OXFORD

Journal of AOAC INTERNATIONAL, 2020, 1–10

doi: 10.1093/jaocint/qsz021
Article

MICROBIOLOGICAL METHODS

Evaluation of the CERTUS Environmental *Listeria* Species Detection Kit for the Detection of *Listeria* Species on Environmental Surfaces: AOAC *Performance Tested Method*^{SM} 101802

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Abstract

Background: CERTUS Environmental *Listeria* species Detection Kit (CERTUS EL Detection Kit) is a real-time, bio-contained assay designed to accurately detect *Listeria* species (*L. grayi*, *L. innocua*, *L. ivanovii*, *L. marthii*, *L. monocytogenes*, *L. seeligeri*, and *L. welshimeri*) from environmental surface matrixes using an antibody-coupled magnetic microparticle with a Surface Enhanced Raman Spectroscopy (SERS) nanoparticle technology test system paired with proprietary CERTUS EL Selective Growth Media and CERTUS Detection Unit.
Objective: The method was evaluated for AOAC® *Performance Tested Method*^{SM} certification.
Methods: Inclusivity and exclusivity, matrix studies, product consistency and stability were conducted to evaluate the CERTUS EL Detection Kit.
Results: In the matrix studies, stainless steel, ceramic tile, plastic (polystyrene) and sealed concrete environmental surfaces (4 × 4" test areas) were tested. No statistically significant differences were found by Probability of Detection analysis (POD) in any of the matrixes when results were compared to the U.S. Food and Drug Administration cultural microbiology reference method for *Listeria*. The CERTUS EL Detection Kit correctly identified all 50 target *Listeria* isolates and correctly excluded all 30 non-target strains that were analyzed. Probability of Detection analysis of CERTUS EL Detection Kit robustness, product consistency (lot-to-lot) and stability studies demonstrated no statistically significant differences, and no variation was observed between instruments.
Conclusions: The data collected in these studies demonstrate that the CERTUS EL Detection Kit is a reliable method for the rapid and specific detection of *Listeria* from stainless steel, ceramic tile, plastic (polystyrene) and sealed concrete environmental surfaces.

Received: 29 October 2019; **Accepted:** 30 October 2019

Scope of Method

(a) *Target organisms*.—*Listeria* species (*L. monocytogenes, L. innocua, L. ivanovii, L. seeligeri, L. welshimeri, L. grayi and L. marthii*).
(b) *Matrixes*.—Stainless steel (18 gauge: 304 food grade with a brushed finish), ceramic tile, plastic (polystyrene) and sealed concrete environmental surfaces (4 × 4" test areas).
(c) *Summary of Validated Performance Claims*.—Performance is equivalent to that of the U.S. Food and Drug Administration *Bacteriological Analytical Manual* (FDA/BAM) Chapter 10 (2017) *Detection of Listeria monocytogenes in Foods and Environmental Samples, and Enumeration of Listeria monocytogenes in Foods* (1) for detection of *Listeria* spp. from selected environmental surfaces.

Definitions

(a) *Probability of Detection*.—Probability of Detection (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.
(b) *Difference of Probabilities of Detection*.—Difference of Probabilities of Detection (dPOD) is the difference between any two POD values. If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

Principle

The CERTUS Environmental *Listeria* species Detection Kit (CERTUS EL Detection Kit) is a homogenous no-wash, real-time sandwich immunoassay. Coupled with proprietary biocontainment (BIO-LOCK™ Sampling Swab and Detection Tube that ensures containment of enriched samples) and CERTUS EL Selective Growth Media, the kit is reliable for the rapid and specific detection of *Listeria* species from environmental swab samples (3). The CERTUS EL Detection Kit relies on antibodies covalently bound to magnetic microparticles and Surface Enhanced Raman Spectroscopy (SERS) nanoparticles that are highly specific to *Listeria* antigens.

Listeria-specific antigens present in the environmental swab samples will bind immunologically to the two antibody-coupled particles forming a complex. The immunological complex is pulled to the side of the Detection Tube by applying an external magnet and interrogated with a laser for the detection of the complexed SERS nanoparticles. The signal generated by the complexed SERS nanoparticle is measured, where its intensity is proportional to the concentration of *Listeria* present in the bio-locked Detection Tube (4).

This process is cycled repeatedly with the raw optical signal automatically interpreted through a proprietary algorithm, where a "Green" result shown on the detection unit and control pad is considered to be negative for the target pathogen and a "Red" result is presumptive positive for the target pathogen.

General Information

Listeria is a Gram-positive, rod-shaped, aerobic (or facultatively anaerobic), non-spore forming bacterium associated with a variety of environments including soils, water, sewage, and silage, as well as plant and animal food products. *Listeria* is unlike many other bacteria because it can grow at refrigeration temperatures. *Listeria* is also transmitted to the consumer mainly by contaminated ready-to-eat foods. The presence and potential persistence of *Listeria* species in food processing facilities are often caused by environmental recontamination at the farm or plant level.

Listeriosis, a serious infection usually caused by eating food contaminated with the bacterium *Listeria monocytogenes*, and is an important public health problem in the United States. The disease primarily affects older adults, pregnant women, newborns and adults with weakened immune systems; which may lead to meningitis, septicemia, encephalitis, fetal loss and death. However, rarely, persons without these risk factors can also be affected. The risk may be reduced by following recommendations for safe food preparation, consumption and storage (4).

Materials and Methods

Test Kit Information

(a) *Kit Name*.—CERTUS EL Detection Kit—60 ct.
(b) *Kit Catalog Number*.—LIST017.
(c) *Ordering Information*.—CERTUS Food Safety, 4809 N. Ravenswood Ave. #113, Chicago, IL 60640. Call: 872.810.4123, Email: orders@certusfoodsafety.com.

Test Kit Components

(a) *CERTUS EL Selective Growth Media, CAT No. List007*.—Quantity of 60 vials per box in working dilution. Store at 2–8°C.
(b) CERTUS EL *BIO-LOCK™ Sampling Swab, CAT No. List003*.—Quantity of 60 swabs per box. Store at room temperature (15–25°C).
(c) CERTUS EL *Detection Tube, CAT No. List004*.—Quantity of 60 tubes per box. Store at room temperature (15–25°C).
(d) CERTUS EL *Positive Control Cap, CAT No. List005*.—Quantity of 1 cap per box. Store at room temperature (15–25°C).
(e) CERTUS EL *Negative Control Cap, CAT No. List006*.—Quantity of 1 cap per box. Store at room temperature (15–25°C).
(f) *Product Instructions*.

Additional supplies and reagents

(a) *CERTUS Detection Unit*.
(b) *Control pad with software for connection to the CERTUS Detection Unit*.

Apparatus

(a) *Exam grade disposable gloves (powder free)*
(b) *Refrigerator*.—Capable of maintaining 2–8°C for storage of pre-measured growth media tubes.
(c) *Test tube rack*.—Capable of holding 25 mm tubes.
(d) *Safety glasses*.

Reference Materials

Organisms used in the method comparison, instrument validation, robustness and stability (lot-to-lot) studies were obtained from the following sources:

(a) *American Type Culture Collection (ATCC)*.—Manassas, VA, USA. www.atcc.org.
(b) *The National Collection of Type Cultures (NCTC)*.—Salisbury, UK. http://www.phe-culturecollections.org.uk/collections/nctc.isp.
(c) *University of Vermont Culture Collection (CWD/LW)*.—Catherine Donnelly, Professor, University of Vermont, Nutrition and Food Sciences, 361 Carrigan Wing, 109 Carrigan Dr., Burlington, VT, USA.

(d) *Cornell University Culture Collection (FSL)*.—Martin Weidmann, Professor, Cornell University, Food Safety Laboratory, 412 Stocking Hall, Ithaca, NY, USA.
(e) *Q Laboratories, Inc. Culture Collection (QL)*.—Patrick Bird, Project Leader, Q Laboratories, Inc., Research and Development, Cincinnati, OH, USA.
(f) *Colorado State University Culture Collection (CSU)*.—Fort Collins, CO, USA.

Safety Precautions

CERTUS EL Detection Kit.—This product is not intended for human diagnostics, or prevention use. Working with post-enriched samples that may be potentially contaminated with *Listeria* should be performed in Biosafety Level 2 (BSL2) regulated laboratories. Do not open the Bio-contained Detection Tubes post enrichment and detection. All other CERTUS EL Detection Kit components are non-hazardous. However, product usage should follow good laboratory practices and all disposable materials must be discarded according to appropriate waste procedures used in the laboratory. To reduce the risk associated with cross- contamination, protective clothing should be worn including safety glasses, mask, and gloves where appropriate. Avoid contact with the skin. For safety information regarding the instrument, *see* the Instrument User Manual.

Enrichment.—*Listeria* is a Biosafety Level 2 organism. Biological samples such as enrichments have the potential to transmit infectious diseases. *L. monocytogenes* is of particular concern for pregnant women, the aged and the immunocompromised. Follow all applicable local, state/provincial, and/or national regulations on disposal of biological wastes. Wear appropriate protective equipment which includes but is 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 (e.g., physical contaminant 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 sterilized following any culture-based confirmatory steps through heat denaturation by autoclaving at 121°C for 15 min.

Sample Collection

(a) Identify the number and description of the sampling sites.
(b) Remove the required number of BIO-LOCK™ Sampling Swabs contained in its individual transport tube and place in suitable carrier. *Note*: The BIO-LOCK™ Sampling Swabs contained in its individual transport tube is sterile and the swab should not be removed until ready to collect a sample.
(c) Carry the container on to the floor to begin site sampling.
(d) Remove the BIO-LOCK™ Sampling Swab from the transport tube.
(e) Swab environmental surfaces using firm and even pressure vertically (approximately 10 times), then flip the sampler and use the other side to swab horizontally (approximately 10 times) and diagonally www.fda.gov/food/foodsciencere search/laboratorymethods.
(f) Place the swab back into its original transport tube.
(g) Record the sampling site location/site ID with a marker on the cap.
(h) Proceed to the next sampling location and repeat steps (d) to (g).
(i) Bring the samples back to the detection unit/sample prep area.

Sample Preparation

(a) *Note:* that samples are to be prepared/placed into the detection unit one at a time.
(b) Place the swabs/transport tubes in a tube rack (tube rack 1).
(c) Open the CERTUS EL Detection Kit and remove the corresponding number of Detection Tube pouches as the number of collected samples.
(d) Open one (1) Detection Tube pouch and remove the plastic cap (do not throw away the Detection Tube pouch. The sample ID number/barcode is required for traceability in the CERTUS App).
(e) Place the opened Detection Tube in a separate tube rack (tube rack 2).
(f) Open the CERTUS Media Kit and remove one (1) media vial.
(g) Pour the contents of the media vial into the opened Detection Tube located in rack 2.
(h) Take one (1) of the labeled caps with an affixed swab from the transport tube rack 1.
(i) Insert the swab into the Detection Tube with added media located in rack 2.
(j) Align the three small ridges of the tube with the holes of the cap.
(k) Lock the tube by pushing the BIO-LOCK™ cap until it clicks.
(l) Repeat steps (d) to (k) for the remaining samples.
(m) Place the fully assembled tubes back in rack 2 and proceed to the Test Site Sample Analysis steps below.

Assay Controls

(a) *Note:* that assay EL controls are required to be run with every new lot of Detection Tubes.
(b) Assay EL Control Standards Preparation.
(c) Open the CERTUS EL Detection Kit and remove two Detection Tube pouches.
(d) Open the removed Detection Tube pouches and remove the plastic caps.
(e) Place the opened Detection Tubes in a tube rack.
(f) Take the Control Standard Caps pouch from the same CERTUS EL Detection Kit used in *Sample Preparation* step (c).
(g) Open the pouch and remove the positive (red) and negative (green) Control Standard Caps (do not throw away the Control Standard's pouch. The barcode will be scanned later in the CERTUS App).
(h) Open the CERTUS Media Kit and take out two media vials.
(i) Open one (1) media vial and pour the contents into one (1) of the Detection Tubes previously placed in the rack.
(j) Take the positive control (red) cap and align the three ridges of the Detection Tubes with the holes on the side of the BIO-LOCK™ caps.
(k) Seal the tube by pushing the BIO-LOCK™ cap until it clicks.
(l) Repeat steps (i) to (k) for the negative control (green) caps.
(m) Place the tubes back in the rack and proceed to Controls and Standards Analysis steps below.

Controls and Standards Analysis

(a) Using the CERTUS Control Pad, set the Detection Unit mode to "*Listeria*".
(b) Once the status has reached the "Ready" mode, prepare to insert the control standard samples.

(c) Use the iPad to select "Positive control" as the sample type and insert the positive control tube in the slot.
(d) Likewise, select "Negative control" as the sample type and insert the negative control tube in the slot.
(e) During each insertion, confirm that both Positive and Negative tubes are inserted completely in the vial tray, with the cap's lip sitting on the catch feature of the tray.
(f) Press "Start" to begin the control sample run.
(g) Monitor the run on the iPad through the CERTUS App home screen. Ideally, the positive run should return a Red/Positive result and the negative run should return a Green/Negative result.
(h) If the control expected results are not returned, users must contact CERTUS customer service to request for a new *EL* Detection test kit.

Test Site Sample Analysis

(a) Using the CERTUS Control Pad, set the Detection Unit mode to "*Listeria*."
(b) Once the status has reached the "Ready" mode, prepare to insert the fully assembled test site samples.
(c) Use the iPad to select "Sample" as the sample type.
(d) Select the correct/corresponding sample ID number from the pouch in step (c) of the sample preparation instructions. The number on the cap should match the site location number selected.
(e) Input additional information in the text field (e.g., name of the technician collecting the sample, additional test/site identifiers, etc.).
(f) Repeat steps (c) to (e) to add the remainder of the collected test site samples.
(g) Prior to each insertion, wipe tubes with an alcohol wipe to ensure there is no media overpour present on the outside of the tubes as this may disrupt sample readability.
(h) During each insertion, confirm that all the sample tubes inserted completely in the vial tray, with the cap's lip sitting on the catch feature of the tray.
(i) Press "Start" to begin the test site sample run.
(j) Monitor the run on the iPad through the CERTUS App home screen. Each tube position's total elapsed analysis time will be displayed on the home screen. A positive test run will return a "Red" result and a negative run will return a "Green" result.
(k) In the case of a presumptive positive result, an automatic email notification will be sent by the instrument to the relevant stakeholders enrolled in the system.

Confirmation

All samples are identified as presumptively positive for *Listeria*. Once the BIO-LOCK™ cap is in place, the tubes are not designed to be re-opened to prevent potential contamination of the environment. However, if confirmation is desired, follow procedures described in the most recent version of the U.S. FDA Bacteriological Analytical Manual, Chapter 10.

To obtain a sample for confirmation the reference laboratory can cut the plastic tube using a standard pipe cutter, hot knife, or another device that can cut through the approximately 2 mm thick polypropylene tube. The following is a suggested protocol to ensure that the tube is opened in a safe manner and the sample is not compromised. *Note*: Centers for Disease Control guidelines for the handling of Biosafety Level 2 organisms should be followed whenever live cultures of *Listeria* are used. *Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition*.

(a) For each tube to be confirmed gently invert the tube two to three times to mix the sample then place upright into a test tube rack.
(b) Prior to opening the tube wipe down the tube and cap with an alcohol wipe.
(c) Depending on the device to be used, e.g., pipe cutter, ensure the device is properly sterilized before and after use.
(d) While wearing appropriate personal protective equipment including latex gloves remove the tube from the test tube rack and tightly hold it around the bottom two-thirds.
(e) Approximately 1 inch from the tube cap-interface slowly cut into the tube using the sterilized cutting device until the cap-swab assembly can be removed from the tube.
(f) Remove the cap-swab assembly and discard as potentially bio-hazardous material per laboratory site operational procedures and place the open tube back into the test tube rack.
(g) The volume remaining in the tube is approximately 15 mL.
(h) Remove the required amount of sample from the tube that will be needed to run the conformation protocol per laboratory site operational procedures.
(i) The open tube and contents can then be discarded as potentially bio-hazardous material per laboratory site operational procedures OR
(j) If the sample is to be retained, the contents of tube can be transferred into a sterile vial with an appropriate closure, labeled appropriately with ID and potentially bio-hazardous marking and stored at 2–8°C for up to 72 h.
(k) Prior to moving to the next sample to be confirmed the latex gloves should be removed discarded per laboratory site operational procedures and new latex gloves worn.

Validation Study

This validation study was conducted under the AOAC Research Institute *Performance Tested Method*℠ protocol and the *AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces* (2). Method developer studies were conducted in contract laboratories of Solus Scientific Solutions Ltd., located in East Kilbride, Scotland, and included the product consistency and stability studies, and robustness testing. The independent laboratory study was conducted by Q Laboratories, located in Cincinnati, Ohio, USA and included the inclusivity/exclusivity study, instrument variation study and matrix studies for all claimed surface matrixes.

The CERTUS *EL* Detection Kit was compared to the FDA/BAM Chapter 10 for stainless steel, ceramic tile, plastic and sealed concrete environmental surfaces.

Independent Laboratory Studies

Inclusivity/Exclusivity Study

This study was carried out with pure cultures to examine the ability of the CERTUS *EL* Detection Kit to detect a variety of claimed *Listeria* strains and to distinguish those strains from closely related non- *Listeria* strains; where at least 50% of the organisms tested originated from foodborne isolates.

For the inclusivity study evaluation of CERTUS *EL* Detection Kit, 50 *Listeria* strains were cultured in 9 mL of Brain Heart Infusion (BHI) broth for 22 ± 2 h at 30 ± 1°C. After incubation, the culture tubes were diluted with sterile BHI broth to 10^3–10^4 CFU/mL.

For the exclusivity portion of the evaluation, 30 species closely related to *Listeria* species were cultured in 9 mL of BHI broth for 22 ± 2 h at 35 ± 1°C. Exclusivity cultures were used

Table 1. CERTUS *EL* Detection Kit inclusivity results

Organism + serotype	Source	Origin	Result	Organism + serotype	Source	Origin	Result
Listeria grayi	NCTC[a] 19120	Animal feces	+[b]	*Listeria monocytogenes* 1/2c	CWD[f] 1552	Not available	+
Listeria grayi	ATCC[c] 25401	Corn stalks	+	*Listeria monocytogenes* 1/2c	CWD 1553	Not available	+
Listeria grayi	ATCC 700545	Not available	+	*Listeria monocytogenes* 1/2a	CWD 1554	Not available	+
Listeria innocua	QL[d] 030911-12	Environmental	+	*Listeria monocytogenes* 1/2a	CWD 1555	Not available	+
Listeria innocua	QL 051111-1	Environmental	+	*Listeria monocytogenes* 4b	CWD1561	Human placenta	+
Listeria innocua	QL 32811.2	Seasoning powder	+	*Listeria monocytogenes* 4b	CWD 1563	Not available	+
Listeria innocua	ATCC 33091	Human feces	+	*Listeria monocytogenes* 4b	CWD 1590	Not available	+
Listeria innocua	QL 32911.1	Environmental	+	*Listeria monocytogenes* 1/2a	CWD 1611	Turkey	+
Listeria innocua	CSU[e] W1-301	Not available	+	*Listeria monocytogenes* 1/2a	CWD 1613	Turkey	+
Listeria marthii	ATCC BAA 1595	Soil	+	*Listeria monocytogenes* 1/2a	CWD 1614	Not available	+
Listeria ivanovii	ATCC 49954	Food, France	+	*Listeria monocytogenes* 1/2b	CWD 1626	Not available	+
Listeria ivanovii	ATCC BAA-678	Sheep fetus	+	*Listeria monocytogenes* 1/2b	CWD 1627	Mother/baby	+
Listeria ivanovii	ATCC Liv004	Not available	+	*Listeria monocytogenes* 1/2a	CWD 1629	Not available	+
Listeria ivanovii	ATCC Liv005	Not available	+	*Listeria monocytogenes* 1/2a	CWD 1630	Turkey	+
Listeria ivanovii	QL 030911-9	Clinical isolate	+	*Listeria monocytogenes*	QL 030911-10	Shellfish	+
Listeria monocytogenes 1/2c	ATCC 7644	Human isolate	+	*Listeria seeligeri* 6b	ATCC 11289	Human feces	+
Listeria monocytogenes 4b	ATCC 13932	Spinal fluid	+	*Listeria seeligeri*	ATCC 11856	Not available	+
Listeria monocytogenes 1/2a	ATCC 15313	Rabbit	+	*Listeria seeligeri* 1/2b	ATCC 35967	Soil	+
Listeria monocytogenes 4a	ATCC 19114	Animal tissue	+	*Listeria seeligeri*	FSL[g] -S4-035	Not available	+
Listeria monocytogenes 4b	ATCC 19115	Human isolate	+	*Listeria seeligeri*	QL 030911-2	Creamer	+
Listeria monocytogenes 4d	ATCC 19117	Sheep	+	*Listeria welshimeri*	ATCC 35897	Not available	+
Listeria monocytogenes 1/2a	ATCC 49594	Not available	+	*Listeria welshimeri* 6a	ATCC 43548	Not available	+
Listeria monocytogenes 4b	ATCC 51778	Dairy products	+	*Listeria welshimeri* 6b	ATCC 43549	Soil	+
Listeria monocytogenes 1/2b	ATCC 51780	Dairy products	+	*Listeria welshimeri* 1/2b	ATCC 43550	Human feces	+
Listeria monocytogenes 4b	ATCC Li2	Human isolate	+	*Listeria welshimeri*	LW[h] 003	Not available	+

[a] NCTC-National Collection of Type Cultures, Salisbury, UK.
[b] + = The target analyte was detected by the CERTUS *EL* Detection Kit.
[c] ATCC-American Type Culture Collection, Manassas, VA.
[d] QL-Q Laboratories Inc. Culture Collection, Cincinnati, OH.
[e] CSU-Colorado State University Culture Collection, Fort Collins, CO.
[f] CWD-University of Vermont Culture Collection, Burlington, VT.
[g] FSL-Cornell University Culture Collection, Ithaca, NY.
[h] LW-University of Vermont Culture Collection, Burlington, VT.

Table 2. CERTUS *EL* Detection Kit exclusivity results

Organism	Source	Origin	Result	Organism	Source	Origin	Result
Bacillus mycoides	ATCC[a] 6462	Soil	–[b]	*Lactobacillus fermentum*	ATCC 9338	Not available	–
Brochothrix thermosphacta	ATCC 11509	Pork sausage	–	*Lactobacillus lactis*	ATCC 4797	Not available	–
Bacillus cereus	ATCC 14579	Not available	–	*Lactobacillus plantarum*	ATCC 8014	Not available	–
Geobacillus stearothermophilus	ATCC 12980	Not available	–	*Micrococcus luteus*	ATCC 7468	Not available	–
Rhodococcus fascians	ATCC 12974	Not available	–	*Proteus mirabilis*	ATCC 7002	Urine	–
Enterococcus hirae	ATCC 8043	Not available	–	*Streptococcus mutans*	ATCC 25715	Not available	–
Enterococcus faecium	ATCC 19434	Not available	–	*Rhodococcus equi*	ATCC 6939	Lung abscess	–
Enterococcus durans	ATCC 19432	Not available	–	*Salmonella* Typhimurium	ATCC 14028	Chicken hearts and livers	–
Enterococcus faecalis	ATCC 29212	Urine	–	*Bacillus subtilis*	ATCC 6051	Not available	–
Kurthia gibsonii	ATCC 43195	Not available	–	*Staphylococcus aureus*	ATCC 29247	Not available	–
Escherichia coli	ATCC 8739	Feces	–	*Staphylococcus epidermidis*	ATCC 12228	Not available	–
Klebsiella oxytoca	ATCC 43165	Clinical isolate	–	*Staphylococcus haemolyticus*	ATCC 29970	Human skin	–
Klebsiella pneumoniae	ATCC 13883	Not available	–	*Staphylococcus warneri*	ATCC 29885	Not available	–
Kurthia zopfii	ATCC 10538	Not available	–	*Streptococcus pneumoniae*	ATCC 6302	Not available	–
Lactobacillus casei	ATCC 11578	Oral cavity	–	*Streptococcus pyogenes*	ATCC 19615	Pharynx of child	–

[a] ATCC-American Type Culture Collection, Manassas, VA.
[b] - = The target analyte was not detected.

without dilution. For each isolate, a BIO-LOCK™ Sampling Swab was inoculated with 10 µL.

Inclusivity and exclusivity cultures were randomized, blind coded and then processed following the CERTUS *EL* Detection Kit product insert.

Of the 50 inclusivity organisms evaluated using the CERTUS *EL* Detection Kit, all 50 organisms were correctly identified. For the 30 exclusivity organisms evaluated, all 30 organisms were correctly excluded. The inclusivity and exclusivity evaluation results are presented in Tables 1 and 2.

Matrix Study

For the four matrixes claimed, this study evaluated 30 un-paired sample replicates comprising of five replicate test portions un-inoculated (0 CFU/test area), 20 replicate test portions at a low inoculation level to yield fractionally positive results (63–88 CFU/test area), and five replicate test portions at a high inoculation level to yield consistently positive results (190–310 CFU/test area).

Different *Listeria* strains were used for the inoculation of the various environmental matrix surfaces. In addition, the stainless steel environmental surface was co-inoculated with a potential competitor at 10 times the level of the *Listeria* strain.

Data demonstrating fractionally positive results for each matrix tested; identified as those in which at least one of the candidate or reference methods yielded five to 15 positive results out of 20 replicates examined for the low inoculation level were required. However, results in which the candidate method was above the fractional range, but the reference method was below the fractional range were also considered acceptable.

For the matrix study evaluation of the candidate CERTUS EL Detection Kit, *L. monocytogenes* 4 b ATCC 19115 (for stainless steel environmental surface matrix), *L. welshimeri* ATCC 35897 (for ceramic tile environmental surface matrix), *L. innocua* ATCC 33090 (for plastic environmental surface matrix) and *L. monocytogenes* ATCC 7644 (for sealed concrete environmental surface matrix) inoculums were prepared by transferring a pure isolated colony of the specified organism from Trypticase Soy Agar with 5% sheep blood (SBA) into BHI broth for 24 ± 2 h at 35 ± 2°C to obtain stationary phase cell densities. The *Listeria* starter cultures were subsequently diluted in BHI broth, to levels that would yield fractional positives at the time of sampling.

Where co-inoculation was required, an *Enterococcus faecalis* ATCC 51299 strain was cultured in BHI broth for 22 ± 2 h at 35 ± 1°C and diluted to 10 times the level of the *Listeria* strain (960–3200 CFU/test area). To determine the inoculation level for the environmental surface, aliquots of each inoculating organism were plated onto Tryptic Soy Agar (TSA) in duplicate.

Prior to inoculation, surfaces were disinfected with a quaternary ammonium based solution, then rinsed with sterile water and treated with UV light for 15–20 min. All 4 × 4" environmental surfaces areas were inoculated with 0.10 mL diluted *Listeria* culture and where appropriate co-inoculated with 0.10 mL competitor organism culture at 10-fold excess; evenly distributed over the entire test area. For the un-inoculated test areas, sterile BHI broth was evenly distributed over the entire test area. Surfaces were dried for 16–24 h at room temperature (20–25°C) prior to sampling. All environmental surfaces were sampled using the BIO-LOCK™ Sampling Swabs according to the AOAC protocol. Samples were stored at 4 ± 2°C for 2 h ± 15 min prior to enrichment. All environmental surface samples were randomized, blind coded and then processed following the CERTUS EL Detection Kit product insert. The CERTUS software indicates the test results on the CERTUS Detection Unit as a presumptive positive or negative for the presence of *Listeria* species in the sampled test portion. Results were obtained at 24 h for a negative result, whereas a positive result was obtained in real time once cultures reached the detection threshold level.

All samples analyzed by the CERTUS EL Detection Kit, regardless of presumptive result, were culturally confirmed as described in the reference method Bam Ch. 10, Section G. Isolation Procedure by directly streaking the primary enrichments to a Modified Oxoid agar and Agar *Listeria* Ottaviani and Agosti plate. Suspect colonies were subsequently identified as described in the reference method BAM Ch. 10, Section H. Identification procedure. Final confirmation was conducted using the Bruker MALDI Biotyper biochemical identification method, AOAC OMA **2017.10** (5).

For the matrix study evaluation of the reference BAM Ch. 10 method, all test areas were prepared as previously stated and swabbed with sampling swabs pre-moistened with 10 mL Dey Engley (D/E) neutralizing broth using vertical, horizontal and diagonal sweeping motions. Sampled swabs were placed in a sterile sampling bag containing sufficient D/E neutralizer to cover the swab. Samples were stored at 4 ± 2°C for 2 h ± 15 min prior to enrichment.

Each sampling swab was enriched in 90 ± 0.9 mL of Buffered *Listeria* Enrichment Broth, with the addition of pyruvate (BLEB+p). The sampling swabs were homogenized by hand massaging for 2 min and subsequently incubated at 30 ± 1°C for 4 h ± 15 min. Following 4 h of incubation, selective supplements acriflavine (10 mg/L), sodium nalidixate (40 mg/L) and cycloheximide (50 mg/L) were added to each test portion and incubated for an additional 20 h ± 30 min. After 24 h of total incubation, the enriched samples were culturally confirmed as described in the reference method BAM Ch. 10, Section G. Isolation Procedure and Section H. Identification procedure, before final confirmation was conducted using the Bruker MALDI Biotyper biochemical identification method, AOAC OMA **2017.10**.

Comparison of the candidate CERTUS EL Detection Kit and reference methods for all matrixes indicated un-inoculated test portions were all negative, high inoculation level test portions were all positive and fractional positive results were achieved at the low inoculation level test portions.

The POD analysis between the candidate CERTUS EL Detection Kit and the reference methods for all matrixes indicated that there was no significant difference at the 5% level between the numbers of positive results by the two methods.

The POD analysis between CERTUS EL Detection Kit presumptive and confirmed results for all matrixes indicated that there was no significant difference at the 5% level. A summary of POD analyses is presented in Tables 3 and 4.

Instrument Variation Study

This study was carried out with pure cultures and verified consistent results between instruments made to a manufacturer's specifications for the CERTUS EL Detection Kit.

For the instrument variation study evaluation of the CERTUS EL Detection Kit, a *L. seeligeri* ATCC 11856 strain was cultured in non-selective BHI broth for 20–24 h at 30 ± 2°C. The starter culture was diluted to approximately 10^2 CFU/mL in sterile BHI broth; levels that would yield fractional positives at the time of sampling.

For each of the three instruments tested, 10 un-paired target organism sample replicates were evaluated through inoculation of a BIO-LOCK™ Sampling Swab with 10 µL diluted culture. All samples were randomized, blind coded and then processed following the CERTUS EL Detection Kit product insert. Results were decoded with POD values and confidence intervals. Data was analyzed for variable detection between instruments.

The POD analysis between instruments when tested against low inoculation *Listeria* cultures on the CERTUS EL Detection Kit indicated that there was no significant difference at the 5% level between the numbers of positive results. A summary of Instrument Variation POD analysis is presented in Table 5.

Table 3. CERTUS *EL* Detection Kit results: presumptive vs confirmed

			CERTUS *EL* Detection Kit presumptive				CERTUS *EL* Detection Kit confirmed				
Matrix	Strain	CFU[a]/test area	N[b]	X[c]	POD_{CP}[d]	95% CI	x	POD_{CC}[e]	95% CI	$dPOD_{CP}$[f]	95% CI[g]
	L. monocytogenes 4b, ATCC[h]	N/A[i]	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	−0.47, 0.47
Stainless steel	19115/10X *E.*	71 Lm[j] & 960 Ef[k]	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	−0.13, 0.13
	faecalis, ATCC 51299	240 Lm & 3200 Ef	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	−0.47, 0.47
		N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	−0.47, 0.47
Ceramic Tile	*L. welshimeri* ATCC 35897	63	20	5	0.25	0.11, 0.47	5	0.25	0.11, 0.47	0.00	−0.13, 0.13
		190	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	−0.47, 0.47
		N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	−0.47, 0.47
Plastic	*L. innocua* ATCC 33090	88	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	−0.13, 0.13
		290	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	−0.47, 0.47
		N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	−0.47, 0.47
Sealed Concrete	*L. monocytogenes* ATCC 7644	79	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	−0.13, 0.13
		310	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	−0.47, 0.47

[a] CFU/Test Area = Results of the CFU/Test area were determined by plating the inoculum for stainless steel and sealed concrete.
[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 (confirmed per BAM Chapter 10) 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] American Type Culture Collection, Manassas, VA.
[i] Not applicable.
[j] *Listeria monocytogenes*.
[k] *Enterococcus faecalis*.

Table 4. Method comparison results: CERTUS *EL* Detection Kit vs BAM Ch. 10

				CERTUS *EL* Detection Kit results			BAM Ch. 10 results				
Matrix	Strain	CFU/test area[a]	N[b]	x[c]	POD_C[d]	95% CI	x	POD_R[e]	95% CI	$dPOD_C$[f]	95% CI[g]
	L. monocytogenes 4b, ATCC[h]	N/A[i]	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	−0.43, 0.43
Stainless steel	19115/10X *E.*	71 Lm[j] & 960 Ef[k]	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	−0.28, 0.28
	faecalis, ATCC 51299	240 Lm & 3200 Ef	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	−0.43, 0.43
		N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	−0.43, 0.43
Ceramic Title	*L. welshimeri* AATCC 35897	63	20	5	0.25	0.11, 0.47	6	0.30	0.15, 0.52	−0.05	−0.31, 0.22
		190	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	−0.43, 0.43
		N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	−0.43, 0.43
Plastic	*L. innocua* ATCC 33090	88	20	9	0.45	0.26, 0.66	7	0.35	0.18, 0.57	0.10	−0.19, 0.37
		290	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	−0.43, 0.43
		N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	−0.43, 0.43
Sealed Concrete	*L. monocytogenes* ATCC 7644	79	20	9	0.45	0.26, 0.66	8	0.40	0.22, 0.61	0.05	−0.24, 0.33
		310	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	−0.43, 0.43

[a] CFU/Test Area = Results of the CFU/Test area were determined by plating the inoculum for stainless steel and sealed concrete.
[b] N = Number of test potions.
[c] x = Number of positive test portions.
[d] POD_C = Candidate method presumptive positive outcomes confirmed positive.
[e] POD_R = Reference method confirmed positive outcomes divided by the total number of trials.
[f] $dPOD_C$ = Difference between the candidate method 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] American Type Culture Collection, Manassas, VA.
[i] Not applicable.
j Listeria monocytogenes.
[k] *Enterococcus faecalis*.

Method Developer Studies

Product Consistency (lot-to-lot) and Stability Studies. This study was carried out with pure cultures and examined three lots of CERTUS *EL* Detection Kits (CERTUS *EL* Selective Growth Media plus BIO-LOCK™ Sampling Swab and Detection Tube) and CERTUS *EL* Control Caps for lot-to-lot consistency and product stability. Individual lots were chosen for accelerated stability evaluation that represented lots near their expiration date, near the middle of the expiration period, and recently manufactured.

Accelerated studies generating stability data are based on the Arrhenius model where 6 months real time storage at 25°C room temperature is equivalent to 20 days accelerated storage at 45°C; and 12 months real time storage at 25°C room temperature is equivalent to 50 days accelerated storage at 45°C (6). As accelerated stability only provides a rough estimate of shelf-life, real time data supporting the entire shelf life of the kit under normal room temperature (15–25°C) storage conditions will also be evaluated. Data demonstrating no statistical difference in detection between lots or time points was required.

For the product consistency (lot-to-lot) and stability study evaluation of CERTUS *EL* Detection Kit, a *L. monocytogenes* 4 b ATCC 19115 strain was cultured in non-selective BHI broth for 20–24 h at 30 ± 2°C upon which the starter culture was diluted to approximately 3–6 × 10^2 CFU/mL in sterile BHI broth.

In addition, a non-target strain *E. faecalis* NCTC 13379 strain was cultured in BHI broth for 20–24 h at 37 ± 1°C and used without dilution. For each of the three CERTUS *EL* Detection Kit lots tested, 10 target and 10 non-target organism sample replicates were evaluated through inoculation of a BIO- LOCK™ Sampling Swab with 10 µL diluted target (approximately 3–6 CFU/swab) and undiluted non-target cultures. All samples were randomized, blind coded and then processed following the CERTUS *EL* Detection Kit product insert.

Due to enrichment occurring in a bio-contained Detection Tube, the target *Listeria* samples were unable to be diluted down to the LOD50 of the method (5 x 10^5 CFU/mL) and were therefore analyzed undiluted. In addition, grown out cultures diluted down to the LOD50 of the method are unable to be analyzed on the CERTUS Detection Unit as it is reliant on generating a positive result in real time from measuring raw optic signals produced by the complexed SERS nanoparticles over multiple cycles, until a culture has reached the detection threshold level as determined by a proprietary algorithm.

For the product consistency (lot-to-lot) and stability study evaluation of CERTUS *EL* Control Cap lots; CERTUS *EL* Positive Control Cap and CERTUS *EL* Negative Control Cap lots were stored at 45°C and samples were removed for evaluation at 0, 20 and 50 days that represented the entire shelf life of the control caps. For each of the accelerated storage time points, five CERTUS *EL* Positive Control Caps and five CERTUS *EL* Negative Control Caps were sampled. All samples were randomized, blind coded and then processed following the CERTUS *EL* Detection Kit product insert.

Comparison of CERTUS *EL* Detection Kit lots that were at their expiration date, near the middle of the expiration period and recently manufactured; the diluted *L. monocytogenes* cultures were all positive, and undiluted *E. faecalis* non-target cultures were all negative. In addition, comparison of CERTUS *EL* Positive and Negative Control Cap lots that were at their expiration date, near the middle of the expiration period and recently manufactured demonstrated expected results. A summary of product consistency (lot-to-lot) and stability POD analyses is presented in Tables 6 and 7.

Table 5. Instrument variation study of CERTUS *EL* Detection Kit

Strain	Instrument number	N[a]	x[b]	PODt[c]	95% CI	
	104	10	5	0.5	0.24	0.76
L. seeligeri ATCC 11856	109	10	4	0.4	0.17	0.69
	106	10	5	0.5	0.24	0.76

[a] N = Number of test portions.
[b] x = Number of positive test portions.
[c] POD_t = Treatment combination confirmed positive outcomes divided by the total number of trials.

Robustness Study

This study was carried out with pure cultures using a two-parameter factorial design, where both high and low

Table 6. Product consistency (lot-to-lot) and stability of CERTUS *EL* Detection Kits, paired lot POD comparison

Lot	Lot age (months)	N[a]	X[b]	POD_A[c]	95% CI		Lot	Lot age (months)	N	x	POD_B[d]	95% CI		$dPOD_{AB}$[e]	95% CI[f]	
Target analyte: *Listeria monocytogenes* 4b ATCC 19115																
DDL230718[g]	6	10	10	1.0	0.72,	1.00	DDL010818[h]	0	10	10	1.0	0.72,	1.00	0.0	−0.28	0.28
DDL130618[i]	12	10	10	1.0	0.72,	1.00	DDL010818	0	10	10	1.0	0.72,	1.00	0.0	−0.28	0.28
DDL130618	12	10	10	1.0	0.72,	1.00	DDL230718	6	10	10	1.0	0.72,	1.00	0.0	−0.28	0.28
Non-target analyte: *Enterococcus faecalis* NCTC 13379																
DDL230718	6	10	0	0.0	0.00,	0.28	DDL010818	0	10	0	0.0	0.00,	0.28	0.0	−0.28	0.28
DDL130618	12	10	0	0.0	0.00,	0.28	DDL010818	0	10	0	0.0	0.00,	0.28	0.0	−0.28	0.28
DDL130618	12	10	0	0.0	0.00,	0.28	DDL230718	6	10	0	0.0	0.00,	0.28	0.0	−0.28	0.28

[a] N = Number of test portions.
[b] x = Number of positive test portions.
[c] POD_A = Positive outcomes divided by the total number of trials first member of pair.
[d] POD_B = Positive outcomes divided by the total number of trials second member of pair.
[e] $dPOD_{AB}$ = Difference in POD between the paired comparison.
[f] 95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[g] Lot DDL230718 was produced July 23, 2018-middle of expiration period.
[h] Lot DDL010818 was produced August 1, 2018-recently manufactured.
[i] Lot DDL130618 was produced June 13, 2018-end of expiration period.

Table 7. Product consistency (lot-to-lot) and stability of CERTUS EL Control Caps, paired lot POD comparison

Lot	Lot age, months	N[a]	X[b]	POD_A[c]	95% CI		Lot	Lot age, months	N	x	POD_B[d]	95% CI		$dPOD_{AB}$[e]	95% CI[f]	
CERTUS EL Positive Control Cap																
DDL020718[g]	6	5	5	1.0	0.57,	1.00	DDL020718	0	5	5	1.0	0.57,	1.00	0.0	−0.43	0.43
DDL020718	12	5	5	1.0	0.57,	1.00	DDL020718	0	5	5	1.0	0.57,	1.00	0.0	−0.43	0.43
DDL020718	12	5	5	1.0	0.57,	1.00	DDL020718	6	5	5	1.0	0.57,	1.00	0.0	−0.43	0.43
CERTUS EL Negative Control Cap																
DDL020718[h]	6	5	0	0.0	0.00,	0.43	DDL020718	0	5	0	0.0	0.00,	0.43	0.0	−0.43	0.43
DDL020718	12	5	0	0.0	0.00,	0.43	DDL020718	0	5	0	0.0	0.00,	0.43	0.0	−0.43	0.43
DDL020718	12	5	0	0.0	0.00,	0.43	DDL020718	6	5	0	0.0	0.00,	0.43	0.0	−0.43	0.43

[a] N = Number of test portions.
[b] x = Number of positive test portions.
[c] POD_A = Positive outcomes divided by the total number of trials first member of pair.
[d] POD_B = Positive outcomes divided by the total number of trials second member of pair.
[e] $dPOD_{AB}$ = Difference in POD between the paired comparison.
[f] 95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[g] Lot DDL020718 CERTUS EL Positive Control Cap was produced July 2, 2018.
[h] Lot DDL020718 CERTUS EL Negative Control Cap was produced July 2, 2018.

Table 8. Robustness study of CERTUS EL Detection Kit, POD comparison

Parameters																
Parameter test combination[a]	Incubation temperature (°C)	Culture time (h)	N[b]	x[c]	POD_E[d]	95% CI		Nominal condition[e]	N	x	POD_N[f]	95% CI		$dPOD_{EN}$[g]	95% CI[h]	
Target analyte: *Listeria monocytogenes* 4b ATCC 19115																
1	27	22	10	8	0.80	0.49,	0.94	5	10	10	1.00	0.72,	1.00	−0.20	−0.51	0.11
2	27	26	10	10	1.00	0.72,	1.00	5	10	10	1.00	0.72,	1.00	0.00	−0.28	0.28
3	33	22	10	10	1.00	0.72,	1.00	5	10	10	1.00	0.72,	1.00	0.00	−0.28	0.28
4	33	26	10	10	1.00	0.72,	1.00	5	10	10	1.00	0.72,	1.00	0.00	−0.28	0.28
5	30	24	10	10	1.00	0.72,	1.00	5	10	10	1.00	0.72,	1.00	0.00	−0.28	0.28
Non-target analyte: *Enterococcus faecalis* NCTC 13379																
1	27	22	10	0	0.00	0.00,	0.28	5	10	0	0.00	0.00,	0.28	0.00	−0.28	0.28
2	27	26	10	0	0.00	0.00,	0.28	5	10	0	0.00	0.00,	0.28	0.00	−0.28	0.28
3	33	22	10	0	0.00	0.00,	0.28	5	10	0	0.00	0.00,	0.28	0.00	−0.28	0.28
4	33	26	10	0	0.00	0.00,	0.28	5	10	0	0.00	0.00,	0.28	0.00	−0.28	0.28
5	30	24	10	0	0.00	0.00,	0.28	5	10	0	0.00	0.00,	0.28	0.00	−0.28	0.28

[a] Each parameter test combination is being compared to the nominal test condition.
[b] N = Number of test portions experimental combination.
[c] x = Number of positive test portions experimental combination.
[d] POD_E = Positive outcomes divided by the total number of trials experimental combination.
[e] Nominal condition = parameter test combination No. 5.
[f] POD_N = Positive outcomes divided by the total number of trials nominal condition.
[g] $dPOD_{EN}$ = Difference in POD between the nominal condition and experimental combinations.
[h] 95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

parameter values were incorporated, plus a combination of the nominal parameter values. All other parameters remained unchanged.

Parameters to be varied included: incubation temperature (27°C, 33°C), and culture time (22 h, 26 h).

> Recommended conditions analyzed were 30°C incubation temperature and 24 h culture time.

For the robustness study evaluation of CERTUS EL Detection kit, a *L. monocytogenes* 4 b ATCC 19115 strain was cultured in non-selective BHI broth for 20–24 h at 30 ± 2°C upon which the starter culture was diluted to approximately 3–5 × 10^2 CFU/mL in sterile BHI broth.

In addition, a non-target strain *E. faecalis* NCTC 13379 strain was cultured in BHI broth for 20–24 h at 37 ± 1°C and used without dilution. For each test combination and the nominal combination, 10 target and 10 non-target organism sample replicates were evaluated on the CERTUS EL Detection Kit through inoculation of a BIO-LOCK™ Sampling Swab with 10 µL diluted target and undiluted non-target cultures. All samples were randomized, blind coded and then processed following the CERTUS EL Detection Kit product insert.

Results were decoded with POD values and confidence intervals calculated for combinations 1–4 which represent changes to assay incubation temperature (27°C, 33°C) and assay time (22 h, 26 h) when compared to the nominal combination 5 (30°C and 24 h). Data was analyzed for variable detection due to changes in parameter settings.

The POD analysis of CERTUS EL Detection Kit when tested against neat *Listeria* and competitor organism indicated that there was no significant difference at the 5% level between

nominal and experimental combinations. A summary of Robustness POD analysis is presented in Table 8.

Discussion

The CERTUS EL Detection Kit successfully recovered *Listeria* species from all four environmental surfaces analyzed. Using POD analysis, no statistically significant differences were observed between the number of positive samples detected by the candidate method and the reference method for all four environmental surfaces tested.

The results of the inclusivity and exclusivity evaluation demonstrated 100% agreement with expected results for the test panels and confirmed the high specificity and selectivity of the CERTUS EL Detection Kit to *Listeria* species. Production of the CERTUS EL Detection Tubes and Control Caps showed consistent performance between lots over the time tested. Robustness testing proved that the performance of the CERTUS EL Detection Kit was not adversely affected by small variations to incubation time and temperature. No variation was seen between the instruments tested.

The CERTUS EL Detection Kit is a quick and simple method to perform, providing results within 24 h of sampling. This method has the advantage of allowing samples to be processed with no dependency on laboratory supplies or equipment, as all materials needed to perform the method are provided within the CERTUS EL Detection Kit. In addition, with the inclusion of proprietary CERTUS™ software, end users are able to track lot information and occurrence of positive results in real time.

Conclusion

The data from the study, with the statistical uncertainty, support the product claims of the CERTUS EL Detection Kit for stainless steel, ceramic tile, plastic and sealed concrete environmental surfaces analyzed within 24 h after sampling. The matrix studies, along with inclusivity/exclusivity, product consistency and stability, robustness and instrument variation testing demonstrate that the CERTUS EL Detection Kit is suitable for *Performance Tested Method*℠ certification.

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Certification Information

The method was independently tested, evaluated, and certified by the AOAC Research Institute as a *Performance Tested Method*℠. *See* https://www.aoac.org/scientific-solutions/research-institute-ptm/for information on certification.

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