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Research Article

MICROBIOLOGICAL METHODS

Evaluation of the Thermo Scientific™ SureTect™ *Listeria monocytogenes* PCR Assay in a Broad Range of Foods and Selected Environmental Surfaces: Pre-Collaborative and Collaborative Study, First Action 2021.05

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Abstract

Background: The Thermo Scientific™ SureTect™ Listeria monocytogenes PCR Assay uses Solaris reagents for performing PCR for the rapid and specific detection of *Listeria monocytogenes* in a broad range of foods and selected environmental surfaces.

Objective: To demonstrate reproducibility of the SureTect Listeria monocytogenes PCR Assay in a collaborative study using a challenging matrix, full-fat cottage cheese (25 g). To extend the scope of the method.

Method: In the collaborative study, the candidate method was compared to the United States Food and Drug Administration/*Bacteriological Analytical Manual* (FDA/BAM) Chapter 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). Eighteen participants from 10 laboratories located within the United States and Europe were solicited for the collaborative study, with 12 participants submitting valid data. Three levels of contamination were evaluated for each matrix. Statistical analysis was conducted according to the probability of detection (POD) statistical model. In addition, to extend the scope, six 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*.

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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.
Conclusions: 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 were reported, highlighting the ease of use, reproducibility, and robustness of the 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 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 monocytogenes PCR Assay is based on use of Solaris™ reagents for performing PCR. Dye-labeled probes target unique DNA sequences specific to *Listeria monocytogenes* and an internal positive control (IPC). Target DNA, if present, is detected by real-time PCR. 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 monocytogenes PCR Assay was validated according to the current AOAC Guidelines (2) in an AOAC *Performance Tested Method*℠ (PTM) study in a broad range of foods and selected environmental surfaces. The SureTect Listeria monocytogenes PCR Assay was awarded PTM certificate 071304 in July 2013 for 12 food matrixes and two environmental surfaces. In addition, to extend the scope, six matrix studies were performed against the United States Food and Drug Administration/*Bacteriological Analytical Manual* (FDA/BAM) Chapter 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 matrix studies were performed by Thermo Fisher Scientific in Basingstoke, UK: cottage cheese (4% fat; 25 g), blue cheese (25 g), Greek yogurt (25 g), plastic surface (1″ × 1″; polystyrene Petri dish), ceramic surface (4″ × 4″; wall/floor tiles), and stainless-steel surface (4″ × 4″; slab, 304 series, brush-finished). *See* Table 1 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 SureTect Listeria monocytogenes PCR Assay to the FDA/BAM Chapter 10 reference method for full-fat cottage cheese (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 Chapter 10 reference method and the SureTect Listeria monocytogenes PCR Assay. The cottage cheese was artificially contaminated with a heat-stressed liquid culture of *L. monocytogenes* American Type Culture Collection (ATCC, Manassas, VA) 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 non-inoculated 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 monocytogenes 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 non-inoculated test portion for determining the total aerobic plate count (APC) following the U.S. Food and Drug Administration, *Bacteriological Analytical Manual* Chapter 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 agar (SBA) from a Q Laboratories (Cincinnati, OH) frozen stock culture stored at −70°C. The organism was incubated for 24 ± 2 h at 35 ± 1°C. Isolated colonies were picked to 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 a 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

Table 1. PTM validation study summary for the Thermo Scientific SureTect Listeria monocytogenes PCR assay

Thermo Scientific SureTect Listeria species PCR Assay—PTM 061302						
Original PTM certificate issued[a]	Matrixes	Sample size	Enrichment media/ dilution	Enrichment time, h	Enrichment temp., °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 finish)	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 dilution	Enrichment time, h	Enrichment temp., °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 dilution	Enrichment time, h	Enrichment temp., °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	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 matrixes 2021	Cottage cheese (4% fat)	25 g	24 LEB/1-in-10	22–30	37 ± 1	FDA/BAM[d]
	Blue cheese	25 g	24 LEB/1-in-10	22–30	37 ± 1	FDA/BAM
	Greek yogurt	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

[a] AOAC PTM Certificate No. 061302.
[b] EN ISO 11290–1.
[c] Matrixes approved with First Action.
[d] FDA/BAM Chapter 10.

incubation, the % injury was determined using the following formula, and the inoculating culture must have a % injury of 50–80:

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

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

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 material was inoculated in a drop-wise manner with an appropriate volume, which was small enough to minimize potential effects on the sample and at a dilution that 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 were directly sampled and packaged in sterile Whirl-Pak®.

Table 2. Participation of each collaborating laboratory

Collaborator ID no.	Participating lab	Analyst	Instrument	Participated[a]
1	1	2	7500 Fast	Y
2	2	2	QS5	Y
3	3	1	QS5	Y
4	4	2	7500 Fast	Y
5	5	1	QS5	Y
6[b]	6	2	7500 Fast	N
7[c]	3	2	7500 Fast	N
8[d]	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] Collaborator voluntarily withdrew prior to testing.
[c] Collaborator could not participate due to shipping issues.
[d] Collaborator dropped due to an incomplete data set for the reference method.

After inoculation, the test matrix was held for 48–72 h at refrigerated temperature (2–8°C) prior to analysis.

The level of each organism 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 each organism 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) provided by AOAC Research Institute.

Test Portion Distribution

All portions were labeled with a randomized, blind-coded three-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 e-mail 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 monocytogenes PCR Assay test portions (25 g) were enriched with 225 mL of 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 monocytogenes 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 monocytogenes PCR Assay. Out of the seven collaborators 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 used.

Regardless of the presumptive result, all test portions were confirmed following the FDA/BAM Chapter 10 reference method. The primary enrichments were streaked onto Oxford agar (OX) and Agar Listeria according to O&A medium at 22 h for SureTect enrichments and 24 h and 48 h for FDA/BAM enrichments. OX plates were incubated at 35°C for up to 48 h, and O&A plates were incubated at 37°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°C for 24–48 h. Isolated colonies were also stabbed to 5% SBA and incubated at 35°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 monocytogenes PCR Assay test portions were also confirmed following an alternative confirmation procedure. Regardless of presumptive results, all SureTect Listeria monocytogenes 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.

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 nalidixic acid, 40 mg/L cycloheximide) and analyzed according to the procedures in the FDA/BAM Chapter 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 were analyzed using the probability of detection (POD) statistical model (6) and conducted using the LCF AOAC Binary Data Interlaboratory Study Workbook v2.3 (Virginia Beach, VA). 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 inter-laboratory correlation coefficient (ICC) were calculated.

AOAC *Official Method*SM 2021.05 *Listeria monocytogenes* in a Broad Range of Foods and Selected Environmental Surfaces Thermo Scientific SureTect Listeria monocytogenes PCR Assay First Action 2021

[Applicable at the time of the submission to the detection of *L. monocytogenes* 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), 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), ice cream (vanilla; 25 g), stainless steel (slab, brushed finish; 4″ × 4″), and plastic (large polystyrene petri dish; 4″ × 4″). 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* (3) which was the most current version 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 and ceramic (wall/floor tiles; 4″ × 4″), plastic (polystyrene Petri dish; 1″ × 1″), and stainless-steel (slab, brushed finish; 4″ × 4″) 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) reference methods.

See Table **2021.05A** for a summary of results of the inter-laboratory study.

See Table **2021.05B** 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) *Listeria monocytogenes* is a food pathogen that can cause listeriosis. Pregnant women, adults aged 65 or over, and people with weakened immune systems 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 monocytogenes PCR Assay is based on the use of Solaris reagents for performing PCR, for the rapid and specific detection of *L. monocytogenes* in a broad range of food types and selected environmental surfaces. The method uses dye-labeled probes to target unique DNA sequences specific to *L. monocytogenes* and an internal positive control (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: product 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.

Table 2021.05A. Summary of results for the detection of *Listeria monocytogenes* in 25 g cottage cheese test portions by the Thermo Scientific SureTect Listeria monocytogenes PCR assay vs. FDA/BAM Chapter 10 in a collaborative study (QuantStudio 5 and 7500 Fast PCR instruments)

Method[a]	Thermo Scientific SureTect Listeria monocytogenes PCR assay		
Inoculation level	Uninoculated	Low	High
Candidate presumptive positive/total no. of samples analyzed	4/144	70/144	144/144
Candidate presumptive LPOD (CP)	0.03 (0.00, 0.70)	0.49 (0.41, 0.57)	1.00 (0.97, 1.00)
s_r[b]	0.17	0.551	0.00
s_L[c]	0.00	0.00	0.00
s_R[d]	0.17	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]	0.00 (−0.03, −0.03)	0.04 (−0.07, 0.15)	0.00 (−0.03, 0.03)
dLPOD (candidate presumptive vs. candidate confirmed)[g,h]	0.03 (−0.01, −0.06)	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] Both the alternative confirmation procedure and the confirmation procedure following the FDA BAM Chapter 5 produced identical results.
[f] 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] Interlaboratory Correlation Coefficient.

(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 monocytogenes PCR Assay and Pathogen Assay File: ListeriaSpp_SureTect_QS5 version 1.0 or later, cat. nos. A36320 (desktop), A36328 (laptop).

(g) *Real-Time PCR Instrument*.—Applied Biosystems 7500 Fast with Applied Biosystems™ RapidFinder Express Software v2.0 or later for use with SureTect Listeria monocytogenes PCR Assay and Pathogen Assay File: Listeria monocytogenes 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.—

(a) *SureTect Listeria monocytogenes 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)*.—One tube.

(4) *Lysis Reagent 2 (clear colorless liquid, red cap)*.—One tube.

Table 2021.05B. Comparative results for the detection of *Listeria monocytogenes* in 25 g cottage cheese test portions by the Thermo Scientific SureTect Listeria monocytogenes PCR assay vs. FDA/BAM Chapter 10 in a collaborative study (QuantStudio 5 and 7500 Fast PCR instruments)

Organism: Listeria monocytogenes			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	$dLPOD_{C,\,R}$	$dLPOD_{CP,\,CC}$
							Uninoculated 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	1	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[a]	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		0.000	12	0	0.000	12	0	0.000	12	0	0.000	0.000	0.000
		16	12	1	0.083	12	0	0.000	12	0	0.000	12	0	0.000	0.000	0.083
		17	12		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	4	0.03	144	0	0.00	144	0	0.00	144	0	1.00	0.00	0.03
LCL*e*	NA				0.00			0.00			0.00			0.00	−0.03	−0.01
UCL*f*	NA				0.07			0.03			0.03			0.03	0.03	0.06
s_r[g]					0.17			0.00			0.00			0.00		
s_L[h]					0.00			0.00			0.00			0.00		
s_R[i]					0.17			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		

(continued)

Table 2021.05B. (continued)

Organism: Listeria monocytogenes			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	$dLPOD_{C,\,R}$	$dLPOD_{CP,\,CC}$
							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
		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		

(continued)

Table 2021.05B. (continued)

Organism: Listeria monocytogenes			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	$dLPOD_{C,R}$	$dLPOD_{CP,CC}$
								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
	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 Chapter 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 or was dropped due to extremely contamination.
[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.

(5) *SureTect Listeria monocytogenes PCR Tubes*.—12 strips of 8 tubes, one pellet each.
(6) *PCR caps*.—12 strips of 8 caps.

(b) *Oxoid 24 Listeria Enrichment Broth (24 LEB), dehydrated*.—Cat. no. CM1107B (base, 500 g).
(c) *Oxoid 24 LEB Selective Supplement*.—Cat. no. SR0243E, 10 ×, 5 mL/bottle.
(d) *Oxoid24 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. Available through the Thermo Fisher Microbiology ordering process.
(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, 500 g).
(g) *Oxoid Brilliance*™ *Listeria Selective Supplement*.—Cat. no. SR0227E.
(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 pipet*.—1–10 mL.
(m) *96-well rack*.
(n) *Filtered pipet 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 pipet*.—10–100 μL or electronic adjustable spacing, multichannel pipet, 10–100 μL.
(r) *Single-channel stepper pipet*.—10–100 μL.
(s) *Filtered pipet 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 pipet*.—5–50 μL.
(y) *Filtered pipet tips*.—10–100 μL.

D. General Instructions.—

(a) *Guidelines for sample enrichment*.
(1) For preparation of master suspensions, follow the instructions of BS EN ISO 6887—Microbiology of food and animal feed. Preparation of test samples, initial suspension and decimal dilutions for microbiological examination (Parts 1–6) (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 vortexing. 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, cantaloupe melon, cooked prawns, cooked sliced turkey, ice cream, pork frankfurters, fresh raw ground/minced beef, fresh raw ground/minced turkey, fresh raw ground/minced pork, pasteurized 2% fat milk, raw pork sausage, raw cod, pasteurized Brie cheese, cooked sliced ham, fresh bagged lettuce, blue cheese, cottage cheese, Greek-style yogurt (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 (two vials) of reconstituted 24 LEB Selective Supplement. Pre-warm to room temperature (23 ± 3°C). Transfer the food sample to a homogenizer bag, and then add the room-temperature media (23 ± 3°C) as indicated. Add 225 mL of prepared media to 25 g sample. Add 10 mL of Oxoid 24 LEB Buffer Supplement per 25 g of 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 of prepared media to 25 g sample. Add 20 mL of Oxoid 24 LEB Buffer Supplement per 25 g of 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.

(c) *Environmental surface swabs and sponges (stainless steel, plastic, ceramic).*—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 both, 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 of reconstituted Oxoid 24 LEB Buffer Supplement per 100 mL of 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, and 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, and then add 10 µL of 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 pipet 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 of Lysis Reagent 2 to the lysis tube.

(*7*) Transfer 10 µL of the enriched sample (or diluted cocoa/chocolate sample) to a lysis tube. For the negative extraction controls, transfer 10 µL of sterile enrichment media to a lysis tube. Ensure that the pipet 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, and 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 **2021.05C**.

(*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.

(*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, and then add 10 µL of 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 pipet 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 of Lysis Reagent 2 to the lysis tube.

(*9*) Transfer 10 µL of the enriched sample to a lysis tube. For the negative extraction controls, transfer 10 µL of sterile enrichment media to a lysis tube. Ensure that the pipet 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, and 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.*

Table 2021.05C. Thermocycler heating steps

Step	Temp., °C	Temp.
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.

(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, and 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 monocytogenes 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, and 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), and 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, and 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 pipet tip. Do not use a tip containing lysate.

(4) Uncap the lysis tubes.

(5) Transfer 20 µL of the 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, and 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, and 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, and 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 monocytogenes 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 monocytogenes PCR tubes in a suitable rack with a PCR carry plate, and 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), and 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, and 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 pipet tip. Do not use a tip containing lysate.

(e) Uncap the lysis tubes.

(f) Transfer 20 µL of the 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.

(g) 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.

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

(i) 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.

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

(k) 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.

(l) 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 2021.05A), click Results, and then click the sub-tab for the desired view of the data.
(1) *Summary*.—Plate format.
(2) *Results*.—Table format.
(3) *Details*.—Amplification plot.
(4) In the RapidFinder Express Software (Figure 2021.05B), 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, and then click View Details to see replicate information about samples.

I. Candidate Confirmation.—

(a) Thermo Scientific SureTect Listeria monocytogenes PCR Assay Test Result Confirmation.
(1) Streak 10 μL of the primary enrichment onto BLA and incubating 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) FDA/BAM Chapter 10.
(b) EN ISO 11290.
(c) USDA/FSIS MLG 8.12 (12).
(d) any other appropriate national reference method.

or using:

(e) an appropriate *Official Method of Analysis* 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.

(4) All Thermo Scientific SureTect Listeria monocytogenes PCR Assay test results in the collaborative study were confirmed following FDA/BAM Chapter 10 in addition to the alternative confirmation.

Results

Collaborative Study

The collaborative study involved a method comparison evaluation of the Thermo Scientific SureTect Listeria monocytogenes PCR Assay to the FDA/BAM Chapter 10 reference method. A total of 18 participants throughout the continental United States and Europe participated in this study. In six of

Result icon	Result
	Positive result
	Negative result
	Result warning

Figure 2021.05A. RapidFinder Analysis Software results icons

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

Figure 2021.05B. RapidFinder Express Software results icons

the laboratories, two separate analysts participated, and in one of the laboratories, three separate analysts participated. The remaining three laboratories each had one participating analyst. Twelve out of the 18 participants submitted valid data. One laboratory voluntarily withdrew from participation prior to initiating testing, one laboratory was removed due to shipping issues, and one laboratory had incomplete 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 monocytogenes PCR Assay and the FDA/BAM Chapter 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 Chapter 10 reference method and using the Thermo Scientific SureTect Listeria monocytogenes PCR Assay, indicated an absence of indigenous *L. monocytogenes*. Ten replicate 25 g test portions (randomly sampled from 50% of the total packages used in the analysis) were screened for the presence of *L. monocytogenes*. All test portions produced negative results for the target analyte.

Table **2021.05A** summarizes the inter-laboratory results. As per criteria outlined in Appendix J of the AOAC Validation Guidelines, fractional positive results were obtained. Detailed results for each laboratory are presented in Table 4. The level of *Listeria monocytogenes* 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 **2021.05B**.

Cottage Cheese (4% Fat)

Detailed results of the LPOD statistical analysis are presented in Table **2021.05B** 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 Thermo Scientific SureTect Listeria monocytogenes PCR Assay with 70 out of 144 test portions ($LPOD_{CC}$ of 0.49) confirming positive (following both the alternative confirmation and the FDA/BAM Chapter 10 reference method). For samples that produced presumptive positive results by the SureTect Listeria monocytogenes PCR Assay, 70 out of 144 samples confirmed positive ($LPOD_C$ of 0.49; value include 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 intervals 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 Chapter 10 reference method).

For the high inoculation level, 144 out of 144 test portions ($LPOD_{CP}$ of 1.00) were reported as presumptive positive by the SureTect Listeria monocytogenes 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 Chapter 10 reference method). For samples that produced presumptive positive results by the SureTect Listeria monocytogenes 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 intervals 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 Chapter 10 reference method).

For the noninoculated controls, four out of 144 samples ($LPOD_{CP}$ of 0.03) produced a presumptive positive result by the

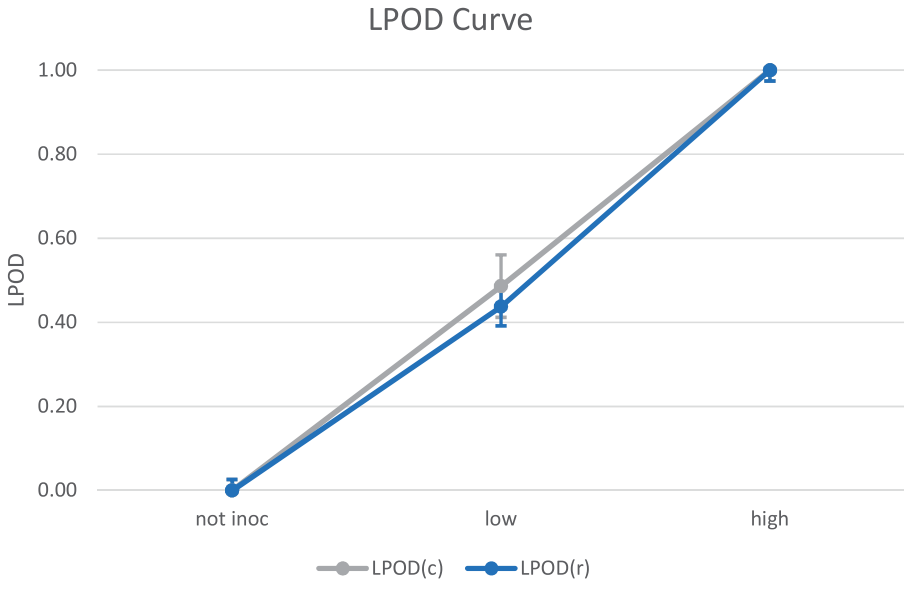

Figure 1. LPOD values of SureTect Listeria monocytogenes PCR Assay and FDA/BAM Chapter 10 for the collaborative study of cottage cheese.

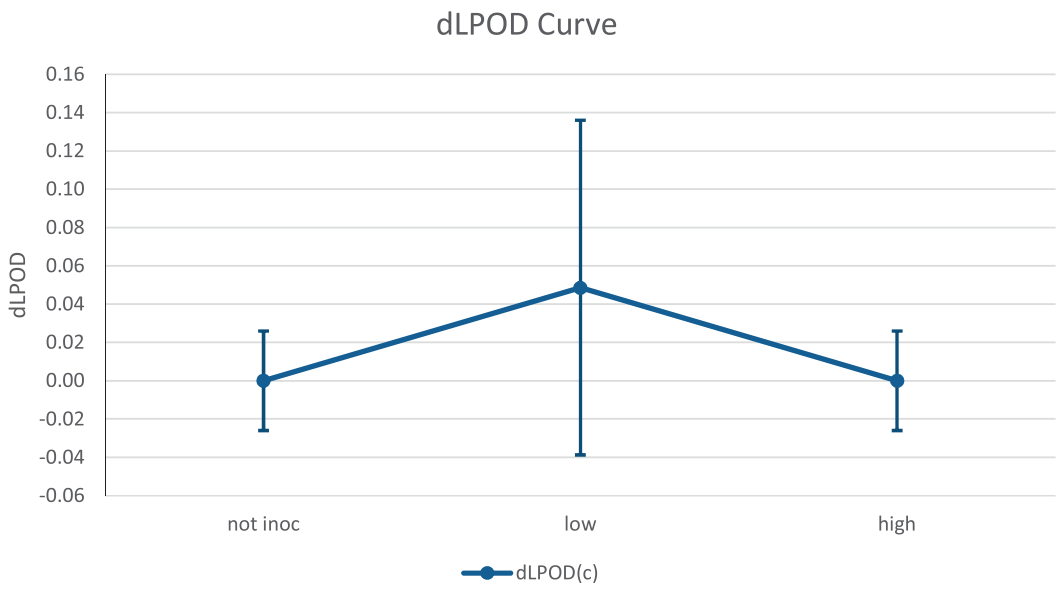

Figure 2. dLPODC values of SureTect Listeria monocytogenes PCR Assay and FDA/BAM Chapter 10 for the collaborative study of cottage cheese.

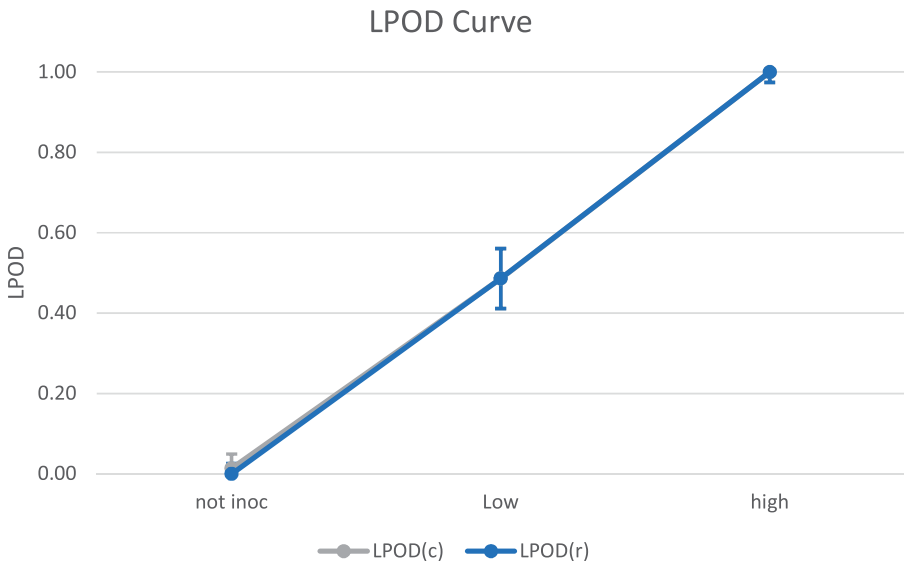

Figure 3. LPOD values of Presumptive Thermo Scientific SureTect Listeria monocytogenes PCR Assay and Confirmed Results for the collaborative study of cottage cheese.

SureTect Listeria monocytogenes 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 Chapter 10 reference method). The four presumptive positive 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 that could not be confirmed 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 intervals 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 Chapter 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

Study overview—Additional matrixes.—The following matrix studies were performed by Thermo Fisher Scientific laboratory in Basingstoke, UK: cottage cheese (4% fat; 25 g), blue cheese (25 g), Greek yogurt (25 g), plastic surface (1″ × 1″; polystyrene Petri

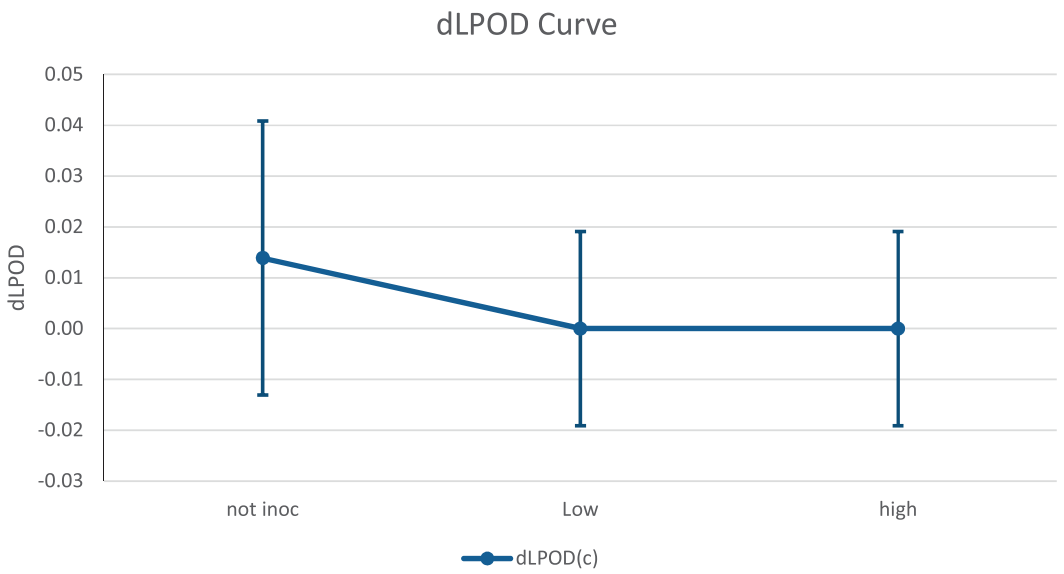

Figure 4. dLPODCP values of Presumptive SureTect Listeria monocytogenes PCR Assay and Confirmed Results for the collaborative study of cottage cheese.

Table 3. Results of aerobic plate count for the 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] 25 g Samples analyzed by the FDA/BAM Chapter 3 reference method.
[b] NA = Not applicable.

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

Collaborator	Temp. 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	NA[a]
10	NA
11	NA
12	NA
13	NA
14	NA
15	3.8
16	3.8
17	3.8
18	NA

[a] NA = Not applicable.

dish), ceramic surface (4″ × 4″; wall/floor tile), and stainless-steel surface (4″ × 4″; slab, brushed finish).

All food matrixes were obtained from local supermarkets or food wholesale companies. For the reference method, the FDA/BAM Chapter 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°C for 22 h. After incubation, samples were tested using the SureTect Listeria monocytogenes PCR Assay on the 7500 Fast and QuantStudio 5 instruments and results were interpreted with RapidFinder Express (v2.0 or later) and, respectively, RapidFinder Analysis (v1.1 or later) software. Presumptive positives were streaked onto a suitable selective plate and confirmed using the Microbact 12 L kit and the FDA/BAM Chapter 10 confirmation method.

The results of the pre-screen showed that none of the matrixes were naturally contaminated. When comparing to the FDA/BAM Chapter 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 yogurt, 60 unpaired portions for plastic (1″ × 1″) surface, 60 unpaired portions for ceramic (4″ × 4″) surface, and 60 unpaired portions for stainless-steel (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 yogurt matrix.

Within each sample set, there were five uninoculated portions (0 CFU/test portion), 20 low level inoculated portions

(0.2–2 cfu/test portion), and five 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 yogurt matrixes, which were spiked with a liquid, heat stressed culture. The cottage cheese matrix was spiked with Research and Development Culture Collection (RDCC; Basingstoke, UK) 3010 *L. monocytogenes*, the blue cheese matrix was spiked with RDCC 3015 *L. monocytogenes*, the Greek yogurt 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*, and the stainless-steel surface was spiked with TCC 813 *L. monocytogenes*.

For the cottage cheese, blue cheese, and Greek yogurt matrixes, spiking cultures were prepared by removing the required strains the −80°C culture collection freezer, subcultured to TSA, and incubating plates at 37 ± 1°C for 24 ± 1 h. After incubation, the test strains were subcultured into 10 mL of tryptone soya broth (TSB) and incubated at 37 ± 1°C for 24 ± 1 h. For the cottage cheese and Greek yogurt, after incubation, the 10 mL of 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 challenging high background 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 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, and stainless-steel environmental surfaces, spiking cultures were prepared by removing the required strains the −80°C culture collection freezer, subcultured to TSA, and incubating plates at 37 ± 1°C for 24 ± 1 h. After incubation, the test strains were subcultured into 10 mL of TSB and incubated at 37 ± 1°C for 24 ± 1 h. After this incubation, 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 onto the plastic (1″ × 1″) surface, ceramic (4″ × 4″) surface, and stainless-steel (4″ × 4″) surface by spot inoculation. The surfaces were allowed to dry at room temperature and 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; Basingstoke, UK) 640 *Enterobacter faecalis*, was also spiked on to the surfaces. 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 pipet 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 yogurt bulk material, the pre-prepared spiking culture was spot-inoculated with a pipet 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.

Five samples for each matrix were not inoculated. For 25 g, 10 g of the 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 *Sample Preparation* section. After enrichment the lysis step was performed using the Applied Biosystems SimpliAmp Thermal Cycler. Samples were then analyzed using the Thermo Scientific SureTect Listeria monocytogenes 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 samples regardless of presumptive results were confirmed using the confirmation procedure previously described in the *Confirmation* section.

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 of the reference method enrichment broth was added, to the 25 g portions 225 mL of reference method enrichment broth was added, and to the 10 g portions 90 mL of 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 of the reference method enrichment broth was added, to the 25 g portions 225 mL of reference method enrichment broth was added, and to the 10 g portions 90 mL of 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 yogurt 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 yogurt was

Table 5. Pre-collaborative: Thermo Scientific SureTect Listeria monocytogenes PCR assay, 7500 Fast, presumptive vs. confirmed and candidate vs. FDA/BAM Ch.10—POD results

			Candidate presumptive (CP)			Candidate confirmed (CC)[a]			Candidate result (C)			Reference method (R)			C vs. R	CP vs. 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]
	Cottage cheese (4% fat; 25 g)	NA[k]	5	0	0.00	5	0	0.00	0	0	0.00	0	0	0.00	0.00	0.00
LCL[l]	*Listeria monocytogenes* RDCC[n]	NA			0.00			0.00			0.00			0.00	−0.43	−0.47
UCL[m]	3010	NA			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		0.36			0.43			0.48			0.43			0.30	−0.15	−0.21
UCL		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
	Blue cheese (25 g) *Listeria mono-*	NA	5	0	0.00	5	0	0.00	0	0	0.00	0	0	0.00	0.00	0.00
LCL	*cytogenes* RDCC 3015	NA			0.00			0.00			0.00			0.00	−0.43	−0.47
UCL		NA			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		0.10			0.34			0.34			0.34			9.18	−0.10	−0.06
UCL		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
	Greek yogurt (25 g) *Listeria*	NA	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL	*monocytogenes* RDCC 1201	NA			0.00			0.00			0.00			0.00	−0.43	−0.47
UCL		NA			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		0.98			0.43			0.43			0.43			0.53	−0.36	−0.13
UCL		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
	Plastic surface (1″ × 1″; polysty-	NA	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL	rene Petri dish) *Listeria mono-*	NA			0.00			0.00			0.00			0.00	−0.43	−0.47
UCL	*cytogenes* TCC[p] 1209	NA			0.43			0.43			0.43			0.43	0.43	0.47
		NA	20	10	0.50	20	10	0.50	20	10	0.50	20	10	0.50	0.00	0.00
LCL		NA			0.30			0.30			0.30			0.30	−0.28	−0.13
UCL		NA			0.70			0.70			0.70			0.70	0.28	0.13
		NA	5	3	0.60	5	3	0.60	5	3	0.60	5	4	0.80	−0.20	0.00
LCL		NA			0.23			0.23			0.23			0.38	−0.62	−0.47
UCL		NA			0.88			0.88			0.88			1.00	0.31	0.47
	Ceramic surface (4″ × 4″; wall/	NA	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL	floor tile) *Listeria monocyto-*	NA			0.00			0.00			0.00			0.00	−0.43	−0.47
UCL	*genes* TCC 1209	NA			0.43			0.43			0.43			0.43	0.43	0.47
		NA	20	6	0.30	20	6	0.30	20	6	0.30	20	1	0.05	0.25	0.00
LCL		NA			0.15			0.15			0.15			0.00	0.01	−0.13
UCL		NA			0.52			0.52			0.52			0.24	0.48	0.13
		NA	5	5	1.00	5	5	1.00	5	5	1.00	5	5	1.00	0.00	0.00
LCL		NA			0.57			0.57			0.57			0.57	−0.43	−0.47
UCL		NA			1.00			1.00			1.00			1.00	0.43	0.47
	Stainless steel surface (4″ × 4″;	NA	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL	slab, bushed finish) *Listeria*	NA			0.00			0.00			0.00			0.00	−0.43	−0.47
UCL	*monocytogenes* TCC l813	NA			0.43			0.43			0.43			0.43	0.43	0.47
		NA	20	10	0.50	20	10	0.50	20	10	0.50	20	14	0.70	−0.20	0.00
LCL		NA			0.30			0.30			0.30			0.48	−0.45	−0.13
UCL		NA			0.70			0.70			0.70			0.86	0.10	0.13

(continued)

Table 5. (continued)

Statistic	Matrix/Organism	MPN[b]	Candidate presumptive (CP)			Candidate confirmed (CC)[a]			Candidate result (C)			Reference method (R)			C vs. R	CP vs. 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]
		NA	5	5	1.00	5	5	1.00	5	5	1.00	5	5	1.00	0.00	0.00
LCL		NA			0.57			0.57			0.57			0.57	−0.43	−0.47
UCL		NA			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 FDA/BAM Chapter 10 reference method.
[b] MPN = Most probable number is calculated using the LCF MPN calculator v. 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] NA = Not applicable.
[k] LCL = Lower confidence limit.
[l] UCL = Upper confidence limit.
[m] RDCC = Research and Development Culture Collection, Basingstoke, UK.
[n] High levels of background microflora were observed on the plates.
[o] TCC = Trials Culture Collection, Basingstoke, UK.

Table 6. Pre-collaborative: Thermo Scientific SureTect Listeria monocytogenes PCR Assay, 7500 Fast, presumptive vs. confirmed and candidate vs. ISO 11290–1—POD results

Statistic	Matrix/organism	MPN[b]	Candidate presumptive (CP)			Candidate confirmed (CC)[a]			Candidate result (C)			Reference method (R)			C vs. R	CP vs. 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]
	Greek yogurt (25 g)	NA[k]	5	0	0.00	5	0	0.00	0	0	0.00	0	0	0.00	0.00	0.00
LCL[l]	*Listeria monocytogenes* RDCC[n]	NA			0.00			0.00			0.00			0.00	−0.43	−0.47
UCL[m]	1201	NA			0.43			0.43			0.43			0.43	0.43	0.47
		1.45	20	13	0.65	20	13	0.70	20	13	0.65	20	15	0.75	−0.10	0.00
LCL		0.98			0.43			0.48			0.43			0.53	−0.36	−0.13
UCL		2.41			0.82			0.86			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

[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 v. 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] NA = Not applicable.
[l] LCL = Lower confidence limit.
[m] UCL = Upper confidence limit.
[n] RDCC = Research and Development Culture Collection, Basingstoke, UK.

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 of the reference method enrichment broth was added, to the 25 g portions 225 mL of reference method enrichment broth was added, and to the 10 g portions 90 mL of 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 the *Official Methods of Analysis*, fractional positive results were obtained for cottage cheese (25 g), blue cheese (25 g), and Greek yogurt (25 g) at 22 h for the SureTect Listeria monocytogenes PCR Assay.

Table 7. Pre-collaborative: Thermo Scientific SureTect Listeria monocytogenes PCR assay, QuantStudio 5, presumptive vs. confirmed and candidate vs. FDA/BAM Ch.10—POD results

			Candidate presumptive (CP)			Candidate confirmed (CC)[a]			Candidate result (C)			Reference method (R)			C vs. R	CP vs. 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]
	Cottage cheese (4% fat; 25 g)	NA[k]	5	0	0.00	5	0	0.00	0	0	0.00	0	0	0.00	0.00	0.00
LCL[l]	*Listeria monocytogenes* RDCC[n]	NA			0.00			0.00			0.00			0.00	−0.43	−0.47
UCL[m]	3010	NA			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		0.36			0.43			0.48			0.43			0.30	−0.15	−0.21
UCL		1.06			0.82			0.86			0.82			0.70	0.41	0.11
		1.14	5	4	0.80	5	4	0.80	5	3	0.60	5	4	0.80	0.00	0.00
LCL		0.72			0.38			0.34			0.23			0.38	−0.47	−0.47
UCL		1.49			1.00			1.00			0.88			1.00	0.47	0.47
	Blue cheese (25 g) *Listeria monocytogenes* RDCC 3015	NA	5	0	0.00	5	0	0.00	0	0	0.00	0	0	0.00	0.00	0.00
LCL		NA			0.00			0.00			0.00			0.00	−0.43	−0.47
UCL		NA			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		0.10			0.34			0.34			0.34			9.18	−0.10	−0.06
UCL		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
	Greek yogurt (25 g) *Listeria monocytogenes* RDCC 1201	NA	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL		NA			0.00			0.00			0.00			0.00	−0.43	−0.47
UCL		NA			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		0.98			0.43			0.43			0.43			0.53	−0.36	−0.13
UCL		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
	Plastic surface (1″ × 1″; Petri dish) *Listeria monocytogenes* TCC[p] 1209	NA	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL		NA			0.00			0.00			0.00			0.00	−0.43	−0.47
UCL		NA			0.43			0.43			0.43			0.43	0.43	0.47
		NA	20	10	0.50	20	10	0.50	20	10	0.50	20	10	0.50	0.00	0.00
LCL		NA			0.30			0.30			0.30			0.30	−0.28	−0.13
UCL		NA			0.70			0.70			0.70			0.70	0.28	0.13
		NA	5	3	0.60	5	3	0.60	5	3	0.60	5	4	0.80	−0.20	0.00
LCL		NA			0.23			0.23			0.23			0.38	−0.62	−0.47
UCL		NA			0.88			0.88			0.88			1.00	0.31	0.47
	Ceramic surface (4″ × 4″; wall/floor tile) *Listeria monocytogenes* TCC 1209	NA	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL		NA			0.00			0.00			0.00			0.00	−0.43	−0.47
UCL		NA			0.43			0.43			0.43			0.43	0.43	0.47
		NA	20	6	0.30	20	6	0.30	20	6	0.30	20	1	0.05	0.25	0.00
LCL		NA			0.15			0.15			0.15			0.00	0.01	−0.13
UCL		NA			0.52			0.52			0.52			0.24	0.48	0.13
		NA	5	5	1.00	5	5	1.00	5	5	1.00	5	5	1.00	0.00	0.00
LCL		NA			0.57			0.57			0.57			0.57	−0.43	−0.47
UCL		NA			1.00			1.00			1.00			1.00	0.43	0.47
	Stainless-steel surface (4″ × 4″; sheet metal token) *Listeria monocytogenes* TCC l813	NA	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL		NA			0.00			0.00			0.00			0.00	−0.43	−0.47
UCL		NA			0.43			0.43			0.43			0.43	0.43	0.47
		NA	20	9	0.45	20	10	0.50	20	10	0.50	20	14	0.70	−0.25	−0.05
LCL		NA			0.26			0.30			0.30			0.48	−0.50	−0.21
UCL		NA			0.66			0.70			0.70			0.86	0.05	0.11

(continued)

Table 7. (continued)

Statistic	Matrix/organism	MPN[b]	Candidate presumptive (CP) N[c]	X[d]	POD_{CP}[e]	Candidate confirmed (CC)[a] N	X	POD_{CC}[f]	Candidate result (C) N	X	POD_C[g]	Reference method (R) N	X	POD_R[h]	C vs. R $dPOD_{C,R}$[i]	CP vs. CC $dPOD_{CP,CC}$[j]
		NA	5	5	1.00	5	5	1.00	5	5	1.00	5	5	1.00	0.00	0.00
LCL		NA			0.57			0.57			0.57			0.57	−0.43	−0.47
UCL		NA			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 FDA/BAM Chapter 10 reference method.
[b] MPN = Most probable number is calculated using the LCF MPN calculator v. 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] NA = 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.

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

Statistic	Matrix/Organism	MPN[b]	Candidate presumptive (CP) N[c]	X[d]	POD_{CP}[e]	Candidate confirmed (CC)[a] N	X	POD_{CC}[f]	Candidate result (C) N	X	POD_C[g]	Reference method (R) N	X	POD_R[h]	C vs. R $dPOD_{C,R}$[i]	CP vs. CC $dPOD_{CP,CC}$[j]
	Greek yogurt (25 g)	NA[k]	5	0	0.00	5	0	0.00	0	0	0.00	0	0	0.00	0.00	0.00
LCL[l]	*Listeria monocytogenes* RDCC[n]	NA			0.00			0.00			0.00			0.00	−0.43	−0.47
UCL[m]	1201	NA			0.43			0.43			0.43			0.43	0.43	0.47
		1.45	20	13	0.65	20	13	0.70	20	13	0.65	20	15	0.75	−0.10	0.00
LCL		0.98			0.43			0.48			0.43			0.53	−0.36	−0.13
UCL		2.41			0.82			0.86			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

[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] NA = Not applicable.
[l] LCL = Lower confidence limit.
[m] UCL = Upper confidence limit.
[n] RDCC = Research and Development Culture Collection, Basingstoke, UK.

Results

Prior to inoculation, an APC result of 2.00×10^7 cfu/g was obtained from the cottage cheese, 9.40×10^6 cfu/g was obtained for the blue cheese, and 4.60×10^6 cfu/g was obtained for the Greek yogurt.

The probability of detection (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 monocytogenes PCR Assay and the FDA/BAM Chapter 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 monocytogenes 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 Thermo Scientific SureTect Listeria monocytogenes PCR Assay. Four false positive (FP) results were observed from the analysis of the SureTect Listeria monocytogenes PCR Assay. The FP rate is 2.77%. During the collaborative study, both the QuantStudio 5 and the 7500 Fast were evaluated. The data suggest 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 support the product claims of the Thermo Scientific SureTect Listeria monocytogenes PCR Assay as a reliable detection method for *L. monocytogenes* 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 monocytogenes PCR Assay adopts First Action *Official Methods*℠ for the detection of *L. monocytogenes* from a broad range of foods and environmental samples.

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Conflict of Interest

None declared.

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