# CERTIFICATION

## AOAC Research Institute

## *Performance Tested Methods*[SM]

Certificate No.

**081401**

**The AOAC Research Institute hereby certifies the method known as:**

## BAX® System Real-Time PCR Assay for Genus *Listeria*

manufactured by

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

This method has been evaluated in the AOAC Research Institute *Performance Tested Methods*[SM] Program and found to perform as stated in the applicability of the method. 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.

Scott Coates

Scott Coates, Senior Director
Signature for AOAC Research Institute

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**Wilmington, DE 19803**

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**METHOD NAME**
BAX® System Real-Time PCR Assay for Genus *Listeria*
Formerly DuPont™ Bax® System Real-Time PCR Assay for Genus *Listeria*

**CATALOG NUMBERS**
BAX System Assay KIT2019 (D15131113), 24 LEB Complete MED2005 (D14654989)

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**APPLICABILITY OF METHOD**
**Target organism – *Listeria* species (including *L. monocytogenes, L. innocua, L. ivanovii, L. welshimeri, L. seeligeri,* and *L. grayi*).**

**Matrixes – USDA/FSIS 8.09: (125 g) – frankfurters (beef) (10 x 10 cm$^2$) – stainless steel, plastic, concrete**
**FDA BAM Ch. 10 – (25 g) – cooked shrimp, bagged lettuce, queso fresco (Mexican style cheese)**
**MODIFICATION MAY 2015 with Actero™ Listeria in conjunction with DuPont™ BAX® System Real-Time PCRF Assay for Genius *Listeria*:**
**FDA BAM Ch. 10 – (25 g) – soft Mexican-style cheese, bagged spinach, frozen cooked shrimp, cold smoked salmon**
**USDA/FSIS 8.09 – (125 g) – Frankfurters (10 x 10 cm$^2$) – stainless steel, plastic, sealed concrete**

**Performance claims – The study data detected no statistical difference between the BAX® System Real-Time PCR Assay for Genus *Listeria* method and the reference methods.**

**REFERENCE METHODS**

**U.S. Department of Agriculture-Food Safety and Inspection Service Microbiology Laboratory Guidebook Chapter 8.09 (2013) (2)**

**U.S. FDA Bacteriological Analytical Manual, Chapter 10 (2013) (3)**

**ORIGINAL CERTIFICATION DATE**
**August 01, 2014**

**CERTIFICATION RENEWAL RECORD**
**Renewed annually through December 2023.**

**METHOD MODIFICATION RECORD**

1. **May 2015**
2. **March 2017 Level 1**
3. **December 2017 Level 2**
4. **January 2018 Level 1**
5. **May 2019 Level 1**
6. **December 2019 Level 1**
7. **December 2021 Level 1**

**SUMMARY OF MODIFICATION**

1. **Modification to include Actero™ Listeria and use of a single step enrichment for 081401. Matrix extension to include additional matrixes.**
2. **Name change from DuPont Nutrition & Health to Qualicon Diagnostics LLC., a Hygiena company.**
3. **Increase in hold time after tablet rehydration.**
4. **Inserts, manuals, and labels updated to Hygiena.**
5. **Editorial insert changes and corporate address.**
6. **Editorial/clerical changes.**
7. **Editorial changes.**

**Under this AOAC *Performance Tested Methods*℠ License Number, 081401 this method is distributed by:**
**NONE**

**Under this AOAC *Performance Tested Methods*℠ License Number, 081401 this method is distributed as:**
**NONE**

**PRINCIPLE OF THE METHOD (1)**

The BAX System uses 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. This automated BAX System method uses fluorescent detection to analyze PCR product. One PCR primer for each target (*Listeria*-specific targets and an internal control) contains a fluorescent dye (two different dyes, one for the internal control and one for the targets) as a constituent of the primer. The primer or a separate oligonucleotide will contain a quencher that will be in close proximity to the dye in the native state but removed from close proximity when the primer has been incorporated into a PCR product which causes an increase in emission signal. This combination of a primer, fluorescent dye and quencher constitute a Scorpion™ probe. The BAX System measures the magnitude and characteristics of fluorescent signal change. Analysis by the BAX System software then evaluates that data to determine a positive or negative result which is displayed as described below.

**DISCUSSION OF THE VALIDATION STUDY (1)**

Overall, the DuPont™ BAX System Real-Time PCR Assay for Genus *Listeria* demonstrates accuracy and reliability statistically equivalent to the reference culture methods used in this study, with a number of additional benefits. *L. grayi* has been included into the inclusivity panel and is easily detectable. Simplified lysis procedure reduces the total lysis time by more than 30 minutes when compared to previously validated BAX System assays for *Listeria*. Furthermore, this real-time assay reduces the automated processing time by about 2 hours in the BAX System Q7 instrument. This reduced time, along with the ease of use and excellent accuracy of the BAX System, make the test method a strong alternative method for *Listeria* species detection.

**BAX® System Real-Time PCR Assay for Genus *Listeria*, AOAC *Performance Tested Methods*℠ Certification Number 081401**

**Table 2. Candidate method presumptive results vs. confirmed results – POD for food 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 BAX® System 28 h | *L. monocytogenes* DD 1309 | 1.2 | 5 | 5 | 1 | (0.57, 1.0) | 5 | 1 | (0.57, 1.0) | 0 | (-0.45, 0.45) |
| | | 0.12 | 20 | 11 | 0.55 | (0.34, 0.74) | 11 | 0.55 | (0.34, 0.74) | 0 | (-0.14, 0.14) |
| | | Uninoculated | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.45, 0.45) |
| Frankfurters BAX® System 48 h | *L. monocytogenes* DD 1309 | 1.2 | 5 | 5 | 1 | (0.57, 1.0) | 5 | 1 | (0.57, 1.0) | 0 | (-0.45, 0.45) |
| | | 0.12 | 20 | 14 | 0.7 | (0.48, 0.85) | 11* | 0.55 | (0.34, 0.74) | 0.15 | (-0.02, 0.32) |
| | | Uninoculated | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.45, 0.45) |
| Frankfurters USDA-FSIS 48 h | *L. monocytogenes* DD 1309 | 1.2 | 5 | 5 | 1 | (0.57, 1.0) | 5 | 1 | (0.57, 1.0) | 0 | (-0.45, 0.45) |
| | | 0.12 | 20 | 3 | 0.15 | (0.05, 0.36) | 3 | 0.15 | (0.05, 0.36) | 0 | (-0.14, 0.14) |
| | | Uninoculated | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.45, 0.45) |
| Bagged spinach BAX® System 24 h | *L. monocytogenes* DD 1283 | 2.9 | 5 | 3 | 0.6 | (0.23, 0.88) | 3 | 0.6 | (0.23, 0.88) | 0 | (-0.46, 0.46) |
| | | 0.29 | 20 | 7 | 0.35 | (0.18, 0.56) | 8 | 0.4 | (0.21, 0.61) | -0.05 | (-0.05, 0.15) |
| | | Uninoculated | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.45, 0.45) |
| Bagged spinach BAX® System 48 hrs | *L. monocytogenes* DD 1283 | 2.9 | 5 | 3 | 0.6 | (0.23, 0.88) | 3 | 0.6 | (0.23, 0.88) | 0 | (-0.46, 0.46) |
| | | 0.29 | 20 | 8 | 0.4 | (0.21, 0.61) | 8 | 0.4 | (0.21, 0.61) | 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 | 2.9 | 5 | 5 | 1 | (0.57, 1.0) | 5 | 1 | (0.57, 1.0) | 0 | (-0.45, 0.45) |
| | | 0.29 | 20 | 4 | 0.2 | (0.08, 0.41) | 4 | 0.2 | (0.08, 0.41) | 0 | (-0.14, 0.14) |
| | | Uninoculated | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.45, 0.45) |
| Queso fresco cheese BAX® System 26 h | *L. innocua* DD 3244 | 10.3 | 5 | 5 | 1.0 | (0.57, 1.0) | 5 | 1.0 | (0.57, 1.0) | 0 | (-0.45, 0.45) |
| | | 1.03 | 20 | 13 | 0.65 | (0.43, 0.81) | 13 | 0.65 | (0.43, 0.81) | 0 | (-0.14, 0.14) |
| | | Uninoculated | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.45, 0.45) |
| Queso fresco cheese BAX® System 48 h | *L. innocua* DD 3244 | 10.3 | 5 | 5 | 1.0 | (0.57, 1.0) | 5 | 1.0 | (0.57, 1.0) | 0 | (-0.45, 0.45) |
| | | 1.03 | 20 | 13 | 0.65 | (0.43, 0.81) | 13 | 0.65 | (0.43, 0.81) | 0 | (-0.14, 0.14) |
| | | 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. innocua* DD 3244 | 10.3 | 5 | 5 | 1.0 | (0.57, 1.0) | 5 | 1.0 | (0.57, 1.0) | 0 | (-0.45, 0.45) |
| | | 1.03 | 20 | 12 | 0.6 | (0.36, 0.78) | 12 | 0.6 | (0.36, 0.78) | 0 | (-0.14, 0.14) |
| | | Uninoculated | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.45, 0.45) |
| Queso fresco cheese[h] BAX® System 26 h | *L. innocua* ATCC 33090 | 3.08 | 5 | 5 | 1 | (0.57, 1.0) | 5 | 1 | (0.57, 1.0) | 0 | (-0.45, 0.45) |
| | | 0.84 | 20 | 15 | 0.75 | (0.53, 0.88) | 15 | 0.75 | (0.53, 0.88) | 0 | (-0.25, 0.25) |
| | | Uninoculated | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.45, 0.45) |
| Queso fresco cheese[h] BAX® System 48 h | *L. innocua* ATCC 33090 | 3.08 | 5 | 5 | 1 | (0.57, 1.0) | 5 | 1 | (0.57, 1.0) | 0 | (-0.45, 0.45) |
| | | 0.84 | 20 | 15 | 0.75 | (0.53, 0.88) | 15 | 0.75 | (0.53, 0.88) | 0 | (-0.25, 0.25) |
| | | Uninoculated | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.45, 0.45) |
| Queso fresco cheese[h] FDA-BAM 48 h | *L. innocua* ATCC 33090 | 3.08 | 5 | 5 | 1 | (0.57, 1.0) | 5 | 1 | (0.57, 1.0) | 0 | (-0.45, 0.45) |
| | | 0.84 | 20 | 18 | 0.9 | (0.69, 0.97) | 18 | 0.9 | (0.69, 0.97) | 0 | (-0.21, 0.21) |
| | | Uninoculated | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.45, 0.45) |
| Cooked shrimp BAX® System 24 h | *L. ivanovii* DD 649 | 11.9 | 5 | 5 | 1 | (0.57, 1.0) | 5 | 1 | (0.57, 1.0) | 0 | (-0.45, 0.45) |
| | | 1.19 | 20 | 14 | 0.7 | (0.48, 0.85) | 15 | 0.75 | (0.53, 0.88) | -0.05 | (-0.05, 0.15) |
| | | Uninoculated | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.45, 0.45) |
| Cooked shrimp BAX® System 48 h | *L. ivanovii* DD 649 | 11.9 | 5 | 5 | 1 | (0.57, 1.0) | 5 | 1 | (0.57, 1.0) | 0 | (-0.45, 0.45) |
| | | 1.19 | 20 | 15 | 0.75 | (0.53, 0.88) | 15 | 0.75 | (0.53, 0.88) | 0 | (-0.14, 0.14) |
| | | Uninoculated | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.45, 0.45) |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Cooked shrimp FDA-BAM 48 h | *L. ivanovii* DD 649 | 11.9 | 5 | 5 | 1 | (0.57, 1.0) | 5 | 1 | (0.57, 1.0) | 0 | (-0.45, 0.45) |
| | | 1.19 | 20 | 14 | 0.70 | (0.48, 0.85) | 14 | 0.70 | (0.48, 0.85) | 0 | (-0.14, 0.14) |
| | | 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 (6), 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]Independent Laboratory Study.
**Note: 11 samples cultured confirmed as described in the USDA-MLG. An additional 3 samples tested postive by BAX and culture confirmed by direct plating from 24 LEB after 48 h of incubation.*

**Table 3. Candidate method presumptive results vs. confirmed results – POD for environmental surfaces**

| Surface | *Strain* | Listeria incoulum level[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 | | |
| 304 2B Stainless steel BAX® System 24 h | *L. monocytogenes* DD 1144 *Pseudomonas aeruginosa* DD6924 | 1120 | 5 | 5 | 1 | (0.57, 1.0) | 5 | 1 | (0.57, 1.0) | 0 | (-0.45, 0.45) |
| | | 112 | 20 | 13 | 0.65 | (0.43, 0.81) | 13 | 0.65 | (0.43, 0.81) | 0 | (-0.14, 0.14) |
| | | Uninoculated | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.45, 0.45) |
| 304 2B Stainless steel BAX® System 48 h | *L. monocytogenes* DD 1144 *Pseudomonas aeruginosa* DD6924 | 1120 | 5 | 5 | 1 | (0.57, 1.0) | 5 | 1 | (0.57, 1.0) | 0 | (-0.45, 0.45) |
| | | 112 | 20 | 13 | 0.65 | (0.43, 0.81) | 13 | 0.65 | (0.43, 0.81) | 0 | (-0.14, 0.14) |
| | | Uninoculated | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.45, 0.45) |
| 304 2B Stainless steel USDA-MLG 48 h | *L. monocytogenes* DD 1144 *Pseudomonas aeruginosa* DD6924 | 1120 | 5 | 5 | 1 | (0.57, 1.0) | 5 | 1 | (0.57, 1.0) | 0 | (-0.45, 0.45) |
| | | 112 | 20 | 13 | 0.65 | (0.43, 0.81) | 13 | 0.65 | (0.43, 0.81) | 0 | (-0.14, 0.14) |
| | | Uninoculated | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.45, 0.45) |
| 304 2B Stainless steel[h] BAX® System 24 h | *L. monocytogenes ATCC 7644 Pseudomonas aeruginosa ATCC 15442* | $3.2 \times 10^4$ | 5 | 5 | 1 | (0.57, 1.0) | 5 | 1 | (0.57, 1.0) | 0 | (-0.45, 0.45) |
| | | $3.2 \times 10^3$ | 20 | 8 | 0.4 | (0.21, 0.61) | 7 | 0.35 | (0.18, 0.56) | 0.05 | (-0.23, 0.32) |
| | | Uninoculated | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.45, 0.45) |
| 304 2B Stainless steel[h] BAX® System 48 h | *L. monocytogenes ATCC 7644 Pseudomonas aeruginosa ATCC 15442* | $3.2 \times 10^4$ | 5 | 5 | 1 | (0.57, 1.0) | 5 | 1 | (0.57, 1.0) | 0 | (-0.45, 0.45) |
| | | $3.2 \times 10^3$ | 20 | 7 | 0.35 | (0.18, 0.56) | 7 | 0.35 | (0.18, 0.56) | 0 | (-0.27, 0.27) |
| | | Uninoculated | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.45, 0.45) |
| 304 2B Stainless steel[h] USDA-MLG 48 h | *L. monocytogenes ATCC 7644 Pseudomonas aeruginosa ATCC 15442* | $3.2 \times 10^4$ | 5 | 5 | 1 | (0.57, 1.0) | 5 | 1 | (0.57, 1.0) | 0 | (-0.45, 0.45) |
| | | $3.2 \times 10^3$ | 20 | 2 | 0.1 | (0.02, 0.30) | 2 | 0.1 | (0.02, 0.30) | 0 | (-0.21, 0.21) |
| | | Uninoculated | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.45, 0.45) |
| Plastic BAX® System 24 h | *L. seeligeri* DD 2874 *Pseudomonas aeruginosa* | 1750 | 5 | 5 | 1 | (0.57, 1.0) | 5 | 1 | (0.57, 1.0) | 0 | (-0.45, 0.45) |
| | | 175 | 20 | 14 | 0.7 | (0.48, 0.85) | 14 | 0.7 | (0.48, 0.85) | 0 | (-0.14, 0.14) |
| | | Uninoculated | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.45, 0.45) |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | DD6924 | | | | | | | | | | |
| Plastic BAX® System 48 h | *L. seeligeri* DD 2874 *Pseudomonas aeruginosa* DD6924 | 1750 | 5 | 5 | 1 | (0.57, 1.0) | 5 | 1 | (0.57, 1.0) | 0 | (-0.45, 0.45) |
| | | 175 | 20 | 14 | 0.7 | (0.48, 0.85) | 14 | 0.7 | (0.48, 0.85) | 0 | (-0.14, 0.14) |
| | | Uninoculated | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.45, 0.45) |
| Plastic USDA-MLG 48 h | *L. seeligeri* DD 2874 *Pseudomonas aeruginosa* DD6924 | 1750 | 5 | 5 | 1 | (0.57, 1.0) | 5 | 1 | (0.57, 1.0) | 0 | (-0.45, 0.45) |
| | | 175 | 20 | 17* | 0.85 | (0.63, 0.94) | 16 | 0.8 | (0.58, 0.91) | 0.05 | (-0.05, 0.15) |
| | | Uninoculated | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.45, 0.45) |
| Concrete BAX® System 24 h | *L. welshimeri* DD 4096 *Enterococcus faecalis* DD10565 | 750 | 5 | 5 | 1 | (0.57, 1.0) | 5 | 1 | (0.57, 1.0) | 0 | (-0.45, 0.45) |
| | | 75 | 20 | 5 | 0.25 | (0.11, 0.46) | 5 | 0.25 | (0.11, 0.46) | 0 | (-0.14, 0.14) |
| | | Uninoculated | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.45, 0.45) |
| Concrete BAX® System 48 h | *L. welshimeri* DD 4096 *Enterococcus faecalis* DD10565 | 750 | 5 | 5 | 1 | (0.57, 1.0) | 5 | 1 | (0.57, 1.0) | 0 | (-0.45, 0.45) |
| | | 75 | 20 | 5 | 0.25 | (0.11, 0.46) | 5 | 0.25 | (0.11, 0.46) | 0 | (-0.14, 0.14) |
| | | Uninoculated | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.45, 0.45) |
| Concrete USDA-MLG 48 h | *L. welshimeri* DD 4096 *Enterococcus faecalis* DD10565 | 750 | 5 | 4 | 0.3 | (0.37, 0.96) | 4 | 0.3 | (0.37, 0.96) | 0 | (-0.45, 0.45) |
| | | 75 | 20 | 6 | 0.3 | (0.14, 0.51) | 6 | 0.3 | (0.14, 0.51) | 0 | (-0.14, 0.14) |
| | | Uninoculated | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.45, 0.45) |

[a]Inoculum level = Inoculum of target strain applied to surface based on non-selective plate counts. Competitor strains were inoculated with 10X the target inoculm level.
[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]Independent Laboratory Study.
**Note: Sample tested using the BAX® System assay at 48 h following the USDA-MGL enrichment method displayed one weak positive result which happened to be next to a strong positive result. This sample was retested in triplicate resulting in all negatives.*

**Table 4. Method comparison results - POD for food matrixes (1)**

| 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 BAX® System 28 h | *L. monocytogenes* DD 1309 | 1.2 | 5 | 5 | 1 | (0.57, 1.0) | 5 | 1 | (0.57, 1.0) | 0 | (-0.45, 0.45) |
| | | 0.12 | 20 | 11 | 0.55 | (0.34, 0.74) | 3 | 0.15 | (0.05, 0.36) | 0.4 | (0.10, 0.61) |
| | | Uninoculated | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.43, 0.43) |
| Frankfurters BAX® System 48 h | *L. monocytogenes* DD 1309 | 1.2 | 5 | 5 | 1 | (0.57, 1.0) | 5 | 1 | (0.57, 1.0) | 0 | (-0.45, 0.45) |
| | | 0.12 | 20 | 14 | 0.7 | (0.48, 0.85) | 3 | 0.15 | (0.05, 0.36) | 0.55 | (0.24, 0.73) |
| | | Uninoculated | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.43, 0.43) |
| Frankfurters USDA-MLG 48 h | *L. monocytogenes* DD 1309 | 1.2 | 5 | 5 | 1 | (0.57, 1.0) | 5 | 1 | (0.57, 1.0) | 0 | (-0.45, 0.45) |
| | | 0.12 | 20 | 3 | 0.15 | (0.05, 0.36) | 3 | 0.15 | (0.05, 0.36) | 0 | (-0.14, 0.14) |
| | | Uninoculated | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.45, 0.45) |
| Bagged spinach BAX® System 24 h | *L. monocytogenes* DD 1283 | 2.9 | 5 | 3 | 0.6 | (0.23, 0.88) | 5 | 1 | (0.57, 1.0) | -0.4 | (-0.76, 0.11) |
| | | 0.29 | 20 | 7 | 0.35 | (0.18, 0.56) | 4 | 0.2 | (0.08, 0.41) | 0.15 | (-0.12, 0.40) |
| | | Uninoculated | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.43, 0.43) |
| Bagged spinach BAX® System 48 h | *L. monocytogenes* DD 1283 | 2.9 | 5 | 3 | 0.6 | (0.23, 0.88) | 5 | 1 | (0.57, 1.0) | -0.4 | (-0.76, 0.11) |
| | | 0.29 | 20 | 8 | 0.4 | (0.21, 0.61) | 4 | 0.2 | (0.08, 0.41) | 0.2 | (-0.08, 0.44) |
| | | Uninoculated | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.43, 0.43) |
| Bagged spinach FDA-BAM 48 h | *L. monocytogenes* DD 1283 | 2.9 | 5 | 5 | 1 | (0.57, 1.0) | 5 | 1 | (0.57, 1.0) | 0 | (-0.45, 0.45) |
| | | 0.29 | 20 | 4 | 0.2 | (0.08, 0.41) | 4 | 0.2 | (0.08, 0.41) | 0 | (-0.14, 0.14) |
| | | Uninoculated | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.45, 0.45) |
| Queso fresco cheese BAX® System 26 h | *L. innocua* DD 3244 | 10.3 | 5 | 5 | 1 | (0.57, 1.0) | 5 | 1 | (0.57, 1.0) | 0 | (-0.45, 0.45) |
| | | 1.03 | 20 | 13 | 0.65 | (0.43, 0.81) | 12 | 0.6 | (0.38, 0.78) | 0.05 | (-0.23, 0.32) |
| | | Uninoculated | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.43, 0.43) |
| Queso fresco cheese | *L. innocua* | 10.3 | 5 | 5 | 1.0 | (0.57, 1.0) | 5 | 1.0 | (0.57, 1.0) | 0 | (-0.45, 0.45) |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| BAX® System 48 h | DD 3244 | 1.03 | 20 | 13 | 0.65 | (0.43, 0.81) | 12 | 0.6 | (0.36, 0.78) | 0.05 | (-0.23, 0.32) |
| | | Uninoculated | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.43, 0.43) |
| Queso fresco cheese FDA-BAM 48 h | *L. innocua* DD 3244 | 10.3 | 5 | 5 | 1.0 | (0.57, 1.0) | 5 | 1.0 | (0.57, 1.0) | 0 | (-0.45, 0.45) |
| | | 1.03 | 20 | 12 | 0.6 | (0.36, 0.78) | 12 | 0.6 | (0.36, 0.78) | 0 | (-0.14, 0.14) |
| | | Uninoculated | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.45, 0.45) |
| Queso fresco cheese[h] BAX® System 26 h | *L. innocua* ATCC 33090 | 3.08 | 5 | 5 | 1 | (0.57, 1.0) | 0 | 0 | (0, 0.43) | 0 | (-0.45, 0.45) |
| | | 0.84 | 20 | 15 | 0.75 | (0.53, 0.88) | 18 | 0.9 | (0.69, 0.97) | -0.15 | (-0.33, 0.09) |
| | | Uninoculated | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.43, 0.43) |
| Queso fresco cheese[h] BAX® System 48 h | *L. innocua* ATCC 33090 | 3.08 | 5 | 5 | 1 | (0.57, 1.0) | 0 | 0 | (0, 0.43) | 0 | (-0.45, 0.45) |
| | | 0.84 | 20 | 15 | 0.75 | (0.53, 0.88) | 18 | 0.9 | (0.69, 0.97) | -0.15 | (-0.33, 0.09) |
| | | Uninoculated | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.43, 0.43) |
| Queso fresco cheese[h] FDA-BAM 48 h | *L. innocua* ATCC 33090 | 3.08 | 5 | 5 | 1 | (0.57, 1.0) | 0 | 0 | (0, 0.43) | 0 | (-0.45, 0.45) |
| | | 0.84 | 20 | 18 | 0.9 | (0.69, 0.97) | 18 | 0.9 | (0.69, 0.97) | 0 | (-0.21, 0.21) |
| | | Uninoculated | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.43, 0.43) |
| Cooked shrimp BAX® System 24 h | *L. ivanovii* DD 649 | 11.9 | 5 | 5 | 1 | (0.57, 1.0) | 5 | 1 | (0.57, 1.0) | 0 | (-0.45, 0.45) |
| | | 1.19 | 20 | 14 | 0.7 | (0.48, 0.85) | 14 | 0.7 | (0.48, 0.85) | | (-0.26, 0.26) |
| | | Uninoculated | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.43, 0.43) |
| Cooked shrimp BAX® System 48 h | *L. ivanovii* DD 649 | 11.9 | 5 | 5 | 1 | (0.57, 1.0) | 5 | 1 | (0.57, 1.0) | 0 | (-0.45, 0.45) |
| | | 1.19 | 20 | 15 | 0.75 | (0.53, 0.88) | 14 | 0.70 | (0.48, 0.85) | 0.05 | (-0.21, 0.30) |
| | | Uninoculated | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.43, 0.43) |
| Cooked shrimp FDA-BAM 48 h | *L. ivanovii* DD 649 | 11.9 | 5 | 5 | 1 | (0.57, 1.0) | 5 | 1 | (0.57, 1.0) | 0 | (-0.45, 0.45) |
| | | 1.19 | 20 | 14 | 0.70 | (0.48, 0.85) | 14 | 0.70 | (0.48, 0.85) | 0 | (-0.14, 0.14) |
| | | Uninoculated | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.45, 0.45) |

[a]MPN = Most Probable Number is based on the POD of reference method test portions using the Least Cost Formulations MPN calculator [6], with 95% confidence interval.
[b]N = Number of test potions.
[c]x = Number of positive test portions.
[d]$POD_C$ = Confirmed candidate method positive outcomes divided by the total number of trials.
[e]$POD_R$ = Confirmed reference method positive outcomes divided by the total number of trials.
[f]$dPOD_C$ = Difference between the candidate method and reference method POD values.
[h]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[g]Independent Laboratory Study.

**Table 5. Method comparison results - POD for environmental matrixes (1)**

| 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 | | |
| 304 2B Stainless steel BAX® System 24 h | *L. monocytogenes* DD 1144 *Pseudomonas aeruginosa* DD6924 | 1120 | 5 | 5 | 1 | (0.57, 1.0) | 5 | 1 | (0.57, 1.0) | 0 | (-0.45, 0.45) |
| | | 112 | 20 | 13 | 0.65 | (0.43, 0.81) | 13 | 0.65 | (0.43, 0.81) | 0 | (-0.27, 0.27) |
| | | Uninoculated | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.43, 0.43) |
| 304 2B Stainless steel BAX® System 48 h | *L. monocytogenes* DD 1144 *Pseudomonas aeruginosa* DD6924 | 1120 | 5 | 5 | 1 | (0.57, 1.0) | 5 | 1 | (0.57, 1.0) | 0 | (-0.45, 0.45) |
| | | 112 | 20 | 13 | 0.65 | (0.43, 0.81) | 13 | 0.65 | (0.43, 0.81) | 0 | (-0.27, 0.27) |
| | | Uninoculated | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.43, 0.43) |
| 304 2B Stainless steel USDA-MLG 48 h | *L. monocytogenes* DD 1144 *Pseudomonas aeruginosa* DD6924 | 1120 | 5 | 5 | 1 | (0.57, 1.0) | 5 | 1 | (0.57, 1.0) | 0 | (-0.45, 0.45) |
| | | 112 | 20 | 13 | 0.65 | (0.43, 0.81) | 13 | 0.65 | (0.43, 0.81) | 0 | (-0.14, 0.14) |
| | | Uninoculated | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.45, 0.45) |
| 304 2B Stainless steel[h] BAX® System 24 h | *L. monocytogenes* *ATCC 7644* *Pseudomonas aeruginosa* *ATCC 15442* | $3.2 \times 10^4$ | 5 | 5 | 1 | (0.57, 1.0) | 5 | 1 | (0.57, 1.0) | 0 | (-0.45, 0.45) |
| | | $3.2 \times 10^3$ | 20 | 8 | 0.4 | (0.21, 0.61) | 2 | 0.10 | (0.02, 0.30) | 0.30 | (0.02, 0.52) |
| | | Uninoculated | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.43, 0.43) |
| 304 2B Stainless steel[h] BAX® System 48 h | *L. monocytogenes* *ATCC 7644* *Pseudomonas aeruginosa* *ATCC 15442* | $3.2 \times 10^4$ | 5 | 5 | 1 | (0.57, 1.0) | 5 | 1 | (0.57, 1.0) | 0 | (-0.45, 0.45) |
| | | $3.2 \times 10^3$ | 20 | 7 | 0.35 | (0.18, 0.56) | 2 | 0.10 | (0.02, 0.30) | 0.25 | (-0.01, 0.47) |
| | | Uninoculated | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.43, 0.43) |
| 304 2B Stainless steel[h] USDA-MLG 48 h | *L. monocytogenes* *ATCC 7644* *Pseudomonas aeruginosa* *ATCC 15442* | $3.2 \times 10^4$ | 5 | 5 | 1 | (0.57, 1.0) | 5 | 1 | (0.57, 1.0) | 0 | (-0.45, 0.45) |
| | | $3.2 \times 10^3$ | 20 | 2 | 0.1 | (0.02, 0.30) | 2 | 0.1 | (0.02, 0.30) | 0 | (-0.21, 0.21) |
| | | Uninoculated | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.43, 0.43) |
| Plastic BAX® System 24 h | *L. seeligeri* DD 2874 *Pseudomonas aeruginosa* DD6924 | 2250 | 5 | 5 | 1 | (0.57, 1.0) | 5 | 1 | (0.57, 1.0) | 0 | (-0.45, 0.45) |
| | | 250 | 20 | 14 | 0.7 | (0.48, 0.85) | 16 | 0.8 | (0.58, 0.91) | -0.1 | (-0.34, 0.16) |
| | | Uninoculated | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.43, 0.43) |
| Plastic BAX® System 48 h | *L. seeligeri* DD 2874 *Pseudomonas aeruginosa* DD6924 | 2250 | 5 | 5 | 1 | (0.57, 1.0) | 5 | 1 | (0.57, 1.0) | 0 | (-0.45, 0.45) |
| | | 250 | 20 | 14 | 0.7 | (0.48, 0.85) | 16 | 0.8 | (0.58, 0.91) | -0.1 | (-0.34, 0.16) |
| | | Uninoculated | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.43, 0.43) |
| Plastic USDA-MLG 48 h | *L. seeligeri* DD 2874 *Pseudomonas aeruginosa* DD6924 | 2250 | 5 | 5 | 1 | (0.57, 1.0) | 5 | 1 | (0.57, 1.0) | 0 | (-0.45, 0.45) |
| | | 250 | 20 | 17* | 0.85 | (0.63, 0.94) | 16 | 0.8 | (0.58, 0.91) | 0.05 | (-0.05, 0.15) |
| | | Uninoculated | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.45, 0.45) |
| Concrete BAX® System 24 h | *L. welshimeri* DD 4096 *Enterococcus* | 750 | 5 | 5 | 1 | (0.57, 1.0) | 4 | 0.8 | (0.37, 0.96) | 0.2 | (-0.26, 0.62) |
| | | 75 | 20 | 5 | 0.25 | (0.11, 0.46) | 6 | 0.3 | (0.14, 0.51) | -0.05 | (-0.31, 0.21) |
| | | Uninoculated | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.43, 0.43) |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *faecalis* DD10565 | | | | | | | | | | |
| Concrete BAX® System 48 h | *L. welshimeri* DD 4096 *Enterococcus faecalis* DD10565 | 750 | 5 | 5 | 1 | (0.57, 1.0) | 4 | 0.8 | (0.37, 0.96) | 0.2 | (-0.26, 0.62) |
| | | 75 | 20 | 5 | 0.25 | (0.11, 0.46) | 6 | 0.3 | (0.14, 0.51) | -0.05 | (-0.30, 0.21) |
| | | Uninoculated | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.43, 0.43) |
| Concrete USDA-MLG 48 h | *L. welshimeri* DD 4096 *Enterococcus faecalis* DD10565 | 750 | 5 | 4 | 0.3 | (0.37, 0.96) | 4 | 0.3 | (0.37, 0.96) | 0 | (-0.45, 0.45) |
| | | 75 | 20 | 6 | 0.3 | (0.14, 0.51) | 6 | 0.3 | (0.14, 0.51) | 0 | (-0.14, 0.14) |
| | | Uninoculated | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.45, 0.45) |

[a]MPN = Most Probable Number is based on the POD of reference method test portions using the Least Cost Formulations MPN calculator [6], with 95% confidence interval.
[b]N = Number of test potions.
[c]x = Number of positive test portions.
[d]$POD_C$ = Confirmed candidate method positive outcomes divided by the total number of trials.
[e]$POD_R$ = Confirmed reference method positive outcomes divided by the total number of trials.
[f]$dPOD_C$ = Difference between the candidate method and reference method POD values.
[h]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[g]Independent Laboratory Study.

**Table 11. BAX System inclusivity**

| Strain ID (DD#) | Collection ID | Other strain designation (if available) | Serotype (if known) | Isolate source | BAX System result |
|---|---|---|---|---|---|
| 566 | *Listeria monocytogenes* | ATCC15313 | | Rabbit | POS |
| 605 | *Listeria monocytogenes* | ATCC19111 | | Poultry | POS |
| 643 | *Listeria murrayi* | ATCC25401 | | Standing corn stalks & leaves | NEG[a] |
| 644 | *Listeria innocua* | ATCC33090 | | Cow brain | POS |
| 647 | *Listeria monocytogenes* | ATCC19118 | 4c | Chicken | POS |
| 648 | *Listeria monocytogenes* | ATCC19114 | 4a | Animal tissue | POS |
| 649 | *Listeria ivanovii* | ATCC19119 | | Sheep | POS |
| 650 | *Listeria seeligeri* | ATCC 35967 | | Soil | POS |
| 652 | *Listeria monocytogenes* | ATCC 19117 | 4c | Chicken | POS |
| 653 | *Listeria monocytogenes* | ATCC 19115 | 4b | Human | POS |
| 654 | *Listeria welshimeri* | ATCC 35897 | | Decaying plant material | POS |
| 892 | *Listeria innocua* | | | Unknown | POS |
| 898 | *Listeria innocua* | | | Unknown | POS |
| 921 | *Listeria innocua* | | | Roast turkey | POS |
| 922 | *Listeria innocua* | | | Ham cured shoulder | POS |
| 924 | *Listeria innocua* | | | Ham cured shoulder | POS |
| 927 | *Listeria innocua* | | | Chopped pork and ham | POS |
| 944 | *Listeria murrayi* | | | Cornstalks and leaves | POS |
| 1063 | *Listeria innocua* | | | Chopped pork and ham | POS |
| 1064 | *Listeria innocua* | | | Chopped pork and ham | POS |
| 1069 | *Listeria monocytogenes* | | | Stuffed gammon joint | POS |
| 1072 | *Listeria monocytogenes* | | | Cheese and ham pancakes | POS |
| 1144 | *Listeria monocytogenes* | | 1/2a | Stilton cheese | POS |
| 1145 | *Listeria monocytogenes* | | 1/2a | Coleslaw salad | POS |
| 1146 | *Listeria monocytogenes* | | 1/2a | Lettuce | POS |
| 1147 | *Listeria monocytogenes* | | 1/2a | Pate | POS |
| 1149 | *Listeria monocytogenes* | | 1/2a | Raw milk | POS |
| 1152 | *Listeria monocytogenes* | | 1/2b | Pate | POS |
| 1156 | *Listeria innocua* | | | Lettuce | POS |
| 1164 | *Listeria ivanovii* | | | Radish | POS |
| 1165 | *Listeria ivanovii* | | | Belgian salami | POS |

| | | | | | |
|---|---|---|---|---|---|
| 1167 | *Listeria ivanovii* | | | Soft cheese | POS |
| 1171 | *Listeria ivanovii* | | | Unknown | POS |
| 1172 | *Listeria welshimeri* | | | Salami | POS |
| 1174 | *Listeria welshimeri* | | | Raw chicken | POS |
| 1175 | *Listeria welshimeri* | | | Sausage | POS |
| 1176 | *Listeria welshimeri* | | | Chicken | POS |
| 1177 | *Listeria welshimeri* | | | Smoked mackerel | POS |
| 1179 | *Listeria welshimeri* | | | Food | POS |
| 1281 | *Listeria monocytogenes* | | 3c | Cooked chicken | POS |
| 1282 | *Listeria monocytogenes* | | 3c | Unknown | POS |
| 1283 | *Listeria monocytogenes* | | 3b | Cooked turkey | POS |
| 1285 | *Listeria monocytogenes* | | 4(not 4b) | Cheese | POS |
| 1286 | *Listeria monocytogenes* | | 3c | Cooked chicken | POS |
| 1287 | *Listeria monocytogenes* | | 3a | Unknown | POS |
| 1288 | *Listeria monocytogenes* | | 3a | Cooked turkey | POS |
| 1289 | *Listeria seeligeri* | | | Crab pate | POS |
| 1291 | *Listeria seeligeri* | | | Lettuce | POS |
| 1292 | *Listeria seeligeri* | | | Cooked chicken | POS |
| 1293 | *Listeria monocytogenes* | | 3a | Pate | POS |
| 1294 | *Listeria monocytogenes* | | 4b | Ice cream | POS |
| 1295 | *Listeria monocytogenes* | | 3b | Pepper quiche | POS |
| 1297 | *Listeria seeligeri* | | | Pate | POS |
| 1298 | *Listeria seeligeri* | | | Chicken roll | POS |
| 1299 | *Listeria monocytogenes* | | 1/2b | Pork liver pate | POS |
| 1300 | *Listeria seeligeri* | | | Cooked ham | POS |
| 1302 | *Listeria monocytogenes* | | 1/2c | Hard boiled eggs | POS |
| 1305 | *Listeria monocytogenes* | | 3a | Boiled ham | POS |
| 1306 | *Listeria monocytogenes* | | 3b | Chicken liver pate | POS |
| 1307 | *Listeria monocytogenes* | | 3b | Pate | POS |
| 1308 | *Listeria monocytogenes* | | 4b | Cheese | POS |
| 1309 | *Listeria monocytogenes* | | 4b | Soft cheese | POS |
| 1310 | *Listeria monocytogenes* | | 3b | Chicken | POS |
| 1311 | *Listeria monocytogenes* | | 1/2c | Cooked meat | POS |
| 1312 | *Listeria monocytogenes* | | | Ice cream | POS |
| 1313 | *Listeria monocytogenes* | | 4b | Cheese | POS |
| 1314 | *Listeria monocytogenes* | | 4(not 4b) | Pate | POS |
| 1315 | *Listeria monocytogenes* | | 1/2c | Pate | POS |
| 1316 | *Listeria monocytogenes* | | 3a | Cooked chicken | POS |
| 1321 | *Listeria monocytogenes* | | 3c | Sandwich | POS |
| 2874 | *Listeria seeligeri* | | | Frozen dessert | POS |
| 3244 | *Listeria innocua* | | | Unknown | POS |
| 3327 | *Listeria seeligeri* | | | Cheese | POS |
| 3329 | *Listeria seeligeri* | | | Unknown | POS |
| 3351 | *Listeria welshimeri* | | | Unknown | POS |
| 3354 | *Listeria welshimeri* | | | Unknown | POS |
| 3359 | *Listeria welshimeri* | | | Radish | POS |
| 3363 | *Listeria murrayi* | | | Unknown | POS |
| 3376 | *Listeria ivanovii* | | | Environmental | POS |
| 3555 | *Listeria grayi* | | | Unknown | POS |
| 3572 | *Listeria innocua* | | | Cow brain | POS |

| | | | | | |
|---|---|---|---|---|---|
| 3573 | *Listeria monocytogenes* | | | Industry sample | POS |
| 3574 | *Listeria monocytogenes* | | | Industry sample | POS |
| 3576 | *Listeria monocytogenes* | | | Industry sample | POS |
| 3577 | *Listeria monocytogenes* | | | Industry sample | POS |
| 3578 | *Listeria monocytogenes* | | | Industry sample | POS |
| 3579 | *Listeria monocytogenes* | | | Industry sample | POS |
| 3580 | *Listeria monocytogenes* | | | Industry sample | POS |
| 3581 | *Listeria monocytogenes* | | | Industry sample | POS |
| 3582 | *Listeria monocytogenes* | | | Industry sample | POS |
| 3678 | *Listeria ivanovii* | | | Unknown | POS |
| 4553 | *Listeria monocytogenes* | | | Smoked ham | POS |
| 4568 | *Listeria monocytogenes* | | | Swab of finger guard | POS |
| 4571 | *Listeria monocytogenes* | | | honey roast ham | POS |
| 5425 | *Listeria monocytogenes* | | | Jalisco cheese isolate | POS |
| 7644 | *Listeria monocytogenes* | | | Unknown | POS |

[a] Strain DD643 demonstrated consistent limit of detection of $10^6$ cfu/mL.

**Table 12. BAX System exclusivity**

| Strain ID (DD#) | Collection ID | Other Strain Designation (if available) | Isolate source | BAX System Result |
|---|---|---|---|---|
| 379 | *Bacillus subtilus* | | Unknown | NEG |
| 383 | *Citrobacter freundii* | | Unknown | NEG |
| 659 | *Lactococcus lactis* | ATCC 19435 | Unknown | NEG |
| 691 | *Streptococcus thermophilus* | ATCC 19258 | Pasteurized milk | NEG |
| 692 | *Streptococcus bovis* | ATCC 33317 | Cow feces | NEG |
| 695 | *Streptococcus pyogenes* | ATCC 12344 | Unknown | NEG |
| 707 | *Salmonella* Newport | ATCC 6962 | Human clinical | NEG |
| 713 | *Bacillus thuringiensis* | ATCC 35646 | Sewage | NEG |
| 714 | *Bacillus thuringiensis* | ATCC 14579 | Unknown | NEG |
| 715 | *Bacillus cereus* | ATCC 14579 | Unknown | NEG |
| 716 | *Bacillus thuringiensis* | ATCC 33679 | Insect larvae | NEG |
| 721 | *Bacillus cereus* | ATCC 13061 | Unknown | NEG |
| 863 | *Staphylococcus aureus* | ATCC 12600 | Human clinical | NEG |
| 877 | *Bacillus cereus* | ATCC 33018 | Powdered infant formula | NEG |
| 878 | *Bacillus cereus* | ATCC 13061 | Unknown | NEG |
| 879 | *Bacillus cereus* | ATCC 11778 | Unknown | NEG |
| 912 | *Staphylococcus aureus* | ATCC 10832 | Unknown | NEG |
| 1011 | *Bacillus subtilus* | | Mashed potato | NEG |
| 1024 | *Bacillus cereus* | ATCC 7004 | Pasteurized milk | NEG |
| 1096 | *Staphylococcus aureus* | | Unknown | NEG |
| 1098 | *Staphylococcus aureus* | | Unknown | NEG |
| 1105 | *Staphylococcus warneri* | | Salami | NEG |
| 1107 | *Staphylococcus xylosus* | | Plockwurst salami | NEG |
| 1111 | *Staphylococcus capitis* | ATCC 35661 | Unknown | NEG |
| 1112 | *Staphylococcus xylosus* | ATCC 35663 | Unknown | NEG |
| 1113 | *Staphylococcus sciuri* | ATCC 29060 | Human non-clinical | NEG |
| 2392 | *Rhodococcus equi* | ATCC 6939 | Equine clinical | NEG |
| 2552 | *Enterococcus faecium* | ATCC 19434 | Unknown | NEG |
| 2553 | *Enterococcus faecium* | ATCC 35667 | Unknown | NEG |

| | | | | |
|---|---|---|---|---|
| 2554 | *Enterococcus faecalis* | ATCC 35550 | Unknown | NEG |
| 2558 | *Citrobacter freundii* | ATCC 43864 | Unknown | NEG |
| 2560 | *Citrobacter koseri* | ATCC 25408 | Human clinical | NEG |
| 2561 | *Citrobacter koseri* | ATCC 27028 | Human clinical | NEG |
| 2624 | *Enterococcus gallinarum* | ATCC 35038 | Chicken intestine | NEG |
| 2625 | *Enterococcus durans* | ATCC 19432 | Unknown | NEG |
| 2626 | *Enterococcus hirae* | ATCC 8043 | Unknown | NEG |
| 2628 | *Salmonella* Kentucky | ATCC 9263 | Unknown | NEG |
| 2636 | *Staphylococcus felis* | ATCC 49168 | Calf ear | NEG |
| 3981 | *Enterococcus faecalis* | ATCC 29212 | Human clinical | NEG |
| 3992 | *Streptococcus mutans* | ATCC 25175 | Carious dentine | NEG |
| 3996 | *Streptococcus equi* | ATCC 33398 | Unknown | NEG |
| 4063 | *Carnobacterium gallinarum* | | Unknown | NEG |
| 4064 | *Carnobacterium divergens* | | Unknown | NEG |
| 7332 | *Lactobacillus curvatus* | ATCC 25601 | Milk | NEG |
| 7344 | *Lactobacillus acidophilus* | ATCC 4356 | Human non-clinical | NEG |
| 9174 | *Micrococcus luteus* | ATCC 272 | Unknown | NEG |

**DISCUSSION OF MODIFICATION DATA APPROVED MAY 2015 (4)**

The Actero™ Listeria Enrichment Broth has been developed for a single-step recovery of *Listeria* from environmental and food samples and subsequent detection by direct plating (Actero Listeria method). In this study, we demonstrated that the Actero Listeria Enrichment Broth can also be used for enrichment of samples to be analyzed using the DuPont BAX System Real-Time PCR assays for *L. monocytogenes* and *Listeria* Genus. The performance of Actero Listeria Enrichment Media was evaluated in the internal and independent laboratory matrix studies and compare to the USDA-FSIS and/or U.S. FDA reference methods for stainless steel, plastic and sealed concrete environmental surfaces and soft Mexican style cheese, frankfurter, fresh bagged spinach, frozen cooked shrimp, cold smoked salmon and pasteurized milk samples. According to the POD statistical model, for all analyzed matrixes and candidate detection methods, the performance was equal or superior to the appropriate reference method. No false negative or false positive results were found for detection of *Listeria* spp. using the BAX System assay and the Actero Listeria method. Only two false negative outcomes (one for stainless steel samples and one for frozen cooked shrimp) and none of false positive results were detected using the BAX® System assay in the environmental and food samples contaminated with *L. monocytogenes*.

The independent laboratory validation study carried out for stainless steel and soft Mexican style cheese confirmed the accuracy and reliability of the candidate method. In conclusion, the results of the validation study demonstrated that the candidate method using single-step enrichment of environmental and food samples in Acetero Listeria Broth followed by the detection with the BAX System assays or/and Actero Listeria method protocol is an effective screening method and is a viable alternative to traditional reference methods of *Listeria* detection.

**Table 7. Actero Listeria Broth Enrichment Followed by Using the BAX System Real-Time PCR Assay for Genus *Listeria* Detection in Environmental Samples – Candidate Method Presumptive Compared to Candidate Method Confirmed Results (4)**

| Matrix | Strain info | I[a] | | N[d] | Candidate Presumptive (CP) | | | Candidate Confirmed (CC) | | | dPOD$_{CP}$[h] | 95% CI[i] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | CFU/ sample | (UCL[b], LCL[c]) | | X[e] | POD$_{CP}$[f] | 95% CI | X | POD$_{CC}$[g] | 95% CI | | |
| Stainless steel | *L. mono*. 1/2c (ready-to-eat food) + *P. aeruginosa (*field isolate) | 0.0 | N/A[k] | 5 | 0 | 0.00 | (0.00; 0.43) | 0 | 0.00 | (0.00; 0.43) | 0.00 | (-0.43; 0.43) |
| | | 5.0 | N/A | 20 | 16 | 0.80 | (0.58; 0.92) | 16 | 0.80 | (0.58; 0.92) | 0.00 | (-0.25; 0.25) |
| | | 63.0 | N/A | 5 | 5 | 1.00 | (0.57; 1.00) | 5 | 1.00 | (0.57; 1.00) | 0.00 | (-0.43; 0.43) |
| Stainless steel[j] | *L. mono*. ATCC 7644 (1/2c) (ready-to-eat food) + *P. aeruginosa* ATCC 15442 | 0.0 | N/A | 5 | 0 | 0.00 | (0.00; 0.43) | 0 | 0.00 | (0.00; 0.43) | 0.00 | (-0.43; 0.43) |
| | | 90.0 | N/A | 20 | 8 | 0.40 | (0.22; 0.61) | 9 | 0.45 | (0.26; 0.66) | -0.05 | (-0.33; 0.24) |
| | | 220 | N/A | 5 | 5 | 1.00 | (0.57; 1.00) | 5 | 1.00 | (0.57; 1.00) | 0.00 | (-0.43; 0.43) |
| Plastic | *L. seeligeri*, (raw milk) + *P. aeruginosa,* (field isolate) | 0.0 | N/A | 5 | 0 | 0.00 | (0.00; 0.43) | 0 | 0.00 | (0.00; 0.43) | 0.00 | (-0.43; 0.43) |
| | | 4.8 | N/A | 20 | 10 | 0.50 | (0.30; 0.70) | 10 | 0.50 | (0.30; 0.70) | 0.00 | (-0.28; 0.28) |
| | | 58.0 | N/A | 5 | 5 | 1.00 | (0.57; 1.00) | 5 | 1.00 | (0.57; 1.00) | 0.00 | (-0.43; 0.43) |
| Sealed concrete | *L. welshimeri* (chicken drip) + *E. faecalis* | 0.0 | N/A | 5 | 0 | 0.00 | (0.00; 0.43) | 0 | 0.00 | (0.00; 0.43) | 0.00 | (-0.43; 0.43) |
| | | 4.3 | N/A | 20 | 11 | 0.55 | (0.34; 0.74) | 11 | 0.55 | (0.34; 0.74) | 0.00 | (-0.28; 0.28) |
| | | 22.0 | N/A | 5 | 5 | 1.00 | (0.57; 1.00) | 5 | 1.00 | (0.57; 1.00) | 0.00 | (-0.43; 0.43) |

[a]I – Inoculum level which was determined only for the environmental samples.
[b]UCL – Upper Confidence Limit.
[c]LCL – Lower Confidence Limit.
[d]N – Number of test portions.
[e]X – Number of positive test portions.
[f]POD$_{CP}$ – Candidate method presumptive positive outcomes divided by the total number of trials.
[g]POD$_{CC}$ – Candidate method confirmed positive outcomes divided by the total number of trials.
[h]dPOD$_{CP}$ – Difference between the candidate method presumptive and candidate method confirmed result POD values.
[i]95% CI – If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[j]The data from the independent matrix study performed by independent laboratory Q Laboratories.
[k]N/A – Not applicable.

**Table 8. Actero Listeria Broth Enrichment Followed by Using the BAX System Real-Time PCR Assay for Genus *Listeria* Detection in Environmental Samples – Candidate Method Compared to Reference Method Results (4)**

| Matrix | Strain info | I[a] | | N[d] | Candidate Method (C) | | | Reference Method (R) | | | $dPOD_{CR}$[h] | 95% CI[i] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | CFU/ sample | (UCL[b], LCL[c]) | | X[e] | $POD_C$[f] | 95% CI | X | $POD_R$[g] | 95% CI | | |
| Stainless steel | *L. mono*. 1/2c (ready-to-eat food) + *P. aeruginosa (*field isolate) | 0.0 | N/A[k] | 5 | 0 | 0.00 | (0.00; 0.43) | 0 | 0.00 | (0.00; 0.43) | 0.00 | (-0.43; 0.43) |
| | | 5.0 | N/A | 20 | 16 | 0.80 | (0.58; 0.92) | 10 | 0.50 | (0.30; 0.70) | 0.30 | (0.00; 0.53) |
| | | 63.0 | N/A | 5 | 5 | 1.00 | (0.57; 1.00) | 4 | 0.80 | (0.38; 0.96) | 0.20 | (-0.26; 0.62) |
| Stainless steel[j] | *L. mono*. ATCC 7644 (1/2c) (ready-to-eat food) + *P. aeruginosa* ATCC 15442 | 0.0 | N/A | 5 | 0 | 0.00 | (0.00; 0.43) | 0 | 0.00 | (0.00; 0.43) | 0.00 | (-0.43; 0.43) |
| | | 90.0 | N/A | 20 | 8 | 0.40 | (0.22; 0.61) | 5 | 0.25 | (0.11; 0.47) | 0.15 | (-0.13; 0.40) |
| | | 220 | N/A | 5 | 5 | 1.00 | (0.57; 1.00) | 5 | 1.00 | (0.57; 1.00) | 0.00 | (-0.43; 0.43) |
| Plastic | *L. seeligeri*, (raw milk) + *P. aeruginosa,* (field isolate) | 0.0 | N/A | 5 | 0 | 0.00 | (0.00; 0.43) | 0 | 0.00 | (0.00; 0.43) | 0.00 | (-0.43; 0.43) |
| | | 4.8 | N/A | 20 | 10 | 0.50 | (0.30; 0.70) | 5 | 0.25 | (0.11; 0.47) | 0.25 | (-0.05; 0.49) |
| | | 58.0 | N/A | 5 | 5 | 1.00 | (0.57; 1.00) | 5 | 1.00 | (0.57; 1.00) | 0.00 | (-0.43; 0.43) |
| Sealed concrete | *L. welshimeri* (chicken drip) + *E. faecalis* | 0.0 | N/A | 5 | 0 | 0.00 | (0.00; 0.43) | 0 | 0.00 | (0.00; 0.43) | 0.00 | (-0.43; 0.43) |
| | | 4.3 | N/A | 20 | 11 | 0.55 | (0.34; 0.74) | 4 | 0.20 | (0.08; 0.42) | 0.35 | (0.05; 0.58) |
| | | 22.0 | N/A | 5 | 5 | 1.00 | (0.57; 1.00) | 5 | 1.00 | (0.57; 1.00) | 0.00 | (-0.43; 0.43) |

[a]I – Inoculum level which was determined only for the environmental samples.
[b]UCL – Upper Confidence Limit.
[c]LCL – Lower Confidence Limit.
[d]N – Number of test portions.
[e]X – Number of positive test portions.
[f]$POD_C$ – Candidate method positive outcomes divided by the total number of trials.
[g]$POD_R$ – Reference method positive outcomes divided by the total number of trials.
[h]$dPOD_{CR}$ – Difference between the candidate method and reference method confirmed result POD values.
[i]95% CI – If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[j]The data from the independent matrix study performed by independent laboratory Q Laboratories.
[k]N/A – Not applicable.

**Table 9. Actero Listeria Broth Enrichment Followed by Using the BAX System Real-Time PCR Assay for Genus *Listeria* Detection in Food Samples – Candidate Method Presumptive Compared to Candidate Method Confirmed Results (4)**

| Matrix | Strain info | MPN[a] | | N[d] | Candidate Presumtive (CP) | | | Candidate Confirmed (CC) | | | dPOD$_{CP}$[h] | 95% CI[i] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | CFU/ sample | (UCL[b], LCL[c]) | | X[e] | POD$_{CP}$[f] | 95% CI | X | POD$_{CC}$[g] | 95% CI | | |
| Soft Mexican style cheese | *L. innocua* (manure/soil) | 0.00 | (0.00; 0.43) | 5 | 0 | 0.00 | (0.00; 0.43) | 0 | 0.00 | (0.00; 0.43) | 0.00 | (-0.43; 0.43) |
| | | 0.39 | (0.17; 0.68) | 20 | 20 | 1.00 | (0.84; 1.00) | 20 | 1.00 | (0.84; 1.00) | 0.00 | (-0.16; 0.16) |
| | | 13.85 | (1.00; 37.90) | 5 | 5 | 1.00 | (0.57; 1.00) | 5 | 1.00 | (0.57; 1.00) | 0.00 | (-0.43; 0.43) |
| Soft Mexican style cheese[j] | *L. innocua* (manure/soil) | 0.00 | (0.00; 0.43) | 5 | 0 | 0.00 | (0.00; 0.43) | 0 | 0.00 | (0.00; 0.43) | 0.00 | (-0.43; 0.43) |
| | | 0.63 | (0.35; 1.04) | 20 | 10 | 0.50 | (0.30; 0.70) | 10 | 0.50 | (0.30; 0.70) | 0.00 | (-0.28; 0.28) |
| | | 3.56 | (1.63; 7.78) | 5 | 5 | 1.00 | (0.57; 1.00) | 5 | 1.00 | (0.57; 1.00) | 0.00 | (-0.43; 0.43) |
| Frankfurter | *L. mono*. 1/2a (milk), heat stressed | 0.00 | (0.00; 0.43) | 5 | 0 | 0.00 | (0.00; 0.43) | 0 | 0.00 | (0.00; 0.43) | 0.00 | (-0.43; 0.43) |
| | | 0.30 | (0.13; 0.53) | 20 | 14 | 0.70 | (0.48; 0.85) | 14 | 0.70 | (0.48; 0.85) | 0.00 | (-0.27; 0.27) |
| | | 3.90 | (1.85; 8.10) | 5 | 5 | 1.00 | (0.57; 1.00) | 5 | 1.00 | (0.57; 1.00) | 0.00 | (-0.43; 0.43) |
| Fresh bagged spinach[k] | *L. mono*. 1/2a (lettuce), + *L. welshimeri* (natural contamination) | 0.00 | (0.00; 0.43) | 5 | 0 | 0.00 | (0.00; 0.43) | 0 | 0.00 | (0.00; 0.43) | 0.00 | (-0.43; 0.43) |
| | | 1.60[L] | (1.00; 2.95) | 20 | 16 | 0.80 | (0.58; 0.92) | 16 | 0.80 | (0.58; 0.92) | 0.00 | (-0.25; 0.25) |
| | | 1000.00 | (4.80; 1000) | 5 | 5 | 1.00 | (0.57; 1.00) | 5 | 1.00 | (0.57; 1.00) | 0.00 | (-0.43; 0.43) |
| Frozen cooked shrimp | *L. seeligeri* (field water), heat stressed | 0.00 | (0.00; 0.43) | 5 | 0 | 0.00 | (0.00; 0.43) | 0 | 0.00 | (0.00; 0.43) | 0.00 | (-0.43; 0.43) |
| | | 0.69 | (0.38; 1.14) | 20 | 12 | 0.60 | (0.39; 0.78) | 12 | 0.60 | (0.39; 0.78) | 0.00 | (-0.28; 0.28) |
| | | 5.34 | (2.45; 11.6) | 5 | 5 | 1.00 | (0.57; 1.00) | 5 | 1.00 | (0.57; 1.00) | 0.00 | (-0.43; 0.43) |
| Cold smoked salmon | *L. innocua*, (turkey/ham/cheese deli sticks) | 0.00 | (0.00; 0.43) | 5 | 0 | 0.00 | (0.00; 0.43) | 0 | 0.00 | (0.00; 0.43) | 0.00 | (-0.43; 0.43) |
| | | 1.30 | (0.82; 2.10) | 20 | 14 | 0.70 | (0.48; 0.85) | 14 | 0.70 | (0.48; 0.85) | 0.00 | (-0.27; 0.27) |
| | | 13.86 | (5.07; 37.90) | 5 | 5 | 1.00 | (0.57; 1.00) | 5 | 1.00 | (0.57; 1.00) | 0.00 | (-0.43; 0.43) |

[a]MPN – Most Probable Number is based on the POD of reference method test portions using the LCF MPN calculator.
[b]UCL – Upper Confidence Limit.
[c]LCL – Lower Confidence Limit.
[d]N – Number of test portions.
[e]X – Number of positive test portions.
[f]POD$_{CP}$ – Candidate method presumptive positive outcomes divided by the total number of trials.
[g]POD$_{CC}$ – Candidate method confirmed positive outcomes divided by the total number of trials.
[h]dPOD$_{CP}$ – Difference between the candidate method presumptive and candidate method confirmed result POD values.
[i]95% CI – If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[j]The data from the independent matrix study performed by independent laboratory, University of Guelph Laboratory Services Division.
[k]Fresh bagged spinach samples naturally contaminated with *L. welshimeri* and artificially contaminated with *L. monocytogenes* MRS0437 were used.
[L]Data of natural contamination with *L. welshimeri* were included in the MPN calculation.

**Table 10. Actero Listeria Broth Enrichment Followed by Using the BAX System Real-Time PCR Assay for Genus *Listeria* Detection in Food Samples – Candidate Method Compared to Reference Method Results (4)**

| Matrix | Strain info | MPN[a] | | N[d] | Candidate Method (C) | | | Reference Method (R) | | | $dPOD_{CR}$[h] | 95% CI[i] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | CFU/ sample | (UCL[b], LCL[c]) | | X[e] | $POD_C$[f] | 95% CI | X | $POD_R$[g] | 95% CI | | |
| Soft Mexican style cheese | *L. innocua* (manure/soil) | 0.00 | (0.00; 0.43) | 5 | 0 | 0.00 | (0.00; 0.43) | 0 | 0.00 | (0.00; 0.43) | 0.00 | (-0.43; 0.43) |
| | | 0.39 | (0.17; 0.68) | 20 | 20 | 1.00 | (0.84; 1.00) | 7 | 0.35 | (0.18; 0.57) | 0.65 | (0.38; 0.82) |
| | | 13.85 | (1.00; 37.90) | 5 | 5 | 1.00 | (0.57; 1.00) | 5 | 1.00 | (0.57; 1.00) | 0.00 | (-0.43; 0.43) |
| Soft Mexican style cheese[j] | *L. innocua* (manure/soil) | 0.00 | (0.00; 0.43) | 5 | 0 | 0.00 | (0.00; 0.43) | 0 | 0.00 | (0.00; 0.43) | 0.00 | (-0.43; 0.43) |
| | | 0.63 | (0.35; 1.04) | 20 | 10 | 0.50 | (0.30; 0.70) | 8 | 0.40 | (0.22; 0.61) | 0.10 | (-0.19; 0.37) |
| | | 3.56 | (1.63; 7.78) | 5 | 5 | 1.00 | (0.57; 1.00) | 5 | 1.00 | (0.57; 1.00) | 0.00 | (-0.43; 0.43) |
| Frankfurter | *L. mono*. 1/2a (milk), heat stressed | 0.00 | (0.00; 0.43) | 5 | 0 | 0.00 | (0.00; 0.43) | 0 | 0.00 | (0.00; 0.43) | 0.00 | (-0.43; 0.43) |
| | | 0.30 | (0.13; 0.53) | 20 | 14 | 0.70 | (0.48; 0.85) | 7 | 0.35 | (0.18; 0.57) | 0.35 | (0.04; 0.58) |
| | | 3.90 | (1.85; 8.10) | 5 | 5 | 1.00 | (0.57; 1.00) | 5 | 1.00 | (0.57; 1.00) | 0.00 | (-0.43; 0.43) |
| Fresh bagged spinach[k] | *L. mono*. 1/2a (lettuce), + *L. welshimeri* (natural contamination) | 0.00 | (0.00; 0.43) | 5 | 0 | 0.00 | (0.00; 0.43) | 0 | 0.00 | (0.00; 0.43) | 0.00 | (-0.43; 0.43) |
| | | 1.60[l] | (1.00; 2.95) | 20 | 16 | 0.80 | (0.58; 0.92) | 15 | 0.75 | (0.53; 0.89) | 0.05 | (-0.21; 0.30) |
| | | 1000.00 | (4.80; 1000) | 5 | 5 | 1.00 | (0.57; 1.00) | 5 | 1.00 | (0.57; 1.00) | 0.00 | (-0.43; 0.43) |
| Frozen cooked shrimp | *L. seeligeri* (field water), heat stressed | 0.00 | (0.00; 0.43) | 5 | 0 | 0.00 | (0.00; 0.43) | 0 | 0.00 | (0.00; 0.43) | 0.00 | (-0.43; 0.43) |
| | | 0.69 | (0.38; 1.14) | 20 | 12 | 0.60 | (0.39; 0.78) | 11 | 0.55 | (0.34; 0.74) | 0.05 | (-0.24; 0.303) |
| | | 5.34 | (2.45; 11.6) | 5 | 5 | 1.00 | (0.57; 1.00) | 5 | 1.00 | (0.57; 1.00) | 0.00 | (-0.43; 0.43) |
| Cold smoked salmon | *L. innocua*, (turkey/ham/cheese deli sticks) | 0.00 | (0.00; 0.43) | 5 | 0 | 0.00 | (0.00; 0.43) | 0 | 0.00 | (0.00; 0.43) | 0.00 | (-0.43; 0.43) |
| | | 1.30 | (0.82; 2.10) | 20 | 14 | 0.70 | (0.48; 0.85) | 14 | 0.70 | (0.48; 0.85) | 0.00 | (-0.27; 0.27) |
| | | 13.86 | (5.07; 37.90) | 5 | 5 | 1.00 | (0.57; 1.00) | 5 | 1.00 | (0.57; 1.00) | 0.00 | (-0.43; 0.43) |

[a] I – Inoculum level which was determined only for the environmental samples.
[b] UCL – Upper Confidence Limit.
[c] LCL – Lower Confidence Limit.
[d] N – Number of test portions.
[e] X – Number of positive test portions.
[f] $POD_C$ – Candidate method positive outcomes divided by the total number of trials.
[g] $POD_R$ – Reference method positive outcomes divided by the total number of trials.
[h] $dPOD_{CR}$ – Difference between the candidate method and reference method confirmed result POD values.
[i] 95% CI – If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[j] The data from the independent matrix study performed by independent laboratory, University of Guelph Laboratory Services Division.
[k] Fresh bagged spinach samples naturally contaminated with *L. welshimeri* and artificially contaminated with *L. monocytogenes* MRS0437 were used.
[l] Data of natural contamination with *L. welshimeri* were included in the MPN calculation.

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