FOOD BIOLOGICAL CONTAMINANTS

Validation of the ANSR® *Listeria* Method for Detection of *Listeria* spp. in Selected Foods

Modification to AOAC *Performance Tested Method*SM 101202

Abstract

ANSR® *Listeria* was previously certified as *Performance Tested Method*SM 101202 for detection of *Listeria* spp. on selected environmental surfaces. This study proposes a matrix extension to the method for detection of *Listeria* spp. in selected food matrixes. The method is an isothermal nucleic acid amplification assay based on the nicking enzyme amplification reaction technology. Following single-step sample enrichment for 16–24 h, the assay is completed in less than 50 min, requiring only simple instrumentation. Inclusivity testing was performed using a panel of 51 strains of *Listeria* spp., representing the species *L. grayi*, *L. innocua*, *L. ivanovii*, *L. monocytogenes*, *L. seeligeri*, and *L. welshimeri*. All strains tested were detected by the ANSR assay. Exclusivity testing of 30 strains representing non-*Listeria* Gram-positive bacteria yielded no evidence of cross-reactivity. Performance of the ANSR method for detection of *Listeria* spp. was compared to that of reference culture procedures for pasteurized liquid egg, pasteurized 2% milk, Mexican-style cheese, ice cream, smoked salmon, lettuce, cantaloupe, and guacamole. Data obtained in these unpaired studies and analyzed using a probability of detection model demonstrated that there were no statistically significant differences in results between the ANSR and reference culture methods, except for milk at 16 h and cantaloupe. In milk and smoked salmon, ANSR sensitivity was low at 16 h and therefore the recommended incubation time is 24 h. In cantaloupe, ANSR was found to be more sensitive than the reference culture method at both 16 and 24 h in independent laboratory testing. The ANSR *Listeria* method can be used as an accurate, rapid, and simple alternative to standard culture methods for detection of *Listeria* spp. in selected food types.

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Scope of Method

(**a**) *Target organisms*.—*Listeria* spp.

(**b**) *Matrixes*.—Pasteurized 2% milk, Mexican-style cheese, ice cream, smoked salmon, lettuce, cantaloupe, guacamole, and pasteurized liquid egg.

(**c**) *Summary of validated performance claims*.—Performance not statistically different from that of the U.S. Food and Drug Administration *Bacteriological Analytical Manual* (FDA/BAM) (1) or U.S. Department of Agriculture-Food Safety and Inspection Service *Microbiology Laboratory Guidebook* (USDA/MLG) (2) reference culture methods as determined by probability of detection (POD) analysis.

Definitions

Probability of detection (POD).—A statistical analysis that measures the proportion of positive analytical outcomes for a qualitative method for a given matrix at a given analyte level or concentration (3).

Introduction

In the original ANSR *Listeria* study, sponge or swab samples taken from inoculated stainless steel, plastic, ceramic tile, sealed concrete, and rubber surfaces were tested in comparison to the USDA/MLG reference method. No statistically significant differences were found in performance of the ANSR and reference methods by POD analysis (4).

Principle

ANSR *Listeria* is a new isothermal nucleic acid amplification assay based on the nicking enzyme amplification reaction

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The method was independently tested, evaluated, and certified by the AOAC Research Institute as a *Performance Tested Method*SM. *See* http://www.aoac.org/testkits/steps.html for information on certification.

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(NEAR™) technology (5). Single-stranded DNA is produced from target *Listeria* ribosomal RNA through the action of reverse transcriptase. This complementary DNA then serves as the 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 in less than 20 min. 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 the results as negative, positive, or invalid based on baseline, rate-of-change, and other criteria. ANSR reagents are provided in ready-to-use lyophilized form, containing all enzymes, oligonucleotide templates, molecular beacon probe, and other factors required for the reverse transcription, amplification, and detection reactions. Each tube of ANSR reagents also contains an internal positive control, signaling in a second fluorescence channel irrespective of the presence of target nucleic acid, and indicating proper functioning of the amplification reagents.

Materials and Methods

Test Kit Information

(**a**) *Kit name*.—ANSR® for *Listeria* test.

(**b**) *Cat. No*.—9821.

(**c**) *Ordering information*.—*In the United States*.—Neogen Corp., 620 Lesher Pl, Lansing, MI 48912; Tel: 517-372-9200, Fax: 517-372-0108, Website: www.neogen.com. *Outside the United States*.—Contact U.S. office for ordering or distributor information.

Test Kit Reagents

(**a**) *ANSR assay reagents*.—Lyophilized reagents in capped strip tubes, eight tubes per strip, 12 strips (96 tests) per kit, in two sealed foil pouches with desiccant packs. Store reagent tubes at 2–8°C, in sealed foil pouches with desiccant.

(**b**) *Lysis reagent*.—Lyophilized reagent, three vials. Store at 2–8°C.

(**c**) *Lysis buffer*.—One bottle, 60 mL. Store at 2–8°C.

(**d**) *Cluster tubes*.—1.2 mL, eight tubes per strip, 12 strips per kit.

(**e**) *Permanent caps*.—Eight caps per strip, 12 strips per kit.

(**f**) *Kit insert*.

Supplies and Reagents

Additional supplies and reagents available from Neogen Corp.:

(**a**) *Incubator/reader*.—Incubator/reader capable of operating at 56 ± 1°C and reading fluorescence in real time in two channels (540/590 nm and 575/620 nm).

(**b**) *Computer and ANSR software*.—For connection to incubator/reader. Minimum requirements for computer: Intel® Core i3 processor, 1 GB RAM, Windows® 7, Ethernet and USB connections.

(**c**) *Heater block*.—With insert for 1.2 mL cluster tubes, 37 ± 2°C.

(**d**) *Heater block*.—With insert for 1.2 mL cluster tubes, 80 ± 2°C.

(**e**) *Micropipettor*.—20–200 µL, adjustable volume.

(**f**) *Pipettor*.—100–1000 µL, adjustable volume.

(**g**) *8-channel micropipettor*.—10–100 µL, adjustable volume.

(**h**) *Pipet tips*.—100 µL, with filter.

(**i**) *Pipet tips*.—1000 µL.

(**j**) *Minute timer*.

(**m**) *ANSR Listeria Enrichment Broth*.—For environmental sample enrichment.

(**n**) LESS broth.—For food sample enrichment.

(**o**) *Buffered Listeria Enrichment Broth (BLEB;* 1*) or MOPS-BLEB (2)*.—For confirmation of positive results.

(**p**) *Listeria selective/differential agar media* (1, 2).—For confirmation of positive results.

(**q**) *Stomacher-type bags*.—For sample enrichment.

(**r**) *Test tubes*.—16 × 125 mm, for sample enrichment.

(**s**) *Sampling sponges*.—Sterile, cellulose, approximately 80 × 40 × 4 mm, pre-moistened with Dey-Engley (DE) neutralizing broth, or equivalent.

(**t**) *Sampling swabs*.—Sterile, polyester, pre-moistened with DE neutralizing broth, or equivalent.

Not available from Neogen Corp.:

(**a**) *Stomacher™*.—Or equivalent, optional.

(**b**) *Pipets*.—Serological, 25 mL.

(**c**) *Incubator*.—36 ± 1°C, for sample enrichment.

(**d**) *Diagnostic reagents*.—As necessary for culture confirmation of positive ANSR results (1, 2).

Standard Reference Materials

Bacterial strains used in this study were obtained from the American Type Culture Collection (ATCC, Manassas, VA), the U.S. Centers for Disease Control and Prevention (Atlanta, GA), and other sources (Tables 1 and 2).

Safety Precautions

Use of this test should be restricted to individuals with appropriate laboratory training in microbiology. Reagents are for laboratory use only. Refer to the Material Safety Data Sheet from Neogen Corp. for more information. Enrichment cultures and ANSR assay lysates should be handled and disposed of as potentially infectious material. The preferred method for disposal of contaminated materials, including cultures, pipet tips, tubes, etc., is autoclaving. Items that cannot be autoclaved should be decontaminated by treatment with disinfectant solution. ANSR reaction tubes should not be autoclaved in areas where they may open and possibly contaminate the environment with amplification products. Alternatively, they may be placed in a sealed container with a small amount of diluted household bleach (10%) and discarded in normal trash.

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

Organism	Serotype	Strain	Source	Origin (if known)	ANSR result 16 h (approximately 10^4 CFU/mL)
L. grayi	—	GT4800	Neogen	Environmental	Positive
L. grayi	—	A203	ATCC 19120	Chinchilla feces	Positive
L. grayi subsp. *murrayi*	—	A198	Neogen	—	Positive
L. innocua	6a	GT3627	H. Seeliger[a]	Cheese	Positive
L. innocua	6a	GT3631	H. Seeliger	Cheese	Positive
L. innocua	6a	A102	ATCC 33090[b]	Cow brain	Positive
L. innocua	6b	GT1026	H. Seeliger	Cheese	Positive
L. innocua	6b	GT1042	H. Seeliger	Cheese	Positive
L. innocua	6b	GT1044	H. Seeliger	Cheese	Positive
L. innocua	6b	GT1050	H. Seeliger	Cheese	Positive
L. innocua	—	GT3785	CDC[c]	—	Positive
L. innocua	—	GT1052	J. Farber[d]	Raw milk	Positive
L. ivanovii	5	GT1028	H. Seeliger	Mouse	Positive
L. ivanovii	5	GT1040	H. Seeliger	Human	Positive
L. ivanovii	5	GT3699	H. Seeliger	Watercress	Positive
L. ivanovii	—	A140	ATCC 19119	Sheep	Positive
L. monocytogenes	1/2a	GT3727	H. Seeliger	Human blood	Positive
L. monocytogenes	1/2a	GT4340	CDC	Fish	Positive
L. monocytogenes	1/2a	GT1038	H. Seeliger	Human blood	Positive
L. monocytogenes	1/2b	GT3635	H. Seeliger	Human blood	Positive
L. monocytogenes	1/2b	GT3728	H. Seeliger	Cheese	Positive
L. monocytogenes	1/2b	GT3856	H. Seeliger	Cheese	Positive
L. monocytogenes	1/2c	GT3698	H. Seeliger	Cheese	Positive
L. monocytogenes	1/2c	GT3648	H. Seeliger	Cheese	Positive
L. monocytogenes	1/2c	GT3730	H. Seeliger	—	Positive
L. monocytogenes	1/2c	GT3741	H. Seeliger	—	Positive
L. monocytogenes	1a	GT3829	C. Donnelly[e]	Raw milk	Positive
L. monocytogenes	1a	GT1072	C. Donnelly	Raw milk	Positive
L. monocytogenes	1a	GT1880	J. Lovett[f]	Brie cheese	Positive
L. monocytogenes	1a	GT3812	J. Lovett	Chocolate milk	Positive
L. monocytogenes	2	A169	ATCC 19112	Human CSF	Positive
L. monocytogenes	3a	GT3720	H. Seeliger	Cheese	Positive
L. monocytogenes	3a	GT1035	H. Seeliger	—	Positive
L. monocytogenes	3b	GT1057	J. Lovett	Brie cheese	Positive
L. monocytogenes	3b	GT3715	H. Seeliger	Human blood	Positive
L. monocytogenes	3b	GT3817	H. Seeliger	Cheese	Positive
L. monocytogenes	3b	GT3857	J. Lovett	Brie cheese	Positive
L. monocytogenes	4a	A170	ATCC 19114	Ruminant brain	Positive
L. monocytogenes	4b	A207	ATCC 13932	Human CSF	Positive
L. monocytogenes	4b	GT1019	Neogen	—	Positive
L. monocytogenes	4b	GT1081	CDC	—	Positive
L. monocytogenes	4c	GT3819	H. Seeliger	Human	Positive
L. seeligeri	1/2b	GT3693	H. Seeliger	Sewage	Positive
L. seeligeri	4a	GT289	H. Seeliger	Cheese	Positive
L. seeligeri	—	A201	ATCC 51334	Vole	Positive
L. seeligeri	6b	GT3708	H. Seeliger	Cheese	Positive

Table 1. (*continued*)

Organism	Serotype	Strain	Source	Origin (if known)	ANSR result 16 h (approximately10^4 CFU/mL)
L. welshimeri	6a	GT293	H. Seeliger	Cheese	Positive
L. welshimeri	—	A199	ATCC 35897	Plant material	Positive
L. welshimeri	—	A200	ATCC 43550	Soil	Positive
L. welshimeri	—	GT1773	Neogen	Environmental isolate	Positive
L. welshimeri	—	GT1729	Neogen	Dairy plant	Positive

[a] Institute of Hygiene and Molecular Microbiology, University of Würzburg, D8700 Würzburg, Germany.

[b] American Type Culture Collection, 10801 University Blvd, Manassas, VA 20110.

[c] Centers for Disease Control and Prevention, 1600 Clifton Rd, Atlanta, GA 30333.

[d] Food Directorate, Health Canada, Banting Research Centre, Tunney's Pasture, Postal Locator 2203G3, Ottawa, Ontario K1A 0L2, Canada.

[e] Department of Nutrition and Food Sciences, University of Vermont, Nutrition and Food Sciences, Room 254, Burlington, VT 05405.

[f] U.S. Food and Drug Administration, 6751 Steger Dr, Cincinnati, OH 45237.

L. monocytogenes is a known hazard to pregnant women and immunocompromised individuals. Consult with your facility safety director for specific instructions.

Sample Preparation

Test samples should be obtained and handled according to standard practices applicable to *Listeria* analysis (1).

Sample Enrichment

Do not autoclave ANSR enrichment media. Use media the same day as prepared. Enrichment media must be pre-warmed to incubation temperature before use. Stomacher-type bags used for sample enrichment should be closed loosely to allow air exchange during incubation.

Pasteurized milk, Mexican-style cheese, ice cream, smoked salmon, bagged lettuce, cantaloupe, guacamole, and pasteurized liquid egg.—Weigh a 25 g sample in a Stomacher-type bag. Add 225 mL of pre-warmed (36°C) LESS broth to the bag. Mix or homogenize for 30 s. Enrich the sample at 36 ± 1°C for 16–24 h. For pasteurized milk and smoked salmon, incubate for 24 ± 2 h.

Environmental sponge samples.—Add the sponge to a sufficient volume of ANSR *Listeria* Enrichment Broth to achieve a broth-to-sample ratio of about 10-to-1 (based on the hydrated volume of the sponge). For typical sampling sponges, 100 mL of broth is recommended. Grasp the bag tightly at the top and shake vigorously from side-to-side. Alternatively, homogenize for 30 s. Incubate at 36 ± 1°C for 16–24 h.

Environmental swab samples.—Add the swab to 10 mL of ANSR *Listeria* Enrichment Broth in a sterile test tube and break off the top of the swab. If using a screw-capped tube, cap the tube and shake by inverting the tube several times. Alternatively, mix by vortexing. Incubate at 36 ± 1°C for 16–24 h.

General Preparation

This assay should be performed in a controlled laboratory environment.

(**a**) Do not hold enriched samples at ambient temperature for more than 8 h prior to testing.

(**b**) Do not use culture media or ANSR reagents beyond their expiration dates. Do not interchange reagents between ANSR kit lots.

(**c**) Remove ANSR reaction tubes from the foil pouch just before use. Avoid prolonged exposure to light. Tap reaction tubes on the bench top to make sure that lyophilized reagents are at the bottom of the tube prior to adding the lysed sample.

(**d**) Complete all assay steps in sequence, avoiding delays between steps.

(**e**) Exercise care in pipetting steps to avoid cross-contamination of samples.

(**f**) Do not remove caps from reaction tubes after the assay is started; this will prevent accidental contamination of the environment with amplification products.

Prior to starting the assay:

(*1*) Preheat the lysis heater blocks to 37 ± 2°C and 80 ± 2°C.

(*2*) Start the ANSR software using the computer connected to the ANSR reader. Enter sample identification and other experiment information. The reader will preheat to 56 ± 1°C.

(*3*) Reconstitute 1 vial of lyophilized lysis reagent with 18 mL of lysis buffer. Swirl gently to mix. This is enough reconstituted lysis reagent for approximately 40 tests. (*Note*: Reconstituted lysis reagent may be used for up to 2 weeks when stored at 2–8°C. Return reconstituted lysis reagent to 2–8°C storage immediately after use.)

Analysis

(*1*) For guacamole, smoked salmon, and pasteurized liquid eggs, dilute an aliquot of the enrichment culture 1:10 in phosphate buffered saline. For all other commodities, use the enrichment culture undiluted.

(*2*) Add 50 µL (diluted/undiluted) of enrichment culture to a 1.2 mL cluster tube.

(*3*) Add 450 µL of reconstituted lysis reagent to the cluster tube.

(*4*) Transfer the cluster tubes to the 37°C heater block and incubate for 10 min.

(*5*) Transfer the cluster tubes to the 80°C heater block and

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

Organism	Strain No.	Source (ATCC No.)	Origin (if known)	ANSR result (approximately 10^9 CFU/mL)	Culture conditions[a]
Bacillus cereus	A208	25621	Cow dung	Negative	
Bacillus megaterium	GT2128	14581	—	Negative	
Bacillus subtilis	GT4402	21556	—	Negative	
Brochothrix thermosphacta	GT664	11509	Pork sausage	Negative	BHI[b] broth, CO_2, 48 h, 25°C
Enterococcus durans	GT407	6056	Human feces	Negative	
Enterococcus faecalis	GT3242	27275	—	Negative	
Enterococcus faecium	GT919	6057	Cheese	Negative	
Enterococcus hirae	GT923	35220	Cow dung	Negative	
Geobacillus stearothermophilus	GT4373	12980	—	Negative	
Gordonia sputi	GT3474	29627	Human	Negative	Nutrient broth, CO_2, 48 h, 37°C
Kocuria rosea	GT1944	185	—	Negative	BHI broth, 48 h, 26°C
Kocuria varians	GT4404	15306	Milk	Negative	
Kurthia gibsonii	GT2129	43195	Meat	Negative	
Kurthia zopfii	GT1941	33403	Turkey cecum	Negative	
Lactobacillus acidophilus	GT256	4356	Human	Negative	
Lactobacillus buchneri	GT4082	11307	Beer	Negative	MRS broth, 48 h, 30°C
Lactobacillus casei	GT805	393	Cheese	Negative	
Lactobacillus fermentum	GT4063	9338	—	Negative	
Lactococcus lactis	GT3516	11454	—	Negative	
Micrococcus luteus	GT1943	381	Water	Negative	
Rhodococcus equi	GT665	6939	Horse	Negative	
Rhodococcus fascians	GT3524	12974	—	Negative	BHI broth, 48 h, 26°C
Staphylococcus aureus	A179	12600	Human pleural fluid	Negative	
Staphylococcus epidermidis	A183	14990	Human	Negative	
Staphylococcus saprophyticus	A185	15305	Human urine	Negative	
Streptococcus equi	GT3596	33398	—	Negative	
Streptococcus gallolyticus	GT668	9809	—	Negative	
Streptococcus mutans	GT412	25175	Human mouth	Negative	
Streptococcus pneumoniae	GT408	6303	—	Negative	
Streptococcus sanguinis	GT411	10556	Human	Negative	

[a] If other than TSB, 16–24 h, 36°C.

[b] BHI = Brain Heart Infusion.

incubate for 20 min. *Note:* Incubation may be extended to a maximum of 60 min for the purpose of managing staggered assay start times.

(*6*) At the end of the second lysis incubation, place the ANSR reaction tubes in the incubator/reader. (*Note*: The strip of tubes may be cut to provide the number of tubes needed.) Remove and discard the caps from the reaction tubes. (*Note*: Steps 7–9 should be completed without delay.)

(*7*) Using an 8-channel micropipettor and filter tips, carefully transfer 50 µL of the lysed samples to the reaction tubes. Mix by rapidly pipetting up and down at least 10 times until the sample appears homogenous in the pipet tip. Avoid excessive bubble formation by not depressing the pipettor plunger beyond the first stop.

(*8*) Place the permanent caps on the reaction tubes and close the lid of the incubator/reader.

(*9*) Click START in the ANSR software to begin the assay.

(*10*) Results will be displayed as POSITIVE, NEGATIVE, or INVALID by the software at the end of the assay. If the result is INVALID, the test must be repeated.

Interpretation of Results

Test interpretation.—The ANSR software will indicate the test results as positive or negative for the presence of *Listeria* spp. in the enriched sample. In addition, the real-time fluorescence curves for both the test and positive control channels can be viewed using the ANSR software.

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

Matrix	Inoculum strain	Inoc. level (CFU/portion)[a]	N[b]	ANSR result			ANSR confirmed result				
				x[c]	POD_{CP}[d]	95% CI	X	POD_{CC}[e]	95% CI	$dPOD_{CP}$[f]	95% CI[g]
Pasteurized 2% milk	*L. welshimeri*	>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[h]	*L. welshimeri*	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	*L. mono* 1/2b	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	*L. mono* 1/2a	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, 0.30
	—	—	5	0	0	0, 0.43	0	0	0, 0.43	0	–0.43, 0.43
Smoked salmon	*L. mono* 4b	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	*L. mono* 1/2a	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
	—	—	5	0	0	0, 0.43	0	0	0, 0.43	0	–0.43, 0.43
Cantaloupe	*L. mono* 1/2b	>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[h]	*L. mono* 1/2b	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, 0.1	0	–0.16, 0.16
	—	—	5	0	0	0, 0.43	0	0	0, 0.43	0	–0.43, 0.43
Pasteurized liquid egg	*L. mono* 4b	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	*L. innocua*	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

[a] Determined by dilution plating of the inoculum cultures.

[b] N = Number of test portions.

[c] x = Number of positive test portions.

[d] POD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials.

[e] POD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials.

[f] $dPOD_{CP}$ = Difference between the candidate method presumptive result and candidate method confirmed result POD values.

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

[h] Trial performed by independent laboratory.

Recommended confirmation procedures.—Streak the enrichment cultures to *Listeria* selective/differential plating media and continue with standard identification procedures for *Listeria* (1, 2). PALCAM and/or MOX agars are recommended. In addition, transfer 0.1 mL of the enrichment culture to 10 mL of BLEB or MOPS-BLEB (1, 2). Incubate the BLEB or MOPS-BLEB cultures for 18–24 h at 35 ± 2°C. Proceed with selective/differential plating and identification procedures as required for samples not confirmed by direct plating.

Internal Validation Studies

Inclusivity Testing

The original inclusivity testing was performed during the ANSR *Listeria* study for environmental samples (4); however, this was done with ANSR *Listeria* Enrichment Broth. In the current study, inclusivity testing was performed with LESS broth. As before, this was performed using a panel of 51 strains of *Listeria* spp., representing the species *L. grayi*, *L. innocua*,

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

Matrix	Inoculum strain	Inoc. level (CFU/portion)[a]	N[b]	ANSR result			ANSR confirmed result				
				x[c]	POD_{CP}[d]	95% CI	X	POD_{CC}[e]	95% CI	$dPOD_{CP}$[f]	95% CI[g]
Pasteurized 2% milk	*L. welshimeri*	>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	0.2, 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[h]	*L. welshimeri*	4.4 (1.7, 11.2)	5	5	1	0.56, 1	5	1	0.57, 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	*L. mono* 1/2b	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	*L. mono* 1/2a	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	–0.20, 0.27
	—	—	5	0	0	0, 0.43	0	0	0, 0.43	0	–0.43, 0.43
Smoked salmon	*L. mono* 4b	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	*L. mono* 1/2a	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	*L. mono* 1/2b	>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[h]	*L. mono* 1/2b	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, 0.1	0	–0.16, 0.16
	—	—	5	0	0	0, 0.43	0	0	0, 0.43	0	–0.43, 0.43
Pasteurized liquid egg	*L. mono* 4b	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	*L. innocua*	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

[a] Determined by dilution plating of the inoculum cultures.

[b] N = Number of test portions.

[c] x = Number of positive test portions.

[d] POD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials.

[e] POD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials.

[f] $dPOD_{CP}$ = Difference between the candidate method presumptive result and candidate method confirmed result POD values.

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

[h] Trial performed by independent laboratory.

L. ivanovii, *L. monocytogenes*, *L. seeligeri*, and *L. welshimeri*. Extra weight was given to *L. monocytogenes* as this is the only species associated with human foodborne listeriosis. Strain source information is shown in Table 1.

(**a**) *Methodology*.—Isolates were taken from –80°C frozen storage and cultured on tryptic soy agar (TSA) for 18–24 h at 36 ± 1°C. Overnight cultures were grown from single isolated colonies and diluted. A tube containing 10 mL of LESS broth was inoculated with the diluted test culture at a level of 10–50 CFU/tube (established based on titers determined for cultures in a pilot phase of the experiment). The cultures were incubated for 16 h at 36 ± 1°C, diluted in LESS broth to a titer of approximately 1×10^4 CFU/mL (about 100-fold above the LOD of the ANSR assay), and tested in the assay. Inclusivity strains were intermixed with exclusivity strains (*see* below), randomized, and blind-coded prior to testing with the ANSR assay.

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

Matrix	Inoculum strain	Inoc. level (CFU/portion)[a]	N[b]	ANSR result			Reference method result				
				x[c]	POD_C[d]	95% CI	X	POD_R[e]	95% CI	$dPOD_C$[f]	95% CI[g]
Pasteurized 2% milk	*L. welshimeri*	>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, 0.30	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[h]	*L. welshimeri*	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	*L. mono* 1/2b	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	*L. mono* 1/2a	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	*L. mono* 4b	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	*L. mono* 1/2a	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	0.70	0.48, 0.85	14	0.70	0.48, 0.85	–0.05	–0.27, 0.27
	—	—	5	0	0	0, 0.43	0	0	0, 0.43	0	–0.43, 0.43
Cantaloupe	*L. mono* 1/2b	>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[h]	*L. mono* 1/2b	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
Pasteurized liquid egg	*L. mono* 4b	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	*L. innocua*	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, 0.30
	—	—	5	0	0	0, 0.43	0	0	0, 0.43	0	–0.43, 0.43

[a] Determined by dilution plating of the inoculum cultures.

[b] N = Number of test portions.

[c] x = Number of positive test portions.

[d] POD_C = Candidate method presumptive positive outcomes confirmed positive.

[e] POD_R = Reference method confirmed positive outcomes divided by the total number of trials.

[f] $dPOD_C$ = Difference between the candidate method and reference method POD values.

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

[h] Trial performed by independent laboratory.

(**b**) *Results*.—Results are shown in Table 1. All 51 strains produced positive results in the ANSR assay.

Exclusivity Testing

Exclusivity testing was performed using a panel of 30 strains representing 13 genera and 30 species of non-*Listeria* Gram-positive bacteria. Strain source information is shown in Table 2.

(**a**) *Methodology*.—Isolates were taken from –80°C frozen storage and cultured on TSA for 18–24 h at 36 ± 1°C, or otherwise, for certain strains, as indicated in Table 2. A single colony was transferred to a tube containing 10 mL of tryptic soy broth (TSB) and incubated for 16–24 h at 36 ± 1°C, or cultured under alternative conditions when required (Table 2). Because TSB and certain other enrichment media produce excessive background fluorescence in the ANSR assay, cells from the overnight cultures were pelleted by centrifugation and

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

Matrix	Inoculum strain	Inoc. level (CFU/portion)[a]	N[b]	ANSR result			Reference method result			dPOD$_C$[f]	95% CI[g]
				x[c]	POD$_C$[d]	95% CI	X	POD$_R$[e]	95% CI		
Pasteurized 2% milk	*L. welshimeri*	>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[h]	*L. welshimeri*	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, 0.50
	—	—	5	0	0	0, 0.43	0	0	0, 0.43	0	–0.43, 0.43
Mexican-style cheese	*L. mono* 1/2b	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	*L. mono* 1/2a	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	*L. mono* 4b	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	*L. mono* 1/2a	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	*L. mono* 1/2b	>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[h]	*L. mono* 1/2b	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
Pasteurized liquid egg	*L. mono* 4b	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	*L. innocua*	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, 0.40
	—	—	5	0	0	0, 0.43	0	0	0, 0.43	0	–0.43, 0.43

[a] Determined by dilution plating of the inoculum cultures.

[b] N = Number of test portions.

[c] x = Number of positive test portions.

[d] POD$_C$ = Candidate method presumptive positive outcomes confirmed positive.

[e] POD$_R$ = Reference method confirmed positive outcomes divided by the total number of trials.

[f] dPOD$_C$ = Difference between the candidate method and reference method POD values.

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

[h] Trial performed by independent laboratory.

re-suspended in an equal volume of LESS broth (titers in the range 1×10^8–1×10^9 CFU/mL). Aliquots of the re-suspended cells were tested in the ASNR assay without dilution.

(**b**) *Results*.—Results are shown in Table 2. All 30 strains produced negative results in the ANSR assay.

Comparative Testing of Inoculated Foods

The ANSR method was compared to the FDA/BAM reference culture procedure (1) for the ability to detect *Listeria* spp. in pasteurized 2% milk, Mexican-style cheese, ice cream, smoked salmon, bagged lettuce, cantaloupe, and guacamole. For pasteurized liquid egg, the ANSR method was compared to the USDA/MLG reference culture procedure (2).

(**a**) *Preparation of inocula and inoculation of foods*.—The inoculated foods were prepared to simulate conditions of natural contamination and typical storage. A different *Listeria* strain was used with each food product (Table 3). Products were inoculated at a target level of 1 CFU/25 g, chosen to produce data sets with fractional positive results (25–75% of test

portions positive by at least one of the methods). Test material at a higher level of inoculation, expected to produce all positive results, was also prepared, along with uninoculated controls.

To prepare the inoculum, a single colony of the test organism was picked from a TSA plate and used to inoculate a 10 mL tube of TSB, which was incubated for 16–24 h at 36 ± 1°C. Serial dilutions were prepared in Butterfield's phosphate-buffered dilution water to the appropriate titer to use for inoculation.

Ice cream was thawed before inoculation. Solid products such as lettuce, smoked salmon, and Mexican-style cheese were shredded or crumbled before inoculation. Whole cantaloupes were surface disinfected, peeled, and diced into ¼ inch pieces. Liquid inoculum was applied with a pipet and the product was mixed extensively by stirring or hand-mixing. All products except ice cream were held at 2–8°C for 48–72 h. Ice cream was re-frozen and held at –20°C for 14 days.

(**b**) *Enrichment and analysis*.—Twenty or twenty-five 25 g test portions were weighed for the ANSR method and another twenty or twenty-five 25 g test portions were weighed for the reference method. Also, for each method, five high level and five uninoculated control portions were prepared. Additional inoculated and uninoculated material was reserved for most probable number analysis on the day of testing for all commodities.

FDA/BAM and USDA/MLG reference method analyses were conducted in accordance with the published methods. ANSR method analysis was performed as described above (*Sample Enrichment, General Preparation, Analysis*, and *Interpretation of Results*) after 16 and 24 h of enrichment for all foods.

ANSR results were confirmed from ANSR-associated enrichment media following selective enrichment and plating in accordance with reference method procedures, regardless of whether the ANSR assay screening result was positive or negative. Confirmation was also performed by streaking the ANSR enrichment culture directly to selective/differential agars.

(**c**) *Results*.—Results of food testing are presented in Tables 3–6. POD analysis was used to determine if a difference in the number of positive results between methods was statistically significant.

POD calculations for ANSR presumptive versus confirmed positive test portions at 16 and 24 h are shown in Tables 3 and 4, respectively. POD calculations for ANSR confirmed versus reference method results for 16 h and 24 h are shown in Tables 5 and 6, respectively. There were no statistically significant differences found by POD analysis in the number of confirmed positive results obtained by the ANSR and reference methods for any of the commodities tested. However, there were notable differences in the number of ANSR positive results at 16 h and 24 h for pasteurized milk and smoked salmon, indicating that 24 h of enrichment is required for optimum sensitivity with these matrixes.

For the high inoculum level, all test portions were positive by the reference method and all were confirmed positive by the ANSR method at both time points. All uninoculated test portions were negative by both the ANSR and reference methods, except for a single lettuce test portion which produced a positive ANSR assay result at 24 h.

(**d**) *Confirmation*.—For smoked salmon, cantaloupe, and pasteurized liquid egg, direct plating was found to be an effective confirmatory technique. For these commodities, 100% of the positive test portions were confirmed by direct plating as well as by plating following secondary enrichment. However, in all other commodities, direct plating was ineffective due to the high level of competitive microbial flora in the various matrixes. Plating was performed subsequent to secondary enrichment, and this method was found to be effective and conclusive as a confirmatory technique.

Ruggedness, Stability, and Lot-to-Lot Consistency Testing

This testing was completed during the validation study for environmental samples (4).

Independent Laboratory Trial

(**a**) *Methodology*.—The same procedures for sample preparation and testing used in the internal trials were followed by the independent laboratory. Pasteurized 2% milk and cantaloupe were the two foods tested.

(**b**) *Results*.—Results are shown in Tables 3–6. In milk, there were no ANSR positives by 16 h and nine confirmed ANSR positives by 24 h. There were four positives by the FDA/BAM reference method. There was a statistically significant difference at 16 h between the number of presumptive and confirmed ANSR positives. In cantaloupe, there were 20 confirmed ANSR positives at both 16 h and 24 h, while there were 14 positives by the FDA/BAM reference method. As a result, there were statistically significant differences between the ANSR confirmed and FDA/BAM reference methods at both time points.

Discussion

Results of the internal and independent laboratory studies showed 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 (16 h) 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 showed 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 than the reference method. There were no unconfirmed positive results from uninoculated control test portions.

In addition to high sensitivity and specificity, the ASNR *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.

Conclusions

Based on results reported here, it is recommended that a matrix extension be granted for *Performance Tested Method* 101202 to include pasteurized 2% milk, Mexican style cheese, ice

cream, smoked salmon, lettuce, cantaloupe, guacamole, and pasteurized liquid egg as validated matrixes.

Acknowledgments

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