CERTIFICATION

AOAC® *Performance Tested*SM

Certificate No.

081802

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

Listeria Right Now™

manufactured by

Neogen Corporation
620 Lesher Place
Lansing, Michigan 48912

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

November 24, 2020
Date

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November 2018 MODIFICATION: Brooke Roman, Mark Mozola, Robert Donofrio, Benjamin Bastin, Nicole Klass, Patrick Bird

SUBMITTING COMPANY
Neogen Corporation
620 Lesher Place
Lansing, Michigan 48912
USA

KIT NAME(S)
Listeria Right Now™

CATALOG NUMBERS
9873

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

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APPLICABILITY OF METHOD
Target organism – *Listeria* spp. (*Listeria* 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 *Bacteriological Analytical Manual* (FDA/BAM) reference culture method (7). In an experiment measuring recovery from a stainless steel surface inoculated with a pure culture of *L. monocytogenes*, 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 *Listeria monocytogenes* in foods. *Bacteriological Analytical Manual*, 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
1. **November 2018 Level 1**
2. **November 2018 Level 2**
3. **November 2019 Level 1**

SUMMARY OF MODIFICATION
1. **Editorial/Clerical changes to inserts.**
2. **Matrix Extension**
3. **Editorial changes**

Under this AOAC® *Performance Tested*℠ License Number, 081802 this method is distributed by:
NONE

Under this AOAC® *Performance Tested*℠ 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
L. aquatica		FSL S10-1188	Cornell Univ.[a]	-	Positive
L. booriae		FSL A5-0281	Cornell Univ.	-	Positive
L. cornellensis		FSL F6-0969	Cornell Univ.	-	Positive[b]
L. fleischmannii		FSL F6-1016	Cornell Univ.	-	Positive
L. floridensis		FSL S10-1187	Cornell Univ.	-	Positive
L. grandensis		FSL F6-0971	Cornell Univ.	-	Positive
L. grayi[c]	-	GT4800	Neogen	Environmental	Positive
L. grayi[c]	-	A203	ATCC[d] 19120	Chinchilla feces	Positive
L. grayi subsp. *Murrayi*[c]	-	A198	Neogen	-	Positive
L. innocua	6a	GT3627	H. Seeliger[e]	Cheese	Positive
L. innocua	6a	A102	ATCC 33090	Cow brain	Positive
L. innocua	6b	GT1026	H. Seeliger	Cheese	Positive
L. innocua	6b	GT1042	H. Seeliger	Cheese	Positive
L. innocua	6b	GT1044	H. Seeliger	Cheese	Positive
L. innocua	6b	GT1050	H. Seeliger	Cheese	Positive
L. innocua	-	GT3785	CDC[f]	-	Positive
L. innocua	-	GT1052	J. Farber[g]	Raw milk	Positive
L. ivanovii	5	GT1028	H. Seeliger	Mouse	Positive
L. ivanovii	5	GT1040	H. Seeliger	Human	Positive
L. ivanovii	5	GT3699	H. Seeliger	Watercress	Positive
L. ivanovii	-	A140	ATCC 19119	Sheep	Positive
L. marthii	-	S4-696	Cornell Univ.	-	Positive

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

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

[a]Department of Food Science, Cornell University, Stocking Hall, Ithaca, NY 14853.
[b]Positive when grown in TSB at 30°C, negative when grown at 36°C.
[c]Grown in TSB-YE (as opposed to TSB) prior to inoculation into LESS Plus broth.
[d]American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110.
[e] Institute of Hygiene and Molecular Microbiology, University of Würzburg, D8700 Würzburg, Germany.
[f]Centers for Disease Control and Prevention, 1600 Clifton Rd., Atlanta, GA 30333.
[g]Food Directorate, Health Canada, Banting Research Centre, Tunney's Pasture, Postal Locator 2203G3, Ottawa, Ontario K1A 0L2, Canada.
[h]Department of Nutrition and Food Sciences, University of Vermont, Nutrition and Food Sciences, Room 254, Burlington, VT 05405.
[i]U.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
Bacillus cereus	A208	25621	Cow dung	BHI broth[b], 5% CO_2, 48 h, 25°C	Negative
Bacillus megaterium	GT2128	14581	-		Negative
Bacillus subtilis	GT4402	21556	-		Negative
Brevibacillus parabrevis	GT803	8186	Dairy product		Negative
Brochothrix thermosphacta	GT664	11509	Pork sausage		Negative
Enterococcus durans	GT407	6056	Human feces		Negative
Enterococcus faecalis	GT3242	27275	-		Negative
Enterococcus faecium	GT919	6057	Cheese		Negative
Enterococcus hirae	GT923	35220	Cow dung		Negative
Geobacillus stearothermophilus	GT4373	12980	-		Negative
Gordonia sputi	GT3474	29627	Human	Nutrient broth, 5% CO_2, 48 h, 37°C	Negative
Kocuria rosea	GT1944	185	-	BHI broth, 48 h, 26°C	Negative
Kocuria varians	GT4404	15306	Milk		Negative
Kurthia gibsonii	GT2129	43195	Meat		Negative
Kurthia zopfii	GT1941	33403	Turkey cecum		Negative
Lactobacillus acidophilus	GT256	4356	Human		Negative
Lactobacillus buchneri	GT4082	11307	Beer	MRS broth[c], 48 h, 30°C	Negative
Lactobacillus casei	GT805	393	Cheese		Negative
Lactobacillus fermentum	GT4063	9338	-		Negative
Lactococcus lactis	GT3516	11454	-		Negative
Micrococcus luteus	GT1943	381	Water		Negative
Rhodococcus equi	GT665	6939	Horse		Negative
Rhodococcus fascians	GT3524	12974	-	BHI broth, 48 h, 26°C	Negative
Staphylococcus aureus	A179	12600	Human pleural fluid		Negative
Staphylococcus epidermidis	A183	14990	Human		Negative
Staphylococcus saprophyticus	A185	15305	Human urine		Negative
Streptococcus equi	GT3596	33398	-		Negative
Streptococcus agalactiae	GT405	13813	-		Negative
Streptococcus mutans	GT412	25175	Human mouth		Negative
Streptococcus pneumoniae	GT408	6303	-		Negative
Streptococcus sanguinis	GT411	10556	Human		Negative

[a]If other than TSB, 24 h, 36°C.
[b]Brain heart infusion broth.
[c]DeMan, Rogosa & Sharpe broth

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

			Listeria Right Now presumptive				*Listeria* Right Now confirmed by culture				
Matrix	Strain	Inoculation level[a]	N[b]	x[c]	POD_{CP}[d]	95% CI	x	POD_{CC}[e]	95% CI	$dPOD_{CP}$[f]	95% CI[g]
Stainless steel, 1" × 1" (swab)	*L. monocytogenes* 1/2a UVM[h] CWD1620/ 10X *E. faecalis* ATCC[i] 29212	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.47, 0.47
		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)	*L. seeligeri* ATCC 35967	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.47, 0.47
		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

[a]Inoculation level = CFU applied to each 1" x 1" surface area (test portion).
[c]x = Number of positive test portions.
[d]POD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials.
[e]POD_{CC} = Candidate method confirmed positive outcomes (per BAM Ch. 10 cultural confirmation procedure) divided by the total number of trials.
[f]$dPOD_{CP}$ = Difference between the candidate method presumptive result and candidate method confirmed result POD values.
[g]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[h]University of Vermont collection, Burlington,VT.
[i]American 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]	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" × 1" (swab)	*L. monocytogenes* 1/2a UVM[h] CWD1620/ 10X *E. faecalis* ATCC[i] 29212	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		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)	*L. seeligeri* ATCC 35967	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		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

[a]Inoculation level = CFU applied to each 1" x 1" surface area (test portion).
[b]N = Number of test potions.
[c]x = Number of positive test portions.
[d]POD$_C$ = Candidate method presumptive positive outcomes confirmed positive per BAM Ch. 10 cultural confirmation procedure.
[e]POD$_R$ = Reference method confirmed positive outcomes divided by the total number of trials.
[f]dPOD$_C$ = Difference between the candidate method and reference method POD values.
[g]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[h]University of Vermont collection, Burlington, VT.
[i]American Type Culture Collection, Manassas, VA

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

Matrix	Strain	Inoculation level[a]	N[b]	*Listeria* Right Now presumptive			*Listeria* Right Now confirmed by PCR			dPOD$_{CP}$[f]	95% CI[g]
				x[c]	POD$_{CP}$[d]	95% CI	x	POD$_{CC}$[e]	95% CI		
Stainless steel, 1" × 1" (swab)	*L. monocytogenes* 1/2a UVM[h] CWD1620/ 10X *E. faecalis* ATCC[i] 29212	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.47, 0.47
		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)	*L. seeligeri* ATCC 35967	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.47, 0.47
		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

[a]Inoculation level = CFU applied to each 1" x 1" surface area (test portion).
[c]x = Number of positive test portions.
[d]POD$_{CP}$ = Candidate method presumptive positive outcomes divided by the total number of trials.
[e]POD$_{CC}$ = Candidate method confirmed positive outcomes (per PCR) divided by the total number of trials.
[f]dPOD$_{CP}$ = Difference between the candidate method presumptive result and candidate method confirmed result POD values.
[g]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[h]University of Vermont collection, Burlington, VT.
[i]American Type Culture Collection, Manassas, VA

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

				Listeria Right Now results			BAM Ch. 10 results				
Matrix	Strain	Inoculation level[a]	N[b]	x[c]	POD_{CP}[d]	95% CI	x	POD_{CC}[e]	95% CI	$dPOD_{CP}$[f]	95% CI[g]
Stainless steel, 1" × 1" (swab)	*L. monocytogenes* 1/2a UVM[h] CWD1620/ 10X *E. faecalis* ATCC[i] 29212	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		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)	*L. seeligeri* ATCC 35967	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		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

[a]Inoculation level = CFU applied to each 1" x 1" surface area (test portion).
[b]N = Number of test potions.
[c]x = Number of positive test portions.
[d]POD_C = Candidate method presumptive positive outcomes confirmed positive per PCR.
[e]POD_R = Reference method confirmed positive outcomes divided by the total number of trials.
[f]$dPOD_C$ = Difference between the candidate method and reference method POD values.
[g]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[h]University of Vermont collection, Burlington, VT.
[i]American 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)

				Listeria Right Now results			BAM Ch. 10 results				
Matrix	Strain	Inoculation level[a]	N[b]	x[c]	POD_{CP}[d]	95% CI	x	POD_{CC}[e]	95% CI	$dPOD_{CP}$[f]	95% CI[g]
Ceramic tile, 1" × 1" (swab)	*L. monocytogenes* 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)	*L. innocua* 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)	*L. monocytogenes* 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

[a]Inoculation level = CFU applied to each 1" x 1" surface area (test portion).
[b]N = Number of test potions.
[c]x = Number of positive test portions.
[d]POD_C = Candidate method presumptive positive outcomes confirmed positive per BAM Ch. 10 cultural confirmation procedure.
[e]POD_R = Reference method confirmed positive outcomes divided by the total number of trials.
[f]$dPOD_C$ = Difference between the candidate method and reference method POD values.
[g]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[h]American Type Culture Collection, Manassas, VA.

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

Matrix	Strain	Inoculation level[a]	N[b]	*Listeria* Right Now presumptive x[c]	*Listeria* Right Now presumptive POD_{CP}[d]	*Listeria* Right Now presumptive 95% CI	*Listeria* Right Now confirmed by PCR x	*Listeria* Right Now confirmed by PCR POD_{CC}[e]	*Listeria* Right Now confirmed by PCR 95% CI	$dPOD_{CP}$[f]	95% CI[g]
Ceramic tile, 1" × 1" (swab)	*L. monocytogenes* 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)	*L. innocua* 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)	*L. monocytogenes* 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

[a]Inoculation level = CFU applied to each 1" x 1" surface area (test portion).
[c]x = Number of positive test portions.
[d]POD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials.
[e]POD_{CC} = Candidate method confirmed positive outcomes (per PCR) divided by the total number of trials.
[f]$dPOD_{CP}$ = Difference between the candidate method presumptive result and candidate method confirmed result POD values.
[g]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[h]American Type Culture Collection, Manassas, VA.

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

				Listeria Right Now results			BAM Ch. 10 results				
Matrix	Strain	Inoculation level[a]	N[b]	x[c]	POD_{CP}[d]	95% CI	x	POD_{CC}[e]	95% CI	$dPOD_{CP}$[f]	95% CI[g]
Ceramic tile, 1" × 1" (swab)	*L. monocytogenes* 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)	*L. innocua* 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)	*L. monocytogenes* 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

[a]Inoculation level = CFU applied to each 1" x 1" surface area (test portion).
[b]N = Number of test potions.
[c]x = Number of positive test portions.
[d]POD_C = Candidate method presumptive positive outcomes confirmed positive per PCR.
[e]POD_R = Reference method confirmed positive outcomes divided by the total number of trials.
[f]$dPOD_C$ = Difference between the candidate method and reference method POD values.
[g]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[h]American Type Culture Collection, Manassas, VA.

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