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Research Article

MICROBIOLOGICAL METHODS

Evaluation of the Thermo Scientific SureTect™ Listeria Species PCR Assay in a Broad Range of Foods and Selected Environmental Surfaces: Pre-Collaborative and Collaborative Study, First Action 2021.06

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Abstract

Background: The Thermo Scientific SureTect™ Listeria species PCR assay utilizes Solaris™ reagents for performing PCR for the rapid and specific detection of *Listeria* species in a broad range of foods and selected environmental surfaces.
Objective: To demonstrate reproducibility of the Thermo Scientific SureTect Listeria species PCR assay in a collaborative study using a challenging matrix, full-fat cottage cheese (25 g), to extend the scope of the method.
Methods: In the collaborative study, the candidate method was compared to the US Food and Drug Administration/*Bacteriological Analytical Manual* (FDA/BAM) Ch. 10 *Listeria* reference method. The candidate method used two PCR thermocyclers, the Applied Biosystems QuantStudio™ 5 Real-Time PCR instrument (QS5) and the Applied Biosystems 7500 Fast Real-Time PCR instrument (7500 Fast). The candidate method included its own confirmation procedure. Eighteen participants from 10 laboratories located within the United States and Europe were solicited for the collaborative study, with 12 participants submitting valid data. Statistical analysis was conducted according to the probability of detection (POD) statistical model. In addition, in order to extend the scope of the method, seven matrix studies were performed comparing the candidate method to the FDA/BAM reference method. One of these matrixes was also compared to the ISO 11290–1:2017 Microbiology of the food chain—Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp.—Part 1: Detection method reference method.
Results: In the collaborative study, the difference in laboratory results indicates equivalence between the candidate method and reference method for the matrix evaluated and the method demonstrated acceptable inter-laboratory reproducibility as determined in the collaborative evaluation. The two PCR instruments used in the study performed equivalently. All presumptive positives were confirmed via the alternative confirmation procedure. In the pre-collaborative studies, the results showed comparable performances between the candidate method and the reference method for all matrixes tested.

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Conclusion: Based on the data generated, the method demonstrated acceptable inter-laboratory reproducibility data and statistical analysis.
Highlights: Due to the COVID-19 pandemic, some participants had to be trained remotely. Additionally, 25 g full-fat cottage cheese is known to be a challenging matrix to test. No unusual cross-contamination, or false-positive/negative data was reported, highlighting the ease of use, reproducibility, and robustness of the candidate method.

Listeria is recognized as a cause of foodborne illness worldwide, which can grow at wide temperature and pH ranges and can tolerate high concentrations of sodium chloride. Due to their ubiquitous nature in soil, water, and in several animals intended for consumption, *Listeria* has been isolated from various food products including dairy, meat, vegetables, and seafood, as well as from food processing facilities (1).

In humans, *Listeria monocytogenes* causes listeriosis, which may lead to meningitis, septicemia, encephalitis, fetal loss and death. Groups at greatest risk include pregnant women, neonates, older adults, and immunocompromised people. The Thermo Scientific SureTect™ Listeria species PCR assay is based upon use of Solaris™ reagents for performing PCR. Dye-labeled probes target unique DNA sequences specific to *Listeria* species, and an internal positive control (IPC). Target DNA, if present, is detected by real-time PCR. There is an option to select between two PCR thermocyclers, the Applied Biosystems QuantStudio™ 5 Real-Time PCR instrument (QS5) and the Applied Biosystems 7500 Fast Real-Time PCR instrument (7500 Fast). Analysis software provides interpretation of results. The IPC template, primers, and probe provide an internal control with each reaction to show that the PCR process has occurred; it is unnecessary to incorporate positive control organisms with routine testing of samples.

Prior to the collaborative study, the SureTect Listeria species PCR assay was validated according to the current guidelines, Appendix J of AOAC *Official Methods of Analysis* (2), in an AOAC *Performance Tested Method*℠ (PTM) study in a broad range of foods and selected environmental surfaces. The SureTect Listeria species PCR assay was awarded PTM certificate 071304 in July 2013 for 10 food matrixes and three environmental surfaces. There were additional matrix studies in 2015 and 2018 to extend the scope. Finally, in 2021 as part of the pre-collaborative study, seven matrix studies were performed against the US Food and Drug Administration/*Bacteriological Analytical Manual* (FDA/BAM) Ch. 10 *Listeria* (3) reference method. In addition, one of the matrixes was also compared to the ISO 11290–1:2017 Microbiology of the food chain—Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp.—Part 1: Detection method (4). The following pre-collaborative matrix studies were performed by Thermo Fisher Scientific (Basingstoke, UK): cottage cheese (4% fat; 25 g), blue cheese (25 g), Greek yoghurt (25 g), plastic surface (1" × 1"; polystyrene Petri dish), ceramic surface (4" × 4"; smooth wall/floor tiles), stainless steel surface (4" × 4"; slab, brush finished), and concrete surface (4" × 4"; slab). *See* Table 1 below for an overview of the previously validated matrixes for PTM certification as well as the new matrix additions.

Experimental

Collaborative Study

The purpose of this collaborative study was to compare the reproducibility of the Thermo Scientific SureTect Listeria species PCR assay to the FDA/BAM Ch. 10 reference method for cottage cheese (4% fat; 25 g).

Study Design

In this collaborative study, 25 g sample sizes of cottage cheese were evaluated. The matrix was obtained from a local retailer and screened for the presence of *Listeria* by the FDA/BAM Ch. 10 reference method and the SureTect Listeria species PCR assay. The cottage cheese was artificially contaminated with a heat-stressed liquid culture of *L. monocytogenes* American Type Culture Collection (ATCC; located in Manassas, VA, USA) 51780. The matrix was inoculated at two levels of contamination: a high inoculation level of approximately 5–10 cfu/test portion and a low inoculation level of approximately 0.2–2 cfu/test portion. A set of noninoculated control test portions (0 cfu/test portion) was also included. The inoculated test portions were held for 48–72 h at refrigerated temperature (2–8°C), prior to initiating testing.

Twelve replicate samples from each of the three inoculation levels were analyzed by each participant. Due to different enrichment media and enrichment conditions, an unpaired study design was followed. A total of 72 samples, 36 for the SureTect Listeria species PCR assay (25 g test portion) and 36 for the reference method (25 g test portion) were sent to each participating technician. Collaborators were also sent a noninoculated test portion for determining the total aerobic plate count (APC) following the US Food and Drug Administration, (2001) *Bacteriological Analytical Manual* Ch. 3: Aerobic Plate Count reference method (5) on the day samples were received.

A detailed collaborative study packet outlining all necessary information related to the study including media preparation, test portion preparation, and documentation of results was sent to each collaborating laboratory prior to the initiation of the study.

Preparation of Inoculum and Test Portions

The *Listeria* strain used in this evaluation was propagated onto tryptone soy agar with 5% sheep blood (SBA) from a Q Laboratories (Cincinnati, OH, USA) frozen stock culture stored at −70°C. The organism was incubated for 24 ± 2 h at 35 ± 1°C. Isolated colonies were picked into 10 mL of brain heart infusion (BHI) broth and incubated for 18–24 h at 35 ± 1°C.

A heat-stressed liquid culture was used to artificially contaminate the cottage cheese. The culture was prepared by heat stressing the overnight BHI broth culture at 55 ± 0.1°C for 10–20 min. The heat-stressed culture was plated to the non-selective agar (tryptic soy agar) and a selective agar (Ottaviani & Agosti [O&A] agar) and incubated for 18–24 h at 35 ± 1°C. Following incubation, the % injury was determined using the following formula, and the inoculating culture must have a % injury of 50–80:

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

where n_{select} = number of colonies on selective agar; and $n_{nonselect}$ = number of colonies on nonselective agar

Table 1. PTM validation study summary for the SureTect Listeria species PCR assay

SureTect Listeria species PCR Assay—PTM 071304						
Original PTM certificate issued[a]	Matrixes	Sample size	Enrichment media/dilution	Enrichment time, h	Enrichment temperature, °C	Reference method
July, 2013	Raw ground beef (80% lean)	25 g	24 LEB/1-in-10	22–30	37 ± 1	ISO[b]
	Raw pork frankfurters	25 g	24 LEB/1-in-10	22–30	37 ± 1	ISO
	Salami	25 g	24 LEB/1-in-20	22–30	37 ± 1	ISO
	Cooked sliced ham	25 g	24 LEB/1-in-10	22–30	37 ± 1	ISO
	Cooked sliced turkey	25 g	24 LEB/1-in-10	22–30	37 ± 1	ISO
	Fresh bagged spinach	25 g	24 LEB/1-in-10	22–30	37 ± 1	ISO
	Cut cantaloupe	25 g	24 LEB/1-in-10	22–30	37 ± 1	ISO
	Processed cheese	25 g	24 LEB/1-in-10	22–30	37 ± 1	ISO
	Smoked salmon	25 g	24 LEB/1-in-10	22–30	37 ± 1	ISO
	Cooked prawns (heads off)	25 g	24 LEB/1-in-10	22–30	37 ± 1	ISO
	Stainless steel (slab, brushed finished)	4” × 4”	24 LEB/100 mL	22–30	37 ± 1	ISO
	Stainless steel (slab, brushed finish)	1” × 1”	24 LEB/10 mL	22–30	37 ± 1	ISO
	Plastic (large polystyrene Petri dish)	4” × 4”	24 LEB/100 mL	22–30	37 ± 1	ISO
Method modification	Matrixes	Sample size	Enrichment media/ dilution	Enrichment time, h	Enrichment temperature, °C	Reference method
February and September, 2015	Raw ground pork	25 g	24 LEB/1-in-10	22–30	37 ± 1	ISO
	Bagged lettuce	25 g	24 LEB/1-in-10	22–30	37 ± 1	ISO
	Raw ground turkey	25 g	24 LEB/1-in-10	22–30	37 ± 1	ISO
	Raw pork sausages	25 g	24 LEB/1-in-10	22–30	37 ± 1	ISO
	Pasteurized 2% fat milk	25 g	24 LEB/1-in-10	22–30	37 ± 1	ISO
	Raw cod	25 g	24 LEB/1-in-10	22–30	37 ± 1	ISO
	Pasteurized brie cheese	25 g	24 LEB/1-in-10	22–30	37 ± 1	ISO
	Ice cream (vanilla)	25 g	24 LEB/1-in-10	22–30	37 ± 1	ISO
Method modification	Matrixes	Sample size	Enrichment media/dilution	Enrichment time, h	Enrichment temperature, °C	Reference method
October, 2018	Sliced deli turkey	25 g	24 LEB/1-in-10	22–30	37 ± 1	ISO
	Bagged lettuce	25 g	24 LEB/1-in-10	22–30	37 ± 1	ISO
	Pasteurized 2% fat milk	25 g	24 LEB/1-in-10	22–30	37 ± 1	ISO
	Stainless steel (slab, brushed finish)	1” × 1”	24 LEB/10 mL	22–30	37 ± 1	ISO
Method modification[c]	Matrixes	Sample size	Enrichment dilution	Enrichment time (h)	Enrichment temp (°C)	Reference method
New matrices 2021	Cottage cheese (4% fat)[d]	25 g	24 LEB/1-in-10	22–30	37 ± 1	FDA/BAM[e]
	Blue cheese	25 g	24 LEB/1-in-10	22–30	37 ± 1	FDA/BAM
	Greek yoghurt	25 g	24 LEB/1-in-10	22–30	37 ± 1	FDA/BAM & ISO
	Plastic (polystyrene Petri dish)	1” × 1”	24 LEB/10 mL	22–30	37 ± 1	FDA/BAM
	Stainless steel (slab, brushed finish)	4” × 4”	24 LEB/100 mL	22–30	37 ± 1	FDA/BAM
	Ceramic (wall/floor tile)	4” × 4”	24 LEB/100 mL	22–30	37 ± 1	FDA/BAM
	Concrete (slab)	4” × 4”	24 LEB/100 mL	22–30	37 ± 1	FDA/BAM

[a] AOAC PTM Certificate No. 071304.
[b] EN ISO 11290–1.
[c] Matrices approved with First Action.
[d] Matrix selected for collaborative study.
[e] FDA/BAM Ch. 10.

The *L. monocytogenes* levels were confirmed by performing 10-fold serial dilutions using phosphate-buffered saline (PBS) to obtain a suitable inoculation level. A bulk material of cottage cheese was prepared and mixed for homogenous inoculation. The bulk sample was inoculated in a spot-wise manner with an appropriate volume, which was small enough to minimize potential effects on the sample, and at a dilution which considered initial die-off, and achieved each of the desired contamination levels at the time of testing: a low level expected to yield fractional positive results (5–15 positive results), and a high level expected to yield all positive results.

For the preparation of the candidate method test portions and reference method test portions, a 25 g sample from the bulk lots was directly sampled and packaged in sterile Whirl-Pak®. After inoculation, the test matrix was held for 48–72 h at refrigerated temperature (2–8°C) prior to analysis.

The level of *L. monocytogenes* in the low-level inoculum was determined by most probable number (MPN) on the day of analysis by evaluating 5 × 50 g, 20 × 25 g (reference method test portions), and 5 × 10 g inoculated samples. The level of *L. monocytogenes* in the high-level inoculum was determined by MPN by evaluating 5 × 25 g (reference method test portions), 5 × 10 g, and 5 × 5 g inoculated test portions. The number of positives from the three test levels was used to calculate the MPN using the Least Cost Formulation (LCF) MPN calculator v1.6 (Virginia Beach, VA, USA) provided by the AOAC Research Institute (RI).

Test Portion Distribution

All portions were labeled with a randomized, blind-coded 3-digit number affixed to the sample container. All collaborators' test portions were shipped on a Tuesday or Wednesday via overnight delivery according to the Category B Dangerous Goods shipment regulations set forth by the International Air Transport Association. Upon receipt, portions were held by the collaborating laboratory at refrigerated temperature (2–8°C) until the same-day analysis was initiated. A temperature recorder was included in each shipment to track the temperature of the package during transit. Participants were instructed to obtain the temperature of their package upon receipt and document the results on the Sample Receipt Confirmation form provided and fax or email it back to the study director.

Test Portion Analysis

Collaborators were instructed to follow the appropriate preparation and analysis as outlined in the study protocol. Two separate sets of 36 test portions (12 high, 12 low and 12 noninoculated controls for each method) were analyzed due to the unpaired study design. The SureTect Listeria species PCR assay test portions (25 g) were enriched with 225 mL ambient tempered Listeria enrichment broth (supplemented 24 LEB), homogenized by hand for at least 30 s, and incubated for 22–30 h at 36–38°C. The lysis step was performed using the Applied Biosystems SimpliAmp™ thermal cycler. The SureTect Listeria species PCR assay analysis was then conducted using the same procedure on either thermocycler: the Applied Biosystems QuantStudio 5 Real-Time PCR instrument and the Applied Biosystems 7500 Fast Real-Time PCR instrument. Both instruments have been included in the PTM evaluations of the SureTect Listeria species PCR assay. Out of the 12 analysts that successfully conducted the testing, six participants conducted analysis using the QuantStudio 5 and six participants conducted analysis using the 7500 Fast instrument. Table 2 presents a summary of the collaborator participation along with instrument utilized.

Regardless of the presumptive result, all test portions were confirmed following the FDA/BAM Ch. 10 reference method. The primary enrichments were streaked onto Oxford agar (OX) and Agar Listeria according to O&A medium formulation at 22 h for SureTect enrichments and 24 h and 48 h for FDA/BAM enrichments. OX plates were incubated at 35 + 1°C for up to 48 h and O&A plates were incubated at 37 + 1°C for up to 48 h. If no visible colonies were present after 24 h of incubation on the plates, they were re-incubated for an additional 24 h. Typical isolated colonies from OX agar were transferred to trypticase soy agar with 0.6% yeast extract (TSA/YE) and incubated at 30 or 35 + 1°C for 24–48 h. Isolated colonies were also stabbed to 5% SBA and incubated at 35 + 1°C for 24–48 h. Presumptive positive culture colonies were confirmed using VITEK2, API Listeria, or Bruker MALDI.

In addition to following the confirmation as outlined in the reference method, all SureTect Listeria species PCR assay test portions were also confirmed following an alternative confirmation procedure. Regardless of presumptive results, all SureTect Listeria species PCR assay primary enrichments were directly streaked to Oxoid *Brilliance*™ Listeria Agar (BLA) and incubated at 37 + 1°C for 22–26 h. From the BLA plates, typical colonies were confirmed using Thermo Scientific Oxoid Microbact™ Listeria 12 L kit.

Table 2. Participation of each collaborating laboratory

Collaborator ID No.	Participating laboratory	Analyst	Instrument	Participated[a]
1	1	2	7500 Fast[b]	Y
2	2	2	QS5[c]	Y
3	3	1	QS5	Y
4	4	2	7500 Fast	Y
5	5	1	QS5	Y
6[d]	6	2	7500 Fast	N
7[e]	3	2	7500 Fast	N
8[f]	5	2	QS5	N
9	7	3	7500 Fast/QS5	Y
10	7	1	QS5	Y

[a] Y= Collaborator analyzed the food type. N= Collaborator did not analyze the food type.
[b] Applied Biosystems 7500 Fast Real-Time PCR instrument.
[c] Applied Biosystems QuantStudio 5 Real-Time PCR instrument.
[d] Collaborator voluntarily withdrew prior to testing.
[e] Collaborator did not participate due to shipping issues.
[f] Collaborator dropped due to an incomplete data set for the reference method.

For the reference method test portions, 25 g samples were enriched in buffered Listeria enrichment broth with pyruvate (supplemented with 10 mg/L acriflavin, 50 mg/L sodium naladixic acid, and 40 mg/L cycloheximide) and analyzed according to the procedures in the FDA/BAM Ch. 10 reference method as described in the preceding paragraph.

Statistical Analysis

Each collaborating laboratory reported the test results on the data sheets provided. The data sheets were submitted to the study director at the end of testing for statistical analysis. Data for each contamination level was analyzed using the POD statistical model (6) and conducted using the LCF AOAC Binary Data Interlaboratory Study Workbook v2.3 (Virginia Beach, VA, USA). The POD was calculated as the number of positive outcomes divided by the total number of trials. Laboratory POD (LPOD) values were calculated as the total POD values for all collaborators. The LPOD was calculated for the candidate presumptive results, $LPOD_{CP,}$ the candidate confirmatory results (including false-negative results), $LPOD_{CC}$, the difference in the candidate presumptive and confirmatory results, $dLPOD_{CP,}$ presumptive candidate results that confirmed positive (excluding false-negative results), $LPOD_{C,}$ the reference method, $LPOD_R$, and the difference in the confirmed candidate and reference methods, $dLPOD_C$. A $dLPOD_C$ confidence interval not containing the point zero would indicate a statistically significant difference between the candidate method and the reference method at the 95% confidence level. In addition to POD values, the repeatability standard deviation (s_r), the among laboratory repeatability standard deviation (s_L), the reproducibility standard deviation (s_R) and the interlaboratory correlation coefficient (ICC) were calculated.

AOAC *Official Method*℠ 2021.06
Listeria Species in a Broad Range of Foods and Selected Environmental Surfaces
Thermo Scientific SureTect Listeria species PCR Assay
First Action 2021

[Applicable at the time of the submission to the detection of *Listeria* in fresh raw ground beef (80% lean, 25 g), raw pork frankfurters (25 g), salami (25 g), cooked sliced ham (25 g), cooked

sliced turkey (25 g), fresh bagged spinach (25 g), cut cantaloupe (25 g), processed cheese (25 g), smoked salmon (25 g), cooked prawns (heads off; 25 g), stainless steel (slab, brushed finish; 4" × 4"), plastic (large polystyrene Petri dish; 4" × 4"). Matrix extensions were approved in September 2015 for SureTect Listeria species PCR assay to add raw ground pork (25 g), bagged lettuce (25 g), raw ground turkey (25 g), raw pork sausages (25 g), pasteurized 2% fat milk (25 g), raw cod (25 g), pasteurized brie cheese (25 g), and ice cream (vanilla; 25 g). Sliced deli turkey was approved in the 2018 matrix extension. All matrixes were compared to ISO 11290–1:1996:/AMD 1:2004 Microbiology of food and animal feeding stuffs—Horizontal method for the detection and enumeration of Listeria monocytogenes—Part 1 Detection Method (version 3; which was the most current at the time; 7). The following matrixes are included for approval as part of the pre-collaborative study: blue cheese (25 g), cottage cheese (4% fat; 25 g), Greek yogurt, ceramic (4" × 4"; smooth wall/floor tiles), plastic (1" × 1"; polystyrene Petri dish), stainless steel (4" × 4"; slab, brushed finish), and concrete (4" × 4"; slab) environmental surfaces. The candidate method was compared to FDA/BAM Ch. 10, except in the case of Greek yogurt, where the candidate method was compared to both FDA/BAM Ch. 10 and EN ISO 11290–1 (2017).]

See Table **2021.06A** for a summary of results of the inter-laboratory study.

See Table **2021.06B** for detailed results of the inter-laboratory study.

Caution

General Safety

(a) Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.
(b) Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.

Chemical Safety

(a) Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials.
(b) Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
(c) Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
(d) Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
(e) Handle chemical wastes in a fume hood.
(f) Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
(g) After emptying a waste container, seal it with the cap provided.
(h) Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
(i) Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

Biological Hazard Safety

(a) Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.
(b) *L. monocytogenes* is a food pathogen that can cause listeriosis. Pregnant women, adults aged 65 or over, and people with a weakened immune system should not come into contact with this organism. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations (8, 9).
(c) Dispose of all inoculated culture media as hazardous microbiological waste, even if shown to be negative for the target organism, according to local guidelines.

A. *Principle*

The Thermo Scientific SureTect Listeria species PCR assay is based upon the use of Solaris reagents for performing PCR, for the rapid and specific detection of *Listeria* species in a broad range of food types and selected environmental surfaces. The method utilizes dye-labeled probes to target unique DNA sequences specific to *Listeria* species, and an IPC. Target DNA, if present, is detected by real-time PCR. The IPC template, primers, and probe provide an internal control with each reaction to show that the PCR process has occurred. Analysis software for the QuantStudio 5 and the 7500 Fast provides interpretation of results.

B. *Apparatus*

Items available from Thermo Fisher Scientific (www.thermofisher.com)

(a) *Homogenizer laboratory blender or dilutor*.—One of the following or equivalent, Cat. Nos. DB5000A, DB4100A, DB4150A,
(b) *Homogenizer bags appropriate for the sample size*.—Cat. Nos. DB4100A or DB4150A; DB4011A, DB4012A, DB4013A, DB4014A or equivalent.
(c) *Incubators fitted with racks for homogenizer bags, set to 35+1°C and 37+1°C*.
(d) *Thermal cycler*.—Applied Biosystems SimpliAmp Cat. No. A24811; or equivalent.
(e) *MicroAmp™ 96-Well Tray/Retainer Set for Veriti™ Systems*.—Cat. No. 4381850.
(f) *Real-Time PCR instrument, 0.1-mL block*.—Applied Biosystems QuantStudio 5, with Thermo Scientific RapidFinder™ Analysis Software v1.1 or later for use with SureTect Listeria species PCR assay and pathogen assay file: ListeriaSpp_SureTect_QS5 version 1.0 or later, Cat. Nos. A36320 (desktop), A36328 (laptop).

Table 2021.06A. Summary of results for the detection of *L. monocytogenes* in 25 g cottage cheese test portions by the SureTect Listeria species PCR assay vs FDA/BAM Ch. 10 in a collaborative study (QuantStudio 5 and 7500 Fast PCR instruments)

Method[a]	SureTect Listeria species PCR assay		
Inoculation level	Non-inoculated	Low	High
Candidate presumptive positive/total no. of samples analyzed	2/144	70/144	144/144
Candidate presumptive $LPOD_{CP}$	0.01 (0.00, 0.05)	0.49 (0.41, 0.57)	1.00 (0.97, 1.00)
s_r[b]	0.00	0.551	0.00
s_L[c]	0.00	0.00	0.00
s_R[d]	0.00	0.51	0.00
ICC[e]	NA[f]	0.00 (−0.07, 0.07)	NA
Candidate confirmed positive/total no. of samples analyzed	0/144	70/144	144/144
Candidate confirmed $LPOD_{CC}$	0.00 (0.00, 0.03)	0.48 (0.40, 0.56)	1.00 (0.97, 1.00)
s_r	0.00	0.51	0.00
s_L	0.00	0.00	0.00
s_R	0.00	0.51	0.00
ICC	NA	0.00 (−0.07, 0.11)	NA
Candidate method positive/total no. of samples analyzed	0/144	70/144	144/144
Candidate presumptive positive that confirmed $LPOD_C$	0.00 (0.00, 0.03)	0.48 (0.40, 0.56)	1.00 (0.97, 1.00)
s_r	0.00	0.51	0.00
s_L	0.00	0.00	0.00
s_R	0.00	0.51	0.00
ICC	NA	0.00 (−0.07, 0.11)	NA
Reference positive/total no. of samples analyzed	1/144	63/144	144/144
Reference $LPOD_R$	0.00 (0.00, 0.03)	0.44 (0.36, 0.52)	1.00 (0.97, 1.00)
s_r	0.00	0.51	0.00
s_L	0.00	0.00	0.00
s_R	0.00	0.51	0.00
ICC	NA	0.00 (−0.07, 0.07)	NA
dLPOD (candidate vs reference)[g]	−1.00 (−1.00, −0.96)	0.04 (−0.07, 0.15)	0.00 (−0.03, 0.03)
dLPOD (candidate presumptive vs candidate confirmed)[g, h]	0.01 (−0.01, 0.04)	0.01 (−0.01, 0.04)	0.00 (−0.02, 0.02)

[a] Results include 95% confidence intervals.
[b] Repeatability standard deviation.
[c] Among-laboratory standard deviation.
[d] Reproducibility standard deviation.
[e] Interlaboratory correlation coefficient.
[f] NA = Not applicable.
[g] A confidence interval for dLPOD that does not contain the value 0 indicates a statistically significant difference between the two methods.
[h] Both the alternative confirmation procedure and the confirmation procedure following the FDA/ BAM Ch. 10 produced identical results.

(g) *Real-Time PCR instrument*.—Applied Biosystems 7500 Fast with Applied Biosystems RapidFinder Express Software v2.0 or later for use with SureTect Listeria species PCR assay and pathogen assay file: Listeria species SureTect 1.0 or later, Cat. Nos. A30304 (desktop), A30299 (laptop).

(h) *MicroAmp 96-Well Tray for VeriFlex™ Block*.—Cat. No. 4379983.

(i) *Precision Plate Holder for SureTect assays*.—Cat. No. PT0690.

(j) *7500 Fast Precision Plate Holder, for 0.1 mL tube strips*.—Cat. No. A29252.

(k) *PCR Carry plate for SureTect assays*.—Cat. No. PT0695.

(l) *VersiPlate PCR Strip Tube Plate, 96-well, low profile*.—Cat. No. AB1800.

(m) *Ultra Clear qPCR Caps, strips of 8*.—Cat. No. AB0866.

(n) If using 7500 Fast Precision Plate Holder for 0.1 mL tube strips—Cat. No. A29252.
  (1) *MicroAmp Fast 8-Tube Strip, 0.1 mL*.—Cat. No. 4358293.
  (2) *MicroAmp Optical 8-Cap Strips*.—Cat. No. 4323032.

C. *Reagents*

Items available from Thermo Fisher Scientific (www.thermofisher.com)

(a) *SureTect Listeria species PCR assay, 96 tests*.—Cat. No. PT0200A.
  (1) *Lysis Reagent 1 Tubes (clear, pale blue liquid containing fine, white particles)*.—12 strips of 8 tubes.
  (2) *Lysis Tube Caps, domed*.—12 strips of 8 tubes.
  (3) *Proteinase K (clear colorless liquid)*.—1 tube.
  (4) *Lysis Reagent 2 (clear colorless liquid, red cap)* .—1 tube.
  (5) *SureTect Listeria species PCR Tubes*.—12 strips of 8 tubes 1 pellet each.
  (6) *PCR caps*.—12 strips of 8 caps.

(b) *Oxoid 24 Listeria Enrichment Broth (24 LEB), dehydrated*.—Cat. No. CM1107B, (base, 500g).

(c) *Oxoid 24 LEB Selective Supplement*.—Cat. No. SR0243E, 10 × 5 mL/bottle.

(d) *Oxoid 24 LEB Buffer Supplement*.—Cat. No. BO1204E, 24 × 10 mL. *Note*: This product may crystallize during storage. If crystals are present, place the tube in a 37°C water bath for 5–10 min, or until all of the crystals are dissolved.

(e) *Dey-Engley Broth or other neutralizing broth, or peptone water*.—As appropriate to the sample type.

(f) *Oxoid Brilliance Listeria Agar Base*.—Cat. No. CM1080 (base), (base, 500g).

(g) *Oxoid Brilliance Listeria Selective Supplement*.—Cat. No. SR0227E.

Table 2021.06B. Comparative results for the detection of *L. monocytogenes* in 25 g cottage cheese test portions by the SureTect Listeria species PCR assay vs FDA/BAM Ch. 10 in a collaborative study (QuantStudio 5 and 7500 Fast PCR instruments)

Organism: *L.monocytogenes* ATCC 51780			Candidate presumptive (CP)			Candidate confirmed (CC)[a]			Candidate result (C)			Reference method (R)			C vs R	CP vs CC
Statistic	Matrix	Collaborator	n[b]	x[c]	POD_{CP}	n	x	POD_{CC}	n	x	POD_C	n	x	POD_R	$dLPOD_{C,\ R}$	$dLPOD_{CP,\ CC}$
Non-inoculated control																
	Cottage Cheese	1	12	0	0.000	12	0	0.000	12	0	0.000	12	0	0.000	0.000	0.000
		2	12	0	0.083	12	0	0.000	12	0	0.000	12	0	0.000	0.000	0.083
		3	12	0	0.000	12	0	0.000	12	0	0.000	12	0	0.000	0.000	0.000
		4	12	0	0.000	12	0	0.000	12	0	0.000	12	0	0.000	0.000	0.000
		5	12	0	0.000	12	0	0.000	12	0	0.000	12	0	0.000	0.000	0.000
		6	12	1	0.083	12	0	0.000	12	0	0.000	12	0	0.000	0.000	0.083
		7	12	0	0.000	12	0	0.000	12	0	0.000	12	0	0.000	0.000	0.000
		8	12	0	0.000	12	0	0.000	12	0	0.000	12	0	0.000	0.000	0.000
		9[d]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		10[d]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		11[d]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		12[d]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		13[d]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		14[d]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		15	12	0	0.000	12	0	0.000	12	0	0.000	12	0	0.000	0.000	0.000
		16	12	0	0.083	12	0	0.000	12	0	0.000	12	0	0.000	0.000	0.083
		17	12	0	0.000	12	0	0.000	12	0	0.000	12	0	0.000	0.000	0.000
		18	12	1	0.083	12	0	0.000	12	0	0.000	12	0	0.000	0.000	0.083
	MPN/test portion															
Estimate	NA	All	144	2	0.01	144	0	0.00	144	0	0.00	144	0	1.00	0.00	0.01
LCL*e*	NA				0.00			0.00			0.00			0.00	−0.03	−0.01
UCL*f*	NA				0.03			0.03			0.03			0.03	0.03	0.04
s_r[g]					0.12			0.00			0.00			0.00		
s_L[h]					0.00			0.00			0.00			0.00		
s_R[i]					0.12			0.00			0.00			0.00		
ICC[j]					0.00			NA			NA			NA		
LCL					−0.07			NA			NA			NA		
UCL					0.07			NA			NA			NA		
Low inoculum level																
	Cottage cheese	1	12	8	0.667	12	8	0.667	12	8	0.667	12	6	0.500	0.167	0.000
		2	12	7	0.583	12	7	0.583	12	7	0.583	12	5	0.417	0.167	0.000
		3	12	4	0.333	12	4	0.333	12	4	0.333	12	6	0.500	−0.167	0.000
		4	12	5	0.417	12	5	0.417	12	5	0.417	12	5	0.417	0.000	0.000
		5	12	6	0.500	12	6	0.500	12	6	0.500	12	6	0.500	0.000	0.000
		6	12	4	0.333	12	4	0.333	12	4	0.333	12	5	0.417	−0.083	0.000
		7	12	5	0.417	12	5	0.417	12	5	0.417	12	3	0.250	0.167	0.000
		8	12	4	0.333	12	4	0.333	12	4	0.333	12	5	0.417	−0.083	0.000
		9	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		10	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		11	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		12	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

(continued)

Table 2021.06B. (continued)

Organism: *L.monocytogenes* ATCC 51780			Candidate presumptive (CP)			Candidate confirmed (CC)[a]			Candidate result (C)			Reference method (R)			C vs R	CP vs CC
Statistic	Matrix	Collaborator	n[b]	x[c]	POD_{CP}	n	x	POD_{CC}	n	x	POD_C	n	x	POD_R	$dLPOD_{C,R}$	$dLPOD_{CP,CC}$
		13	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		14	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		15	12	7	0.583	12	7	0.583	12	7	0.583	12	6	0.500	0.083	0.000
		16	12	7	0.583	12	7	0.583	12	7	0.583	12	5	0.417	0.167	0.000
		17	12	6	0.500	12	6	0.500	12	6	0.500	12	5	0.417	0.083	0.000
		18	12	7	0.583	12	7	0.583	12	7	0.583	12	6	0.500	0.083	0.000
	MPN/test portion															
Estimate	0.62	All	144	70	0.49	144	70	0.49	144	70	0.49	144	63	0.44	0.05	0.00
LCL	0.65				0.41			0.41			0.41			0.36	−0.07	−0.02
UCL	1.69				0.57			0.57			0.57			0.52	0.16	0.02
s_r					0.51			0.51			0.51			0.51		
s_L					0.00			0.00			0.00			0.00		
s_R					0.51			0.51			0.51			0.51		
ICC					0.00			0.00			0.00			0.00		
LCL					−0.07			−0.07			−0.07			−0.07		
UCL					0.07			0.07			0.07			0.07		
								High inoculum level								
	Cottage cheese	1	12	12	1.000	12	12	1.000	12	12	1.000	12	12	1.000	0.00	0.00
		2	12	12	1.000	12	12	1.000	12	12	1.000	12	12	1.000	0.00	0.00
		3	12	12	1.000	12	12	1.000	12	12	1.000	12	12	1.000	0.00	0.00
		4	12	12	1.000	12	12	1.000	12	12	1.000	12	12	1.000	0.00	0.00
		5	12	12	1.000	12	12	1.000	12	12	1.000	12	12	1.000	0.00	0.00
		6	12	12	1.000	12	12	1.000	12	12	1.000	12	12	1.000	0.00	0.00
		7	12	12	1.000	12	12	1.000	12	12	1.000	12	12	1.000	0.00	0.00
		8	12	12	1.000	12	12	1.000	12	12	1.000	12	12	1.000	0.00	0.00
		9[a]	12	12	NA	12	12	NA	12	12	NA	12	12	NA	NA	NA
		10	12	12	NA	12	12	NA	12	12	NA	12	12	NA	NA	NA
		11	12	12	NA	12	12	NA	12	12	NA	12	12	NA	NA	NA
		12	12	12	NA	12	12	NA	12	12	NA	12	12	NA	NA	NA
		13	12	12	NA	12	12	NA	12	12	NA	12	12	NA	NA	NA
		14	12	12	NA	12	12	NA	12	12	NA	12	12	NA	NA	NA
		15	12	12	1.000	12	12	1.000	12	12	1.000	12	12	1.000	0.00	0.00
		16	12	12	1.000	12	12	1.000	12	12	1.000	12	12	1.000	0.00	0.00
		17	12	12	1.000	12	12	1.000	12	12	1.000	12	12	1.000	0.00	0.00
		18	12	12	1.000	12	12	1.000	12	12	1.000	12	12	1.000	0.00	0.00

(continued)

Table 2021.06B. (continued)

Organism: *L.monocytogenes* ATCC 51780			Candidate presumptive (CP)			Candidate confirmed (CC)[a]			Candidate result (C)			Reference method (R)			C vs R	CP vs CC
Statistic	Matrix	Collaborator	n^b	x^c	POD_{CP}	n	x	POD_{CC}	n	x	POD_C	n	x	POD_R	$dLPOD_{C,\,R}$	$dLPOD_{CP,\,CC}$
	MPN/test portion															
Estimate	2.08	All	144	144	1.00	144	144	1.00	144	144	1.00	144	144	1.00	0.00	0.00
LCL	1.74				0.97			0.97			0.97			0.97	−0.03	−0.02
UCL	7.57				1.00			1.00			1.00			1.00	0.03	0.02
s_r					0.00			0.00			0.00			0.00		
s_L					0.00			0.00			0.00			0.00		
s_R					0.00			0.00			0.00			0.00		
ICC					NA			NA			NA			NA		
LCL					NA			NA			NA			NA		
UCL					NA			NA			NA			NA		

[a] The alternative confirmation and the FDA/BAM Ch. 10 confirmation produced identical results.
[b] n = Number of test portions.
[c] x = Number of positive test portions.
[d] NA = Collaborator did not complete testing.
[e] LCL = Lower confidence limit.
[f] UCL = Upper confidence limit.
[g] s_r = Repeatability standard deviation.
[h] s_L = Among-laboratory standard deviation.
[i] s_R = Reproducibility standard deviation.
[j] ICC = Interlaboratory correlation coefficient.

(h) *Oxoid Brilliance Listeria Differential Supplement*.—Cat. No. SR0228E.

(i) *Oxoid Microbact Listeria 12L Kit*.—Cat. No. MB1128A.

(j) *Oxoid Microbact Listeria 12L Haemolysin Reagent*.—Cat. No. MB1249A.

Additional items required

(k) *Disposable gloves*.

(l) *Variable volume single-channel pipette, 1–10 mL*.

(m) *Rack, 96-well*.

(n) *Filtered pipette tips, 1–10 mL*.

(o) *Sample tubes, 1.5 mL*.

(p) *Sterile sampling swabs or sponges*.—Remel™ bio-spo sponge or equivalent.

(q) *Single channel pipette*.—10–100 µL or electronic, adjustable spacing, multichannel pipette, 10–100 µL.

(r) *Single-channel stepper pipette*.—10–100 µL.

(s) *Filtered pipette tips*.—10–100 µL.

(t) *Compact PCR tube rack, mixed colors*.

(u) *Tool for capping and decapping (optional)*.

(v) *Timer*.

(w) *Vortex mixer*.

(x) *Eight-channel pipette*.—5–50 µL.

(y) *Filtered pipette tips*.—10–100 µL.

D. *General Instructions*

(a) *Guidelines for sample enrichment*.—

(1) For preparation of master suspensions, follow the instructions of ISO 6887-1:2017: Microbiology of the food chain—Preparation of test samples, initial suspension and decimal dilutions for microbiological examination—Part 1: General rules for the preparation of the initial suspension and decimal dilutions (10) standard. Comply with good laboratory practices (refer to ISO 7218:2007: Microbiology of food and animal feeding stuffs—General requirements and guidance for microbiological examinations (11) standard).

(2) Follow the manufacturer's instructions for preparation of culture media.

(3) When following the short enrichment protocol, ensure that the enrichment broth is pre-warmed for 18–24 h before adding to the sample.

(4) Use filtered homogenizer bags to help with fat and particle separation.

(5) For consistent PCR results, use a ventilated incubator.

(6) Follow the specified temperature allowances.

(b) *Guidelines for sample lysis*.—

(1) Follow the specified temperature allowances.

(2) For downstream PCR on the 7500 Fast instrument or the QuantStudio 5 instrument: Prepare a mock-purified sample using sterile enrichment media as a negative extraction control. (The negative extraction control is required for RapidFinder Express Software; it is optional but recommended for RapidFinder Analysis Software.) Add the enriched sample or negative extraction control to the bottom of the lysis tube.

(3) To prevent crushing tubes, use the MicroAmp 96-Well Tray/Retainer Set provided with the SimpliAmp thermal cycler. See the SimpliAmp thermal cycler user guide (Pub. No. MAN0009889). Alternatively, use at least four complete tube strips in the heat block. We

recommend spacing the strips evenly across the heat block. If needed, add empty SureTect tubes to make four complete strips.

(c) *Guidelines for PCR.*—

(1) *Important:* After the lysate has been added to the pellets, ensure that the pellet rehydrates immediately by tapping the tubes on the laboratory bench. Start the PCR run within 30 min.

(2) Tube and cap strips can be cut when less than a full strip is required. Do not cut the strips of caps or tubes too close to the wall of the tube or the cap lid, otherwise the lid might not seal adequately during PCR.

(3) After the PCR tubes have been opened, add lysate within 10 min.

(4) Particulate matter from the lysate can inhibit the PCR. To ensure that no particles are transferred from the Lysis Reagent 1 Tube to the PCR tube, remove lysate from the top half of the liquid, taking care not to disrupt the particles at the bottom of the tube. If the particles become disturbed, allow the particles to resettle for 1–2 min before lysate removal.

(5) Ensure that the pellet is fully dissolved by using a vortex mixer. The solution changes from blue to green when the pellet is dissolved.

(6) For ease of use, a multi-channel pipettor can be used to transfer multiple lysates to the PCR tubes.

(7) Follow "Good laboratory practices for PCR". For more information go to: https://www.thermofisher.com/st/js/home/life-science/pcr/pcr-learning-center.html.

E. Sample Enrichment

(a) *Smoked salmon, processed cheese, fresh bagged spinach, cut cantaloupe, cooked prawns, cooked sliced turkey, ice cream, raw pork frankfurters, raw ground beef, raw ground turkey, raw ground pork, pasteurized 2% fat milk, raw pork sausages, raw cod, pasteurized Brie cheese, cooked sliced ham, fresh bagged lettuce, blue cheese, cottage cheese, Greek yoghurt (25 g), 1-in-10 ratio of sample to media.*—Prepare 24 LEB according to the manufacturer's instructions. Prepare the media by combining 1 L of 24 LEB and 10 mL (2 vials) of reconstituted 24 LEB Selective Supplement. Pre-warm to room temperature (23 ± 3°C). Transfer the food sample to a homogenizer bag, then add the room-temperature media (23 ± 3°C) as indicated. Add 225 mL prepared media to 25 g sample. Add 10 mL Oxoid 24 LEB Buffer Supplement per 25 g sample to the media in the homogenizer bag. For soft samples, homogenize 30 s to 1 min using a homogenizer. For samples containing hard particles, such as bone, squeeze the bag by hand until the sample is mixed thoroughly with the media. Incubate at 37 ± 1°C for 22–30 h.

(b) *Salami (25 g), 1-in-20 ratio of sample to media.*—Prepare media as indicated in **D(a).** Add 475 mL prepared media to 25 g sample. Add 20 mL Oxoid 24 LEB Buffer Supplement per 25 g sample to the media in the homogenizer bag. For soft samples, homogenize 30 s to 1 min using a homogenizer. For samples containing hard particles, squeeze the bag by hand until the sample is mixed thoroughly with the media. Incubate at 37 ± 1°C for 22–30 h.

(c) *Environmental surface swabs and sponges (stainless steel, plastic, ceramic, and concrete).*—Pre-moisten sterile sampling swab or sponge. For sampling areas that have been cleaned or treated with disinfectants and other cleaning agents, use a neutralizing broth, such as Dey-Engley broth. For other areas, use sterile peptone water or another equivalent diluent. Rub the swab or sponge in both a horizontal and vertical direction across the entire sampling area. Place the sample in the original packaging or other material that is suitable for transport. Samples may be held for up to 2 h at room temperature (23 ± 5°C) or 8 h in the refrigerator prior to adding the samples to media. Prepare media as indicated in **D(a)**. Add swabs to 10 mL prepared media. Add sponges to 100 mL prepared media. Add 4.4 mL reconstituted Oxoid 24 LEB Buffer Supplement per 100 mL media to the homogenizer bag. Homogenize thoroughly. Incubate at 37 ± 1°C for 22–30 h.

For all samples, Remove the enriched sample from the incubator, briefly mix the liquid in the homogenizer bag/tube by hand, transfer an aliquot of sample from the filtered side of the bag to a new tube, then close the tube and briefly mix.

Retain enough sample for confirmation or repeat testing.

F. Lysate Preparation

(a) *Lysis using SimpliAmp thermal cycler.*—

(1) Equilibrate the Lysis Reagent 1 Tubes to room temperature (23 ± 5°C).

(2) Check that there is no liquid around the plastic seal and the reagents are collected at the bottom of the tube.

(3) Allow the tubes to remain at room temperature (23 ± 5°C) for approximately 10 min before opening.

(4) Remove the plastic seal from each Lysis Reagent 1 Tube, then add 10 µL Proteinase K to the tube. These tubes are referred to as Lysis Tubes in the rest of the procedure.

(5) *Important:* Avoid contamination of the Proteinase K stock tube. Use a new filtered pipette tip each time Proteinase K is withdrawn from the stock tube. Use a 10–100 µL repeat pipettor to reduce the number of tips required.

(6) Add 10 µL Lysis Reagent 2 to the Lysis Tube.

(7) Transfer 10 µL enriched sample (or diluted cocoa/chocolate sample) to a Lysis Tube. For the negative extraction controls, transfer 10 µL sterile enrichment media to a Lysis Tube. Ensure that the pipette tip reaches the bottom of the Lysis Tube, to facilitate complete mixing of the sample with Lysis Reagent 1.

(8) Seal the tubes with the domed Lysis Tube Caps, then incubate the samples in the SimpliAmp thermal cycler using the following program.

(9) *Important:* To prevent crushing the tubes in the SimpliAmp thermal cycler, use the MicroAmp 96-Well Tray/Retainer Set or include at least four complete SureTect Lysis tube strips.

(10) Ensure that the lid heater is on and set to 105°C, and the volume is set to maximum. *See* Table 2021.06C.

(11) Proceed directly to PCR. Optional: Store the samples at 2–8°C for up to 24 h, including any time stored at 4°C in the thermal cycler.

(b) *Lysis using heat blocks.*—

(1) Ensure that two heating blocks are set to 37 ± 2°C, and 95 ± 2°C.

(2) Equilibrate the Lysis Reagent 1 Tubes to room temperature (23 ± 5°C).

(3) Place the required number of Lysis Reagent 1 Tubes in a suitable rack.

Table 2021.06C. Thermocycler heating steps for the SimpliAmp thermal cycler

Step	Temperature, °C	Time
1	37	10 min
2	95	5 min
3	10	2 min
4	4	Hold[a]

[a] For convenience, samples can be held at 4°C until proceeding to PCR or transfer to storage at 2–8°C.

(4) Check that there is no liquid around the plastic seal and the reagents are collected at the bottom of each tube.
(5) Allow the tubes to remain at room temperature (23 ± 5°C) for approximately 10 min before opening.
(6) Remove the plastic seal from each Lysis Reagent 1 Tube, then add 10 μL Proteinase K to the tube. These tubes are referred to as Lysis Tubes in the rest of the procedure.
(7) *Important:* Avoid contamination of the Proteinase K stock tube. Use a new filtered pipette tip each time Proteinase K is withdrawn from the stock tube. Use a 10–100 μL repeat pipettor to reduce the number of tips required.
(8) Add 10 μL Lysis Reagent 2 to the Lysis Tube.
(9) Transfer 10 μL enriched sample to a Lysis Tube. For the negative extraction controls, transfer 10 μL sterile enrichment media to a Lysis Tube. Ensure that the pipette tip reaches the bottom of the Lysis Tube, to facilitate complete mixing of the sample with Lysis Reagent 1.
(10) Seal the tubes with domed Lysis Tube Caps, then incubate the samples in the appropriate heating blocks:
  (a) 37 ± 2°C for 10 min.
  (b) 95 ± 2°C for 5 min.
  (c) Ambient temperature for 2 min. For convenience, samples can be transferred to storage at 2–8°C for up to 24 h.
(11) Proceed directly to PCR. Optional: Store the samples at 2–8°C for up to 24 h.

G. *Analysis*

(a) *PCR with the QuantStudio 5 instrument and RapidFinder Analysis Software v1.1 or later.*—
  (1) The plate layout is determined by the user. See the "Help" function in the software for detailed instructions. In the home screen of RapidFinder Analysis Software, click "Create Experiment," then enter or edit the well parameters. Select ListeriaSpp_SureTect_QS5 version 2.1 or later for the assay. Before starting this procedure, ensure that you are familiar with "Guidelines for PCR."
  (2) Following the plate layout previously set up in the software, place the required number of SureTect Listeria species PCR Tubes in the MicroAmp 96-Well Tray for VeriFlex Block. Place the block on the MicroAmp Splash-Free 96-Well Base. Press the PCR tubes to the tray to ensure they sit firmly, then tap the tubes on the bench to ensure that the pellets are located at the bottom of the tubes.
  (3) Allow the PCR tubes to remain on the bench for approximately 5 min, to bring to room temperature (23 ± 5°C), then open one strip of PCR tubes by removing the seal.
  Important:
    (a) If all sample lysates can be applied to the PCR tubes in 10 min, then open *all* strips of the PCR tubes.
    (b) If all sample lysates cannot be applied to the PCR tubes in 10 min, then open *only one* strip of the PCR tubes, then proceed to the next step.
    (c) PCR pellets are pale yellow. If the pellet is collapsed or not pale yellow, do not use.
    (d) If the pellet is not positioned at the bottom of a tube, gently move the pellet to the bottom of the tube with a sterile, empty pipette tip. Do not use a tip containing lysate.
  (4) Uncap the Lysis Tubes.
  (5) Transfer 20 μL lysate or mock-purified sample (negative extraction control reaction) to the appropriate PCR tube to rehydrate the pellet. Tap the rack to ensure that the lysate is at the bottom of the tube and touching the pellet.
  Important: Remove lysate from the top half of the liquid to ensure that no lysis particles are transferred from the Lysis Tube to the PCR tube. Do not touch the pellet when adding the lysate.
  (6) Seal the PCR tubes with the flat optical PCR Caps provided with the kit. Ensure that the tubes are properly sealed by pressing down firmly over each opening. Do not use the capping tool to seal the PCR tubes.
  (7) If *only one* strip of PCR tubes was opened, then repeat steps 2–5 for the remaining strips of PCR tubes.
  (8) Mix all PCR tubes thoroughly for 10–15 s to ensure that the pellet is fully rehydrated. This can be performed using a vortex mixer. Ensure that the liquid is at the bottom of the tube before placing in the PCR instrument. If needed, hold the tubes upright, and flick sharply downward or spin down using a centrifuge.
  Important: Start the PCR run within 30 min of addition of sample lysates to the PCR tubes.
  (9) Eject the instrument drawer. Use the MicroAmp 96-Well Tray for VeriFlex Block to transfer the tubes to the instrument in the same configuration as the plate layout determined in the software, then close the instrument drawer.
  (10) In the "Run" tab of the experiment file in RapidFinder Analysis Software, select the instrument's serial number from the "Instrument" drop-down list.
  (11) Click "Start Run," then follow the software prompts. Data analysis is automated by the software. For detailed instructions, and options for reporting, export, and storage of results, see the "Help" function in the software.

(b) *PCR with the 7500 Fast instrument and RapidFinder Express Software v2.0 or later.*—
  (1) RapidFinder Express Software determines the "Run Layout" (plate layout) for your samples based on the information entered and creates a run file. Refer to the "Help" function in the software for more details. On the main page of RapidFinder Express Software, select "Create/Edit a Run File," then enter or edit the

"Run File" information at the prompts. If desired, you can manually customize the plate layout in the software. Select Listeria species SureTect 2.0 or later for the assay. Before starting this procedure, ensure that you are familiar with "Guidelines for PCR".

(2) Following the plate layout previously set up in the software, place the required number of SureTect Listeria species PCR Tubes in a suitable rack with a PCR carry plate, then tap the rack of tubes on the bench to ensure that the pellets are located at the bottom of the tubes. If required by the plate layout, place empty SureTect PCR Tubes in the rack to balance the tray when the tubes are placed in the instrument.

(3) Allow the PCR tubes to remain on the bench for approximately 5 min, to bring to room temperature (23 ± 5°C), then open one strip of PCR tubes by removing the seal.
Important:
- (a) If all sample lysates can be applied to the PCR tubes in 10 min, then open *all* strips of the PCR tubes.
- (b) If all sample lysates cannot be applied to the PCR tubes in 10 min, then open *only one* strip of the PCR tubes, then proceed to the next step.
- (c) PCR pellets are pale yellow. If the pellet is collapsed or not pale yellow, do not use.
- (d) If the pellet is not positioned at the bottom of a tube, gently move the pellet to the bottom of the tube with a sterile, empty pipette tip. Do not use a tip containing lysate.

(4) Uncap the Lysis Tubes.

(5) Transfer 20 µL lysate or mock-purified sample (negative extraction control reaction) to the appropriate PCR tube to rehydrate the pellet. Tap the rack to ensure that the lysate is at the bottom of the tube and touching the pellet.
Important: Remove lysate from the top half of the liquid to ensure that no lysis particles are transferred from the Lysis Tube to the PCR tube. Do not touch the pellet when adding the lysate.

(6) Seal the PCR tubes with the flat optical PCR Caps provided with the kit. Ensure that the tubes are properly sealed by pressing down firmly over each opening. Do not use the capping tool to seal the PCR tubes.

(7) If *only one* strip of PCR tubes was opened, then repeat steps 2–5 for the remaining strips of PCR tubes.

(8) Mix all PCR tubes thoroughly for 10–15 s to ensure that the pellet is fully rehydrated. This can be performed using a vortex mixer. Ensure that the liquid is at the bottom of the tube before placing in the PCR instrument. If needed, hold the tubes upright, and flick sharply downward or spin down using a centrifuge.
Important: Start the PCR run within 30 min of addition of sample lysates to the PCR tubes.

(9) In the RapidFinder Express Software, select "Start Instrument Run" on the main page, select the appropriate run file, and follow the software prompts.

(10) Use the PCR carry plate to transfer the tubes to the instrument in the same configuration as the run layout. Use the Precision Plate Holder for SureTect assays. Be sure to load empty SureTect PCR tube strips as directed by the software.

(11) Close the tray to the instrument and follow the RapidFinder Express Software prompts to start the run. Data analysis is automated by the software.

H. Test Result Report and Interpretation of Results

(a) In the home screen of the RapidFinder Analysis Software (Figure 2021.06A), click "Results," then click the sub-tab for the desired view of the data.
- (1) *Summary*.—Plate format.
- (2) *Results*.—Table format.
- (3) *Details*.—Amplification plot.

Positive results indicate the presence of target DNA. Negative results indicate the absence of target DNA. A warning result indicates there is inhibition in the PCR reaction. See package insert for troubleshooting steps.

(b) In the RapidFinder Express Software (Figure 2021.06B), select "View Results" on the main page, select the appropriate run file, and follow the prompts to view results. To display a list of results in table format, click "Table View." Select a sample, then click "View Details" to see replicate information about samples. Positive results indicate the presence of target DNA. Negative results indicate the absence of target DNA. A warning result indicates there is inhibition in the PCR reaction. See package insert for troubleshooting steps.

I. Candidate Confirmation

(a) *Thermo Scientific SureTect Listeria species PCR assay test result confirmation.*—
- (1) Streak 10 µL of the primary enrichment onto BLA (or equivalent) and incubate for 22–26 h at 36–38°C.
- (2) Confirm characteristic and well-isolated *Listeria* colonies using:
  - (a) Microbact Listeria 12 L Kit.
- (3) Characteristic colonies can also be confirmed using the methods described in, depending on the legislation territory or matrix:
  - (a) the FDA/BAM Ch. 10
  - (b) EN ISO 11290-1
  - (c) USDA/FSIS MLG 8.12 (12)
  - (d) Any other appropriate national reference method or using:
  - (e) an appropriate *Official Methods of AOAC INTERNATIONAL* validated confirmation method
  - (f) an EN ISO 16140-6:2019 (13) validated confirmation method.

  In the event of discordant results (presumptive positive with the alternative method, not confirmed by one of the means described above), the laboratory must employ adequate means to ensure the validity of the result obtained.

All Thermo Scientific SureTect Listeria species PCR assay test results in the collaborative study were confirmed following FDA/BAM Ch. 10 in addition to the alternative confirmation.

Results

Collaborative Study

The collaborative study involved a method comparison evaluation of the SureTect Listeria species PCR assay to the FDA/BAM Ch. 10 reference method. A total of 18 participants throughout the continental United States and Europe participated in this study. In six of the laboratories, two separate analysts participated and in one of the laboratories, three separate analysts

Result icon	Result
	Positive result
	Negative result
	Result warning

Figure 2021.06A. RapidFinder Analysis Software results icons.

Result icon[a]	Result
	Positive result
	Negative result
	Result warning

[a]RapidFinder™ Express displays results pictorially.

Figure 2021.06B. RapidFinder Express Software results icons.

participated. The remaining three laboratories each had one participating analyst. Twelve out of the 18 participants from seven laboratories submitted valid data. One laboratory voluntarily withdrew from participation prior to initiating testing, one laboratory did not participate due to shipping issues, and one laboratory had incomplete reference method data for all test portions that were analyzed. For the laboratories that did participate, each participant analyzed 36 unpaired test portions for the SureTect Listeria species PCR assay and the FDA/BAM Ch. 10 reference method: 12 inoculated with a high level of *Listeria*, 12 inoculated with a low level of *Listeria*, and 12 uninoculated controls. In addition to the test portions, all participants set up an APC to determine the total microbial load of the test matrix. The average APC result obtained by the collaborators was 4.0×10^7 cfu/g. The highest count documented out of all the participants was 8.1×10^7 cfu/g and the lowest was 4.9×10^5 cfu/g.

A background screen of the matrix, following the FDA/BAM Ch. 10 reference method and using the Thermo Scientific SureTect Listeria monocytogenes PCR assay, indicated an absence of indigenous *Listeria* species. Ten replicate 25 g test portions (randomly sampled from 50% of the total packages used in the analysis) were screened for the presence of *Listeria*. All test portions produced negative results for the target analyte.

Table 2021.06A summarizes the inter-laboratory results. As per criteria outlined in Appendix J of AOAC *Official Methods of Analysis*, fractional positive results were obtained. Detailed results for each laboratory are presented in Table 2021.06B. The level of *Listeria* was determined by MPN on the day of initiation of analysis by the coordinating laboratory. The MPN levels obtained, with a 95% confidence interval, were 0.62 MPN/test portion (0.65, 1.69) for the low inoculum level and 2.08 MPN/test portion (1.74, 7.57) for the high inoculum level. MPN results are presented in the second column of Table 2021.06B.

Cottage Cheese (4% Fat)

Detailed results of the LPOD statistical analysis are presented in Table 2021.06B and Figures 1–4. For the low inoculation level, 70 out of 144 test portions ($LPOD_{CP}$ of 0.49) were reported as presumptive positive by the SureTect Listeria species PCR assay with 70 out of 144 test portions ($LPOD_{CC}$ of 0.48) confirming positive (following both the alternative confirmation and the FDA/BAM Ch. 10 reference method). For samples that produced presumptive positive results by the SureTect Listeria species PCR assay, 70 out of 144 samples confirmed positive ($LPOD_C$ of 0.48; value includes only presumptive positive results that confirmed positive). For the reference method, 63 out of 144 test portions were reported as positive ($LPOD_R$ of 0.44). A $dLPOD_C$ value of 0.01 with 95% confidence interval of (−0.2, 0.03) was obtained between the candidate and reference method, indicating no statistically significant difference between the two methods. A $dLPOD_{CP}$ value of −0.01 with 95% confidence interval of (−0.03, 0.02) was obtained between presumptive and confirmed results indicating no statistically significant difference between the presumptive and confirmed results (following both the alternative confirmation and the FDA/BAM Ch. 10 reference method).

For the high inoculum level, 144 out of 144 test portions ($LPOD_{CP}$ of 1.00) were reported as presumptive positive by the SureTect Listeria species PCR assay. There were 144 out of 144 reported test portions ($LPOD_{CC}$ of 1.00) that confirmed positive (following both the alternative confirmation and the FDA/BAM Ch. 10 reference method). For samples that produced presumptive positive results by the SureTect Listeria species PCR assay, 144 out of 144 samples confirmed positive ($LPOD_C$ of 1.00). For the reference method, 144 out of 144 test portions were reported as positive ($LPOD_R$ of 1.00). A $dLPOD_C$ value of 0.00 with 95% confidence interval of (−0.03, 0.03) was obtained between the candidate and reference method, indicating no statistically significant difference between the two methods. A $dLPOD_{CP}$ value of 0.00 with 95% confidence interval of (−0.02, 0.02) was obtained between presumptive and confirmed results indicating no statistically significant difference between the presumptive and confirmed results (following both the alternative confirmation and the FDA/BAM Ch. 10 reference method).

For the noninoculated controls, two out of 144 samples ($LPOD_{CP}$ of 0.01) produced a presumptive positive result by the SureTect Listeria species PCR assay with 0 out of 144 test portions ($LPOD_{CC}$ of 0.00) confirming positive (following both the alternative confirmation and the FDA/BAM Ch. 10 reference method). The two presumptive positives PCR results may have arisen from cross-contamination when preparing the enriched samples for PCR while manipulating multiple highly contaminated enrichment bags. During routine testing, as observed during the collaborative study, such presumptive positives would not be confirmed and would be investigated following the guidance outlined in the manufacturer's instructions. For the reference method, 0 out of 144 test portions were reported as positive ($LPOD_R$ of 0.00). A $dLPOD_C$ value of 0.00 with 95% confidence interval of (−0.03, 0.03) was obtained between the candidate and reference method, indicating no statistically significant difference between the two methods. A $dLPOD_{CP}$ value of 0.00 with 95% confidence interval of (−0.02, 0.02) was

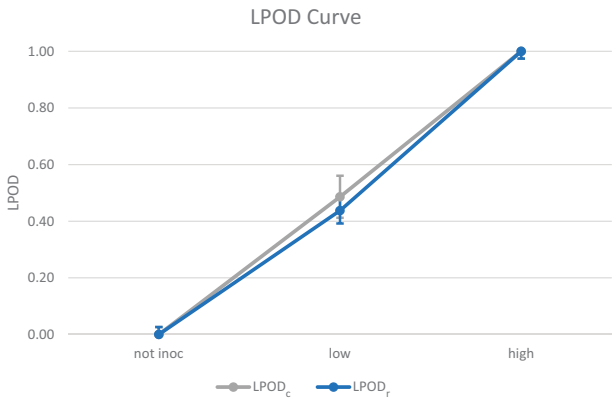

Figure 1. LPOD values of SureTect Listeria species PCR assay and FDA/BAM Ch. 10 for the collaborative study of cottage cheese.

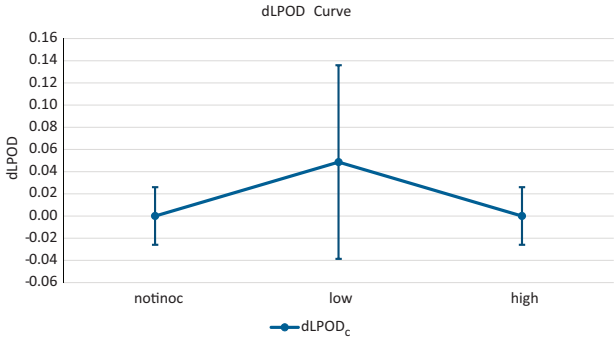

Figure 2. dLPOD$_C$ values of SureTect Listeria species PCR assay and FDA/BAM Ch. 10 for the collaborative study of cottage cheese.

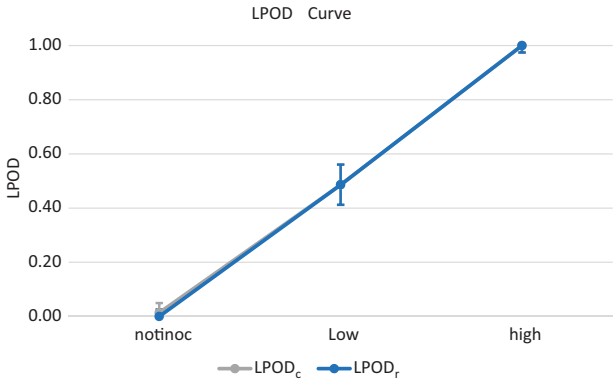

Figure 3. LPOD values of presumptive SureTect Listeria species PCR assay and confirmed results for the collaborative study of cottage cheese.

obtained between presumptive and confirmed results indicating no statistically significant difference between the presumptive and confirmed results (following both the alternative confirmation and the FDA/BAM Ch. 10 reference method).

Results of the APC for the collaborating laboratories are listed in Table 3. Details of the shipment temperatures for the collaborating laboratories are listed in Table 4.

Pre-Collaborative Study

The following matrix studies were performed by the Thermo Fisher Scientific laboratory in Basingstoke, UK: cottage cheese

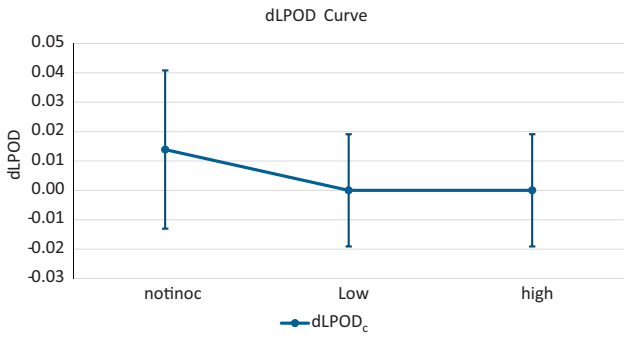

Figure 4. dLPOD$_{CP}$ values of presumptive SureTect Listeria species PCR assay and confirmed results for the collaborative study of cottage cheese.

Table 3. Results of aerobic plate count in collaborative study of cottage cheese

Collaborator	Cottage cheese CFU/g[a]
1	2.5×10^6
2	2.5×10^6
3	1.8×10^7
4	1.3×10^7
5	2.6×10^7
6	4.9×10^5
7	6.3×10^5
8	1.6×10^8
9	NA[b]
10	NA
11	NA
12	NA
13	NA
14	NA
15	1.1×10^8
16	8.1×10^7
17	7.8×10^6
18	6.4×10^7

[a] Samples (25 g) analyzed by the FDA/BAM Ch. 3 method.
[b] NA = Not applicable.

(4% fat; 25 g), blue cheese (25 g), Greek yoghurt (25 g), plastic surface (1" × 1"; polystyrene Petri dish), ceramic surface (4" × 4"; smooth wall/floor tile), stainless steel surface (4" × 4"; slab, brushed finish), and concrete surface (4" × 4"; slab).

All food matrixes were obtained from local supermarkets or food wholesale companies. The environmental surfaces were obtained from local companies. For the reference method, the FDA/BAM Ch. 10 was used in these studies. For Greek yogurt only, the candidate method was compared to both the FDA/BAM Ch. 10 and EN ISO 11290–1 reference methods.

To pre-screen the 25 g food samples, relevant portions of the samples were enriched by performing a 1-in-10 dilution with supplemented 24 LEB. The bags were then incubated at 37 + 1°C for 22 h. After incubation, samples were tested using the SureTect Listeria species PCR assay on the 7500 Fast and QuantStudio 5 instruments and results were interpreted with RapidFinder Express (v2.0 or later) and RapidFinder Analysis (v1.1 or later) software, respectively. Presumptive positives were streaked onto a suitable selective plate and confirmed using the Microbact 12 L kit and the FDA/BAM Ch. 10 confirmation method. The environmental surfaces were not pre-screened.

Table 4. Shipment temperatures for the collaborative study of cottage cheese

Collaborator	Temperature measured by recorder, °C
1	3.88
2	3.88
3	3.72
4	3.77
5	3.72
6	3.61
7	3.63
8	4.77
9	N/A[a]
10	N/A
11	N/A
12	N/A
13	N/A
14	N/A
15	3.8
16	3.8
17	3.8
18[b]	N/A

[a] N/A = Not applicable.
[b] Collaborator participated but temperature recording data was unavailable. APC result showed comparable results to other collaborators.

The noninoculated environmental surface samples acted as a screen.

The results of the pre-screen showed that none of the matrixes were naturally contaminated. When comparing to the FDA/BAM Ch. 10 reference method, the matrix study consisted of evaluating a total of 60 unpaired 25 g portions for cottage cheese, 60 unpaired 25 g portions for blue cheese, 60 unpaired 25 g portions for Greek yoghurt, 60 unpaired portions for plastic (1" × 1") surface, 60 unpaired portions for ceramic (4" × 4") surface, 60 unpaired portions for stainless steel (4" × 4") surface, and 60 unpaired portions for concrete (4" × 4") surface.

When comparing to the EN ISO 11290–1:2017 reference method, the matrix study consisted of evaluating a total of 60 unpaired 25 g portions for the Greek yoghurt matrix.

Within each sample set, there were 5 noninoculated portions (0 cfu/test portion), 20 low-level inoculated portions (0.2–2 cfu/test portion), and 5 high-level inoculated portions (2–10 cfu/test portion).

Organism Preparation and Inoculation

All inoculated matrixes tested were spiked with a liquid, unstressed culture, except the cottage cheese and Greek yoghurt matrixes, which were spiked with a liquid, heat-stressed culture. The cottage cheese matrix was spiked with Research & Development Culture Collection (RDCC; Basingstoke, UK) 3010 *L. monocytogenes*, the blue cheese matrix was spiked with RDCC 3015 *L. monocytogenes*, the Greek yoghurt matrix was spiked with RDCC 1201 *L. monocytogenes*, the plastic surface was spiked with Trials Culture Collection (TCC; Basingstoke, UK) 1209 *L. monocytogenes*, the ceramic surface was spiked with TCC 1209 *L. monocytogenes*, the stainless steel surface was spiked with TCC 813 *L. monocytogenes*, and the concrete surface was spiked with Oxoid Culture Collection (OCC; Basingstoke, UK) 1575 *L. innocua*.

For the cottage cheese, blue cheese, and Greek yoghurt matrixes, spiking cultures were prepared by removing the required strains from the −80°C culture collection freezer, subculturing to TSA, and incubating plates at 37 ± 1°C for 24 ± 1 h. After incubation, the test strains were subcultured into 10 mL tryptone soya broth (TSB) and incubated at 37 ± 1°C for 24 ± 1 h. For the cottage cheese and Greek yoghurt, after incubation, the 10 mL TSB was transferred to a water bath where a heat-stressing procedure was applied. The culture for blue cheese did not undergo heat stress due to a high background of microflora. Strains were diluted to the equivalent of a 0.5 McFarland standard using sterile saline and then further diluted to 10^{-6} in maximum recovery diluent (MRD). The liquid culture was spiked by spot inoculation into the bulk material for each food matrix, mixed by physically manipulating the bag, and then stored for 48–72 h at 2–8°C. Additionally, 50 µL of the 10^{-4} and 10^{-5} dilutions were subcultured onto TSA in triplicate and then incubated at 37 ± 1°C for 24 ± 1 h. The plate results were used to enumerate the cfu in the bulk material which was then used to calculate the amount of spiked material to combine with the non-inoculated material to create the 25 g test portions.

For the plastic, ceramic, stainless steel, and concrete environmental surface, spiking cultures were prepared by removing the required strains from the −80°C culture collection freezer, subculturing to TSA, and incubating plates at 37°C for 24 ± 1 h. After incubation, the test strains were subcultured into 10 mL TSB and incubated at 37 ± 1°C for 24 ± 1 h. The strains were diluted to the equivalent of a 0.5 McFarland standard using sterile saline and then further diluted to 10^{-6} in MRD. To prevent the strain from dying off on the surface while in storage, 10% milk powder was added to the relevant dilution before spiking. The liquid culture was spiked by spot inoculation onto the plastic (1" × 1") surface, ceramic (4" × 4") surface, stainless steel (4" × 4") surface, and concrete (4" × 4") surface. The surfaces were allowed to dry at room temperature then transferred to a dark cupboard and stored overnight. Additionally, 50 µL of the 10^{-4} and 10^{-5} dilutions were subcultured onto TSA in triplicate and then incubated at 37 ± 1°C for 24 ± 1 h. The plate results were used to enumerate the cfu on the surface of the environmental samples which was then used to calculate the amount spiked onto each surface. A non-target background microorganism, Oxoid Culture Collection (OCC) 640 *Enterobacter faecalis*, was also spiked on to the surfaces through spot inoculation. To prevent die off, 10% milk powder was added to the 10^{-4} dilution and 50 µL was spiked onto each surface.

For the blue cheese bulk material, the matrix was cut into small pieces measuring ≤2 cm. The pre-prepared spiking culture was spot inoculated with a pipette onto the surface of the food and the food was then mixed by hand inside a sterile bag to evenly distribute the inoculum. The bag opening was folded over and sealed with a rubber band with as much air excluded as possible, with the complete bulk stored at 4°C until the day of use.

For the cottage cheese and Greek yoghurt bulk material, the pre-prepared spiking culture was spot inoculated with a pipette onto the surface of the food and the food was then mixed by hand inside a sterile bag to evenly distribute the inoculum. The bag opening was folded over and sealed with a rubber band with as much air excluded as possible, with the complete bulk stored at 4°C until the day of use.

Samples were inoculated so that on the day of testing the level of inoculum was such that fractionally positive results, 5–15 positives per 20 replicates for low spike, and 5/5 positives per five replicates for high spike, were targeted.

Table 5. Pre-collaborative study: SureTect Listeria species PCR assay, 7500 Fast, presumptive versus confirmed and candidate versus FDA/BAM Ch.10—POD results

			Candidate presumptive (CP)			Candidate confirmed (CC)[a]			Candidate result (C)			Reference method (R)			C versus R	CP versus CC
Statistic	Matrix/organism	MPN[b]	N[c]	X[d]	POD_{CP}[e]	N	X	POD_{CC}[f]	N	X	POD_C[g]	N	X	POD_R[h]	$dPOD_{C,R}$[i]	$dPOD_{CP,CC}$[j]
		N/A[k]	5	0	0.00	5	0	0.00	0	0	0.00	0	0	0.00	0.00	0.00
LCL[l]		N/A			0.00			0.00			0.00			0.00	−0.43	−0.47
UCL[m]		N/A			0.43			0.43			0.43			0.43	0.43	0.47
		0.65	20	13	0.65	20	14	0.70	20	13	0.65	20	10	0.50	0.15	−0.05
LCL	Cottage cheese (4% fat; 25 g)	0.36			0.43			0.48			0.43			0.30	−0.15	−0.21
UCL	*L.monocytogenes* RDCC[n] 3010	1.06			0.82			0.86			0.82			0.70	0.41	0.11
		1.14	5	3	0.60	5	4	0.80	5	3	0.60	5	4	0.80	−0.20	−0.20
LCL		0.72			0.23			0.38			0.23			0.38	−0.62	−0.76
UCL		1.49			0.88			1.00			0.88			1.00	0.31	0.36
		N/A	5	0	0.00	5	0	0.00	0	0	0.00	0	0	0.00	0.00	0.00
LCL		N/A			0.00			0.00			0.00			0.00	−0.43	−0.47
UCL		N/A			0.43			0.43			0.43			0.43	0.43	0.47
		0.25	20	11	0.55	20	8	0.47	20	11	0.55	20	7	0.35	0.20	0.18
LCL	Blue cheese (25 g)	0.10			0.34			0.34			0.34			9.18	−0.10	−0.06
UCL	*L. monocytogenes* RDCC 3015	0.42			0.74			0.74			0.74			0.57	0.46	0.41
		0.19[o]	5	4	0.80	5	3	0.60	5	4	0.80	5	3	0.60	0.20	0.20
LCL		0.06			0.38			0.23			0.38			0.23	−0.31	−0.36
UCL		0.35			1.00			0.88			1.00			0.88	0.62	0.76
		N/A	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL		N/A			0.00			0.00			0.00			0.00	−0.43	−0.47
UCL		N/A			0.43			0.43			0.43			0.43	0.43	0.47
		1.45	20	12	0.60	20	13	0.65	20	12	0.60	20	15	0.75	−0.15	−0.05
LCL	Greek yogurt (25 g)	0.98			0.39			0.43			0.39			0.53	−0.40	−0.21
UCL	*L.monocytogenes* RDCC 1201	2.41			0.78			0.82			0.78			0.89	0.13	0.11
		4.38	5	5	1.00	5	5	1.00	5	5	1.00	5	5	1.00	0.00	0.00
LCL		1.72			0.57			0.57			0.57			0.57	−0.43	−0.47
UCL		11.15			1.00			1.00			1.00			1.00	0.43	0.47
		N/A	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL		N/A			0.00			0.00			0.00			0.00	−0.43	−0.47
UCL		N/A			0.43			0.43			0.43			0.43	0.43	0.47
		N/A	20	10	0.50	20	10	0.50	20	10	0.50	20	10	0.50	0.00	0.00
LCL	Plastic surface (1” × 1”; Petri dish)	N/A			0.30			0.30			0.30			0.30	−0.28	−0.13
UCL	*L.monocytogenes* TCC[p] 1209	N/A			0.70			0.70			0.70			0.70	0.28	0.13
		N/A	5	3	0.60	5	3	0.60	5	3	0.60	5	4	0.80	−0.20	0.00
LCL		N/A			0.23			0.23			0.23			0.38	−0.62	−0.47
UCL		N/A			0.88			0.88			0.88			1.00	0.31	0.47
		N/A	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL		N/A			0.00			0.00			0.00			0.00	−0.43	−0.47
UCL	Ceramic surface (4” × 4”; wall/floor tile)	N/A			0.43			0.43			0.43			0.43	0.43	0.47
		N/A	20	5	0.25	20	6	0.30	20	5	0.25	20	1	0.05	0.20	−0.05
LCL	*L.monocytogenes* TCC 1209	N/A			0.15			0.15			0.15			0.00	−0.03	−0.21
UCL		N/A			0.52			0.52			0.52			0.24	0.42	0.11

(continued)

Table 5. (continued)

Statistic	Matrix/organism	MPN[b]	Candidate presumptive (CP)			Candidate confirmed (CC)[a]			Candidate result (C)			Reference method (R)			C versus R	CP versus CC
			N[c]	X[d]	POD_{CP}[e]	N	X	POD_{CC}[f]	N	X	POD_C[g]	N	X	POD_R[h]	$dPOD_{C,R}$[i]	$dPOD_{CP,CC}$[j]
		N/A	5	5	1.00	5	5	1.00	5	5	1.00	5	5	1.00	0.00	0.00
LCL		N/A			0.57			0.57			0.57			0.57	−0.43	−0.47
UCL		N/A			1.00			1.00			1.00			1.00	0.43	0.47
		N/A	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL		N/A			0.00			0.00			0.00			0.00	−0.43	−0.47
UCL		N/A			0.43			0.43			0.43			0.43	0.43	0.47
	Stainless steel surface (4” × 4”; sheet metal token)	N/A	20	9	0.45	20	10	0.50	20	10	0.50	20	14	0.70	−0.25	−0.05
LCL		N/A			0.26			0.30			0.30			0.48	−0.50	−0.21
UCL	*L. monocytogenes* TCC 813	N/A			0.66			0.70			0.70			0.86	0.05	0.11
		N/A	5	5	1.00	5	5	1.00	5	5	1.00	5	5	1.00	0.00	0.00
LCL		N/A			0.57			0.57			0.57			0.57	−0.43	−0.47
UCL		N/A			1.00			1.00			1.00			1.00	0.43	0.47
		N/A	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL		N/A			0.00			0.00			0.00			0.00	−0.43	−0.47
UCL		N/A			0.43			0.43			0.43			0.43	0.43	0.47
		N/A	20	7	0.35	20	7	0.35	20	7	0.35	20	10	0.50	−0.15	0.00
LCL	Concrete surface (4” × 4”; slab)	N/A			0.18			0.18			0.18			0.30	−0.41	−0.13
UCL	*L. innocua* OCC[q] 1575	N/A			0.57			0.57			0.57			0.70	0.15	0.13
		N/A	5	3	0.60	5	3	0.60	5	3	0.60	5	4	0.80	−0.20	0.00
LCL		N/A			0.23			0.23			0.23			0.38	−0.62	−0.47
UCL		N/A			0.88			0.88			0.88			1.00	0.31	0.47

[a] Results obtained following the alternative confirmation were identical to results obtain from confirmation by FDA/BAM Ch. 10 reference method.
[b] MPN = Most probable number is calculated using the LCF MPN calculator ver. 1.6 provided by AOAC RI, with 95% confidence interval.
[c] N = Number of test portions.
[d] X = Number of positive test portions.
[e] POD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials.
[f] POD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials.
[g] POD_C = Candidate method confirmed positive outcomes divided by the total number of trials.
[h] POD_R = Reference method confirmed positive outcomes divided by the total number of trials.
[i] $dPOD_{CP}$= Difference between the candidate method presumptive result and candidate method confirmed result POD values.
[j] $dPOD_C$= Difference between the confirmed candidate method result and reference method confirmed result POD values.
[k] N/A = Not applicable.
[l] LCL = Lower confidence limit.
[m] UCL = Upper confidence limit.
[n] RDCC = Research and Development Culture Collection, Basingstoke, UK.
[o] High levels of background microflora were observed on the plates.
[p] TCC = Trials Culture Collection, Basingstoke, UK.
[q] OCC = Oxoid Culture Collection, Basingstoke, UK.

Table 6. Pre-collaborative study: SureTect Listeria species PCR assay, 7500 Fast, presumptive versus confirmed and candidate versus ISO 11290–1—POD results

			Candidate presumptive (CP)			Candidate confirmed (CC)[a]			Candidate result (C)			Reference method (R)			C versus R	CP versus CC
Statistic	Matrix/organism	MPN[b]	N[c]	X[d]	POD_{CP}[e]	N	X	POD_{CC}[f]	N	X	POD_C[g]	N	X	POD_R[h]	$dPOD_{C,R}$[i]	$dPOD_{CP,CC}$[j]
		N/A[k]	5	0	0.00	5	0	0.00	0	0	0.00	0	0	0.00	0.00	0.00
LCL[l]		N/A			0.00			0.00			0.00			0.00	−0.43	−0.47
UCL[m]		N/A			0.43			0.43			0.43			0.43	0.43	0.47
	Greek yogurt (25 g) *L. monocytogenes* RDCC[n] 1201	1.45	20	12	0.60	20	13	0.70	20	13	0.65	20	15	0.75	−0.15	−0.05
LCL		0.98			0.39			0.48			0.43			0.53	−0.40	−0.21
UCL		2.41			0.78			0.86			0.82			0.89	0.13	0.11
		4.38	5	5	1.00	5	5	1.00	5	5	1.00	5	5	1.00	0.00	0.00
LCL		1.72			0.57			0.57			0.57			0.57	−0.43	−0.47
UCL		11.15			1.00			1.00			1.00			1.00	0.43	0.47

[a] Results obtained following the alternative confirmation were identical to results obtain from confirmation by ISO 11290–1 reference method.
[b] MPN = Most probable number is calculated using the LCF MPN calculator ver. 1.6 provided by AOAC RI, with 95% confidence interval.
[c] N = Number of test portions.
[d] X = Number of positive test portions.
[e] POD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials.
[f] POD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials.
[g] POD_C = Candidate method confirmed positive outcomes divided by the total number of trials.
[h] POD_R = Reference method confirmed positive outcomes divided by the total number of trials.
[i] $dPOD_{CP}$ = Difference between the candidate method presumptive result and candidate method confirmed result POD values.
[j] $dPOD_C$ = Difference between the confirmed candidate method result and reference method confirmed result POD values.
[k] N/A = Not applicable.
[l] LCL = Lower confidence limit.
[m] UCL = Upper confidence limit.
[n] RDCC = Research and Development Culture Collection, Basingstoke, UK.

Table 7. Pre-collaborative study: SureTect Listeria species PCR assay, QuantStudio 5, presumptive versus confirmed and candidate versus FDA/BAM Ch.10—POD results

			Candidate presumptive (CP)			Candidate confirmed (CC)[a]			Candidate result (C)			Reference method (R)			C versus R	CP versus CC
Statistic	Matrix/Organism	MPN[b]	N[c]	X[d]	POD_{CP}[e]	N	X	POD_{CC}[f]	N	X	POD_C[g]	N	X	POD_R[h]	$dPOD_{C,R}$[i]	$dPOD_{CP,CC}$[j]
		N/A[k]	5	0	0.00	5	0	0.00	0	0	0.00	0	0	0.00	0.00	0.00
LCL[l]		N/A			0.00			0.00			0.00			0.00	−0.43	−0.47
UCL[m]		N/A			0.43			0.43			0.43			0.43	0.43	0.47
		0.65	20	14	0.70	20	14	0.70	20	14	0.70	20	10	0.50	0.20	0.00
LCL	Cottage cheese (4% fat; 25 g)	0.36			0.48			0.48			0.48			0.30	−0.10	−0.13
UCL	*L. monocytogenes* RDCC[n] 3010	1.06			0.86			0.86			0.86			0.70	0.45	0.13
		1.14	5	4	0.80	5	4	0.80	5	4	0.80	5	4	0.80	0.00	0.00
LCL		0.72			0.38			0.38			0.38			0.38	−0.43	−0.47
UCL		1.49			1.00			1.00			1.00			1.00	0.43	0.47
		N/A	5	0	0.00	5	0	0.00	0	0	0.00	0	0	0.00	0.00	0.00
LCL		N/A			0.00			0.00			0.00			0.00	−0.43	−0.47
UCL		N/A			0.43			0.43			0.43			0.43	0.43	0.47
		0.25	20	11	0.55	20	8	0.47	20	11	0.55	20	7	0.35	0.20	0.18
LCL	Blue cheese (25 g)	0.10			0.34			0.34			0.34			9.18	−0.10	−0.06
UCL	*L. monocytogenes* RDCC 3015	0.42			0.74			0.74			0.74			0.57	0.46	0.41
		0.19[o]	5	4	0.80	5	3	0.60	5	4	0.80	5	3	0.60	0.20	0.20
LCL		0.06			0.38			0.23			0.38			0.23	−0.31	−0.36
UCL		0.35			1.00			0.88			1.00			0.88	0.62	0.76
		N/A	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL		N/A			0.00			0.00			0.00			0.00	−0.43	−0.47
UCL		N/A			0.43			0.43			0.43			0.43	0.43	0.47
		1.45	20	13	0.65	20	13	0.65	20	13	0.65	20	15	0.75	−0.10	0.00
LCL	Greek yogurt (25 g)	0.98			0.43			0.43			0.43			0.53	−0.36	−0.13
UCL	*L. monocytogenes* RDCC 1201	2.41			0.82			0.82			0.82			0.89	0.18	0.13
		4.38	5	5	1.00	5	5	1.00	5	5	1.00	5	5	1.00	0.00	0.00
LCL		1.72			0.57			0.57			0.57			0.57	−0.43	−0.47
UCL		11.15			1.00			1.00			1.00			1.00	0.43	0.47
		N/A	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL		N/A			0.00			0.00			0.00			0.00	−0.43	−0.47
UCL		N/A			0.43			0.43			0.43			0.43	0.43	0.47
		N/A	20	10	0.50	20	10	0.50	20	10	0.50	20	10	0.50	0.00	0.00
LCL	Plastic surface (1” × 1”; Petri dish)	N/A			0.30			0.30			0.30			0.30	−0.28	−0.13
UCL	*L. monocytogenes* TCC[p] 1209	N/A			0.70			0.70			0.70			0.70	0.28	0.13
		N/A	5	3	0.60	5	3	0.60	5	3	0.60	5	4	0.80	−0.20	0.00
LCL		N/A			0.23			0.23			0.23			0.38	−0.62	−0.47
UCL		N/A			0.88			0.88			0.88			1.00	0.31	0.47
		N/A	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL	Ceramic surface (4” × 4”;	N/A			0.00			0.00			0.00			0.00	−0.43	−0.47
UCL	wall/floor tile)	N/A			0.43			0.43			0.43			0.43	0.43	0.47
	L. monocytogenes TCC 1209	N/A	20	6	0.30	20	6	0.30	20	6	0.30	20	1	0.05	0.25	0.00
LCL		N/A			0.15			0.15			0.15			0.00	0.01	−0.13

(continued)

Table 7. (continued)

Statistic	Matrix/Organism	MPN[b]	Candidate presumptive (CP)			Candidate confirmed (CC)[a]			Candidate result (C)			Reference method (R)			C versus R	CP versus CC
			N[c]	X[d]	POD_{CP}[e]	N	X	POD_{CC}[f]	N	X	POD_C[g]	N	X	POD_R[h]	$dPOD_{C,R}$[i]	$dPOD_{CP,CC}$[j]
UCL		N/A			0.52			0.52			0.52			0.24	0.48	0.13
		N/A	5	5	1.00	5	5	1.00	5	5	1.00	5	5	1.00	0.00	0.00
LCL		N/A			0.57			0.57			0.57			0.57	−0.43	−0.47
UCL		N/A			1.00			1.00			1.00			1.00	0.43	0.47
		N/A	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL		N/A			0.00			0.00			0.00			0.00	−0.43	−0.47
UCL		N/A			0.43			0.43			0.43			0.43	0.43	0.47
	Stainless steel surface (4” × 4”; sheet metal token)	N/A	20	10	0.50	20	10	0.50	20	10	0.50	20	14	0.70	−0.20	0.00
LCL		N/A			0.30			0.30			0.30			0.48	−0.45	−0.13
UCL	*L. monocytogenes* TCC 813	N/A			0.70			0.70			0.70			0.86	0.10	0.13
		N/A	5	5	1.00	5	5	1.00	5	5	1.00	5	5	1.00	0.00	0.00
LCL		N/A			0.57			0.57			0.57			0.57	−0.43	−0.47
UCL		N/A			1.00			1.00			1.00			1.00	0.43	0.47
		N/A	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL		N/A			0.00			0.00			0.00			0.00	−0.43	−0.47
UCL		N/A			0.43			0.43			0.43			0.43	0.43	0.47
		N/A	20	7	0.35	20	7	0.35	20	7	0.35	20	10	0.50	−0.15	0.00
LCL	Concrete surface (4” × 4”; slab)	N/A			0.18			0.18			0.18			0.30	−0.41	−0.13
UCL	*L. innocua* OCC[q] 1575	N/A			0.57			0.57			0.57			0.70	0.15	0.13
		N/A	5	3	0.60	5	3	0.60	5	3	0.60	5	4	0.80	−0.20	0.00
LCL		N/A			0.23			0.23			0.23			0.38	−0.62	−0.47
UCL		N/A			0.88			0.88			0.88			1.00	0.31	0.47

[a] Results obtained following the alternative confirmation were identical to results obtain from confirmation by FDA/BAM Ch. 10 reference method.
[b] MPN = Most probable number is calculated using the LCF MPN calculator ver. 1.6 provided by AOAC RI, with 95% confidence interval.
[c] N = Number of test portions.
[d] X = Number of positive test portions.
[e] POD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials.
[f] POD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials.
[g] POD_C = Candidate method confirmed positive outcomes divided by the total number of trials.
[h] POD_R = Reference method confirmed positive outcomes divided by the total number of trials.
[i] $dPOD_{CP}$ = Difference between the candidate method presumptive result and candidate method confirmed result POD values.
[j] $dPOD_C$ = Difference between the confirmed candidate method result and reference method confirmed result POD values.
[k] N/A = Not applicable.
[l] LCL = Lower confidence limit.
[m] UCL = Upper confidence limit.
[n] RDCC = Research and Development Culture Collection, Basingstoke, UK.
[o] High levels of background microflora were observed on the plates.
[p] TCC = Trials Culture Collection, Basingstoke, UK.
[q] OCC = Oxoid Culture Collection, Basingstoke, UK.

Table 8. Pre-collaborative study: Thermo Scientific SureTect Listeria species PCR assay, QuantStudio 5, presumptive vs. confirmed and candidate vs. ISO 11290–1—POD Results

			Candidate presumptive (CP)			Candidate confirmed (CC)[a]			Candidate result (C)			Reference method (R)			C versus R	CP versus CC
Statistic	Matrix/organism	MPN[b]	N[c]	X[d]	POD_{CP}[e]	N	X	POD_{CC}[f]	N	X	POD_C[g]	N	X	POD_R[h]	$dPOD_{C,R}$[i]	$dPOD_{CP,CC}$[j]
		N/A[k]	5	0	0.00	5	0	0.00	0	0	0.00	0	0	0.00	0.00	0.00
LCL[l]		N/A			0.00			0.00			0.00			0.00	−0.43	−0.47
UCL[m]		N/A			0.43			0.43			0.43			0.43	0.43	0.47
	Greek yogurt (25 g)	1.45	20	13	0.65	20	13	0.70	20	13	0.65	20	15	0.75	−0.15	0.00
LCL	*L.monocytogenes*	0.98			0.43			0.48			0.43			0.53	−0.40	−0.13
UCL	RDCC[n] 1201	2.41			0.82			0.86			0.82			0.89	0.13	0.13
		4.38	5	5	1.00	5	5	1.00	5	5	1.00	5	5	1.00	0.00	0.00
LCL		1.72			0.57			0.57			0.57			0.57	−0.43	−0.47
UCL		11.15			1.00			1.00			1.00			1.00	0.43	0.47

[a] Results obtained following the alternative confirmation were identical to results obtain from confirmation by ISO 11290–1 reference method.
[b] MPN = Most probable number is calculated using the LCF MPN calculator ver. 1.6 provided by AOAC RI, with 95% confidence interval.
[c] N = Number of test portions.
[d] X = Number of positive test portions.
[e] POD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials.
[f] POD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials.
[g] POD_C = Candidate method confirmed positive outcomes divided by the total number of trials.
[h] POD_R = Reference method confirmed positive outcomes divided by the total number of trials.
[i] $dPOD_{CP}$ = Difference between the candidate method presumptive result and candidate method confirmed result POD values.
[j] $dPOD_C$ = Difference between the confirmed candidate method result and reference method confirmed result POD values.
[k] N/A = Not applicable.
[l] LCL = Lower confidence limit.
[m] UCL = Upper confidence limit.
[n] RDCC = Research and Development Culture Collection, Basingstoke, UK.

Five samples for each matrix were not inoculated. For 25 g, 10 g inoculated bulk sample was combined with 15 g non-inoculated product to form each 25 g sample portion.

For all matrixes, enrichment was carried out as detailed above in the section **E** section above. After enrichment the lysis step was performed using the SimpliAmp thermal cycler. Samples were then analyzed using the SureTect Listeria species PCR assay on the 7500 Fast and QuantStudio 5 instruments and results were interpreted with RapidFinder Express (v2.0 or later) and RapidFinder Analysis (v1.1 or later) software, respectively. Following the final incubation time point, all positive samples regardless of presumptive results, were confirmed using the confirmation procedure previously described in section **I**.

Most Probable Number Analysis

The level of RDCC 3010 *L. monocytogenes* in the low-level inoculum for all 25 g test portions of cottage cheese was determined by MPN on the day of analysis by evaluating 5 × 50 g, 20 × 25 g (reference method test portions), and 5 × 10 g inoculated samples. The level of RDCC 3010 *L. monocytogenes* in the high-level inoculum for all 25 g test portions of cottage cheese was determined by MPN on the day of analysis by evaluating 5 × 50 g, 20 × 25 g (reference method test portions), and 5 × 10 g inoculated samples. To the 50 g portions 450 mL reference method enrichment broth was added, to the 25 g portions 225 mL reference method enrichment broth was added, and to the 10 g portions 90 mL reference method enrichment broth was added.

The level of RDCC 3015 *L. monocytogenes* in the low-level inoculum for all 25 g test portions of blue cheese was determined by MPN on the day of analysis by evaluating 5 × 50 g, 20 × 25 g (reference method test portions), and 5 × 10 g inoculated samples. The level of RDCC 3015 *L. monocytogenes* in the high-level inoculum for all 25 g test portions of blue cheese was determined by MPN on the day of analysis by evaluating 5 × 50 g, 20 × 25 g (reference method test portions), and 5 × 10 g inoculated samples. To the 50 g portions 450 mL reference method enrichment broth was added, to the 25 g portions 225 mL reference method enrichment broth was added, and to the 10 g portions 90 mL reference method enrichment broth was added.

The level of RDCC 1201 *L. monocytogenes* in the low-level inoculum for all 25 g test portions of Greek yoghurt was determined by MPN on the day of analysis by evaluating 5 × 50 g, 20 × 25 g (reference method test portions), and 5 × 10 g inoculated samples. The level of RDCC 1201 *L. monocytogenes* in the high-level inoculum for all 25 g test portions of Greek yoghurt was determined by MPN on the day of analysis by evaluating 5 × 50 g, 20 × 25 g (reference method test portions), and 5 × 10 g inoculated samples. To the 50 g portions 450 mL reference method enrichment broth was added, to the 25 g portions 225 mL reference method enrichment broth was added, and to the 10 g portions 90 mL reference method enrichment broth was added.

The number of positives from the three test levels was used to calculate the MPN using the LCF MPN calculator (version 1.6) provided by AOAC RI. As per criteria outlined in Appendix J of AOAC *Official Methods of Analysis*, fractional positive results were obtained for cottage cheese (25 g), blue cheese (25 g), and Greek yoghurt (25 g) at 22 h for the SureTect Listeria species PCR assay.

Results

Prior to inoculation, an APC result of 2.00 × 107 cfu/g was obtained from the cottage cheese, 9.40 × 106 cfu/g was obtained for the blue cheese, and 4.60 × 106 cfu/g was obtained for the Greek yoghurt. For the blue cheese, a high level of background microflora was observed.

The POD was calculated as the number of positive outcomes divided by the total number of trials. The POD was calculated for the candidate presumptive results, POD_{CP}, the candidate confirmatory results, POD_{CC}, the difference in the candidate presumptive and confirmatory results, $dPOD_{CP}$, presumptive candidate results that confirmed positive, POD_C, the reference method, POD_R, and the difference in the confirmed candidate and reference methods, $dPOD_C$. The POD analysis between the SureTect Listeria species PCR assay and the FDA/BAM Ch. 10 reference method indicated that there was no significant difference at the 5% level between the number of positive results by the methods at all time points evaluated, regardless of the reference method. The POD analysis between the SureTect Listeria species PCR assay presumptive and confirmed results indicated that there was no significant difference at the 5% level for all methods at all time points following both the traditional confirmation procedure and the alternative confirmation procedure. A summary of POD analyses is presented in Tables 5–8.

Discussion

Collaborative Study

No negative feedback was provided regarding the performance of the SureTect Listeria species PCR assay. Two false-positive (FP) results in the uninoculated samples were observed from the analysis of the SureTect Listeria species PCR assay. The FP rate is 1.39%. The FP PCR results may have arisen from cross-contamination when preparing the enriched samples for PCR while manipulating multiple highly contaminated enrichment bags. During the collaborative study, both the QuantStudio 5 and the 7500 Fast were evaluated. The data suggests the instruments are considered equivalent.

Overall, the data generated during this evaluation demonstrate the reproducibility of this method. No statistically significant differences were observed between the presumptive method and the confirmed results. All candidate method presumptive positives were confirmed by the alternative method confirmation procedure.

Pre-Collaborative Study

The data from these studies supports the product claims of the Thermo Scientific SureTect Listeria species PCR assay as a reliable detection method for *Listeria* species in a broad range of foods and environmental samples. The POD analysis results for the matrix studies demonstrated that there were no statistically significant differences between the candidate method and any of the reference methods for all samples tested.

Recommendations

It is recommended that the Thermo Scientific SureTect Listeria species PCR assay receives First Action *Official Methods*^{SM} for the detection of *Listeria* species from a broad range of foods and environmental samples.

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Conflict of Interest

None declared.

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