



# CERTIFICATION

## AOAC Research Institute *Performance Tested Methods*<sup>SM</sup>

Certificate No.  
**081401**

The AOAC Research Institute hereby certifies the method known as:

### **BAX<sup>®</sup> System Real-Time PCR Assay for Genus *Listeria***

manufactured by

**Hygiena  
2 Boulden Circle  
New Castle, DE 19720  
USA**

This method has been evaluated and certified according to the policies and procedures of the AOAC *Performance Tested Methods*<sup>SM</sup> Program. This certificate indicates an AOAC Research Institute Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC Research Institute *Performance Tested Methods*<sup>SM</sup> certification mark on the above-mentioned method for the period below. Renewal may be granted by the Expiration Date under the rules stated in the licensing agreement.

A handwritten signature in black ink, appearing to read "Bradley A. Stawick".

Bradley A. Stawick, Senior Director  
Signature for AOAC Research Institute

Issue Date	December 10, 2024
Expiration Date	December 31, 2025

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<b>METHOD NAME</b> BAX® System Real-Time PCR Assay for Genus <i>Listeria</i> Formerly DuPont™ Bax® System Real-Time PCR Assay for Genus <i>Listeria</i>	<b>CATALOG NUMBERS</b> BAX System Assay KIT2019 (D15131113), 24 LEB Complete MED2005 (D14654989)
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<b>INDEPENDENT LABORATORY</b> Q Laboratories, Inc. 1400 Harrison Avenue Cincinnati, OH 45214 USA
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<b>APPLICABILITY OF METHOD</b> Target organism – <i>Listeria</i> species (including <i>L. monocytogenes</i> , <i>L. innocua</i> , <i>L. ivanovii</i> , <i>L. welshimeri</i> , <i>L. seeligeri</i> , and <i>L. grayi</i> ).  Matrixes – USDA/FSIS 8.09: (125 g) – frankfurters (beef) (10 x 10 cm <sup>2</sup> ) – stainless steel, plastic, concrete FDA BAM Ch. 10 – (25 g) – cooked shrimp, bagged lettuce, queso fresco (Mexican style cheese) MODIFICATION MAY 2015 with Actero™ <i>Listeria</i> in conjunction with DuPont™ BAX® System Real-Time PCR Assay for Genus <i>Listeria</i> : FDA BAM Ch. 10 – (25 g) – soft Mexican-style cheese, bagged spinach, frozen cooked shrimp, cold smoked salmon USDA/FSIS 8.09 – (125 g) – Frankfurters (10 x 10 cm <sup>2</sup> ) – stainless steel, plastic, sealed concrete  Performance claims – Performance indistinguishable from or better than the corresponding reference method.	<b>REFERENCE METHODS</b>  U.S. Department of Agriculture-Food Safety and Inspection Service Microbiology Laboratory Guidebook Chapter 8.09 (2013) (2)  U.S. FDA Bacteriological Analytical Manual, Chapter 10 (2013) (3)
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<b>ORIGINAL CERTIFICATION DATE</b> August 01, 2014	<b>CERTIFICATION RENEWAL RECORD</b> Renewed through December 2025.
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<b>METHOD MODIFICATION RECORD</b>	<b>SUMMARY OF MODIFICATION</b>
1. May 2015	1. Modification to include Actero™ <i>Listeria</i> and use of a single step enrichment for 081401. Matrix extension to include additional matrixes.
2. March 2017 Level 1	2. Name change from DuPont Nutrition & Health to Qualicon Diagnostics LLC., a Hygiena company.
3. December 2017 Level 2	3. Increase in hold time after tablet rehydration.
4. January 2018 Level 1	4. Inserts, manuals, and labels updated to Hygiena.
5. May 2019 Level 1	5. Editorial insert changes and corporate address.
6. December 2019 Level 1	6. Editorial changes.
7. December 2021 Level 1	7. Editorial changes.
8. January 2024 Level 1	8. Editorial changes.
9. December 2024 Level 1	9. Editorial changes.

Under this AOAC <i>Performance Tested Methods</i> <sup>SM</sup> License Number, 081401 this method is distributed by: NONE	Under this AOAC <i>Performance Tested Methods</i> <sup>SM</sup> License Number, 081401 this method is distributed as: NONE
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**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 the genus *Listeria*, 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 (*Listeria*-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 Genus *Listeria* demonstrates accuracy and reliability statistically equivalent to the reference culture methods used in this study, with a number of additional benefits. *L. grayi* has been included into the inclusivity panel and is easily detectable. Simplified lysis procedure reduces the total lysis time by more than 30 minutes when compared to previously validated BAX System assays for *Listeria*. Furthermore, this real-time assay reduces the automated processing time by about 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 for *Listeria* species detection.

Table 2. Candidate method presumptive results vs. confirmed results – POD for food matrixes

Matrix	Strain	MPN <sup>a</sup> /test portion	N <sup>b</sup>	Candidate method presumptive			Candidate method confirmed			dPO D <sub>CP</sub> <sup>f</sup>	95% CI <sup>g</sup>
				X <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	x	POD <sub>CC</sub> <sup>e</sup>	95% CI		
Frankfurters BAX® System 28 h	<i>L. monocytogenes</i> DD 1309	1.2	5	5	1	(0.57, 1.0)	5	1	(0.57, 1.0)	0	(-0.45, 0.45)
		0.12	20	11	0.55	(0.34, 0.74)	11	0.55	(0.34, 0.74)	0	(-0.14, 0.14)
		Uninoculated	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	1.2	5	5	1	(0.57, 1.0)	5	1	(0.57, 1.0)	0	(-0.45, 0.45)
		0.12	20	14	0.7	(0.48, 0.85)	11*	0.55	(0.34, 0.74)	0.15	(-0.02, 0.32)
		Uninoculated	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	1.2	5	5	1	(0.57, 1.0)	5	1	(0.57, 1.0)	0	(-0.45, 0.45)
		0.12	20	3	0.15	(0.05, 0.36)	3	0.15	(0.05, 0.36)	0	(-0.14, 0.14)
		Uninoculated	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	2.9	5	3	0.6	(0.23, 0.88)	3	0.6	(0.23, 0.88)	0	(-0.46, 0.46)
		0.29	20	7	0.35	(0.18, 0.56)	8	0.4	(0.21, 0.61)	- 0.05	(-0.05, 0.15)
		Uninoculated	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Bagged spinach BAX® System 48 hrs	<i>L. monocytogenes</i> DD 1283	2.9	5	3	0.6	(0.23, 0.88)	3	0.6	(0.23, 0.88)	0	(-0.46, 0.46)
		0.29	20	8	0.4	(0.21, 0.61)	8	0.4	(0.21, 0.61)	0	(-0.14, 0.14)
		Uninoculated	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Bagged spinach FDA-BAM 48 h	<i>L. monocytogenes</i> DD 1283	2.9	5	5	1	(0.57, 1.0)	5	1	(0.57, 1.0)	0	(-0.45, 0.45)
		0.29	20	4	0.2	(0.08, 0.41)	4	0.2	(0.08, 0.41)	0	(-0.14, 0.14)
		Uninoculated	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. innocua</i> DD 3244	10.3	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		1.03	20	13	0.65	(0.43, 0.81)	13	0.65	(0.43, 0.81)	0	(-0.14, 0.14)
		Uninoculated	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. innocua</i> DD 3244	10.3	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		1.03	20	13	0.65	(0.43, 0.81)	13	0.65	(0.43, 0.81)	0	(-0.14, 0.14)
		Uninoculated	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. innocua</i> DD 3244	10.3	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		1.03	20	12	0.6	(0.36, 0.78)	12	0.6	(0.36, 0.78)	0	(-0.14, 0.14)
		Uninoculated	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Queso fresco cheese <sup>h</sup> BAX® System 26 h	<i>L. innocua</i> ATCC 33090	3.08	5	5	1	(0.57, 1.0)	5	1	(0.57, 1.0)	0	(-0.45, 0.45)
		0.84	20	15	0.75	(0.53, 0.88)	15	0.75	(0.53, 0.88)	0	(-0.25, 0.25)
		Uninoculated	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Queso fresco cheese <sup>h</sup> BAX® System 48 h	<i>L. innocua</i> ATCC 33090	3.08	5	5	1	(0.57, 1.0)	5	1	(0.57, 1.0)	0	(-0.45, 0.45)
		0.84	20	15	0.75	(0.53, 0.88)	15	0.75	(0.53, 0.88)	0	(-0.25, 0.25)
		Uninoculated	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Queso fresco cheese <sup>h</sup> FDA-BAM 48 h	<i>L. innocua</i> ATCC 33090	3.08	5	5	1	(0.57, 1.0)	5	1	(0.57, 1.0)	0	(-0.45, 0.45)
		0.84	20	18	0.9	(0.69, 0.97)	18	0.9	(0.69, 0.97)	0	(-0.21, 0.21)
		Uninoculated	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Cooked shrimp BAX® System 24 h	<i>L. ivanovii</i> DD 649	11.9	5	5	1	(0.57, 1.0)	5	1	(0.57, 1.0)	0	(-0.45, 0.45)
		1.19	20	14	0.7	(0.48, 0.85)	15	0.75	(0.53, 0.88)	- 0.05	(-0.05, 0.15)
		Uninoculated	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Cooked shrimp BAX® System 48 h	<i>L. ivanovii</i> DD 649	11.9	5	5	1	(0.57, 1.0)	5	1	(0.57, 1.0)	0	(-0.45, 0.45)
		1.19	20	15	0.75	(0.53, 0.88)	15	0.75	(0.53, 0.88)	0	(-0.14, 0.14)
		Uninoculated	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)

Cooked shrimp FDA-BAM 48 h	<i>L. ivanovii</i> DD 649	11.9	5	5	1	(0.57, 1.0)	5	1	(0.57, 1.0)	0	(-0.45, 0.45)
		1.19	20	14	0.70	(0.48, 0.85)	14	0.70	(0.48, 0.85)	0	(-0.14, 0.14)
		Uninoculated	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)

<sup>a</sup>Most Probable Number is based on the POD of reference method test portions using the Least Cost Formulations MPN calculator (6), with 95% confidence interval.

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

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

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

<sup>f</sup>dPOD<sub>CP</sub> = Difference between the candidate method presumptive result and candidate method confirmed result POD values. <sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

<sup>h</sup>Independent Laboratory Study.

\*Note: 11 samples cultured confirmed as described in the USDA-MLG. An additional 3 samples tested positive by BAX and culture confirmed by direct plating from 24 LEB after 48 h of incubation.

**Table 3. Candidate method presumptive results vs. confirmed results – POD for environmental surfaces**

Surface	Strain	Listeria inoculum level <sup>a</sup> /test portion	N <sup>b</sup>	Candidate method presumptive			Candidate method confirmed			dPOD <sub>CP</sub> <sup>f</sup>	95% CI <sup>g</sup>
				X <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	x	POD <sub>CC</sub> <sup>e</sup>	95% CI		
304 2B Stainless steel BAX® System 24 h	<i>L. monocytogenes</i> DD 1144 <i>Pseudomonas aeruginosa</i> DD6924	1120	5	5	1	(0.57, 1.0)	5	1	(0.57, 1.0)	0	(-0.45, 0.45)
		112	20	13	0.65	(0.43, 0.81)	13	0.65	(0.43, 0.81)	0	(-0.14, 0.14)
		Uninoculated	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
304 2B Stainless steel BAX® System 48 h	<i>L. monocytogenes</i> DD 1144 <i>Pseudomonas aeruginosa</i> DD6924	1120	5	5	1	(0.57, 1.0)	5	1	(0.57, 1.0)	0	(-0.45, 0.45)
		112	20	13	0.65	(0.43, 0.81)	13	0.65	(0.43, 0.81)	0	(-0.14, 0.14)
		Uninoculated	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
304 2B Stainless steel USDA-MLG 48 h	<i>L. monocytogenes</i> DD 1144 <i>Pseudomonas aeruginosa</i> DD6924	1120	5	5	1	(0.57, 1.0)	5	1	(0.57, 1.0)	0	(-0.45, 0.45)
		112	20	13	0.65	(0.43, 0.81)	13	0.65	(0.43, 0.81)	0	(-0.14, 0.14)
		Uninoculated	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
304 2B Stainless steel <sup>h</sup> BAX® System 24 h	<i>L. monocytogenes</i> ATCC 7644 <i>Pseudomonas aeruginosa</i> ATCC 15442	3.2 x10 <sup>4</sup>	5	5	1	(0.57, 1.0)	5	1	(0.57, 1.0)	0	(-0.45, 0.45)
		3.2 x10 <sup>3</sup>	20	8	0.4	(0.21, 0.61)	7	0.35	(0.18, 0.56)	0.05	(-0.23, 0.32)
		Uninoculated	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
304 2B Stainless steel <sup>h</sup> BAX® System 48 h	<i>L. monocytogenes</i> ATCC 7644 <i>Pseudomonas aeruginosa</i> ATCC 15442	3.2 x10 <sup>4</sup>	5	5	1	(0.57, 1.0)	5	1	(0.57, 1.0)	0	(-0.45, 0.45)
		3.2 x10 <sup>3</sup>	20	7	0.35	(0.18, 0.56)	7	0.35	(0.18, 0.56)	0	(-0.27, 0.27)
		Uninoculated	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
304 2B Stainless steel <sup>h</sup> USDA-MLG 48 h	<i>L. monocytogenes</i> ATCC 7644 <i>Pseudomonas aeruginosa</i> ATCC 15442	3.2 x10 <sup>4</sup>	5	5	1	(0.57, 1.0)	5	1	(0.57, 1.0)	0	(-0.45, 0.45)
		3.2 x10 <sup>3</sup>	20	2	0.1	(0.02, 0.30)	2	0.1	(0.02, 0.30)	0	(-0.21, 0.21)
		Uninoculated	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Plastic BAX® System 24 h	<i>L. seeligeri</i> DD 2874 <i>Pseudomonas aeruginosa</i>	1750	5	5	1	(0.57, 1.0)	5	1	(0.57, 1.0)	0	(-0.45, 0.45)
		175	20	14	0.7	(0.48, 0.85)	14	0.7	(0.48, 0.85)	0	(-0.14, 0.14)
		Uninoculated	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)

	DD6924										
Plastic BAX® System 48 h	<i>L. seeligeri</i> DD 2874 <i>Pseudomonas aeruginosa</i> DD6924	1750	5	5	1	(0.57, 1.0)	5	1	(0.57, 1.0)	0	(-0.45, 0.45)
		175	20	14	0.7	(0.48, 0.85)	14	0.7	(0.48, 0.85)	0	(-0.14, 0.14)
		Uninoculated	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Plastic USDA-MLG 48 h	<i>L. seeligeri</i> DD 2874 <i>Pseudomonas aeruginosa</i> DD6924	1750	5	5	1	(0.57, 1.0)	5	1	(0.57, 1.0)	0	(-0.45, 0.45)
		175	20	17*	0.85	(0.63, 0.94)	16	0.8	(0.58, 0.91)	0.05	(-0.05, 0.15)
		Uninoculated	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Concrete BAX® System 24 h	<i>L. welshimeri</i> DD 4096 <i>Enterococcus faecalis</i> DD10565	750	5	5	1	(0.57, 1.0)	5	1	(0.57, 1.0)	0	(-0.45, 0.45)
		75	20	5	0.25	(0.11, 0.46)	5	0.25	(0.11, 0.46)	0	(-0.14, 0.14)
		Uninoculated	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Concrete BAX® System 48 h	<i>L. welshimeri</i> DD 4096 <i>Enterococcus faecalis</i> DD10565	750	5	5	1	(0.57, 1.0)	5	1	(0.57, 1.0)	0	(-0.45, 0.45)
		75	20	5	0.25	(0.11, 0.46)	5	0.25	(0.11, 0.46)	0	(-0.14, 0.14)
		Uninoculated	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Concrete USDA-MLG 48 h	<i>L. welshimeri</i> DD 4096 <i>Enterococcus faecalis</i> DD10565	750	5	4	0.3	(0.37, 0.96)	4	0.3	(0.37, 0.96)	0	(-0.45, 0.45)
		75	20	6	0.3	(0.14, 0.51)	6	0.3	(0.14, 0.51)	0	(-0.14, 0.14)
		Uninoculated	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)

<sup>a</sup>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.

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

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

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

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

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

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

<sup>h</sup>Independent Laboratory Study.

<sup>i</sup>Note: Sample tested using the BAX® System assay at 48 h 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 comparison results - POD for food matrixes (1)**

Matrix	Strain	MPN <sup>a</sup> /test portion	N <sup>b</sup>	Test method			Reference method			dPOD <sup>f</sup>	95% CI <sup>g</sup>
				X <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	x	POD <sub>CC</sub> <sup>e</sup>	95% CI		
Frankfurters BAX® System 28 h	<i>L. monocytogenes</i> DD 1309	1.2	5	5	1	(0.57, 1.0)	5	1	(0.57, 1.0)	0	(-0.45, 0.45)
		0.12	20	11	0.55	(0.34, 0.74)	3	0.15	(0.05, 0.36)	0.4	(0.10, 0.61)
		Uninoculated	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	1.2	5	5	1	(0.57, 1.0)	5	1	(0.57, 1.0)	0	(-0.45, 0.45)
		0.12	20	14	0.7	(0.48, 0.85)	3	0.15	(0.05, 0.36)	0.55	(0.24, 0.73)
		Uninoculated	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	1.2	5	5	1	(0.57, 1.0)	5	1	(0.57, 1.0)	0	(-0.45, 0.45)
		0.12	20	3	0.15	(0.05, 0.36)	3	0.15	(0.05, 0.36)	0	(-0.14, 0.14)
		Uninoculated	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	2.9	5	3	0.6	(0.23, 0.88)	5	1	(0.57, 1.0)	-0.4	(-0.76, 0.11)
		0.29	20	7	0.35	(0.18, 0.56)	4	0.2	(0.08, 0.41)	0.15	(-0.12, 0.40)
		Uninoculated	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	2.9	5	3	0.6	(0.23, 0.88)	5	1	(0.57, 1.0)	-0.4	(-0.76, 0.11)
		0.29	20	8	0.4	(0.21, 0.61)	4	0.2	(0.08, 0.41)	0.2	(-0.08, 0.44)
		Uninoculated	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	2.9	5	5	1	(0.57, 1.0)	5	1	(0.57, 1.0)	0	(-0.45, 0.45)
		0.29	20	4	0.2	(0.08, 0.41)	4	0.2	(0.08, 0.41)	0	(-0.14, 0.14)
		Uninoculated	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. innocua</i> DD 3244	10.3	5	5	1	(0.57, 1.0)	5	1	(0.57, 1.0)	0	(-0.45, 0.45)
		1.03	20	13	0.65	(0.43, 0.81)	12	0.6	(0.38, 0.78)	0.05	(-0.23, 0.32)
		Uninoculated	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.43, 0.43)
Queso fresco cheese	<i>L. innocua</i>	10.3	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)

BAX® System	Strain	MPN <sup>a</sup> /test portion	N <sup>b</sup>	X <sup>c</sup>	POD <sub>c</sub> <sup>d</sup>	95% CI	x	POD <sub>R</sub> <sup>e</sup>	95% CI	dPOD <sub>c</sub> <sup>f</sup>	95% CI <sup>g</sup>
BAX® System 48 h	DD 3244	1.03	20	13	0.65	(0.43, 0.81)	12	0.6	(0.36, 0.78)	0.05	(-0.23, 0.32)
		Uninoculated	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.43, 0.43)
Queso fresco cheese FDA-BAM 48 h	<i>L. innocua</i> DD 3244	10.3	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		1.03	20	12	0.6	(0.36, 0.78)	12	0.6	(0.36, 0.78)	0	(-0.14, 0.14)
		Uninoculated	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Queso fresco cheese <sup>h</sup> BAX® System 26 h	<i>L. innocua</i> ATCC 33090	3.08	5	5	1	(0.57, 1.0)	0	0	(0, 0.43)	0	(-0.45, 0.45)
		0.84	20	15	0.75	(0.53, 0.88)	18	0.9	(0.69, 0.97)	-0.15	(-0.33, 0.09)
		Uninoculated	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.43, 0.43)
Queso fresco cheese <sup>h</sup> BAX® System 48 h	<i>L. innocua</i> ATCC 33090	3.08	5	5	1	(0.57, 1.0)	0	0	(0, 0.43)	0	(-0.45, 0.45)
		0.84	20	15	0.75	(0.53, 0.88)	18	0.9	(0.69, 0.97)	-0.15	(-0.33, 0.09)
		Uninoculated	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.43, 0.43)
Queso fresco cheese <sup>h</sup> FDA-BAM 48 h	<i>L. innocua</i> ATCC 33090	3.08	5	5	1	(0.57, 1.0)	0	0	(0, 0.43)	0	(-0.45, 0.45)
		0.84	20	18	0.9	(0.69, 0.97)	18	0.9	(0.69, 0.97)	0	(-0.21, 0.21)
		Uninoculated	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.43, 0.43)
Cooked shrimp BAX® System 24 h	<i>L. ivanovii</i> DD 649	11.9	5	5	1	(0.57, 1.0)	5	1	(0.57, 1.0)	0	(-0.45, 0.45)
		1.19	20	14	0.7	(0.48, 0.85)	14	0.7	(0.48, 0.85)		(-0.26, 0.26)
		Uninoculated	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.43, 0.43)
Cooked shrimp BAX® System 48 h	<i>L. ivanovii</i> DD 649	11.9	5	5	1	(0.57, 1.0)	5	1	(0.57, 1.0)	0	(-0.45, 0.45)
		1.19	20	15	0.75	(0.53, 0.88)	14	0.70	(0.48, 0.85)	0.05	(-0.21, 0.30)
		Uninoculated	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.43, 0.43)
Cooked shrimp FDA-BAM 48 h	<i>L. ivanovii</i> DD 649	11.9	5	5	1	(0.57, 1.0)	5	1	(0.57, 1.0)	0	(-0.45, 0.45)
		1.19	20	14	0.70	(0.48, 0.85)	14	0.70	(0.48, 0.85)	0	(-0.14, 0.14)
		Uninoculated	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)

<sup>a</sup>MPN = Most Probable Number is based on the POD of reference method test portions using the Least Cost Formulations MPN calculator [6], with 95% confidence interval.

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

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

<sup>d</sup>POD<sub>c</sub> = Confirmed candidate method positive outcomes divided by the total number of trials.

<sup>e</sup>POD<sub>R</sub> = Confirmed reference method positive outcomes divided by the total number of trials.

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

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

<sup>h</sup>Independent Laboratory Study.

Table 5. Method comparison results - POD for environmental matrices (1)

Matrix	Strain	MPN <sup>a</sup> /test portion	N <sup>b</sup>	Test method			Reference method			dPOD <sub>c</sub> <sup>f</sup>	95% CI <sup>g</sup>
				X <sup>c</sup>	POD <sub>c</sub> <sup>d</sup>	95% CI	x	POD <sub>R</sub> <sup>e</sup>	95% CI		
304 2B Stainless steel BAX® System 24 h	<i>L. monocytogenes</i> DD 1144 <i>Pseudomonas aeruginosa</i> DD6924	1120	5	5	1	(0.57, 1.0)	5	1	(0.57, 1.0)	0	(-0.45, 0.45)
		112	20	13	0.65	(0.43, 0.81)	13	0.65	(0.43, 0.81)	0	(-0.27, 0.27)
		Uninoculated	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 1144 <i>Pseudomonas aeruginosa</i> DD6924	1120	5	5	1	(0.57, 1.0)	5	1	(0.57, 1.0)	0	(-0.45, 0.45)
		112	20	13	0.65	(0.43, 0.81)	13	0.65	(0.43, 0.81)	0	(-0.27, 0.27)
		Uninoculated	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 1144 <i>Pseudomonas aeruginosa</i> DD6924	1120	5	5	1	(0.57, 1.0)	5	1	(0.57, 1.0)	0	(-0.45, 0.45)
		112	20	13	0.65	(0.43, 0.81)	13	0.65	(0.43, 0.81)	0	(-0.14, 0.14)
		Uninoculated	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
304 2B Stainless steel <sup>h</sup> BAX® System 24 h	<i>L. monocytogenes</i> ATCC 7644 <i>Pseudomonas aeruginosa</i> ATCC 15442	3.2 x10 <sup>4</sup>	5	5	1	(0.57, 1.0)	5	1	(0.57, 1.0)	0	(-0.45, 0.45)
		3.2 x10 <sup>3</sup>	20	8	0.4	(0.21, 0.61)	2	0.10	(0.02, 0.30)	0.30	(0.02, 0.52)
		Uninoculated	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.43, 0.43)
304 2B Stainless steel <sup>h</sup> BAX® System 48 h	<i>L. monocytogenes</i> ATCC 7644 <i>Pseudomonas aeruginosa</i> ATCC 15442	3.2 x10 <sup>4</sup>	5	5	1	(0.57, 1.0)	5	1	(0.57, 1.0)	0	(-0.45, 0.45)
		3.2 x10 <sup>3</sup>	20	7	0.35	(0.18, 0.56)	2	0.10	(0.02, 0.30)	0.25	(-0.01, 0.47)
		Uninoculated	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.43, 0.43)
304 2B Stainless steel <sup>h</sup> USDA-MLG 48 h	<i>L. monocytogenes</i> ATCC 7644 <i>Pseudomonas aeruginosa</i> ATCC 15442	3.2 x10 <sup>4</sup>	5	5	1	(0.57, 1.0)	5	1	(0.57, 1.0)	0	(-0.45, 0.45)
		3.2 x10 <sup>3</sup>	20	2	0.1	(0.02, 0.30)	2	0.1	(0.02, 0.30)	0	(-0.21, 0.21)
		Uninoculated	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.43, 0.43)
Plastic BAX® System 24 h	<i>L. seeligeri</i> DD 2874 <i>Pseudomonas aeruginosa</i> DD6924	2250	5	5	1	(0.57, 1.0)	5	1	(0.57, 1.0)	0	(-0.45, 0.45)
		250	20	14	0.7	(0.48, 0.85)	16	0.8	(0.58, 0.91)	-0.1	(-0.34, 0.16)
		Uninoculated	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.43, 0.43)
Plastic BAX® System 48 h	<i>L. seeligeri</i> DD 2874 <i>Pseudomonas aeruginosa</i> DD6924	2250	5	5	1	(0.57, 1.0)	5	1	(0.57, 1.0)	0	(-0.45, 0.45)
		250	20	14	0.7	(0.48, 0.85)	16	0.8	(0.58, 0.91)	-0.1	(-0.34, 0.16)
		Uninoculated	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.43, 0.43)
Plastic USDA-MLG 48 h	<i>L. seeligeri</i> DD 2874 <i>Pseudomonas aeruginosa</i> DD6924	2250	5	5	1	(0.57, 1.0)	5	1	(0.57, 1.0)	0	(-0.45, 0.45)
		250	20	17*	0.85	(0.63, 0.94)	16	0.8	(0.58, 0.91)	0.05	(-0.05, 0.15)
		Uninoculated	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Concrete BAX® System 24 h	<i>L. welshimeri</i> DD 4096 <i>Enterococcus</i>	750	5	5	1	(0.57, 1.0)	4	0.8	(0.37, 0.96)	0.2	(-0.26, 0.62)
		75	20	5	0.25	(0.11, 0.46)	6	0.3	(0.14, 0.51)	-0.05	(-0.31, 0.21)
		Uninoculated	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.43, 0.43)

	<i>faecalis</i> DD10565										
Concrete BAX® System 48 h	<i>L. welshimeri</i> DD 4096	750	5	5	1	(0.57, 1.0)	4	0.8	(0.37, 0.96)	0.2	(-0.26, 0.62)
		75	20	5	0.25	(0.11, 0.46)	6	0.3	(0.14, 0.51)	-0.05	(-0.30, 0.21)
	<i>Enterococcus faecalis</i> DD10565	Uninoculated	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.43, 0.43)
Concrete USDA-MLG 48 h	<i>L. welshimeri</i> DD 4096	750	5	4	0.3	(0.37, 0.96)	4	0.3	(0.37, 0.96)	0	(-0.45, 0.45)
		75	20	6	0.3	(0.14, 0.51)	6	0.3	(0.14, 0.51)	0	(-0.14, 0.14)
	<i>Enterococcus faecalis</i> DD10565	Uninoculated	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)

<sup>a</sup>MPN = Most Probable Number is based on the POD of reference method test portions using the Least Cost Formulations MPN calculator [6], with 95% confidence interval.

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

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

<sup>d</sup>POD<sub>c</sub> = Confirmed candidate method positive outcomes divided by the total number of trials.

<sup>e</sup>POD<sub>R</sub> = Confirmed reference method positive outcomes divided by the total number of trials.

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

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

<sup>h</sup>Independent Laboratory Study.

Table 11. BAX System inclusivity					
Strain ID (DD#)	Collection ID	Other strain designation (if available)	Serotype (if known)	Isolate source	BAX System result
566	<i>Listeria monocytogenes</i>	ATCC15313		Rabbit	POS
605	<i>Listeria monocytogenes</i>	ATCC19111		Poultry	POS
643	<i>Listeria murrayi</i>	ATCC25401		Standing corn stalks & leaves	NEG <sup>h</sup>
644	<i>Listeria innocua</i>	ATCC33090		Cow brain	POS
647	<i>Listeria monocytogenes</i>	ATCC19118	4c	Chicken	POS
648	<i>Listeria monocytogenes</i>	ATCC19114	4a	Animal tissue	POS
649	<i>Listeria ivanovii</i>	ATCC19119		Sheep	POS
650	<i>Listeria seeligeri</i>	ATCC 35967		Soil	POS
652	<i>Listeria monocytogenes</i>	ATCC 19117	4c	Chicken	POS
653	<i>Listeria monocytogenes</i>	ATCC 19115	4b	Human	POS
654	<i>Listeria welshimeri</i>	ATCC 35897		Decaying plant material	POS
892	<i>Listeria innocua</i>			Unknown	POS
898	<i>Listeria innocua</i>			Unknown	POS
921	<i>Listeria innocua</i>			Roast turkey	POS
922	<i>Listeria innocua</i>			Ham cured shoulder	POS
924	<i>Listeria innocua</i>			Ham cured shoulder	POS
927	<i>Listeria innocua</i>			Chopped pork and ham	POS
944	<i>Listeria murrayi</i>			Cornstalks and leaves	POS
1063	<i>Listeria innocua</i>			Chopped pork and ham	POS
1064	<i>Listeria innocua</i>			Chopped pork and ham	POS
1069	<i>Listeria monocytogenes</i>			Stuffed gammon joint	POS
1072	<i>Listeria monocytogenes</i>			Cheese and ham pancakes	POS
1144	<i>Listeria monocytogenes</i>		1/2a	Stilton cheese	POS
1145	<i>Listeria monocytogenes</i>		1/2a	Coleslaw salad	POS
1146	<i>Listeria monocytogenes</i>		1/2a	Lettuce	POS
1147	<i>Listeria monocytogenes</i>		1/2a	Pate	POS
1149	<i>Listeria monocytogenes</i>		1/2a	Raw milk	POS
1152	<i>Listeria monocytogenes</i>		1/2b	Pate	POS
1156	<i>Listeria innocua</i>			Lettuce	POS
1164	<i>Listeria ivanovii</i>			Radish	POS
1165	<i>Listeria ivanovii</i>			Belgian salami	POS

1167	<i>Listeria ivanovii</i>			Soft cheese	POS
1171	<i>Listeria ivanovii</i>			Unknown	POS
1172	<i>Listeria welshimeri</i>			Salami	POS
1174	<i>Listeria welshimeri</i>			Raw chicken	POS
1175	<i>Listeria welshimeri</i>			Sausage	POS
1176	<i>Listeria welshimeri</i>			Chicken	POS
1177	<i>Listeria welshimeri</i>			Smoked mackerel	POS
1179	<i>Listeria welshimeri</i>			Food	POS
1281	<i>Listeria monocytogenes</i>		3c	Cooked chicken	POS
1282	<i>Listeria monocytogenes</i>		3c	Unknown	POS
1283	<i>Listeria monocytogenes</i>		3b	Cooked turkey	POS
1285	<i>Listeria monocytogenes</i>		4(not 4b)	Cheese	POS
1286	<i>Listeria monocytogenes</i>		3c	Cooked chicken	POS
1287	<i>Listeria monocytogenes</i>		3a	Unknown	POS
1288	<i>Listeria monocytogenes</i>		3a	Cooked turkey	POS
1289	<i>Listeria seeligeri</i>			Crab pate	POS
1291	<i>Listeria seeligeri</i>			Lettuce	POS
1292	<i>Listeria seeligeri</i>			Cooked chicken	POS
1293	<i>Listeria monocytogenes</i>		3a	Pate	POS
1294	<i>Listeria monocytogenes</i>		4b	Ice cream	POS
1295	<i>Listeria monocytogenes</i>		3b	Pepper quiche	POS
1297	<i>Listeria seeligeri</i>			Pate	POS
1298	<i>Listeria seeligeri</i>			Chicken roll	POS
1299	<i>Listeria monocytogenes</i>		1/2b	Pork liver pate	POS
1300	<i>Listeria seeligeri</i>			Cooked ham	POS
1302	<i>Listeria monocytogenes</i>		1/2c	Hard boiled eggs	POS
1305	<i>Listeria monocytogenes</i>		3a	Boiled ham	POS
1306	<i>Listeria monocytogenes</i>		3b	Chicken liver pate	POS
1307	<i>Listeria monocytogenes</i>		3b	Pate	POS
1308	<i>Listeria monocytogenes</i>		4b	Cheese	POS
1309	<i>Listeria monocytogenes</i>		4b	Soft cheese	POS
1310	<i>Listeria monocytogenes</i>		3b	Chicken	POS
1311	<i>Listeria monocytogenes</i>		1/2c	Cooked meat	POS
1312	<i>Listeria monocytogenes</i>			Ice cream	POS
1313	<i>Listeria monocytogenes</i>		4b	Cheese	POS
1314	<i>Listeria monocytogenes</i>		4(not 4b)	Pate	POS
1315	<i>Listeria monocytogenes</i>		1/2c	Pate	POS
1316	<i>Listeria monocytogenes</i>		3a	Cooked chicken	POS
1321	<i>Listeria monocytogenes</i>		3c	Sandwich	POS
2874	<i>Listeria seeligeri</i>			Frozen dessert	POS
3244	<i>Listeria innocua</i>			Unknown	POS
3327	<i>Listeria seeligeri</i>			Cheese	POS
3329	<i>Listeria seeligeri</i>			Unknown	POS
3351	<i>Listeria welshimeri</i>			Unknown	POS
3354	<i>Listeria welshimeri</i>			Unknown	POS
3359	<i>Listeria welshimeri</i>			Radish	POS
3363	<i>Listeria murrayi</i>			Unknown	POS
3376	<i>Listeria ivanovii</i>			Environmental	POS
3555	<i>Listeria grayi</i>			Unknown	POS
3572	<i>Listeria innocua</i>			Cow brain	POS

3573	<i>Listeria monocytogenes</i>			Industry sample	POS
3574	<i>Listeria monocytogenes</i>			Industry sample	POS
3576	<i>Listeria monocytogenes</i>			Industry sample	POS
3577	<i>Listeria monocytogenes</i>			Industry sample	POS
3578	<i>Listeria monocytogenes</i>			Industry sample	POS
3579	<i>Listeria monocytogenes</i>			Industry sample	POS
3580	<i>Listeria monocytogenes</i>			Industry sample	POS
3581	<i>Listeria monocytogenes</i>			Industry sample	POS
3582	<i>Listeria monocytogenes</i>			Industry sample	POS
3678	<i>Listeria ivanovii</i>			Unknown	POS
4553	<i>Listeria monocytogenes</i>			Smoked ham	POS
4568	<i>Listeria monocytogenes</i>			Swab of finger guard	POS
4571	<i>Listeria monocytogenes</i>			honey roast ham	POS
5425	<i>Listeria monocytogenes</i>			Jalisco cheese isolate	POS
7644	<i>Listeria monocytogenes</i>			Unknown	POS

<sup>a</sup>Strain DD643 demonstrated consistent limit of detection of 10<sup>6</sup> cfu/mL.

Table 12. BAX System exclusivity				
Strain ID (DD#)	Collection ID	Other Strain Designation (if available)	Isolate source	BAX System Result
379	<i>Bacillus subtilis</i>		Unknown	NEG
383	<i>Citrobacter freundii</i>		Unknown	NEG
659	<i>Lactococcus lactis</i>	ATCC 19435	Unknown	NEG
691	<i>Streptococcus thermophilus</i>	ATCC 19258	Pasteurized milk	NEG
692	<i>Streptococcus bovis</i>	ATCC 33317	Cow feces	NEG
695	<i>Streptococcus pyogenes</i>	ATCC 12344	Unknown	NEG
707	<i>Salmonella</i> Newport	ATCC 6962	Human clinical	NEG
713	<i>Bacillus thuringiensis</i>	ATCC 35646	Sewage	NEG
714	<i>Bacillus thuringiensis</i>	ATCC 14579	Unknown	NEG
715	<i>Bacillus cereus</i>	ATCC 14579	Unknown	NEG
716	<i>Bacillus thuringiensis</i>	ATCC 33679	Insect larvae	NEG
721	<i>Bacillus cereus</i>	ATCC 13061	Unknown	NEG
863	<i>Staphylococcus aureus</i>	ATCC 12600	Human clinical	NEG
877	<i>Bacillus cereus</i>	ATCC 33018	Powdered infant formula	NEG
878	<i>Bacillus cereus</i>	ATCC 13061	Unknown	NEG
879	<i>Bacillus cereus</i>	ATCC 11778	Unknown	NEG
912	<i>Staphylococcus aureus</i>	ATCC 10832	Unknown	NEG
1011	<i>Bacillus subtilis</i>		Mashed potato	NEG
1024	<i>Bacillus cereus</i>	ATCC 7004	Pasteurized milk	NEG
1096	<i>Staphylococcus aureus</i>		Unknown	NEG
1098	<i>Staphylococcus aureus</i>		Unknown	NEG
1105	<i>Staphylococcus warneri</i>		Salami	NEG
1107	<i>Staphylococcus xylosus</i>		Plockwurst salami	NEG
1111	<i>Staphylococcus capitis</i>	ATCC 35661	Unknown	NEG
1112	<i>Staphylococcus xylosus</i>	ATCC 35663	Unknown	NEG
1113	<i>Staphylococcus sciuri</i>	ATCC 29060	Human non-clinical	NEG
2392	<i>Rhodococcus equi</i>	ATCC 6939	Equine clinical	NEG
2552	<i>Enterococcus faecium</i>	ATCC 19434	Unknown	NEG
2553	<i>Enterococcus faecium</i>	ATCC 35667	Unknown	NEG

2554	<i>Enterococcus faecalis</i>	ATCC 35550	Unknown	NEG
2558	<i>Citrobacter freundii</i>	ATCC 43864	Unknown	NEG
2560	<i>Citrobacter koseri</i>	ATCC 25408	Human clinical	NEG
2561	<i>Citrobacter koseri</i>	ATCC 27028	Human clinical	NEG
2624	<i>Enterococcus gallinarum</i>	ATCC 35038	Chicken intestine	NEG
2625	<i>Enterococcus durans</i>	ATCC 19432	Unknown	NEG
2626	<i>Enterococcus hirae</i>	ATCC 8043	Unknown	NEG
2628	<i>Salmonella Kentucky</i>	ATCC 9263	Unknown	NEG
2636	<i>Staphylococcus felis</i>	ATCC 49168	Calf ear	NEG
3981	<i>Enterococcus faecalis</i>	ATCC 29212	Human clinical	NEG
3992	<i>Streptococcus mutans</i>	ATCC 25175	Carious dentine	NEG
3996	<i>Streptococcus equi</i>	ATCC 33398	Unknown	NEG
4063	<i>Carnobacterium gallinarum</i>		Unknown	NEG
4064	<i>Carnobacterium divergens</i>		Unknown	NEG
7332	<i>Lactobacillus curvatus</i>	ATCC 25601	Milk	NEG
7344	<i>Lactobacillus acidophilus</i>	ATCC 4356	Human non-clinical	NEG
9174	<i>Micrococcus luteus</i>	ATCC 272	Unknown	NEG

#### DISCUSSION OF MODIFICATION DATA 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 7. Actero <i>Listeria</i> Broth Enrichment Followed by Using the BAX System Real-Time PCR Assay for Genus <i>Listeria</i> Detection in Environmental Samples – Candidate Method Presumptive Compared to Candidate Method Confirmed Results (4)													
Matrix	Strain info	I <sup>a</sup>			N <sup>d</sup>	Candidate Presumptive (CP)			Candidate Confirmed (CC)			dPOD <sub>CP</sub> <sup>h</sup>	95% CI <sup>i</sup>
		CFU/sample	(UCL <sup>b</sup> , LCL <sup>c</sup> )			X <sup>e</sup>	POD <sub>CP</sub> <sup>f</sup>	95% CI	X	POD <sub>CC</sub> <sup>g</sup>	95% CI		
Stainless steel	<i>L. mono.</i> 1/2c (ready-to-eat food) + <i>P. aeruginosa</i> (field isolate)	0.0	N/A <sup>k</sup>	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 <sup>j</sup>	<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	8	0.40	(0.22; 0.61)	9	0.45	(0.26; 0.66)	-0.05	(-0.33; 0.24)	
		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. seeligeri</i> , (raw milk) + <i>P. aeruginosa</i> , (field isolate)	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	10	0.50	(0.30; 0.70)	10	0.50	(0.30; 0.70)	0.00	(-0.28; 0.28)	
		58.0	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. welshimeri</i> (chicken drip) + <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)	
		4.3	N/A	20	11	0.55	(0.34; 0.74)	11	0.55	(0.34; 0.74)	0.00	(-0.28; 0.28)	
		22.0	N/A	5	5	1.00	(0.57; 1.00)	5	1.00	(0.57; 1.00)	0.00	(-0.43; 0.43)	

<sup>a</sup>I – Inoculum level which was determined only for the environmental samples.

<sup>b</sup>UCL – Upper Confidence Limit.

<sup>c</sup>LCL – Lower Confidence Limit.

<sup>d</sup>N – Number of test portions.

<sup>e</sup>X – Number of positive test portions.

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

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

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

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

<sup>j</sup>The data from the independent matrix study performed by independent laboratory Q Laboratories.

<sup>k</sup>N/A – Not applicable.

**Table 8. Actero *Listeria* Broth Enrichment Followed by Using the BAX System Real-Time PCR Assay for Genus *Listeria* Detection in Environmental Samples – Candidate Method Compared to Reference Method Results (4)**

Matrix	Strain info	I <sup>a</sup>		N <sup>d</sup>	Candidate Method (C)			Reference Method (R)			dPOD <sub>CR</sub> <sup>h</sup>	95% CI <sup>i</sup>
		CFU/sample	(UCL <sup>b</sup> , LCL <sup>c</sup> )		X <sup>e</sup>	POD <sub>C</sub> <sup>f</sup>	95% CI	X	POD <sub>R</sub> <sup>g</sup>	95% CI		
Stainless steel	<i>L. mono.</i> 1/2c (ready-to-eat food) + <i>P. aeruginosa</i> (field isolate)	0.0	N/A <sup>k</sup>	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 <sup>j</sup>	<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	8	0.40	(0.22; 0.61)	5	0.25	(0.11; 0.47)	0.15	(-0.13; 0.40)
		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. seeligeri</i> , (raw milk) + <i>P. aeruginosa</i> , (field isolate)	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	10	0.50	(0.30; 0.70)	5	0.25	(0.11; 0.47)	0.25	(-0.05; 0.49)
		58.0	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. welshimeri</i> (chicken drip) + <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)
		4.3	N/A	20	11	0.55	(0.34; 0.74)	4	0.20	(0.08; 0.42)	0.35	(0.05; 0.58)
		22.0	N/A	5	5	1.00	(0.57; 1.00)	5	1.00	(0.57; 1.00)	0.00	(-0.43; 0.43)

<sup>a</sup>I – Inoculum level which was determined only for the environmental samples.

<sup>b</sup>UCL – Upper Confidence Limit.

<sup>c</sup>LCL – Lower Confidence Limit.

<sup>d</sup>N – Number of test portions.

<sup>e</sup>X – Number of positive test portions.

<sup>f</sup>POD<sub>C</sub> – Candidate method positive outcomes divided by the total number of trials.

<sup>g</sup>POD<sub>R</sub> – Reference method positive outcomes divided by the total number of trials.

<sup>h</sup>dPOD<sub>CR</sub> – Difference between the candidate method and reference method confirmed result POD values.

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

<sup>j</sup>The data from the independent matrix study performed by independent laboratory Q Laboratories.

<sup>k</sup>N/A – Not applicable.

**Table 9. Actero *Listeria* Broth Enrichment Followed by Using the BAX System Real-Time PCR Assay for Genus *Listeria* Detection in Food Samples – Candidate Method Presumptive Compared to Candidate Method Confirmed Results (4)**

Matrix	Strain info	MPN <sup>a</sup>		N <sup>d</sup>	Candidate Presumptive (CP)			Candidate Confirmed (CC)			dPOD <sub>CP</sub> <sup>h</sup>	95% CI <sup>i</sup>
		CFU/sample	(UCL <sup>b</sup> , LCL <sup>c</sup> )		X <sup>e</sup>	POD <sub>CP</sub> <sup>f</sup>	95% CI	X	POD <sub>CC</sub> <sup>g</sup>	95% CI		
Soft Mexican style cheese	<i>L. innocua</i> (manure/soil)	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)
		0.39	(0.17; 0.68)	20	20	1.00	(0.84; 1.00)	20	1.00	(0.84; 1.00)	0.00	(-0.16; 0.16)
		13.85	(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 <sup>l</sup>	<i>L. innocua</i> (manure/soil)	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)
		0.63	(0.35; 1.04)	20	10	0.50	(0.30; 0.70)	10	0.50	(0.30; 0.70)	0.00	(-0.28; 0.28)
		3.56	(1.63; 7.78)	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. 1/2a</i> (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 <sup>k</sup>	<i>L. mono. 1/2a</i> (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)
		1.60 <sup>l</sup>	(1.00; 2.95)	20	16	0.80	(0.58; 0.92)	16	0.80	(0.58; 0.92)	0.00	(-0.25; 0.25)
		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. seeligeri</i> (field water), 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)
		0.69	(0.38; 1.14)	20	12	0.60	(0.39; 0.78)	12	0.60	(0.39; 0.78)	0.00	(-0.28; 0.28)
		5.34	(2.45; 11.6)	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. innocua</i> , (turkey/ham/cheese deli sticks)	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)
		1.30	(0.82; 2.10)	20	14	0.70	(0.48; 0.85)	14	0.70	(0.48; 0.85)	0.00	(-0.27; 0.27)
		13.86	(5.07; 37.90)	5	5	1.00	(0.57; 1.00)	5	1.00	(0.57; 1.00)	0.00	(-0.43; 0.43)

<sup>a</sup>MPN – Most Probable Number is based on the POD of reference method test portions using the LCF MPN calculator.

<sup>b</sup>UCL – Upper Confidence Limit.

<sup>c</sup>LCL – Lower Confidence Limit.

<sup>d</sup>N – Number of test portions.

<sup>e</sup>X – Number of positive test portions.

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

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

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

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

<sup>l</sup>The data from the independent matrix study performed by independent laboratory, University of Guelph Laboratory Services Division.

<sup>k</sup>Fresh bagged spinach samples naturally contaminated with *L. welshimeri* and artificially contaminated with *L. monocytogenes* MRS0437 were used.

<sup>l</sup>Data of natural contamination with *L. welshimeri* were included in the MPN calculation.

**Table 10. Actero *Listeria* Broth Enrichment Followed by Using the BAX System Real-Time PCR Assay for Genus *Listeria* Detection in Food Samples – Candidate Method Compared to Reference Method Results (4)**

Matrix	Strain info	MPN <sup>a</sup>			Candidate Method (C)			Reference Method (R)			dPOD <sub>CR</sub> <sup>h</sup>	95% CI <sup>i</sup>
		CFU/ sample	(UCL <sup>b</sup> , LCL <sup>c</sup> )	N <sup>d</sup>	X <sup>e</sup>	POD <sub>C</sub> <sup>f</sup>	95% CI	X	POD <sub>R</sub> <sup>g</sup>	95% CI		
Soft Mexican style cheese	<i>L. innocua</i> (manure/soil)	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)
		0.39	(0.17; 0.68)	20	20	1.00	(0.84; 1.00)	7	0.35	(0.18; 0.57)	0.65	(0.38; 0.82)
		13.85	(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 <sup>j</sup>	<i>L. innocua</i> (manure/soil)	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)
		0.63	(0.35; 1.04)	20	10	0.50	(0.30; 0.70)	8	0.40	(0.22; 0.61)	0.10	(-0.19; 0.37)
		3.56	(1.63; 7.78)	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 <sup>k</sup>	<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)
		1.60 <sup>l</sup>	(1.00; 2.95)	20	16	0.80	(0.58; 0.92)	15	0.75	(0.53; 0.89)	0.05	(-0.21; 0.30)
		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. seeligeri</i> (field water), 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)
		0.69	(0.38; 1.14)	20	12	0.60	(0.39; 0.78)	11	0.55	(0.34; 0.74)	0.05	(-0.24; 0.303)
		5.34	(2.45; 11.6)	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. innocua</i> , (turkey/ham/cheese deli sticks)	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)
		1.30	(0.82; 2.10)	20	14	0.70	(0.48; 0.85)	14	0.70	(0.48; 0.85)	0.00	(-0.27; 0.27)
		13.86	(5.07; 37.90)	5	5	1.00	(0.57; 1.00)	5	1.00	(0.57; 1.00)	0.00	(-0.43; 0.43)

<sup>a</sup>I – Inoculum level which was determined only for the environmental samples.

<sup>b</sup>UCL – Upper Confidence Limit.

<sup>c</sup>LCL – Lower Confidence Limit.

<sup>d</sup>N – Number of test portions.

<sup>e</sup>X – Number of positive test portions.

<sup>f</sup>POD<sub>C</sub> – Candidate method positive outcomes divided by the total number of trials.

<sup>g</sup>POD<sub>R</sub> – Reference method positive outcomes divided by the total number of trials.

<sup>h</sup>dPOD<sub>CR</sub> – Difference between the candidate method and reference method confirmed result POD values.

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

<sup>j</sup>The data from the independent matrix study performed by independent laboratory, University of Guelph Laboratory Services Division.

<sup>k</sup>Fresh bagged spinach samples naturally contaminated with *L. welshimeri* and artificially contaminated with *L. monocytogenes* MRS0437 were used.

<sup>l</sup>Data of natural contamination with *L. welshimeri* were included in the MPN calculation.

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