



# CERTIFICATION

**AOAC<sup>®</sup> Performance Tested<sup>SM</sup>**

Certificate No.

**101202**

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

**ANSR<sup>®</sup> for *Listeria***

manufactured by

**Neogen Corporation**

**620 Leshar Place**

**Lansing, Michigan 48912**

This method has been evaluated in the AOAC<sup>®</sup> *Performance Tested Methods*<sup>SM</sup> Program and found to perform as stated by the manufacturer contingent to the comments contained in the manuscript. This certificate means that an AOAC<sup>®</sup> Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC *Performance Tested*<sup>SM</sup> 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*

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Scott Coates, Senior Director  
Signature for AOAC Research Institute

\_\_\_\_\_  
November 24, 2019

Date

**METHOD AUTHORS**

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MODIFICATION MAY 2015: Oscar Caballero, Susan Alles, Quynh-Nhi Le, R. Lucas Gray, Edan Hosking, Lisa Pinkava, Paul Norton, Jerry Tolan, Mark Mozola, and Jennifer Rice

MODIFICATION JUNE 2017: Susan Alles, Evan Meister, Edan Hosking, Eric Tovar, Rebecca Shaulis, Mark Schonfeld, Lei Zhang, Lin Li, Preetha Biswas, Mark Mozola, and Robert Donofrio

**SUBMITTING COMPANY**

Neogen Corporation  
620 Leshar Pl.  
Lansing, MI 48912

**KIT NAME(S)**

ANSR® for *Listeria*

**CATALOG NUMBERS**

9821, 9871

**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(S)** – *Listeria* spp. Detection of *L. grayi* is variable depending on the ability of the individual strains to grow in the selective enrichment medium.

**MATRICES** – (MLG 8.07): stainless steel (4 x 4 sponge), plastic (1 x 1 swab), sealed concrete (4 x 4 sponge), ceramic tile (1 x 1 swab), rubber (4 x 4 sponge)

SEPTEMBER 2013 LEVEL 3 MODIFICATION: (BAM Ch. 10 2011, 25 g samples) Pasteurized 2% milk, Mexican-style cheese, ice cream, smoked salmon, lettuce, cantaloupe, guacamole (MLG 8.09): pasteurized liquid egg (25 g)

MAY 2015 LEVEL 3 MODIFICATION: BAM Ch. 10 (2011): Queso fresco (25 g), cantaloupe (25 g)

MLG 8.09: hot dogs (125 g)

JUNE 2017 LEVEL 2 MODIFICATION: BAM Ch. 10 (2016): Queso fresco (25 g), cantaloupe (25 g), stainless steel (4 x 4 sponge)

MLG 8.10: hot dogs (125 g)

**PERFORMANCE CLAIMS** - Performance equivalent to that of the FDA/BAM or the USDA/MLG reference culture methods as determined by POD analysis.

**REFERENCE METHODS****Original Validation**

USDA-FSIS (2009) *Microbiology Laboratory Guidebook*, chapter 8.07

[http://www.fsis.usda.gov/PDF/MLG\\_8\\_07.pdf](http://www.fsis.usda.gov/PDF/MLG_8_07.pdf) (3)

Matrix Extension September 2013 and May 2015

US FDA (2011) *Bacteriological Analytical Manual*, chapter 10

<http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm071400.htm>

USDA-FSIS (2012) *Microbiology Laboratory Guidebook*, chapter 8.09 (5)

<http://www.fsis.usda.gov/wps/wcm/connect/1710bee8-76b9-4e6c-92fc-fdc290dbfa92/MLG-8.pdf?MOD=AJPERES> (6)

Matrix Extension June 2017

USDA-FSIS (2017) Isolation and identification of *Listeria monocytogenes* from red meat, poultry and egg products, ready-to-eat siluriformes (fish) and environmental samples. *Microbiology Laboratory Guidebook*, chapter 8.10

<https://www.fsis.usda.gov/wps/wcm/connect/1710bee8-76b9-4e6c-92fc-fdc290dbfa92/MLG-8.pdf?MOD=AJPERES> (Accessed February 2017) (9)

US FDA (2017) Detection and enumeration of *Listeria monocytogenes* in foods.

*Bacteriological Analytical Manual*, chapter 10

<https://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm070149.htm>

(10)

**ORIGINAL CERTIFICATION DATE**

October 24, 2012

**CERTIFICATION RENEWAL RECORD**

Renewed Annually Through December 2020

**METHOD MODIFICATION RECORD**

1. September 23, 2013
2. May 2015 Level 3
3. June 2017 Level 2
4. December 2018 Level 1
5. November 2019 Level 1

**SUMMARY OF MODIFICATION**

1. Matrix modification (Level 3) to include pasteurized liquid egg, pasteurized 2% milk, Mexican-style cheese, ice cream, smoked salmon, bagged lettuce, cantaloupe, and guacamole
2. Minor formulation/procedural changes and alternative enrichment medium
3. Reagent formulation change to the amplification reagent master mix
4. Editorial changes
5. Editorial changes

Under this AOAC® *Performance Tested*<sup>SM</sup> License Number, 101202 this method is distributed by:  
NONE

Under this AOAC® *Performance Tested*<sup>SM</sup> License Number, 101202 this method is distributed as:  
NONE

**PRINCIPLE OF THE METHOD (1)**

ANSR *Listeria* is a new isothermal nucleic acid amplification assay based on the nicking enzyme amplification reaction (NEAR™) technology [2]. Single-stranded DNA is produced from target *Listeria* ribosomal RNA through the action of reverse transcriptase. This complementary DNA then serves as target for the amplification reaction. 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. The second product is homologous to the target sequence and is detected using a specific molecular beacon probe. A fluorescent signal is generated in real time, with amplification and detection complete within 20 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. The ANSR reagents are provided in a ready-to-use lyophilized form and include all enzymes, oligonucleotide templates, molecular beacon probe, and other factors required for the reverse transcription, amplification, and detection reactions. Each tube of ANSR reagent 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)**

Results of the internal and independent laboratory studies show that ANSR *Listeria* is an effective procedure for detection of *Listeria* spp. in sponge or swab samples from a variety of environmental surfaces. Inclusivity was 94% in testing of 51 strains of *Listeria* spp. representing 6 species, and 100% with *L. grayi* excluded. Exclusivity was 100% in testing of 32 strains of non-*Listeria*. Method sensitivity was comparable to that of the USDA-FSIS method as determined by chi-square and POD analysis. In 4 out of 7 trials there were no statistically significant differences in the number of positive results obtained with the ANSR and reference culture methods. In the two trials with stainless steel, there were significantly more positives by the ANSR method at 16 h (second trial) and at 24 h (both trials) as determined by both chi-square and POD analysis. Considering all data from fractional positive trials, sensitivity relative to the USDA-FSIS reference method was 112% at 16 h and 128% at 24 h. There were no unconfirmed positive results from uninoculated control test portions.

Results of assay ruggedness testing showed that the timing of the second lysis step (80°C) is critical and that the specified incubation period of 10 min must be observed. Aberrant assay results, specifically false-negative results and invalid assays, were observed when the incubation time for this step was reduced to 5 min. The most likely cause of the aberrant results is incomplete inactivation of endogenous nucleases in the enriched test sample and their effect on the ANSR assay chemistry.

For the ANSR method, enriched test portions were confirmed by two methods: direct streaking from the ANSR *Listeria* Enrichment Broth cultures to USDA-FSIS method selective/differential agars, and subculture to Fraser broth followed by streaking to the same agars. Considering all data and both confirmation methods, there were 164 culture-positive test portions from the ANSR method analyses at both 16 h and 24 h. Of these, 147 (90%) were confirmed by streaking from the primary enrichment cultures at 16 h and all 164 were confirmed from the primary enrichment cultures at 24 h. Significantly more test portions were confirmed using the subculture method only in the internal stainless steel trial at the 16 h time point (Table 3).

In addition to high sensitivity and specificity, the ANSR *Listeria* method offers the advantages of single-step enrichment, minimal labor and assay hardware requirements, and assay results within 40 min following sample enrichment.

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

Organism	Serotype	Strain	Source	Origin (if known)	ANSR Result 12 h (~ 10 <sup>4</sup> cfu/mL)	ANSR Result 24 h (diluted to ~ 10 <sup>4</sup> cfu/mL)
<i>L. grayi</i>	-	GT4800	Neogen	Environmental	Negative	Negative <sup>a</sup>
<i>L. grayi</i>	-	A203	ATCC 19120	Chinchilla feces	Negative	Negative <sup>a</sup>
<i>L. grayi</i> subsp. <i>murrayi</i>	-	A198	Neogen	-	Negative	Negative <sup>a, b</sup>
<i>L. innocua</i>	6a	GT3627	H. Seeliger <sup>c</sup>	Cheese	Positive	Positive
<i>L. innocua</i>	6a	GT3631	H. Seeliger	Cheese	Positive	Positive
<i>L. innocua</i>	6a	A102	ATCC 33090 <sup>d</sup>	Cow brain	Positive	Positive
<i>L. innocua</i>	6b	GT1026	H. Seeliger	Cheese	Positive	Positive
<i>L. innocua</i>	6b	GT1042	H. Seeliger	Cheese	Positive	Positive
<i>L. innocua</i>	6b	GT1044	H. Seeliger	Cheese	Positive	Positive
<i>L. innocua</i>	6b	GT1050	H. Seeliger	Cheese	Positive	Positive
<i>L. innocua</i>	-	GT3785	CDC <sup>e</sup>	-	Positive	Positive
<i>L. innocua</i>	-	GT1052	J. Farber <sup>f</sup>	Raw milk	Positive	Positive
<i>L. ivanovii</i>	5	GT1028	H. Seeliger	Mouse	Positive	Positive
<i>L. ivanovii</i>	5	GT1040	H. Seeliger	Human	Positive	Positive
<i>L. ivanovii</i>	5	GT3699	H. Seeliger	Watercress	Positive	Positive
<i>L. ivanovii</i>	-	A140	ATCC 19119	Sheep	Positive	Positive

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<i>L. monocytogenes</i>	1/2a	GT3727	H. Seeliger	Human blood	Positive	Positive
<i>L. monocytogenes</i>	1/2a	GT4340	CDC	Fish	Positive	Positive
<i>L. monocytogenes</i>	1/2a	GT1038	H. Seeliger	Human blood	Positive	Positive
<i>L. monocytogenes</i>	1/2b	GT3635	H. Seeliger	Human blood	Positive	Positive
<i>L. monocytogenes</i>	1/2b	GT3728	H. Seeliger	Cheese	Positive	Positive
<i>L. monocytogenes</i>	1/2b	GT3856	H. Seeliger	Cheese	Positive	Positive
<i>L. monocytogenes</i>	1/2c	GT3698	H. Seeliger	Cheese	Positive	Positive
<i>L. monocytogenes</i>	1/2c	GT3648	H. Seeliger	Cheese	Positive	Positive
<i>L. monocytogenes</i>	1/2c	GT3730	H. Seeliger	-	Positive	Positive
<i>L. monocytogenes</i>	1/2c	GT3741	H. Seeliger	-	Positive	Positive
<i>L. monocytogenes</i>	1a	GT3829	C. Donnelly <sup>b</sup>	Raw milk	Positive	Positive
<i>L. monocytogenes</i>	1a	GT1072	C. Donnelly	Raw milk	Positive	Positive
<i>L. monocytogenes</i>	1a	GT1880	J. Lovett <sup>h</sup>	Brie cheese	Positive	Positive
<i>L. monocytogenes</i>	1a	GT3812	J. Lovett	Chocolate milk	Positive	Positive
<i>L. monocytogenes</i>	2	A169	ATCC 19112	Human CSF	Positive	Positive
<i>L. monocytogenes</i>	3a	GT3720	H. Seeliger	Cheese	Positive	Positive
<i>L. monocytogenes</i>	3a	GT1035	H. Seeliger	-	Positive	Positive
<i>L. monocytogenes</i>	3b	GT1057	J. Lovett	Brie cheese	Positive	Positive
<i>L. monocytogenes</i>	3b	GT3715	H. Seeliger	Human blood	Positive	Positive
<i>L. monocytogenes</i>	3b	GT3817	H. Seeliger	Cheese	Positive	Positive
<i>L. monocytogenes</i>	3b	GT3857	J. Lovett	Brie cheese	Positive	Positive
<i>L. monocytogenes</i>	4a	A170	ATCC 19114	Ruminant brain	Positive	Positive
<i>L. monocytogenes</i>	4b	A207	ATCC 13932	Human CSF	Positive	Positive
<i>L. monocytogenes</i>	4b	GT1019	Neogen	-	Positive	Positive
<i>L. monocytogenes</i>	4b	GT1081	CDC	-	Positive	Positive
<i>L. monocytogenes</i>	4c	GT3819	H. Seeliger	Human	Positive	Positive
<i>L. seeligeri</i>	1/2b	GT3693	H. Seeliger	Sewage	Positive	Positive
<i>L. seeligeri</i>	4a	GT289	H. Seeliger	Cheese	Positive	Positive
<i>L. seeligeri</i>	-	A201	ATCC 51334	Vole	Positive	Positive
<i>L. seeligeri</i>	6b	GT3708	H. Seeliger	Cheese	Positive	Positive
<i>L. welshimeri</i>	6a	GT293	H. Seeliger	Cheese	Positive	Positive
<i>L. welshimeri</i>	-	A199	ATCC 35897	Plant material	Positive	Positive
<i>L. welshimeri</i>	-	A200	ATCC 43550	Soil	Positive	Positive
<i>L. welshimeri</i>	-	GT1773	Neogen	Environmental isolate	Positive	Positive
<i>L. welshimeri</i>	-	GT1729	Neogen	Dairy plant	Positive	Positive

<sup>a</sup> Positive in ANSR assay when grown in tryptic soy broth.

<sup>b</sup> Positive in ANSR assay when grown in ANSR *Listeria* Enrichment Broth in the presence of food matrix.

<sup>c</sup> Institute of Hygiene and Molecular Microbiology, University of Würzburg, D8700 Würzburg, Germany.

<sup>d</sup> American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110.

<sup>e</sup> Centers for Disease Control and Prevention, 1600 Clifton Rd., Atlanta, GA 30333.

<sup>f</sup> Food Directorate, Health Canada, Banting Research Centre, Tunney's Pasture, Postal Locator 2203G3, Ottawa, Ontario K1A 0L2, Canada.

<sup>g</sup> Department of Nutrition and Food Sciences, University of Vermont, Nutrition and Food Sciences, Room

254, Burlington, VT 05405.

<sup>h</sup> U.S. Food and Drug Administration, 6751 Steger Dr., Cincinnati, OH 45237.**Table 2. Results of exclusivity testing for the ANSR *Listeria* test. (1)**

Organism	Strain #	Source (ATCC #)	Origin (if known)	ANSR Result (~ 10 <sup>9</sup> cfu/mL)	Culture Conditions <sup>a</sup>
<i>Bacillus cereus</i>	A208	25621	Cow dung	Negative	
<i>Bacillus coagulans</i>	GT811	7050	Milk	Negative	
<i>Bacillus megaterium</i>	GT2128	14581	-	Negative	
<i>Bacillus subtilis</i>	GT4402	21556	-	Negative	
<i>Brevibacillus parabrevis</i>	GT803	8186	Cheese	Negative	
<i>Brochothrix thermosphacta</i>	GT664	11509	Pork sausage	Negative	BHI broth, CO <sub>2</sub> , 48 h, 25°C
<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	Negative	Nutrient broth, CO <sub>2</sub> , 48 h, 37°C
<i>Kocuria rosea</i>	GT1944	185	-	Negative	BHI broth, 48 h, 26°C
<i>Kocuria varians</i>	GT4404	15306	Milk	Negative	
<i>Kurthia gibsonii</i>	GT2129	43195	Meat	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	MRS broth, 48 h, 30°C
<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	-	Negative	BHI broth, 48 h, 26°C
<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 gallolyticus</i>	GT668	9809	-	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	

<sup>a</sup> If other than TSB, 16-24 h, 36°C.

**Table 3. Results of comparative testing of environmental samples with the ANSR *Listeria* and USDA-FSIS reference methods. (1)**

Food Type	Inoculum Strain	Inoculation Level (cfu/surface) <sup>a</sup>	No. Samples	Number of Positive Samples				USDA Ref. Method	Sensitivity (%) <sup>d</sup> (16 h/24 h)	$\chi^2$ <sup>e</sup> (16 h/24 h)
				ANSR Method						
				Assay 16 h <sup>b</sup>	Conf. 16 h <sup>c</sup>	Assay 24 h <sup>b</sup>	Conf. 24 h <sup>c</sup>			
Stainless steel #1	<i>L. monocytogenes</i> 1/2a + <i>Enterococcus faecalis</i>	46 3,900	20	6	15	15	15	6	100/250	0.00/7.92
		460 3,900	20	20	20	20	20	20	100/100	-/-
	-	5	0	0	0	0	0	-	-/-	
Stainless steel #2	<i>L. monocytogenes</i> 1/2a + <i>Enterococcus faecalis</i>	140 5,100	20	5	5	5	5	0	-	5.57/5.57
		720 5,100	20	19	19	19	11	173/173	8.32/8.32	
		7,200 5,100	5	5	5	5	5	100/100	-/-	
	-	5	0	0	0	0	0	-	-/-	
Stainless steel <sup>f</sup>	<i>L. monocytogenes</i> 1/2a + <i>Enterococcus faecalis</i>	100 920	20	13	12	13	12	14	86/86	0.43/0.43
		150 920	5	5	5	5	5	100/100	-/-	
	-	5	0	0	0	0	0	-	-/-	
Plastic	<i>L. innocua</i>	87	20	16	16	16	16	14	114/114	0.52/0.52
		870	5	5	5	5	5	100/100	-/-	
	-	5	0	0	0	0	0	-	-/-	
Sealed concrete	<i>L. welshimeri</i>	20	20	10	14	14	14	15	67/93	2.60/0.12
		170	20	19	20	20	20	20	95/100	1.00/-
	-	5	0	0	0	0	0	-	-/-	
Ceramic tile	<i>L. monocytogenes</i> 4b	180	20	17	17	17	17	13	131/131	2.08/2.08
		1,800	5	5	5	5	5	100/100	-/-	
	-	5	0	0	0	0	0	-	-/-	
Rubber	<i>L. monocytogenes</i> 1/2b	950	20	6	6	7	6	8	75/75	0.43/0.43
		9,500	5	5	5	5	5	100/100	-/-	
	-	5	0	0	0	0	0	-	-/-	

<sup>a</sup> Determined by dilution plating of the inoculum cultures.

<sup>b</sup> Number of test portions positive by ANSR assay not considering subsequent culture confirmation.

<sup>c</sup> Number test portions positive by the ANSR assay and confirmed by culture from ANSR-associated enrichments.

<sup>d</sup> Sensitivity (relative) = Number of ANSR confirmed positives divided by number of reference method positives.

<sup>e</sup>  $\chi^2$  by Mantel-Haenszel formula [3];  $\chi^2 > 3.84$  indicates a statistically significant difference at  $P < 0.05$ .

<sup>f</sup> Trial performed by independent laboratory.

**DISCUSSION OF MODIFICATION APPROVED SEPTEMBER 2013 (4)**

Results of the internal and independent laboratory studies show that ANSR *Listeria* is an effective procedure for detection of *Listeria* spp. in pasteurized 2% milk, Mexican-style cheese, ice cream, smoked salmon, lettuce, cantaloupe, guacamole, and pasteurized liquid egg. Method sensitivity was comparable to that of the FDA/BAM and USDA/MLG methods as determined by POD analysis, except for milk and cantaloupe. In the case of milk, both internal and independent laboratory results showed low sensitivity at 16 h and indicated that enrichment for 24 h is required. While internal results for cantaloupe show no statistically significant difference at either time point, the independent laboratory findings show a significant difference between ANSR and the FDA/BAM reference method at both time points, with ANSR having a greater number of positives (20) than the reference method (14). There were no unconfirmed positive results from uninoculated control test portions.

In addition to high sensitivity and specificity, the ANSR *Listeria* method offers the advantages of single-step enrichment, minimal labor and assay hardware requirements, and assay results in less than 50 min. following sample enrichment.

**Table 1. Results of inclusivity testing for the ANSR *Listeria* test using LESS broth enrichment. (4)**

Organism	Serotype	Strain	Source	Origin (if known)	ANSR Result 16 h (~ 10 <sup>4</sup> cfu/mL)
<i>L. grayi</i>	-	GT4800	Neogen	Environmental	Positive
<i>L. grayi</i>	-	A203	ATCC 19120	Chinchilla feces	Positive
<i>L. grayi</i> subsp. <i>murrayi</i>	-	A198	Neogen	-	Positive
<i>L. innocua</i>	6a	GT3627	H. Seeliger <sup>a</sup>	Cheese	Positive
<i>L. innocua</i>	6a	GT3631	H. Seeliger	Cheese	Positive
<i>L. innocua</i>	6a	A102	ATCC 33090 <sup>b</sup>	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 <sup>c</sup>	-	Positive
<i>L. innocua</i>	-	GT1052	J. Farber <sup>d</sup>	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. 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	GT3635	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	GT3698	H. Seeliger	Cheese	Positive
<i>L. monocytogenes</i>	1/2c	GT3648	H. Seeliger	Cheese	Positive
<i>L. monocytogenes</i>	1/2c	GT3730	H. Seeliger	-	Positive
<i>L. monocytogenes</i>	1/2c	GT3741	H. Seeliger	-	Positive
<i>L. monocytogenes</i>	1a	GT3829	C. Donnelly <sup>e</sup>	Raw milk	Positive
<i>L. monocytogenes</i>	1a	GT1072	C. Donnelly	Raw milk	Positive
<i>L. monocytogenes</i>	1a	GT1880	J. Lovett <sup>f</sup>	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. 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>	-	A199	ATCC 35897	Plant material	Positive
<i>L. welshimeri</i>	-	A200	ATCC 43550	Soil	Positive
<i>L. welshimeri</i>	-	GT1773	Neogen	Environmental isolate	Positive
<i>L. welshimeri</i>	-	GT1729	Neogen	Dairy plant	Positive

<sup>a</sup> Institute of Hygiene and Molecular Microbiology, University of Würzburg, D8700 Würzburg, Germany.

<sup>b</sup> American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110.

<sup>c</sup> Centers for Disease Control and Prevention, 1600 Clifton Rd., Atlanta, GA 30333.

<sup>d</sup> Food Directorate, Health Canada, Banting Research Centre, Tunney's Pasture, Postal Locator 2203G3, Ottawa, Ontario K1A 0L2, Canada.

<sup>e</sup> Department of Nutrition and Food Sciences, University of Vermont, Nutrition and Food Sciences, Room 254, Burlington, VT 05405.

<sup>f</sup> U.S. Food and Drug Administration, 6751 Steger Dr., Cincinnati, OH 45237.

**Table 2. Results of exclusivity testing for the ANSR *Listeria* test using LESS broth enrichment. (4)**

Organism	Strain #	Source (ATCC #)	Origin (if known)	ANSR Result (~ 10 <sup>9</sup> cfu/mL)	Culture Conditions <sup>a</sup>	
<i>Bacillus cereus</i>	A208	25621	Cow dung	Negative	BHI broth, CO <sub>2</sub> , 48 h, 25°C	
<i>Bacillus megaterium</i>	GT2128	14581	-	Negative		
<i>Bacillus subtilis</i>	GT4402	21556	-	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	Negative		Nutrient broth, CO <sub>2</sub> , 48 h, 37°C
<i>Kocuria rosea</i>	GT1944	185	-	Negative	BHI broth, 48 h, 26°C	
<i>Kocuria varians</i>	GT4404	15306	Milk	Negative		
<i>Kurthia gibsonii</i>	GT2129	43195	Meat	Negative		
<i>Kurthia zopfii</i>	GT1941	33403	Turkey cecum	Negative		
<i>Lactobacillus acidophilus</i>	GT256	4356	Human	Negative		MRS broth, 48 h, 30°C
<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	-	Negative	BHI broth, 48 h, 26°C	
<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 gallolyticus</i>	GT668	9809	-	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	
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<sup>a</sup> If other than TSB, 16-24 h, 36°C.

**Table 3. Probability of detection calculations for ANSR *Listeria* presumptive and confirmed results, 16 h results. (4)**

Matrix	Inoculum Strain	Inoc. Level (cfu/portion) <sup>a</sup>	N <sup>b</sup>	ANSR Presumptive Result			ANSR Confirmed Result			dPOD <sub>CP</sub> <sup>f</sup>	95% CI <sup>g</sup>
				x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	x	POD <sub>CC</sub> <sup>e</sup>	95% CI		
Pasteurized 2% Milk	<i>L. welshimeri</i>	>11 (2.25, 45)	5	3	0.60	0.23, 0.88	5	1	0.56, 1	-0.40	-0.76, 0.11
		<0.75 (0.04, 2.4)	25	3	0.12	0.04, 0.29	9	0.36	0.2, 0.55	-0.24	-0.44, -0.00
	-	-	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
Pasteurized 2% Milk <sup>h</sup>	<i>L. welshimeri</i>	4.4 (1.7, 11.2)	5	4	0.80	0.38, 1	5	1	0.57, 1	-0.20	-0.62, 0.28
		0.26 (0.1, 0.48)	20	0	0	0, 0.16	6	0.30	0.15, 0.52	-0.30	-0.52, -0.07
	-	-	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
Mexican-Style Cheese	<i>L. mono. 1/2b</i>	23 (4.5, 105)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
		0.89 (0.04, 4.5)	20	8	0.40	0.22, 0.61	9	0.45	0.26, 0.66	-0.05	-0.33, 0.24
	-	-	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
Ice Cream	<i>L. mono. 1/2a</i>	5.7 (1.15, 23.5)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
		2.3 (0.35, 9.5)	25	20	0.80	0.6, 0.91	18	0.72	0.52, 0.86	0.08	-0.15, 0.3
	-	-	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
Smoked Salmon	<i>L. mono. 4b</i>	30 (9.25, 105)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
		0.89 (0.04, 4.5)	20	6	0.30	0.15, 0.52	9	0.45	0.26, 0.66	-0.15	-0.41, 0.14
	-	-	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
Lettuce	<i>L. mono. 1/2a</i>	37 (9.25, 105)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
		1.5 (0.3, 4.5)	20	14	0.70	0.48, 0.85	13	0.65	0.43, 0.82	0.05	-0.23, 0.32
	-	-	20	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
Cantaloupe	<i>L. mono. 1/2b</i>	> 275 (45, 1025)	5	4	0.80	0.37, 1	5	1	0.56, 1	-0.20	-0.62, 0.28
		5.8 (1.15, 23.5)	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0	-0.28, 0.28
	-	-	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
Cantaloupe <sup>h</sup>	<i>L. mono. 1/2b</i>	4.38 (1.72, 11.2)	5	5	1	0.57, 1	5	1	0.57, 1	0	-0.43, 0.43
		1.30 (0.82, 2.1)	20	20	1	0.84, 1	20	1	0.84, 1	0	-0.16, 0.16
	-	-	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
Past. Liquid Egg	<i>L. mono. 4b</i>	115 (22.5, 500)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
		0.9 (0.04, 4.5)	20	13	0.65	0.43, 0.82	13	0.65	0.43, 0.82	0	-0.28, 0.28
	-	-	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
Guacamole	<i>L. innocua</i>	23 (4.5, 105)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
		0.89 (0.04, 4.5)	20	5	0.25	0.11, 0.47	5	0.25	0.11, 0.47	0	-0.26, 0.26
	-	-	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43

<sup>a</sup> Determined by dilution plating of the inoculum cultures.

<sup>b</sup> N = Number of test portions.

<sup>c</sup> x = Number of positive test portions.

<sup>d</sup> POD<sub>CP</sub> = Candidate method presumptive positive outcomes divided by the total number of trials.

<sup>e</sup> POD<sub>CC</sub> = Candidate method confirmed positive outcomes divided by the total number of trials.

<sup>f</sup> dPOD<sub>CP</sub> = Difference between the candidate method presumptive result and candidate method confirmed result POD values.

<sup>g</sup> 95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

<sup>h</sup> Trial performed by independent laboratory.

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

Matrix	Inoculum Strain	Inoc. Level (cfu/portion) <sup>a</sup>	N <sup>b</sup>	ANSR Presumptive Result			ANSR Confirmed Result			dPOD <sub>CP</sub> <sup>f</sup>	95% CI <sup>g</sup>
				x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	x	POD <sub>CC</sub> <sup>e</sup>	95% CI		
Pasteurized 2% Milk	<i>L. welshimeri</i>	>11 (2.25, 45)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
		<0.75 (0.04, 2.4)	25	9	0.36	0.20, 0.55	9	0.36	0.20, 0.55	0	-0.25, 0.25
	-	-	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
Pasteurized 2% Milk <sup>h</sup>	<i>L. welshimeri</i>	4.4 (1.7, 11.2)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
		0.26 (0.1, 0.48)	20	9	0.45	0.26, 0.66	10	0.50	0.3, 0.7	-0.05	-0.33, 0.24
	-	-	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
Mexican-Style Cheese	<i>L. mono. 1/2b</i>	23 (4.5, 105)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
		0.89 (0.04, 4.5)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0	-0.28, 0.28
	-	-	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
Ice Cream	<i>L. mono. 1/2a</i>	5.7 (1.15, 23.5)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
		2.3 (0.35, 9.5)	25	19	0.76	0.57, 0.89	18	0.72	0.52, 0.86	0.04	-0.2, 0.27
	-	-	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
Smoked Salmon	<i>L. mono. 4b</i>	30 (9.25, 105)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
		0.89 (0.04, 4.5)	20	8	0.40	0.22, 0.61	9	0.45	0.26, 0.66	-0.05	-0.33, 0.24
	-	-	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
Lettuce	<i>L. mono. 1/2a</i>	37 (9.25, 105)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
		1.5 (0.3, 4.5)	20	14	0.70	0.48, 0.85	15	0.75	0.53, 0.89	-0.05	-0.31, 0.22
	-	-	5	1	0.20	0, 0.62	0	0	0, 0.43	0.20	-0.28, 0.62
Cantaloupe	<i>L. mono. 1/2b</i>	> 275 (45, 1025)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
		5.8 (1.15, 23.5)	20	13	0.65	0.43, 0.82	13	0.65	0.43, 0.82	0	-0.28, 0.28
	-	-	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
Cantaloupe <sup>h</sup>	<i>L. mono. 1/2b</i>	4.38 (1.72, 11.2)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
		1.30 (0.82, 2.1)	20	20	1	0.84, 1	20	1	0.84, 1	0	-0.16, 0.16
	-	-	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
Past. Liquid Egg	<i>L. mono 4b</i>	115 (22.5, 500)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
		0.9 (0.04, 4.5)	20	13	0.65	0.43, 0.82	13	0.65	0.43, 0.82	0	-0.28, 0.28
	-	-	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
Guacamole	<i>L. innocua</i>	23 (4.5, 105)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
		0.89 (0.04, 4.5)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0	-0.28, 0.28
	-	-	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43

<sup>a</sup> Determined by dilution plating of the inoculum cultures.<sup>b</sup> N = Number of test portions.<sup>c</sup> x = Number of positive test portions.<sup>d</sup> POD<sub>CP</sub> = Candidate method presumptive positive outcomes divided by the total number of trials.<sup>e</sup> POD<sub>CC</sub> = Candidate method confirmed positive outcomes divided by the total number of trials.<sup>f</sup> dPOD<sub>CP</sub> = Difference between the candidate method presumptive result and candidate method confirmed result POD values.<sup>g</sup> 95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.<sup>h</sup> Trial performed by independent laboratory.

Table 5. Probability of detection calculations for ANSR *Listeria* confirmed and reference method results, 16 h results. (4)

Matrix	Inoculum Strain	Inoc. Level (cfu/portion) <sup>a</sup>	N <sup>b</sup>	ANSR Result			Reference Method Result			dPOD <sub>c</sub> <sup>f</sup>	95% CI <sup>g</sup>
				x <sup>c</sup>	POD <sub>c</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI		
Pasteurized 2% Milk	<i>L. welshimeri</i>	>11 (2.25, 45)	5	3	0.60	0.23, 0.88	5	1	0.56, 1	-0.40	-0.76, 0.11
		<0.75 (0.04, 2.4)	25	3	0.12	0.04, 0.3	5	0.20	0.09, 0.39	-0.08	-0.29, 0.13
	-	-	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
Pasteurized 2% Milk <sup>h</sup>	<i>L. welshimeri</i>	4.4 (1.7, 11.2)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
		0.26 (0.1, 0.48)	20	0	0	0, 0.16	4	0.20	0.08, 0.42	-0.20	-0.42, 0.00
	-	-	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
Mexican-Style Cheese	<i>L. mono. 1/2b</i>	23 (4.5, 105)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
		0.89 (0.04, 4.5)	20	8	0.40	0.22, 0.61	6	0.30	0.15, 0.52	0.10	-0.18, 0.36
	-	-	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
Ice Cream	<i>L. mono. 1/2a</i>	5.7 (1.15, 23.5)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
		2.3 (0.35, 9.5)	25	18	0.72	0.52, 0.86	22	0.90	0.7, 0.96	-0.16	-0.37, 0.07
	-	-	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
Smoked Salmon	<i>L. mono. 4b</i>	30 (9.25, 105)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
		0.89 (0.04, 4.5)	20	6	0.30	0.15, 0.52	7	0.35	0.18, 0.57	-0.05	-0.32, 0.23
	-	-	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
Lettuce	<i>L. mono. 1/2a</i>	37 (9.25, 105)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
		1.5 (0.3, 4.5)	20	13	0.65	0.43, 0.82	14	0.70	0.48, 0.85	-0.05	-0.32, 0.23
	-	-	20	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
Cantaloupe	<i>L. mono. 1/2b</i>	> 275 (45, 1025)	5	4	0.80	0.37, 1	5	1	0.56, 1	-0.20	-0.62, 0.28
		5.8 (1.15, 23.5)	20	11	0.55	0.34, 0.74	13	0.65	0.43, 0.82	-0.10	-0.37, 0.19
	-	-	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
Cantaloupe <sup>h</sup>	<i>L. mono. 1/2b</i>	4.38 (1.72, 11.2)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
		1.30 (0.82, 2.1)	20	20	1	0.84, 1	14	0.70	0.48, 0.85	0.30	0.08, 0.52
	-	-	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
Past. Liquid Egg	<i>L. mono 4b</i>	115 (22.5, 500)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
		0.9 (0.04, 4.5)	20	13	0.65	0.43, 0.82	15	0.75	0.53, 0.89	-0.10	-0.36, 0.18
	-	-	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
Guacamole	<i>L. innocua</i>	23 (4.5, 105)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
		0.89 (0.04, 4.5)	20	5	0.25	0.11, 0.47	4	0.20	0.08, 0.42	0.05	-0.21, 0.3
	-	-	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43

<sup>a</sup> Determined by dilution plating of the inoculum cultures.<sup>b</sup> N = Number of test portions.<sup>c</sup> x = Number of positive test portions.<sup>d</sup> POD<sub>c</sub> = Candidate method presumptive positive outcomes confirmed positive.<sup>e</sup> POD<sub>R</sub> = Reference method confirmed positive outcomes divided by the total number of trials.<sup>f</sup> dPOD<sub>c</sub> = Difference between the candidate method and reference method POD values.<sup>g</sup> 95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.<sup>h</sup> Trial performed by independent laboratory

Table 6. Probability of detection calculations for ANSR *Listeria* confirmed and reference method results, 24 h results. (4)

Matrix	Inoculum Strain	Inoc. Level (cfu/portion) <sup>a</sup>	N <sup>b</sup>	ANSR Result			Reference Method Result			dPOD <sup>c</sup>	95% CI <sup>g</sup>
				x <sup>c</sup>	POD <sub>c</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI		
Pasteurized 2% Milk	<i>L. welshimeri</i>	>11(2.25, 45)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
		<0.75 (0.04, 2.4)	25	9	0.36	0.2, 0.55	5	0.20	0.09, 0.39	0.16	-0.09, 0.38
	-	-	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
Pasteurized 2% Milk <sup>h</sup>	<i>L. welshimeri</i>	4.4 (1.7, 11.2)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
		0.26 (0.1, 0.48)	20	9	0.45	0.26, 0.66	4	0.20	0.08, 0.42	0.25	-0.04, 0.5
	-	-	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
Mexican-Style Cheese	<i>L. mono. 1/2b</i>	23 (4.5, 105)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
		0.89 (0.04, 4.5)	20	9	0.45	0.26, 0.66	6	0.30	0.15, 0.52	0.15	-0.14, 0.41
	-	-	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
Ice Cream	<i>L. mono. 1/2a</i>	5.7 (1.15, 23.5)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
		2.3 (0.35, 9.5)	25	18	0.72	0.52, 0.86	22	0.90	0.7, 0.96	-0.18	-0.37, 0.07
	-	-	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
Smoked Salmon	<i>L. mono. 4b</i>	30 (9.25, 105)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
		0.89 (0.04, 4.5)	20	8	0.40	0.22, 0.61	7	0.35	0.18, 0.57	0.05	-0.23, 0.32
	-	-	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
Lettuce	<i>L. mono. 1/2a</i>	37 (9.25, 105)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
		1.5 (0.3, 4.5)	20	14	0.70	0.48, 0.85	14	0.70	0.48, 0.85	0	-0.27, 0.27
	-	-	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
Cantaloupe	<i>L. mono. 1/2b</i>	>275 (45, 1025)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
		5.8 (1.15, 23.5)	20	13	0.65	0.43, 0.82	13	0.65	0.43, 0.82	0	-0.28, 0.28
	-	-	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
Cantaloupe <sup>h</sup>	<i>L. mono. 1/2b</i>	4.38 (1.72, 11.2)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
		1.30 (0.82, 2.1)	20	20	1	0.84, 1	14	0.70	0.48, 0.85	0.30	0.08, 0.52
	-	-	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
Past. Liquid Egg	<i>L. mono 4b</i>	115 (22.5, 500)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
		0.9 (0.04, 4.5)	20	13	0.65	0.43, 0.82	15	0.75	0.53, 0.89	-0.10	-0.36, 0.18
	-	-	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
Guacamole	<i>L. innocua</i>	23 (4.5, 105)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
		0.89 (0.04, 4.5)	20	7	0.35	0.18, 0.57	4	0.20	0.08, 0.42	0.15	-0.12, 0.4
	-	-	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43

<sup>a</sup> Determined by dilution plating of the inoculum cultures.<sup>b</sup> N = Number of test portions.<sup>c</sup> x = Number of positive test portions.<sup>d</sup> POD<sub>c</sub> = Candidate method presumptive positive outcomes confirmed positive.<sup>e</sup> POD<sub>R</sub> = Reference method confirmed positive outcomes divided by the total number of trials.<sup>f</sup> dPOD<sub>c</sub> = Difference between the candidate method and reference method POD values.<sup>g</sup> 95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.<sup>h</sup> Trial performed by independent laboratory.

**DISCUSSION OF MODIFICATION APPROVED MAY 2015 (7)**

The results presented here demonstrate that method modifications including reformulation of lyophilized reagent components for increased solubility and immediate capping and vortexing of ANSR reagents do not affect performance of the assay. In comparative testing of three foods and sponge samples from a stainless steel surface, performance of the ANSR method after 24 h of enrichment was comparable to that of the FDA/BAM or USDA-FSIS/MLG methods for all matrices tested, as determined by POD analysis. After 16 h of enrichment, cantaloupe and stainless steel also yielded results that were not statistically different from the reference methods. Results of robustness testing showed that the modified assay can withstand perturbations to multiple assay parameters and still yield accurate results. A second phase of the study examined LESS Plus as an alternative enrichment medium for all sample types. No significant differences in performance were seen between the ANSR method with LESS Plus enrichment and the reference methods for any matrix at either enrichment time point. However, method sensitivity was higher at 24 h for hot dogs and therefore the longer enrichment period is recommended for this food type.

The choice of matrices for this study was carefully decided in order to ensure appropriate representation of the matrices previously validated. The three matrices and one surface tested here (meat, dairy, produce, and stainless steel) provided a challenging mix of products for this study.

The enhanced reagent formulation for ANSR *Listeria* provides increased pellet solubility, thus eliminating the need for pipette mixing of the lysed sample and ANSR reagents. This improves operator ease of use and minimizes the opportunity for cross contamination or potential amplicon contamination of the work environment. The use of LESS Plus also provides a convenient, universal enrichment alternative to the end user. In addition to high sensitivity and specificity, the ANSR *Listeria* method offers the advantages of single-step enrichment, minimal labor and assay hardware requirements, and assay results within 40 min following sample enrichment.

**Table 1. Results of inclusivity testing for the ANSR *Listeria* test using LESS Plus enrichment media (7)**

Organism	Serotype	Strain	Source	Origin (if known)	ANSR Result (~ 10 <sup>4</sup> cfu/mL)
<i>L. grayi</i> <sup>a</sup>	-	GT4800	Neogen	Environmental	Positive
<i>L. grayi</i> <sup>a</sup>	-	A203	ATCC <sup>b</sup> 19120	Chinchilla feces	Positive
<i>L. grayi</i> subsp. <i>murrayi</i> <sup>a</sup>	-	A198	Neogen	-	Positive
<i>L. innocua</i>	6a	GT3627	H. Seeliger <sup>c</sup>	Cheese	Positive
<i>L. innocua</i>	6a	GT3631	H. Seeliger	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 <sup>d</sup>	-	Positive
<i>L. innocua</i>	-	GT1052	J. Farber <sup>e</sup>	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. 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	GT3635	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	GT3698	H. Seeliger	Cheese	Positive
<i>L. monocytogenes</i>	1/2c	GT3648	H. Seeliger	Cheese	Positive
<i>L. monocytogenes</i>	1/2c	GT3730	H. Seeliger	-	Positive
<i>L. monocytogenes</i>	1/2c	GT3741	H. Seeliger	-	Positive
<i>L. monocytogenes</i>	1a	GT3829	C. Donnelly <sup>f</sup>	Raw milk	Positive
<i>L. monocytogenes</i>	1a	GT1072	C. Donnelly	Raw milk	Positive
<i>L. monocytogenes</i>	1a	GT1880	J. Lovett <sup>g</sup>	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 <sup>a</sup>
<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. 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>	-	A199	ATCC 35897	Plant material	Positive
<i>L. welshimeri</i>	-	A200	ATCC 43550	Soil	Positive
<i>L. welshimeri</i>	-	GT1773	Neogen	Environmental isolate	Positive
<i>L. welshimeri</i>	-	GT1729	Neogen	Dairy plant	Positive

**Table 2. Results of exclusivity testing for the ANSR *Listeria* test (7)**

Organism	Strain #	Source (ATCC #)	Origin (if known)	ANSR Result (~ 10 <sup>9</sup> cfu/mL)	Culture Conditions <sup>o</sup>
<i>Bacillus cereus</i>	A208	25621	Cow dung	Negative	BHI broth, 5% CO <sub>2</sub> , 48 h, 25°C
<i>Bacillus megaterium</i>	GT2128	14581	-	Negative	
<i>Bacillus subtilis</i>	GT4402	21556	-	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	Negative	
<i>Kocuria rosea</i>	GT1944	185	-	Negative	
<i>Kocuria varians</i>	GT4404	15306	Milk	Negative	BHI broth, 48 h, 26°C
<i>Kurthia gibsonii</i>	GT2129	43195	Meat	Negative	
<i>Kurthia zopfii</i>	GT1941	33403	Turkey cecum	Negative	MRS broth, 48 h, 30°C
<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	-	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 gallolyticus</i>	GT668	9809	-	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	

<sup>o</sup> If other than TSB, 24 h, 36°C.

**Table 3. Probability of detection calculations for ANSR *Listeria* presumptive and confirmed results, original enrichments, 16 h time point (7)**

Matrix/Sample Size	Inoculum Strain	Inoc. Level (cfu/portion) <sup>a</sup>	N <sup>b</sup>	ANSR Presumptive Result			ANSR Confirmed Result			dPOD <sub>CP</sub> <sup>f</sup>	95% CI <sup>g</sup>
				χ <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	x	POD <sub>CC</sub> <sup>e</sup>	95% CI		
Hot dogs, 125 g	<i>L. ivanovii</i>	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.25 (0.09, 0.48)	20	0	0	0, 0.17	3	0.15	0.05, 0.36	-0.15	-0.36, 0.05
		27 (6.4, 117)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Queso fresco, 25 g	<i>L. monocytogenes</i> 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	5	0.25	0.11, 0.47	7	0.35	0.18, 0.57	-0.10	-0.36, 0.18
		11 (2.5, 53)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Cantaloupe, 25 g	<i>L. monocytogenes</i> 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	6	0.30	0.14, 0.52	6	0.30	0.14, 0.52	0	-0.27, 0.27
		5.5 (2.1, 15)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Stainless steel	<i>L. monocytogenes</i> 1/2a + <i>Enterococcus faecium</i>	0/2,600	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		360/2,600	20	16	0.80	0.58, 0.92	15	0.75	0.53, 0.89	0.05	-0.21, 0.3
		1,100/13,000	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43

<sup>a</sup> Determined by most probable number analysis except for stainless steel where determination was by titer of inoculum.

<sup>b</sup> N = Number of test portions.

<sup>c</sup> χ = Number of positive test portions.

<sup>d</sup>POD<sub>CP</sub> = Candidate method presumptive positive outcomes.

<sup>e</sup>POD<sub>CC</sub> = Candidate method presumptive positive outcomes confirmed positive.

<sup>f</sup>dPOD<sub>CP</sub> = Difference between the candidate method presumptive and candidate method confirmed POD values.

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

**Table 4. Probability of detection calculations for ANSR *Listeria* presumptive and confirmed results, original enrichments, 24 h time point (7)**

Matrix/Sample Size	Inoculum Strain	Inoc. Level (cfu/portion) <sup>a</sup>	N <sup>b</sup>	ANSR Presumptive Result			ANSR Confirmed Result			dPOD <sub>CP</sub> <sup>f</sup>	95% CI <sup>g</sup>
				χ <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	x	POD <sub>CC</sub> <sup>e</sup>	95% CI		
Hot dogs, 125 g	<i>L. ivanovii</i>	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.25 (0.09, 0.48)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0	-0.28, 0.28
		27 (6.4, 117)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Queso fresco, 25 g	<i>L. monocytogenes</i> 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	8	0.40	0.22, 0.61	9	0.45	0.26, 0.66	-0.05	-0.33, 0.24
		11 (2.5, 53)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Cantaloupe, 25 g	<i>L. monocytogenes</i> 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	6	0.30	0.14, 0.52	6	0.30	0.14, 0.52	0	-0.27, 0.27
		5.5 (2.1, 15)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Stainless steel	<i>L. monocytogenes</i> 1/2a + <i>Enterococcus faecium</i>	0/2,600	5	2	0.40	0.12, 0.77	0	0	0, 0.43	0.40	-0.12, 0.77
		360/2,600	20	18	0.90	0.7, 0.97	16	0.80	0.58, 0.92	0.10	-0.13, 0.33
		1,100/13,000	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43

<sup>a</sup> Determined by most probable number analysis.

<sup>b</sup> N = Number of test portions.

<sup>c</sup> χ = Number of positive test portions.

<sup>d</sup>POD<sub>CP</sub> = Candidate method presumptive positive outcomes.

<sup>e</sup>POD<sub>CC</sub> = Candidate method presumptive positive outcomes confirmed positive.

<sup>f</sup>dPOD<sub>CP</sub> = Difference between the candidate method presumptive and candidate method confirmed POD values.

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

**Table 5. Probability of detection calculations for ANSR *Listeria* confirmed and reference method results, original enrichments, 16 h time point (7)**

Matrix/Sample Size	Inoculum Strain	Inoc. Level (cfu/portion) <sup>a</sup>	N <sup>b</sup>	ANSR Confirmed Result			Reference Method Result			dPOD <sup>f</sup>	95% CI <sup>g</sup>
				X <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	x	POD <sub>CC</sub> <sup>e</sup>	95% CI		
Hot dogs, 125 g	<i>L. ivanovii</i>	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.25 (0.09, 0.48)	20	0	0	0, 0.17	5	0.25	0.11, 0.47	-0.25	-0.47, -0.04
		27 (6.4, 117)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Queso fresco, 25 g	<i>L. monocytogenes</i> 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	5	0.25	0.11, 0.47	13	0.65	0.43, 0.82	-0.40	-0.62, -0.09
		11 (2.5, 53)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Cantaloupe, 25 g	<i>L. monocytogenes</i> 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	6	0.30	0.14, 0.52	9	0.45	0.26, 0.66	-0.15	-0.41, 0.14
		5.5 (2.1, 15)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Stainless steel	<i>L. monocytogenes</i> 1/2a + <i>Enterococcus faecium</i>	0/2,600	5	0	0	0, 0.49	0	0	0, 0.49	0	-0.43, 0.43
		360/2,600	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0	-0.26, 0.26
		1,100/13,000	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43

<sup>a</sup> Determined by most probable number analysis.

<sup>b</sup>N = Number of test portions.

<sup>c</sup>X = Number of positive test portions.

<sup>d</sup>POD<sub>CP</sub> = Candidate method presumptive positive outcomes confirmed positive.

<sup>e</sup>POD<sub>CC</sub> = Reference method confirmed positive outcomes.

<sup>f</sup>dPOD<sub>CP</sub> = Difference between the candidate method and reference method POD values.

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

**Table 6. Probability of detection calculations for ANSR *Listeria* confirmed and reference method results, original enrichments, 24 h time point (7)**

Matrix/Sample Size	Inoculum Strain	Inoc. Level (cfu/portion) <sup>a</sup>	N <sup>b</sup>	ANSR Confirmed Result			Reference Method Result			dPOD <sup>f</sup>	95% CI <sup>g</sup>
				X <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	x	POD <sub>CC</sub> <sup>e</sup>	95% CI		
Hot dogs, 125 g	<i>L. ivanovii</i>	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.25 (0.09, 0.48)	20	7	0.35	0.18, 0.57	5	0.25	0.11, 0.47	0.10	-0.18, 0.36
		27 (6.4, 117)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Queso fresco, 25 g	<i>L. monocytogenes</i> 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	8	0.40	0.22, 0.61	13	0.65	0.43, 0.82	-0.25	-0.50, 0.05
		11 (2.5, 53)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Cantaloupe, 25 g	<i>L. monocytogenes</i> 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	6	0.30	0.14, 0.52	9	0.45	0.26, 0.66	-0.15	-0.41, 0.14
		5.5 (2.1, 15)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Stainless steel	<i>L. monocytogenes</i> 1/2a + <i>Enterococcus faecium</i>	0/2,600	5	0	0	0, 0.49	0	0	0, 0.49	0	-0.49, 0.49
		360/2,600	20	16	0.80	0.58, 0.92	15	0.75	0.53, 0.89	0.05	-0.21, 0.30
		1,100/13,000	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43

<sup>a</sup> Determined by most probable number analysis.

<sup>b</sup>N = Number of test portions.

<sup>c</sup>X = Number of positive test portions.

<sup>d</sup>POD<sub>CP</sub> = Candidate method presumptive positive outcomes confirmed positive.

<sup>e</sup>POD<sub>CC</sub> = Reference method confirmed positive outcomes.

<sup>f</sup>dPOD<sub>CP</sub> = Difference between the candidate method and reference method POD values.

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

**Table 7. Probability of detection calculations for ANSR *Listeria* presumptive and confirmed results, LESS Plus enrichment, 16 h time point (7)**

Matrix/Sample Size	Inoculum Strain	Inoc. Level (cfu/portion) <sup>a</sup>	N <sup>b</sup>	ANSR Presumptive Result			ANSR Confirmed Result			dPOD <sub>CP</sub> <sup>f</sup>	95% CI <sup>g</sup>
				x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	x	POD <sub>CC</sub> <sup>e</sup>	95% CI		
Hot dogs, 125 g	<i>L. ivanovii</i>	0	5	1	0.20	0, 0.62	0	0	0, 0.43	0.20	-0.28, 0.62
		0.25 (0.09, 0.48)	20	4	0.20	0.08, 0.42	3	0.15	0.05, 0.36	0.05	-0.19, 0.29
		27 (6.4, 117)	5	5	1	0.57, 1	5	1	0.57, 1	0	-0.43, 0.43
Queso fresco, 25 g	<i>L. monocytogenes</i> 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	0.57, 1	5	1	0.57, 1	0	-0.43, 0.43
Cantaloupe, 25 g	<i>L. monocytogenes</i> 4b	0	5	1	0.20	0, 0.62	0	0	0, 0.43	0.20	-0.28, 0.62
		0.31 (0.16, 0.49)	20	10	0.50	0.30, 0.70	9	0.45	0.26, 0.66	0.05	-0.24, 0.33
		5.5 (2.1, 15)	5	5	1	0.57, 1	5	1	0.57, 1	0	-0.43, 0.43
Stainless steel	<i>L. monocytogenes</i> 1/2a + <i>Enterococcus faecium</i>	0/2,600	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		360/2,600	20	16	0.80	0.58, 0.92	16	0.80	0.58, 0.92	0	-0.25, 0.25
		1,100/13,000	5	5	1	0.57, 1	5	1	0.57, 1	0	-0.43, 0.43
Stainless steel <sup>h</sup>	<i>L. monocytogenes</i> 1/2a + <i>Enterococcus faecalis</i>	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	0.57, 1	5	1	0.57, 1	0	-0.43, 0.43

<sup>a</sup> Determined by most probable number analysis except for stainless steel where determination was by titer of inoculum.

<sup>b</sup> N = Number of test portions.

<sup>c</sup> x = Number of positive test portions.

<sup>d</sup> POD<sub>CP</sub> = Candidate method presumptive positive outcomes.

<sup>e</sup> POD<sub>CC</sub> = Candidate method presumptive positive outcomes confirmed positive.

<sup>f</sup> dPOD<sub>CP</sub> = Difference between the candidate method presumptive and candidate method confirmed POD values.

<sup>g</sup> 95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

<sup>h</sup> Trial performed by independent laboratory.

**Table 8. Probability of detection calculations for ANSR *Listeria* presumptive and confirmed results, LESS Plus enrichment, 24 h time point (7)**

Food Type	Inoculum Strain	Inoc. Level (cfu/portion) <sup>a</sup>	N <sup>b</sup>	ANSR Presumptive Result			ANSR Confirmed Result			dPOD <sub>CP</sub> <sup>f</sup>	95% CI <sup>g</sup>
				X <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	x	POD <sub>CC</sub> <sup>e</sup>	95% CI		
Hot dogs, 125 g	<i>L. ivanovii</i>	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.25 (0.09, 0.48)	20	5	0.25	0.11, 0.47	5	0.25	0.11, 0.47	0	-0.26, 0.26
		27 (6.4, 117)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Hot dogs, 125 g <sup>h</sup>	<i>L. monocytogenes</i> 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
Queso fresco, 25 g	<i>L. monocytogenes</i> 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
Queso fresco, 25 g <sup>h</sup>	<i>L. monocytogenes</i> 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	<i>L. monocytogenes</i> 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	10	0.50	0.30, 0.70	9	0.45	0.26, 0.66	0.05	-0.24, 0.33
		5.5 (2.1, 15)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Stainless steel	<i>L. monocytogenes</i> 1/2a + <i>Enterococcus faecium</i>	0/2,600	5	1	0.20	0, 0.62	0	0	0, 0.43	0	-0.28, 0.62
		360/2,600	20	16	0.80	0.58, 0.92	16	0.80	0.58, 0.92	0	-0.25, 0.25
		1,100/13,000	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Stainless steel <sup>h</sup>	<i>L. monocytogenes</i> 1/2a + <i>Enterococcus faecalis</i>	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

Determined by most probable number analysis.

<sup>b</sup>N = Number of test portions.

<sup>c</sup>X = Number of positive test portions.

<sup>d</sup>POD<sub>CP</sub> = Candidate method presumptive positive outcomes.

<sup>e</sup>POD<sub>CC</sub> = Candidate method presumptive positive outcomes confirmed positive.

<sup>f</sup>dPOD<sub>CP</sub> = Difference between the candidate method presumptive and candidate method confirmed POD values.

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

<sup>h</sup>Trial performed by independent laboratory.

**Table 9. Probability of detection calculations for ANSR *Listeria* confirmed and reference method results, LESS Plus enrichment, 16 h time point (7)**

Food Type	Inoculum Strain	Inoc. Level (cfu/portion) <sup>a</sup>	N <sup>b</sup>	ANSR Confirmed Result			Reference Method Result			dPOD <sup>f</sup>	95% CI <sup>g</sup>
				X <sup>c</sup>	POD <sub>c</sub> <sup>d</sup>	95% CI	x	POD <sub>r</sub> <sup>e</sup>	95% CI		
Hot dogs, 125 g	<i>L. ivanovii</i>	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.25 (0.09, 0.48)	20	3	0.15	0.05, 0.36	5	0.25	0.11, 0.47	-0.10	-0.34, 0.15
		27 (6.4, 117)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Queso fresco, 25 g	<i>L. monocytogenes</i> 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
Cantaloupe, 25 g	<i>L. monocytogenes</i> 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	9	0.45	0.26, 0.66	8	0.40	0.22, 0.61	0.05	-0.24, 0.33
		5.5 (2.1, 15)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Stainless steel	<i>L. monocytogenes</i> 1/2a + <i>Enterococcus faecium</i>	0/2,600	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		360/2,600	20	16	0.80	0.58, 0.92	15	0.75	0.53, 0.89	0.05	-0.21, 0.30
		1,100/13,000	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Stainless steel <sup>h</sup>	<i>L. monocytogenes</i> 1/2a + <i>Enterococcus faecalis</i>	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

<sup>a</sup> Determined by most probable number analysis.

<sup>b</sup> N = Number of test portions.

<sup>c</sup> X = Number of positive test portions.

<sup>d</sup> POD<sub>CP</sub> = Candidate method presumptive positive outcomes confirmed positive.

<sup>e</sup> POD<sub>CC</sub> = Reference method confirmed positive outcomes.

<sup>f</sup> dPOD<sub>CP</sub> = Difference between the candidate method and reference method POD values.

<sup>g</sup> 95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

<sup>h</sup> Trial performed by independent laboratory.

**Table 10. Probability of detection calculations for ANSR *Listeria* confirmed and reference method results, LESS Plus enrichment, 24 h time point (7)**

Food Type	Inoculum Strain	Inoc. Level (cfu/portion) <sup>a</sup>	N <sup>b</sup>	ANSR Confirmed Result			Reference Method Result			dPOD <sup>c</sup>	95% CI <sup>g</sup>
				X <sup>c</sup>	POD <sub>C</sub> <sup>d</sup>	95% CI	x	POD <sub>R</sub> <sup>e</sup>	95% CI		
Hot dogs, 125 g	<i>L. ivanovii</i>	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.25 (0.09, 0.48)	20	5	0.25	0.11, 0.47	5	0.25	0.11, 0.47	0	-0.26, 0.26
		27 (6.4, 117)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Hot dogs, 125 g <sup>h</sup>	<i>L. monocytogenes</i> 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
Queso fresco, 25 g	<i>L. monocytogenes</i> 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
Queso fresco, 25 g <sup>h</sup>	<i>L. monocytogenes</i> 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	<i>L. monocytogenes</i> 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	9	0.45	0.26, 0.66	8	0.40	0.22, 0.61	0.05	-0.24, 0.33
		5.5 (2.1, 15)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Stainless steel	<i>L. monocytogenes</i> 1/2a + <i>Enterococcus faecium</i>	0/2,600	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		360/2,600	20	16	0.80	0.58, 0.92	15	0.75	0.53, 0.89	0.05	-0.21, 0.30
		1,100/13,000	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
Stainless steel <sup>h</sup>	<i>L. monocytogenes</i> 1/2a + <i>Enterococcus faecalis</i>	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

<sup>a</sup> Determined by most probable number analysis.<sup>b</sup> N = Number of test portions.<sup>c</sup> x = Number of positive test portions.<sup>d</sup> POD<sub>C</sub> = Candidate method presumptive positive outcomes confirmed positive.<sup>e</sup> POD<sub>CC</sub> = Reference method confirmed positive outcomes.<sup>f</sup> dPOD<sub>C</sub> = Difference between the candidate method and reference method POD values.<sup>g</sup> 95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.<sup>h</sup> Trial performed by independent laboratory.**DISCUSSION OF MODIFICATION APPROVED JUNE 2017 (8)**

Results presented here validate performance of the proposed modification to the ANSR for *Listeria* reagents including addition of salmon sperm DNA and an accompanying volume adjustment to the pre-lyophilization master mix. In comparative testing of three foods and sponge samples from a stainless steel surface, performance of the ANSR method after either 16 h or 24 h of enrichment was comparable to that of the FDA/BAM or USDA/MLG methods for all matrices tested as determined by POD analysis. For hot dogs, as more ANSR positives were obtained at 24 h than at 16 h, the longer enrichment time is indicated for this matrix. The proposed modification to the reagent master mix results in stronger internal positive control curves and a decreased probability of obtaining invalid assay results due to positive control failure. In our experience, this is especially true for enriched samples containing low quantities of non-target DNA (from microbial and/or matrix sources) as the internal positive control reaction is partly dependent on the presence of non-target DNA. Only one invalid assay result was obtained in these trials.

In addition to high sensitivity and specificity, the ANSR *Listeria* method offers the advantages of single-step enrichment, minimal labor and assay hardware requirements, and assay results within 50 min following sample enrichment.

**Table 1. Results of inclusivity testing for the ANSR for Listeria assay (8)**

Organism	Serotype	Strain	Source	Origin (if known)	ANSR Result
<i>L. aquatica</i>		FSL S10-1188	Cornell Univ. <sup>a</sup>	-	Positive
<i>L. booriae</i>		FSL A5-0281	Cornell Univ.	-	Positive
<i>L. cornellensis</i>		FSL F6-0969	Cornell Univ.	-	Positive
<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> <sup>b</sup>	-	GT4800	Neogen	Environmental	Positive
<i>L. grayi</i> <sup>b</sup>	-	A203	ATCC <sup>c</sup> 19120	Chinchilla feces	Positive
<i>L. grayi</i> subsp. <i>murrayi</i> <sup>b</sup>	-	A198	Neogen	-	Positive
<i>L. innocua</i>	6a	GT3627	H. Seeliger <sup>d</sup>	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 <sup>e</sup>	-	Positive
<i>L. innocua</i>	-	GT1052	J. Farber <sup>f</sup>	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 <sup>g</sup>	Raw milk	Positive
<i>L. monocytogenes</i>	1a	GT1072	C. Donnelly	Raw milk	Positive
<i>L. monocytogenes</i>	1a	GT1880	J. Lovett <sup>h</sup>	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. rocourtia</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

<sup>a</sup> Department of Food Science, Cornell University, Stocking Hall, Ithaca, NY 14853.

<sup>b</sup> Grown in TSB-YE (as opposed to TSB) prior to inoculation into LESS Plus broth.

<sup>c</sup> American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110.

<sup>d</sup> Institute of Hygiene and Molecular Microbiology, University of Würzburg, D8700 Würzburg, Germany.

<sup>e</sup> Centers for Disease Control and Prevention, 1600 Clifton Rd., Atlanta, GA 30333.

<sup>f</sup> Food Directorate, Health Canada, Banting Research Centre, Tunney's Pasture, Postal Locator 2203G3, Ottawa, Ontario K1A 0L2, Canada.

<sup>g</sup> Department of Nutrition and Food Sciences, University of Vermont, Nutrition and Food Sciences, Room 254, Burlington, VT 05405.

<sup>h</sup> U.S. Food and Drug Administration, 6751 Steger Dr., Cincinnati, OH 45237.

Table 2. Results of exclusivity testing for the ANSR for *Listeria* assay (8)

Organism	Strain #	Source (ATCC #)	Origin (if known)	Culture Conditions <sup>a</sup>	ANSR Result
<i>Bacillus cereus</i>	A208	25621	Cow dung		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	BHI broth, 5% CO <sub>2</sub> , 48 h, 25°C	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 <sub>2</sub> , 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		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	MRS broth, 48 h, 30°C	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

<sup>a</sup> If other than TSB, 24 h, 36°C.

**Table 3. Probability of detection calculations for ANSR *Listeria* presumptive and confirmed results, 16 h time point (8)**

Matrix/Sample Size	Inoculum Strain	Inoc. Level (CFU/portion) <sup>a</sup>	N <sup>b</sup>	ANSR Presumptive Result			ANSR Confirmed Result			dPOD <sub>CP</sub> <sup>f</sup>	95% CI <sup>g</sup>
				X <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	x	POD <sub>CC</sub> <sup>e</sup>	95% CI		
Hot dogs, 125 g	<i>L. seeligeri</i> ATCC35967	0	5	0	0	0, 0.434	0	0	0, 0.434	0	-0.434, 0.434
		0.19 (0.04, 0.35)	20	2	0.100	0.028, 0.301	4	0.200	0.081, 0.416	-0.100	-0.328, 0.134
		2.5 (1.2, 5.4)	5	5	1	0.565, 1	5	1	0.565, 1	0	-0.434, 0.434
Queso fresco, 25 g	<i>L. monocytogenes</i> 1/2b GT3856	0	5	0	0	0, 0.434	0	0	0, 0.434	0	-0.434, 0.434
		0.34 (0.14, 0.59)	30	8	0.267	0.142, 0.444	8	0.267	0.142, 0.444	0	-0.217, 0.217
		11 (2.5, 46)	5	5	1	0.565, 1	5	1	0.565, 1	0	-0.434, 0.434
Cantaloupe, 25 g	<i>L. monocytogenes</i> 4b GT4342	0	5	0	0	0, 0.434	0	0	0, 0.434	0	-0.434, 0.434
		1.7 (1.0, 3.3)	20	15	0.750	0.531, 0.888	15	0.750	0.531, 0.888	0	-0.259, 0.259
		275 (64, 1176)	5	5	1	0.565, 1	5	1	0.565, 1	0	-0.434, 0.434
Stainless steel	<i>L. monocytogenes</i> 1/2a GT1021 + <i>Enterococcus faecalis</i>	0	5	0	0	0, 0.434	0	0	0, 0.434	0	-0.434, 0.434
		33/975	20	11	0.550	0.342, 0.742	11	0.550	0.342, 0.742	0	-0.283, 0.283
		335/9750	5	5	1	0.565, 1	5	1	0.565, 1	0	-0.434, 0.434

<sup>a</sup> Determined by most probable number analysis except for stainless steel where determination was by titer of inoculum.

<sup>b</sup> N = Number of test portions.

<sup>c</sup> x = Number of positive test portions.

<sup>d</sup> POD<sub>CP</sub> = Candidate method presumptive positive outcomes.

<sup>e</sup> POD<sub>CC</sub> = Candidate method presumptive positive outcomes confirmed positive.

<sup>f</sup> dPOD<sub>CP</sub> = Difference between the candidate method presumptive and candidate method confirmed POD values.

<sup>g</sup> 95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

**Table 4. Probability of detection calculations for ANSR *Listeria* presumptive and confirmed results, 24 h time point (8)**

Matrix/Sample Size	Inoculum Strain	Inoc. Level (CFU/portion) <sup>a</sup>	N <sup>b</sup>	ANSR Presumptive Result			ANSR Confirmed Result			dPOD <sub>CP</sub> <sup>f</sup>	95% CI <sup>g</sup>
				X <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	x	POD <sub>CC</sub> <sup>e</sup>	95% CI		
Hot dogs, 125 g	<i>L. seeligeri</i> ATCC35967	0	5	0	0	0, 0.434	0	0	0, 0.434	0	-0.434, 0.434
		0.19 (0.04, 0.35)	20	5	0.250	0.112, 0.469	5	0.250	0.112, 0.469	0	-0.259, 0.259
		2.5 (1.2, 5.4)	5	5	1	0.565, 1	5	1	0.565, 1	0	-0.434, 0.434
Queso fresco, 25 g	<i>L. monocytogenes</i> 1/2b GT3856	0	5	0	0	0, 0.434	0	0	0, 0.434	0	-0.434, 0.434
		0.34 (0.14, 0.59)	30	9	0.300	0.167, 0.479	8	0.267	0.142, 0.444	0.033	-0.189, 0.248
		11 (2.5, 46)	5	5	1	0.565, 1	5	1	0.565, 1	0	-0.434, 0.434
Cantaloupe, 25 g	<i>L. monocytogenes</i> 4b GT4342	0	5	0	0	0, 0.434	0	0	0, 0.434	0	-0.434, 0.434

		1.7 (1.0, 3.3)	20	15	0.750	0.531, 0.888	15	0.750	0.531, 0.888	0	-0.259, 0.259
		275 (64, 1176)	5	5	1	0.565, 1	5	1	0.565, 1	0	-0.434, 0.434
Stainless steel	<i>L. monocytogenes</i> 1/2a GT1021 + <i>Enterococcus faecalis</i>	0	5	1	0.200	0, 0.624	0	0	0, 0.434	0.200	-0.278, 0.624
		33/975	20	12	0.600	0.387, 0.781	12	0.600	0.387, 0.781	0	-0.280, 0.280
		335/9750	5	5	1	0.565, 1	5	1	0.565, 1	0	-0.434, 0.434

<sup>a</sup> Determined by most probable number analysis except for stainless steel where determination was by titer of inoculum.

<sup>b</sup> N = Number of test portions.

<sup>c</sup> x = Number of positive test portions.

<sup>d</sup> POD<sub>CP</sub> = Candidate method presumptive positive outcomes.

<sup>e</sup> POD<sub>CC</sub> = Candidate method presumptive positive outcomes confirmed positive.

<sup>f</sup> dPOD<sub>CP</sub> = Difference between the candidate method presumptive and candidate method confirmed POD values.

<sup>g</sup> 95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

**Table 5. Probability of detection calculations for ANSR *Listeria* confirmed and reference method results, 16 h time point (8)**

Matrix/Sample Size	Inoculum Strain	Inoc. Level (CFU/portion) <sup>a</sup>	N <sup>b</sup>	ANSR Confirmed Result			Reference Method Result			dPOD <sup>f</sup>	95% CI <sup>g</sup>
				X <sup>c</sup>	POD <sub>C</sub> <sup>d</sup>	95% CI	x	POD <sub>R</sub> <sup>e</sup>	95% CI		
Hot dogs, 125 g	<i>L. seeligeri</i> ATCC35967	0	5	0	0	0, 0.434	0	0	0, 0.434	0	-0.434, 0.434
		0.19 (0.04, 0.35)	20	2	0.100	0.028, 0.301	2	0.100	0.028, 0.301	0	-0.213, 0.213
		2.5 (1.2, 5.4)	5	5	1	0.565, 1	5	1	0.565, 1	0	-0.434, 0.434
Queso fresco, 25 g	<i>L. monocytogenes</i> 1/2b GT3856	0	5	0	0	0, 0.434	0	0	0, 0.434	0	-0.434, 0.434
		0.34 (0.14, 0.59)	30	8	0.267	0.142, 0.444	9	0.300	0.167, 0.479	-0.033	-0.251, 0.189
		11 (2.5, 46)	5	5	1	0.565, 1	5	1	0.565, 1	0	-0.434, 0.434
Cantaloupe, 25 g	<i>L. monocytogenes</i> 4b GT4342	0	5	0	0	0, 0.434	0	0	0, 0.434	0	-0.434, 0.434
		1.7 (1.0, 3.3)	20	15	0.750	0.531, 0.888	16	0.800	0.584, 0.919	-0.050	-0.299, 0.206
		275 (64, 1176)	5	5	1	0.565, 1	5	1	0.565, 1	0	-0.434, 0.434
Stainless steel	<i>L. monocytogenes</i> 1/2a GT1021 + <i>Enterococcus faecalis</i>	0	5	0	0	0, 0.434	0	0	0, 0.434	0	-0.434, 0.434
		33/975	20	11	0.550	0.342, 0.742	6	0.300	0.145, 0.519	0.25	-0.052, 0.528
		335/9750	5	5	1	0.565, 1	5	1	0.565, 1	0	-0.434, 0.434

<sup>a</sup> Determined by most probable number analysis except for stainless steel where determination was by titer of inoculum.

<sup>b</sup> N = Number of test portions.

<sup>c</sup> x = Number of positive test portions.

<sup>d</sup> POD<sub>C</sub> = Candidate method presumptive positive outcomes confirmed positive.

<sup>e</sup> POD<sub>R</sub> = Reference method confirmed positive outcomes.

<sup>f</sup> dPOD<sub>C</sub> = Difference between the candidate method and reference method POD values.

<sup>g</sup> 95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

Table 6. Probability of detection calculations for ANSR *Listeria* confirmed and reference method results, 24 h time point (8)

Matrix/Sample Size	Inoculum Strain	Inoc. Level (CFU/portion) <sup>a</sup>	N <sup>b</sup>	ANSR Confirmed Result			Reference Method Result			dPOD <sup>c</sup>	95% CI <sup>g</sup>
				X <sup>c</sup>	POD <sub>C</sub> <sup>d</sup>	95% CI	x	POD <sub>R</sub> <sup>e</sup>	95% CI		
Hot dogs, 125 g	<i>L. seeligeri</i> ATCC35967	0	5	0	0	0, 0.434	0	0	0, 0.434	0	-0.434, 0.434
		0.19 (0.04, 0.35)	20	5	0.250	0.112, 0.469	2	0.100	0.028, 0.301	0.150	-0.094, 0.380
		2.5 (1.2, 5.4)	5	5	1	0.565, 1	5	1	0.565, 1	0	-0.434, 0.434
Queso fresco, 25 g	<i>L. monocytogenes</i> 1/2b GT3856	0	5	0	0	0, 0.434	0	0	0, 0.434	0	-0.434, 0.434
		0.34 (0.14, 0.59)	30	8	0.267	0.142, 0.444	9	0.300	0.167, 0.479	-0.033	-0.251, 0.189
		11 (2.5, 46)	5	5	1	0.565, 1	5	1	0.565, 1	0	-0.434, 0.434
Cantaloupe, 25 g	<i>L. monocytogenes</i> 4b GT4342	0	5	0	0	0, 0.434	0	0	0, 0.434	0	-0.434, 0.434
		1.7 (1.0, 3.3)	20	15	0.750	0.531, 0.888	16	0.800	0.584, 0.919	-0.050	-0.299, 0.206
		275 (64, 1176)	5	5	1	0.565, 1	5	1	0.565, 1	0	-0.434, 0.434
Stainless steel	<i>L. monocytogenes</i> 1/2a GT1021 + <i>Enterococcus faecalis</i>	0	5	0	0	0, 0.434	0	0	0, 0.434	0	-0.434, 0.434
		33/975	20	12	0.600	0.387, 0.781	6	0.300	0.145, 0.519	0.300	-0.006, 0.538
		335/9750	5	5	1	0.565, 1	5	1	0.565, 1	0	-0.434, 0.434

<sup>a</sup> Determined by most probable number analysis except for stainless steel where determination was by titer of inoculum.

<sup>b</sup> N = Number of test portions.

<sup>c</sup> x = Number of positive test portions.

<sup>d</sup> POD<sub>C</sub> = Candidate method presumptive positive outcomes confirmed positive.

<sup>e</sup> POD<sub>R</sub> = Reference method confirmed positive outcomes.

<sup>f</sup> dPOD<sub>C</sub> = Difference between the candidate method and reference method POD values.

<sup>g</sup> 95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

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