CERTIFICATION

AOAC® *Performance Tested*SM

Certificate No.

030502

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

BAX® System PCR Assay for Genus *Listeria*
BAX® System X5 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 Methods*SM 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 Tested*SM 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 03, 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 03, 2019
Date

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KIT NAME(S)
DuPont™ Bax® System PCR Assay for Genus *Listeria*
March 01, 2017, BAX® System PCR Assay for Genus *Listeria*
BAX® System X5 PCR Assay for Genus *Listeria*

CATALOG NUMBERS
BAX® System Assay KIT2016, BAX® System X5 Assay KIT2024

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[4] US FDA, CFSAN, College Park, MD, USA: July 2013 and January 2016 Modifications

APPLICABILITY OF METHOD
Target organism – *Listeria* species

Matrices – (swab, 1 x 1 in) - Cast Iron, Plastic, Air Filter Material, Unpainted wood, drain swabs
(sponge, 4 x 4 in) - Ceramic tile, Sealed Concrete, rubber, Painted wood surfaces
July 2008 Modification: USDA-FSIS - frankfurters, and environmental sponges
FDA BAM 10- spinach, smoked salmon
AOAC 993.12 processed cheese
January 2016 Modification:
MLG 8.09 – frankfurters, stainless steel (4 x 4 in sponge), plastic (4 x 4 in sponge)
FDA BAM Ch.10 – bagged spinach and queso cheese

Performance claims - Sensitivity equivalent to the respective reference methods.

REFERENCE METHODS
Cook, L. V. 2002. "USDA/FSIS microbiology laboratory guide-book," 3rd ed., revision 3, USDA/FSIS, http://www.fsis.usda.gov/Ophs/Microlab/Mlg8.03.pdf (2)
Food and Drug Administration, FDA Bacteriological Analytical Manual, http://www.cfsan.fda.gov/~ebam/bam-10.html date of access 9 Feb 2008. (4)
***AOAC Official Methods of Analysis of AOAC INTERNATIONAL* 18th Ed., AOAC INTERNATIONAL, Arlington, VA, date of access 9 Feb 2008 (5)**
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 (8)

ORIGINAL CERTIFICATION DATE
March 10, 2005

CERTIFICATION RENEWAL RECORD
Renewed Annually through December 2020

METHOD MODIFICATION RECORD
1. **July 2008**
2. **July 2013**
3. **January 2016**
4. **March 2017 Level 1**
5. **January 2018 Level 1**
6. **May 2019 Level 1**
7. **December 2019 Level 1**

SUMMARY OF MODIFICATIONS
1. **Addition of a two-stage enrichment, matrix extension**
2. **Addition of Thermal Block for automated sample lysis**
3. **Certification of BAX® X5 Instrument**
4. **Name change from DuPont Nutrition & Health to Qualicon Diagnostics LLC., a Hygiena company**
5. **Update of inserts and labels to Hygiena**
6. **Insert edits and corporate address change**
7. **Editorial/clerical changes.**

Under this AOAC® *Performance Tested*SM License Number, 030502 this method is distributed by:
NONE

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NONE

PRINCIPLE OF THE METHOD (1)

PCR amplification - The BAX® system uses the Polymerase Chain Reaction (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, thus significantly reducing the potential for contamination with one or more molecules of amplified PCR product.

Fluorescent detection - The automated BAX® system uses fluorescent detection to analyze PCR product. Each PCR tablet contains a fluorescent dye, which binds with double-stranded DNA and emits a signal in response to excitation light. During the detection phase, the temperature of the sample is slowly increased to denature the DNA, which in turn, releases the dye and causes a drop in emission signal. The BAX® system measures the denaturation temperature and analyzes the magnitude of the fluorescent signal change to determine a positive or negative result.

DISCUSSION OF THE ORIGINAL VALIDATION STUDY (1)

Results from the method comparison studies demonstrate BAX® system performance that is equivalent to the reference methods for detection of *Listeria species* from environmental surfaces with the exception of stainless steel.

The inclusivity/exclusivity study showed 100% agreement (when excluding *L. grayi* strains) and 97.2% when *L. grayi* is included with expected results for the test panel. As DNA sequence based typing schemes of *Listeria* indicate that *L. grayi* clusters distantly from all other species of *Listeria* (9), debate as to whether it is truly a *Listeria* species is ongoing within the bacterial taxonomy community. Other phenotypic and genotypic studies have also raised questions as to whether *L. grayi* should be included in the genus *Listeria* or placed in a new genus, *Murraya* (12).

Lot-to-lot and stability studies showed consistent performance. The ruggedness study demonstrated that the BAX® system was not sensitive to changes in lysis temperature, lysis sample volume, or PCR sample volume and that enrichment conditions variations within the stated acceptable ranges for the variables did not alter assay results.

Original Validation Data (1) **Table 4. BAX ® system inclusivity**				
dd#	**Collection ID**	**Isolate source**	**BAX® L genus**	**API *Listeria* confirmation**
3361	*L. grayi*	ice cream	neg	
3355	*L. grayi*	unknown	neg	
645	**L. grayi**	chinchilla feces	neg	
643	*L. grayi*	corn stalks	neg	
1162	*L. innocua*	raw chicken	**pos**	*L. innocua*
1163	*L. innocua*	egg mayonnaise	**pos**	*L. innocua*
1154	*L. innocua*	pate'	**pos**	*L. innocua*
1156	*L. innocua*	lettuce	**pos**	*L. innocua*
1157	*L. innocua*	beef burger	**pos**	*L. innocua*
1158	*L. innocua*	black pudding	**pos**	*L. innocua*
1159	*L. innocua*	celery salad	**pos**	***L. innocua***
1063	*L. innocua*	chopped ham	**pos**	*L. innocua*
1064	*L. innocua*	chopped ham	**pos**	*L. innocua*
1065	*L. innocua*	pork	**pos**	*L. innocua*
1066	*L. innocua*	Stilton cheese	**pos**	*L. innocua*
921	*L. innocua*	roast turkey	**pos**	*L. innocua*
922	*L. innocua*	ham cured shoulder	**pos**	*L. innocua*
924	*L. innocua*	ham cured shoulder	**pos**	*L. innocua*
927	*L. innocua*	chopped ham	**pos**	*L. innocua*
3326	*L. innocua*	unknown	**pos**	*L. innocua*
2086	*L. innocua*	Liederkranz	**pos**	*L. innocua*
2087	*L. innocua*	unknown	**pos**	*L. innocua*
3244	*L. innocua*	unknown	**pos**	*L. innocua*
3241	*L. innocua*	unknown	**pos**	*L. innocua*
3237	*L. innocua*	unknown	**pos**	*L. innocua*
3073	*L. innocua*	unknown	**pos**	*L. innocua*
1155	*L. innocua*	Camembert cheese	**pos**	*L. innocua*
3336	*L. innocua*	unknown	**pos**	*L. seeligeri*
1165	*L. ivanovii*	Belgian salami	**pos**	*L. ivanovii*
1166	*L. ivanovii*	salami	**pos**	*L. ivanovii*
1167	*L. ivanovii*	soft cheese	**pos**	*L. ivanovii*
1164	*L. ivanovii*	radish	**pos**	*L. seeligeri*
1168	*L. ivanovii*	unknown	**pos**	*L. ivanovii*
1169	*L. ivanovii*	unknown	**pos**	*L. ivanovii*
1171	*L. ivanovii*	unknown	**pos**	*L. ivanovii*
892	*L. ivanovii*	unknown	**pos**	*L. ivanovii*
893	*L. ivanovii*	unknown	**pos**	*L. ivanovii*
898	*L. ivanovii*	unknown	**pos**	*L. ivanovii*
907	*L. ivanovii*	unknown	**pos**	*L. ivanovii*
649	*L. ivanovii*	sheep	**pos**	*L. ivanovii*
3325	*L. ivanovii*	unknown	**pos**	*L. ivanovii*

1465	*L. ivanovii*	unknown	**pos**	*L. ivanovii*
1466	*L. ivanovii*	unknown	**pos**	*L. ivanovii*
2088	*L. ivanovii*	unknown	**pos**	*L. ivanovii*
3072	*L. ivanovii*	unknown	**pos**	*L. ivanovii*
3373	*L. ivanovii*	unknown	**pos**	*L. ivanovii*
3375	*L. ivanovii*	unknown	**pos**	*L. ivanovii*
3678	*L. ivanovii*	unknown	**pos**	*L. ivanovii*
3557	*L. ivanovii*	unknown	**pos**	*L. ivanovii*
3555	*L. ivanovii*	unknown	**pos**	*L. ivanovii*
3376	*L. ivanovii*	environmental	**pos**	*L. ivanovii*
3370	*L. ivanovii*	unknown	**pos**	*L. ivanovii*
3571	*L. ivanovii*	decaying vegetable	**pos**	*L. welshimeri*
3371	*L. ivanovii*	unknown	**pos**	*L. seeligeri*
3340	*L. ivanovii*	unknown	**pos**	*L. ivanovii*
3372	*L. ivanovii*	unknown	**pos**	*L. seeligeri*
1147	*L. monocytogenes 1/2a*	pate'	**pos**	*L. monocytogenes*
1149	*L. monocytogenes 1/2a*	raw milk	**pos**	*L. monocytogenes*
1152	*L. monocytogenes 1/2b*	pate'	**pos**	*L. monocytogenes*
1281	*L. monocytogenes 3c*	cooked chicken	**pos**	*L. monocytogenes*
1282	*L. monocytogenes 3c*	unknown	**pos**	*L. monocytogenes*
1069	*L. monocytogenes*	stuffed gammon joint	**pos**	*L. monocytogenes*
1072	*L. monocytogenes*	ham and cheese pancakes	**pos**	*L. monocytogenes*
1144	*L. monocytogenes 1/2a*	Stilton cheese	**pos**	*L. monocytogenes*
1145	*L. monocytogenes 1/2a*	coleslaw	**pos**	*L. monocytogenes*
1146	*L. monocytogenes 1/2a*	lettuce	**pos**	*L. monocytogenes*
1283	*L. monocytogenes 3b*	cooked turkey	**pos**	*L. monocytogenes*
1285	*L. monocytogenes 4 (not 4b)*	cheese	**pos**	*L. monocytogenes*
1286	*L. monocytogenes 3c*	cooked chicken	**pos**	*L. monocytogenes*
1287	*L. monocytogenes 3a*	unknown	**pos**	*L. monocytogenes*
1293	*L. monocytogenes 3a*	pate'	**pos**	*L. monocytogenes*
1294	*L. monocytogenes 4b*	ice cream	**pos**	*L. monocytogenes*
1295	*L. monocytogenes 3b*	pepper quiche	**pos**	*L. monocytogenes*
1299	*L. monocytogenes 1/2b*	pork liver pate	**pos**	*L. monocytogenes*
1302	*L. monocytogenes 1/2c*	hard boiled eggs	**pos**	*L. monocytogenes*
1314	*L. monocytogenes 4 (not 4b)*	pate'	**pos**	*L. monocytogenes*
1315	*L. monocytogenes 1/2c*	pate'	**pos**	*L. monocytogenes*
1316	*L. monocytogenes 3a*	cooked chicken	**pos**	*L. monocytogenes*
1321	*L. monocytogenes 3c*	sandwich	**pos**	*L. monocytogenes*
1305	*L. monocytogenes 3a*	boiled ham	**pos**	*L. monocytogenes*
1306	*L. monocytogenes 3b*	chicken liver pate'	**pos**	*L. monocytogenes*
1307	*L. monocytogenes 3b*	pate'	**pos**	*L. monocytogenes*
1308	*L. monocytogenes 4b*	cheese	**pos**	*L. monocytogenes*
1309	*L. monocytogenes 4b*	soft cheese	**pos**	*L. monocytogenes*
1310	*L. monocytogenes 3b*	chicken	**pos**	*L. monocytogenes*
1311	*L. monocytogenes 1/2c*	cooked meat	**pos**	*L. monocytogenes*
1312	*L. monocytogenes 1/2c*	ice cream	**pos**	*L. monocytogenes*
1313	*L. monocytogenes 4b*	cheese	**pos**	*L. monocytogenes*

1288	*L. monocytogenes 3a*	cooked turkey	**pos**	*L. monocytogenes*
3721	*L. monocytogenes*	pizza	**pos**	*L. monocytogenes*
4553	*L. monocytogenes*	English honey	**pos**	*L. monocytogenes*
4568	*L. monocytogenes*	environmental	**pos**	*L. monocytogenes*
4571	*L. monocytogenes*	English honey	**pos**	*L. monocytogenes*
4646	*L. monocytogenes*	ham line swab	**pos**	*L. monocytogenes*
5425	*L. monocytogenes 4b*	Jalisco-brand cheese	**pos**	*L. monocytogenes*
5849	*L. monocytogenes*	dairy environment	**pos**	*L. monocytogenes*
5850	*L. monocytogenes*	sliced cooked meat	**pos**	*L. monocytogenes*
5851	*L. monocytogenes*	dairy environment	**pos**	*L. monocytogenes*
5852	*L. monocytogenes*	dairy environment	**pos**	*L. monocytogenes*
6138	*L. monocytogenes*	dairy environment	**pos**	*L. monocytogenes*
6369	*L. monocytogenes*	smoked salmon	**pos**	*L. monocytogenes*
6484	*L. monocytogenes*	dairy environment	**pos**	*L. monocytogenes*
6610	*L. monocytogenes*	hamburger	**pos**	*L. monocytogenes*
6891	*L. monocytogenes*	Brie cheese	**pos**	*L. monocytogenes*
3328	*L. seeligeri*	cheese	**pos**	*L. seeligeri*
3327	*L. seeligeri*	cheese	**pos**	*L. seeligeri*
1297	*L. seeligeri*	pate'	**pos**	*L. seeligeri*
1298	*L. seeligeri*	chicken roll	**pos**	*L. seeligeri*
1300	*L. seeligeri*	cooked ham	**pos**	*L. seeligeri*
1301	*L. seeligeri*	corned beef	**pos**	*L. seeligeri*
1303	*L. seeligeri*	pate'	**pos**	*L. seeligeri*
2090	*L. seeligeri*	unknown	**pos**	*L seel/innocua/ivanovii*
1304	*L. seeligeri*	chicken	**pos**	*L.seel/welsh/innocua*
2875	*L. seeligeri*	unknown	**pos**	*L. seeligeri*
2874	*L. seeligeri*	unknown	**pos**	*L. seeligeri*
1289	*L. seeligeri*	crab pate'	**pos**	*L. seeligeri*
1291	*L. seeligeri*	lettuce	**pos**	*L. seeligeri*
1292	*L. seeligeri*	cooked chicken	**pos**	*L. seeligeri*
3374	*L. seeligeri*	unknown	**pos**	*L. seeligeri*
3382	*L. seeligeri*	environmental	**pos**	*L. seeligeri*
4049	*L. seeligeri*	unknown	**pos**	*L. seeligeri*
3800	*L. seeligeri*	three bean salad	**pos**	*L. seeligeri*
3801	*L. seeligeri*	egg mayonnaise	**pos**	*L. seeligeri*
3332	*L. seeligeri*	brie	**pos**	*L. seeligeri*
3331	*L. seeligeri*	unknown	**pos**	*L. seeligeri*
3329	*L. seeligeri*	unknown	**pos**	*L. seeligeri*
4050	*L. seeligeri*	dairy environment	**pos**	*L. seeligeri*
4319	*L. seeligeri*	coleslaw	**pos**	*L. seeligeri*
4340	*L. seeligeri*	lettuce	**pos**	*L. seeligeri*
4474	*L. seeligeri*	environmental	**pos**	*L. seeligeri*
6136	*L. seeligeri*	apple pie	**pos**	*L. seeligeri*
6479	*L. seeligeri*	salmon	**pos**	*L. seeligeri*
6482	*L. seeligeri*	smoked salmon	**pos**	*L. seeligeri*
6483	*L. seeligeri*	smoked salmon	**pos**	*L. seeligeri*
6765	*L. seeligeri*	rice salad	**pos**	*L. seeligeri*

7040	*L. seeligeri*	cheese	**pos**	*L. seeligeri*
7131	*L. seeligeri*	cheese	**pos**	*L. seeligeri*
7309	*L. seeligeri*	salmon-watercress	**pos**	*L. seeligeri*
7925	*L. seeligeri*	cured ham	**pos**	*L. seeligeri*
8046	*L. seeligeri*	cured ham	**pos**	*L. seeligeri*
9988	*L. seeligeri*	unknown	**pos**	*L. seeligeri*
10696	*L. seeligeri*	unknown	**pos**	*L. seeligeri*
10697	*L. seeligeri*	unknown	**pos**	*L. seeligeri*
1172	*L. welshimeri*	salami	**pos**	*L. welshimeri*
1174	*L. welshimeri*	raw chicken	**pos**	*L. welshimeri*
1175	*L. welshimeri*	sausage	**pos**	*L. welshimeri*
1176	*L. welshimeri*	chicken	**pos**	*L. welshimeri*
1177	*L. welshimeri*	smoked mackerel	**pos**	*L. welshimeri*
1178	*L. welshimeri*	unknown	**pos**	*L. welshimeri*
1179	*L. welshimeri*	food	**pos**	*L. welshimeri*
1180	*L. welshimeri*	food	**pos**	*L. welshimeri*
3242	*L. welshimeri*	unknown	**pos**	*L. welshimeri*
3233	*L. welshimeri*	unknown	**pos**	*L. welshimeri*
3360	*L. welshimeri*	potato	**pos**	*L. welshimeri*
3359	*L. welshimeri*	radish	**pos**	*L. welshimeri*
3409	*L. welshimeri*	chicken	**pos**	*L. welshimeri*
3558	*L. welshimeri*	ground veal	**pos**	*L. welshimeri*
3411	*L. welshimeri*	hamburger	**pos**	*L. welshimeri*
3354	*L. welshimeri*	unknown	**pos**	*L. welshimeri*
3353	*L. welshimeri*	unknown	**pos**	*L. welshimeri*
3351	*L. welshimeri*	unknown	**pos**	*L. welshimeri*
4096	*L. welshimeri*	raw chicken	**pos**	*L. welshimeri*
4105	*L. welshimeri*	cooked chicken	**pos**	*L. monocytogenes*
4697	*L. welshimeri*	cheese+ tomato	**pos**	*L. welshimeri*
5196	*L. welshimeri*	forza-lardons	**pos**	*L. welshimeri*
5199	*L. welshimeri*	fleury michon-lardons	**pos**	*L. welshimeri*
5203	*L. welshimeri*	dobedo-le savouray	**pos**	*L. seeligeri*
5205	*L. welshimeri*	forza-lardons	**pos**	*L. welshimeri*
5453	*L. welshimeri*	smoked salmon	**pos**	*L. seeligeri*
5828	*L. welshimeri*	lemon grass	**pos**	*L. welshimeri*
5912	*L. welshimeri*	raw chicken	**pos**	*L. welshimeri*
5915	*L. welshimeri*	chicken ready meal	**pos**	*L. welshimeri*
6587	*L. welshimeri*	smoked salmon	**pos**	*L. seeligeri*
6620	*L. welshimeri*	cooked flounder	**pos**	*L. welshimeri*
6621	*L. welshimeri*	cooked crab meat	**pos**	*L. welshimeri*
2	*L. welshimeri*	hamburger	**pos**	*L. welshimeri*
4851	*L. welshimeri*	milk	**pos**	*L. seeligeri*
4855	*L. welshimeri*	mixed salad	**pos**	*L. seeligeri*

Table 5. BAX ® system exclusivity (1)			
dd#	**Collection ID**	**Isolate source**	**BAX® *Listeria* genus test result**
1024	*Bacillus cereus*	unknown	neg
879	*Bacillus cereus*	unknown	neg
878	*Bacillus cereus*	unknown	neg
877	*Bacillus cereus*	powdered infant formula	neg
721	*Bacillus cereus*	unknown	neg
715	*Bacillus cereus*	unknown	neg
1011	*Bacillus subtilus*	mashed potatoes	neg
379	*Bacillus subtilus*	unknown	neg
713	*Bacillus thuringiensis*	unknown	neg
716	*Bacillus thuringiensis*	diseased insect larvae	neg
714	*Bacillus thuringiensis*	Mediterranean flour moth	neg
1114	*Brochothrix campestris*	soil	neg
4064	*Carnobacterium divergens*	unknown	neg
4063	*Carnobacterium gallinarum*	unknown	neg
2558	*Citrobacter freundii*	unknown	neg
383	*Citrobacter freundii*	unknown	neg
2561	*Citrobacter koseri*	blood	neg
2560	*Citrobacter koseri*	throat	neg
2625	*Enterococcus durans*	unknown	neg
2554	*Enterococcus faecalis*	unknown	neg
3981	*Enterococcus faecalis*	urine	neg
2553	*Enterococcus faecium*	unknown	neg
2552	*Enterococcus faecium*	unknown	neg
2624	*Enterococcus gallinarum*	chicken intestine	neg
2626	*Enterococcus hirae*	unknown	neg
2626	*Enterococcus hirae*	unknown	neg
7344	*Lactobacillus acidophilus*	human	neg
7332	*Lactobacillus curvatus*	milk	neg
620	*Lactobacillus rhamnosus*	unknown	neg
659	*Lactococcus lactis*	unknown	neg
9174	*Micrococcus luteus*	unknown	neg
2392	*Rhodococcus equi*	lung abscess from foal	neg
2628	*Salmonella kentucky*	unknown	neg
707	*Salmonella newport*	fatal case of food poisoning	neg
1098	*Staphylococcus aureus*	unknown	neg
1096	*Staphylococcus aureus*	unknown	neg
912	*Staphylococcus aureus*	unknown	neg
863	*Staphylococcus aureus*	unknown	neg
1111	*Staphylococcus capitis*	unknown	neg
2636	*Staphylococcus felis*	cat's ear	neg
1113	*Staphylococcus sciuri*	human skin	neg
1113	*Staphylococcus sciuri*	human skin	neg
1105	*Staphylococcus warneri*	German salami	neg

1112	*Staphylococcus xylosus*	unknown	neg
1107	*Staphylococcus xylosus*	lockwurst	neg
692	*Streptococcus bovis*	cow dung	neg
3996	*Streptococcus equi*	unknown	neg
3992	*Streptococcus mutans*	carious dentine	neg
695	*Streptococcus pyogenes*	unknown	neg
692	*Streptococcus thermophilus*	cow dung	neg

Table 3a Summary table of Listeria recovery rates from surfaces. (1)						
Surface	**Challenge Strain**	**Sample**	**cfu/ surface**	**USDA/FSIS culture**	**BAX® – 24 / 28 hour (24 ± 2 hr swab, 28 ± 2 hr sponge)**	**MOX/API *Listeria* culture positive**
		Type		Number positive/Total	Number positive / Total (Number confirmed / Number BAX® assay positive)[a]	BAX® enrichment [b]
Ceramic †	*L. innocua DD 104*	Sponge	39	1/20	4/20 (4/4)	5/20
			390	12/20	19/20 (19/19)	19/20
			0	0/5	0/5	0/5
Cast Iron †	*L. monocytogenes DD 1288*	Swab	156	9/20	11/20 (11/11)	12/20
			780	18/20	19/20 (19/19)	19/20
			0	0/5	0/5	0/5
Plastic †	*L. monocytogenes DD 1306*	Swab	15	8/20	6/20 (6/6)	6/20
			99	19/20	20/20 (20/20)	20/20
			0	0/5	0/5	0/5
Concrete *	*L. ivanovii DD 3557*	Sponge	211	19/20	20/20 (20/20)	20/20
			319	20/20	20/20 (20/20)	20/20
			0	0/5	0/5	0/5
Rubber *	*L. welshimeri DD 3359*	Sponge	2^{c}	3/20	2/20 (2/2)	2/20
			21^{d}	12/20	13/20 (13/13)	14/20
			0	0/5	0/5	0/5
Air filter †	*L. monocytogenes DD 1305*	Swab	60	18/20	15/20 (15/15)	15/20
			300	20/20	20/20 (20/20)	20/20
			0	0/5	0/5	0/5
Wood, painted †	*L. seeligeri DD 2874*	Sponge	1×10^5	17/20	17/20 (17/17)	17/20
			5×10^5	20/20	20/20 (20/20)	20/20
			0	0/5	0/5	0/5
Wood, unpainted ‡	*L. welshimeri* ATCC 35897	Swab	1.5×10^4	16/20	15/20 (15/15)	18/20
			0	0/5	0/5	0/5
Totals (uninoculated)				40/40	40/40	40/40
Totals (inoculated)				212/300	221/300	227/300
Steel, stainless ‡, e	*L. monocytogenes* ATCC 7644	Sponge	5×10^5	14/20	2/20	4/20

† Experiment conducted at the DuPont Central Research and Development Experimental Station * Experiment conducted at DuPont Qualicon

‡ Experiment conducted by rtech laboratories

a Figures in parenthesis are the number of assays which are BAX® assay positive for which culture confirmation was successful

b Figure represents the number of enrichments from which a confirmed *Listeria* isolate was recovered (therefore if there were 4 confirmed BAX® positive enrichments and 5 culture positive enrichments there was 1 false negative for that treatment)

[c] 6.5×10^5 cfu *Pseudomonas aeruginosa* added to each plot as competitive flora

[d] 2.5×10^5 cfu *Pseudomonas aeruginosa* added to each plot as competitive flora

e Results from rtech do not support a claim of equivalence, therefore this surface is not included in the AOAC validated product claim.

Table 3b Method performance for the detection of *Listeria* species from environmental surfaces by the BAX® System. (1)										
Surface	Level (cfu applied per analytical unit)	Total Samples (each treatment)	BAX® Presumptive (# positive)	BAX® Enrichment Confirmed (# positive)	Reference Method (# positive)	Sensitivity	Specificity	False Negative	False Positive	X^2 Value
Ceramic	39	20	4	5	1	0.80	1.00	0.20	0.00	1.33
	390	20	19	19	12	1.00	1.00	0.00	0.00	1.16
Cast Iron	156	20	11	12	9	0.92	1.00	0.08	0.00	0.05
	780	20	19	19	18	1.00	1.00	0.00	0.00	0
Plastic	15	20	6	6	8	1.00	1.00	0.00	0.00	0.07
	99	20	20	20	19	1.00	1.00	0.00	0.00	0
Concrete	211	20	20	20	19	1.00	1.00	0.00	0.00	0
	319	20	20	20	20	1.00	1.00	0.00	0.00	0
Rubber	2	20	2	2	3	1.00	1.00	0.00	0.00	0
	21	20	13	14	12	0.93	1.00	0.07	0.00	0
Air filter	60	20	15	15	18	1.00	1.00	0.00	0.00	0.12
	300	20	20	20	20	1.00	1.00	0.00	0.00	0
Wood, painted	1×10^5	20	17	17	17	1.00	1.00	0.00	0.00	0
	5×10^5	20	20	20	20	1.00	1.00	0.00	0.00	0
Wood, unpainted	1.5×10^4	20	15	18	16	0.83	1.00	0.17	0.00	0
Steel, stainless	5×10^5	20	2	4	14	0.50	1.00	0.50	0.00	10

DISCUSSION OF JULY 2008 MODIFICATION (3)

No statistically significant differences were obtained when comparing the BAX ® test kit and the appropriate reference method for any of the studies performed. Chi-square values calculated for these studies ranged from 0.10 to 1.26 (with a value of > 3.84 indicative of significance at the 95% confidence level) demonstrating that the test and reference methods performed in an indistinguishable manner. All BAX ® positive results were able to be confirmed by culture, and none of the BAX ® negative samples were positive by culture, thus no false negative or false positive results were obtained by the test method. Sensitivity and specificity were thus 100% for all matrices tested. For naturally occurring flora from smoked salmon and environmental sponges, a wide variety of species were isolated as determined by Micro-ID *Listeria* characterization.

All indeterminate (*Listeria monocytogenes/innocua* Micro-ID *Listeria* code 44044) results resolved as *L. monocytogene*s upon characterization with the Riboprinter ™ for salmon derived isolates. The environmental sponge samples also were positive for a diverse population of *Listeria* species, but were not subjected to molecular subtyping at the request of the provider of the samples.

***Table 2a. Method performance BAX ® Instrument* (3)**

			Samples Positive					Sensitivity rate, %[2]	False Neg %[3]	False Pos %[4]	Specificity rate, %[5]
			Total	BAX system		Reference OMA	Chi sq.[1]	BAX®	BAX®	BAX®	BAX®
Sample Type	Sample Type	MPN/ 25g		Pos.	Conf.						
Spinach	Spiked	0.58	20	7	7	6	0.11	100	0	0	100
	Control		5	0	0	0				0	
Frankfurters	Spiked	0.88	20	15	15	13	0.46	100	0	0	100
	Control		5	0	0	0				0	
Frankfurters (Independent Laboratory Study)	Spiked	0.58	20	7	7	8	0.10	100	0	0	100
	Control		5	0	0	0				0	
Processed Cheese	Spiked	2.3	20	12	12	14	0.43	100	0	0	100
	Control		5	0	0	0				0	
Salmon	Natural	1.2	20	17	17	14	1.26	100	0	0	100
Drain	Natural	N/A	20	7	7	8	0.10	100	0	0	100

[1] Mantel-Haenszel Chi-Square test statistic

[2] False negative rate is calculated as BAX (-) Ref (+) BAX enrichment samples / Tot Ref (+) samples

[3] False positive rate is calculated as BAX (+) Ref (-) / Tot Ref (-) samples

[4] Sensitivity is calculated as 100% – false negative rate = 100%

[5] Specificity is calculated as 100% – false positive rate = 100%

***Table 2b. Method performance BAX ® Q7 Instrument* (3)**

			Samples Positive					Sensitivity rate, %[2]	False Neg %[3]	False Pos %[4]	Specificity rate, %[5]
			Total	BAX system		Reference OMA					
Sample Type	Sample Type	MPN/ 25g		Pos.	Conf.		Chi sq.[1]	BAX®	BAX®	BAX®	BAX®
Spinach	Spiked	0.58	20	7	7	6	0.11	100	0	0	100
	Control		5	0	0	0				0	
Frankfurters	Spiked	0.88	20	15	15	13	0.46	100	0	0	100
	Control		5	0	0	0				0	
Frankfurters (Independent Laboratory Study)	Spiked	0.58	20	7	7	8	0.10	100	0	0	100
	Control		5	0	0	0				0	
Processed Cheese	Spiked	2.3	20	12	12	14	0.43	100	0	0	100
	Control		5	0	0	0				0	
Salmon	Natural	1.2	20	17	17	14	1.26	100	0	0	100
Drain	Natural	N/A	20	7	7	8	0.10	100	0	0	100

[1] Mantel-Haenszel Chi-Square test statistic
[2] False negative rate is calculated as BAX (-) Ref (+) BAX enrichment samples / Tot Ref (+) samples
[3] False positive rate is calculated as BAX (+) Ref (-) / Tot Ref (-) samples
[4] Sensitivity is calculated as 100% – false negative rate = 100%
[5] Specificity is calculated as 100% – false positive rate = 100%

Table 5. Inclusivity Results (3)

Strain	Other Collection Designation	Species	Source	BAX Classic	BAX Q7
1283		*L. monocytogenes 3b*	Cooked turkey	POS	POS
3411		*L. welshimeri*	Hamburger	POS	POS
3558		*L. welshimeri*	Ground veal	POS	POS
1175		*L. welshimeri*	Sausage	POS	POS
1176		*L. welshimeri*	Chicken	POS	POS
1177		*L. welshimeri*	Smoked mackerel	POS	POS
1063		*L. innocua*	Chopped ham	POS	POS
1064		*L. innocua*	Chopped ham	POS	POS
1066		*L. innocua*	Stilton cheese	POS	POS
1154		*L. innocua*	Paté	POS	POS
1156		*L. innocua*	Lettuce	POS	POS
649	*ATCC 19119*	*L. ivanovii*	Sheep	POS	POS
1164		*L. ivanovii*	Radish	POS	POS
1165		*L. ivanovii*	Belgian salami	POS	POS
3072		*L. ivanovii*	Unknown	POS	POS
1281		*L. monocytogenes* 3c	cooked chicken	POS	POS
1287		*L. monocytogenes* 3a	unknown	POS	POS
1313		*L. monocytogenes* 4b	cheese	POS	POS
1316		*L. monocytogenes* 3a	Cooked chicken	POS	POS
3359		*L. welshimeri*	Radish	POS	POS
3360		*L. welshimeri*	Potato	POS	POS
3409		*L. welshimeri*	Chicken	POS	POS
1157		*L. innocua*	Black pudding	POS	POS
1158		*L. innocua*	Celery salad	POS	POS
1159		*L. innocua*	Raw chicken	POS	POS
1288		*L. monocytogenes 3a*	Cooked turkey	POS	POS
1294		*L. monocytogenes 4b*	Ice cream	POS	POS
1299		*L. monocytogenes 1/2b*	Pork liver paté	POS	POS
1302		*L. monocytogenes 1/2c*	Hard-boiled eggs	POS	POS
1308		*L. monocytogenes 4b*	Cheese	POS	POS
1309		*L. monocytogenes 4b*	Soft cheese	POS	POS
1144		*L. monocytogenes 1/2a*	Stilton cheese	POS	POS
5425		*L. monocytogenes 4b*	Jalisco cheese	POS	POS
1289		*L. seeligeri*	Crab paté	POS	POS
1291		*L. seeligeri*	Lettuce	POS	POS
1297		*L. seeligeri*	Paté	POS	POS
1298		*L. seeligeri*	Chicken roll	POS	POS
1300		*L. seeligeri*	Cooked ham	POS	POS
1301		*L. seeligeri*	Corned beef	POS	POS
3373		*L. ivanovii*	Unknown	POS	POS
1293		*L. monocytogenes 3a*	Paté	POS	POS
1295		*L. monocytogenes 3b*	Pepper quiche	POS	POS
1305		*L. monocytogenes 3a*	Boiled ham	POS	POS
1306		*L. monocytogenes 3b*	Chicken liver paté	POS	POS
1307		*L. monocytogenes 3b*	Paté	POS	POS
1310		*L. monocytogenes 3b*	Chicken	POS	POS
1311		*L. monocytogenes 1/2c*	Cooked meat	POS	POS
1312		*L. monocytogenes 1/2c*	Ice cream	POS	POS
1314		*L. monocytogenes 4 (not 4b)*	Paté	POS	POS
1315		*L. monocytogenes 1/2c*	Paté	POS	POS

Table 6. BAX system exclusivity (3)

dd# 1024	Other Collection Designation	collection ID	isolate source	BAX® *Listeria* genus test result
879	ATCC 7004	*Bacillus cereus*	Unknown	NEG
878	ATCC 11778	*Bacillus cereus*	Unknown	NEG
877	ATCC 13061	*Bacillus cereus*	Unknown	NEG
721	ATCC 33018	*Bacillus cereus*	Powdered infant formula	NEG
715	ATCC 13061	*Bacillus cereus*	Unknown	NEG
1011	ATCC 14579	*Bacillus cereus*	Unknown	NEG
379		*Bacillus subtilus*	Mashed potatoes	NEG
713		*Bacillus subtilus*	Unknown	NEG
716	ATCC 35646	*Bacillus thuringiensis*	Unknown	NEG
714	ATCC 33679	*Bacillus thuringiensis*	Diseased insect larvae	NEG
1114	ATCC 10792	*Bacillus thuringiensis*	Mediterranean flour moth	NEG
4064	ATCC 43754	*Brochothrix campestris*	Soil	NEG
4063		*Carnobacterium divergens*	Unknown	NEG
2558		*Carnobacterium gallinarum*	Unknown	NEG
383	ATCC 43864	*Citrobacter freundii*	Unknown	NEG
2561	ATCC 8090	*Citrobacter freundii*	Unknown	NEG
2560	ATCC 27028	*Citrobacter koseri*	Blood	NEG
2625	ATCC 25408	*Citrobacter koseri*	Throat	NEG
2554	ATCC 19432	*Enterococcus durans*	Unknown	NEG
3981	ATCC 35550	*Enterococcus faecalis*	Unknown	NEG
2553	ATCC 29212	*Enterococcus faecalis*	Urine	NEG
2552	ATCC 35667	*Enterococcus faecium*	Unknown	NEG
2624	ATCC 19434	*Enterococcus faecium*	Unknown	NEG
2626	ATCC 35038	*Enterococcus gallinarum*	Chicken intestine	NEG
7344	ATCC 8043	*Enterococcus hirae*	Unknown	NEG
7332	ATCC 9649	*Lactobacillus acidophilus*	Human	NEG
620	ATCC 25601	*Lactobacillus curvatus*	Milk	NEG
659	ATCC 7469	*Lactobacillus rhamnosus*	Unknown	NEG
9174	ATCC 19435	*Lactococcus lactis*	Unknown	NEG
2392	ATCC 272	*Micrococcus luteus*	Unknown	NEG
2628	ATCC 6939	*Rhodococcus equi*	Lung abscess from foal	NEG
707	ATCC 9263	*Salmonella* Kentucky	Unknown	NEG
1098	ATCC 6962	*Salmonella* Newport	Fatal case of food poisoning	NEG
1096		*Staphylococcus aureus*	Unknown	NEG
912		*Staphylococcus aureus*	Unknown	NEG
863	ATCC 10832	*Staphylococcus aureus*	Unknown	NEG
1111	ATCC 12600	*Staphylococcus aureus*	Unknown	NEG
2636	ATCC 35661	*Staphylococcus capitis*	Unknown	NEG
1113	ATCC 49168	*Staphylococcus felis*	Cat's ear	NEG
1105	ATCC 29060	*Staphylococcus sciuri*	Human skin	NEG
1112		*Staphylococcus warneri*	German salami	NEG
1107	ATCC 35663	*Staphylococcus xylosus*	Unknown	NEG
692		*Staphylococcus xylosus*	Lockwurst	NEG
3996	ATCC 33317	*Streptococcus bovis*	Cow dung	NEG
3992	ATCC 33398	*Streptococcus equi*	Unknown	NEG
695	ATCC 25175	*Streptococcus mutans*	Carious dentine	NEG
693	ATCC 12344	*Streptococcus pyogenes*	Unknown	NEG

DISCUSSION OF JULY 2013 MODIFICATION (6)

The results of the method comparison between the digital DuPont™ Thermal Block and the analog heating/cooling blocks are provided in Table 3 below. For all sample types and BAX® System assays evaluated, the results for samples processed with the DuPont™ Thermal Block and the original heating/cooling blocks demonstrated no significant statistical difference as indicated by POD analysis (the 95% confidence interval of the dPOD included 0 in all cases). For additional figures illustrating the target responses of the individual BAX® System assays, see Appendix B.

All 544 samples inoculated with high levels of the target organism returned positive results with the BAX® System using both sample preparation methods, and all 544 samples tested as unspiked negative controls returned negative results with the BAX® System using both sample preparation methods with the exception of the non-inoculated poultry rinse samples that gave positive results *for Campylobacer jejuni*, while giving negative results for the target *C. coli* that was spiked into the test samples. For samples inoculated with low levels of target organism, the two preparation methods returned identical results for 530 of the 544 samples tested. The results for the 14 samples that returned different results between the two methods are summarized in Table 3. Because the low-spike samples were tested at levels near the limit of detection for the BAX® System assays, some discrepancy between the two methods is expected based on factors such as the distribution of the target organism within the sample.

Analysis of target response in cases where a fractional response was not generated, while demonstrating significant differences from a statistical standpoint in some cases, were not indicative of any difference that would likely be significant in a practical sense. All average differences were less than 10% for melt curve based target peak height, or target peak area to target plus internal control peak areas (for the Yeast and Mold assay) and all average C_t differences were less than 1 for all real time assay.

Because the difference in results between the two methods demonstrated no significant statistical difference as indicated by the POD analysis, these differences are found to be acceptable in this study for demonstrating equivalency between the two methods.

Table 3. BAX® System Results – DuPont™ Thermal Block vs. Analog Heating/Cooling Blocks (6)

BAX® System Assay	Sample Type	Spike Level	Test Portions	Heating/Cooling Blocks			DuPont™ Thermal Block			$dPOD_{TB}$ [d]	95% CI[e]
				X[a]	POD_{2B}[b]	95% CI[e]	X[a]	POD_{TB}[c]	95% CI[e]		
Genus *Listeria*	Queso fresco cheese	High	17	17	1	0.82, 1.0	17	1	0.82, 1.0	0	-0.18, 0.18
		Low	17	9	0.53	0.31, 0.74	12	0.71	0.47, 0.87	-0.18	-0.45, 0.14
		Control	17	0	0	0 , 0.19	0	0	0, 0.19	0	-0.19, 0.19
	Frankfurters	High	17	17	1	0.82, 1.0	17	1	0.82, 1.0	0	-0.18, 0.18
		Low	17	16	0.94	0.73, 0.99	17	1	0.82, 1.0	-0.059	-0.27, 0.13
		Control	17	0	0	0, 0.19	0	0	0, 0.19	0	-0.19, 0.19
	Smoked salmon	High	17	17	1	0.82, 1.0	17	1	0.82, 1.0	0	-0.18, 0.18
		Low	17	17	1	0.82, 1.0	17	1	0.82, 1.0	0	-0.18, 0.18
		Control	17	0	0	0, 0.19	0	0	0, 0.19	0	-0.19 , 0.19
		High	17	17	1	0.82, 1.0	17	1	0.82, 1.0	0	-0.18, 0.18
										0	-0.18, 0.18
										0	-0.19, 0.19

DISCUSSION OF JANUARY 2016 MODIFICATION (7)

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 *Listeria* spp. from frankfurters, bagged spinach, queso fresco and plastic and stainless steel 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 January 2016 (7)

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)
Stainless Steel USDA-MLG 48 h	*L. ivanovii* DD 649 *C. freundii* DD 3907[h]	1.8×10^5	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		1.8×10^4	20	6	0.30	(0.14, 0.52)	5	0.25	(0.11, 0.47)	0.05	(-0.22, 0.31)
		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.
[h]*C. freundii* was applied at levels targeting 10 X the *Listeria* level. For the high level samples this was 9×10^5 CFU/mL and for the fractional level samples this was 9×10^4 CFU/mL

Table 3. Method comparison results - POD for food and environmental matrixes (7)

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)
Stainless Steel USDA-MLG 48 h	*L. ivanovii* DD 649 *C. freundii* DD 3907[h]	1.8 X 10^5	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		1.8 X 10^4	20	5	0.25	(0.11, 0.47)	5	0.25	(0.11, 0.47)	0.05	(-0.22, 0.31)
		Uninoculated	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.14, 0.14)

[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.
[h]*C. freundii* was applied at levels targeting 10 X the *Listeria* level. For the high level samples this was 9 X 10^5 CFU/mL and for the fractional level samples this was 9 X 10^4 CFU/mL.

Table 4. Inclusivity Results for the BAX® System X5 PCR Assay for Genus *Listeria* (7)

DuPont Strain ID	Genus *Listeria* Serotype and Serogroup	Result	DuPont Strain ID	Genus *Listeria* Serotype and Serogroup	Result
566	*Listeria monocytogenes*	Pos	4553	*Listeria monocytogenes*	Pos
605	*Listeria monocytogenes*	Pos	4568	*Listeria monocytogenes*	Pos
647	*Listeria monocytogenes*	Pos	4571	*Listeria monocytogenes*	Pos
648	*Listeria monocytogenes*	Pos	5425	*Listeria monocytogenes*	Pos
652	*Listeria monocytogenes*	Pos	7644	*Listeria monocytogenes*	Pos
653	*Listeria monocytogenes*	Pos	643	*Listeria murrayi/ grayi*	Neg
1069	*Listeria monocytogenes*	Pos	644	*Listeria innocua*	Pos
1072	*Listeria monocytogenes*	Pos	649	*Listeria invanovii*	Pos
1144	*Listeria monocytogenes*	Pos	650	*Listeria seeligeri*	Pos
1145	*Listeria monocytogenes*	Pos	654	*Listeria welshimeri*	Pos
1146	*Listeria monocytogenes*	Pos	892	*Listeria innocua*	Pos
1147	*Listeria monocytogenes*	Pos	898	*Listeria innocua*	Pos
1149	*Listeria monocytogenes*	Pos	921	*Listeria innocua*	Pos
1152	*Listeria monocytogenes*	Pos	922	*Listeria innocua*	Pos
1281	*Listeria monocytogenes*	Pos	924	*Listeria innocua*	Pos
1282	*Listeria monocytogenes*	Pos	927	*Listeria innocua*	Pos
1283	*Listeria monocytogenes*	Pos	944	*Listeria murrayi/grayi*	Neg
1285	*Listeria monocytogenes*	Pos	1063	*Listeria innocua*	Pos
1286	*Listeria monocytogenes*	Pos	1064	*Listeria innocua*	Pos
1287	*Listeria monocytogenes*	Pos	1156	*Listeria innocua*	Pos
1288	*Listeria monocytogenes*	Pos	1164	*Listeria ivanovii*	Pos
1293	*Listeria monocytogenes*	Pos	1165	*Listeria ivanovii*	Pos
1294	*Listeria monocytogenes*	Pos	1167	*Listeria ivanovii*	Pos
1295	*Listeria monocytogenes*	Pos	1171	*Listeria ivanovii*	Pos
1299	*Listeria monocytogenes*	Pos	1172	*Listeria welshimeri*	Pos
1302	*Listeria monocytogenes*	Pos	1174	*Listeria welshimeri*	Pos
1305	*Listeria monocytogenes*	Pos	1175	*Listeria welshimeri*	Pos
1306	*Listeria monocytogenes*	Pos	1176	*Listeria welshimeri*	Pos
1307	*Listeria monocytogenes*	Pos	1177	*Listeria welshimeri*	Pos
1308	*Listeria monocytogenes*	Pos	1179	*Listeria welshimeri*	Pos
1309	*Listeria monocytogenes*	Pos	1289	*Listeria seeligeri*	Pos
1310	*Listeria monocytogenes*	Pos	1291	*Listeria seeligeri*	Pos
1311	*Listeria monocytogenes*	Pos	1292	*Listeria seeligeri*	Pos
1312	*Listeria monocytogenes*	Pos	1297	*Listeria seeligeri*	Pos
1313	*Listeria monocytogenes*	Pos	1298	*Listeria seeligeri*	Pos
1314	*Listeria monocytogenes*	Pos	1300	*Listeria seeligeri*	Pos
1315	*Listeria monocytogenes*	Pos	2874	*Listeria seeligeri*	Pos
1316	*Listeria monocytogenes*	Pos	3244	*Listeria innocua*	Pos
1321	*Listeria monocytogenes*	Pos	3327	*Listeria seeligeri*	Pos
3573	*Listeria monocytogenes*	Pos	3329	*Listeria seeligeri*	Pos
3574	*Listeria monocytogenes*	Pos	3351	*Listeria welshimeri*	Pos
3576	*Listeria monocytogenes*	Pos	3354	*Listeria welshimeri*	Pos
3577	*Listeria monocytogenes*	Pos	3359	*Listeria welshimeri*	Pos
3578	*Listeria monocytogenes*	Pos	3363	*Listeria murrayi/grayi*	Neg
3579	*Listeria monocytogenes*	Pos	3376	*Listeria ivanovii*	Pos
3580	*Listeria monocytogenes*	Pos	3555	*Listeria grayi*	Pos
3581	*Listeria monocytogenes*	Pos	3572	*Listeria innocua*	Pos
3582	*Listeria monocytogenes*	Pos	3678	*Listeria ivanovii*	Pos

Table 5. Exclusivity Results for the BAX® System X5 PCR Assay for Genus *Listeria* (7)

DuPont Strain ID	*Non-GenusListeria* Serotype and Serogroup	Result	DuPont Strain ID	*Non-Genus Listeria* Serotype and Serogroup	Result
379	*Bacillus subtilus*	Neg	1111	*Staphylococcus capitus*	Neg
383	*Citrobacter freundii*	Neg	1112	*Staphylococcus xylosus*	Neg
659	*Lactococcus lactis*	Neg	1113	*Staphylococcus sciuri*	Neg
691	*Streptococcus thermophilus*	Neg	2392	*Rhodococcus equi*	Neg
692	*Streptococcus bovis*	Neg	2552	*Enterococcus faecium*	Neg
695	*Streptococcus pyogenes*	Neg	2553	*Enterococcus faecium*	Neg
707	*Salmonella newport*	Neg	2554	*Enterococcus faecalis*	Neg
713	*Bacillus thuringiensis*	Neg	2558	*Citrobacter freundii*	Neg
714	*Bacillus thuringiensis*	Neg	2560	*Citrobacter koseri*	Neg
715	*Bacillus cereus*	Neg	2561	*Citrobacter koseri*	Neg
716	*Bacillus thuringiensis*	Neg	2624	*Enterococcus gallinarum*	Neg
721	*Bacillus cereus*	Neg	2625	*Enterococcus durans*	Neg
863	*Staphylococcus aureus*	Neg	2626	*Enterococcus hirae*	Neg
877	*Bacillus cereus*	Neg	2628	*Salmonella kentucky*	Neg
878	*Bacillus cereus*	Neg	2636	*Staphylococcus felis*	Neg
879	*Bacillus cereus*	Neg	3981	*Enterococcus faecalis*	Neg
912	*Staphylococcus aureus*	Neg	3992	*Streptococcus mutans*	Neg
1011	*Bacillus subtilus*	Neg	3996	*Streptococcus equi*	Neg
1024	*Bacillus cereus*	Neg	4063	*Carnobacterium gallinarum*	Neg
1096	*Staphylococcus aureus*	Neg	4064	*Carnobacterium divergens*	Neg
1098	*Staphylococcus aureus*	Neg	7332	*Lactobacillus curvatus*	Neg
1105	*Staphylococcus warneri*	Neg	7334	*Lactobacillus acidophilus*	Neg
1107	*Staphylococcus xylosus*	Neg	9174	*Micrococcus luteus*	Neg

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