CERTIFICATION

AOAC Research Institute
Performance Tested Methods℠

Certificate No.

070202

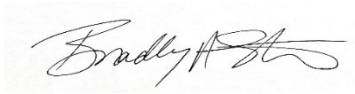
The AOAC Research Institute hereby certifies the method known as

BAX® System PCR Assay for *Listeria monocytogenes*
BAX® System X5 PCR Assay for *Listeria monocytogenes*

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*℠ 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*℠ 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.



Bradley A. Stawick, AOAC Research Institute Senior Director

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SUBMITTING COMPANY
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METHOD NAMES
BAX® System PCR Assay for *Listeria monocytogenes* and
BAX® System X5 PCR Assay for *Listeria monocytogenes*
Formerly DuPont™ BAX® System PCR Assay for *Listeria monocytogenes*

CATALOG NUMBERS
BAX® System Assay KIT2017, BAX® System X5 Assay KIT2023

INDEPENDENT LABORATORY
R-Tech Laboratories
P.O. Box 64101
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APPLICABILITY OF METHOD
Target organism – *Listeria monocytogenes*.

Matrixes – (25 g) – chocolate ice cream, milk (2%), vanilla yogurt, apple juice, cabbage slaw, fish sticks, frankfurters, langostinos, orange juice, peas, pepperoni, spinach, strawberries, Surimi, deli turkey, ground pork, queso fresco, plastic (4x4 in)

Performance claims – Method performs equivalent or better than reference method.

REFERENCE METHODS

USDA, FSIS, MLG.c2002 Ch 8, Isolation and Identification of *Listeria monocytogenes* from Red Meat, Poultry, Egg and Environmental Samples (9)
US FDA, CFSAN. BAM. c2001 Ch 10, *Listeria monocytogenes* (10)

***Official Methods of Analysis* (2002), 17th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, sec 993.12 (11)**

ORIGINAL CERTIFICATION DATE
July 24, 2002

CERTIFICATION RENEWAL RECORD
Renewed through December 2026.

METHOD MODIFICATION RECORD
1. **July 2013**
2. **December 2015 Level 2**
3. **March 2017 Level 1**
4. **December 2017 Level 1**
5. **May 2019 Level 1**
6. **December 2019 Level 1**
7. **November 2022 Level 1**
8. **December 2023 Level 1**
9. **December 2024 Level 1**

SUMMARY OF MODIFICATION
1. **Addition of Thermal Block for automated sample lysis.**
2. **Certified use of BAX® X5 with kit.**
3. **Name change from DuPont Nutrition & Health to Qualicon Diagnostics LLC., a Hygiena company.**
4. **Update inserts, manuals, and labels to Hygiena.**
5. **Editorial updates to inserts and corporate address change.**
6. **Editorial changes.**
7. **Editorial changes.**
8. **Editorial changes.**
9. **Editorial changes.**

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NONE

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NONE

PRINCIPLE OF THE METHOD (1)
The BAX® System uses the Polymerase Chain Reaction (PCR) [2] 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 automated BAX System then uses fluorescent detection [3] to analyze PCR product for positive or negative results. (The non-automated BAX system, which uses gel-detection methods, has already received AOAC-RI Performance Tested certification [4].)
PCR offers the potential for rapid and definitive detection of *L. monocytogenes* [5], and has been demonstrated to be effective for other organisms [6, 7]. In order to realize this potential, however, some of the drawbacks of PCR must be overcome. The first of these is the complexity of the PCR procedure itself. The second is the potential for false positives due to contamination with one or more molecules of amplified PCR product in the laboratory. This most often happens during the many pipetting steps associated with traditional PCR. The BAX System with automated detection alleviates these drawbacks by combining the reagents needed for the PCR process into a stable, dry, manufactured tablet already packaged inside the PCR tubes. After amplification, these tubes remain sealed for the detection phase, thus significantly reducing the potential for contamination.
The BAX System combines primers, polymerase and nucleotides needed for PCR into a single tablet. The specificity of a PCR assay is determined by the DNA sequences of the primers employed. The basis for primer design for the BAX System assay is a collection of 1572 strains of *L. monocytogenes*, which have been analyzed by ribotyping [8]. This large, well characterized culture collection has facilitated the design of primers that can detect all strains of *L. monocytogenes*, while remaining highly specific for *L. monocytogenes* and no other species.

PRINCIPLE OF THE METHOD Cont. (1)

The product of the PCR reaction is automatically analyzed by fluorescent detection. Each PCR tablet contains fluorescent dye, which binds with double-stranded DNA and emits a signal in response to light. During the automated detection phase, the temperature of the samples is modified to denature the DNA, which releases the dye and changes the signal. These changes are measured, and the BAX System proprietary software analyzes that data to generate melting curves and determine a positive or negative result.

To perform the BAX System assay, the user begins with a two-stage enrichment of the food sample. An aliquot of the secondary enrichment is combined with a buffer containing a proteolytic enzyme, then heated to rupture the wall of the bacterial cells and release their DNA. The lysed mixture is used to hydrate a BAX System PCR tablet. The PCR process then selectively amplifies DNA from *L. monocytogenes*. Repeated cycles of denaturing, annealing and extending produce an exponential increase in the number of target DNA fragments, creating millions of copies within a matter of hours. If the target sequence is not present, no detectable amplification takes place. Results from this assay are available about 5 hours after enrichment, involving less than an hour of user time. Detailed instructions are included in the *User Guide* that is shipped with the instrument. Table 1 summarizes the process.

DISCUSSION OF THE VALIDATION STUDY (1)

The data in these studies, within their statistical uncertainty, support the product claims and demonstrate that the BAX system is comparable or superior to standard reference methods for detecting *L. monocytogenes* in a wide variety of foods.

Table 2. BAX system inclusivity (1)

Strain DD#	Strain ID	BAX Result	Strain DD#	Strain ID	BAX Result	Strain DD#	Strain ID	BAX Result
1961	*L. monocytogenes*	Pos	1316	*L. monocytogenes*	Pos	5820	*L. monocytogenes*	Pos
4613	*L. monocytogenes*	Pos	3408	*L. monocytogenes*	Pos	3599	*L. monocytogenes*	Pos
6450	*L. monocytogenes*	Pos	3401	*L. monocytogenes*	Pos	1287	*L. monocytogenes*	Pos
5855	*L. monocytogenes*	Pos	3569	*L. monocytogenes*	Pos	3333	*L. monocytogenes*	Pos
3544	*L. monocytogenes*	Pos	3592	*L. monocytogenes*	Pos	940	*L. monocytogenes*	Pos
3545	*L. monocytogenes*	Pos	1058	*L. monocytogenes*	Pos	3394	*L. monocytogenes*	Pos
6444	*L. monocytogenes*	Pos	5457	*L. monocytogenes*	Pos	1145	*L. monocytogenes*	Pos
4698	*L. monocytogenes*	Pos	6783	*L. monocytogenes*	Pos	1313	*L. monocytogenes*	Pos
1320	*L. monocytogenes*	Pos	1327	*L. monocytogenes*	Pos	936	*L. monocytogenes*	Pos
6616	*L. monocytogenes*	Pos	4693	*L. monocytogenes*	Pos	3570	*L. monocytogenes*	Pos
4676	*L. monocytogenes*	Pos	5839	*L. monocytogenes*	Pos	1048	*L. monocytogenes*	Pos
3350	*L. monocytogenes*	Pos	4868	*L. monocytogenes*	Pos	6611	*L. monocytogenes*	Pos
1057	*L. monocytogenes*	Pos	3549	*L. monocytogenes*	Pos	6139	*L. monocytogenes*	Pos
3395	*L. monocytogenes*	Pos	2365	*L. monocytogenes*	Pos	3563	*L. monocytogenes*	Pos
4695	*L. monocytogenes*	Pos	3574	*L. monocytogenes*	Pos	5823	*L. monocytogenes*	Pos
1325	*L. monocytogenes*	Pos	1288	*L. monocytogenes*	Pos	3578	*L. monocytogenes*	Pos
3560	*L. monocytogenes*	Pos	1284	*L. monocytogenes*	Pos	3405	*L. monocytogenes*	Pos
6478	*L. monocytogenes*	Pos	1305	*L. monocytogenes*	Pos	4560	*L. monocytogenes*	Pos
3335	*L. monocytogenes*	Pos	3576	*L. monocytogenes*	Pos	6367	*L. monocytogenes*	Pos
6612	*L. monocytogenes*	Pos	3565	*L. monocytogenes*	Pos	3573	*L. monocytogenes*	Pos
2363	*L. monocytogenes*	Pos	6130	*L. monocytogenes*	Pos	7856	*L. monocytogenes*	Pos
2077	*L. monocytogenes*	Pos	4864	*L. monocytogenes*	Pos	3410	*L. monocytogenes*	Pos
2364	*L. monocytogenes*	Pos	4589	*L. monocytogenes*	Pos	4568	*L. monocytogenes*	Pos
3579	*L. monocytogenes*	Pos	2360	*L. monocytogenes*	Pos	4700	*L. monocytogenes*	Pos
3402	*L. monocytogenes*	Pos	3575	*L. monocytogenes*	Pos	3568	*L. monocytogenes*	Pos
3559	*L. monocytogenes*	Pos	3548	*L. monocytogenes*	Pos	1307	*L. monocytogenes*	Pos
1964	*L. monocytogenes*	Pos	3379	*L. monocytogenes*	Pos	1464	*L. monocytogenes*	Pos
3580	*L. monocytogenes*	Pos	5464	*L. monocytogenes*	Pos	6442	*L. monocytogenes*	Pos
941	*L. monocytogenes*	Pos	5857	*L. monocytogenes*	Pos	4854	*L. monocytogenes*	Pos
3406	*L. monocytogenes*	Pos	3407	*L. monocytogenes*	Pos	6764	*L. monocytogenes*	Pos
4618	*L. monocytogenes*	Pos	3556	*L. monocytogenes*	Pos	2882	*L. monocytogenes*	Pos
3561	*L. monocytogenes*	Pos	4616	*L. monocytogenes*	Pos	4617	*L. monocytogenes*	Pos
5425	*L. monocytogenes*	Pos	1144	*L. monocytogenes*	Pos	3593	*L. monocytogenes*	Pos
4571	*L. monocytogenes*	Pos	3365	*L. monocytogenes*	Pos	3564	*L. monocytogenes*	Pos
3598	*L. monocytogenes*	Pos	1149	*L. monocytogenes*	Pos	7304	*L. monocytogenes*	Pos
3403	*L. monocytogenes*	Pos	957	*L. monocytogenes*	Pos	1072	*L. monocytogenes*	Pos
3582	*L. monocytogenes*	Pos	3385	*L. monocytogenes*	Pos	4856	*L. monocytogenes*	Pos
6415	*L. monocytogenes*	Pos	3393	*L. monocytogenes*	Pos	1152	*L. monocytogenes*	Pos
2362	*L. monocytogenes*	Pos	6760	*L. monocytogenes*	Pos	2081	*L. monocytogenes*	Pos
3323	*L. monocytogenes*	Pos	3581	*L. monocytogenes*	Pos	1286	*L. monocytogenes*	Pos
4553	*L. monocytogenes*	Pos	3583	*L. monocytogenes*	Pos	3392	*L. monocytogenes*	Pos
8023	*L. monocytogenes*	Pos	4567	*L. monocytogenes*	Pos	6138	*L. monocytogenes*	Pos
5458	*L. monocytogenes*	Pos	5830	*L. monocytogenes*	Pos	6754	*L. monocytogenes*	Pos

7822	*L. monocytogenes*	Pos	3567	*L. monocytogenes*	Pos	1423	*L. monocytogenes*	Pos
4640	*L. monocytogenes*	Pos	3366	*L. monocytogenes*	Pos	1281	*L. monocytogenes*	Pos
1283	*L. monocytogenes*	Pos	1326	*L. monocytogenes*	Pos	3075	*L. monocytogenes*	Pos
3391	*L. monocytogenes*	Pos	4641	*L. monocytogenes*	Pos	1148	*L. monocytogenes*	Pos
3597	*L. monocytogenes*	Pos	8810	*L. monocytogenes*	Pos	3339	*L. monocytogenes*	Pos
891	*L. monocytogenes*	Pos	3595	*L. monocytogenes*	Pos	4651	*L. monocytogenes*	Pos
1310	*L. monocytogenes*	Pos	1306	*L. monocytogenes*	Pos	1419	*L. monocytogenes*	Pos
1294	*L. monocytogenes*	Pos	6763	*L. monocytogenes*	Pos	4569	*L. monocytogenes*	Pos
6535	*L. monocytogenes*	Pos	3596	*L. monocytogenes*	Pos	6447	*L. monocytogenes*	Pos
4590	*L. monocytogenes*	Pos	4564	*L. monocytogenes*	Pos	1293	*L. monocytogenes*	Pos
4561	*L. monocytogenes*	Pos	6420	*L. monocytogenes*	Pos	3577	*L. monocytogenes*	Pos
1328	*L. monocytogenes*	Pos	1295	*L. monocytogenes*	Pos	5862	*L. monocytogenes*	Pos
4684	*L. monocytogenes*	Pos	6891	*L. monocytogenes*	Pos	5858	*L. monocytogenes*	Pos
6455	*L. monocytogenes*	Pos	4646	*L. monocytogenes*	Pos	6610	*L. monocytogenes*	Pos
2361	*L. monocytogenes*	Pos	939	*L. monocytogenes*	Pos	3546	*L. monocytogenes*	Pos
6474	*L. monocytogenes*	Pos	6369	*L. monocytogenes*	Pos	1299	*L. monocytogenes*	Pos
1322	*L. monocytogenes*	Pos	3334	*L. monocytogenes*	Pos	647	*L. monocytogenes*	Pos
2359	*L. monocytogenes*	Pos	6766	*L. monocytogenes*	Pos	1151	*L. monocytogenes*	Pos
1153	*L. monocytogenes*	Pos	5821	*L. monocytogenes*	Pos	6439	*L. monocytogenes*	Pos
6618	*L. monocytogenes*	Pos	3390	*L. monocytogenes*	Pos	3566	*L. monocytogenes*	Pos
3547	*L. monocytogenes*	Pos	3594	*L. monocytogenes*	Pos	4675	*L. monocytogenes*	Pos
8816	*L. monocytogenes*	Pos	1282	*L. monocytogenes*	Pos	6366	*L. monocytogenes*	Pos
1324	*L. monocytogenes*	Pos						

Table 3. BAX system exclusivity (1)

Strain DD#	Strain ID	BAX Result	Strain DD#	Strain ID	BAX Result	Strain DD#	Strain ID	BAX Result
3361	*Listeria grayi*	Neg	5907	*Listeria murrayi*	Neg	3351	*Listeria welshimeri*	Neg
3355	*Listeria grayi*	Neg	3363	*Listeria murrayi*	Neg	1173	*Listeria welshimeri*	Neg
10698	*Listeria grayi*	Neg	3357	*Listeria murrayi*	Neg	1175	*Listeria welshimeri*	Neg
1157	*Listeria innocua*	Neg	3815	*Listeria murrayi*	Neg	1180	*Listeria welshimeri*	Neg
927	*Listeria innocua*	Neg	3802	*Listeria murrayi*	Neg	1172	*Listeria welshimeri*	*Neg
1161	*Listeria innocua*	Neg	3803	*Listeria murrayi*	Neg	2538	*Listeria welshimeri*	*Neg
1063	*Listeria innocua*	Neg	4095	*Listeria murrayi*	Neg	1024	*Bacillus cereus*	Neg
1156	*Listeria innocua*	Neg	4323	*Listeria murrayi*	Neg	9204	*Bacillus subtilis*	Neg
1066	*Listeria innocua*	Neg	5861	*Listeria murrayi*	Neg	7329	*Carnobacterium piscicola*	Neg
1154	*Listeria innocua*	Neg	1301	*Listeria seeligeri*	Neg	1991	*E. coli 0157:H7*	Neg
1158	*Listeria innocua*	Neg	1298	*Listeria seeligeri*	Neg	2122	*E.coli*	Neg
1155	*Listeria innocua*	Neg	1297	*Listeria seeligeri*	Neg	4088	*E.coli*	Neg
2087	*Listeria innocua*	Neg	1291	*Listeria seeligeri*	Neg	2468	*Enterococcus species*	Neg
1064	*Listeria innocua*	*Neg	2090	*Listeria seeligeri*	Neg	3981	*Enterococcus faecalis*	Neg
1159	*Listeria innocua*	*Neg	949	*Listeria seeligeri*	Neg	9509	*Enterococcus faecalis*	*Neg
1465	*Listeria ivanovii*	Neg	1289	*Listeria seeligeri*	Neg	8031	*Enterococcus faecium*	Neg
1466	*Listeria ivanovii*	Neg	1300	*Listeria seeligeri*	Neg	958	*Enterococcus faecium*	Neg
1170	*Listeria ivanovii*	Neg	1304	*Listeria seeligeri*	Neg	9111	*Kurthia gibsoni*	Neg
1166	*Listeria ivanovii*	Neg	1292	*Listeria seeligeri*	Neg	10836	*Lactobacillus delbruecki*	Neg
1171	*Listeria ivanovii*	Neg	1303	*Listeria seeligeri*	*Neg	10205	*Lactococcus lactis*	Neg
1165	*Listeria ivanovii*	Neg	1179	*Listeria welshimeri*	Neg	9178	*Micrococcus luteus*	Neg
1164	*Listeria ivanovii*	Neg	1174	*Listeria welshimeri*	Neg	1095	*Staphylococcus carnosus*	Neg
1169	*Listeria ivanovii*	Neg	943	*Listeria welshimeri*	Neg	1106	*Staphylococcus xylosis*	Neg
1167	*Listeria ivanovii*	Neg	3233	*Listeria welshimeri*	Neg	1396	*Staphylococcus aureus*	Neg
1168	*Listeria ivanovii*	*Neg	1177	*Listeria welshimeri*	Neg	3515	*Staphylococcus epidermis*	Neg
3364	*Listeria murrayi*	Neg	1176	*Listeria welshimeri*	Neg	3990	*Streptococcus equi*	Neg

* Initial positive repeated four times with negative results.

Table 4. Food type, reference method and strain (1)

Food Type	Reference Method	*L. monocytogenes* Strain
Ice cream (chocolate)	AOAC Method 993.12 (19.10)	DD4693
Milk (2%)	AOAC Method 993.12 (19.10)	DD1144
Yogurt (vanilla)	AOAC Method 993.12 (19.10)	DD3394
Apple juice	FDA-BAM (19.9)	DD5457
Cabbage slaw	FDA-BAM (19.9)	DD6420
Fish sticks	FDA-BAM (19.9)	DD4641
Frankfurters	FDA-BAM (19.9)	DD8810
Langostinos	FDA-BAM (19.9)	DD6760
Orange juice	FDA-BAM (19.9)	ATCC 49594
Peas	FDA-BAM (19.9)	DD5457
Pepperoni	FDA-BAM (19.9)	DD6369
Spinach	FDA-BAM (19.9)	ATCC 49594
Strawberries	FDA-BAM (19.9)	ATCC 49594

Surimi (imitation crab)	FDA-BAM (19.9)	ATCC 12496
Deli turkey	USDA-FSIS (19.8)	ATCC 19116
Ground pork	USDA-FSIS (19.8)	DD1324

Table 7. Summary of repeatability results – automated BAX System (1)

Spike target	MPN/ actual spike	Food type	BAX results	Ref results	Chi-square value	Ref method
High 10-50 cfu/25g	3.75/25g	Milk (2%)	20/20	20/20	0	AOAC
	2.3/25g	Apple juice	18/20	17/20	0	BAM
	27.5/25g	Ice cream (chocolate)	20/20	20/20	0	AOAC
	1.875/25g	Deli turkey	18/20	18/20	0	FSIS
	11.5/25g	Fish sticks	20/20	20/20	0	BAM
	11.5/25g	Frankfurters	20/20	20/20	0	BAM
	6/25g	Langostinos	20/20	20/20	0	BAM
	2.35/25g	Orange juice	20/20	20/20	0	BAM
	11.5/25g	Peas	20/20	20/20	0	BAM
	3.8/25g	Pepperoni	15/20	15/20	0	BAM
	11.5/25g	Cabbage slaw	20/20	20/20	0	BAM
	0.35/25g	Spinach	14/20	6/20	4.90	BAM
	2.325/25g	Strawberries	17/20	10/20	5.14	BAM
	27.5/25g	Surimi	20/20	20/20	0	BAM
	3.75/25g	Yogurt (vanilla)	20/20	19/20	0	AOAC
High spike totals			**282/300**	**265/300**		
Low spike 1-10 cfu/25/g	1.08/25g	Milk (2%)	9/20	10/20	0	AOAC
	0.6/25g	Apple juice	6/20	8/20	0.50	BAM
	2.33/25g	Ice cream (chocolate)	16/20	16/20	0	AOAC
	3.75/25g	Deli turkey	8/20	7/20	0	FSIS
	0.975/25g	Fish sticks	19/20	19/20	0	BAM
	0.4/25g	Ground pork	20/20	17/20	--	FSIS
	1.8/25g	Frankfurters	16/20	16/20	0	BAM
	2.33/25g	Langostinos	8/20	9/20	0	BAM
	1.075/25g	Orange juice	13/20	15/20	0.50	BAM
	0.7/25g	Peas	13/20	11/20	0.50	BAM
	2.3/25g	Pepperoni	12/20	12/20	0	BAM
	0.4/25g	Cabbage slaw	11/20	11/20	0	BAM
	<0.075/25g	Spinach	6/20	1/20	2.29	BAM
	2.325/25g	Strawberries	13/20	8/20	5.14	BAM
	6/25g	Surimi	18/20	18/20	0	BAM
	0.228/25g	Yogurt (vanilla)	6/20	4/20	0.25	AOAC
Low spike totals			**194/320**	**182/320**		
Combined spike totals			**476/620**	**447/620**		

DISCUSSION OF MODIFICATION APPROVED DECEMBER 2015 (17)

The comparison study demonstrated that the alternative method as performed using the X5 BAX instrument is equivalent to the USDA-FSIS, and FDA-BAM reference methods for the detection of *L. monocytogenes* from frankfurters, bagged spinach, queso fresco and plastic surfaces as determined by the specified AOAC statistical criteria. Inclusivity and exclusivity results were as expected with 100% inclusivity for target strains tested and 100% exclusivity for non-target strains tested. The test kit also demonstrated positive results across a wide titer of target in both pure culture and in enriched food matrix.

Modification Data Approved December 2015 (17)

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

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 USDA-FSIS 48 h	*L. monocytogenes* DD 1305	58	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		5.8	20	6	0.3	(0.14, 0.52)	6	0.3	(0.14, 0.52)	0	(-0.27, 0.27)
		Uninoculated	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Bagged spinach FDA-BAM 48 h	*L. monocytogenes* DD 1283	15	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		1.5	20	8	0.4	(0.22, 0.61)	9	0.45	(0.26, 0.66)	-0.05	(-0.32, 0.23)
		Uninoculated	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Queso fresco cheese FDA-BAM 48 h	*L. monocytogenes* DD 5426	140	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		14	20	13	0.65	(0.43, 0.82)	13	0.65	(0.43, 0.82)	0	(-0.27, 0.27)
		Uninoculated	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Plastic USDA-MLG 48 h	*L. monocytogenes* DD 1309	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, 0.89)	15	0.75	(0.53, 0.89)	0	(-0.26, 0.26)
		Uninoculated	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)

[a]Most Probable Number is based on the POD of reference method test portions using the Least Cost Formulations MPN calculator (7), with 95% confidence interval.

[b]N = Number of test potions.

[c]x = Number of positive test portions.

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

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

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

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

Table 3. Method comparison results - POD for food and environmental matrixes (17)											
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 USDA-MLG 48 h	*L. monocytogenes* DD 1305	58	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		5.8	20	6	0.3	(0.14, 0.52)	6	0.3	(0.14, 0.52)	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	*L. monocytogenes* DD 1283	15	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		1.5	20	8	0.4	(0.22, 0.61)	9	0.45	(0.26, 0.66)	-0.05	(-0.15, 0.05)
		Uninoculated	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Queso fresco cheese FDA-BAM 48 h	*L. monocytogenes* DD 5426	140	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		14	20	13	0.65	(0.43, 0.82)	13	0.65	(0.42, 0.82)	0	(-0.14, 0.14)
		Uninoculated	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.43, 0.43)
Plastic USDA-MLG 48 h	*L. monocytogenes* DD 1309	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, 0.89)	15	0.75	(0.53, 0.89)	0	(-0.14, 0.14)
		Uninoculated	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)

[a]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.

[b]N = Number of test potions.

[c]x = Number of positive test portions.

[d]POD$_C$ = Confirmed candidate method positive outcomes divided by the total number of trials.

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

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

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

Table 4. Inclusivity Results for the BAX System X5 PCR Assay for *L. monocytogenes* (17)

DuPont Strain ID	*L monocytogenes* Serotype where available	Result	DuPont Strain ID	*L. monocytogenes* Serotype where available	Result
566	*Listeria monocytogenes 1/2a*	Pos	1306	*Listeria monocytogenes 3b*	Pos
605	*Listeria monocytogenes 1/2a*	Pos	1307	*Listeria monocytogenes 3b*	Pos
647	*Listeria monocytogenes 4e*	Pos	1308	*Listeria monocytogenes 4b*	Pos
648	*Listeria monocytogenes 4a*	Pos	1309	*Listeria monocytogenes 4b*	Pos
652	*Listeria monocytogenes 4c*	Pos	1310	*Listeria monocytogenes 3b*	Pos
653	*Listeria monocytogenes 4b*	Pos	1311	*Listeria monocytogenes 1/2c*	Pos
1069	*Listeria monocytogenes*	Pos	1312	*Listeria monocytogenes 1/2c*	Pos
1072	*Listeria monocytogenes*	Pos	1313	*Listeria monocytogenes 4b*	Pos
1144	*Listeria monocytogenes 1/2a*	Pos	1314	*Listeria monocytogenes 4 (not 4b)*	Pos
1145	*Listeria monocytogenes1/2a*	Pos	1315	*Listeria monocytogenes 1/2c*	Pos
1146	*Listeria monocytogenes 1/2a*	Pos	1316	*Listeria monocytogenes 3a*	Pos
1147	*Listeria monocytogenes 1/2a*	Pos	1321	*Listeria monocytogenes 3c*	Pos
1149	*Listeria monocytogenes 1/2a*	Pos	3573	*Listeria monocytogenes*	Pos
1152	*Listeria monocytogenes 1/2b*	Pos	3574	*Listeria monocytogenes*	Pos
1281	*Listeria monocytogenes 3c*	Pos	3576	*Listeria monocytogenes*	Pos
1282	*Listeria monocytogenes 3c*	Pos	3577	*Listeria monocytogenes*	Pos
1283	*Listeria monocytogenes 3b*	Pos	3578	*Listeria monocytogenes*	Pos
1285	*Listeria monocytogenes 4 (not 4b)*	Pos	3579	*Listeria monocytogenes*	Pos
1286	*Listeria monocytogenes 3c*	Pos	3580	*Listeria monocytogenes*	Pos
1287	*Listeria monocytogenes 3a*	Pos	3581	*Listeria monocytogenes*	Pos
1288	*Listeria monocytogenes 3a*	Pos	3582	*Listeria monocytogenes*	Pos
1293	*Listeria monocytogenes 3a*	Pos	4553	*Listeria monocytogenes*	Pos
1294	*Listeria monocytogenes 4b*	Pos	4568	*Listeria monocytogenes*	Pos
1295	*Listeria monocytogenes 3b*	Pos	4571	*Listeria monocytogenes*	Pos
1299	*Listeria monocytogenes 1/2b*	Pos	5425	*Listeria monocytogenes 4b*	Pos
1305	*Listeria monocytogenes 3a*	Pos	7644	*Listeria monocytogenes 4b*	Pos

Table 5. Exclusivity Results for the BAX System X5 PCR Assay for *L. monocytogenes* (17)

DuPont Strain ID	*Genus and Species*	Result	DuPont Strain ID	*Genus and Species*	Result
379	*Bacillus subtilus*	Neg	2558	*Citrobacter freundii*	Neg
383	*Citrobacter freundii*	Neg	2560	*Citrobacter koseri*	Neg
659	*Lactococcus lactis*	Neg	2561	*Citrobacter koseri*	Neg
691	*Streptococcus thermophilus*	Neg	2624	*Enterobacter gallinarum*	Neg
692	*Streptococcus bovis*	Neg	2625	*Enterobacter durans*	Neg
695	*Streptococcus pyogenes*	Neg	2626	*Enterobacter hirae*	Neg
707	*Salmonella newport*	Neg	2628	*Salmonella kentucky*	Neg
713	*Bacillus thuringiensis*	Neg	2636	*Staphylococcus felis*	Neg
714	*Bacillus thuringiensis*	Neg	3981	*Enterococcus faecalis*	Neg
715	*Bacillus cereus*	Neg	3992	*Streptococcus mutans*	Neg
716	*Bacillus thuringiensis*	Neg	3996	*Streptococcus equi*	Neg
721	*Bacillus cereus*	Neg	4063	*Carnobacterium gallinarum*	Neg
863	*Staphylococcus aureus*	Neg	4064	*Carnobacterium divergens*	Neg
877	*Bacillus cereus*	Neg	7332	*Lactobacillus curvatus*	Neg
878	*Bacillus cereus*	Neg	7334	*Lactobacillus acidophilus*	Neg
879	*Bacillus cereus*	Neg	9174	*Micrococcus luteus*	Neg
912	*Staphylococcus aureus*	Neg	643	*Listeria murrayi/grayi*	Neg
1011	*Bacillus subtilus*	Neg	649	*Listeria ivanovii*	Neg
1024	*Bacillus cereus*	Neg	654	*Listeria welshimeri*	Neg
1096	*Staphylococcus aureus*	Neg	944	*Listeria murrayi/grayi*	Neg
1098	*Staphylococcus aureus*	Neg	1156	*Listeria innocua*	Neg
1105	*Staphylococcus warneri*	Neg	1164	*Listeria ivanovii*	Neg
1107	*Staphylococcus xylosus*	Neg	1172	*Listeria welshimeri*	Neg
1111	*Staphylococcus capitis*	Neg	2874	*Listeria seeligeri*	Neg
1112	*Staphylococcus xylosus*	Neg	3327	*Listeria seeligeri*	Neg
1113	*Staphylococcus sciuri*	Neg	3329	*Listeria seeligeri*	Neg
2392	*Rhodococcus equi*	Neg	3359	*Listeria welshimeri*	Neg
2552	*Enterococcus faecium*	Neg	3363	*Listeria murrayi/grayi*	Neg
2553	*Enterococcus faecium*	Neg	3376	*Listeria ivanovii*	Neg
2554	*Enterococcus faecalis*	Neg	3572	*Listeria innocua*	Neg

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