CERTIFICATION

AOAC® *Performance Tested*SM

Certificate No.

080901

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

BAX® System PCR Assay for *L. monocytogenes* 24E

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 05, 2020 – December 31, 2021). 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 05, 2020
Date

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ESL Building 400
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Wilmington, DE 19880-0400

CURRENT SPONSOR
Hygiena
2 Boulden Circle
New Castle, DE 19720
USA

KIT NAME(S)
DuPont™ BAX® System PCR Assay for *L. monocytogenes* 24E March 01, 2017, BAX® System PCR Assay for *L. monocytogenes* 24E

CATALOG NUMBERS
BAX® Assay KIT2002 (D13608125), 24 LEB Complete MED2005 (D14654989), 24 LEB Buffer Supplement MED2000 (D15407304)

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APPLICABILITY OF METHOD
Target organism – *Listeria monocytogenes*

Matrixes – Bagged spinach, processed cheese, frankfurters, cooked shrimp, and stainless steel

Performance claims - Equivalent or superior to the reference methods.

REFERENCE METHODS
United States Department of Agriculture/Food Safety Inspection Services Microbiological Laboratory Guidelines (2)
U.S. Food and Drug Administration, FDA Bacteriological Analytical Manual (3)

ORIGINAL CERTIFICATION DATE
August 03, 2009

CERTIFICATION RENEWAL RECORD
Renewed Annually through December 2021

METHOD MODIFICATION RECORD
1. **March 2017 Level 1**
2. **January 2018 Level 1**
3. **May 2019 Level 1**
4. **December 2019 Level 1**

SUMMARY OF MODIFICATION
1. **Name change from DuPont Nutrition & Health to Qualicon Diagnostics LLC., a Hygiena company**
2. **Editorial updates to Inserts, labels, manuals updated to Hygiena**
3. **Editorial updates to inserts and corporate address**
4. **Editorial/clerical changes.**

Under this AOAC® *Performance Tested*℠ License Number, 080901 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 L. monocytogenes, 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 VALIDATION STUDY (1)

The results of the method comparison study demonstrate that the BAX® system assay for detecting *L. monocytogenes* is comparable to the reference methods for detecting *L. monocytogenes* in a variety of sample types. Chi-square values for the sample types tested showed equivalent (<3.84) or better (≥3.84) *L. monocytogenes* detection with the BAX® system compared to the reference method at a 95% confidence level. The results for frankfurter and stainless steel samples from the independent laboratory support the results of the internal study. In all cases where there is a non-significant difference, sampling statistics are likely the cause. While there are arithmetic differences, in these cases a statistical analysis is critical since when testing a variety of food and/or environmental matrixes, it would be unlikely that all un-paired study results would be the same across this many studies. The two cases where there is a statistically significant difference in method performance (the shrimp and Queso Fresco matrixes) both favor the test method.

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 obtaining best results, a minimum enrichment time of 26 hours is recommended for this matrix.

As the BAX® system returned positive results for all *L. monocytogenes* strains and negative results for all non-*L. monocytogenes* and non-*Listeria* strains tested, the results of inclusivity/exclusivity testing suggest 100% inclusivity and 100% exclusivity for this assay.

Table 3a. Summary table of results (1)

Food/Surface Type	Type	Instrument	Inoculation cfu/sample	MPN / sample	Reference Method culture	BAX® 24E	*L. monocytogenes* confirmed culture positive
			At time of inoculation by direct plating	MPN at time of testing by reference method	Number positive/Total	Number positive / Total (Number confirmed / Number BAX® assay positive)[a]	BAX® enrichment [b]
Frankfurters	Spiked	BAX and Q7	0.57	0.57	9/20	6/20 (6/6)	6
	Control	BAX and Q7	-	0	0/5	0/5	0
Spinach	Spiked	BAX and Q7	3.4	0.23	15/20	13/20 (13/13)	15
	Control	BAX and Q7	-	0	0/5	0/5	0
Stainless Steel	Spiked	BAX and Q7	1.2×10^5	NA	17/20	19/20 (19/19)	19
	Spiked	BAX and Q7	2.8	NA	6/20	3/20 (3/3)	3
	Control	BAX and Q7	-	0	0/5	0/5	0
Cooked Shrimp	Spiked	BAX	0.98	0.53	11/20	19/20 (19/19)	20
		Q7				18/20 (18/18)	
	Control	BAX and Q7	-	0	0/5	0/5	0
Queso Fresco Cheese (26 h)	Spiked	BAX and Q7	2.3×10^2	1.3	10/20	20/20	20
	Control	BAX and Q7	-	0	0/5	0/5	0

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

[b] Figure represents the number of enrichments from which a reference method confirmed *Listeria* isolate was recovered

Table 3b. Method performance for the detection of *Listeria monocytogenes*. (1)												
Food/Surface	Strain tested	Level (cfu applied per unit)	MPN / 25 g	Instrument	BAX® Presumptive (# positive)	BAX® Enrichment Confirmed (# positive)	Reference Method (# positive)	Sensitivity[1]	Specificity [2]	False Negative[3]	False Positive[4]	X^2 Value[5]
Frankfurters	*L. monocytogenes* 4b DD 1309	0.57	0.57	BAX	6/20	6/20	9/20	1.00	1.00	0	0	0.936
				BAX Q7	6/20	6/20	9/20	1.00	1.00	0	0	0.936
	Control	0	0	BAX & BAX Q7	0/5	0/5	0/5	-	1.00	0	0	-
Spinach	*L. monocytogenes* 3b DD 1283	3.4	0.23	BAX	13/20	15/20	14/20	0.87	1.00	0.13	0	0.111
				BAX Q7	13/20	15/20	14/20	0.87	1.00	0.13	0	0.111
	Control	0	N/A	BAX & BAX Q7	0/5	0/5	0/5	-	1.00	0	0	-
Stainless steel	*L. monocytogenes* 4b DD 1308	1.2×10^5	N/A	BAX	19/20	19/20	17/20	1.00	1.00	0	0	1.08
				BAX Q7	19/20	19/20	17/20	1.00	1.00	0	0	1.08
	L. monocytogenes 4b DD 1308	2.8	N/A	BAX	3/20	3/20	6/20	1.00	1.00	0	0	1.26
				BAX Q7	3/20	3/20	6/20	1.00	1.00	0	0	1.26
	Control	0	0	BAX & BAX Q7	0/5	0/5	0/5	-	1.00	0	0	-
Cooked shrimp	*L. monocytogenes* 1/2a DD 1144	0.98	0.53	BAX	19/20	20/20	11/20	0.95	1.00	0.05	0	5.99
				BAX Q7	18/20	20/20	11/20	0.90	1.00	0.10	0	5.99
	Control	0	0	BAX & BAX Q7	0/5	0/5	0/5	-	1.00	0	0	-
Queso fresco cheese	*L. monocytogenes* 1/2a DD 605	2.3×10^2	1.3	BAX	20/20	20/20	10/20	1.00	1.00	0	0	13.0
				BAX Q7	20/20	20/20	10/20	1.00	1.00	0	0	13.0
	Control	0	0	BAX & BAX Q7	0/5	0/5	0/5	-	1.00	0	0	-
Composite data	-	-	-	BAX	80/145	81/145	67/145	0.99	1.00	0.01	0	2.32
Composite data	-	-	-	BAX Q7	79/145			0.98	1.00	0.02	0	1.98

[1] Sensitivity is calculated as 100% – false negative rate enrichments

[2] Specificity is calculated as 100% – false positive rate of results

[3] False negative is the number of BAX (-) Ref (+) BAX enrichment samples / Tot Ref (+) BAX enrichment

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

[5] Mantel -Haenszel Chi-Square test statistic used for calculating significance

[1] Sensitivity is calculated as 100% – false negative rate enrichments

[2] Specificity is calculated as 100% – false positive rate of results

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

[5] Mantel -Haenszel Chi-Square test statistic used for calculating significance

Table 5. BAX® system inclusivity (1)				
			BAX® System 24E L. monocytogenes	
DD#	Collection ID	Isolate source	Q7 Result	Classic Result
566	*Listeria monocytogenes*	Rabbit	POS	POS
605	*Listeria monocytogenes*	Poultry	POS	POS
647	*Listeria monocytogenes*	Chicken	POS	POS
648	*Listeria monocytogenes*	Animal tissue	POS	POS
652	*Listeria monocytogenes*	Chicken	POS	POS
653	*Listeria monocytogenes*	Human	POS	POS
1069	*Listeria monocytogenes*	Stuffed gammon joint	POS	POS
1072	*Listeria monocytogenes*	Cheese and ham pancakes	POS	POS
1144	*Listeria monocytogenes*	Stilton cheese	POS	POS
1145	*Listeria monocytogenes*	Coleslaw salad	POS	POS
1146	*Listeria monocytogenes*	Lettuce	POS	POS
1147	*Listeria monocytogenes*	Pate	POS	POS
1149	*Listeria monocytogenes*	Raw milk	POS	POS
1152	*Listeria monocytogenes*	Pate	POS	POS
1281	*Listeria monocytogenes*	Cooked chicken	POS	POS
1282	*Listeria monocytogenes*	Unknown	POS	POS
1283	*Listeria monocytogenes*	Cooked turkey	POS	POS
1285	*Listeria monocytogenes*	Cheese	POS	POS
1286	*Listeria monocytogenes*	Cooked chicken	POS	POS
1287	*Listeria monocytogenes*	Unknown	POS	POS
1288	*Listeria monocytogenes*	Cooked turkey	POS	POS
1293	*Listeria monocytogenes*	Pate	POS	POS
1294	*Listeria monocytogenes*	Ice cream	POS	POS
1295	*Listeria monocytogenes*	Pepper quiche	POS	POS
1299	*Listeria monocytogenes*	Pork liver pate	POS	POS
1302	*Listeria monocytogenes*	Hard boiled eggs	POS	POS
1305	*Listeria monocytogenes*	Boiled ham	POS	POS
1306	*Listeria monocytogenes*	Chicken liver pate	POS	POS
1307	*Listeria monocytogenes*	Pate	POS	POS
1308	*Listeria monocytogenes*	Cheese	POS	POS
1309	*Listeria monocytogenes*	Soft cheese	POS	POS
1310	*Listeria monocytogenes*	Chicken	POS	POS
1311	*Listeria monocytogenes*	Cooked meat	POS	POS
1312	*Listeria monocytogenes*	Ice cream	POS	POS
1313	*Listeria monocytogenes*	Cheese	POS	POS
1314	*Listeria monocytogenes*	Pate	POS	POS
1315	*Listeria monocytogenes*	Pate	POS	POS
1316	*Listeria monocytogenes*	Cooked chicken	POS	POS
1321	*Listeria monocytogenes*	Sandwich	POS	POS
3573	*Listeria monocytogenes*	Industry sample	POS	POS
3574	*Listeria monocytogenes*	Industry sample	POS	POS
3576	*Listeria monocytogenes*	Industry sample	POS	POS
3577	*Listeria monocytogenes*	Industry sample	POS	POS

3578	*Listeria monocytogenes*	Industry sample	POS	POS
3579	*Listeria monocytogenes*	Industry sample	POS	POS
3580	*Listeria monocytogenes*	Industry sample	POS	POS
3581	*Listeria monocytogenes*	Industry sample	POS	POS
3582	*Listeria monocytogenes*	Industry sample	POS	POS
4553	*Listeria monocytogenes*	Smoked ham	POS	POS
4568	*Listeria monocytogenes*	Swab of finger guard	POS	POS
4571	*Listeria monocytogenes*	honey roast ham	POS	POS
5425	*Listeria monocytogenes*	Jalisco cheese isolate	POS	POS
7644	*Listeria monocytogenes*	Unknown	POS	POS

Table 6. BAX® system exclusivity (1)

			BAX® System 24E L. monocytogenes	
DD#	**Collection ID**	**Isolate source**	**Q7 Result**	**Classic Result**
715	*Bacillus cereus*	unknown	NEG	NEG
721	*Bacillus cereus*	unknown	NEG	NEG
877	*Bacillus cereus*	powdered infant formula	NEG	NEG
878	*Bacillus cereus*	unknown	NEG	NEG
879	*Bacillus cereus*	unknown	NEG	NEG
1024	*Bacillus cereus*	unknown	NEG	NEG
379	*Bacillus subtilus*	unknown	NEG	NEG
1011	*Bacillus subtilus*	mashed potatoes	NEG	NEG
713	*Bacillus thuringiensis*	unknown	NEG	NEG
714	*Bacillus thuringiensis*	Mediterranean flour moth	NEG	NEG
716	*Bacillus thuringiensis*	diseased insect larvae	NEG	NEG
1114	*Brochothrix campestris*	soil	NEG	NEG
4064	*Carnobacterium divergens*	unknown	NEG	NEG
4063	*Carnobacterium gallinarum*	unknown	NEG	NEG
383	*Citrobacter freundii*	unknown	NEG	NEG
2558	*Citrobacter freundii*	unknown	NEG	NEG
2560	*Citrobacter koseri*	throat	NEG	NEG
2561	*Citrobacter koseri*	blood	NEG	NEG
2625	*Enterococcus durans*	unknown	NEG	NEG
2554	*Enterococcus faecalis*	unknown	NEG	NEG
3981	*Enterococcus faecalis*	urine	NEG	NEG
2552	*Enterococcus faecium*	unknown	NEG	NEG
2553	*Enterococcus faecium*	unknown	NEG	NEG
2624	*Enterococcus gallinarum*	chicken intestine	NEG	NEG
2626	*Enterococcus hirae*	unknown	NEG	NEG
2626	*Enterococcus hirae*	unknown	NEG	NEG
7344	*Lactobacillus acidophilus*	human	NEG	NEG
7332	*Lactobacillus curvatus*	milk	NEG	NEG
620	*Lactobacillus rhamnosus*	unknown	NEG	NEG
659	*Lactococcus lactis*	unknown	NEG	NEG

1156	*Listeria innocua*	lettuce	NEG	NEG
3244	*Listeria innocua*	unknown	NEG	NEG
3572	*Listeria innocua*	cow brain	NEG	NEG
649	*Listeria ivanovii*	sheep	NEG	NEG
1164	*Listeria ivanovii*	radish	NEG	NEG
3376	*Listeria ivanovii*	environmental	NEG	NEG
643	*Listeria murrayi/grayi*	corn stalks	NEG	NEG
944	*Listeria murrayi/grayi*	corn stalks	NEG	NEG
3363	*Listeria murrayi/grayi*	unknown	NEG	NEG
2874	*Listeria seeligeri*	frozen dessert	NEG	NEG
3327	*Listeria seeligeri*	cheese	NEG	NEG
3329	*Listeria seeligeri*	unknown	NEG	NEG
654	*Listeria welshimeri*	decaying plant material	NEG	NEG
1172	*Listeria welshimeri*	salami	NEG	NEG
3359	*Listeria welshimeri*	radish	NEG	NEG
9174	*Micrococcus luteus*	unknown	NEG	NEG
2392	*Rhodococcus equi*	lung abscess from foal	NEG	NEG
2628	*Salmonella kentucky*	unknown	NEG	NEG
707	*Salmonella newport*	fatal case of food poisoning	NEG	NEG
863	*Staphylococcus aureus*	unknown	NEG	NEG
912	*Staphylococcus aureus*	unknown	NEG	NEG
1096	*Staphylococcus aureus*	unknown	NEG	NEG
1098	*Staphylococcus aureus*	unknown	NEG	NEG
1111	*Staphylococcus capitis*	unknown	NEG	NEG
2636	*Staphylococcus felis*	cat's ear	NEG	NEG
1113	*Staphylococcus sciuri*	human skin	NEG	NEG
1105	*Staphylococcus warneri*	German salami	NEG	NEG
1107	*Staphylococcus xylosus*	lockwurst	NEG	NEG
1112	*Staphylococcus xylosus*	unknown	NEG	NEG
692	*Streptococcus bovis*	cow dung	NEG	NEG
3996	*Streptococcus equi*	unknown	NEG	NEG
3992	*Streptococcus mutans*	carious dentine	NEG	NEG
695	*Streptococcus pyogenes*	unknown	NEG	NEG
692	*Streptococcus thermophilus*	cow dung	NEG	NEG

REFERENCES CITED

1. Wallace, M.,Fallon, D., DeMarco, D., and Varkey, S.., Evaluation of the DuPont™ BAX® System PCR Assay for *L. monocytogenes* 24E , AOAC® *Performance Tested*℠ certification number 080901.
2. United States Department of Agriculture/Food Safety Inspection Services Microbiological Laboratory Guidelines,, available at: http://www.fsis.usda.gov/PDF/MLG_8_06.pdf, date of access 5/21/08
3. U.S. Food and Drug Administration, FDA Bacteriological Analytical Manual, available at: http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm071400.htm, date of access 6/12/09