



# CERTIFICATION

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

Certificate No.

**010403**

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

**GeneQuence<sup>TM</sup> Listeria Microwell Test**

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 23, 2019 – December 31, 2020). Renewal may be granted at the end of one year under the rules stated in the licensing agreement.

A handwritten signature in black ink that reads "Scott Coates".

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Scott Coates, Senior Director  
Signature for AOAC Research Institute

\_\_\_\_\_  
November 23, 2019

Date

**METHOD AUTHORS**

**ORIGINAL VALIDATION:** Omar A. Oyarzabal, Nicole M. Behnke, and Mark A. Mozola

**MODIFICATION NOVEMBER 2005:** Linda X. Peng, Susan Alles, Ann Stafford, and Mark Mozola

**MODIFICATION JUNE 2006:** Susan Alles, Linda X. Peng, and Mark Mozola

**SUBMITTING COMPANY**

Neogen Corporation  
620 Leshar Place  
Lansing, Michigan 48912 USA

**KIT NAME(S)**

GeneQuence™ *Listeria* Microwell Test

**CATALOG NUMBERS**

6708, 6708B, 6700, 6700B

**INDEPENDENT LABORATORY**

Richter International  
Columbus, Ohio  
USA

**AOAC EXPERTS AND PEER REVIEWERS**

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<sup>4</sup> Modifications: November 2005, June 2006

**APPLICABILITY OF METHOD**

Target organism – *Listeria* spp.

Matrices – (25 g) - Parmesan cheese, Brie cheese, pasteurized milk, ice cream, cottage cheese, mayonnaise, ground beef, deli turkey, hot dogs, ground pork, deli ham, lettuce, mixed vegetables, alfalfa sprouts, raw shrimp, pasteurized crab meat, smoked salmon  
stainless steel, ceramic, cast iron, plastic, concrete, painted wood

**Performance claims -**

Sensitivity	≥ 97%
Specificity	≥ 98%
Agreement with FDA (BAM) culture method	≥ 94%
Agreement with USDA FSIS culture method	≥ 97%

**REFERENCE METHODS**

U. S. Department of Agriculture Food Safety and Inspection Service. 1998. Isolation and Identification of *Listeria monocytogenes* from Red Meat, Poultry, Egg and Environmental Samples. Chapter 8, Microbiology Laboratory Guidebook, 3<sup>rd</sup> ed. Revision #3, 4/29/02  
<http://www.fsis.usda.gov/OPHS/microlab/mlgchp803.pdf>. (2)  
U. S. Food and Drug Administration. 1998. *Listeria monocytogenes*. Chapter 5, Bacteriological Analytical Manual, 8<sup>th</sup> ed.  
<http://www.cfsan.fda.gov/~ebam/bam-10.html>) (3)  
U. S. Food and Drug Administration. 2001. *Listeria monocytogenes*. Chapter 10, Bacteriological Analytical Manual, online.  
<http://www.cfsan.fda.gov/~ebam/bam-10.html> (6)

**ORIGINAL CERTIFICATION DATE**

April 02, 2004

**CERTIFICATION RENEWAL RECORD**

Renewed annually through December 2020

**METHOD MODIFICATION RECORD**

1. November 2005 Level 2
2. June 2006 Level 2
3. December 2018 Level 1

**SUMMARY OF MODIFICATION**

1. Change in enrichment procedure
2. Enrichment protocol to include LESS broth at 30°C for 24-28 hrs
3. Change in inactive ingredients in Blue-K Advanced substrate

Under this AOAC® Performance Tested<sup>SM</sup> License Number, 010403 this method is distributed by:

NONE

Under this AOAC® Performance Tested<sup>SM</sup> License Number, 010403 this method is distributed as:

NONE

**PRINCIPLE OF THE METHOD (1)**

The GENE-TRAK® *Listeria* Microwell Test is a DNA probe-based diagnostic in kit format, which permits rapid and accurate detection of *Listeria* spp. in foods. Following sample pre-enrichment and selective enrichment, target bacteria are lysed enzymatically at 37°C and *Listeria*-specific oligonucleotide probes are added for a 60-minute hybridization incubation at 45°C. If *Listeria* ribosomal RNA (rRNA) is present in the test sample, the detector probe, labeled with horseradish peroxidase (HRP), and polydeoxyadenylic acid (poly dA)-tailed capture probe will hybridize to the target organism rRNA sequences. Concurrently, base pairing between the poly dA-tailed capture probe and polydeoxythymidylic acid (poly dT) coated polystyrene microwells facilitates solid phase capture of probe-target molecules. Unbound probe is removed by washing, and then substrate chromogen is added to react with HRP to yield a blue color. The reaction is stopped by the addition of sulfuric acid, which changes the color of the substrate from blue to yellow. A microwell plate or microwell strip reader (A450) measures absorbance. An absorbance in excess of the threshold value indicates the presence of *Listeria* in the test sample. Positive assay results must be confirmed by standard culture methods.

#### DISCUSSION OF THE VALIDATION STUDY (1)

Results of the 15-food trial clearly show that the microwell format assay is capable of detecting low levels of *Listeria* spp. in a variety of foods. The assay has also proved to be very specific, with no cross reactions observed in exclusivity testing.

The DNA method has a high agreement with both the FDA and the USDA culture methods. In internal trials, the false negative rate for all food commodities was 2.8%. In testing of meat and poultry samples, the DNA assay had a false positive rate of 0.5%, and a false negative rate of 4.0%. In comparison, the USDA method had a false negative rate of 1.3%. In testing of dairy products, seafood, and fruits and vegetables, the DNA assay had a false positive rate of 2.1%, and a false negative rate of 1.9%. In comparison, the FDA method showed a false negative rate of 7.7%.

In comparing the DNA method and the reference culture method for the ability to detect *Listeria* spp. in foods, there was no significant difference in detection for any food tested, with the single exclusion of brie cheese for which the DNA method showed a statistically significant advantage over the FDA method for the high level samples ( $\chi^2 = 4.17$ ; Table 4).

Results of the independent laboratory validation study confirm the results from the internal trials. No false negative or false positive results were obtained in the testing of cottage cheese and mayonnaise samples. Results of this study further showed that the DNA hybridization method is applicable to the detection of *Listeria* spp. in environmental samples, with a sensitivity equivalent to that of the USDA culture method.

Table 1a. Inclusivity testing results from BLEB primary enrichment. (1)

Strain #	Organism	Serotype (if known)	Source	OD*		Results
				1:10 Dilution***	1:100 Dilution**	
A169	<i>L. monocytogenes</i>	2	ATCC 19112	2.950	0.542	Positive
A207	<i>L. monocytogenes</i>	4b	ATCC 13932	2.950	0.154	Positive
A170	<i>L. monocytogenes</i>	4a	ATCC 19114	2.950	0.621	Positive
GT3829	<i>L. monocytogenes</i>	1a	C. Donnelly	2.950	0.713	Positive
GT1072	<i>L. monocytogenes</i>	1a	C. Donnelly	2.950	1.122	Positive
GT1880	<i>L. monocytogenes</i>	1a	J. Lovett	2.950	0.472	Positive
GT3812	<i>L. monocytogenes</i>	1a	J. Lovett	2.950	0.514	Positive
GT1021	<i>L. monocytogenes</i>	1/2a	CDC	2.406	0.355	Positive
GT3727	<i>L. monocytogenes</i>	1/2a	H. Seeliger	2.950	0.202	Positive
GT4340	<i>L. monocytogenes</i>	1/2a	CDC	2.934	0.489	Positive
GT1038	<i>L. monocytogenes</i>	1/2a	H. Seeliger	2.950	0.243	Positive
GT3635	<i>L. monocytogenes</i>	1/2b	H. Seeliger	2.769	0.261	Positive
GT3728	<i>L. monocytogenes</i>	1/2b	H. Seeliger	1.577	0.154	Positive
GT3856	<i>L. monocytogenes</i>	1/2b	H. Seeliger	2.950	0.274	Positive
GT3698	<i>L. monocytogenes</i>	1/2c	H. Seeliger	2.950	0.961	Positive
GT3648	<i>L. monocytogenes</i>	1/2c	H. Seeliger	2.950	0.410	Positive
GT3730	<i>L. monocytogenes</i>	1/2c	H. Seeliger	2.950	0.461	Positive
GT3741	<i>L. monocytogenes</i>	1/2c	H. Seeliger	2.950	1.205	Positive
GT3720	<i>L. monocytogenes</i>	3a	H. Seeliger	2.460	0.160	Positive
GT1035	<i>L. monocytogenes</i>	3a	H. Seeliger	2.950	0.266	Positive
GT1057	<i>L. monocytogenes</i>	3b	J. Lovett	2.029	0.169	Positive
GT3715	<i>L. monocytogenes</i>	3b	H. Seeliger	2.950	1.879	Positive
GT3817	<i>L. monocytogenes</i>	3b	H. Seeliger	2.022	0.125	Positive
GT3857	<i>L. monocytogenes</i>	3b	J. Lovett	2.196	0.305	Positive
GT1019	<i>L. monocytogenes</i>	4b	GENE-TRAK	2.950	0.813	Positive
GT1081	<i>L. monocytogenes</i>	4b	CDC	2.950	0.173	Positive
GT3819	<i>L. monocytogenes</i>	4c	H. Seeliger	2.950	0.317	Positive
GT4800	<i>L. grayi</i>		GENE-TRAK	1.145	0.157	Positive

Table 1a. Inclusivity testing results from BLEB primary enrichment, continued. (1)

Strain #	Organism	Serotype (if known)	Source	OD*		Results
				1:10 Dilution**	1:100 Dilution**	
GT674	<i>L. grayi</i>		Inst. Pasteur	2.944	0.846	Positive
GT3881	<i>L. grayi</i> subsp. <i>murrayi</i>		GENE-TRAK	1.908	0.201	Positive
GT3785	<i>L. innocua</i>		CDC	0.993	0.162	Positive
GT3627	<i>L. innocua</i>	6a	H. Seeliger	2.240	0.209	Positive
GT3631	<i>L. innocua</i>	6a	H. Seeliger	2.950	0.540	Positive
GT1026	<i>L. innocua</i>	6b	H. Seeliger	1.444	0.141	Positive
GT1042	<i>L. innocua</i>	6b	H. Seeliger	2.355	0.246	Positive
GT1044	<i>L. innocua</i>	6b	H. Seeliger	2.950	0.285	Positive
A102	<i>L. innocua</i>	6a	ATCC 33090	2.950	0.287	Positive
GT1050	<i>L. innocua</i>	6b	H. Seeliger	2.049	0.166	Positive
GT1052	<i>L. innocua</i>		J. Farber	2.934	0.389	Positive
A140	<i>L. ivanovii</i>		ATCC 19119	2.551	0.282	Positive
GT1028	<i>L. ivanovii</i>	5	H. Seeliger	2.950	2.348	Positive
GT1040	<i>L. ivanovii</i>	5	H. Seeliger	2.950	0.849	Positive
GT3699	<i>L. ivanovii</i>	5	H. Seeliger	2.950	0.351	Positive
GT3794	<i>L. seeligeri</i>	1	J. Lovett	2.950	1.725	Positive
A201	<i>L. seeligeri</i>		ATCC 51334	2.950	2.943	Positive
GT3693	<i>L. seeligeri</i>	1/2b	H. Seeliger	2.950	2.943	Positive
GT289	<i>L. seeligeri</i>	4a	H. Seeliger	2.950	2.619	Positive
A199	<i>L. welshimeri</i>		ATCC 35897	2.045	0.945	Positive
A200	<i>L. welshimeri</i>		ATCC 43550	2.950	2.314	Positive
GT1773	<i>L. welshimeri</i>		Environmental isolate	2.950	1.479	Positive
GT1729	<i>L. welshimeri</i>		Dairy plant	2.950	2.943	Positive
GT293	<i>L. welshimeri</i>	6a	H. Seeliger	2.950	0.424	Positive

\* OD values  $\geq 0.10$  were considered positive.

\*\* Tested 1:10 and 1:100 dilutions of a PBS resuspension of growth from an OXA plate.

Table 1b. Inclusivity testing results from UVM primary enrichment. (1)

Strain #	Organism	Serotype (if known)	Source	OD*		Results
				1:10 Dilution***	1:100 Dilution**	
A169	<i>L. monocytogenes</i>	2	ATCC 19112	2.949	2.374	Positive
A207	<i>L. monocytogenes</i>	4b	ATCC 13932	2.949	2.948	Positive
A170	<i>L. monocytogenes</i>	4a	ATCC 19114	2.949	1.543	Positive
GT3829	<i>L. monocytogenes</i>	1a	C. Donnelly	2.949	1.815	Positive
GT1072	<i>L. monocytogenes</i>	1a	C. Donnelly	2.949	2.948	Positive
GT1880	<i>L. monocytogenes</i>	1a	J. Lovett	2.949	0.485	Positive
GT3812	<i>L. monocytogenes</i>	1a	J. Lovett	2.949	1.099	Positive
GT1021	<i>L. monocytogenes</i>	1/2a	CDC	2.949	0.521	Positive
GT3727	<i>L. monocytogenes</i>	1/2a	H. Seeliger	2.949	1.286	Positive
GT4340	<i>L. monocytogenes</i>	1/2a	CDC	2.949	2.379	Positive
GT1038	<i>L. monocytogenes</i>	1/2a	H. Seeliger	2.949	2.288	Positive
GT3635	<i>L. monocytogenes</i>	1/2b	H. Seeliger	2.949	0.775	Positive
GT3728	<i>L. monocytogenes</i>	1/2b	H. Seeliger	2.949	0.763	Positive
GT3856	<i>L. monocytogenes</i>	1/2b	H. Seeliger	2.949	0.629	Positive
GT3698	<i>L. monocytogenes</i>	1/2c	H. Seeliger	2.949	2.563	Positive
GT3648	<i>L. monocytogenes</i>	1/2c	H. Seeliger	2.949	1.178	Positive
GT3730	<i>L. monocytogenes</i>	1/2c	H. Seeliger	2.949	1.391	Positive
GT3741	<i>L. monocytogenes</i>	1/2c	H. Seeliger	2.949	2.948	Positive
GT3720	<i>L. monocytogenes</i>	3a	H. Seeliger	2.949	1.564	Positive
GT1035	<i>L. monocytogenes</i>	3a	H. Seeliger	2.949	2.478	Positive
GT1057	<i>L. monocytogenes</i>	3b	J. Lovett	2.949	0.769	Positive
GT3715	<i>L. monocytogenes</i>	3b	H. Seeliger	2.949	2.948	Positive
GT3817	<i>L. monocytogenes</i>	3b	H. Seeliger	2.949	0.733	Positive
GT3857	<i>L. monocytogenes</i>	3b	J. Lovett	2.949	0.817	Positive
GT1019	<i>L. monocytogenes</i>	4b	GENE-TRAK	2.949	2.948	Positive
GT1081	<i>L. monocytogenes</i>	4b	CDC	2.949	2.948	Positive
GT3819	<i>L. monocytogenes</i>	4c	H. Seeliger	2.949	1.689	Positive
GT4800	<i>L. grayi</i>		GENE-TRAK	2.949	0.404	Positive

Table 1b. Inclusivity testing results from UVM primary enrichment, continued. . (1)

Strain #	Organism	Serotype (if known)	Source	OD*		Results
				1:10 Dilution**	1:100 Dilution**	
GT674	<i>L. grayi</i>		Inst. Pasteur	2.949	1.044	Positive
GT3881	<i>L. grayi</i> subsp. <i>murrayi</i>		GENE-TRAK	0.418	0.133	Positive
GT3785	<i>L. innocua</i>		CDC	2.949	2.058	Positive
GT3627	<i>L. innocua</i>	6a	H. Seeliger	2.949	0.759	Positive
GT3631	<i>L. innocua</i>	6a	H. Seeliger	2.949	1.752	Positive
GT1026	<i>L. innocua</i>	6b	H. Seeliger	2.949	0.878	Positive
GT1042	<i>L. innocua</i>	6b	H. Seeliger	2.949	0.600	Positive
GT1044	<i>L. innocua</i>	6b	H. Seeliger	2.949	0.521	Positive
A102	<i>L. innocua</i>	6a	ATCC 33090	2.949	1.265	Positive
GT1050	<i>L. innocua</i>	6b	H. Seeliger	2.949	1.394	Positive
GT1052	<i>L. innocua</i>		J. Farber	2.949	1.189	Positive
A140	<i>L. ivanovii</i>		ATCC 19119	2.949	0.627	Positive
GT1028	<i>L. ivanovii</i>	5	H. Seeliger	2.949	0.354	Positive
GT1040	<i>L. ivanovii</i>	5	H. Seeliger	2.949	2.948	Positive
GT3699	<i>L. ivanovii</i>	5	H. Seeliger	2.949	0.712	Positive
GT3794	<i>L. seeligeri</i>	1	J. Lovett	2.949	2.363	Positive
A201	<i>L. seeligeri</i>		ATCC 51334	2.949	2.948	Positive
GT3693	<i>L. seeligeri</i>	1/2b	H. Seeliger	2.949	0.532	Positive
GT289	<i>L. seeligeri</i>	4a	H. Seeliger	2.949	2.948	Positive
A199	<i>L. welshimeri</i>		ATCC 35897	2.949	2.948	Positive
A200	<i>L. welshimeri</i>		ATCC 43550	2.949	2.948	Positive
GT1773	<i>L. welshimeri</i>		Environmental isolate	2.949	2.948	Positive
GT1729	<i>L. welshimeri</i>		Dairy plant	2.949	2.948	Positive
GT293	<i>L. welshimeri</i>	6a	H. Seeliger	2.949	2.948	Positive

\* OD values  $\geq 0.10$  were considered positive.

\*\* Tested 1:10 and 1:100 dilutions of a PBS resuspension of growth from an OXA plate.

Table 2. Exclusivity testing results. (1)

Strain #	Organism	Source	OD*	Results
GT803	<i>Bacillus brevis</i>	ATCC 8186	0.007	Negative
A208	<i>Bacillus cereus</i>	ATCC 25621	0.004	Negative
GT811	<i>Bacillus coagulans</i>	ATCC 7050	0.034	Negative
GT10	<i>Bacillus fragilis</i>	ATCC 23745	0.001	Negative
GT2128	<i>Bacillus megaterium</i>	ATCC 14581	0.005	Negative
GT804	<i>Bacillus subtilis</i>	ATCC 23059	0.002	Negative
GT4373	<i>Bacillus stearothermophilus</i>	ATCC 12980	0.010	Negative
GT664	<i>Brocothrix thermosphacta</i>	ATCC 11509	0.000	Negative
GT918	<i>Enterococcus durans</i>	ATCC 11576	0.019	Negative
GT406	<i>Enterococcus faecalis</i>	ATCC 19433	0.015	Negative
GT919	<i>Enterococcus faecium</i>	ATCC 6057	0.016	Negative
GT923	<i>Enterococcus hirae</i>	ATCC 35220	0.022	Negative
GT2129	<i>Kurthia gibsonii</i>	ATCC 43195	0.004	Negative
GT1941	<i>Kurthia zopfii</i>	ATCC 33403	0.002	Negative
GT256	<i>Lactobacillus acidophilus</i>	ATCC 4356	0.007	Negative
GT4082	<i>Lactobacillus buchneri</i>	ATCC 11305	0.006	Negative
GT805	<i>Lactobacillus casei</i>	ATCC 393	0.010	Negative
GT4063	<i>Lactobacillus fermentum</i>	ATCC 9338	0.019	Negative
GT3516	<i>Lactococcus lactis</i>	ATCC 11454	0.019	Negative
GT1943	<i>Micrococcus luteus</i>	ATCC 381	0.028	Negative
GT1944	<i>Micrococcus roseus</i>	ATCC 186	0.008	Negative
GT4404	<i>Micrococcus varians</i>	ATCC 15306	0.017	Negative
GT665	<i>Rodococcus equi</i>	ATCC 6939	0.029	Negative
GT3524	<i>Rodococcus fascians</i>	ATCC 12974	0.030	Negative
GT3474	<i>Rodococcus sputi</i>	ATCC 29627	0.015	Negative
A179	<i>Staphylococcus aureus</i>	ATCC 12600	0.020	Negative
A183	<i>Staphylococcus epidermidis</i>	ATCC 14990	0.016	Negative
A185	<i>Staphylococcus saprophyticus</i>	ATCC 15305	0.015	Negative
GT668	<i>Streptococcus bovis</i>	ATCC 9809	0.005	Negative
Strain #	Organism	Source	OD	Results
GT3596	<i>Streptococcus equi</i>	ATCC 33398	0.042	Negative
GT412	<i>Streptococcus mutans</i>	ATCC 25175	0.018	Negative
GT408	<i>Streptococcus pneumoniae</i>	ATCC 6303	0.013	Negative
GT411	<i>Streptococcus sanguis</i>	ATCC 10556	0.015	Negative

\* OD values &lt; 0.10 were considered negative



**Table 3. Results of inoculated food experiments. Comparison between the DNA method and the USDA FSIS method. . (1)**

Foods	Organism	No. Samples	MPN (CFU/g)	Total Positive <sup>a</sup>	Samples Positive			Chi Square <sup>d</sup>
					Assay <sup>b</sup>	Confirmed <sup>c</sup>	USDA FSIS	
Ground beef (Trial 1)	<i>L. monocytogenes</i> , sero 4b	20	11.00	20	20	20	20	NA
		20	0.04	18	18	18	18	NA
		10	0.00	2	1	1	2	0.00
		20	4.60	20	20	20	20	NA
Ground beef (Trial 2)	<i>L. monocytogenes</i> , sero 4b	20	0.28	18	13	13	18	3.20
		20	0.00	0	0	0	0	NA
Deli turkey (Trial 1)	<i>L. monocytogenes</i> , sero 1a	20	2.40	20	20	20	20	NA
		20	<0.03	5	5	5	5	NA
		10	0.00	0	0	0	0	NA
		20	0.38	20	20	20	20	NA
Deli turkey (Trial 2)	<i>L. monocytogenes</i> , sero 1a	20	0.23	14	14	14	14	NA
		10	0.00	0	0	0	0	NA
Hot dogs	<i>L. monocytogenes</i> , sero 4b	20	0.75	16	16	16	16	NA
		20	<0.03	5	5	5	5	NA
		10	0.00	0	0	0	0	NA
Ground pork (Trial 1)	<i>L. monocytogenes</i> , sero 1/2a	20	2.40	19	19	19	18	0.00
		20	<0.03	8	7	7	6	0.00
		10	0.00	1	1	1	1	NA

Foods	Organism	No. Samples	MPN (CFU/g)	Total Positive <sup>a</sup>	Assay <sup>b</sup>	Samples Positive Confirmed <sup>c</sup>	USDA FSIS	Chi Square <sup>d</sup>
		20	0.00	0	1	0	0	NA
Ground pork (Trial 2)	Naturally contaminated, uninoculated	20	0.23	18	16	16	18	0.50
		20	0.00	0	0	0	0	NA
Deli ham	<i>L. monocytogenes</i> , sero 1/2b	20	0.15	19	19	19	19	NA
		20	<0.03	2	2	2	2	NA
		10	0.00	0	0	0	0	NA
<b>Total:</b>		<b>420</b>	-	<b>225</b>	<b>217</b>	<b>216</b>	<b>222</b>	-

<sup>a</sup> Number of samples confirmed positive by one or more methods; <sup>b</sup> Number of samples positive by DNA hybridization assay not considering subsequent culture confirmation; <sup>c</sup> Number of samples positive by DNA hybridization assay and confirmed by plating from associated cultures; <sup>d</sup> Chi square value by McNemar's test comparing confirmed DNA hybridization assay and reference culture method. Chi square values  $\geq 3.84$  indicates a statistically significant difference at  $P \leq 0.05$ .

Table 4. Results of inoculated food experiments. Comparison between the DNA method and the FDA (BAM) method. . (1)

Foods	Organism	No. Samples	MPN (CFU/g)	Total Positive <sup>a</sup>	Samples Positive			Chi Square <sup>e</sup>	
					Assay <sup>b</sup>	Confirmed <sup>c</sup>	FDA Method		
Parmesan	<i>L. monocytogenes</i> , sero 3b	20	0.93	20	19	19	17	0.25	
		20	0.23	14	14	14	12	0.50	
		10	0.00	0	0	0	0	NA	
Brie cheese	<i>L. monocytogenes</i> , sero 1/2a	20	0.04	20	20	20	14	4.17	
		20	0.06	19	19	19	18	0.00	
		10	0.00	0	0	0	0	NA	
Pasteurized milk	<i>L. monocytogenes</i> , sero 1/2a	20	0.23	19	19	19	19	1.33	
		20	0.03	1	2	1	1	0.00	
		10	0.00	0	1	0	0	NA	
Ice cream	<i>L. monocytogenes</i> , sero 4c	20	0.40	13	13	13	13	NA	
		20	<0.03	0	2	0	0	NA	
		10	0.00	0	0	0	0	NA	
Raw shrimp	<i>L. monocytogenes</i> , sero 1/2c	20	0.04	9	8	8	5	0.80	
		20	<0.03	0	0	0	0	NA	
		10	0.00	0	0	0	0	NA	
Foods	Organism	No. Samples	MPN (CFU/g)	Total Positive <sup>a</sup>	Samples Positive			Chi Square <sup>d</sup>	
Pasteurized crab meat	<i>L. monocytogenes</i> , sero 1/2c	20	0.75	20	20	20	20	20	NA
		20	0.04	18	18	18	18	18	NA
		10	0.00	0	0	0	0	0	NA
Smoked salmon	<i>L. monocytogenes</i> , sero 1/2c	20	2.40	20	20	20	20	20	NA
		20	0.23	18	15	15	18	1.33	
		10	0.00	0	0	0	0	0	NA
Lettuce	<i>L. ivanovii</i>	20	0.43	20	20	20	20	20	NA
		20	0.15	19	19	19	19	19	NA
		10	0.00	0	0	0	0	0	NA
Mixed vegetables	<i>L. seeligeri</i> , sero 1 ( <i>L. monocytogenes</i> , natural contaminant)	20	0.09	20	20	20	20	20	NA
		20	0.75	18	18	18	15	1.33	
		10	0.43	9	9	9	8	0.00	
Alfalfa sprouts	<i>L. monocytogenes</i> , sero 3a	20	11.00	20	20	20	19	0.00	
		20	0.38	15	14	14	11	0.80	
		10	0.00	0	0	0	0	NA	
<b>Total:</b>		<b>500</b>	-	<b>312</b>	<b>310</b>	<b>306</b>	<b>288</b>	-	

<sup>a</sup> Number of samples confirmed positive by one or more methods; <sup>b</sup> Number of samples positive by DNA hybridization assay not considering subsequent culture confirmation; <sup>c</sup> Number of samples positive by DNA hybridization assay and confirmed by plating from associated cultures; <sup>d</sup> Chi square value by McNemar's test comparing confirmed DNA hybridization assay and reference culture method. Chi square values  $\geq 3.84$  indicates a statistically significant difference at  $P \leq 0.05$

**DISCUSSION OF MODIFICATION APPROVED NOVEMBER 2005 (4)**

Results of the 14-food trial clearly show that the microwell format assay is capable of detecting low levels of *Listeria* spp. in a variety of foods. The assay has also proved to be very specific, with no cross-reactions observed in exclusivity testing and no false positive results produced in food testing.

In internal trials, the sensitivity of the DNA hybridization method was 95.6% compared to a combined sensitivity of 87.1% for the FDA and USDA-FSIS culture methods. The specificity of the DNA hybridization assay was 100%.

In comparing the DNA hybridization method and the reference culture methods for the ability to detect *Listeria* spp. in foods, there were no significant differences in detection for any of the foods tested, with the exception of the low-level ice cream samples and both the low and high-level parmesan cheese samples. For these three data sets, the DNA hybridization method produced significantly more positives than the FDA culture method, as determined by chi-square analysis. As discussed previously, the FDA plates showed a heavy load of background flora, presumably hampering the recovery of listeriae from some samples. The DNA hybridization method uses a somewhat different secondary enrichment and confirmation scheme, which may have been advantageous. In addition, a positive signal by the DNA hybridization assay is dependent only on the final titer of *Listeria* spp. in the secondary enrichment, and not the ratio of listeriae to competitors.

Results of the independent laboratory study are consistent with those of the internal trials. Comparable performance between the DNA hybridization and reference culture methods was demonstrated for all 3 foods tested. There were a few unconfirmed positive assay results, but most of these occurred on inoculated samples and thus confirmation failure is a plausible alternative explanation.

The trial with the ceramic tile environmental surface yielded more positives by the DNA hybridization method in comparison to the USDA-FSIS culture procedure. This was also found to be the case with 5 of the 6 surfaces tested in the internal validation study. It appears that the LESS broth enrichment protocol used with the hybridization method is more productive than the UVM enrichment procedure used in the USDA-FSIS method in recovering *Listeria* spp. from environmental surfaces with this experimental model.

Strain #	Organism	Serotype (if known)	Source	Origin (if known)	OD*		Result
					1:10 Dilution **	1:100 Dilution **	
GT4800	<i>L. grayi</i>	-	GENE-TRAK	Environmental	1.853	0.194	Positive
A203	<i>L. grayi</i>	-	ATCC 19120	Chinchilla feces	2.032	0.280	Positive
GT3881	<i>L. grayi</i> subsp. <i>murrayi</i>	-	GENE-TRAK	-	1.800	0.218	Positive
GT3627	<i>L. innocua</i>	6a	H. Seeliger	Cheese	2.985	0.785	Positive
GT3631	<i>L. innocua</i>	6a	H. Seeliger	Cheese	2.985	0.621	Positive
A102	<i>L. innocua</i>	6a	ATCC 33090	Cow brain	2.985	0.670	Positive
GT1026	<i>L. innocua</i>	6b	H. Seeliger	Cheese	2.985	0.938	Positive
GT1042	<i>L. innocua</i>	6b	H. Seeliger	Cheese	1.843	0.265	Positive
GT1044	<i>L. innocua</i>	6b	H. Seeliger	Cheese	2.608	0.341	Positive
GT1050	<i>L. innocua</i>	6b	H. Seeliger	Cheese	2.985	1.346	Positive
GT3785	<i>L. innocua</i>	-	CDC	-	2.103	0.323	Positive
GT1052	<i>L. innocua</i>	-	J. Farber	Raw milk	2.985	0.573	Positive
GT1028	<i>L. ivanovii</i>	5	H. Seeliger	Mouse	2.985	2.223	Positive
GT1040	<i>L. ivanovii</i>	5	H. Seeliger	Human	2.985	1.980	Positive
GT3699	<i>L. ivanovii</i>	5	H. Seeliger	Watercress	2.985	1.786	Positive
A140	<i>L. ivanovii</i>	-	ATCC 19119	Sheep	2.985	2.257	Positive
GT1021	<i>L. monocytogenes</i>	1/2a	CDC	Human	2.985	0.511	Positive
GT3727	<i>L. monocytogenes</i>	1/2a	H. Seeliger	Human blood	2.985	0.461	Positive
GT4340	<i>L. monocytogenes</i>	1/2a	CDC	Fish	2.985	0.582	Positive
GT1038	<i>L. monocytogenes</i>	1/2a	H. Seeliger	Human blood	2.985	1.311	Positive
GT3635	<i>L. monocytogenes</i>	1/2b	H. Seeliger	Human blood	2.985	0.719	Positive
GT3728	<i>L. monocytogenes</i>	1/2b	H. Seeliger	Cheese	2.985	0.560	Positive
GT3856	<i>L. monocytogenes</i>	1/2b	H. Seeliger	Cheese	2.985	1.853	Positive
GT3698	<i>L. monocytogenes</i>	1/2c	H. Seeliger	Cheese	2.985	0.676	Positive
GT3648	<i>L. monocytogenes</i>	1/2c	H. Seeliger	Cheese	2.985	0.574	Positive
GT3730	<i>L. monocytogenes</i>	1/2c	H. Seeliger	-	2.985	1.098	Positive
GT3741	<i>L. monocytogenes</i>	1/2c	H. Seeliger	-	2.985	1.135	Positive
GT3829	<i>L. monocytogenes</i>	1a	C. Donnelly	Raw milk	2.985	0.807	Positive
GT1072	<i>L. monocytogenes</i>	1a	C. Donnelly	Raw milk	2.985	1.152	Positive
GT1880	<i>L. monocytogenes</i>	1a	J. Lovett	Brie cheese	2.985	1.790	Positive
GT3812	<i>L. monocytogenes</i>	1a	J. Lovett	Chocolate milk	2.985	1.330	Positive
A169	<i>L. monocytogenes</i>	2	ATCC 19112	Human CSF	2.985	0.960	Positive
GT3720	<i>L. monocytogenes</i>	3a	H. Seeliger	Cheese	2.985	0.662	Positive
GT1035	<i>L. monocytogenes</i>	3a	H. Seeliger	-	2.985	0.734	Positive
GT1057	<i>L. monocytogenes</i>	3b	J. Lovett	Brie cheese	2.438	0.333	Positive
GT3715	<i>L. monocytogenes</i>	3b	H. Seeliger	Human blood	1.931	0.269	Positive
GT3817	<i>L. monocytogenes</i>	3b	H. Seeliger	Cheese	2.521	0.297	Positive

GT3857	<i>L. monocytogenes</i>	3b	J. Lovett	Brie cheese	2.985	0.469	Positive
A170	<i>L. monocytogenes</i>	4a	ATCC 19114	Ruminant brain	2.985	1.176	Positive
A207	<i>L. monocytogenes</i>	4b	ATCC 13932	Human CSF	2.985	0.724	Positive
GT1019	<i>L. monocytogenes</i>	4b	GENE-TRAK	-	2.985	0.785	Positive
GT1081	<i>L. monocytogenes</i>	4b	CDC	-	2.283	0.343	Positive
GT3819	<i>L. monocytogenes</i>	4c	H. Seeliger	Human	2.580	0.275	Positive
GT3794	<i>L. seeligeri</i>	1	J. Lovett	Brie cheese	2.985	0.875	Positive
GT3693	<i>L. seeligeri</i>	1/2b	H. Seeliger	Sewage	2.985	2.097	Positive
GT289	<i>L. seeligeri</i>	4a	H. Seeliger	Cheese	2.985	1.716	Positive
A201	<i>L. seeligeri</i>	-	ATCC 51334	Vole	2.985	2.504	Positive
GT293	<i>L. welshimeri</i>	6a	H. Seeliger	Cheese	2.985	1.504	Positive
A199	<i>L. welshimeri</i>	-	ATCC 35897	Decaying plant matter	2.985	0.980	Positive
A200	<i>L. welshimeri</i>	-	ATCC 43550	Soil	2.985	2.130	Positive
GT1773	<i>L. welshimeri</i>	-	-	Environmental isolate	2.985	1.609	Positive
GT1729	<i>L. welshimeri</i>	-	-	Dairy plant	2.985	1.308	Positive

\* OD values > 0.10 were considered positive.

\*\* Tested 1:10 and 1:100 dilutions of the secondary enrichment culture.

**Table 1b. *Listeria* spp. inclusivity testing results from UVM to PALCAM enrichment.(4)**

Strain #	Organism	Serotype (if known)	Source	Origin (if known)	OD*		Result
					1:10 Dilution**	1:100 Dilution**	
GT4800	<i>L. grayi</i>	-	GENE-TRAK	Environmental	0.693	0.133	Positive
A203	<i>L. grayi</i>	-	ATCC 19120	Chinchilla feces	1.116	0.191	Positive
GT3881	<i>L. grayi</i> subsp. <i>murrayi</i>	-	GENE-TRAK	-	0.329	0.081	Pos/Neg
GT3627	<i>L. innocua</i>	6a	H. Seeliger	Cheese	1.654	0.299	Positive
GT3631	<i>L. innocua</i>	6a	H. Seeliger	Cheese	2.249	0.325	Positive
A102	<i>L. innocua</i>	6a	ATCC 33090	Cow brain	2.193	0.300	Positive
GT1026	<i>L. innocua</i>	6b	H. Seeliger	Cheese	0.548	0.103	Positive
GT1042	<i>L. innocua</i>	6b	H. Seeliger	Cheese	0.331	0.075	Pos/Neg
GT1044	<i>L. innocua</i>	6b	H. Seeliger	Cheese	0.666	0.112	Positive
GT1050	<i>L. innocua</i>	6b	H. Seeliger	Cheese	2.888	0.410	Positive
GT3785	<i>L. innocua</i>	-	CDC	-	0.632	0.110	Positive
GT1052	<i>L. innocua</i>	-	J. Farber	Raw milk	1.566	0.257	Positive
GT1028	<i>L. ivanovii</i>	5	H. Seeliger	Mouse	2.140	0.335	Positive
GT1040	<i>L. ivanovii</i>	5	H. Seeliger	Human	1.037	0.160	Positive
GT3699	<i>L. ivanovii</i>	5	H. Seeliger	Watercress	1.250	0.233	Positive
A140	<i>L. ivanovii</i>	-	ATCC 19119	Sheep	1.060	0.163	Positive
GT1021	<i>L. monocytogenes</i>	1/2a	CDC	Human	1.745	0.636	Positive
GT3727	<i>L. monocytogenes</i>	1/2a	H. Seeliger	Human blood	0.756	0.124	Positive
GT4340	<i>L. monocytogenes</i>	1/2a	CDC	Fish	0.226	0.072	Pos/Neg
GT1038	<i>L. monocytogenes</i>	1/2a	H. Seeliger	Human blood	1.507	0.227	Positive
GT3635	<i>L. monocytogenes</i>	1/2b	H. Seeliger	Human blood	1.376	0.226	Positive
GT3728	<i>L. monocytogenes</i>	1/2b	H. Seeliger	Cheese	0.650	0.152	Positive
GT3856	<i>L. monocytogenes</i>	1/2b	H. Seeliger	Cheese	1.955	0.271	Positive
GT3698	<i>L. monocytogenes</i>	1/2c	H. Seeliger	Cheese	0.596	0.123	Positive
GT3648	<i>L. monocytogenes</i>	1/2c	H. Seeliger	Cheese	0.649	0.115	Positive
GT3730	<i>L. monocytogenes</i>	1/2c	H. Seeliger	-	1.886	0.260	Positive
GT3741	<i>L. monocytogenes</i>	1/2c	H. Seeliger	-	2.358	0.324	Positive
GT3829	<i>L. monocytogenes</i>	1a	C. Donnelly	Raw milk	0.966	0.158	Positive
GT1072	<i>L. monocytogenes</i>	1a	C. Donnelly	Raw milk	0.846	0.157	Positive
GT1880	<i>L. monocytogenes</i>	1a	J. Lovett	Brie cheese	2.990	0.516	Positive
GT3812	<i>L. monocytogenes</i>	1a	J. Lovett	Chocolate milk	0.493	0.078	Pos/Neg
A169	<i>L. monocytogenes</i>	2	ATCC 19112	Human CSF	0.886	0.147	Positive
GT3720	<i>L. monocytogenes</i>	3a	H. Seeliger	Cheese	0.261	0.071	Pos/Neg
GT1035	<i>L. monocytogenes</i>	3a	H. Seeliger	-	1.348	0.218	Positive
GT1057	<i>L. monocytogenes</i>	3b	J. Lovett	Brie cheese	1.619	0.234	Positive
GT3715	<i>L. monocytogenes</i>	3b	H. Seeliger	Human blood	0.779	0.157	Positive
GT3817	<i>L. monocytogenes</i>	3b	H. Seeliger	Cheese	0.442	0.087	Pos/Neg
GT3857	<i>L. monocytogenes</i>	3b	J. Lovett	Brie cheese	1.624	0.244	Positive
A170	<i>L. monocytogenes</i>	4a	ATCC 19114	Ruminant brain	2.331	0.231	Positive
A207	<i>L. monocytogenes</i>	4b	ATCC 13932	Human CSF	0.968	0.151	Positive
GT1019	<i>L. monocytogenes</i>	4b	GENE-TRAK	-	0.644	0.114	Positive
GT1081	<i>L. monocytogenes</i>	4b	CDC	-	0.436	0.090	Pos/Neg

GT3819	<i>L. monocytogenes</i>	4c	H. Seeliger	Human	0.554	0.100	Positive
GT3794	<i>L. seeligeri</i>	1	J. Lovett	Brie cheese	2.990	0.672	Positive
GT3693	<i>L. seeligeri</i>	1/2b	H. Seeliger	Sewage	2.990	1.850	Positive
GT289	<i>L. seeligeri</i>	4a	H. Seeliger	Cheese	2.990	1.481	Positive
A201	<i>L. seeligeri</i>	-	ATCC 51334	Vole	2.990	1.259	Positive
GT293	<i>L. welshimeri</i>	6a	H. Seeliger	Cheese	2.990	0.438	Positive
A199	<i>L. welshimeri</i>	-	ATCC 35897	Decaying plant matter	0.654	0.097	Pos/Neg
A200	<i>L. welshimeri</i>	-	ATCC 43550	Soil	2.990	0.678	Positive
GT1773	<i>L. welshimeri</i>	-	-	Environmental isolate	0.577	0.091	Pos/Neg
GT1729	<i>L. welshimeri</i>	-	-	Dairy plant	2.990	0.867	Positive

\* OD values > 0.10 were considered positive.

\*\* Tested 1:10 and 1:100 dilutions of the secondary enrichment culture.

**Table 1c. *Listeria* spp. inclusivity testing results from LESS broth enrichment (4)**

Strain #	Organism	Serotype (if known)	Source	Origin (if known)	OD*		Result
					1:10 Dilution**	1:100 Dilution**	
GT4800	<i>L. grayi</i>	-	GENE-TRAK	Environmental	2.951	0.459	Positive
A203	<i>L. grayi</i>	-	ATCC 19120	Chinchilla feces	2.951	0.608	Positive
GT3881	<i>L. grayi</i> subsp. <i>murrayi</i>	-	GENE-TRAK	-	2.951	0.928	Positive
GT3627	<i>L. innocua</i>	6a	H. Seeliger	Cheese	2.951	0.574	Positive
GT3631	<i>L. innocua</i>	6a	H. Seeliger	Cheese	2.218	0.192	Positive
A102	<i>L. innocua</i>	6a	ATCC 33090	Cow brain	2.951	0.491	Positive
GT1026	<i>L. innocua</i>	6b	H. Seeliger	Cheese	2.951	1.028	Positive
GT1042	<i>L. innocua</i>	6b	H. Seeliger	Cheese	2.951	0.489	Positive
GT1044	<i>L. innocua</i>	6b	H. Seeliger	Cheese	2.951	2.401	Positive
GT1050	<i>L. innocua</i>	6b	H. Seeliger	Cheese	2.951	1.929	Positive
GT3785	<i>L. innocua</i>	-	CDC	-	2.951	0.540	Positive
GT1052	<i>L. innocua</i>	-	J. Farber	Raw milk	2.951	2.514	Positive
GT1028	<i>L. ivanovii</i>	5	H. Seeliger	Mouse	2.951	2.951	Positive
GT1040	<i>L. ivanovii</i>	5	H. Seeliger	Human	2.951	2.951	Positive
GT3699	<i>L. ivanovii</i>	5	H. Seeliger	Watercress	2.951	2.951	Positive
A140	<i>L. ivanovii</i>	-	ATCC 19119	Sheep	2.951	2.951	Positive
GT1021	<i>L. monocytogenes</i>	1/2a	CDC	Human	2.951	2.951	Positive
GT3727	<i>L. monocytogenes</i>	1/2a	H. Seeliger	Human blood	2.951	2.951	Positive
GT4340	<i>L. monocytogenes</i>	1/2a	CDC	Fish	2.951	2.951	Positive
GT1038	<i>L. monocytogenes</i>	1/2a	H. Seeliger	Human blood	2.951	2.951	Positive
GT3635	<i>L. monocytogenes</i>	1/2b	H. Seeliger	Human blood	2.951	2.247	Positive
GT3728	<i>L. monocytogenes</i>	1/2b	H. Seeliger	Cheese	2.951	1.362	Positive
GT3856	<i>L. monocytogenes</i>	1/2b	H. Seeliger	Cheese	2.951	2.413	Positive
GT3698	<i>L. monocytogenes</i>	1/2c	H. Seeliger	Cheese	2.951	2.951	Positive
GT3648	<i>L. monocytogenes</i>	1/2c	H. Seeliger	Cheese	2.951	1.275	Positive
GT3730	<i>L. monocytogenes</i>	1/2c	H. Seeliger	-	2.951	2.951	Positive
GT3741	<i>L. monocytogenes</i>	1/2c	H. Seeliger	-	2.951	2.951	Positive
GT3829	<i>L. monocytogenes</i>	1a	C. Donnelly	Raw milk	2.951	2.951	Positive
GT1072	<i>L. monocytogenes</i>	1a	C. Donnelly	Raw milk	2.951	2.951	Positive
GT1880	<i>L. monocytogenes</i>	1a	J. Lovett	Brie cheese	2.951	2.951	Positive
GT3812	<i>L. monocytogenes</i>	1a	J. Lovett	Chocolate milk	2.951	2.951	Positive
A169	<i>L. monocytogenes</i>	2	ATCC 19112	Human CSF	2.951	0.528	Positive
GT3720	<i>L. monocytogenes</i>	3a	H. Seeliger	Cheese	2.951	0.641	Positive
GT1035	<i>L. monocytogenes</i>	3a	H. Seeliger	-	2.951	2.875	Positive
GT1057	<i>L. monocytogenes</i>	3b	J. Lovett	Brie cheese	2.951	1.893	Positive
GT3715	<i>L. monocytogenes</i>	3b	H. Seeliger	Human blood	2.951	1.201	Positive
GT3817	<i>L. monocytogenes</i>	3b	H. Seeliger	Cheese	2.951	2.951	Positive
GT3857	<i>L. monocytogenes</i>	3b	J. Lovett	Brie cheese	2.951	2.880	Positive
A170	<i>L. monocytogenes</i>	4a	ATCC 19114	Ruminant brain	2.951	2.951	Positive
A207	<i>L. monocytogenes</i>	4b	ATCC 13932	Human CSF	2.951	2.951	Positive
GT1019	<i>L. monocytogenes</i>	4b	GENE-TRAK	-	2.951	2.951	Positive
GT1081	<i>L. monocytogenes</i>	4b	CDC	-	2.951	2.280	Positive
GT3819	<i>L. monocytogenes</i>	4c	H. Seeliger	Human	2.951	2.510	Positive
GT3794	<i>L. seeligeri</i>	1	J. Lovett	Brie cheese	2.951	1.878	Positive
GT3693	<i>L. seeligeri</i>	1/2b	H. Seeliger	Sewage	2.951	2.951	Positive
GT289	<i>L. seeligeri</i>	4a	H. Seeliger	Cheese	2.951	2.951	Positive
A201	<i>L. seeligeri</i>	-	ATCC 51334	Vole	2.951	2.951	Positive

GT293	<i>L. welshimeri</i>	6a	H. Seeliger	Cheese	2.951	2.472	Positive
A199	<i>L. welshimeri</i>	-	ATCC 35897	Decaying plant matter	2.951	2.160	Positive
A200	<i>L. welshimeri</i>	-	ATCC 43550	Soil	2.951	2.951	Positive
GT1773	<i>L. welshimeri</i>	-	-	Environmental isolate	2.951	2.951	Positive
GT1729	<i>L. welshimeri</i>	-	-	Dairy plant	2.951	2.951	Positive

\* OD values > 0.10 were considered positive.

\*\* Tested 1:10 and 1:100 dilutions of the secondary enrichment culture.

**Table 2. Exclusivity testing results. (4)**

Strain #	Organism	Source	Origin (if known)	OD*	Result
A208	<i>Bacillus cereus</i>	ATCC 25621	Cow dung	0.004	Negative
GT811	<i>Bacillus coagulans</i>	ATCC 7050	Milk	0.034	Negative
GT2128	<i>Bacillus megaterium</i>	ATCC 14581	-	0.005	Negative
GT804	<i>Bacillus subtilis</i>	ATCC 23059	Soil	0.002	Negative
GT803	<i>Brevibacillus parabrevis</i>	ATCC 8186	Cheese	0.007	Negative
GT664	<i>Brocothrix thermosphacta</i>	ATCC 11509	Pork sausage	0.000	Negative
GT918	<i>Enterococcus durans</i>	ATCC 11576	Cheese	0.019	Negative
GT406	<i>Enterococcus faecalis</i>	ATCC 19433	-	0.015	Negative
GT919	<i>Enterococcus faecium</i>	ATCC 6057	Cheese	0.016	Negative
GT923	<i>Enterococcus hirae</i>	ATCC 35220	Cow dung	0.022	Negative
GT4373	<i>Geobacillus stearothermophilus</i>	ATCC 12980	-	0.010	Negative
GT3474	<i>Gordonia sputi</i>	ATCC 29627	Human	0.015	Negative
GT1944	<i>Kocuria rosea</i>	ATCC 185	-	0.008	Negative
GT4404	<i>Kocuria varians</i>	ATCC 15306	Milk	0.017	Negative
GT2129	<i>Kurthia gibsonii</i>	ATCC 43195	Meat	0.004	Negative
GT1941	<i>Kurthia zopfii</i>	ATCC 33403	Turkey caecum	0.002	Negative
GT256	<i>Lactobacillus acidophilus</i>	ATCC 4356	Human	0.007	Negative
GT4082	<i>Lactobacillus buchneri</i>	ATCC 11307	Beer	0.006	Negative
GT805	<i>Lactobacillus casei</i>	ATCC 393	Cheese	0.010	Negative
GT4063	<i>Lactobacillus fermentum</i>	ATCC 9338	-	0.019	Negative
GT3516	<i>Lactococcus lactis</i>	ATCC 11454	-	0.019	Negative
GT1943	<i>Micrococcus luteus</i>	ATCC 381	Water	0.028	Negative
GT665	<i>Rhodococcus equi</i>	ATCC 6939	Horse	0.029	Negative
GT3524	<i>Rhodococcus fascians</i>	ATCC 12974	-	0.030	Negative
A179	<i>Staphylococcus aureus</i>	ATCC 12600	Human pleural fluid	0.020	Negative
A183	<i>Staphylococcus epidermidis</i>	ATCC 14990	Human	0.016	Negative
A185	<i>Staphylococcus saprophyticus</i>	ATCC 15305	Human urine	0.015	Negative
GT3596	<i>Streptococcus equi</i>	ATCC 33398	-	0.042	Negative
GT668	<i>Streptococcus gallolyticus</i>	ATCC 9809	-	0.005	Negative
GT412	<i>Streptococcus mutans</i>	ATCC 25175	Human mouth	0.018	Negative
GT408	<i>Streptococcus pneumoniae</i>	ATCC 6303	-	0.013	Negative
GT411	<i>Streptococcus sanguinis</i>	ATCC 10556	Human	0.015	Negative

\* OD values < 0.10 were considered negative.

**Table 3. Results of inoculated food experiments. Comparison between the DNA hybridization method and the USDA-FSIS or FDA methods (4)**

Foods	Organism	No. Samples	MPN (CFU/g)	Total Positive <sup>a</sup>	Samples Positive				Chi Square <sup>d</sup>
					DNAH Assay <sup>b</sup>	DNAH Confirmed <sup>c</sup>	USDA-FSIS	FDA	Chi Square <sup>d</sup>
Deli Turkey Trial 1	<i>L. monocytogenes</i> , sero 1a	20	1.49	20	20	20	20		-
		20	< 0.03	2	1	1	1		-
		10	0.00	0	0	0	0		-
Deli Turkey Trial 2	<i>L. monocytogenes</i> , sero 1a	20	0.43	20	20	20	20		-

		20	0.092	14	14	14	14		-
		10	0.00	0	0	0	0		-
Deli Ham	<i>L. monocytogenes</i> , sero 4b	20	0.93	20	20	20	20		-
		20	0.23	19	19	19	19		-
		10	0.00	0	0	0	0		-
Hot Dogs	<i>L. monocytogenes</i> , sero 4b	20	0.23	20	20	20	20		-
		20	0.23	17	17	17	17		-
		10	0.00	0	0	0	0		-
Raw Ground Beef	<i>L. monocytogenes</i> , sero 4b	20	4.6	20	20	20	20		-
		20	0.28	20	17	17	18		0.00
		20	0.00	0	0	0	0		-
Raw Frozen Ground Pork	<i>L. monocytogenes</i> , sero 1/2a	20	0.75	19	16	16	19		1.33
		20	<0.03	6	5	5	6		0.00
		20	0.00	0	0	0	0		-
Smoked Salmon	<i>L. monocytogenes</i> , sero 1/2c	20	0.092	15	15	15		15	-
		20	<0.03	7	7	7		7	-
		10	0.00	0	0	0		0	-
Cooked Crab Meat	<i>L. monocytogenes</i> , sero 1/2b	20	<0.03	9	9	9		8	0.00
		20	<0.03	1	1	1		1	-
		10	0.00	0	0	0		0	-
Ice Cream	<i>L. monocytogenes</i> , sero 4c	20	0.93	20	20	20		20	-
		20	0.092	17	17	17		9	6.13
		10	0.00	0	0	0		0	-
Pasteurized Milk	<i>L. monocytogenes</i> , sero 1a	20	0.93	20	20	20		20	-
		20	<0.03	8	8	8		6	0.50
		10	0.00	0	0	0		0	-
Brie Cheese	<i>L. monocytogenes</i> , sero 1/2a	20	1.49	19	19	19		18	0.00
		20	<0.03	10	8	8		6	0.17
		10	0.00	0	0	0		0	-
Parmesan Cheese	<i>L. monocytogenes</i> , sero 3c	20	0.43	20	20	20		8	10.08
		20	0.092	18	18	18		3	13.07
		10	0.00	0	0	0		0	-
						<b>Samples Positive</b>			

Foods	Organism	No. Samples	MPN (CFU/g)	Total Positive <sup>a</sup>	DNAH Assay <sup>b</sup>	DNAH Confirmed <sup>c</sup>	USDA-FSIS	FDA	Chi Square <sup>d</sup>
Lettuce	<i>L. monocytogenes</i> , sero 4b	20	0.75	20	20	20		20	-
		20	0.21	15	15	15		15	-
		20	0.00	0	0	0		0	-
Frozen Peas	<i>L. monocytogenes</i> , sero 6b	20	0.75	19	16	16		13	0.44
		20	< 0.03	18	12	12		13	0.00
		10	0.00	0	0	0		0	-
Soy Flour	<i>L. monocytogenes</i> , sero 4a	20	0.092	12	11	11		11	0.00
		20	0.036	5	5	5		5	-
		10	0.00	0	0	0		0	-
<b>Total:</b>		<b>780</b>		<b>450</b>	<b>430</b>	<b>430</b>	<b>194</b>	<b>198</b>	

<sup>a</sup> Number of samples confirmed positive by one or more methods; <sup>b</sup> Number of samples positive by DNA hybridization assay not considering subsequent culture confirmation; <sup>c</sup> Number of samples positive by DNA hybridization assay and confirmed by plating from associated cultures; <sup>d</sup> Chi square value by McNemar's test comparing confirmed DNA hybridization assay and reference culture method.  $\chi^2 \geq 3.84$  indicates a statistically significant difference at  $p \leq 0.05$ .

**Table 4. Results of environmental surface experiments. (4)**

Surface	Sample	Inoc Strain	Initial Inoc Level <sup>a</sup>	# Samples	DNAH Assay <sup>b</sup>	DNAH Conf <sup>c</sup>	USDA-FSIS	Chi Square <sup>d</sup>
Stainless Steel	Swab	<i>L. mono.1/2c</i>	$2.9 \times 10^4$	20	20	20	2	29.2
				5	0	0	0	-
Plastic	Swab	<i>L. mono. 4b</i>	$6.9 \times 10^4$	20	14	14	2	12.6
				5	0	0	0	-
Cast Iron	Swab	<i>L. innocua</i>	$4.2 \times 10^3$	20	16	16	7	6.55
				5	0	0	0	-
Ceramic Tile	Sponge	<i>L. mono. 1a</i> <i>10x S. aureus</i>	$3.0 \times 10^3$ $1.5 \times 10^4$	20	17	17	13	1.20
				5	0	0	0	-
Sealed Concrete Trial 1	Sponge	<i>L. welshimeri</i> + cocktail <sup>e</sup>	$6.2 \times 10^5$ $2.7 \times 10^7$	20	6	6	11	1.64
				5	0	0	0	-
Sealed Concrete Trial 2	Sponge	<i>L. welshimeri</i> + cocktail <sup>e</sup>	$1.3 \times 10^4$ $9.2 \times 10^4$	20	17	17	18	0.00
				5	0	0	0	-
Painted Wood	Sponge	<i>L. mono. 1/2b</i>	$1.2 \times 10^2$	20	20	20	9	12.5
				5	0	0	0	-
<b>Total:</b>				<b>175</b>	<b>110</b>	<b>110</b>	<b>62</b>	

<sup>a</sup> Inoculation level in cfu/surface; <sup>b</sup> Number of samples positive by DNA hybridization assay not considering subsequent culture confirmation; <sup>c</sup> Number of samples positive by DNA hybridization assay and confirmed by plating from associated cultures; <sup>d</sup> Chi square value by independent chi square test comparing confirmed DNA hybridization assay and reference culture method.  $\chi^2 \geq 3.84$  indicates a statistically significant difference at  $p \leq 0.05$ ; <sup>e</sup> Competitor cocktail of *Staphylococcus aureus*, *Enterococcus faecalis* and *Bacillus licheniformis*.



Table 5. Results of independent laboratory testing. (4)

Foods	Organism	No. Samples	MPN (CFU/g)	Total Positive <sup>a</sup>	Samples Positive				Chi Square <sup>d</sup>
					DNAH Assay <sup>b</sup>	DNAH Confirmed <sup>c</sup>	USDA-FSIS	FDA	Chi Square <sup>d</sup>
Cottage Cheese	<i>L. welshimeri</i>	20	0.46	20	19	19		20	0.00
		20	0.064	12	12	12		12	-
		5	< 0.003	0	0	0		0	-
Cabbage	<i>L. innocua</i>	20	0.043	10	10	10		10	-
		20	0.0092	7	8	6		7	0.00
		5	< 0.003	0	0	0		0	-
Smoked Salmon	<i>L. monocytogenes</i> sero. 1/2c	20	0.15	20	20	20		20	-
		20	0.092	18	19	18		18	-
		5	< 0.003	0	1	0		0	-
Ceramic Tile	<i>L. monocytogenes</i> sero. 1a (+ 10x <i>S. aureus</i> )	20	4.5 x 10 <sup>3</sup> <sup>e</sup> (1.0 x 10 <sup>5</sup> )	f	14	13	2		10.7
		20	1.8 x 10 <sup>3</sup> <sup>e</sup> (4.2 x 10 <sup>4</sup> )	f	13	13	7		2.50
		5	-	f	1	0	0		-

<sup>a</sup> Number of samples confirmed positive by one or more methods; <sup>b</sup> Number of samples positive by DNA hybridization assay not considering subsequent culture confirmation; <sup>c</sup> Number of samples positive by DNA hybridization assay and confirmed by plating from associated cultures; <sup>d</sup> Chi square value by McNemar's test comparing confirmed DNA hybridization assay and reference culture method (independent chi square test used for ceramic tile data).  $\chi^2 \geq 3.84$  indicates a statistically significant difference at  $p \leq 0.05$ ;

<sup>e</sup> Initial inoculation level in cfu/surface; <sup>f</sup> Not applicable, unpaired samples.

#### DISCUSSION OF MODIFICATION APPROVED JUNE 2006 (5)

Results of the in-house and independent validation studies presented here show that the DNA probe method is an effective procedure for detection of *Listeria* spp. in environmental samples, employing enrichment in LESS broth for as little as 24 h. Overall, the probe method was more productive than the USDA reference culture procedure.

Table 1. *Listeria* spp. inclusivity testing results from LESS broth enrichment, 24 h.

Strain #	Organism	Serotype (if known)	Source	Origin (if known)	OD*		Result
					1:10 Dilution**	1:100 Dilution**	
GT4800	<i>L. grayi</i>	-	GENE-TRAK	Environmental	2.947	0.690	Positive
A203	<i>L. grayi</i>	-	ATCC 19120	Chinchilla feces	2.947	1.466	Positive
GT3881	<i>L. grayi</i> subsp. <i>murrayi</i>	-	GENE-TRAK	-	2.947	1.734	Positive
GT3627	<i>L. innocua</i>	6a	H. Seeliger	Cheese	2.947	0.233	Positive
GT3631	<i>L. innocua</i>	6a	H. Seeliger	Cheese	2.947	0.506	Positive
A102	<i>L. innocua</i>	6a	ATCC 33090	Cow brain	2.947	0.619	Positive
GT1026	<i>L. innocua</i>	6b	H. Seeliger	Cheese	2.947	1.788	Positive
GT1042	<i>L. innocua</i>	6b	H. Seeliger	Cheese	2.947	2.168	Positive
GT1044	<i>L. innocua</i>	6b	H. Seeliger	Cheese	2.947	1.016	Positive
GT1050	<i>L. innocua</i>	6b	H. Seeliger	Cheese	2.947	1.562	Positive
GT3785	<i>L. innocua</i>	-	CDC	-	2.947	0.765	Positive
GT1052	<i>L. innocua</i>	-	J. Farber	Raw milk	2.947	2.075	Positive
GT1028	<i>L. ivanovii</i>	5	H. Seeliger	Mouse	2.947	2.947	Positive
GT1040	<i>L. ivanovii</i>	5	H. Seeliger	Human	2.947	2.947	Positive
GT3699	<i>L. ivanovii</i>	5	H. Seeliger	Watercress	2.947	2.224	Positive
A140	<i>L. ivanovii</i>	-	ATCC 19119	Sheep	2.947	2.947	Positive
GT1021	<i>L. monocytogenes</i>	1/2a	CDC	Human	2.947	2.441	Positive
GT3727	<i>L. monocytogenes</i>	1/2a	H. Seeliger	Human blood	2.947	2.716	Positive
GT4340	<i>L. monocytogenes</i>	1/2a	CDC	Fish	2.947	1.090	Positive
GT1038	<i>L. monocytogenes</i>	1/2a	H. Seeliger	Human blood	2.947	1.490	Positive
GT3635	<i>L. monocytogenes</i>	1/2b	H. Seeliger	Human blood	2.947	2.947	Positive
GT3728	<i>L. monocytogenes</i>	1/2b	H. Seeliger	Cheese	2.947	1.917	Positive
GT3856	<i>L. monocytogenes</i>	1/2b	H. Seeliger	Cheese	2.947	2.059	Positive
GT3698	<i>L. monocytogenes</i>	1/2c	H. Seeliger	Cheese	2.947	2.947	Positive
GT3648	<i>L. monocytogenes</i>	1/2c	H. Seeliger	Cheese	2.947	0.882	Positive
GT3730	<i>L. monocytogenes</i>	1/2c	H. Seeliger	-	2.947	0.767	Positive
GT3741	<i>L. monocytogenes</i>	1/2c	H. Seeliger	-	2.947	2.947	Positive
GT3829	<i>L. monocytogenes</i>	1a	C. Donnelly	Raw milk	2.947	2.947	Positive
GT1072	<i>L. monocytogenes</i>	1a	C. Donnelly	Raw milk	2.947	2.947	Positive
GT1880	<i>L. monocytogenes</i>	1a	J. Lovett	Brie cheese	2.947	2.947	Positive
GT3812	<i>L. monocytogenes</i>	1a	J. Lovett	Chocolate milk	2.947	1.569	Positive
A169	<i>L. monocytogenes</i>	2	ATCC 19112	Human CSF	2.947	0.930	Positive
GT3720	<i>L. monocytogenes</i>	3a	H. Seeliger	Cheese	2.947	0.752	Positive
GT1035	<i>L. monocytogenes</i>	3a	H. Seeliger	-	2.947	1.068	Positive
GT1057	<i>L. monocytogenes</i>	3b	J. Lovett	Brie cheese	2.947	2.864	Positive
GT3715	<i>L. monocytogenes</i>	3b	H. Seeliger	Human blood	2.947	0.387	Positive
GT3817	<i>L. monocytogenes</i>	3b	H. Seeliger	Cheese	2.947	1.976	Positive
GT3857	<i>L. monocytogenes</i>	3b	J. Lovett	Brie cheese	2.947	1.970	Positive
A170	<i>L. monocytogenes</i>	4a	ATCC 19114	Ruminant brain	2.947	2.931	Positive
A207	<i>L. monocytogenes</i>	4b	ATCC 13932	Human CSF	2.947	2.947	Positive
GT1019	<i>L. monocytogenes</i>	4b	GENE-TRAK	-	2.947	2.947	Positive
GT1081	<i>L. monocytogenes</i>	4b	CDC	-	2.947	1.756	Positive
GT3819	<i>L. monocytogenes</i>	4c	H. Seeliger	Human	2.947	1.513	Positive
GT3794	<i>L. seeligeri</i>	1	J. Lovett	Brie cheese	2.947	2.465	Positive
GT3693	<i>L. seeligeri</i>	1/2b	H. Seeliger	Sewage	2.947	2.053	Positive
GT289	<i>L. seeligeri</i>	4a	H. Seeliger	Cheese	2.947	1.817	Positive
A201	<i>L. seeligeri</i>	-	ATCC 51334	Vole	2.947	2.419	Positive
GT293	<i>L. welshimeri</i>	6a	H. Seeliger	Cheese	2.947	2.947	Positive
A199	<i>L. welshimeri</i>	-	ATCC 35897	Decaying plant matter	2.947	2.947	Positive
A200	<i>L. welshimeri</i>	-	ATCC 43550	Soil	2.947	2.947	Positive
GT1773	<i>L. welshimeri</i>	-	-	Environmental isolate	2.947	2.947	Positive
GT1729	<i>L. welshimeri</i>	-	-	Dairy plant	2.947	1.587	Positive

\* OD values &gt; 0.10 were considered positive.

\*\* Tested 1:10 and 1:100 dilutions of the enrichment culture.

**Table 2. Results of environmental surface experiments. (5)**

Surface	Sample	Inoc Strain	Initial Inoc Level <sup>a</sup>	# Samples	DNAH Assay <sup>b</sup>	DNAH Conf <sup>c</sup>	USDA	Chi Square <sup>d</sup>
Stainless Steel	Swab	<i>L. mono.1/2c</i>	$2.9 \times 10^4$	20	17	20	2	19.65
				5	0	0	0	-
Plastic	Swab	<i>L. mono. 4b</i>	$6.9 \times 10^4$	20	10	14	2	5.83
				5	0	0	0	-
Cast Iron	Swab	<i>L. innocua</i>	$4.2 \times 10^3$	20	14	16	7	3.61
				5	0	0	0	-
Ceramic Tile	Sponge	<i>L. mono. 1a</i>	$3.0 \times 10^3$	20	12	17	13	0.00
		10x <i>S. aureus</i>	$1.5 \times 10^4$	5	0	0	0	-
Sealed Concrete	Sponge	<i>L. welshimeri</i>	$7.7 \times 10^4$	20	19	19	19	-
				5	0	0	0	-
<b>Total:</b>				<b>125</b>	<b>72</b>	<b>86</b>	<b>43</b>	

<sup>a</sup> Inoculation level in cfu/surface; <sup>b</sup> Number of samples positive by DNA hybridization assay not considering subsequent culture confirmation; <sup>c</sup> Number of samples positive by plating from DNA probe method cultures; <sup>d</sup> Chi square value by independent chi square test comparing confirmed DNA hybridization assay and reference culture method.  $\chi^2 \geq 3.84$  indicates a statistically significant difference at  $p \leq 0.05$ .

**Table 3. Results of independent laboratory testing. (5)**

		Initial Inoc Level <sup>a</sup>	DNAH Assay <sup>b</sup>	DNAH Confirmed <sup>c</sup>	USDA	Chi Square <sup>d</sup>
Ceramic Tile	<i>L. monocytogenes 1a</i>	20	$5.3 \times 10^2$	19	20	2.77
		20	$4.3 \times 10^2$	17	18	0.57
		5	-	0	0	0

<sup>a</sup> Initial inoculation level in cfu/surface; <sup>b</sup> Number of samples positive by DNA hybridization assay not considering subsequent culture confirmation; <sup>c</sup> Number of samples positive by plating from DNA probe method cultures; <sup>d</sup> Chi square value by McNemar's test comparing confirmed DNA hybridization assay and reference culture method.  $\chi^2 \geq 3.84$  indicates a statistically significant difference at  $p \leq 0.05$ .

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