



CERTIFICATION

AOAC[®] Performance TestedSM

Certificate No.

121402

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

BAX[®] System Real-Time PCR Assay for *L. monocytogenes*

manufactured by

Hygiena

2 Boulden Circle

New Castle, DE 19720

USA

This method has been evaluated in the AOAC[®] Performance Tested MethodsSM Program and found to perform as stated by the manufacturer contingent to the comments contained in the manuscript. This certificate means that an AOAC[®] Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC Performance TestedSM 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 (December 06, 2020 – December 31, 2021). Renewal may be granted at the end of one year under the rules stated in the licensing agreement.

Scott Coates

Scott Coates, Senior Director
Signature for AOAC Research Institute

December 06, 2020

Date

METHOD AUTHORS

ORIGINAL VALIDATION: Bridget Andaloro, Dawn Fallon, Jeff Rohrebeck, Steven Hoelzer, Julie Weller, Eugene Davis, George Tice, and F. Morgan Wallace
 MODIFICATION MAY 2015: Sergiy Olishkevskyy, Benoit Crevier, Renaud Tremblay, Morgan Wallace

SUBMITTING COMPANY

DuPont Nutrition & Health
 Experimental Station 400
 200 Powder Mill Road
 P. O. Box 8352
 Wilmington, DE 19803 USA

CURRENT SPONSOR

Hygiena
 2 Boulden Circle
 New Castle, DE 19720
 USA

KIT NAME(S)

DuPont™ BAX® System Real-Time PCR Assay for *L. monocytogenes*
 March 01, 2017, BAX® System Real-Time PCR Assay for *L. monocytogenes*

CATALOG NUMBERS

BAX® System Assay KIT2005 (D15134303), 24 LEB Complete MED2005 (D14654989)

INDEPENDENT LABORATORY

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APPLICABILITY OF METHOD

Target organism – *Listeria monocytogenes*

Matrixes - (25 g) - Frankfurters (beef), bagged spinach, queso fresco, cooked shrimp
 (10 x 10 cm sq) - stainless steel, plastic, concrete

REFERENCE METHODS

U.S. Department of Agriculture-Food Safety and Inspection Service Microbiology Laboratory Guidebook Chapter 8.09 (2013) (2)
 U.S. Food and Drug Administration Bacteriological Analytical Manual, Chapter 10 (2011) (3)

MODIFICATION MAY 2015:

FDA BAM Ch. 10: (25 g) - soft Mexican-style cheese, bagged spinach, frozen cooked shrimp, cold smoked salmon
 USDA/FSIS MLM 8.09: frankfurters, stainless steel, plastic, sealed concrete

Performance claims - Performance indistinguishable from or better than the corresponding reference method.

ORIGINAL CERTIFICATION DATE

December 22, 2014

CERTIFICATION RENEWAL RECORD

Renewed annually through December 2021

METHOD MODIFICATION RECORD

1. May 2015
2. March 2017 Level 1
3. December 2017 Level 2
4. January 2018 Level 1
5. May 2019 Level 1
6. December 2019 Level 1

SUMMARY OF MODIFICATION

1. Modification to include Actero™ *Listeria* and use of a single step enrichment for 121402. Matrix extension to include additional matrixes.
2. Name change from DuPont Nutrition & Health to Qualicon Diagnostics LLC., a Hygiena company
3. Change in hold time after tablet rehydration
4. Editorial updates to inserts, manuals, labels to Hygiena
5. Editorial insert updates and corporate address change
6. Editorial insert updates.

Under this AOAC® Performance TestedSM License Number, 121402 this method is distributed by:
 NONE

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 NONE

PRINCIPLE OF THE METHOD (1)

The BAX® System uses PCR to amplify a specific fragment of bacterial DNA, which is stable and unaffected by growth environment. The fragment is a genetic sequence that is unique to *L. monocytogenes*, thus providing a highly reliable indicator that the organism is present. The BAX® System simplifies the PCR process by combining the requisite primers, polymerase and nucleotides into a stable, dry, manufactured tablet already packaged inside the PCR tubes. After amplification, these tubes remain sealed for the detection phase, significantly reducing the potential for contamination with one or more molecules of amplified PCR product. This automated BAX® System method uses fluorescent detection to analyze PCR product. One PCR primer for each target (*L. monocytogenes*-specific targets and an internal control) contains a fluorescent dye (two different dyes, one for the internal control and one for the targets) as a constituent of the primer. The primer or a separate oligonucleotide will contain a quencher that will be in close proximity to the dye in the native state, but removed from close proximity when the primer has been incorporated into a PCR product which causes an increase in emission signal. This combination of a primer, fluorescent dye and quencher constitute a Scorpion™ probe. The BAX® System measures the magnitude and characteristics of fluorescent signal change. Analysis by the BAX® System software then evaluates that data to determine a positive or negative result which is displayed as described below.

DISCUSSION OF THE VALIDATION STUDY (1)

Overall, the DuPont™ BAX® System Real-Time PCR Assay for *Listeria monocytogenes* demonstrates accuracy and reliability statistically equivalent to the reference culture methods used in this study. Simplified lysis procedure reduces the total lysis time by more than 30 minutes when compared to previously validated BAX® System assays for *Listeria monocytogenes*. Furthermore, this real-time assay reduces the automated processing time by more than 2 hours in the BAX® System Q7 instrument. This reduced time, along with the ease of use and excellent accuracy of the BAX® System, make the test method a strong alternative method to culture for *Listeria monocytogenes* detection.

Table 2. Method results POD Food Matrixes – Candidate Method Compared to Culture Results from Candidate Enrichments (1)

Matrix	Strain	MPN ^a /test portion	N ^b	Candidate Method Presumptive			Candidate Method Confirmed			dPOD _{CP} ^f	95% CI ^g
				X ^c	POD _{CP} ^d	95% CI	x	POD _{CC} ^e	95% CI		
Frankfurters BAX® System 28 h	<i>L. monocytogenes</i> DD 1309	2.9 (1.7, 5.2)	5	5	1.0	(0.57, 1.0)	5	1	(0.57, 1.0)	0	(-0.45, 0.45)
		0.27 (0.12, 0.51)	20	5	0.25	(0.11, 0.46)	6	0.30	(0.14, 0.52)	-0.050	(-0.15, 0.05)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Frankfurters BAX® System 30 h	<i>L. monocytogenes</i> DD 1309	2.9 (1.7, 5.2)	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		0.27 (0.12, 0.51)	20	6	0.30	(0.14, 0.52)	6	0.30	(0.14, 0.52)	0	(-0.14, 0.14)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Frankfurters BAX® System 48 h	<i>L. monocytogenes</i> DD 1309	2.9 (1.7, 5.2)	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		0.27 (0.12, 0.51)	20	6	0.30	(0.14, 0.52)	6	0.30	(0.14, 0.52)	0	(-0.14, 0.14)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Frankfurters USDA-FSIS 48 h	<i>L. monocytogenes</i> DD 1309	2.9 (1.7, 5.2)	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		0.27 (0.12, 0.51)	20	6	0.30	(0.14, 0.52)	6	0.30	(0.14, 0.52)	0	(-0.14, 0.14)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Bagged Spinach BAX® System 24 h	<i>L. monocytogenes</i> DD 1283	4.6 (2.6, 8.1)	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		0.4 (0.23, 0.81)	20	9	0.45	(0.25, 0.65)	10	0.50	(0.30, 0.70)	-0.050	(-0.15, 0.05)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Bagged Spinach BAX® System 48 h	<i>L. monocytogenes</i> DD 1283	4.6 (2.6, 8.1)	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		0.4 (0.23, 0.81)	20	10	0.50	(0.30, 0.70)	10	0.50	(0.30, 0.70)	0	(-0.14, 0.14)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Bagged Spinach FDA- 48 h	<i>L. monocytogenes</i> DD 1283	4.6 (2.6, 8.1)	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		0.4 (0.23, 0.81)	20	9	0.45	(0.25, 0.65)	9	0.45	(0.25, 0.65)	0	(-0.14, 0.14)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Queso Fresco Cheese BAX® System 26 h	<i>L. monocytogenes</i> DD 5425	6.4 (4.1, 9.9)	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		0.64 (0.4, 0.9)	20	5	0.25	(0.11, 0.46)	6	0.30	(0.14, 0.52)	-0.050	(-0.15, 0.05)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Queso Fresco Cheese BAX® System 48 h	<i>L. monocytogenes</i> DD 5425	6.4 (4.1, 9.9)	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		0.64 (0.4, 0.9)	20	6	0.30	(0.14, 0.52)	6	0.30	(0.14, 0.52)	0	(-0.14, 0.14)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)

Queso Fresco Cheese FDA-BAM 48 h	<i>L. monocytogenes</i> DD 5425	6.4 (4.1, 9.9)	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		0.64 (0.4, 0.9)	20	11*	0.55	(0.34, 0.74)	9	0.45	(0.25, 0.65)	0.10	(-0.04, 0.24)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Queso Fresco Cheese ^h BAX® System 26 h	<i>L. monocytogenes</i> ATC C 19115	5.09	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		0.95	20	8	0.40	(0.21, 0.61)	8	0.40	(0.21, 0.61)	0	(-0.28, 0.28)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Queso Fresco Cheese ^h BAX® System 48 h	<i>L. monocytogenes</i> ATC C 19115	5.09	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		0.95	20	8	0.40	(0.21, 0.61)	8	0.40	(0.21, 0.61)	0	(-0.28, 0.28)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Queso Fresco Cheese ^h FDA-BAM 48 h	<i>L. monocytogenes</i> ATC C 19115	5.09	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		0.95	20	11	0.55	(0.34, 0.74)	9	0.45	(0.25, 0.65)	0.10	(-0.19, 0.37)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Cooked Shrimp BAX® System 48 h	<i>L. monocytogenes</i> DD 6618	4.8 (2.8, 8.3)	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		0.47 (0.2, 0.8)	20	12	0.60	(0.38, 0.78)	12	0.60	(0.38, 0.78)	0	(-0.14, 0.14)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Cooked Shrimp FDA-BAM 48 h	<i>L. monocytogenes</i> DD 6618	4.8 (2.8, 8.3)	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		0.47 (0.2, 0.8)	20	7	0.35	(0.18, 0.56)	7	0.35	(0.18, 0.56)	0	(-0.14, 0.14)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)

^aMPN = Most Probable Number plus 95% CIs for food samples calculated using the Least Cost Formulations MPN Calculator (6).

^bN = Number of test portions.

^cx = Number of positive test portions.

^dPOD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials.

^ePOD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials.

^fdPOD_{CP} = Difference between the candidate method presumptive result and candidate method confirmed result POD values.

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

^hIndependent Laboratory Study.

*Note: 11 samples tested positive by BAX® System method, however two samples displayed very weak positive results and when retested in triplicate all three tests from each of the two samples displayed negative results.

Table 3. Method results POD Environmental Surfaces – Candidate Method Compared to Culture Results from Candidate Enrichments (1)

Surface and Enrichment Media	Strain	<i>Listeria Incolum</i> level ^g /test portion	N ^b	Candidate Method Presumptive			Candidate Method Confirmed			dPOD _{cp} ^f	95% CI ^g
				X ^c	POD _{cp} ^d	95% CI	x	POD _{cc} ^e	95% CI		
304 2B Stainless Steel 24E enrichment broth 24 h	<i>L. monocytogenes</i> DD 1302 <i>P. aeruginosa</i> DD 13148	1060	5	5	1	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		106	20	19	0.95	(0.76, 0.99)	19	0.95	(0.76, 0.99)	0	(-0.14, 0.14)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
304 2B Stainless Steel 24E enrichment broth 48 h	<i>L. monocytogenes</i> DD 1302 <i>P. aeruginosa</i> DD 13148	1060	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		106	20	19	0.95	(0.76, 0.99)	19	0.95	(0.76, 0.99)	0	(-0.14, 0.14)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
304 2B Stainless Steel USDA-MLG enrichment protocol 48 h	<i>L. monocytogenes</i> DD 1302 <i>P. aeruginosa</i> DD 13148	1060	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		106	20	14	0.70	(0.48, 0.84)	14	0.70	(0.48, 0.84)	0	(-0.14, 0.14)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Stainless Steel Actero™ enrichment broth 20 h	<i>L. monocytogenes</i> 1/2c MSR0119 <i>P. aeruginosa</i> MSR0132	63.0	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		5.0	20	16	0.80	(0.58, 0.92)	16	0.80	(0.58, 0.92)	0	(-0.25, 0.25)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
304 2B Stainless Steel ^h 24E enrichment broth 24 h	<i>L. monocytogenes</i> ATCC 7644 <i>P. aeruginosa</i> ATCC 15442	170	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		60	20	16	0.80	(0.58, 0.91)	16	0.80	(0.58, 0.91)	0	(-0.24, 0.24)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
304 2B Stainless Steel ^h BAX® System 48 h	<i>L. monocytogenes</i> ATCC 7644 <i>P. aeruginosa</i> ATCC 15442	170	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		60	20	16	0.80	(0.58, 0.91)	16	0.80	(0.58, 0.91)	0	(-0.24, 0.24)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)

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304 2B Stainless Steel ^h USDA-MLG 48 h	<i>L. monocytogenes</i> ATCC 7644 <i>P. aeruginosa</i> ATCC 15442	170	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		60	20	14	0.70	(0.48, 0.85)	14	0.70	(0.48, 0.85)	0	(-0.14, 0.14)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Stainless Steel ^h Actero™ 20 h	<i>L. monocytogenes</i> ATCC 7644 <i>P. aeruginosa</i> ATCC15442	220	5	5	1.0	(0.57, 1.0)	5	1.0	(0.38, 1.0)	0.80	(-0.45, 0.45)
		90	20	9	0.45	(0.26, 0.66)	9	0.45	(0.26, 0.66)	0	(-0.14, 0.14)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Plastic BAX® System 24 h	<i>L. monocytogenes</i> DD 1309 <i>E. faecalis</i> DD10565	640	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		64	20	19	0.95	(0.76, 0.99)	19	0.95	(0.76, 0.99)	0	(-0.14, 0.14)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Plastic BAX® System 48 h	<i>L. monocytogenes</i> DD 1309 <i>E. faecalis</i> DD10565	640	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		64	20	19	0.95	(0.76, 0.99)	19	0.95	(0.76, 0.99)	0	(-0.14, 0.14)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Plastic USDA-MLG 48 h	<i>L. monocytogenes</i> DD 1309 <i>E. faecalis</i> DD10565	640	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		64	20	15	0.75	(0.53, 0.88)	15	0.75	(0.53, 0.88)	0	(-0.14, 0.14)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Plastic Actero™ 20 h	<i>L. monocytogenes</i> 4b MSR0446 <i>E. faecalis</i> ATCC 19433	65.0	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		4.0	20	8	0.40	(0.22, 0.61)	8	0.40	(0.22, 0.61)	0	(-0.14, 0.14)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Concrete BAX® System 24 h	<i>L. monocytogenes</i> DD 7856 <i>E. faecalis</i> DD10565	970	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		97	20	15	0.75	(0.53, 0.88)	16	0.80	(0.58, 0.91)	-0.050	(-0.15, 0.05)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)

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Concrete BAX® System 48 h	<i>L. monocytogenes</i> DD 7856 <i>E. faecalis</i> DD10565	970	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		97	20	16	0.80	(0.58, 0.91)	16	0.80	(0.58, 0.91)	0	(-0.14, .014)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Concrete USDA-MLG 48 h	<i>L. monocytogenes</i> DD 7856 <i>E. faecalis</i> DD10565	970	5	4	0.30	(0.37, 0.96)	4	0.30	(0.37, 0.96)	0	(-0.45, 0.45)
		97	20	1	0.05	(0, 0)	1	0.05	(0, 0)	0	(-0.07, 0.07)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Concrete Actero™ 20 h	<i>L. monocytogenes</i> 1/2a MSR0447	36	5	4	0.30	(0.37, 0.96)	4	0.30	(0.37, 0.96)	0	(-0.45, 0.45)
		4.8	20	12	0.60	(0.39, 0.78)	12	0.60	(0.39, 0.78)	0	(-0.28, 0.28)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)

^a Inoculum level = Inoculum of target strain applied to surface based on non-selective plate counts. Competitor strains were inoculated with ~10X the target inoculum level.

^bN = Number of test portions.

^cx = Number of positive test portions.

^dPOD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials.

^ePOD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials.

^fdPOD_{CP} = Difference between the candidate method presumptive result and candidate method confirmed result POD values.

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

^hIndependent Laboratory Study.

*Note: Sample tested using the BAX® System assay at 48 hours following the USDA-MGL enrichment method displayed one weak positive result which happened to be next to a strong positive result. This sample was retested in triplicate resulting in all negatives

Table 4. Method results POD Food Matrixes – Candidate Method Compared to Reference Culture Method (1)											
Matrix	Strain	MPN ^a /test portion	N ^b	Test Method			Reference Method			dPOD _c ^f	95% CI ^g
				X ^c	POD _c ^d	95% CI	x	POD _R ^e	95% CI		
Frankfurters BAX® System 28 h	<i>L. monocytogenes</i> DD 1309	2.9 (1.7, 5.2)	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.43, 0.43)
		0.27 (0.12, 0.51)	20	5	0.25	(0.11, 0.46)	6	0.30	(0.14, 0.52)	-0.050	(-0.31, 0.22)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.43, 0.43)
Frankfurters BAX® System 30 h	<i>L. monocytogenes</i> DD 1309	2.9 (1.7, 5.2)	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.43, 0.43)
		0.27 (0.12, 0.51)	20	6	0.30	(0.14, 0.52)	6	0.30	(0.14, 0.52)	0	(-0.26, 0.26)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.43, 0.43)
Frankfurters BAX® System 48 h	<i>L. monocytogenes</i> DD 1309	2.9 (1.7, 5.2)	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.43, 0.43)
		0.27 (0.12, 0.51)	20	6	0.30	(0.14, 0.52)	6	0.30	(0.14, 0.52)	0	(-0.26, 0.26)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.43, 0.43)
Frankfurters USDA-MLG 48 h	<i>L. monocytogenes</i> DD 1309	2.9 (1.7, 5.2)	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		0.27 (0.12, 0.51)	20	6	0.30	(0.14, 0.52)	6	0.30	(0.14, 0.52)	0	(-0.14, 0.14)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Bagged Spinach BAX® System 24 h	<i>L. monocytogenes</i> DD 1283	4.6 (2.6, 8.1)	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		0.4 (0.23, 0.81)	20	9	0.45	(0.25, 0.65)	9	0.45	(0.25, 0.65)	0	(-0.28, 0.28)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.43, 0.43)
Bagged Spinach BAX® System 48 h	<i>L. monocytogenes</i> DD 1283	4.6 (2.6, 8.1)	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		0.4 (0.23, 0.81)	20	10	0.50	(0.30, 0.70)	9	0.45	(0.25, 0.65)	0.050	(-0.23, 0.32)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.43, 0.43)
Bagged Spinach FDA-BAM 48 h	<i>L. monocytogenes</i> DD 1283	4.6 (2.6, 8.1)	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		0.4 (0.23, 0.81)	20	9	0.45	(0.25, 0.65)	9	0.45	(0.25, 0.65)	0	(-0.14, 0.14)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Queso Fresco Cheese BAX® System 26 h	<i>L. monocytogenes</i> DD 5425	6.4 (4.1, 9.9)	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		0.64 (0.4, 0.9)	20	5	0.25	(0.11, 0.46)	9	0.45	(0.25, 0.65)	-0.20	(-0.44, 0.09)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.43, 0.43)
Queso Fresco Cheese BAX® System 48 h	<i>L. monocytogenes</i> DD 5425	6.4 (4.1, 9.9)	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		0.64 (0.4, 0.9)	20	6	0.30	(0.14, 0.52)	9	0.45	(0.25, 0.65)	-0.15	(-0.41, 0.14)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.43, 0.43)

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Queso Fresco Cheese FDA-BAM 48 h	<i>L. monocytogenes</i> DD 5425	6.4 (4.1, 9.9)	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		0.64 (0.4, 0.9)	20	11*	0.55	(0.34, 0.74)	9	0.45	(0.25, 0.65)	0.10	(-0.04, 0.24)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Queso Fresco Cheese ^b BAX® System 26 h	<i>L. monocytogenes</i> ATC C 19115	5.09	5	5	1.0	(0.57, 1.0)	0	0	(0, 0.43)	0	(-0.45, 0.45)
		0.95	20	8	0.40	(0.22, 0.61)	9	0.45	(0.26, 0.66)	-0.050	(-0.33, 0.24)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.43, 0.43)
Queso Fresco Cheese ^b BAX® System 48 h	<i>L. monocytogenes</i> ATC C 19115	5.09	5	5	1	(0.57, 1.0)	0	0	(0, 0.43)	0	(-0.45, 0.45)
		0.95	20	8	0.40	(0.22, 0.61)	9	0.45	(0.26, 0.66)	-0.050	(-0.33, 0.24)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.43, 0.43)
Queso Fresco Cheese ^b FDA-BAM 48 h	<i>L. monocytogenes</i> ATC C 19115	5.09	5	5	1.0	(0.57, 1.0)	0	0	(0, 0.43)	0	(-0.45, 0.45)
		0.95	20	11	0.55	(0.34, 0.74)	9	0.45	(0.25, 0.65)	0.10	(-0.19, 0.37)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.43, 0.43)
Cooked Shrimp BAX® System 48 h	<i>L. monocytogenes</i> DD 6618	4.8 (2.8, 8.3)	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		0.47 (0.2, 0.8)	20	12	0.60	(0.38, 0.78)	7	0.35	(0.18, 0.56)	0.25	(-0.05, 0.50)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.43, 0.43)
Cooked Shrimp FDA-BAM 48 h	<i>L. monocytogenes</i> DD 6618	4.8 (2.8, 8.3)	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		0.47 (0.2, 0.8)	20	7	0.35	(0.18, 0.56)	7	0.35	(0.18, 0.56)	0	(-0.14, 0.14)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)

^aMPN = Most Probable Number plus 95% CIs for food samples calculated using the Least Cost Formulations MPN Calculator (6)

^bN = Number of test portions.

^cx = Number of positive test portions.

^dPOD_c = Confirmed candidate method positive outcomes divided by the total number of trials.

^ePOD_r = Confirmed reference method positive outcomes divided by the total number of trials.

^fdPOD_c = Difference between the candidate method and reference method POD values.

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

^hIndependent Laboratory Study.

*Note: Sample tested using the BAX® System assay at 48 hours following the USDA-MGL enrichment method displayed one weak positive result which happened to be next to a strong positive result. This sample was retested in triplicate resulting in all negatives.

Table 5. Method Results POD Environmental Surfaces – Candidate Method Compared to Reference Culture Method (1)											
Matrix	Strain	<i>Listeria</i> Inoculum level ^a /test portion	N ^b	Test Method			Reference Method			dPOD _c ^f	95% CI ^g
				χ ^c	POD _c ^d	95% CI	x	POD _R ^e	95% CI		
304 2B Stainless Steel BAX® System 24 h	<i>L. monocytogenes</i> DD 1302 <i>P. aeruginosa</i> DD 13148	1060	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		106	20	19	0.95	(0.76, 0.99)	14	0.70	(0.78, 0.85)	0.25	(0.01, 0.47)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.43, 0.43)
304 2B Stainless Steel BAX® System 48 h	<i>L. monocytogenes</i> DD 1302 <i>P. aeruginosa</i> DD 13148	1060	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		106	20	19	0.95	(0.76, 0.99)	14	0.70	(0.78, 0.85)	0.25	(0.01, 0.47)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.43, 0.43)
304 2B Stainless Steel USDA-MLG 48 h	<i>L. monocytogenes</i> DD 1302 <i>P. aeruginosa</i> DD 13148	1060	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		106	20	14	0.70	(0.48, 0.84)	14	0.70	(0.48, 0.84)	0	(-0.14, 0.14)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Stainless Steel Actero™ 20 h	<i>L. monocytogenes</i> 1/2c MSR0119 <i>P. aeruginosa</i> MSR0132	63	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		5.0	20	16	0.80	(0.58, 0.92)	10	0.50	(0.30, 0.70)	0.30	(0.00, 0.53)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
304 2B Stainless Steel ^h BAX® System 24 h	<i>L. monocytogenes</i> ATCC 7644 <i>P. aeruginosa</i> ATCC 15442	170	5	5	1.0	(0.57, 1.0)	5	0.80	(0.38, 1.0)	0	(-0.45, 0.45)
		60	20	16	0.80	(0.54, 0.92)	14	0.70	(0.48, 0.85)	0.10	(-0.16, 0.34)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.43, 0.43)
304 2B Stainless Steel ^h BAX® System 48 h	<i>L. monocytogenes</i> ATCC 7644 <i>P. aeruginosa</i> ATCC 15442	170	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.43, 0.43)
		60	20	16	0.80	(0.54, 0.92)	14	0.70	(0.48, 0.85)	0.10	(-0.17, 0.35)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.43, 0.43)
304 2B Stainless Steel ^h USDA-MLG 48 h	<i>L. monocytogenes</i> ATCC 7644 <i>P. aeruginosa</i> ATCC 15442	170	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		60	20	14	0.70	(0.48, 0.85)	14	0.70	(0.48, 0.85)	0	(-0.27, 0.27)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Stainless Steel ^h Actero™ 20 h	<i>L. monocytogenes</i> ATCC 7644 <i>P. aeruginosa</i> ATCC15442	220	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.43, 0.43)
		90	20	9	0.45	(0.22, 0.61)	5	0.25	(0.11, 0.47)	0.15	(-0.09, 0.45)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.43, 0.43)
Plastic BAX® System 24 h	<i>L. monocytogenes</i> DD 1309 <i>E. faecalis</i>	640	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		64	20	19	0.95	(0.76, 0.99)	15	0.75	(0.53, 0.88)	0.20	(-0.03, 0.42)

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	DD10565	Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Plastic BAX® System 48 h	<i>L. monocytogenes</i> DD 1309 <i>E. faecalis</i> DD10565	640	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		64	20	19	0.95	(0.76, 0.99)	15	0.75	(0.53, 0.88)	0.20	(-0.03, 0.42)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Plastic USDA-MLG 48 h	<i>L. monocytogenes</i> DD 1309 <i>E. faecalis</i> DD10565	640	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		64	20	15	0.75	(0.53, 0.88)	15	0.75	(0.53, 0.88)	0	(-0.45, 0.45)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0.20	(-0.43, 0.43)
Plastic Actero™ 20 h	<i>L. monocytogenes</i> 4b MSR0446 <i>E. faecalis</i> ATCC 19433	65	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.43, 0.43)
		4.0	20	8	0.40	(0.22, 0.61)	4	0.20	(0.08, 0.42)	0.20	(-0.08, 0.44)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0.20	(-0.43, 0.43)
Concrete BAX® System 24 h	<i>L. monocytogenes</i> DD 7856 <i>E. faecalis</i> DD10565	970	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		97	20	15	0.75	(0.53, 0.88)	1	0.050	(0, 0)	0.70	(0.48, 0.85)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.43, 0.43)
Concrete BAX® System 48 h	<i>L. monocytogenes</i> DD 7856 <i>E. faecalis</i> DD10565	970	5	5	1.0	(0.57, 1.0)	4	0.80	(0.37, 0.96)	0.20	(-0.26, 0.62)
		97	20	16	0.80	(0.58, 0.91)	1	0.050	(0, 0)	0.75	(0.52, 0.87)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.43, 0.43)
Concrete USDA-MLG 48 h	<i>L. monocytogenes</i> DD 7856 <i>E. faecalis</i> DD10565	970	5	4	0.30	(0.37, 0.96)	4	0.30	(0.37, 0.96)	0	(-0.45, 0.45)
		97	20	1	0.05	(0, 0)	1	0.05	(0, 0)	0	(-0.14, 0.14)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Concrete Actero™ 20 h	<i>L. monocytogenes</i> 1/2a MSR0447	36	5	5	10	(0.57, 1.0)	5	1	(0.57, 1.0)	0	(-0.45, 0.45)
		4.8	20	12	0.60	(0.39, 0.78)	10	0.5	(0.30, 0.70)	0.10	(-0.19, 0.37)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.43, 0.43)

^aInoculum level = Inoculum of target strain applied to surface based on non-selective plate counts. Competitor strains were inoculated with ~10X the target inoculum level.

^bN = Number of test portions.

^cχ = Number of positive test portions.

^dPOD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials.

^ePOD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials.

^fdPOD_{CP} = Difference between the candidate method presumptive result and candidate method confirmed result POD values.

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

^hIndependent Laboratory Study.

Table 11. BAX® System Inclusivity Results (1)

Strain ID	Strain Name	BAX Result	Strain ID	Strain Name	BAX Result
566	<i>Listeria monocytogenes</i>	POS	1306	<i>Listeria monocytogenes</i>	POS
605	<i>Listeria monocytogenes</i>	POS	1307	<i>Listeria monocytogenes</i>	POS
647	<i>Listeria monocytogenes</i>	POS	1308	<i>Listeria monocytogenes</i>	POS
648	<i>Listeria monocytogenes</i>	POS	1309	<i>Listeria monocytogenes</i>	POS
652	<i>Listeria monocytogenes</i>	POS	1310	<i>Listeria monocytogenes</i>	POS
653	<i>Listeria monocytogenes</i>	POS	1311	<i>Listeria monocytogenes</i>	POS
1069	<i>Listeria monocytogenes</i>	POS	1312	<i>Listeria monocytogenes</i>	POS
1072	<i>Listeria monocytogenes</i>	POS	1313	<i>Listeria monocytogenes</i>	POS
1144	<i>Listeria monocytogenes</i>	POS	1314	<i>Listeria monocytogenes</i>	POS
1145	<i>Listeria monocytogenes</i>	POS	1315	<i>Listeria monocytogenes</i>	POS
1145	<i>Listeria monocytogenes</i>	POS	1316	<i>Listeria monocytogenes</i>	POS
1145	<i>Listeria monocytogenes</i>	POS	1321	<i>Listeria monocytogenes</i>	POS
1149	<i>Listeria monocytogenes</i>	POS	3573	<i>Listeria monocytogenes</i>	POS
1152	<i>Listeria monocytogenes</i>	POS	3574	<i>Listeria monocytogenes</i>	POS
1281	<i>Listeria monocytogenes</i>	POS	3576	<i>Listeria monocytogenes</i>	POS
1282	<i>Listeria monocytogenes</i>	POS	3577	<i>Listeria monocytogenes</i>	POS
1283	<i>Listeria monocytogenes</i>	POS	3578	<i>Listeria monocytogenes</i>	POS
1285	<i>Listeria monocytogenes</i>	POS	3579	<i>Listeria monocytogenes</i>	POS
1286	<i>Listeria monocytogenes</i>	POS	3580	<i>Listeria monocytogenes</i>	POS
1287	<i>Listeria monocytogenes</i>	POS	3581	<i>Listeria monocytogenes</i>	POS
1288	<i>Listeria monocytogenes</i>	POS	3582	<i>Listeria monocytogenes</i>	POS
1293	<i>Listeria monocytogenes</i>	POS	4553	<i>Listeria monocytogenes</i>	POS
1294	<i>Listeria monocytogenes</i>	POS	4568	<i>Listeria monocytogenes</i>	POS
1295	<i>Listeria monocytogenes</i>	POS	4571	<i>Listeria monocytogenes</i>	POS
1299	<i>Listeria monocytogenes</i>	POS	5425	<i>Listeria monocytogenes</i>	POS
1302	<i>Listeria monocytogenes</i>	POS	7644	<i>Listeria monocytogenes</i>	POS
1305	<i>Listeria monocytogenes</i>	POS			

Table 12. BAX® System Exclusivity Results (1)					
Strain ID	Strain Name	BAX Result	Strain ID	Strain Name	BAX Result
379	<i>Bacillus subtilis</i>	NEG	1164	<i>Listeria ivanovii</i>	NEG
383	<i>Citrobacter freundii</i>	NEG	1172	<i>Listeria welshimeri</i>	NEG
643	<i>Listeria murrayi / grayi</i>	NEG	2392	<i>Rhodococcus equi</i>	NEG
649	<i>Listeria ivanovii</i>	NEG	2552	<i>Enterococcus faecium</i>	NEG
654	<i>Listeria welshimeri</i>	NEG	2553	<i>Enterococcus faecium</i>	NEG
659	<i>Lactococcus lactis</i>	NEG	2554	<i>Enterococcus faecalis</i>	NEG
691	<i>Streptococcus thermophilus</i>	NEG	2558	<i>Citrobacter freundii</i>	NEG
692	<i>Streptococcus bovis</i>	NEG	2560	<i>Citrobacter koseri</i>	NEG
695	<i>Streptococcus pyogenes</i>	NEG	2561	<i>Citrobacter koseri</i>	NEG
707	<i>Salmonella</i> Newport	NEG	2624	<i>Enterococcus gallinarum</i>	NEG
713	<i>Bacillus thuringiensis</i>	NEG	2625	<i>Enterococcus durans</i>	NEG
714	<i>Bacillus thuringiensis</i>	NEG	2626	<i>Enterococcus hirae</i>	NEG
715	<i>Bacillus cereus</i>	NEG	2628	<i>Salmonella</i> Kentucky	NEG
716	<i>Bacillus thuringiensis</i>	NEG	2636	<i>Staphylococcus felis</i>	NEG
721	<i>Bacillus cereus</i>	NEG	2874	<i>Listeria seeligeri</i>	NEG
863	<i>Staphylococcus aureus</i>	NEG	3244	<i>Listeria innocua</i>	NEG
877	<i>Bacillus cereus</i>	NEG	3327	<i>Listeria seeligeri</i>	NEG
878	<i>Bacillus cereus</i>	NEG	3329	<i>Listeria seeligeri</i>	NEG
879	<i>Bacillus cereus</i>	NEG	3359	<i>Listeria welshimeri</i>	NEG
912	<i>Staphylococcus aureus</i>	NEG	3363	<i>Listeria murrayi / grayi</i>	NEG
944	<i>Listeria murrayi / grayi</i>	NEG	3376	<i>Listeria ivanovii</i>	NEG
1011	<i>Bacillus subtilis</i>	NEG	3572	<i>Listeria innocua</i>	NEG
1024	<i>Bacillus cereus</i>	NEG	3981	<i>Enterococcus faecalis</i>	NEG
1096	<i>Staphylococcus aureus</i>	NEG	3992	<i>Streptococcus mutans</i>	NEG
1098	<i>Staphylococcus aureus</i>	NEG	3996	<i>Streptococcus equi</i>	NEG
1105	<i>Staphylococcus warnerii</i>	NEG	4063	<i>Carnobacterium gallinarum</i>	NEG
1107	<i>Staphylococcus xylosus</i>	NEG	4064	<i>Carnobacterium divergens</i>	NEG
1111	<i>Staphylococcus capitis</i>	NEG	7332	<i>Lactobacillus curvatus</i>	NEG
1112	<i>Staphylococcus xylosus</i>	NEG	7344	<i>Lactobacillus acidophilus</i>	NEG
1113	<i>Staphylococcus sciuri</i>	NEG	9174	<i>Micrococcus luteus</i>	NEG
1156	<i>Listeria innocua</i>	NEG			

DISCUSSION OF THE MODIFICATION APPROVED MAY 2015 (4)

The Actero™ *Listeria* Enrichment Broth has been developed for a single-step recovery of *Listeria* from environmental and food samples and subsequent detection by direct plating (Actero™ *Listeria* method). In this study, we demonstrated that the Actero™ *Listeria* Enrichment Broth can also be used for enrichment of samples to be analyzed using the DuPont™ BAX® System Real-Time PCR assays for *L. monocytogenes* and *Listeria* Genus. The performance of Actero™ *Listeria* Enrichment Media was evaluated in the internal and independent laboratory matrix studies and compare to the USDA-FSIS and/or U.S. FDA reference methods for stainless steel, plastic and sealed concrete environmental surfaces and soft Mexican style cheese, frankfurter, fresh bagged spinach, frozen cooked shrimp, cold smoked salmon and pasteurized milk samples.

According to the POD statistical model, for all analyzed matrixes and candidate detection methods, the performance was equal or superior to the appropriate reference method. No false negative or false positive results were found for detection of *Listeria* spp. using the BAX® System assay and the Actero™ *Listeria* method. Only two false negative outcomes (one for stainless steel samples and one for frozen cooked shrimp) and none of false positive results were detected using the BAX® System assay in the environmental and food samples contaminated with *L. monocytogenes*.

The independent laboratory validation study carried out for stainless steel and soft Mexican style cheese confirmed the accuracy and reliability of the candidate method.

In conclusion, the results of the validation study demonstrated that the candidate method using single-step enrichment of environmental and food samples in ACTERO™ *Listeria* Broth followed by the detection with the BAX® System assays or/and Actero™ *Listeria* method protocol is an effective screening method and is a viable alternative to traditional reference methods of *Listeria* detection.

Table 3. Actero™ Listeria Broth Enrichment Followed by Using the BAX® System Assay for *L. monocytogenes* Detection in Environmental Samples – Candidate Method Presumptive Compared to Candidate Method Confirmed Results (4)

Matrix	Strain info	I ^a		N ^d	Candidate Presumptive (CP)			Candidate Confirmed (CC)			dPOD _{CP} ^h	95% CI ⁱ
		CFU/ sample	(UCL ^b , LCL ^c)		X ^e	POD _{CP} ^f	95% CI	X	POD _{CC} ^g	95% CI		
Stainless steel	<i>L. mono.</i> 1/2c (ready-to-eat food) + <i>P. aeruginosa</i> (field isolate)	0.0	N/A ^k	5	0	0.00	(0.00; 0.43)	0	0.00	(0.00; 0.43)	0.00	(-0.43; 0.43)
		5.0	N/A	20	16	0.80	(0.58; 0.92)	16	0.80	(0.58; 0.92)	0.00	(-0.25; 0.25)
		63.0	N/A	5	5	1.00	(0.57; 1.00)	5	1.00	(0.57; 1.00)	0.00	(-0.43; 0.43)
Stainless steel ^j	<i>L. mono.</i> ATCC 7644 (1/2c) (ready-to-eat food) + <i>P. aeruginosa</i> ATCC 15442	0.0	N/A	5	0	0.00	(0.00; 0.43)	0	0.00	(0.00; 0.43)	0.00	(-0.43; 0.43)
		90.0	N/A	20	9	0.45	(0.26; 0.66)	9	0.45	(0.26; 0.66)	0.00	(-0.28; 0.28)
		220	N/A	5	5	1.00	(0.57; 1.00)	5	1.00	(0.57; 1.00)	0.00	(-0.43; 0.43)
Plastic	<i>L. mono.</i> 4b (sheep's head) + <i>E. faecalis</i>	0.0	N/A	5	0	0.00	(0.00; 0.43)	0	0.00	(0.00; 0.43)	0.00	(-0.43; 0.43)
		4.0	N/A	20	8	0.40	(0.22; 0.61)	8	0.40	(0.22; 0.61)	0.00	(-0.28; 0.28)
		65.0	N/A	5	5	1.00	(0.57; 1.00)	5	1.00	(0.57; 1.00)	0.00	(-0.43; 0.43)
Sealed concrete	<i>L. mono.</i> 1/2a (goat's brain tissues)	0.0	N/A	5	0	0.00	(0.00; 0.43)	0	0.00	(0.00; 0.43)	0.00	(-0.43; 0.43)
		4.8	N/A	20	12	0.60	(0.39; 0.78)	12	0.60	(0.39; 0.78)	0.00	(-0.28; 0.28)
		36.0	N/A	5	5	1.00	(0.57; 1.00)	5	1.00	(0.57; 1.00)	0.00	(-0.43; 0.43)

^aI – Inoculum level which was determined only for the environmental samples.

^bUCL – Upper Confidence Limit.

^cLCL – Lower Confidence Limit.

^dN – Number of test portions.

^eX – Number of positive test portions.

^fPOD_{CP} – Candidate method presumptive positive outcomes divided by the total number of trials.

^gPOD_{CC} – Candidate method confirmed positive outcomes divided by the total number of trials.

^hdPOD_{CP} – Difference between the candidate method presumptive and candidate method confirmed result POD values.

ⁱ95% CI – If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

^jThe data from the independent matrix study performed by independent laboratory, Q Laboratories.

^kN/A – Not applicable.

Table 4. Actero™ Listeria Broth Enrichment Followed by Using the BAX® System Assay for *L. monocytogenes* Detection in Environmental Samples - Candidate Method Compared to Reference Method Results (4)

Matrix	Strain info	I ^a		N ^d	Candidate Method (C)			Reference Method (R)			dPOD _{CR} ^h	95% CI ⁱ
		CFU/ sample	(UCL ^b , LCL ^c)		X ^e	POD _C ^f	95% CI	X	POD _R ^g	95% CI		
Stainless steel	<i>L. mono.</i> 1/2c (ready-to-eat food) + <i>P. aeruginosa</i> (field isolate)	0.0	N/A ^k	5	0	0.00	(0.00; 0.43)	0	0.00	(0.00; 0.43)	0.00	(-0.43; 0.43)
		5.0	N/A	20	16	0.80	(0.58; 0.92)	10	0.50	(0.30; 0.70)	0.30	(0.00; 0.53)
		63.0	N/A	5	5	1.00	(0.57; 1.00)	4	0.80	(0.38; 0.96)	0.20	(-0.26; 0.62)
Stainless steel ^j	<i>L. mono.</i> ATCC 7644 (1/2c) (ready-to-eat food)+ <i>P. aeruginosa</i> ATCC 15442	0.0	N/A	5	0	0.00	(0.00; 0.43)	0	0.00	(0.00; 0.43)	0.00	(-0.43; 0.43)
		90.0	N/A	20	9	0.45	(0.26; 0.66)	5	0.25	(0.11; 0.47)	0.20	(-0.09; 0.45)
		220	N/A	5	5	1.00	(0.57; 1.00)	5	1.00	(0.57; 1.00)	0.00	(-0.43; 0.43)
Plastic	<i>L. mono.</i> 4b (sheep's head) + <i>E. faecalis</i>	0.0	N/A	5	0	0.00	(0.00; 0.43)	0	0.00	(0.00; 0.43)	0.00	(-0.43; 0.43)
		4.0	N/A	20	8	0.40	(0.22; 0.61)	4	0.20	(0.08; 0.42)	0.20	(-0.08; 0.44)
		65.0	N/A	5	5	1.00	(0.57; 1.00)	5	1.00	(0.57; 1.00)	0.00	(-0.43; 0.43)
Sealed concrete	<i>L. mono</i> 1/2a (goat's brain tissues)	0.0	N/A	5	0	0.00	(0.00; 0.43)	0	0.00	(0.00; 0.43)	0.00	(-0.43; 0.43)
		4.8	N/A	20	12	0.60	(0.39; 0.78)	10	0.50	(0.30; 0.70)	0.10	(-0.19; 0.37)
		36.0	N/A	5	5	1.00	(0.57; 1.00)	5	1.00	(0.57; 1.00)	0.00	(-0.43; 0.43)

^aI – Inoculum level which was determined only for the environmental samples.

^bUCL – Upper Confidence Limit.

^cLCL – Lower Confidence Limit.

^dN – Number of test portions.

^eX – Number of positive test portions.

^fPOD_C – Candidate method positive outcomes divided by the total number of trials.

^gPOD_R – Reference method positive outcomes divided by the total number of trials.

^hdPOD_{CR} – Difference between the candidate method and reference method confirmed result POD values.

ⁱ95% CI – If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

^jThe data from the independent matrix study performed by independent laboratory Q Laboratories.

^kN/A – Not applicable.

Table 5. Actero™ Listeria Broth Enrichment Followed by Using the BAX® System Assay for *L. monocytogenes* Detection in Food Samples - Candidate Method Presumptive Compared to Candidate Method Confirmed Results (4)

Matrix	Strain info	MPN ^a		N ^d	Candidate Presumptive (CP)			Candidate Confirmed (CC)			dPOD _{CP} ^h	95% CI ⁱ
		CFU/sample	(UCL ^b , LCL ^c)		X ^e	POD _{CP} ^f	95% CI	X	POD _{CC} ^g	95% CI		
Soft Mexican style cheese	<i>L. mono.</i> 4b (sheep's head)	0.00	(0.00; 0.43)	5	0	0.00	(0.00; 0.43)	0	0.00	(0.00; 0.43)	0.00	(-0.43; 0.43)
		0.30	(0.13; 0.50)	20	19	0.95	(0.76; 0.99)	19	0.95	(0.76; 0.99)	0.00	(-0.19; 0.19)
		13.90	(1.00; 37.90)	5	5	1.00	(0.57; 1.00)	5	1.00	(0.57; 1.00)	0.00	(-0.43; 0.43)
Soft Mexican style cheese ^j	<i>L. mono.</i> 4b (sheep's head)	0.00	(0.00; 0.43)	5	0	0.00	(0.00; 0.43)	0	0.00	(0.00; 0.43)	0.00	(-0.43; 0.43)
		0.41	(0.22; 0.69)	20	7	0.35	(0.18; 0.57)	7	0.35	(0.18; 0.57)	0.00	(-0.28; 0.28)
		6.60	(2.80; 15.00)	5	5	1.00	(0.57; 1.00)	5	1.00	(0.57; 1.00)	0.00	(-0.43; 0.43)
Frankfurter	<i>L. mono.</i> 1/2a (milk), heat stressed	0.00	(0.00; 0.43)	5	0	0.00	(0.00; 0.43)	0	0.00	(0.00; 0.43)	0.00	(-0.43; 0.43)
		0.30	(0.13; 0.53)	20	14	0.70	(0.48; 0.85)	14	0.70	(0.48; 0.85)	0.00	(-0.27; 0.27)
		3.90	(1.85; 8.10)	5	5	1.00	(0.57; 1.00)	5	1.00	(0.57; 1.00)	0.00	(-0.43; 0.43)
Fresh bagged spinach ^k	<i>L. mono.</i> 1/2a (lettuce), + <i>L. welshimeri</i> (natural contamination)	0.00	(0.00; 0.43)	5	0	0.00	(0.00; 0.43)	0	0.00	(0.00; 0.43)	0.00	(-0.43; 0.43)
		1.40 ^l	(0.89; 2.38)	20	15	0.75	(0.53; 0.89)	15	0.75	(0.53; 0.89)	0.00	(-0.26; 0.26)
		1000.00	(4.80; 1000)	5	5	1.00	(0.57; 1.00)	5	1.00	(0.57; 1.00)	0.00	(-0.43; 0.43)
Frozen cooked shrimp	<i>L. mono.</i> 1/2a (dead goat), heat stressed	0.00	(0.00; 0.43)	5	0	0.00	(0.00; 0.43)	0	0.00	(0.00; 0.43)	0.00	(-0.43; 0.43)
		1.25	(0.78; 2.20)	20	11	0.55	(0.34; 0.74)	12	0.60	(0.39; 0.78)	-0.05	(-0.24; 0.33)
		6.65	(3.06; 14.45)	5	5	1.00	(0.57; 1.00)	5	1.00	(0.57; 1.00)	0.00	(-0.43; 0.43)
Cold smoked salmon	<i>L. mono.</i> 4b (Mexican style cheese)	0.00	(0.00; 0.43)	5	0	0.00	(0.00; 0.43)	0	0.00	(0.00; 0.43)	0.00	(-0.43; 0.43)
		0.76	(0.44; 1.27)	20	10	0.50	(0.30; 0.70)	10	0.50	(0.30; 0.70)	0.00	(-0.28; 0.28)
		1000.00	(4.80; 1000)	5	5	1.00	(0.57; 1.00)	5	1.00	(0.57; 1.00)	0.00	(-0.43; 0.43)

^aMPN – Most Probable Number is based on the POD of reference method test portions using the LCF MPN calculator.

^bUCL – Upper Confidence Limit.

^cLCL – Lower Confidence Limit.

^dN – Number of test portions.

^eX – Number of positive test portions.

^fPOD_{CP} – Candidate method presumptive positive outcomes divided by the total number of trials.

^gPOD_{CC} – Candidate method confirmed positive outcomes divided by the total number of trials.

^hdPOD_{CP} – Difference between the candidate method presumptive and candidate method confirmed result POD values.

ⁱ95% CI – If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

^jThe data from the independent matrix study performed by independent laboratory, University of Guelph Laboratory Services Division.

^kFresh bagged spinach samples naturally contaminated with *L. welshimeri* and artificially contaminated with *L. monocytogenes* MRS0437 were used.

^lData of natural contamination with *L. welshimeri* were included in the MPN calculation.

Table 6. Actero™ Listeria Broth Enrichment Followed by Using the BAX® System Assay for *L. monocytogenes* Detection in Food Samples - Candidate Method Compared to Reference Method Results (4)

Matrix	Strain info	MPN ^a		N ^d	Candidate Method (C)			Reference Method (R)			dPOD _{CR} ^h	95% CI ⁱ
		CFU/sample	(UCL ^b , LCL ^c)		X ^e	POD _C ^f	95% CI	X	POD _R ^g	95% CI		
Soft Mexican style cheese	<i>L. mono.</i> 4b (sheep's head)	0.00	(0.00; 0.43)	5	0	0.00	(0.00; 0.43)	0	0.00	(0.00; 0.43)	0.00	(-0.43; 0.43)
		0.30	(0.13; 0.50)	20	19	0.95	(0.76; 0.99)	6	0.30	(0.15; 0.52)	0.65	(0.36; 0.81)
		13.90	(1.00; 37.90)	5	5	1.00	(0.57; 1.00)	5	1.00	(0.57; 1.00)	0.00	(-0.43; 0.43)
Soft Mexican style cheese ^l	<i>L. mono.</i> 4b (sheep's head)	0.00	(0.00; 0.43)	5	0	0.00	(0.00; 0.43)	0	0.00	(0.00; 0.43)	0.00	(-0.43; 0.43)
		0.41	(0.22; 0.69)	20	7	0.35	(0.18; 0.57)	5	0.25	(0.11; 0.47)	0.10	(-0.18; 0.36)
		6.60	(2.80; 15.00)	5	5	1.00	(0.57; 1.00)	5	1.00	(0.57; 1.00)	0.00	(-0.43; 0.43)
Frankfurter	<i>L. mono.</i> 1/2a (milk), heat stressed	0.00	(0.00; 0.43)	5	0	0.00	(0.00; 0.43)	0	0.00	(0.00; 0.43)	0.00	(-0.43; 0.43)
		0.30	(0.13; 0.53)	20	14	0.70	(0.48; 0.85)	7	0.35	(0.18; 0.57)	0.35	(0.04; 0.58)
		3.90	(1.85; 8.10)	5	5	1.00	(0.57; 1.00)	5	1.00	(0.57; 1.00)	0.00	(-0.43; 0.43)
Fresh bagged spinach ^k	<i>L. mono.</i> 1/2a (lettuce), + <i>L. welshimeri</i> (natural contamination)	0.00	(0.00; 0.43)	5	0	0.00	(0.00; 0.43)	0	0.00	(0.00; 0.43)	0.00	(-0.43; 0.43)
		1.40 ^l	(0.89; 2.38)	20	15	0.75	(0.53; 0.89)	15	0.75	(0.53; 0.89)	0.00	(-0.26; 0.26)
		1000.00	(4.80; 1000)	5	5	1.00	(0.57; 1.00)	5	1.00	(0.57; 1.00)	0.00	(-0.43; 0.43)
Frozen cooked shrimp	<i>L. mono.</i> 1/2a (dead goat), heat stressed	0.00	(0.00; 0.43)	5	0	0.00	(0.00; 0.43)	0	0.00	(0.00; 0.43)	0.00	(-0.43; 0.43)
		1.25	(0.78; 2.20)	20	11	0.55	(0.34; 0.74)	16	0.80	(0.58; 0.92)	-0.25	(-0.49; 0.04)
		6.65	(3.06; 14.45)	5	5	1.00	(0.57; 1.00)	5	1.00	(0.57; 1.00)	0.00	(-0.43; 0.43)
Cold smoked salmon	<i>L. mono.</i> 4b (Mexican style cheese)	0.00	(0.00; 0.43)	5	0	0.00	(0.00; 0.43)	0	0.00	(0.00; 0.43)	0.00	(-0.43; 0.43)
		0.76	(0.44; 1.27)	20	10	0.50	(0.30; 0.70)	10	0.50	(0.30; 0.70)	0.00	(-0.28; 0.28)
		1000.00	(4.80; 1000)	5	5	1.00	(0.57; 1.00)	5	1.00	(0.57; 1.00)	0.00	(-0.43; 0.43)

^aI – Inoculum level which was determined only for the environmental samples.

^bUCL – Upper Confidence Limit.

^cLCL – Lower Confidence Limit.

^dN – Number of test portions.

^eX – Number of positive test portions.

^fPOD_C – Candidate method positive outcomes divided by the total number of trials.

^gPOD_R – Reference method positive outcomes divided by the total number of trials.

^hdPOD_{CR} – Difference between the candidate method and reference method confirmed result POD values.

ⁱ95% CI – If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

^jThe data from the independent matrix study performed by independent laboratory, University of Guelph Laboratory Services Division.

^kFresh bagged spinach samples naturally contaminated with *L. welshimeri* and artificially contaminated with *L. monocytogenes* MRS0437 were used.

^lData of natural contamination with *L. welshimeri* were included in the MPN calculation.

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