



CERTIFICATION

AOAC[®] Performance TestedSM

Certificate No.

081401

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

BAX[®] System Real-Time PCR Assay for Genus *Listeria*

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 04, 2019 – December 31, 2020). 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 04, 2019

Date

METHOD AUTHORS

ORIGINAL VALIDATION: Timothy Dambaugh, Seth Blumerman, Daniel DeMarco, Stephen Varkey, Bridget Andaloro, Dawn Fallon, Jeff Rohrbeck, Steven Hoelzer, Julie Kraynak, Eugene Davis, George Tice, F. Morgan Wallace, Pathrick Bird, and Erin Crowely
MODIFICATION MAY 2015: Sergiy Olishevskyy, Benoit Crevier, Renaud Tremblay, and Morgan Wallace

SUBMITTING COMPANY

DuPont
 Experimental Station 400
 200 Powder Mill Road
 Wilmington, DE 19803

CURRENT SPONSOR

Hygiena
 2 Boulden Circle
 New Castle, DE 19720

KIT NAME(S)

DuPont™ Bax® System Real-Time PCR Assay for Genus *Listeria*
 March 01, 2017, BAX® System Real-Time PCR Assay for Genus *Listeria*

CATALOG NUMBERS

BAX® System Assay KIT2019 (D15131113), 24 LEB Complete MED2005 (D14654989)

INDEPENDENT LABORATORY

Q Laboratories, Inc.
 1400 Harrison Avenue
 Cincinnati, OH 45214
 USA

AOAC EXPERTS AND PEER REVIEWERS

Yi Chen¹, Michael Brodsky², Joseph Odumeru³, Wayne Ziemer⁴
¹ US FDA, Center for Food Safety and Applied Nutrition, College Park, MD, USA
² Brodsky Consultants, Thornhill, Ontario, Canada
³ University of Guelph, Guelph, Ontario, Canada
⁴ Consultant, Loganville, GA, USA (May 2015 Modification only)

APPLICABILITY OF METHOD

Target organism – *Listeria* species (including *L. monocytogenes*, *L. innocua*, *L. ivanovii*, *L. welshimeri*, *L. seeligeri*, and *L. grayi*).

Matrices - USDA/FSIS 8.09: frankfurters (beef), stainless steel, plastic, concrete

FDA BAM Ch. 10: cooked shrimp, bagged lettuce, queso fresco (Mexican style cheese)

Matrix modification (May 2015) with Actero™ *Listeria* in conjunction with DuPont™ BAX® System Real-Time PCR Assay for Genus *Listeria*:

FDA BAM Ch. 10: soft Mexican-style cheese, bagged spinach, frozen cooked shrimp, cold smoked salmon

USDA/FSIS 8.09: Frankfurters, stainless steel, plastic, sealed concrete

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

REFERENCE METHODS

U.S. Department of Agriculture-Food Safety and Inspection Service Microbiology Laboratory Guidebook Chapter 8.09 (2013) <http://www.fsis.usda.gov/wps/wcm/connect/1710bee8-76b9-4e6c-92fc-fdc290dbfa92/MLG-8.pdf?MOD=AJPERES> (2)

U.S. Food and Drug Administration Bacteriological Analytical Manual, Chapter 10 (2013) <http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm071400.htm> (3)

ORIGINAL CERTIFICATION DATE

August 01, 2014

CERTIFICATION RENEWAL RECORD

Renewed Annually through December 2020

METHOD MODIFICATION RECORD

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

SUMMARY OF MODIFICATION

1. Modification to include Actero™ *Listeria* and use of a single step enrichment for 081401. Matrix extension to include additional matrices.
2. Name change from DuPont Nutrition & Health to Qualicon Diagnostics LLC., a Hygiena company
3. Increase in hold time after tablet rehydration
4. Inserts, manuals, and labels updated to Hygiena
5. Editorial insert changes and corporate address
6. Editorial/clerical changes.

Under this AOAC® *Performance Tested*SM License Number, 081401 this method is distributed by:
 NONE

Under this AOAC® *Performance Tested*SM License Number, 081401 this method is distributed as:
 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 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 4. Method comparison results - POD for food matrixes (1)											
Matrix	Strain	MPN ^a /test portion	N ^b	Test method			Reference method			dPOD ^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	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 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)	1 2	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 ^h 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 ^h 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 ^h 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)

^aMPN = 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.

^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.

^h95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

^gIndependent Laboratory Study.

Table 5. Method comparison results - POD for environmental matrixes (1)												
Matrix	Strain	MPN ^a /test portion	N ^b	Test method				Reference method		dPOD _d ^f	95% CI ^g	
				X ^c	POD _e ^d	95% CI	x	POD _h ^e	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 ^h BAX® System 24 h	<i>L. monocytogenes</i> ATCC 7644 <i>Pseudomonas aeruginosa</i> ATCC 15442	3.2 x10 ⁴	5	5	1	(0.57, 1.0)	5	1	(0.57, 1.0)	0	(-0.45, 0.45)	
		3.2 x10 ³	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 ^h BAX® System 48 h	<i>L. monocytogenes</i> ATCC 7644 <i>Pseudomonas aeruginosa</i> ATCC 15442	3.2 x10 ⁴	5	5	1	(0.57, 1.0)	5	1	(0.57, 1.0)	0	(-0.45, 0.45)	
		3.2 x10 ³	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 ^h USDA-MLG 48 h	<i>L. monocytogenes</i> ATCC 7644 <i>Pseudomonas aeruginosa</i> ATCC 15442	3.2 x10 ⁴	5	5	1	(0.57, 1.0)	5	1	(0.57, 1.0)	0	(-0.45, 0.45)	
		3.2 x10 ³	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 faecalis</i> DD10565	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)	
Concrete BAX® System 48 h	<i>L. welshimeri</i> DD 4096 <i>Enterococcus faecalis</i> DD10565	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)	
		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 <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)	

^aMPN = 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.

^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.

^h95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

^gIndependent Laboratory Study.

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 matrices 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™ Listeria Broth Enrichment Followed by Using the BAX® System Real-Time PCR Assay for Genus *Listeria* 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	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)

^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 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 ^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	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)

^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 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 ^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. 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 ^j	<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.</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)
		1.60 ^l	(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)

^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 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 ^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. 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 ^l	<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 ^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)
		1.60 ^l	(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)

^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.

^lThe 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.

REFERENCES CITED

- Dambaugh, T., Blumberman, S., DeMarco, D., Varkey, S., Andaloro, B., Fallon, D., Rohrbeck, J., Hoelzer, S., Kravak, J., Davis, E., Tice, G., Wallace, M., Bird, P., and Crowley, E., Evaluation of the DuPont™ Bax® System Real-Time PCR Assay for Genus *Listeria*, AOAC® Performance TestedSM certification number 081401.
- U.S. Department of Agriculture-Food Safety and Inspection Service Microbiology Laboratory Guidebook Chapter 8.09 (2013) <http://www.fsis.usda.gov/wps/wcm/connect/1710bee8-76b9-4e6c-92fc-fdc290dbfa92/MLG-8.pdf?MOD=AJPERES>
- U.S. Food and Drug Administration Bacteriological Analytical Manual, Chapter 10 (2013) <http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm071400.htm>
- Olishevskyy, Sergiy, Crevier, Benoit, Tremblay, Renaud, and Wallace, Morgan. Evaluation of the Actero™ Listeria Enrichment Media for Detecting *Listeria* In Environmental and Food Samples, Using the DuPont™ BAX® System Real-Time PCR Test Kits and Actero™ Listeria Method AOAC® Performance TestedSM certification number 111201, 081401, and 121402. Approved May 2015