



# CERTIFICATION

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

Certificate No.  
**050903**

The AOAC Research Institute hereby certifies the method known as:

### **BAX<sup>®</sup> System PCR Assay for Genus *Listeria* 24E**

manufactured by

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

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

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

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

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<b>METHOD NAME</b> BAX® System PCR Assay for Genus <i>Listeria</i> 24E Formerly DuPont™ BAX® System PCR Assay for Genus <i>Listeria</i> 24E	<b>CATALOG NUMBERS</b> BAX® Assay KIT2003 (D13608135), 24 LEB Complete MED2005 (D14654989), 24 LEB Buffer Supplement MED2000 (D15407304)	
<b>INDEPENDENT LABORATORY</b> rtech Laboratories 1200 W. Country Road F Arden Hills, MN 55112 USA		
<b>APPLICABILITY OF METHOD</b> Target organism – <i>Listeria</i> species.  Matrixes – Bagged spinach, processed cheese, frankfurters, cooked shrimp, and stainless steel  Performance claims – The method performed equivalent or superior to the reference methods.	<b>REFERENCE METHODS</b>  Cook, L.V. 2002. USDA/FSIS Microbiology Laboratory Guidebook, 3 <sup>rd</sup> Ed., revision 3. USDA/FSIS (2)  FDA Bacteriological Analytical Manual. U.S. Food and Drug Administration (3)	
<b>ORIGINAL CERTIFICATION DATE</b> May 15, 2009	<b>CERTIFICATION RENEWAL RECORD</b> Renewed through December 2025.	
<b>METHOD MODIFICATION RECORD</b> <ol style="list-style-type: none"> <li>1. March 2017 Level 1</li> <li>2. January 2018 Level 1</li> <li>3. May 2019 Level 1</li> <li>4. December 2023 Level 1</li> <li>5. December 2024 Level 1</li> </ol>	<b>SUMMARY OF MODIFICATION</b> <ol style="list-style-type: none"> <li>1. Name change from DuPont Nutrition &amp; Health to Qualicon D LLC., a Hygiena company.</li> <li>2. Inserts, labels, and manuals updated to Hygiena.</li> <li>3. Editorial updates to insert and corporate address.</li> <li>4. Editorial changes.</li> <li>5. Editorial changes.</li> </ol>	
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**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, 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 VALIDATION STUDY (1)**

The results of the method comparison study demonstrate that the BAX system assay for detecting Genus *Listeria* 24E is comparable to the reference methods for detecting *Listeria* spp. in a variety of sample types. Chi-square values for the sample types tested showed equivalent (<3.84) or better (≥3.84) *Listeria* detection with the BAX system compared to the reference method at a 95% confidence level. With regard to false positives reported with stainless steel samples, contaminating cells were dried on the surface, thus injuring them. While sub-lethally injured cells can remain viable in selective culture, they may not grow due to other selective pressures.

The results for frankfurter and stainless steel samples from the independent laboratory support the results of the internal study.

All test samples were incubated for 24 hours, with the exception of Queso Fresco cheese samples, which were incubated for 26 hours. Preparatory studies indicated slower growth of *Listeria* in this food type. Thus, in the interest of maximum detection, a minimum enrichment time of 26 hours is recommended for this matrix.

As the BAX system returned positive results for all *Listeria* strains, with the exception of some *L. grayi/murrayi* strains, and negative results for all non-*Listeria* strains tested, the results of inclusivity/exclusivity testing suggest 100% inclusivity (excluding the known exceptions) and 100% exclusivity for this assay. As DNA sequence-based typing schemes of *Listeria* indicate that *L. grayi* clusters distantly from all other species of *Listeria* (3), debate as to whether this organism is truly a *Listeria* species is ongoing. 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* (4).

**Table 3a. Summary table of results. (1)**

Food/Surface Type	Challenge Strain	Type	cfu/ analytical unit MPN	Reference Method culture Number positive/Total	BAX 24E Number positive / Total (Number confirmed / Number BAX assay positive) <sup>a</sup>	MOX/API <i>Listeria</i> culture positive BAX enrichment <sup>b</sup>
Frankfurters	<i>L. monocytogenes</i> 4b DD 1309	Spiked	0.57	9/20	6/20 (6/6)	6
		control	0	0/5	0/5	0
Spinach	<i>L. monocytogenes</i> 3b DD 1283	Spiked	0.23	14/20	14/20 (14/14)	15
		Control	0	0/5	0/5	0
Stainless Steel - Study 1	<i>L. monocytogenes</i> DD 1308	Spiked	1.2 x 10 <sup>5</sup>	17/20	19/20 (19/19)	19
		Control	0	0/5	0/5	0
Stainless Steel - Study 2	<i>L. monocytogenes</i> DD 1308	Spiked	2.8	6/20	3/20 (3/3)	3
		Control	0	0/5	0/5	0
Stainless Steel - Study 3	<i>L. ivanovii</i> DD 649	Spiked	1.08 x 10 <sup>5</sup>	0/20	18/20 (16/18)	16
		Control	0	0/5	0/5	0
Cooked Shrimp-Study 1	<i>Listeria</i> (naturally contaminated) <sup>c</sup>	Spiked	11.5	14/20	10/20 (10/10)	10
		Control	0	0/5	0/5	0
Cooked Shrimp-Study 2	<i>L. monocytogenes</i> DD 1144	Spiked	6	11/20	19/20 (19/19)	20
		Control	0	0/5	0/5	0
Queso Fresco Cheese (26 h) - Study 1	<i>L. monocytogenes</i> DD 605	Spiked	>27.5	12/20	20/20 (20/20)	20
		Control	0	0/5	0/5	0
Queso Fresco Cheese (26 h) - Study 2	<i>L. innocua</i> DD 3244	Spiked	0.725	13/20	18/20 (18/18)	18

<sup>a</sup> Figures in parenthesis are the number of assays which are BAX assay positive for which culture confirmation was successful

<sup>b</sup> Figure represents the number of enrichments from which a confirmed *Listeria* isolate was recovered

<sup>c</sup> See footnote a in Table 2

**Table 3b Method performance for the detection of *Listeria* species by the BAX system. (1)**

Food/Surface	Strain tested	cfu/ analytical unit	MPN/ 25 g	Instrument	BAX Presumptive (# positive)	BAX Enrichment (# positive)	Reference Method (# positive)	Sensitivity <sup>1</sup>	Specificity <sup>2</sup>	False Negative <sup>3</sup>	False Positive <sup>4</sup>	X <sup>2</sup> Value <sup>5</sup>
Frankfurters	<i>L. monocytogenes</i> 4b DD 1309	0.57	0.57	Classic Q7	6/20	6/20	9/20	1.00	1.00	0	0	0.936
					6/20	6/20	9/20	1.00	1.00	0	0	0.936
					0/5	0/5	0/5	-	1.00	0	0	
Spinach	<i>L. monocytogenes</i> 3b DD 1283	3.4	0.23	Classic Q7	14/20	15/20	14/20	0.93	1.00	0.07	0	0
					14/20	15/20	14/20	0.93	1.00	0.07	0	0
					0/5	0/5	0/5	-	1.00	0	0	
Stainless steel	<i>L. monocytogenes</i> DD 1308	1.2 X10 <sup>5</sup>	N/A	Classic Q7	19/20	19/20	17/20	1.00	1.00	0	0	1.08
					19/20	19/20	17/20	1.00	1.00	0	0	1.08
					0/5	0/5	0/5	-	1.00	0	0	
	<i>L. monocytogenes</i> DD 1308	2.8	N/A	Classic Q7	3/20	3/20	6/20	1.00	1.00	0	0	1.26
					3/20	3/20	6/20	1.00	1.00	0	0	1.26
					0/5	0/5	0/5	-	1.00	0	0	
Cooked shrimp	<i>L. ivanovii</i> DD 649	1.08 X10 <sup>5</sup>	N/A	Classic Q7	18/20	16/20	0/20	0.94	0.25	0.06	0.75	23.4
					18/20	16/20	0/20	1.00	0.50	0	0.50	26.0
					0/15	0/15	0/15	-	1.00	0	0	
	<i>L. monocytogenes</i> DD 1144	0.98	6	Classic Q7	19/20	20/20	11/20	0.95	1.00	0.05	0	8.32
					19/20	20/20	11/20	0.95	1.00	0.05	0	8.32
					0/5	0/5	0/5	-	1.00	0	0	
Queso fresco cheese	<i>Listeria</i> (nat.urally contaminated)	N/A	11.5	Classic Q7	10/20	10/20	14/20	1.00	1.00	0	0	1.63
					9/20	10/20	14/20	0.90	1.00	0.05	0	2.49
					0/5	0/5	0/5	-	1.00	0	0	
	<i>L. monocytogenes</i> DD 605	2.31 X10 <sup>2</sup>	>27.5	Classic Q7	20/20	20/20	12/20	1.00	1.00	0	0	13.0
					20/20	20/20	12/20	1.00	1.00	0	0	13.0
					0/5	0/5	0/5	-	1.00	0	0	
<i>L. innocua</i> DD 3244	7.1	0.725	Classic Q7	18/20	18/20	13/20	1.00	1.00	0	0	3.49	
				18/20	18/20	13/20	1.00	1.00	0	0	3.49	
				0/10	0/10	0/10	-	1.00	0	0		

<sup>1</sup> Sensitivity is calculated as 100% – false negative rate

<sup>2</sup> Specificity is calculated as 100% – false positive rate

<sup>3</sup> False negative is the number of BAX (-) Ref (+) BAX enrichment samples / Tot Ref (+) BAX enrichments

<sup>4</sup> False positive rate is calculated as BAX (+) Ref (-) / Tot Ref (-) BAX enrichments

<sup>5</sup> Mantel -Haenszel Chi-Square test statistic used for calculating significance of results

Table 5 BAX system inclusivity (1)						
dd#	Collection ID	Other Strain Designation (if available)	Serotype (if known)	Isolate source	BAX System 24E Genus <i>Listeria</i>	
					Q7 Result	Classic Result
566	<i>Listeria monocytogenes</i>	ATCC15313		Rabbit	POS	POS
605	<i>Listeria monocytogenes</i>	ATCC19111		Poultry	POS	POS
643	<i>Listeria murrayi</i>	ATCC25401		Standing corn stalks & leaves	NEG	NEG
644	<i>Listeria innocua</i>	ATCC33090		Cow brain	POS	POS
647	<i>Listeria monocytogenes</i>	ATCC19118		Chicken	POS	POS
648	<i>Listeria monocytogenes</i>	ATCC19114		Animal tissue	POS	POS
649	<i>Listeria ivanovii</i>	ATCC19119		Sheep	POS	POS
650	<i>Listeria seeligeri</i>	ATCC 35967		Soil	POS	POS
652	<i>Listeria monocytogenes</i>	ATCC 19117		Chicken	POS	POS
653	<i>Listeria monocytogenes</i>	ATCC 19115		Human	POS	POS
654	<i>Listeria welshimeri</i>	ATCC 35897		Decaying plant material	POS	POS
892	<i>Listeria innocua</i>			Unknown	POS	POS
898	<i>Listeria innocua</i>			Unknown	POS	POS
921	<i>Listeria innocua</i>			Roast turkey	POS	POS
922	<i>Listeria innocua</i>			Ham cured shoulder	POS	POS
924	<i>Listeria innocua</i>			Ham cured shoulder	POS	POS
927	<i>Listeria innocua</i>			Chopped pork and ham	POS	POS
944	<i>Listeria murrayi</i>			Cornstalks and leaves	NEG	POS
1063	<i>Listeria innocua</i>			Chopped pork and ham	POS	POS
1064	<i>Listeria innocua</i>			Chopped pork and ham	POS	POS
1069	<i>Listeria monocytogenes</i>			Stuffed gammon joint	POS	POS
1072	<i>Listeria monocytogenes</i>			Cheese and ham pancakes	POS	POS
1144	<i>Listeria monocytogenes</i>		1/2a	Stilton cheese	POS	POS
1145	<i>Listeria monocytogenes</i>		1/2a	Coleslaw salad	POS	POS
1146	<i>Listeria monocytogenes</i>			Lettuce	POS	POS
1147	<i>Listeria monocytogenes</i>			Pate	POS	POS
1149	<i>Listeria monocytogenes</i>		1/2a	Raw milk	POS	POS
1152	<i>Listeria monocytogenes</i>		1/2b	Pate	POS	POS
1156	<i>Listeria innocua</i>			Lettuce	POS	POS
1164	<i>Listeria ivanovii</i>			Radish	POS	POS
1165	<i>Listeria ivanovii</i>			Belgian salami	POS	POS
1167	<i>Listeria ivanovii</i>			Soft cheese	POS	POS
1171	<i>Listeria ivanovii</i>			Unknown	POS	POS
1172	<i>Listeria welshimeri</i>			Salami	POS	POS
1174	<i>Listeria welshimeri</i>			Raw chicken	POS	POS
1175	<i>Listeria welshimeri</i>			Sausage	POS	POS
1176	<i>Listeria welshimeri</i>			Chicken	POS	POS
1177	<i>Listeria welshimeri</i>			Smoked mackerel	POS	POS
1179	<i>Listeria welshimeri</i>			Food	POS	POS
1281	<i>Listeria monocytogenes</i>		3c	Cooked chicken	POS	POS
1282	<i>Listeria monocytogenes</i>		3c	Unknown	POS	POS
1283	<i>Listeria monocytogenes</i>		3b	Cooked turkey	POS	POS
1285	<i>Listeria monocytogenes</i>			Cheese	POS	POS
1286	<i>Listeria monocytogenes</i>		3c	Cooked chicken	POS	POS
1287	<i>Listeria monocytogenes</i>		3a	Unknown	POS	POS
1288	<i>Listeria monocytogenes</i>		3a	Cooked turkey	POS	POS
1289	<i>Listeria seeligeri</i>			Crab pate	POS	POS
1291	<i>Listeria seeligeri</i>			Lettuce	POS	POS
1292	<i>Listeria seeligeri</i>			Cooked chicken	POS	POS
1293	<i>Listeria monocytogenes</i>		3a	Pate	POS	POS
1294	<i>Listeria monocytogenes</i>		4b	Ice cream	POS	POS
1295	<i>Listeria monocytogenes</i>		3b	Pepper quiche	POS	POS
1297	<i>Listeria seeligeri</i>			Pate	POS	POS
1298	<i>Listeria seeligeri</i>			Chicken roll	POS	POS
1299	<i>Listeria monocytogenes</i>		1/2b	Pork liver pate	POS	POS
1300	<i>Listeria seeligeri</i>			Cooked ham	POS	POS
1302	<i>Listeria monocytogenes</i>			Hard boiled eggs	POS	POS
1305	<i>Listeria monocytogenes</i>		3a	Boiled ham	POS	POS

1306	<i>Listeria monocytogenes</i>		3b	Chicken liver pate	POS	POS
1307	<i>Listeria monocytogenes</i>		3b	Pate	POS	POS
1308	<i>Listeria monocytogenes</i>			Cheese	POS	POS
1309	<i>Listeria monocytogenes</i>			Soft cheese	POS	POS
1310	<i>Listeria monocytogenes</i>		3b	Chicken	POS	POS
1311	<i>Listeria monocytogenes</i>			Cooked meat	POS	POS
1312	<i>Listeria monocytogenes</i>			Ice cream	POS	POS
1313	<i>Listeria monocytogenes</i>		4b	Cheese	POS	POS
1314	<i>Listeria monocytogenes</i>			Pate	POS	POS
1315	<i>Listeria monocytogenes</i>			Pate	POS	POS
1316	<i>Listeria monocytogenes</i>		3a	Cooked chicken	POS	POS
1321	<i>Listeria monocytogenes</i>			Sandwich	POS	POS
2874	<i>Listeria seeligeri</i>			Frozen dessert	POS	POS
3244	<i>Listeria innocua</i>			Unknown	POS	POS
3327	<i>Listeria seeligeri</i>			Cheese	POS	POS
3329	<i>Listeria seeligeri</i>			Unknown	POS	POS
3351	<i>Listeria welshimeri</i>			Unknown	POS	POS
3354	<i>Listeria welshimeri</i>			Unknown	POS	POS
3359	<i>Listeria welshimeri</i>			Radish	POS	POS
3363	<i>Listeria murrayi</i>			Unknown	POS	POS
3376	<i>Listeria ivanovii</i>			Environmental	POS	POS
3555	<i>Listeria grayi</i>			Unknown	NEG	NEG
3572	<i>Listeria innocua</i>			Cow brain	POS	POS
3573	<i>Listeria monocytogenes</i>			Industry sample	POS	POS
3574	<i>Listeria monocytogenes</i>			Industry sample	POS	POS
3576	<i>Listeria monocytogenes</i>			Industry sample	POS	POS
3577	<i>Listeria monocytogenes</i>			Industry sample	POS	POS
3578	<i>Listeria monocytogenes</i>			Industry sample	POS	POS
3579	<i>Listeria monocytogenes</i>			Industry sample	POS	POS
3580	<i>Listeria monocytogenes</i>			Industry sample	POS	POS
3581	<i>Listeria monocytogenes</i>			Industry sample	POS	POS
3582	<i>Listeria monocytogenes</i>			Industry sample	POS	POS
3678	<i>Listeria ivanovii</i>			Unknown	POS	POS
4553	<i>Listeria monocytogenes</i>			Smoked ham	POS	POS
4568	<i>Listeria monocytogenes</i>			Swab of finger guard	POS	POS
4571	<i>Listeria monocytogenes</i>			honey roast ham	POS	POS
5425	<i>Listeria monocytogenes</i>			Jalisco cheese isolate	POS	POS
7644	<i>Listeria monocytogenes</i>			Unknown	POS	POS

Table 6 BAX system exclusivity (1)				BAX System 24E Genus <i>Listeria</i>	
dd#	Other Strain Designation (if available)	Collection ID	Isolate source	Q7 Result	Classic Result
715	ATCC 14579	<i>Bacillus cereus</i>	unknown	NEG	NEG
721	ATCC 13061	<i>Bacillus cereus</i>	unknown	NEG	NEG
877	ATCC 33018	<i>Bacillus cereus</i>	powdered infant formula	NEG	NEG
878	ATCC 13061	<i>Bacillus cereus</i>	unknown	NEG	NEG
879	ATCC 11778	<i>Bacillus cereus</i>	unknown	NEG	NEG
1024	ATCC 7004	<i>Bacillus cereus</i>	unknown	NEG	NEG
379		<i>Bacillus subtilis</i>	unknown	NEG	NEG
1011		<i>Bacillus subtilis</i>	mashed potatoes	NEG	NEG
713	ATCC 35646	<i>Bacillus thuringiensis</i>	unknown	NEG	NEG
714	ATCC 10792	<i>Bacillus thuringiensis</i>	Mediterranean flour moth	NEG	NEG
716	ATCC 33679	<i>Bacillus thuringiensis</i>	diseased insect larvae	NEG	NEG
1114	ATCC 43754	<i>Brochothrix campestris</i>	soil	NEG	NEG
4064		<i>Carnobacterium divergens</i>	unknown	NEG	NEG
4063		<i>Carnobacterium gallinarum</i>	unknown	NEG	NEG
383	ATCC 8090	<i>Citrobacter freundii</i>	unknown	NEG	NEG
2558	ATCC 43864	<i>Citrobacter freundii</i>	unknown	NEG	NEG
2560		<i>Citrobacter koseri</i>	throat	NEG	NEG
2561		<i>Citrobacter koseri</i>	blood	NEG	NEG
2625		<i>Enterococcus durans</i>	unknown	NEG	NEG
2554		<i>Enterococcus faecalis</i>	unknown	NEG	NEG
3981		<i>Enterococcus faecalis</i>	urine	NEG	NEG
2552		<i>Enterococcus faecium</i>	unknown	NEG	NEG
2553		<i>Enterococcus faecium</i>	unknown	NEG	NEG
2624		<i>Enterococcus gallinarum</i>	chicken intestine	NEG	NEG
2626		<i>Enterococcus hirae</i>	unknown	NEG	NEG
2626		<i>Enterococcus hirae</i>	unknown	NEG	NEG
7344		<i>Lactobacillus acidophilus</i>	human	NEG	NEG
7332		<i>Lactobacillus curvatus</i>	milk	NEG	NEG
620		<i>Lactobacillus rhamnosus</i>	unknown	NEG	NEG
659		<i>Lactococcus lactis</i>	unknown	NEG	NEG
9174		<i>Micrococcus luteus</i>	unknown	NEG	NEG
2392		<i>Rhodococcus equi</i>	lung abscess from foal	NEG	NEG
2628		<i>Salmonella kentucky</i>	unknown	NEG	NEG
707		<i>Salmonella newport</i>	fatal case of food poisoning	NEG	NEG
863		<i>Staphylococcus aureus</i>	unknown	NEG	NEG
912		<i>Staphylococcus aureus</i>	unknown	NEG	NEG
1096		<i>Staphylococcus aureus</i>	unknown	NEG	NEG
1098		<i>Staphylococcus aureus</i>	unknown	NEG	NEG
1111		<i>Staphylococcus capitis</i>	unknown	NEG	NEG
2636		<i>Staphylococcus felis</i>	cat's ear	NEG	NEG
1113		<i>Staphylococcus sciuri</i>	human skin	NEG	NEG
1113		<i>Staphylococcus sciuri</i>	human skin	NEG	NEG
1105		<i>Staphylococcus warneri</i>	German salami	NEG	NEG
1107		<i>Staphylococcus xylosum</i>	lockwurst	NEG	NEG
1112		<i>Staphylococcus xylosum</i>	unknown	NEG	NEG
692		<i>Streptococcus bovis</i>	cow dung	NEG	NEG
3996		<i>Streptococcus equi</i>	unknown	NEG	NEG
3992		<i>Streptococcus mutans</i>	carious dentine	NEG	NEG
695		<i>Streptococcus pyogenes</i>	unknown	NEG	NEG
692		<i>Streptococcus thermophilus</i>	cow dung	NEG	NEG

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