) 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.

^hAmerican Type Culture Collection, Manassas, VA.

Table 4. Method Comparison Results: *Listeria* Right Now (PCR Confirmation) vs. BAM Ch. 10 (9)

Matrix	Strain	Inoculation level ^a	N ^b	<i>Listeria</i> Right Now results			BAM Ch. 10 results				
				x ^c	POD _{CP} ^d	95% CI	x	POD _{CC} ^e	95% CI	dPOD _{CP} ^f	95% CI ^g
Ceramic tile, 1" × 1" (swab)	<i>L. monocytogenes</i> 4b ATCC ^h 19115	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		56	20	10	0.50	0.30, 0.70	7	0.35	0.18, 0.57	0.15	-0.15, 0.41
		140	5	5	1	0.57, 1	5	1	0.57, 1	0	-0.43, 0.43
Plastic, 1" x 1" (swab)	<i>L. innocua</i> ATCC 33091	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		44	20	9	0.45	0.26, 0.66	8	0.40	0.22, 0.61	0.05	-0.24, 0.33
		110	5	5	1	0.57, 1	5	1	0.57, 1	0	-0.43, 0.43
Rubber, 1" x 1" (swab)	<i>L. monocytogenes</i> 1/2b ATCC BAA-751	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		72	20	14	0.70	0.48, 0.85	12	0.60	0.39, 0.78	0.10	-0.18, 0.36
		210	5	5	1	0.57, 1	5	1	0.57, 1	0	-0.43, 0.43

^aInoculation level = CFU applied to each 1" x 1" surface area (test portion).

^bN = Number of test portions.

^cx = Number of positive test portions.

^dPOD_C = Candidate method presumptive positive outcomes confirmed positive per PCR.

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

^fdPOD_C = Difference between the candidate method and reference method 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.

^hAmerican Type Culture Collection, Manassas, VA.

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