CERTIFICATION

AOAC® *Performance Tested*SM

Certificate No.

061506

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

ANSR® for *Listeria monocytogenes*

manufactured by

Neogen Corporation
620 Lesher Place
Lansing, MI 48912
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 (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, 2019
Date

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SUBMITTING COMPANY
Neogen Corporation
620 Lesher Place
Lansing, MI 48912

KIT NAME(S)
ANSR® for *Listeria monocytogenes* #061506

CATALOG NUMBERS
9824

INDEPENDENT LABORATORY
Q Laboratories, Inc.
1400 Harrison Avenue, Cincinnati, OH 45214
USA

AOAC EXPERTS AND PEER REVIEWERS
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APPLICABILITY OF METHOD
Target organism – *Listeria monocytogenes*

Matrices – BAM Ch. 10 (25 g): queso fresco, cantaloupe, guacamole, pasteurized liquid egg, sprout irrigation water
MLG 8.09: hot dogs (125 g), stainless steel (sponge, 4 x 4 in)

Performance claims - Performance equivalent to that of the reference methods.

REFERENCE METHODS
US FDA (2011) *Bacteriological Analytical Manual*, chapter 10 (Accessed February, 2015) http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm071400.htm (2)
USDA-FSIS (2014) *Microbiology Laboratory Guidebook*, chapter 8.09 (Accessed January, 2015) http://www.fsis.usda.gov/wps/wcm/connect/1710bee8-76b9-4e6c-92fc-fdc290dbfa92/MLG-8.pdf?MOD=AJPERES (3)

ORIGINAL CERTIFICATION DATE
June 30, 2015

CERTIFICATION RENEWAL RECORD
Renewed annually through December 2020

METHOD MODIFICATION RECORD
1. **December 2018 Level 1**
2. **November 2019 Level 1**

SUMMARY OF MODIFICATION
1. **Editorial changes for clarity**
2. **Editorial changes for clarity**

Under this AOAC® *Performance Tested*SM License Number, 061506 this method is distributed by:
NONE

Under this AOAC® *Performance Tested*SM License Number, 061506 this method is distributed as:
NONE

PRINCIPLE OF THE METHOD (1)
ANSR *Listeria monocytogenes* is an isothermal nucleic acid amplification assay based on nicking enzyme amplification reaction (NEAR™) technology (3). The amplification mechanism involves binding of an oligonucleotide "template" to a specific sequence of target DNA. The template contains a recognition site for a specific endonuclease. The nicked strand is recognized as damaged and repaired by the action of a thermostable DNA polymerase, displacing the original strand with the newly-synthesized repaired portion. This displaced DNA "product" then binds to a second template and the same reactions lead to formation of a second product. Amplification products are detected using a specific molecular beacon probe. A fluorescent signal is generated in real time, with amplification and detection complete within 10 minutes. The entire assay is conducted at a constant temperature of 56°C using a temperature-controlled fluorescence detection instrument. Assay software analyzes the fluorescent signal over time; a data interpretation algorithm interprets results as negative, positive, or invalid based on baseline, rate-of-change, and other criteria. Each ANSR reagent tube also contains an internal positive control, signaling in a second fluorescence channel irrespective of the presence of target DNA, and indicating proper functioning of the amplification reagents.

DISCUSSION OF THE VALIDATION STUDY (1)
The results presented here demonstrate that overall performance of the ANSR *Listeria monocytogenes* assay is equivalent to that of either the USDA-FSIS/MLG or FDA/BAM reference methods. Based on internal as well as independent laboratory studies for hot dogs, Mexican-style cheese, cantaloupe, guacamole, pasteurized liquid egg, sprout irrigation water, and sponge samples from a stainless steel surface, ANSR *Listeria monocytogenes* is an effective procedure for detection of *L. monocytogenes* after 24 h of enrichment. Further, the data supports testing of stainless steel sponge samples after only 16 h of enrichment. In inclusivity testing, 96% of *L. monocytogenes* strains tested were positive, while in exclusivity testing, 100% of strains tested were negative. Results of robustness testing showed that the modified assay can withstand minor deliberate perturbations to multiple assay parameters and still yield accurate results.
In addition to high sensitivity and specificity, the ANSR *Listeria monocytogenes* method offers the advantages of single-step enrichment, a universal enrichment medium, minimal labor and assay hardware requirements, and assay results within 40 minutes following sample enrichment. When used with sample enrichment in LESS Plus broth, the ANSR *Listeria* and ANSR *Listeria monocytogenes* assays can be employed either separately, or in combination, as part of an efficient *Listeria* testing program. Samples can be screened using the *Listeria* spp. assay to provide results at an indicator level, and then positives may be re-tested with the *Listeria monocytogenes* assay to provide a rapid and high confidence indication of the presence of the pathogenic species.

Table 1. Results of inclusivity testing for the ANSR *Listeria monocytogenes* test using LESS Plus enrichment media (1)

Organism	Serotype	Strain #	Source	Origin (if known)	ANSR Result, (~10^6 CFU/mL)
L. monocytogenes	1/2a	GT2669	H. Seeliger[b]	Cheese	Positive
L. monocytogenes	1/2a	GT1030	H. Seeliger	Cheese	Positive
L. monocytogenes	1/2b	GT1038	H. Seeliger	Blood	Positive
L. monocytogenes	3b	GT3396	H. Seeliger	Cheese	Positive
L. monocytogenes	1/2a	GT3727	H. Seeliger	Blood	Positive
L. monocytogenes	1/2a	GT4340	CDC[c]	Butterfish	Positive
L. monocytogenes	1/2a	GT4343	CDC	Sliced ham	Positive
L. monocytogenes	1/2a	GTA194	ATCC[a] 51773	-	Positive
L. monocytogenes	1/2b	GT3635	H. Seeliger	Blood	Positive
L. monocytogenes	1/2b	GT3728	H. Seeliger	Cheese	Positive
L. monocytogenes	1/2b	GT4341	CDC	Butterfish	Positive
L. monocytogenes	1/2b	A195	ATCC 51780	-	Positive
L. monocytogenes	1/2c	GT3648	H. Seeliger	Cheese	Positive
L. monocytogenes	1/2c	GT2400	H. Seeliger	Blood	Positive
L. monocytogenes	1/2c	GT3730	H. Seeliger	-	Positive
L. monocytogenes	1/2b	GT3741	H. Seeliger	-	Positive
L. monocytogenes	1a	GT1072	C. Donnelly[d]	Raw milk	Positive
L. monocytogenes	1a	GT1082	C. Donnelly	Raw milk	Positive
L. monocytogenes	1a	GT1880	J. Lovett[e]	Brie cheese	Positive
L. monocytogenes	1a	GT3812	J. Lovett	Chocolate milk	Positive
L. monocytogenes	1a	GT3829	C. Donnelly	Raw milk	Positive
L. monocytogenes	1b	GT3784	CDC	-	Negative[f]
L. monocytogenes	2	A169	ATCC 19112	-	Positive
L. monocytogenes	3a	GT1035	H. Seeliger	-	Positive
L. monocytogenes	-	GT3147	Neogen	Environmental	Positive
L. monocytogenes	3a	GT3720	H. Seeliger	Cheese	Positive
L. monocytogenes	3b	GT1057	J. Lovett	Brie cheese	Positive
L. monocytogenes	3b	GT3715	H. Seeliger	Blood	Positive
L. monocytogenes	3b	GT3745	H. Seeliger	-	Positive
L. monocytogenes	3b	GT3857	J. Lovett	Brie cheese	Positive
L. monocytogenes	3c	GT3695	H. Seeliger	Cheese	Positive
L. monocytogenes	4a	GT1046	H. Seeliger	Human	Positive
L. monocytogenes	4a	A170	ATCC 19114	-	Positive
L. monocytogenes	4b	GT1019	Neogen	-	Positive
L. monocytogenes	4b	GT1022	CDC	Human	Positive
L. monocytogenes	4b	GT1081	C. Donnelly	-	Positive
L. monocytogenes	4b	GT3733	H. Seeliger	Human	Positive
L. monocytogenes	4b	GT3746	H. Seeliger	Human	Positive
L. monocytogenes	4b	GT3957	H. Seeliger	Cheese	Positive
L. monocytogenes	4b	GT4342	CDC	Sausage	Positive
L. monocytogenes	4b	A171	ATCC 19115	Human	Positive
L. monocytogenes	4b	A207	ATCC 13932	Human CSF	Positive
L. monocytogenes	3b	GT3817	H. Seeliger	Cheese	Positive

L. monocytogenes	4b	GT2402	H. Seeliger	Blood	Positive
L. monocytogenes	6b	GT672	H. Seeliger	Cheese	Positive
L. monocytogenes	7	GT278	H. Seeliger	-	Positive
L. monocytogenes	7a	GT354	Neogen	-	Positive
L. monocytogenes	-	3707	ATCC 35152	Guinea pig	Negative[f]
L. monocytogenes	-	GT1013	USDA	Beef	Positive
L. monocytogenes	-	4790	ATCC 35152	Guinea pig	Positive

[a] American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110.
[b] Institute of Hygiene and Molecular Microbiology, University of Würzburg, D8700 Würzburg, Germany.
[c] Centers for Disease Control and Prevention, 1600 Clifton Rd., Atlanta, GA 30333.
[d] Department of Nutrition and Food Sciences, University of Vermont, Nutrition and Food Sciences, 355 MLS Carrigan Wing , Burlington, VT 05405.
[e] U.S. Food and Drug Administration, 6751 Steger Dr., Cincinnati, OH 45237.
[f] Strains were confirmed by PCR to be lacking the assay target gene.

Table 2. Results of exclusivity testing for the ANSR *Listeria monocytogenes* test

Organism	Strain No.	Source	Origin (if known)	ANSR result (~10^9 CFU/mL)	Culture conditions[a]
Bacillus cereus	A208	ATCC 25621	Cow dung	Negative	
Bacillus megaterium	GT2128	ATCC 14581	-	Negative	
Bacillus subtilis	GT4402	ATCC[b] 21556	Grain	Negative	
Brochothrix thermosphacta	GT664	ATCC 11509	Pork sausage	Negative	BHI Broth, 26°C for 48 h, 5% CO2
Enterococcus durans	GT407	ATCC 6056	Human feces	Negative	
Enterococcus faecalis	GT3242	ATCC 27275	-	Negative	
Enterococcus faecium	GT919	ATCC 6057	Cheese	Negative	
Enterococcus hirae	GT923	ATCC 35220	Cow dung	Negative	
Geobacillus stearothermophilus	GT4373	ATCC 12980	-	Negative	
Kocuria varians	GT4404	ATCC 15306	Milk	Negative	Nutrient Broth, 48 h
Kurthia gibsonii	GT2129	ATCC 43195	Meat	Negative	
Kurthia zopfii	GT1941	ATCC 33403	Turkey cecum	Negative	
Lactobacillus acidophilus	GT256	ATCC 4356	Human	Negative	
Lactobacillus fermentum	GT4063	ATCC 9338	-	Negative	MRS Broth, 35°C for 48 h, 5% CO2
Lactococcus lactis	GT3516	ATCC 11454	-	Negative	
Listeria grayi	GT4800	Neogen[c]	Environmental	Negative	
Listeria grayi	A203	ATCC 19120	Chinchilla feces	Negative	
Listeria grayi	GT259	Land O'Lakes[d]	Dairy plant	Negative	
Listeria innocua	GT1029	H. Seeliger[e]	Milk	Negative	
Listeria innocua	GT1026	H. Seeliger	Cheese	Negative	
Listeria innocua	GT1042	H. Seeliger	Cheese	Negative	
Listeria innocua	GT1050	H. Seeliger	Cheese	Negative	
Listeria innocua	GT1044	H. Seeliger	Cheese	Negative	
Listeria innocua	GT1052	J. Farber[f]	Milk	Negative	
Listeria innocua	GT3627	H. Seeliger	Cheese	Negative	
Listeria innocua	3785	ATCC 33090	Cow brain	Negative	
Listeria innocua	GT3631	CDC[g]	Cheese	Negative	
Listeria innocua	A102	ATCC 33090	Cow brain	Negative	
Listeria ivanovii	A140	ATCC 19119	Sheep	Negative	

Listeria ivanovii	GT1028	H. Seeliger	Mouse brain	Negative	
Listeria ivanovii	GT4339	CDC	Stuffed lamb chop	Negative	
Listeria seeligeri	A201	ATCC 51334	Bank vole intestines	Negative	
Listeria seeligeri	GT3794	J. Lovett[h]	Brie cheese	Negative	
Listeria seeligeri	GT3821	H. Seeliger	Soft cheese	Negative	
Listeria welshimeri	A199	ATCC 35897	Decaying plant material	Negative	
Listeria welshimeri	A200	ATCC 43550	Soil	Negative	
Listeria welshimeri	GT3862	C. Donnelly[i]	Milk	Negative	
Micrococcus luteus	GT1943	ATCC 381	Water	Negative	
Rhodococcus equi	GT665	ATCC 6939	Horse	Negative	
Rhodococcus fascians	GT3524	ATCC 12974	-	Negative	BHI broth, 35°C for 48 h
Staphylococcus aureus	A179	ATCC 12600	Pleural fluid	Negative	
Staphylococcus epidermidis	A183	ATCC 14990	Human nose	Negative	
Staphylococcus saprophyticus	A185	ATCC 15305	Human urine	Negative	
Streptococcus equi	GT3596	ATCC 33398	-	Negative	CBAB/ BHI Broth
Streptococcus gallolyticus	GT668	ATCC 9809	-	Negative	
Streptococcus mutans	GT412	ATCC 25175	Human mouth	Negative	BHI Broth for 48 h
Streptococcus sanguinis	GT411	ATCC 10556	Human	Negative	

[a] If other than TSB, 16–24 h, 36°C.
[b] American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110.
[c] Neogen Corp., 620 Lesher Place, Lansing, MI 48912.
[d] Land O' Lakes, 1200 County Road F West, Arden Hills, MN 55112.
[e] Institute of Hygiene and Molecular Microbiology, University of Würzburg, D8700 Würzburg, Germany.
[f] Health Canada, Bureau of Microbial Hazards, Ottawa K1A 0L2, Canada.
[g] Centers for Disease Control and Prevention, 1600 Clifton Rd., Atlanta, GA 30333.
[h] U.S. Food and Drug Administration, 6751 Steger Dr., Cincinnati, OH 45237.
[i] Department of Nutrition and Food Sciences, University of Vermont, Nutrition and Food Sciences, 355 MLS Carrigan Wing , Burlington, VT 05405.

Table 3. Probability of detection calculations for ANSR *Listeria monocytogenes* presumptive and confirmed results, 16 h time point (1)

Matrix/sample size	Inoculum strain	Inoc. level (CFU/portion)[a]	N[b]	ANSR presumptive result			ANSR confirmed result			$dPOD_{CP}$[f]	95% CI[g]
				X[c]	POD_{CP}[d]	95% CI	x	POD_{CC}[e]	95% CI		
Hot dogs, 125 g	*L. monocytogenes* 4b	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.69 (0.36, 1.2)	20	5	0.25	0.11, 0.47	17	0.85	0.64, 0.95	-0.60	-0.77, -0.30
		> 58	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Mexican-style cheese, 25 g	*L. monocytogenes* 1/2b	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.74 (0.42, 1.4)	20	11	0.55	0.34, 0.74	14	0.70	0.48, 0.85	-0.15	-0.41, 0.14
		11 (2.5, 53)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Cantaloupe, 25 g	*L. monocytogenes* 4b	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.31 (0.16, 0.49)	20	8	0.40	0.22, 0.61	9	0.45	0.26, 0.66	-0.05	-0.33, 0.24
		5.5 (2.1, 15)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Guacamole, 25 g	*L. monocytogenes* 1/2c	0	5	1	0.20	0, 0.62	0	0	0, 0.43	0.20	-0.28, 0.62
		0.29 (0.10, 0.60)	20	3	0.15	0.05, 0.36	4	0.20	0.08, 0.42	-0.05	-0.29, 0.19
		27 (6.5, 117)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Pasteurized liquid egg[h], 25 g	*L. monocytogenes* 1/2a	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		1.7 (1.0, 3.3)	20	10	0.50	0.3, 0.7	12	0.60	0.39, 0.78	-0.10	-0.37, 0.19
		1.9 (1.2, 3.7)	20	13	0.65	0.43, 0.82	16	0.80	0.58, 0.92	-0.15	-0.4, 0.12
		37 (11, 122)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Sprout irrigation water, 25 mL	*L. monocytogenes* 1/2b	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.40 (0.15, 0.72)	20	2	0.10	0.03, 0.3	4	0.20	0.08, 0.42	-0.10	-0.33, 0.13
		3.7 (2.1, 23)	20	20	1	0.84, 1	20	1	0.84, 1	0	-0.16, 0.16
		11 (2.3, 47)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Stainless steel	*L. monocytogenes* 1/2a + *E. faecium*	0/31,000	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		3,700/312,000	20	18	0.90	0.70, 0.97	16	0.80	0.58, 0.92	0.10	-0.13, 0.33
		370,000/3,120,000	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Stainless steel[i]	*L. monocytogenes 1/2a + E. faecalis*	0/0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		430/1,200	20	17	0.85	0.64, 0.95	17	0.85	0.64, 0.95	0	-0.23, 0.23
		820/7,300	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43

[a] Determined by most probable number analysis except for stainless steel where determination was by titer of inoculum.
[b] N = Number of test portions.
[c] x = Number of positive test portions.
[d] POD_{CP} = Candidate method presumptive positive outcomes
[e] POD_{CC} = Candidate method presumptive positive outcomes confirmed positive.
[f] $dPOD_{CP}$ = Difference between the candidate method presumptive and candidate method confirmed 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] 24 h for pasteurized liquid egg.
[i] Trial performed by independent laboratory.

Table 4. Probability of detection calculations for ANSR *Listeria monocytogenes* presumptive and confirmed results, 24 h time point (1)

Food type	Inoculum strain	Inoc. level (CFU/portion)[a]	N[b]	ANSR presumptive result			ANSR confirmed result			dPOD$_{CP}$[f]	95% CI[g]
				X[c]	POD$_{CP}$[d]	95% CI	x	POD$_{CC}$[e]	95% CI		
Hot dogs, 125 g	*L. monocytogenes* 4b	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.69 (0.36, 1.2)	20	19	0.95	0.76, 1	20	1	0.84, 1	-0.05	-0.24, 0.12
		> 58	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Hot dogs, 125 g[h]	*L. monocytogenes* 4b	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.92 (0.55, 1.6)	20	13	0.65	0.43, 0.82	13	0.65	0.43, 0.82	0	-0.28, 0.28
		7.4 (3.2, 17)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Mexican-style cheese, 25 g	*L. monocytogenes* 1/2b	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.74 (0.42, 1.4)	20	14	0.70	0.48, 0.85	14	0.70	0.48, 0.85	0	-0.27, 0.27
		11 (2.5, 53)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Mexican-style cheese, 25 g[h]	*L. monocytogenes* 1/2b	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.69 (0.41, 1.1)	20	10	0.50	0.3, 0.7	10	0.50	0.3, 0.7	0	-0.28, 0.28
		1.9 (0.89, 4.1)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Cantaloupe, 25 g	*L. monocytogenes* 4b	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.31 (0.16, 0.49)	20	8	0.40	0.22, 0.61	9	0.45	0.26, 0.66	-0.05	-0.33, 0.24
		5.5 (2.1, 15)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Guacamole, 25 g	*L. monocytogenes* 1/2c	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.29 (0.10, 0.60)	20	4	0.20	0.08, 0.42	4	0.20	0.08, 0.42	0	-0.25, 0.25
		27 (6.5, 117)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Pasteurized liquid egg, 25[i] g	*L. monocytogenes* 1/2a	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		1.7 (1.0, 3.3)	20	12	0.60	0.39, 0.78	12	0.60	0.39, 0.78	0	-0.28, 0.28
		1.9 (1.2, 3.7)	20	17	0.85	0.64, 0.95	16	0.80	0.58, 0.92	0.05	-0.19, 0.29
		37 (11, 122)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Sprout irrigation water, 25 mL	*L. monocytogenes* 1/2b	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.40 (0.15, 0.72)	20	4	0.20	0.08, 0.42	4	0.20	0.08, 0.42	0	-0.25, 0.25
		3.7 (2.1, 23)	20	20	1	0.84, 1	19	0.95	0.76, 1	0.05	-0.12, 0.24
		11 (2.3, 47)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Stainless steel	*L. monocytogenes* 1/2a + *E. faecium*	0/31,000	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		3,700/312,000	20	19	0.95	0.76, 1	17	0.85	0.64, 0.95	0.10	-0.11, 0.32
		370,000/3,120,000	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Stainless steel[h]	*L. monocytogenes* 1/2a + *E. faecalis*	0/0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		430/1,200	20	17	0.85	0.64, 0.95	17	0.85	0.64, 0.95	0	-0.23, 0.23

		820/7,300	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43

[a] Determined by MPN analysis.
[b] N = Number of test portions.
[c] x = Number of positive test portions.
[d] POD_{CP} = Candidate method presumptive positive outcomes
[e] POD_{CC} = Candidate method presumptive positive outcomes confirmed positive.
[f] $dPOD_{CP}$ = Difference between the candidate method presumptive and candidate method confirmed 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] Trial performed by independent laboratory.
[i] 30 h for pasteurized liquid egg.

Table 5. Probability of detection calculations for ANSR *Listeria monocytogenes* confirmed and reference method results, 16 h time point (1)

Food type	Inoculum strain	Inoc. level (CFU/portion)[a]	N[b]	ANSR confirmed result			Reference method result			$dPOD_C$[f]	95% CI[g]
				X[c]	POD_C[d]	95% CI	x	POD_R[e]	95% CI		
Hot dogs, 125 g	*L. monocytogenes* 4b	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.69 (0.36, 1.2)	20	5	0.25	0.11, 0.47	7	0.35	0.18, 0.57	-0.10	-0.36, 0.18
		> 58	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Mexican-style cheese, 25 g	*L. monocytogenes* 1/2b	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.74 (0.42, 1.4)	20	11	0.55	0.34, 0.74	13	0.65	0.43, 0.82	-0.10	-0.37, 0.19
		11 (2.5, 53)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Cantaloupe, 25 g	*L. monocytogenes* 4b	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.31 (0.16, 0.49)	20	8	0.40	0.22, 0.61	9	0.45	0.26, 0.66	-0.05	-0.33, 0.24
		5.5 (2.1, 15)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Guacamole, 25 g	*L. monocytogenes* 1/2c	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.29 (0.10, 0.60)	20	3	0.15	0.05, 0.36	5	0.25	0.11, 0.47	-0.10	-0.34, 0.15
		27 (6.5, 117)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Pasteurized liquid egg[h], 25 g	*L. monocytogenes* 1/2a	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		1.7 (1.0, 3.3)	20	10	0.50	0.3, 0.7	16	0.80	0.58, 0.92	-0.30	-0.53, -0.01
		1.9 (1.2, 3.7)	20	9	0.45	0.26, 0.66	17	0.85	0.64, 0.95	-0.40	-0.62, -0.10
		37 (11, 122)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Sprout irrigation water, 25 mL	*L. monocytogenes* 1/2b	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.40 (0.15, 0.72)	20	2	0.10	0.03, 0.3	7	0.35	0.18, 0.57	-0.25	-0.48, 0.01
		3.7 (2.1, 23)	20	20	1	0.84, 1	19	0.95	0.76, 1	0.05	-0.12, 0.24
		11 (2.3, 47)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Stainless steel	*L. monocytogenes* 1/2a + *E. faecium*	0/31,000	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		3,700/312,000	20	16	0.80	0.58, 0.92	12	0.60	0.39, 0.78	0.20	-0.08, 0.44
		370,000/3,120,000	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43

Stainless steel[i]	*L. monocytogenes* 1/2a + *E. faecalis*	0/0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		430/1,200	20	17	0.85	0.64, 0.95	14	0.70	0.48, 0.85	0.15	-0.11, 0.39
		820/7,300	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43

[a] Determined by most probable number analysis.
[b] N = Number of test portions.
[c] x = Number of positive test portions.
[d] POD_{CP} = Candidate method presumptive positive outcomes confirmed positive.
[e] POD_{CC} = Reference method confirmed positive outcomes.
[f] $dPOD_{CP}$ = 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] 24 h for pasteurized liquid egg.
[i] Trial performed by independent laboratory.

Table 6. Probability of detection calculations for ANSR *Listeria monocytogenes* confirmed and reference method results, 24 h time point (1)

Food type	Inoculum strain	Inoc. level (CFU/portion)[a]	N[b]	ANSR confirmed result			Reference method result			$dPOD_C$[f]	95% CI[g]
				X[c]	POD_C[d]	95% CI	x	POD_R[e]	95% CI		
Hot dogs, 125 g	*L. monocytogenes* 4b	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.69 (0.36, 1.2)	20	19	0.95	0.76, 1	7	0.35	0.18, 0.57	0.60	0.31, 0.78
		> 58	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Hot dogs, 125 g[h]	*L. monocytogenes* 4b	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.92 (0.55, 1.6)	20	13	0.65	0.43, 0.82	12	0.60	0.39, 0.78	0.05	-0.23, 0.32
		7.4 (3.2, 17)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Mexican-style cheese, 25 g	*L. monocytogenes* 1/2b	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.74 (0.42, 1.4)	20	14	0.70	0.48, 0.85	13	0.65	0.43, 0.82	0.05	-0.23, 0.32
		11 (2.5, 53)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Mexican-style cheese, 25 g[h]	*L. monocytogenes* 1/2b	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.69 (0.41, 1.1)	20	10	0.50	0.3, 0.7	10	0.50	0.3, 0.7	0	-0.28, 0.28
		1.9 (0.89, 4.1)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Cantaloupe, 25 g	*L. monocytogenes* 4b	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.31 (0.16, 0.49)	20	8	0.40	0.22, 0.61	9	0.45	0.26, 0.66	-0.05	-0.33, 0.24
		5.5 (2.1, 15)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Guacamole, 25 g	*L. monocytogenes* 1/2c	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.29 (0.10, 0.60)	20	4	0.20	0.08, 0.42	5	0.25	0.11, 0.47	-0.05	-0.30, 0.21

		27 (6.5, 117)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Pasteurized liquid egg[i], 25 g	*L. monocytogenes* 1/2a	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		1.7 (1.0, 3.3)	20	12	0.60	0.39, 0.78	16	0.80	0.58, 0.92	-0.20	-0.44, 0.08
		1.9 (1.2, 3.7)	20	16	0.80	0.58, 0.92	17	0.85	0.64, 0.95	-0.05	-0.29, 0.19
		37 (11, 122)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Sprout irrigation water, 25 mL	*L. monocytogenes* 1/2b	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.40 (0.15, 0.72)	20	4	0.20	0.08, 0.42	7	0.35	0.18, 0.57	-0.15	-0.4, 0.12
		3.7 (2.1, 23)	20	20	1	0.84, 1	19	0.95	0.76, 1	0.05	-0.12, 0.24
		11 (2.3, 47)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Stainless steel	*L. monocytogenes* 1/2a + *Enterococcus faecium*	0/31,000	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		3,700/312,000	20	17	0.85	0.64, 0.95	12	0.60	0.39, 0.78	0.25	-0.03, 0.48
		370,000/3,120,000	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Stainless steel[h]	*L. monocytogenes* 1/2a + *Enterococcus faecalis*	0/0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		430/1,200	20	17	0.85	0.64, 0.95	14	0.70	0.48, 0.85	0.15	-0.11, 0.39
		820/7,300	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43

[a] Determined by most probable number analysis.
[b] N = Number of test portions.
[c] x = Number of positive test portions.
[d] POD_{CP} = Candidate method presumptive positive outcomes confirmed positive.
[e] POD_{CC} = Reference method confirmed positive outcomes.
[f] $dPOD_{CP}$ = 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] Trial performed by independent laboratory.
[i] 30 h for pasteurized liquid egg.

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