AOAC GovVal Test Kit/Reference Method Comparison Program

# MicroSEQ® *Listeria monocytogenes* Detection Kit

## GovVal Validation Comparison to the Health Canada MFHPB-30 Reference Method for Detection of *Listeria* in Ready-to-Eat Meats and on Stainless Steel

***Performance Tested Method*SM 011002**


## Abstract

The Applied Biosystems *Performance Tested Method*SM for detecting *Listeria monocytogenes* in food and environmental samples was compared to the Health Canada reference method (MFHPB-30) for the analysis of five ready-to-eat (RTE) meats (deli turkey, hot dogs, liver paté, deli ham, and raw fermented sausage) and a stainless steel environmental surface. The MicroSEQ® method includes the MicroSEQ *Listeria monocytogenes* Detection Kit and the option of two different sample preparation kits, either the automated high-throughput PrepSEQ™ Nucleic Acid Extraction Kit or the manual low- to mid-throughput PrepSEQ Rapid Spin Sample Preparation Kit. For each sample matrix, 20 replicates were analyzed at two inoculum levels: for RTE meats, a low-level inoculum at 0.2–2 CFU/25 g and a high-level inoculum at 2–5 CFU/25 g; and for environmental surfaces, a low-level inoculum at 0.2–2 CFU/5 $cm^2$ sampling area and a high-level inoculum at 2–5 CFU/5 $cm^2$ sampling area. Five control replicates were also analyzed at 0 CFU/25 g (uninoculated) for food or 0 CFU/5 $cm^2$ sampling area for environmental surface. Both sample preparation methods returned identical results. There was no statistically significant difference in the number of positive samples detected by the MicroSEQ *Listeria monocytogenes* method and the MFHPB-30 reference method for four of the five RTE meats and the one stainless steel surface tested. For deli turkey, there was a statistically significant difference in the number of positive results detected by the MicroSEQ method and the Health Canada MFHPB-30 reference method for the low inoculation level, with the MicroSEQ method detecting more positives. Because the MicroSEQ method uses real-time PCR to detect pathogens, it provides faster time-to-results compared to culture methods while at the same time demonstrating equivalent detection. The MicroSEQ method detects *L. monocytogenes* within 2 to 3 h following 24 to 28 h enrichment compared to culture methods that take at least 5 days for presumptive positive results.


The method was independently tested, evaluated, and certified by the AOAC Research Institute (RI) as part of the GovVal validation program. Candidate test kit methods were evaluated using a validation protocol approved by AOAC, the Canadian Food Inspection Agency, and Health Canada, using blind-coded, randomized samples. Sample preparation and independent testing of many test kit methods, as well as the MFHPB-30 reference method, were done by an AOAC RI-approved independent laboratory, Q Laboratories, Inc. Results were reviewed and approved by the AOAC General Referee on Microbiology. For more information on the GovVal program, visit http://www.aoac.org/News/AOAC_govval.htm

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## Scope of Method

The MicroSEQ® *Listeria monocytogenes* Detection Kit was previously validated in the *Performance Tested Methods*SM program for the detection of *L. monocytogenes* in roast beef, cured bacon, lox (smoked salmon), lettuce, pasteurized whole cow's milk, dry infant formula, ice cream, salad dressing, and mayonnaise. The method was shown to be comparable to the International Organization for Standardization (ISO) 11290-1 reference method (1). The current study extends the validation to include ready-to-eat (RTE) meats (liver paté, hot dogs, raw fermented sausage, sliced deli turkey, and sliced deli ham) and stainless steel surfaces in comparison to the Health Canada, Health Products and Food Branch *Compendium of Analytical Methods* reference method (2).

## Definitions

(**a**) *Probability of detection (POD).*—The POD is the proportion of positive analytical outcomes for a qualitative method for a given matrix at a given analyte level or concentration. POD is concentration-dependent. Several POD measures can be calculated: $POD_R$ (reference method POD); $POD_C$ (confirmed candidate method POD); $POD_{CP}$ (candidate method presumptive result POD); and $POD_{CC}$ (candidate method confirmation result POD).

The POD estimate is calculated as the number of positive outcomes divided by the total number of trials. Estimate the


**Figure 1. Key to symbols used to display sample and control results.**

POD with a 95% confidence interval for the candidate method, the reference method and, if included, the presumptive and confirmed results.

(**b**) *Difference of POD (dPOD).*—The dPOD is the difference between any two POD values. Estimate the $dPOD_{CP}$ as the difference between the candidate presumptive result POD ($POD_{CP}$) and the candidate confirmed result POD ($POD_{CC}$) values. Calculate the 95% confidence interval on the $dPOD_{CP}$:

$$dPOD_{CP} = POD_{CP} - POD_{CC}$$

Estimate the $dPOD_C$ as the difference between the candidate method and reference method POD values. Calculate the 95% confidence interval on the $dPOD_C$:

$$dPOD_C = POD_C - POD_R$$

If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level (3).

(**c**) *Chi-square.*—A Chi-square value <3.84 indicates that the proportions positive for the alternative and reference methods are not statistically different at the 5% level of significance. This criterion must be satisfied for each level of each food type. However, a significant difference between the proportions positive for the two methods is acceptable provided that the alternative method demonstrates superior recovery to the reference method.

(**d**) *Unpaired test portions.*—Mantel-Haenszel:

$$\chi^2 = (n - 1)(ad - bc)^2/[(a + b)(a + c)(b + d)(c + d)]$$

where $n$ = total number of samples tested by the two methods, a = number of samples positive by the test method, b = number of samples positive by the reference method, c = number of samples negative by test method, and d = number of samples negative by the reference method (4).

## Principle of the Method

### *Real-Time PCR Amplification*

The MicroSEQ Pathogen Detection System is based on TaqMan® real-time PCR technology (5), providing two levels of specificity for confident pathogen detection by combining PCR amplification (6) and signal detection in a single reaction. The first level of specificity is provided by target specific PCR primers that identify the DNA sequence of the organism in the sample. The identification of the organism is confirmed simultaneously by TaqMan probes, which represent the second level of specificity. As a result, a fluorescent signal is emitted only if the unique genetic signature of the pathogen has been recognized. By addressing the unique genetic signature of the target organism, the real-time PCR system delivers results independent of environmental factors, such as temperature or pH.

In addition, the MicroSEQ Pathogen Detection System contains an Internal Positive Control (IPC) in the reaction mix to monitor the presence of PCR inhibitors. Amplification of IPC demonstrates the absence of PCR inhibition, providing more confident negative results (reducing false-negatives). After PCR amplification and detection, reaction tubes remain sealed, thus significantly reducing the potential for contamination (false-positives).

### *Data Analysis*

The RapidFinder™ Express software simplifies real-time PCR setup and processing by providing a software-guided workflow and automated analysis of results. Designed specifically for pathogen detection in food and environmental samples, RapidFinder Express software guides the user through each step of the assay and performs all required calculations. During PCR, real-time fluorescence data are collected cycle-by-cycle for each reaction. Positive signals result in an increase of the target-specific fluorescent signal, while the fluorescence of negative signals remains below the threshold applied by RapidFinder Express software. When the assay is complete, RapidFinder Express software transforms the individual fluorescent signals and displays them in an intuitive, easy-to-read, color-coded format.

## General Information

Two species, *L. monocytogenes* and *L. ivanovii,* are known pathogens and cause listeriosis in humans. *L. ivanovii* has only been reported in a small number of human listeriosis cases and appears to be more host-adapted to ruminants. *L. monocytogenes* is a human pathogen that is ubiquitous in the environment, and can be found in soil, wastewater, animals, and vegetation. *L. monocytogenes* is robust and can survive extremes of temperature, pH, and salt concentration. *L. monocytogenes* has been associated with such foods as raw milk, soft Hispanic and surface-ripened cheeses, raw vegetables, raw meats (all types), RTE delicatessen meats, and raw and smoked fish and seafood. The ability to grow at temperatures as low as 3°C permits *Listeria* multiplication in refrigerated foods.

## Test Kit Information

(**a**) *Kit name.*—MicroSEQ *Listeria monocytogenes* Detection Kit.

(**b**) *Cat. No.*—4403874.

(**c**) *Ordering information.*—Applied Biosystems, Life Technologies, Tel: 650-638-5800, Fax: 650-638-5787, Website: www.appliedbiosystems.com/foodsafety.

## Test Kit Components

(**a**) *Part 1 of 2: MicroSEQ Listeria monocytogenes Detection Kit.*—Sealable pouch. Contains 12 eight-tube strips placed in a

**Table 1. Aerobic plate counts**

| Food matrix | Properties | CFU/g |
|---|---|---|
| Liver paté | Chicken liver, refrigerated. With onion, garlic, bay leaves, thyme, salt, black pepper. | $6.8 \times 10^4$ |
| Hot dogs | Turkey/chicken/pork, 16% fat, vacuum-packed, refrigerated, sodium diacetate, sodium nitrite. | $1.7 \times 10^3$ |
| Raw fermented sausage | 12% fat. Mini Sopressata pork salami with salt, nonfat dry milk, burgundy wine, lactic acid starter culture, sugar, pepper, spices, sodium erythorbate, garlic, sodium nitrite, sodium nitrate. Dry cured in casing. Ambient temperature storage. | $3.8 \times 10^3$ |
| Sliced deli turkey | 1% fat, vacuum-packaged, refrigerated, with sodium propionate, sodium diacetate, sodium benzoate, sodium ascorbate, and sodium nitrite. | $1.2 \times 10^3$ |
| Sliced deli ham | 2% fat, vacuum-packaged, refrigerated with water added. | $1.3 \times 10^3$ |

96-well support base, a bag of optical caps, a product insert, and a desiccant pack. Each tube contains a lyophilized real-time PCR reaction mix and is sealed with non-optical caps. The lyosphere includes enzyme, buffers, magnesium, nucleotides, and supplements, together with primers and a FAM™-dye-labeled probe for the detection of *L. monocytogenes*, an IPC template together with primers, and a VIC®-dye-labeled probe for the detection of the IPC. Store at 2–8°C.

(**b**) *Part 2 of 2*: *Pathogen detection negative control, one tube*.—Contains an aqueous buffer prepared with PCR-grade water and monitors for contamination and reagent integrity. Store at 2–8°C.

## Additional Supplies and Reagents

(**a**) *Fast Reaction Tubes (eight tubes/strip)*.—Applied BioSystems, 125 Strips, product number (p/n) 4358293.

(**b**) *Optical Caps (eight caps/strip)*.—Applied Biosystems, 300 strips, p/n 4323032.

(**c**) *Automated sample preparation*.—PrepSEQ™ Nucleic Acid Extraction Kit, p/n 4480466. The Kit is shipped as three separate parts.

*Part 1 of 3*.—Seven bottles, buffers and reagents for cell lysis and nucleic acid purification. Store at ambient temperature.

*Part 2 of 3*.—Two tubes, magnetic particles. Store at 2–8°C.

*Part 3 of 3*.—One tube, proteinase K. Store at –20°C.

(**d**) *Manual sample preparation*.—PrepSEQ Rapid Spin Sample Preparation Kit – Extra Clean with Proteinase K, p/n 4413269. The Kit is shipped as three separate parts.

*Part 1 of 3*.—Plastic tubes and clarification columns for 100 preparations. Store at ambient temperature.

*Part 2 of 3*.—One bottle, lysis buffer. Store at 2–8°C.

*Part 3 of 3*.—One tube, proteinase K. Store at –20°C.

(**e**) *Pipet tips, aerosol resistant*.—Different sizes, from 0.5 to 1000 µL. Aerosol-resistant for low risk of contamination.

## Apparatus

(**a**) *Incubators (Major Laboratory Supplier; MLS)*.—For maintaining bacterial enrichment media at 37 ± 1°C.

(**b**) *Stomacher (MLS)*.—For mixing food samples with culture media.

(**c**) *Benchtop microcentrifuge (MLS)*.—For sample concentration and sample preparation. Capable of centrifuging 1.5 mL microcentrifuge tubes at 14 000–16 000 × *g*.

(**d**) *Vortex (MLS)*.—For sample mixing.

(**e**) *Pipettors (MLS)*.—For transferring reagents. Set of adjustable pipets that cover volumes from 0.5 to 1000 µL.

(**f**) *Automated sample preparation instrument and supplies*.—To be used with PrepSEQ Nucleic Acid Extraction Kit:

(*1*) *Deep well magnetic particle processor*.—MagMAX™ Express-96, p/n 4400079. High throughput instrument for isolation of high-quality nucleic acids from virtually any sample.

(*2*) *Deep well tip combs*.—MagMAX Express-96, p/n 4388487, 100 combs.

(*3*) *Deep well plates*.—MagMAX Express-96, p/n 4388476, 50 plates.

(*4*) *Standard plates*.—MagMAX Express-96, p/n 4388475, 48 plates.

(*5*) *96-Well magnetic-ring stand*.—p/n AM10050. For preventing the transfer of magnetic particles to the PCR reaction if magnetic particles are present in the elution plate.

(*6*) *Pipettor*.—Multichannel pipettor for transferring 100–300 µL into 96-well plates.

(*7*) *Clear adhesive film*.— MicroAmp™, p/n 4306311, 100 films. For sealing 96-well plates for transport and storage.

(**g**) *Manual sample preparation equipment and supplies*.—To be used with PrepSEQ Rapid Spin Sample Preparation Kit.

*Heat block (MLS)*.—Set at 56°C. If necessary, verify actual temperature is 56 ± 1°C. For proteinase K digestion.

*Heat block (MLS)*.—Set at 95–100°C. For bacteria cell lysis, and proteinase K heat inactivation.

(**h**) *Real-time PCR system*.—One of the following:

*Applied Biosystems 7500 Fast Real-Time PCR System, p/n 4363914*.—Real-time PCR system for automated detection of pathogens with notebook computer and RapidFinder Express v1.0 Software.

*Applied Biosystems 7500 Fast Real-Time PCR System, p/n 4363915*.—Real-time PCR system for automated detection of pathogens with PC tower computer and RapidFinder Express v1.0 Software.

## Reference Test Cultures

(**a**) *L. monocytogenes* 1/2a, American Type Culture collection (ATCC) 49594.

(**b**) *L. monocytogenes* 1/2b, ATCC BAA-751.

(**c**) *L. innocua*, ATCC 33091 (human feces).

(**d**) *L. monocytogenes* 3b, CWD 1591 (University of Vermont).

(**e**) *L. monocytogenes* 3c, FSL-J1-049 (Cornell University).

(**f**) *L. monocytogenes* 4b, ATCC 19115 (human).

(**g**) *L. monocytogenes* 4d, ATCC 19117 (sheep).

(**h**) *Enterococcus faecalis,* ATCC 29212 (urine).

**Table 2. Heat–stress injury**

| Test organism | Matrix | CFU/OXA | CFU/BHI | Degree injury, % |
|---|---|---|---|---|
| *L. monocytogenes* 1/2a ATCC 49594 | Liver paté | $2.4 \times 10^8$ | $1.1 \times 10^9$ | 78 |
| *L. monocytogenes* 1/2b ATCC BAA–751 | Hot dogs | $4.4 \times 10^8$ | $1.5 \times 10^9$ | 71 |
| *L. monocytogenes* 3b CWD 1591 | Fermented sausage | $3.8 \times 10^8$ | $1.2 \times 10^9$ | 68 |
| *L. monocytogenes* 3c FSL–J1–049 | Sliced deli turkey | $6.6 \times 10^8$ | $1.5 \times 10^9$ | 56 |
| *L. monocytogenes* 4b ATCC 19115 | Sliced deli ham | $7.2 \times 10^8$ | $1.8 \times 10^9$ | 60 |
| *L. innocua* ATCC 33091 | Hot dogs | $3.5 \times 10^8$ | $9.4 \times 10^8$ | 63 |

## Standard Solutions

(**a**) *Buffered Listeria Enrichment Broth (BLEB).*—p/n 1.09628.0500, EMD Chemicals, Inc. (Gibbstown, NJ). The MicroSEQ *Listeria monocytogenes* Detection Kit was evaluated using BLEB purchased from EMD Chemicals; other sources of BLEB might not give equivalent results.

(**b**) *BLEB supplements.*—Acriflavine (10 mg/L); sodium nalidixate (40 mg/L); and cycloheximide (50 mg/L). p/n 1.11781; EMD Chemicals, Inc. (San Diego, CA).

(**c**) *Dey-Engley (D/E) neutralizing broth.*—p/n 281910; Becton, Dickinson, and Co. (Sparks, MD).

## Safety Precautions

Lysis buffer and magnetic particles contain guanidine thiocyanate. Proteinase K buffer contains guanidine hydrochloride. Contact with acids or bleach liberates toxic gases. Harmful if inhaled, absorbed through the skin, or swallowed. Causes eye, skin, and respiratory tract irritation. Read the Material Safety Data Sheet (MSDS), and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Rapid Spin lysis buffer (Applied Biosystems, Foster City, CA) is not considered hazardous according to 29 CFR 1910.1200 (Occupational Safety and Health Administration Hazard Communication Standard). As a precaution, wear personal protective equipment. Avoid contact with skin, eyes, and clothing.

The reagents used in the MicroSEQ *Listeria* spp. Detection System pose no hazards when used as directed. Some wastes produced by the operation of the system are potentially hazardous. To minimize the hazards of chemical waste, read and understand the MSDS, minimize contact with chemicals, and wear appropriate protective equipment, such as safety glasses, gloves, or protective clothing, when handling chemicals.

Use the real-time PCR system as specified by Applied Biosystems. Using this instrument in a manner not specified by Applied Biosystems may result in personal injury or damage to the instrument. Moving parts can crush and cut. Keep hands clear of moving parts while operating the system. Disconnect power before servicing the instrument. Wear appropriate eyewear, clothing, and gloves at all times during operation.

*Listeria* Selective Enrichment Supplement contains cycloheximide and acriflavine hydrochloride. This material is considered hazardous, and may be fatal if swallowed. It causes severe eye irritation, irritation to the skin and respiratory tract, and may cause birth defects. Do not ingest. Avoid prolonged contact with eyes, skin, and clothing. Avoid breathing dust. Keep container closed. Use only with adequate ventilation, and wash thoroughly after handling. Read the MSDS and wear appropriate personal protective equipment. Disposal should be in accordance with applicable regional, national, and local laws and regulations.

Biological samples such as enrichments have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations on disposal of biological wastes. Wear appropriate protective equipment, including but not limited to protective eyewear, face shield, clothing/laboratory coat, and gloves. All work should be conducted in properly equipped facilities utilizing the appropriate safety equipment (for example, physical containment devices). Individuals should be trained in accordance with applicable regulatory and company/institution requirements before working with potentially infectious materials. All enrichment broths should be autoclaved following any culture-based confirmatory steps.

## General Preparation

(**a**) *PrepSEQ Nucleic Acid Extraction Kit.*—Add 30 mL 2-propanol to the empty bottle labeled "Binding Solution."

Add 74 mL 95% ethanol to the bottles labeled "Wash Buffer Concentrate."

Examine the magnetic particles for white precipitate. If white precipitate is present, incubate at 37°C for 10 min with intermittent mixing. Vortex the magnetic particles until well- dispersed.

(**b**) *PrepSEQ Rapid Spin Sample Preparation Kit.*—Set one heat block to 56°C and the other heat block to 95–100°C.

## Sample Preparation

(**a**) *Pre-enrichment.*—Add a 25 g sample to 225 mL BLEB, homogenize by stomaching, and incubate at 37 ± 1°C for 4 h. At the 4th h of incubation, add acriflavine (10 mg/L), sodium nalidixate (40 mg/L), and cycloheximide (50 mg/L) to the samples, then reincubate for a total of 24–28 h.

Use a cotton swab pre-wetted with 0.5 mL D/E neutralizing broth to wipe the 5 $cm^2$ (1 × 1″ in.) surface area to be tested. Add the swab to 10 mL BLEB and twirl in the broth for 60 s. Incubate the enrichment at 37 ± 1°C for 4 h. At the 4th h of incubation, add acriflavine (10 mg/L), sodium nalidixate (40 mg/L), and cycloheximide (50 mg/L) to the environmental sample and reincubate for a total of 28–32 h.

(**b**) *PrepSEQ Nucleic Acid Extraction Kit.*—Remove 1 mL

**Table 3. Summary of the methods comparison results[a]**

| | Deli turkey | | Hot dogs | | Liver paté | | Fermented sausage | | Deli ham | | Stainless steel | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Inoculation level | MFHPB-30 | MicroSEQ | MFHPB-30 | MicroSEQ | MFHPB-30 | MicroSEQ | MFHPB-30 | MicroSEQ | MFHPB-30 | MicroSEQ | MFHPB-30 | MicroSEQ |
| Low | 13/20 | 20/20 | 9/20 | 9/20 | 10/20 | 10/20 | 11/20 | 10/20 | 13/20 | 16/20 | 11/20 | 8/20 |
| High | 20/20 | 20/20 | 14/20 | 9/20 | 15/20 | 13/20 | 17/20 | 18/20 | 16/20 | 18/20 | 13/20 | 16/20 |
| Control | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 |

[a] Results are confirmed positive.

aliquot of each enriched sample and centrifuge for 3 min in a 1.5 mL microcentrifuge tube. After removing the supernatant liquid, resuspend the pellet in 200 µL proteinase K buffer by pipetting up and down and/or vortexing. Then, add 10 µL proteinase K to the sample and mix to disperse the proteinase K. Transfer the sample to a 96-well deep well plate to be loaded onto the MagMAX Express-96 deep well magnetic particle processor (MME). Switch on the MME instrument and select the program "44000799DWPrepSEQGP."

Prepare the elution plate using a 96-well standard plate by adding 140 µL elution buffer to each well position that contains sample in the lysis plate. Similarly, prepare two identical wash plates using two 96-well deep well plates by adding 300 µL wash buffer to each well position that contains sample in the lysis plate. Prepare the tip comb plate by placing the tip comb into a 96-well standard plate. Load the tip-comb plate, elution plate, two wash plates, and lysis plate as prompted by the MME instrument, and start the run by pressing the Start button.

After 20 min, the MME instrument will pause. Remove the lysis plate and add 300 µL lysis buffer to each well containing sample. Return the lysis plate to the MME instrument and resume the run by pressing the Start button. After 18 min, the MME instrument will pause. Remove the lysis plate and add 30 µL magnetic particles, followed by 300 µL binding solution to each well containing sample. Return the lysis plate to the MME instrument and start the run by pressing the Start button. After 30 min, the MME instrument will complete the run. Recover the elution plate containing the extracted DNA/RNA samples from the instrument and seal with clear adhesive film. The samples can be stored at 4°C for up to 1 week, or at –20°C for long term storage.

(**c**) *PrepSEQ Rapid Spin Sample Preparation Kit.*—Place a clarification column into a 1.5 mL microcentrifuge tube for each sample to be tested. Remove a 0.75 mL aliquot of enriched sample and load onto the clarification column and centrifuge for 3 min at 16 000 × *g*. Discard the spent clarification column, aspirate, and discard the supernatant liquid, leaving behind a bacterial pellet mixed with a carrier matrix material. Care must be taken to remove all residual liquid from the tube. Resuspend the bacterial pellet in 55 µL of a Rapid Spin Lysis Buffer-PK mixture (made by combining 50 µL Rapid Spin Lysis Buffer with 5 µL 20 mg/mL proteinase K) by pipetting up and down, or by vortexing. For samples with high fat content, immediately transfer the resuspended pellet to a clean microcentrifuge tube before the next step (*PrepSEQ Rapid Spin Extra-Clean protocol*). Place the tube into a heating block set at 56°C for 30 min, then transfer to a second heating block set at 95–100°C for 10 min. Allow the tube to cool at ambient temperature for 1 min, then centrifuge for 1 min to remove condensation from the top of the tube. Add 250 µL water to each sample. Vortex the tube briefly, then centrifuge for 1 min to separate particulate materials from the aqueous phase that contains the bacterial DNA. The samples can be stored at 4°C for up to 72 h, or at –20°C for long-term storage.

## Analysis

(**a**) *Prepare equipment*.—Turn on power to the real-time PCR instrument, followed by the power to the instrument computer; then launch RapidFinder Express Software by double-clicking the RapidFinder Express Software icon on the

**Table 4. MicroSEQ *Listeria monocytogenes*, presumptive versus confirmed—POD**

| | | | | MicroSEQ *Listeria* spp. Presumptive | | | MicroSEQ *Listeria* spp. Confirmed | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Matrix | Strain | MPN/25 g[a] | *N*[b] | x[c] | $POD_{CP}$[d] | 95% CI | x | $POD_{CC}$[e] | 95% CI | $dPOD_{CP}$[f] | 95% CI[g] |
| Liver paté | *L. monocytogenes* 1/2a | <0.075 | 5 | 0 | 0 | (0.00, 0.43) | 0 | 0 | (0.00, 0.43) | 0 | (–0.43, 0.43) |
| | | 0.693 (0.357–1.204) | 20 | 10 | 0.5 | (0.30, 0.70) | 10 | 0.5 | (0.30, 0.70) | 0 | (–0.28, 0.28) |
| | | 1.386 (0.799–2.2302) | 20 | 13 | 0.65 | (0.43, 0.82) | 13 | 0.65 | (0.43, 0.82) | 0 | (–0.28, 0.28) |
| Hot dogs | *L. monocytogenes* 1/2b plus 10× *L. innocua* 4ab | <0.075 | 5 | 0 | 0 | (0.00, 0.43) | 0 | 0 | (0.00, 0.43) | 0 | (–0.43, 0.43) |
| | | 0.598 (0.288–1.050) | 20 | 9 | 0.45 | (0.26, 0.66) | 9 | 0.45 | (0.26, 0.66) | 0 | (–0.28, 0.28) |
| | | 1.21 (0.693–2.302) | 20 | 9 | 0.45 | (0.26, 0.66) | 9 | 0.45 | (0.26, 0.66) | 0 | (–0.28, 0.28) |
| Raw fermented sausage | *L. monocytogenes* 3b | <0.075 | 5 | 0 | 0 | (0.00, 0.43) | 0 | 0 | (0.00, 0.43) | 0 | (–0.43, 0.43) |
| | | 0.7975 (0.431–1.386) | 20 | 10 | 0.5 | (0.30, 0.70) | 10 | 0.5 | (0.30, 0.70) | 0 | (–0.28, 0.28) |
| | | 1.8975 (1.095–3.288) | 20 | 18 | 0.9 | (0.70, 0.97) | 18 | 0.9 | (0.70, 0.97) | 0 | (–0.21, 0.21) |
| Sliced deli turkey | *L. monocytogenes* 3c | <0.075 | 5 | 0 | 0 | (0.00, 0.43) | 0 | 0 | (0.00, 0.43) | 0 | (–0.43, 0.43) |
| | | 0.598 (0.288–1.050) | 20 | 20 | 1 | (0.84, 1.00) | 20 | 1 | (0.84, 1.00) | 0 | (–1.0, 1.0) |
| | | 1.610 (0.932–2.775) | 20 | 20 | 1 | (0.84, 1.00) | 20 | 1 | (0.84, 1.00) | 0 | (–1.0, 1.0) |
| Sliced deli ham | *L. monocytogenes* 4b | <0.075 | 5 | 0 | 0 | (0.00, 0.43) | 0 | 0 | (0.00, 0.43) | 0 | (–0.43, 0.43) |
| | | 1.050 (0.598–1.897) | 20 | 16 | 0.8 | (0.58, 0.92) | 16 | 0.8 | (0.58, 0.92) | 0 | (–0.25, 0.25) |
| | | 1.610 (0.932–2.775) | 20 | 18 | 0.9 | (0.70, 0.97) | 18 | 0.9 | (0.70, 0.97) | 0 | (–0.21, 0.21) |
| Stainless steel | *L. monocytogenes* 4d plus 10× *E. faecalis* | NA[h] | 5 | 0 | 0 | (0.00, 0.43) | 0 | 0 | (0.00, 0.43) | 0 | (–0.43, 0.43) |
| | | NA | 20 | 8 | 0.4 | (0.22, 0.61) | 8 | 0.4 | (0.22, 0.61) | 0 | (–0.28, 0.28) |
| | | NA | 20 | 16 | 0.8 | (0.58, 0.92) | 16 | 0.8 | (0.58, 0.92) | 0 | (–0.25, 0.25) |

[a] MPN = Most probable number, based on the POD of reference method test portions across laboratories using the AOAC MPN calculator, with 95% confidence interval.

[b] N = Number of test portions.

[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] NA = Not applicable.

Windows desktop. The RapidFinder Main Page will open and provide you with complete guidance during plate setup, plate run, and data analysis.

(**b**) *Plate setup*.—

*Create a run file*.—On the Main Page of RapidFinder Express software, click "Create/Edit a Run File." Click "Next" or "Back" to scroll through pages in the Run File, or "Close" to return to the Main Page. In Run File, follow the directions to name the run file, to specify the number of samples, replicates and controls, and to enter individual sample names. After the Run File is completed, select "Next" to open the Pipet Samples section, or close to return to the Main Page. The Pipet Samples section can also be opened from the Main Page.

*Pipet samples*.—On the Main Page of RapidFinder Express software click "Pipet Samples." Select a Run File, then select "Next." Choose an option to print the pipetting procedure, display the online pipetting procedure, or to display the run layout. The RapidFinder Express software determines the location for each sample to be loaded into the 7500 Fast real-time PCR system, and may include blank tubes to balance tubes across the instrument block. Follow the directions on each page to pipet samples, negative controls, and positive controls, then click "Next" to continue. After the Pipet Samples section is completed, select "Next" to open the Start Instrument Run section, or "Close" to return to the Main Page. The Start Instrument Run section can also be opened from the Main Page.

(**c**) *Run a plate*.—Load the eight-strip tubes into the instrument as shown in the run layout. On the Main Page of RapidFinder Express software click "Start Instrument Run." Select a Run File, then select "Next." Click "Start" on the Start Instrument Run page. When the run is completed, click "Close," and remove the eight-strip tubes.

## Interpretation and Test Result Report

On the Main Page of RapidFinder Express software click "View Results." Select a run file to view. RapidFinder Express

**Table 5. Method comparison results**

| | | | | MicroSEQ *Listeria* spp. Method | | | MFHPB-30 Reference Method | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Matrix | Strain | MPN/25 g[a] | N[b] | x[c] | $POD_C$[d] | 95% CI | x | $POD_R$[e] | 95% CI | $dPOD_C$[f] | 95% CI[g] |
| Liver paté | *L. monocytogenes* 1/2a | <0.075 | 5 | 0 | 0 | (0.00, 0.43) | 0 | 0 | (0.00, 0.43) | 0 | (–0.43, 0.43) |
| | | 0.693 (0.357–1.204) | 20 | 10 | 0.5 | (0.30, 0.70) | 10 | 0.5 | (0.30, 0.70) | 0 | (–0.28, 0.28) |
| | | 1.386 (0.799–2.2302) | 20 | 13 | 0.65 | (0.43, 0.82) | 15 | 0.75 | (0.53, 0.89) | 0.10 | (–0.16, 0.38) |
| Hot dogs | *L. monocytogenes* 1/2b plus 10× *L. innocua* 4ab | <0.075 | 5 | 0 | 0 | (0.00, 0.43) | 0 | 0 | (0.00, 0.43) | 0 | (–0.43, 0.43) |
| | | 0.598 (0.288–1.050) | 20 | 9 | 0.45 | (0.26, 0.66) | 9 | 0.45 | (0.26, 0.66) | 0 | (–0.28, 0.28) |
| | | 1.21 (0.693–2.302) | 20 | 9 | 0.45 | (0.26, 0.66) | 14 | 0.7 | (0.48, 0.85) | 0.25 | (0.01, 0.55) |
| Raw fermented sausage | *L. monocytogenes* 3b | <0.075 | 5 | 0 | 0 | (0.00, 0.43) | 0 | 0 | (0.00, 0.43) | 0 | (–0.43, 0.43) |
| | | 0.7975 (0.431–1.386) | 20 | 10 | 0.5 | (0.30, 0.70) | 11 | 0.55 | (0.34, 0.74) | 0.05 | (–0.23, 0.34) |
| | | 1.8975 (1.095–3.288) | 20 | 18 | 0.9 | (0.70, 0.97) | 17 | 0.85 | (0.64, 0.95) | 0.05 | (–0.17, 0.27) |
| Sliced deli turkey | *L. monocytogenes* 3c | <0.075 | 5 | 0 | 0 | (0.00, 0.43) | 0 | 0 | (0.00, 0.43) | 0 | (–0.43, 0.43) |
| | | 0.598 (0.288–1.050) | 20 | 20 | 1 | (0.84, 1.00) | 13 | 0.65 | (0.43, 0.82) | 0.35 | (0.12, 0.57) |
| | | 1.610 (0.932–2.775) | 20 | 20 | 1 | (0.84, 1.00) | 20 | 1 | (0.84, 1.00) | 0 | (–1.0, 1.0) |
| Sliced deli ham | *L. monocytogenes* 4b | <0.075 | 5 | 0 | 0 | (0.00, 0.43) | 0 | 0 | (0.00, 0.43) | 0 | (–0.43, 0.43) |
| | | 1.050 (0.598–1.897) | 20 | 16 | 0.8 | (0.58, 0.92) | 13 | 0.65 | (0.43, 0.82) | 0.15 | (–0.13, 0.40) |
| | | 1.610 (0.932–2.775) | 20 | 18 | 0.9 | (0.70, 0.97) | 16 | 0.8 | (0.58, 0.92) | 0.10 | (–0.13, 0.33) |
| Stainless steel | *L. monocytogenes* 4d plus 10× *E. faecalis* | NA[h] | 5 | 0 | 0 | (0.00, 0.43) | 0 | 0 | (0.00, 0.43) | 0 | (–0.43, 0.43) |
| | | NA | 20 | 8 | 0.4 | (0.22, 0.61) | 11 | 0.55 | (0.34, 0.74) | 0.15 | (–0.11, 0.45) |
| | | NA | 20 | 16 | 0.8 | (0.58, 0.92) | 13 | 0.65 | (0.43, 0.82) | 0.15 | (–0.13, 0.40) |

[a] MPN = Most probable number, based on the POD of reference method test portions across laboratories using the AOAC MPN calculator, with 95% confidence interval.

[b] N = Number of test portions.

[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 and reference method POD values.

[g] 95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

[h] NA = Not applicable.

software displays the results for each sample by location (Plate View) or as a table (Table View).

In Plate View, RapidFinder Express software uses a simple system of different color coded symbols to display the result for each sample, differentiating between samples and controls (Figure 1).

In the Table View, RapidFinder Express software provides a list of results and assessment text for each sample. Click "Help" for a description of assessment text and suggested actions for any warning results. Real-time PCR amplification plots can also be reviewed by clicking "View in SDS."

On the View Results page of RapidFinder Express software, click the "Report" tab. Choose from the list of options to include in the report and then click "Print Results Report," or export the report by clicking on "Export Results Report."

## Confirmation

Appropriate confirmation procedures are described in MFHPB-30, http://www.hc-sc.gc.ca/fn-an/alt_formats/hpfb-dgpsa/pdf/res-rech/mfhpb30-eng.pdf.

## Validation Study

This validation study was conducted under the AOAC Research Institute GovVal program. The study was designed to compare previously AOAC-approved rapid methods to the Health Protection Branch MFHPB-30 reference method for the detection of *L. monocytogenes* in RTE meats and *Listeria* species on stainless steel surfaces. The specific matrixes evaluated were liver paté, hot dogs, raw fermented sausage, sliced deli turkey, sliced deli ham, and stainless steel. The study included 20 replicate test portions, each at two contamination levels, where fractionally positive results (5–15 positive results per 20 replicate portions tested) were obtained by at

**Table 6. Method comparison results—Chi–square, unpaired test portions**

| Matrix | Strain | MPN/25 g[a] | N[b] | MicroSEQ *Listeria* spp. Method | | MFHPB-30 Method | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Presumptive positive | Confirmed positive | Positive | Chi–square[c] | Relative sensitivity[d] |
| Liver paté | *L. monocytogenes* 1/2a | <0.075 | 5 | 0 | 0 | 0 | — | — |
| | | 0.693 (0.357–1.204) | 20 | 10 | 10 | 10 | 0 | 1.00 |
| | | 1.386 (0.799–2.2302) | 20 | 13 | 13 | 15 | 0.46 | 0.87 |
| Hot dogs | *L. monocytogenes* 1/2b plus 10× *L. innocua* 4ab | <0.075 | 5 | 0 | 0 | 0 | — | — |
| | | 0.598 (0.288–1.050) | 20 | 9 | 9 | 9 | 0 | 1.00 |
| | | 1.21 (0.693–2.302) | 20 | 9 | 9 | 14 | 2.49 | 0.64 |
| Raw fermented sausage | *L. monocytogenes* 3b | <0.075 | 5 | 0 | 0 | 0 | — | — |
| | | 0.7975 (0.431–1.386) | 20 | 10 | 10 | 11 | 0.10 | 0.91 |
| | | 1.8975 (1.095–3.288) | 20 | 18 | 18 | 17 | 0.22 | 1.06 |
| Sliced deli turkey | *L. monocytogenes* 3c | <0.075 | 5 | 0 | 0 | 0 | — | — |
| | | 0.598 (0.288–1.050) | 20 | 20 | 20 | 13 | 8.27 | 1.54 |
| | | 1.610 (0.932–2.775) | 20 | 20 | 20 | 20 | 0 | 1.00 |
| Sliced deli ham | *L. monocytogenes* 4b | <0.075 | 5 | 0 | 0 | 0 | — | — |
| | | 1.050 (0.598–1.897) | 20 | 16 | 16 | 13 | 1.10 | 1.23 |
| | | 1.610 (0.932–2.775) | 20 | 18 | 18 | 16 | 0.76 | 1.12 |
| Stainless steel | *L. monocytogenes* 4d plus 10× *E. faecalis* | NA[e] | 5 | 0 | 0 | 0 | — | — |
| | | NA | 20 | 8 | 8 | 11 | 0.88 | 0.73 |
| | | NA | 20 | 16 | 16 | 13 | 1.10 | 1.23 |

[a] MPN = Most probable number, based on the POD of reference method test portions across laboratories using the AOAC MPN calculator, with 95% confidence interval.

[b] N = Number of test portions.

[c] Chi–square = Mantel–Haenszel: $\chi^2 = (n - 1)(ad - bc)^2/[(a + b)(a + c)(b + d)(c + d)]$, where $n$ = total number of samples tested by the two methods, a = number of samples positive by the test method, b = number of samples negative by the test method, c = number of samples positive by the reference method, and d = number of samples negative by the reference method.

[d] Relative sensitivity = a/c, where a = number of samples confirmed positive by the test method and c = number of samples positive by the reference method.

[e] NA = Not applicable.

least one method at one level. Five uncontaminated controls were included per method. Replicates were taken from a singularly inoculated lot of material. A different serotype of *L. monocytogenes* was used for each matrix. All samples were prepared, packaged into test portions, blind-coded, and shipped to the method developers' laboratory by Q Laboratories. In addition, Q Laboratories performed reference method analyses on all matrixes.

### *Methodology*

All foods were prescreened using MFHPB-30 to ensure that no naturally contaminating *L. monocytogenes* was present. An aerobic plate count was conducted to evaluate the level of background flora present in each product (Table 1).

Strains of *L. monocytogenes*, *L. innocua*, and *E. faecalis* were cultured by inoculating Brain Heart Infusion (BHI) broth and incubating at 37 ± 2°C for 24 h. Following incubation, the strains to be used for inoculating the food matrixes were heat-stressed by incubating at 55°C for 10 min in a water bath to achieve 50–80% injury. The degree of injury of the culture was estimated by plating an aliquot of diluted culture onto OXA and BHI agars. The agars were incubated at 37°C for 24 h, and the colonies were counted. The degree of injury was estimated as:

$$(1-\frac{n_{select}}{n_{nonselect}})\times 100$$

where $n_{select}$ = number of CFUs on selective agar, and $n_{nonselect}$ = number of CFUs on nonselective agar. The resulting estimated injury for each strain is shown in Table 2.

Each bulk food matrix was divided into three subsamples: one was set aside as an uninoculated control; one was inoculated with the heat-stressed *L. monocytogenes* culture at a target concentration of 0.2–2 CFU/25 g; and one was inoculated with the heat-stressed *L. monocytogenes* culture at a target concentration of 2–5 CFU/25 g. Hot dogs were inoculated with heat-stressed *L. innocua* as the competitor at 10 times the concentration of *L. monocytogenes*. Following inoculation, the subsamples were stabilized at 4°C for approximately 48 h during shipping to ensure that all samples were received by Monday of the testing week. Test portions (30 g each) were

packaged from each subsample. Each sample set consisted of five replicate test portions of uninoculated material, 20 replicate test portions of low-level inoculated material, and 20 replicate test portions of high-level inoculated material. The test portions were randomized, blind-coded, and shipped refrigerated to participants via overnight courier. All test portions were packaged and shipped according to International Air Transport Association (IATA) Category B Biological Substances guidelines.

Stainless steel surface pieces were treated with ethanol, rinsed with distilled water, and allowed to dry. *L. monocytogenes* 4d and *E. faecalis* were cultured in BHI at 37°C for 20 h. Cultures were diluted and mixed together in 10% nonfat dry milk such that the *Listeria* was in a concentration range expected to yield fractional positives, and the *Enterococcus* was at a concentration 10 times higher than the *Listeria.* A second surface was prepared and inoculated at twice the expected fractional level. The uninoculated surface areas received 10% nonfat milk only. Surfaces were dried at room temperature for 16–24 h. Swabs were pre-moistened with D/E neutralizing broth before use. For each sample set, 20 replicate test areas, $1 \times 1''$ each, were swabbed per inoculation level, plus five replicate uninoculated test areas. The swabs were randomized, blind-coded, and shipped refrigerated to participants via overnight courier. All test portions were packaged and shipped according to IATA Category B Biological Substances guidelines.

Upon receipt, the temperatures of the samples were checked, recorded on the Sample Receipt Submission form provided, and sent to the independent laboratory for verification. Sample sets were stored at 2–8°C upon arrival until the time of analysis. Analyses began approximately 72 h after inoculation of the matrix. The MicroSEQ *Listeria* spp. method and the reference method were carried out as written. In the case of unpaired test portions, all MicroSEQ *Listeria* spp. enrichments, regardless of presumptive results, were struck to selective agar for confirmation of typical colonies as described in the MFHPB-30 method.

The most probable number (MPN) was estimated for each level by the independent laboratory and was calculated based on the POD of the reference method across all laboratories using the AOAC MPN calculator (7). Enrichments, isolation, and confirmations were carried out according to the MFHPB-30 reference method.

## Results

The MicroSEQ *Listeria* species method gave identical results for all six matrixes evaluated in this study when the samples were prepared using either the automated PrepSEQ Nucleic Acid Extraction Kit or the manual PrepSEQ Rapid Spin Sample Preparation Kit. Therefore, the results are described collectively as the MicroSEQ *Listeria* species method. A background screen of each matrix showed no natural contamination of the target organisms. All sample matrixes were fractionally positive (5–15 positives out of 20 replicates) by one or both of the test or reference methods for at least one inoculum level. A summary of the results is presented in Table 3, and detailed analyses are shown Tables 4–6.

### *Deli Turkey*

For deli turkey, there were 20 confirmed positives for the low-level inoculum (0.2–2 CFU/25 g) and 20 confirmed positives for the high-level inoculum (2–5 CFU/25 g) for the MicroSEQ *Listeria monocytogenes* method. For the Health Canada MFHPB-30 reference method, there were 13 confirmed positives for the low-level inoculum and 20 for the high-level inoculum. All uninoculated samples were negative for both methods. There was no significant difference for the high inoculum level between the MicroSEQ *Listeria monocytogenes* method and the Health Canada MFHPB-30 reference method. There was a statistically significant difference in the number of positive results detected by the MicroSEQ method and the Health Canada MFHPB-30 reference method for the low inoculation level, with the MicroSEQ method detecting more positives.

### *Hot Dogs*

For hot dogs, there were nine confirmed positives for the low-level inoculum (0.2–2 CFU/25 g) and nine confirmed positives for the high-level inoculum (2–5 CFU/25 g) for the MicroSEQ *Listeria monocytogenes* method. For the Health Canada MFHPB-30 reference method, there were eight confirmed positives for the low-level inoculum and 14 for the high-level inoculum. All uninoculated samples were negative for both methods. The Mantel-Haenszel Chi-square analysis between the two methods indicated no significant difference for both inoculum levels.

### *Liver Paté*

For liver paté, there were 10 confirmed positives for the low-level inoculum (0.2–2 CFU/25 g) and 15 confirmed positives for the high-level inoculum (2–5 CFU/25 g) for the MicroSEQ *Listeria monocytogenes* method. For the Health Canada MFHPB-30 reference method, there were 10 confirmed positives for the low-level inoculum and 13 for the high-level inoculum. All uninoculated samples were negative for both methods. There was no significant difference for both inoculum levels between the MicroSEQ *Listeria monocytogenes* method and the Health Canada MFHPB-30 reference method.

### *Raw Fermented Sausage*

For raw fermented sausage, there were 10 confirmed positives for the low-level inoculum (0.2–2 CFU/25 g) and 18 confirmed positives for the high-level inoculum (2–5 CFU/25 g) for the MicroSEQ *Listeria monocytogenes* method. For the Health Canada MFHPB-30 reference method, there were 11 confirmed positives for the low-level inoculum and 17 for the high-level inoculum. All uninoculated samples were negative for both methods. There was no significant difference for both inoculum levels between the MicroSEQ *Listeria monocytogenes* method and the Health Canada MFHPB-30 reference method.

### *Deli Ham*

For deli ham, there were 16 confirmed positives for the low-level inoculum (0.2–2 CFU/25 g) and 18 confirmed positives for the high-level inoculum (2–5 CFU/25 g) for the MicroSEQ *Listeria monocytogenes* method. For the Health Canada MFHPB-30 reference method, there were 13 confirmed positives for the low-level inoculum and 16 for the high-level inoculum. All uninoculated samples were negative for both methods. There was no significant difference for both inoculum levels between the MicroSEQ *Listeria monocytogenes* method and the Health Canada MFHPB-30 reference method.

### *Stainless Steel*

For stainless steel, there were eight confirmed positives for the low-level inoculum (0.2–2 CFU/5 $cm^2$) and 14 confirmed positives for the high-level inoculum (2–5 CFU/5 $cm^2$) for the MicroSEQ *Listeria monocytogenes* method. For the Health Canada MFHPB-30 reference method, there were 11 confirmed positives for the low-level inoculum and 13 for the high-level inoculum. All uninoculated samples were negative for both methods. There was no significant difference for both inoculum levels between the MicroSEQ *Listeria monocytogenes* method and the Health Canada MFHPB-30 reference method.

## Discussion

Two sample preparation methods were used with the MicroSEQ *Listeria monocytogenes* Detection Kit, the automated PrepSEQ Nucleic Acid Extraction Kit and the manual PrepSEQ Rapid Spin Sample Preparation Kit. Both methods gave the exact same results for all six matrixes evaluated. The results from the MicroSEQ method for the five food matrixes, and the environmental surface were confirmed by culture, resulting in no false-negative or false-positive results. A Mantel-Haenszel Chi-square analysis (for unmatched test portions) between the MicroSEQ method and the reference method indicated that there was no significant difference between the number of positive results given by the two methods being compared for deli ham, hot dogs, liver paté, fermented sausage, and the stainless steel environmental surface. Chi-square analysis for deli turkey did show a significant difference between the reference and candidate method for the low-level inoculum, with the candidate method detecting more positives. These samples were confirmed by culture, indicating they were true positives. The cause for increased detection of *Listeria* in deli turkey with the candidate method compared to the reference method could be due to sample variations because this was an unpaired study.

## Conclusions

The MicroSEQ *Listeria monocytogenes* method is a rapid method for the detection of *L. monocytogenes* in food and environmental surfaces, providing results within 2 to 3 h after enrichment. Due to the high selectivity of real-time PCR, the MicroSEQ method shows equivalence to culture reference methods with shorter enrichment times: 24–28 h for food matrixes and 28–32 h for environmental surfaces. The data in these studies, within their statistical uncertainty, support the product claims of the MicroSEQ *Listeria* spp. Detection Kit in the food matrixes and environmental surfaces tested.

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