



CERTIFICATION

AOAC Research Institute *Performance Tested Methods*SM

Certificate No.

080901

The AOAC Research Institute hereby certifies the method 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 and certified according to the policies and procedures of the AOAC *Performance Tested Methods*SM 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*SM 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, AOAC Research Institute Senior Director

Issue Date

January 06, 2026

Expiration Date

December 31, 2026

| | | |
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| AUTHORS F. Morgan Wallace, Dawn Fallon, Daniel DeMarco, and Stephen Varkey | SUBMITTING COMPANY DuPont ESL Building 400 Route 141 & Henry Clay Road Wilmington, DE 19880-0400 | CURRENT SPONSOR Hygiena 2 Boulden Circle New Castle, DE 19720 USA |
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| METHOD NAME BAX® System PCR Assay for <i>L. monocytogenes</i> 24E Formerly DuPont™ BAX® System PCR Assay for <i>L. monocytogenes</i> 24E | CATALOG NUMBERS BAX® Assay KIT2002, 24 LEB Complete MED2005, 24 LEB Buffer Supplement MED2000 |
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| INDEPENDENT LABORATORY rtech Laboratories 1200 W. Country Road F Arden Hills, MN 55112 USA |
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| APPLICABILITY OF METHOD Target organism – <i>Listeria monocytogenes</i> . 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) |
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| ORIGINAL CERTIFICATION DATE August 03, 2009 | CERTIFICATION RENEWAL RECORD Renewed through December 2026. |
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| METHOD MODIFICATION RECORD | SUMMARY OF MODIFICATION |
| 1. March 2017 Level 1 | 1. Name change from DuPont Nutrition & Health to Qualicon Diagnostics LLC., a Hygiena company. |
| 2. January 2018 Level 1 | 2. Editorial updates to Inserts, labels, manuals updated to Hygiena. |
| 3. May 2019 Level 1 | 3. Editorial updates to inserts and corporate address. |
| 4. December 2019 Level 1 | 4. Editorial changes. |
| 5. December 2023 Level 1 | 5. Editorial changes. |
| 6. December 2024 Level 1 | 6. Editorial changes. |

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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 *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 | MPN / | Reference Method | BAX 24E | <i>L. monocytogenes</i> |
|----------------------------|---------|------------|-----------------------|---------------|------------------|--|-----------------------------|
| | | | cfu/sample | sample | culture | | confirmed culture |
| | | | At time of | MPN at time | Number | Number positive / Total | BAX enrichment ^b |
| | | | inoculation by | of testing by | positive/Total | (Number confirmed / | |
| | | | direct plating | reference | | Number BAX® assay positive) ^a | |
| | | | method | | | | |
| 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 x 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 x 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 <i>Listeria monocytogenes</i> . (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 | <i>L. monocytogenes</i> 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 | <i>L. monocytogenes</i> 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 | <i>L. monocytogenes</i> 4b DD 1308 | 1.2 x 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 |
| | <i>L. monocytogenes</i> 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 | <i>L. monocytogenes</i> 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 | <i>L. monocytogenes</i> 1/2a DD 605 | 2.3 x 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 | 81/145 | 67/145 | 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 significance of results

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

² Specificity is calculated as 100% – false positive rate significance 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

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

⁵ Mantel -Haenszel Chi-Square test statistic used for calculating

| Table 5. BAX system inclusivity (1) | | | | |
|-------------------------------------|-------------------------------|-------------------------|--|----------------|
| DD# | Collection ID | Isolate source | BAX System 24E <i>L. monocytogenes</i> | |
| | | | Q7 Result | Classic Result |
| 566 | <i>Listeria monocytogenes</i> | Rabbit | POS | POS |
| 605 | <i>Listeria monocytogenes</i> | Poultry | POS | POS |
| 647 | <i>Listeria monocytogenes</i> | Chicken | POS | POS |
| 648 | <i>Listeria monocytogenes</i> | Animal tissue | POS | POS |
| 652 | <i>Listeria monocytogenes</i> | Chicken | POS | POS |
| 653 | <i>Listeria monocytogenes</i> | Human | 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> | Stilton cheese | POS | POS |
| 1145 | <i>Listeria monocytogenes</i> | 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> | Raw milk | POS | POS |
| 1152 | <i>Listeria monocytogenes</i> | Pate | POS | POS |
| 1281 | <i>Listeria monocytogenes</i> | Cooked chicken | POS | POS |
| 1282 | <i>Listeria monocytogenes</i> | Unknown | POS | POS |
| 1283 | <i>Listeria monocytogenes</i> | Cooked turkey | POS | POS |
| 1285 | <i>Listeria monocytogenes</i> | Cheese | POS | POS |
| 1286 | <i>Listeria monocytogenes</i> | Cooked chicken | POS | POS |
| 1287 | <i>Listeria monocytogenes</i> | Unknown | POS | POS |
| 1288 | <i>Listeria monocytogenes</i> | Cooked turkey | POS | POS |
| 1293 | <i>Listeria monocytogenes</i> | Pate | POS | POS |
| 1294 | <i>Listeria monocytogenes</i> | Ice cream | POS | POS |
| 1295 | <i>Listeria monocytogenes</i> | Pepper quiche | POS | POS |
| 1299 | <i>Listeria monocytogenes</i> | Pork liver pate | POS | POS |
| 1302 | <i>Listeria monocytogenes</i> | Hard boiled eggs | POS | POS |
| 1305 | <i>Listeria monocytogenes</i> | Boiled ham | POS | POS |
| 1306 | <i>Listeria monocytogenes</i> | Chicken liver pate | POS | POS |
| 1307 | <i>Listeria monocytogenes</i> | 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> | 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> | Cheese | POS | POS |
| 1314 | <i>Listeria monocytogenes</i> | Pate | POS | POS |
| 1315 | <i>Listeria monocytogenes</i> | Pate | POS | POS |
| 1316 | <i>Listeria monocytogenes</i> | Cooked chicken | POS | POS |
| 1321 | <i>Listeria monocytogenes</i> | Sandwich | 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 |
| 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) | | | | |
|-------------------------------------|------------------------|-------------------------|--|----------------|
| DD# | Collection ID | Isolate source | BAX System 24E <i>L. monocytogenes</i> | |
| | | | Q7 Result | Classic Result |
| 715 | <i>Bacillus cereus</i> | unknown | NEG | NEG |
| 721 | <i>Bacillus cereus</i> | unknown | NEG | NEG |
| 877 | <i>Bacillus cereus</i> | powdered infant formula | NEG | NEG |
| 878 | <i>Bacillus cereus</i> | unknown | NEG | NEG |

| | | | | |
|------|-----------------------------------|------------------------------|-----|-----|
| 879 | <i>Bacillus cereus</i> | unknown | NEG | NEG |
| 1024 | <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 | <i>Bacillus thuringiensis</i> | unknown | NEG | NEG |
| 714 | <i>Bacillus thuringiensis</i> | Mediterranean flour moth | NEG | NEG |
| 716 | <i>Bacillus thuringiensis</i> | diseased insect larvae | NEG | NEG |
| 1114 | <i>Brochothrix campestris</i> | soil | NEG | NEG |
| 4064 | <i>Carnobacterium divergens</i> | unknown | NEG | NEG |
| 4063 | <i>Carnobacterium gallinarum</i> | unknown | NEG | NEG |
| 383 | <i>Citrobacter freundii</i> | unknown | NEG | NEG |
| 2558 | <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 |
| 1156 | <i>Listeria innocua</i> | lettuce | NEG | NEG |
| 3244 | <i>Listeria innocua</i> | unknown | NEG | NEG |
| 3572 | <i>Listeria innocua</i> | cow brain | NEG | NEG |
| 649 | <i>Listeria ivanovii</i> | sheep | NEG | NEG |
| 1164 | <i>Listeria ivanovii</i> | radish | NEG | NEG |
| 3376 | <i>Listeria ivanovii</i> | environmental | NEG | NEG |
| 643 | <i>Listeria murrayi/grayi</i> | corn stalks | NEG | NEG |
| 944 | <i>Listeria murrayi/grayi</i> | corn stalks | NEG | NEG |
| 3363 | <i>Listeria murrayi/grayi</i> | unknown | NEG | NEG |
| 2874 | <i>Listeria seeligeri</i> | frozen dessert | NEG | NEG |
| 3327 | <i>Listeria seeligeri</i> | cheese | NEG | NEG |
| 3329 | <i>Listeria seeligeri</i> | unknown | NEG | NEG |
| 654 | <i>Listeria welshimeri</i> | decaying plant material | NEG | NEG |
| 1172 | <i>Listeria welshimeri</i> | salami | NEG | NEG |
| 3359 | <i>Listeria welshimeri</i> | radish | 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 |
| 1105 | <i>Staphylococcus warneri</i> | German salami | NEG | NEG |
| 1107 | <i>Staphylococcus xylosus</i> | lockwurst | NEG | NEG |
| 1112 | <i>Staphylococcus xylosus</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 |

REFERENCES CITED

- Wallace, M., Fallon, D., DeMarco, D., and Varkey, S., Evaluation of the DuPont™ BAX® System PCR Assay for *L. monocytogenes* 24E , AOAC Performance Tested MethodsSM certification number 080901.
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