



# CERTIFICATION

**AOAC Research Institute**  
***Performance Tested Methods*<sup>SM</sup>**

Certificate No.  
**061302**

The AOAC Research Institute hereby certifies the method known as:

**Thermo Scientific<sup>TM</sup> SureTect<sup>TM</sup> Listeria monocytogenes PCR Assay**

manufactured by

**Oxoid Ltd. part of Thermo Fisher Scientific**  
**Wade Road**  
**Basingstoke**  
**Hampshire, RG248PW**

This method has been evaluated and certified according to the policies and procedures of the AOAC *Performance Tested Methods*<sup>SM</sup> Program. This certificate indicates an AOAC Research Institute Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC Research Institute *Performance Tested Methods*<sup>SM</sup> certification mark on the above-mentioned method for the period below. Renewal may be granted by the Expiration Date under the rules stated in the licensing agreement.

A handwritten signature in black ink, appearing to read "Bradley A. Stawick".

Bradley A. Stawick, Senior Director  
Signature for AOAC Research Institute

Issue Date  
Expiration Date

November 15, 2024  
December 31, 2025

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#### SUBMITTING COMPANY

Oxoid Ltd. part of Thermo Fisher Scientific  
Wade Road  
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#### METHOD NAME

Thermo Scientific™ SureTect™ *Listeria monocytogenes* PCR Assay

#### CATALOG NUMBER

A56843, PT0300A

#### INDEPENDENT LABORATORY

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#### MODIFICATION JUNE 2023

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#### APPLICABILITY OF METHOD

Target Organism – *Listeria monocytogenes*.

Matrixes – Original Validation (25 g) – raw ground beef, pork frankfurters, salami, cooked sliced turkey, fresh bagged spinach, ice cream, smoked salmon, cooked prawns, fresh cantaloupe, processed cheese.

(Sponge with neutralizing broth) – stainless steel, plastic  
MODIFICATION FEBRUARY 2015 (25 g) raw ground turkey, raw ground pork, pasteurized 2% milk, raw pork sausage, raw cod, pasteurized brie cheese, cooked sliced ham, and bagged lettuce

October 2018 Modification – (25 g) – sliced deli turkey, bagged lettuce, pasteurized 2% fat milk; (1 x 1 in swab) stainless steel

MODIFICATION JUNE 2023 – deli salad (Piemontaise) (25 g), pork rillettes (25 g), raw milk (25 mL), smoked salmon (25 g), ready-to-cook vegetables (25 g), and process water (25 mL)

Performance claims – Performance not significantly different between the Thermo Scientific™ SureTect™ *Listeria monocytogenes* PCR Assay method and the reference methods.

#### REFERENCE METHODS

Microbiology of food and animal feeding stuffs-Horizontal method for the detection of *Listeria monocytogenes* ISO ref method 11290-1:1996 including Amendment 1:2004 (3)

ISO Horizontal method for the detection of *L. monocytogenes* and *L. species* in ISO 11290-1:2017 (11)

U.S. Food and Drug Administration (2019) *Bacteriological Analytical Manual*, Chapter 10: *Detection of Listeria monocytogenes in Foods and Environmental Samples, and Enumeration of Listeria monocytogenes in Foods* (16)

<b>ORIGINAL CERTIFICATION DATE</b> June 21, 2013	<b>CERTIFICATION RENEWAL RECORD</b> Renewed annually through December 2025.
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METHOD MODIFICATION RECORD	SUMMARY OF MODIFICATION
1. February 2015	1. Matrix Extension.
2. October 2015	2. Validation of the Applied Biosystems 7500 Fast Instrument.
3. December 2017 Level 1	3. Editorial changes to update company name.
4. April 2018 Level 2	4. Evaluation of workflow and lyophilization steps.
5. October 2018 Level 2	5. Validation of the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR (with Applied Biosystems™ RapidFinder™ Analysis Software v2.0 or greater).
6. December 2018 Level 1	6. Updated user manual to include complete AOAC workflow, update template, and minor edits.
7. November 2019 Level 1	7. Editorial/clerical changes.
8. October 2020 Level 2	8. Modification to upgrade the software for Thermo Scientific RapidFinder Analysis (RFA) PCR software to v1.1 (designed for use the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR).
9. October 2020 Level 2	9. Modification to upgrade the software for the Applied Biosystems™ RapidFinder™ Express (RFE) to v2.0 (designed for use with the Applied Biosystems™ 7500 Fast™ Real-Time PCR).
10. January 2021 Level 2	10. Modification to use manual heat block or automated SimpliAmp with 7500Fast or QS5 PCR Instruments.
11. October 2021 Level 1	11. Editorial changes to insert to update software version number and add additional information from January 2021 modification.
12. July 2022 Level 2	12. Changes made to improve handling steps and visual indicators.
13. December 2022 Level 1	13. Editorial/clerical changes.
14. June 2023 Level 2	14. New formulation to the Thermo Scientific™ Oxoid™ <i>Brilliance</i> ™ Listeria Agar as the confirmation agar. Matrix extension to include deli salad, pork rillettes, raw milk, smoked salmon, ready-to-cook vegetables, and process water.
15. January 2024 Level 1	15. Editorial/clerical changes.
16. January 2024 Level 2	16. Addition of automated lysis procedure and PCR setup procedure.

Under this AOAC Performance Tested Methods <sup>SM</sup> License Number, 061302 this method is distributed by: <b>NONE</b>	Under this AOAC Performance Tested Methods <sup>SM</sup> License Number, 061302 this method is distributed as: <b>NONE</b>
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**Thermocyclers characteristics to run the Real-Time PCR:**

1. Applied Biosystems™ 7500 Fast Real-Time PCR Instrument and equivalents manufactured by Thermo Fisher Scientific and/or subsidiaries with the following characteristics

Characteristics	7500 Fast Real-Time PCR Instrument
Optics	12v 75w halogen bulb
Filters	5 excitation and 5 emission filters
Sample ramp rate	Standard mode: ± 1.6°C/sec Fast mode: ± 3.5°C/sec
Thermal range	4-100°C
Thermal accuracy	± 0.5°C
Thermal uniformity	± 1°C
Format	96-well, 0.1-mL block

2. Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument and equivalents manufactured by Thermo Fisher Scientific and/or subsidiaries with the following characteristics

Characteristics	QuantStudio™ 5 Real-Time PCR Instrument
Optics	Bright white LED
Filters	6 excitation and 6 emission filters
Sample ramp rate	Average: 3.66°C/sec Maximum: 9.0°C/sec
Thermal range	4-99°C
Thermal accuracy	± 0.25°C
Thermal uniformity	± 0.4°C
Format	96-well, 0.1-mL block

**Table 1. PTM Validation Study Summary for the Thermo Scientific SureTect Listeria monocytogenes PCR Assay (15)**

Thermo Scientific SureTect Listeria monocytogenes PCR Assay – PTM 061302

Original PTM certificate issued <sup>a</sup>		Test Portion size	Enrichment media/dilution	Enrichment time, h	Enrichment temp., °C	Reference method
July, 2013 Initial validation	Raw ground beef (80% lean)	25 g	24 LEB/1-in-10	22 - 30	37±1	ISO <sup>b</sup>
	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" x 4"	24 LEB/100 mL	22 - 30	37±1	ISO
	Stainless steel (slab, brushed finish)	1" x 1"	24 LEB/10 mL	22 - 30	37±1	ISO
	Plastic (large polystyrene petri dish)	4" x 4"	24 LEB/100 mL	22 - 30	37±1	ISO
Method modification		Test Portion size	Enrichment dilution	Enrichment time (h)	Enrichment temp (°C)	Reference method
February & September 2015 7500 Fast PCR Instrument validation and matrix extensions	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		Test Portion size	Enrichment dilution	Enrichment time (h)	Enrichment temp (°C)	Reference method
October 2018 Q55 PCR instrument validation and matrix extensions	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" x 1"	24 LEB/10 mL	22 - 30	37±1	ISO
Method modification <sup>c</sup>		Test Portion size	Enrichment dilution	Enrichment time (h)	Enrichment temp (°C)	Reference method
February 2021 Matrix extensions with BAM reference method	Cottage cheese (4% fat) <sup>d</sup>	25 g	24 LEB/1-in-10	22 - 30	37±1	BAM <sup>e</sup>
	Blue cheese	25 g	24 LEB/1-in-10	22 - 30	37±1	BAM
	Greek yoghurt	25 g	24 LEB/1-in-10	22 - 30	37±1	BAM & I
	Plastic (polystyrene Petri dish)	1" x 1"	24 LEB/10 mL	22 - 30	37±1	BAM
	Stainless steel (slab, brushed finish)	4" x 4"	24 LEB/100 mL	22 - 30	37±1	BAM
	Ceramic (wall/floor tile)	4" x 4"	24 LEB/100 mL	22 - 30	37±1	BAM
	Concrete (slab)	4" x 4"	24 LEB/100 mL	22 - 30	37±1	BAM
Method modification		Test Portion size	Enrichment dilution	Enrichment time (h)	Enrichment temp (°C)	Reference method
New matrixes pending approval 2023, New Brilliance Listeria Agar (ISO)	Deli salad (Piemontaise)	25 g	24 LEB/1-in-10	20 - 28	37±1	ISO
	Rillettes	25 g	24 LEB/1-in-10	20 - 28	37±1	ISO
	Raw milk	25 ml	24 LEB/1-in-10	20 - 28	37±1	ISO
	Cooked salmon	25 g	24 LEB/1-in-10	20 - 28	37±1	ISO
	Ready-to-cook vegetables	25 g	24 LEB/1-in-10	20 - 28	37±1	ISO
Process water	25 ml	24 LEB/1-in-10	20 - 28	37±1	ISO	

<sup>a</sup>AOAC Research Institute Certified Methods Search.<sup>b</sup>EN ISO 11290-1.<sup>c</sup>Matrixes approved with First Action.<sup>d</sup>Matrix selected for collaborative study.<sup>e</sup>BAM Chapter 10.

**PRINCIPLE OF THE METHOD (1)**

The Thermo Scientific SureTect Listeria monocytogenes PCR assay is a Real-Time Polymerase Chain Reaction (PCR) test intended to be used in conjunction with the Thermo Scientific PikoReal™ Real-Time PCR Instrument and SureTect Software for the detection and identification of *L. monocytogenes* in food and environmental samples.

The assay is supplied as a kit containing all necessary reagents, including pre-filled Lysis Tubes and lyophilized PCR pellets, containing all necessary PCR reagents (target-specific primers, dye labelled probes and PCR master mix components) to easily conduct the PCR analysis. The PCR probes are short oligonucleotides with a quencher molecule at one end that, when not bound to target DNA, greatly reduces fluorescence from the dye label at the opposite end of the probe molecule. The oligonucleotides target unique DNA sequences found only in *L. monocytogenes*. If *L. monocytogenes* is present, the target DNA sequence will be amplified and the increasing fluorescent signal generated will be detected by the PikoReal Real-Time instrument and interpreted by the Thermo Scientific SureTect Software. In addition to detection of any target DNA, the SureTect Listeria monocytogenes PCR pellets contain probe, primers and DNA templates for an internal amplification control (IAC). During PCR cycling, the IAC template is amplified whether any target DNA is present or not. Since the probe used for the IAC contains a different colored fluorescent dye than that in the probe to detect target DNA, detection by the PikoReal Instrument occurs through a separate dye channel.

The result is that after a successful PCR run, the instrument will detect amplification of the IAC DNA sequence. In the absence of any target DNA being detected by the assay, the presence of the IAC amplification curve confirms that the PCR process has occurred successfully.

The assays used in the Thermo Scientific SureTect System are based on Solaris™ qPCR technology. The PCR probes have a molecule called Minor Groove Binder (MGB) attached to one end, which enhances the probe-template DNA bond and yields a better signal-to-noise ratio by lowering the background fluorescence. Results from this assay system are achieved 80 minutes after loading the prepared sample into the PikoReal Instrument and are displayed on the attached PC screen as simple positive or negative symbols with PCR amplification plots that are easily accessible for review. All results interpreted by the SureTect Software can be stored, printed or downloaded by the user, as required.

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

The data presented and discussed in this report, within the statistical uncertainty of the analysis, supports the product claims of the SureTect Listeria monocytogenes assay for recovery of *L. monocytogenes* from fresh cantaloupe, salami, smoked salmon, fresh bagged spinach, cooked sliced turkey, pork frankfurters, ice cream, cooked prawns, processed cheese and raw ground beef as well as stainless steel and plastic surfaces. Additional studies conducted as part of the validation show that the assay has excellent inclusivity and is unaffected by high levels of non-target bacteria.

**Table 1: Inclusivity of the Thermo Scientific SureTect Listeria monocytogenes Assay (1)**

Isolate	Serotype	TCC <sup>a</sup> No	Source	Result
<i>Listeria monocytogenes</i>	2	867	CSF: Clinical	Positive
<i>Listeria monocytogenes</i>	7	2184	Faecal sample	Positive
<i>Listeria monocytogenes</i>	1/2a	860	Poultry	Positive
<i>Listeria monocytogenes</i>	1/2a	1215	Chorizo sausage	Positive
<i>Listeria monocytogenes</i>	1/2a	1216	Sandwich	Positive
<i>Listeria monocytogenes</i>	1/2a	1217	Carrow cheese	Positive
<i>Listeria monocytogenes</i>	1/2a	1218	Butter	Positive
<i>Listeria monocytogenes</i>	1/2a	1219	Pilau rice	Positive
<i>Listeria monocytogenes</i>	1/2a	1220	Sandwich	Positive
<i>Listeria monocytogenes</i>	1/2b	1205	Cake	Positive
<i>Listeria monocytogenes</i>	1/2b	1206	Whipped Cream	Positive
<i>Listeria monocytogenes</i>	1/2b	1207	Cheese	Positive
<i>Listeria monocytogenes</i>	1/2b	1208	Cheese	Positive
<i>Listeria monocytogenes</i>	1/2b	1209	Cream	Positive
<i>Listeria monocytogenes</i>	1/2b	1210	Cheese	Positive
<i>Listeria monocytogenes</i>	1/2c	858	Clinical sample	Positive
<i>Listeria monocytogenes</i>	1/2c	1195	Ox tongue	Positive
<i>Listeria monocytogenes</i>	1/2c	1196	Roast beef	Positive
<i>Listeria monocytogenes</i>	1/2c	1197	Topside beef	Positive
<i>Listeria monocytogenes</i>	1/2c	1198	Wiltshire ham	Positive
<i>Listeria monocytogenes</i>	1/2c	1199	Ham sandwich	Positive
<i>Listeria monocytogenes</i>	3a	812	Environmental	Positive
<i>Listeria monocytogenes</i>	3a	813	Environmental	Positive
<i>Listeria monocytogenes</i>	3a	840	Butter	Positive
<i>Listeria monocytogenes</i>	3a	870	Clinical sample	Positive
<i>Listeria monocytogenes</i>	3a	888	Food	Positive
<i>Listeria monocytogenes</i>	3a	889	Food	Positive
<i>Listeria monocytogenes</i>	3b	2179	Unknown	Positive
<i>Listeria monocytogenes</i>	3c	2180	Unknown	Positive
<i>Listeria monocytogenes</i>	4a	2181	Unknown	Positive
<i>Listeria monocytogenes</i>	4b	864	Meningitis	Positive
<i>Listeria monocytogenes</i>	4b	865	CSF: Meningitis	Positive
<i>Listeria monocytogenes</i>	4b	1224	Food- blood	Positive
<i>Listeria monocytogenes</i>	4b	1225	Chicken	Positive
<i>Listeria monocytogenes</i>	4b	1226	Dressed crab	Positive
<i>Listeria monocytogenes</i>	4b	1227	Turkey breast	Positive
<i>Listeria monocytogenes</i>	4c	2183	Bird: heart disease	Positive
<i>Listeria monocytogenes</i>	4d	863	Sheep	Positive
<i>Listeria monocytogenes</i>	4e	868	Chicken	Positive
<i>Listeria monocytogenes</i>	4e	883	Veterinary sample	Positive
<i>Listeria monocytogenes</i>	4e	884	Unknown	Positive
<i>Listeria monocytogenes</i>	4e	885	Unknown	Positive
<i>Listeria monocytogenes</i>	Untyped	874	Unknown food source-isolated by food lab	Positive
<i>Listeria monocytogenes</i>	Untyped	856	Unknown food source-isolated by food lab	Positive

<i>Listeria monocytogenes</i>	Untyped	857	Unknown food source-isolated by food lab	Positive
<i>Listeria monocytogenes</i>	Untyped	859	Tartare de Salmon	Positive
<i>Listeria monocytogenes</i>	Untyped	866	Unknown ATCC isolate	Positive
<i>Listeria monocytogenes</i>	Untyped	871	Unknown food source-isolated by food lab	Positive
<i>Listeria monocytogenes</i>	Untyped	873	Unknown food source-isolated by food lab	Positive
<i>Listeria monocytogenes</i>	Untyped	875	Unknown food source-isolated by food lab	Positive
<i>Listeria monocytogenes</i>	Untyped	881	Unknown food source-isolated by food lab	Positive
<i>Listeria monocytogenes</i>	Untyped	882	Unknown food source-isolated by food lab	Positive
<i>Listeria monocytogenes</i>	4b	1841	ATCC <sup>®</sup> 19115	Positive

<sup>a</sup>TCC. Trials Culture Collection Number - Proprietary to Thermo Fisher Scientific, Microbiology Division

**Table 2: Exclusivity of the Thermo Scientific SureTect Listeria monocytogenes assay (1)**

Isolate	Source	TCC No	Result
<i>Listeria ivanovii</i> subsp. <i>londoniensis</i>	NCTC	869	Negative
<i>Listeria ivanovii</i>	Food-unknown	880	Negative
<i>Listeria ivanovii</i>	Food-unknown	1183	Negative
<i>Listeria ivanovii</i>	Food-unknown	1184	Negative
<i>Listeria innocua</i>	Chicken sandwich	1177	Negative
<i>Listeria innocua</i> 6a	NCTC	2186	Negative
<i>Listeria welshimeri</i>	Chicken sandwich	1185	Negative
<i>Listeria welshimeri</i> 6b	Institut Pasteur	2188	Negative
<i>Listeria welshimeri</i> 6b	NCTC	1978	Negative
<i>Listeria seeligeri</i> 1/2b	NCTC	1979	Negative
<i>Listeria seeligeri</i>	Food-unknown	1191	Negative
<i>Listeria seeligeri</i>	Cheese	1190	Negative
<i>Listeria grayii</i>	Environmental sample	1172	Negative
<i>Listeria grayii</i>	Unknown	872	Negative
<i>Listeria grayii</i>	Butter	1174	Negative
<i>Bacillus mycoides</i>	NCTC	2300	Negative
<i>Brochothrix thermosphacta</i>	Pork sausage	2192	Negative
<i>Carnobacterium divergens</i>	Brie	2258	Negative
<i>Carnobacterium gallinarum</i>	Unknown	2259	Negative
<i>Carnobacterium piscicola</i>	Cooked ham	2260	Negative
<i>Citrobacter freundii</i>	NCTC	1911	Negative
<i>Enterobacter aerogenes</i>	Unknown	2200	Negative
<i>Erysipelothrix rhusiopathiae</i>	Unknown	2262	Negative
<i>Escherichia fergusonii</i>	Sausages	2263	Negative
<i>Escherichia coli</i>	NCTC	1809	Negative
<i>Klebsiella pneumoniae</i>	NCTC	1892	Negative
<i>Kurthia gibsonii</i>	Pork sausage	2193	Negative
<i>Lactobacillus casei</i> subsp. <i>casei</i>	Fermented catsup	2194	Negative
<i>Lactobacillus delbrueckii</i> subsp. <i>lactis</i>	Emmenthal cheese	2195	Negative
<i>Lactobacillus plantarum</i>	Red Cheshire cheese	2196	Negative
<i>Micrococcus luteus</i>	NCIMB	OCC <sup>b</sup> 2352	Negative
<i>Proteus vulgaris</i>	Unknown	1424	Negative
<i>Propionibacterium freundenreichii</i>	Swiss cheese production	2304	Negative
<i>Rhodococcus equi</i>	NCTC	2358	Negative
<i>Salmonella enterica</i> subsp. <i>enterica</i> Typhimurium	NCTC	1911	Negative
<i>Staphylococcus aureus</i>	Food-unknown	2240	Negative
<i>Streptococcus salivarius</i>	NCTC	2352	Negative
<i>Bacillus cereus</i>	Milk	2299	Negative

<sup>a</sup>TCC. Trials Culture Collection Number - Proprietary to Thermo Fisher Scientific, Microbiology Division, Basingstoke, UK

<sup>b</sup>OCC. Oxoid Culture Collection - Proprietary to Thermo Fisher Scientific, Microbiology Division, Basingstoke, UK

Table 3: SureTect Listeria monocytogenes assay Presumptive vs. SureTect Confirmation Procedure Confirmed Result-POD Analysis (1)

Matrix	Strain	MPN <sup>a</sup> /test portion	N <sup>b</sup>	SureTect Method Presumptive			SureTect Method Confirmation			dPOD <sub>cp</sub> <sup>f</sup>	95% CI <sup>g</sup>
				X <sup>c</sup>	POD <sub>cp</sub> <sup>d</sup>	95% CI	X	POD <sub>cc</sub> <sup>e</sup>	95% CI		
Cantaloupe	<i>Listeria monocytogenes</i> (TCC 2180)	N/A <sup>h</sup>	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.70 (0.39, 1.29)	20	13	0.65	(0.43, 0.82)	13	0.65	(0.43, 0.82)	0.00	(-0.28, 0.28)
		3.00 (1.25, 7.00)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Salami	<i>Listeria monocytogenes</i> (TCC 1215)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.29 (0.14, 0.51)	20	10	0.50	(0.30, 0.70)	11	0.55	(0.34, 0.74)	-0.05	(-0.33, 0.24)
		0.40 (0.23, 0.91)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Stainless steel surface (sponge)	<i>Listeria monocytogenes</i> (TCC 813) and 10x <i>Enterococcus faecalis</i> (OCC 640)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		N/A	20	11	0.55	(0.34, 0.74)	11	0.55	(0.34, 0.74)	0.00	(-0.28, 0.28)
		N/A	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Smoked salmon	<i>Listeria monocytogenes</i> (TCC 859)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.60 (0.36, 0.98)	20	7	0.35	(0.18, 0.57)	8	0.40	(0.22, 0.61)	-0.05	(-0.32, 0.23)
		1.25 (0.58, 2.69)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Spinach	<i>Listeria monocytogenes</i> (TCC 2179)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.38 (0.18, 0.66)	20	8	0.40	(0.22, 0.61)	8	0.40	(0.22, 0.61)	0.00	(-0.28, 0.28)
		0.39 (0.17, 0.84)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Cooked sliced turkey (Chilled)	<i>Listeria monocytogenes</i> (TCC 1225)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.06 (0.64, 1.78)	20	19	0.95	(0.76, 1.00)	19	0.95	(0.76, 1.00)	0.00	(-0.19, 0.19)
		4.37 (1.71, 11.19)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Pork frankfurters	<i>Listeria monocytogenes</i> (TCC 884)	N/A <sup>h</sup>	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.72 (0.41, 1.19)	20	14	0.70	(0.48, 0.85)	14	0.70	(0.48, 0.85)	0.00	(-0.27, 0.27)
		1.0 (0.46, 2.25)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)

Ice cream (vanilla)	<i>Listeria monocytogenes</i> (TCC 1206)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.24 (0.11, 0.50)	20	12	0.60	(0.39, 0.78)	12	0.60	(0.39, 0.78)	0.00	(-0.28, 0.28)
		0.64 (0.28, 1.42)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Plastic surface (sponge)	<i>Listeria monocytogenes</i> (TCC 812)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		N/A	20	16	0.80	(0.58, 0.92)	16	0.80	(0.58, 0.92)	0.00	(-0.25, 0.25)
		N/A	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Cooked prawns (chilled)	<i>Listeria monocytogenes</i> (TCC 865)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.00 (0.57, 1.96)	20	15	0.75	(0.53, 0.89)	15	0.75	(0.53, 0.89)	0.00	(-0.26, 0.26)
		1.88 (0.84, 4.18)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Processed cheese	<i>Listeria monocytogenes</i> (TCC 1217)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.53 (0.28, 0.90)	20	6	0.30	(0.15, 0.52)	6	0.30	(0.15, 0.52)	0.00	(-0.27, 0.27)
		1.48 (0.65, 3.37)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Raw ground beef (12% fat)	<i>Listeria monocytogenes</i> (TCC 1196)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.1 (0.71, 1.88)	20	13	0.65	(0.43, 0.82)	13	0.65	(0.43, 0.82)	0.00	(-0.28, 0.28)
		1.9 (0.84, 4.18)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Fresh bagged lettuce <sup>1</sup>	<i>Listeria monocytogenes</i> (LI0549)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.37 (0.24, 0.60)	20	10	0.50	(0.29, 0.70)	10	0.50	(0.29, 0.70)	0.00	(-0.28, 0.28)
		2.19 (0.93, 5.12)	5	5	1.00	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0.00	(-0.43, 0.43)
Pork frankfurters <sup>1</sup>	<i>Listeria monocytogenes</i> (ATCC <sup>®</sup> 19115)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.58 (0.36, 0.98)	20	12	0.60	(0.38, 0.78)	12	0.60	(0.38, 0.78)	0.00	(-0.28, 0.28)
		2.96 (1.25, 7.00)	5	5	5	(0.56, 1.00)	5	5	(0.56, 1.00)	0.00	(-0.43, 0.43)
Stainless steel surface 4"x4" <sup>1</sup>	<i>Listeria monocytogenes</i> (LI7163) and 10x <i>Enterococcus faecalis</i> (ATCC <sup>®</sup> 29212)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		N/A	20	8	0.40	(0.21, 0.61)	8	0.40	(0.21, 0.61)	0.00	(-0.28, 0.28)
		N/A	5	5	1.00	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0.00	(-0.43, 0.43)

<sup>a</sup>MPN = Most Probable number is based on the POD of the reference method test portions using the Least Cost Formulations MPN calculator with 95% confidence interval.

<sup>b</sup>N = Number of test portions

<sup>c</sup>X = Number of positive test portions

<sup>d</sup>PODcp = Candidate method presumptive positive outcomes divided by the total number of portions

<sup>e</sup>PODcc = Candidate confirmation method positive outcomes divided by the total number of portions

<sup>f</sup>dPODcp = Difference between the candidate presumptive result and the candidate method confirmed result POD values

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

<sup>h</sup>N/A = Not applicable

<sup>i</sup>Independent laboratory study



**Table 4: SureTect Listeria monocytogenes assay Presumptive vs. Reference Confirmation Procedure Confirmed Result-POD Analysis (1)**

Matrix	Strain	MPN <sup>a</sup> /test portion	N <sup>b</sup>	SureTect Method Presumptive			Reference Confirmation (CC2)			dPOD <sub>cp</sub> <sup>f</sup>	95% CI <sup>g</sup>
				χ <sup>c</sup>	POD <sub>cp</sub> <sup>d</sup>	95% CI	X	POD <sub>cc2</sub> <sup>e</sup>	95% CI		
Cantaloupe	<i>Listeria monocytogenes</i> (TCC 2180)	N/A <sup>h</sup>	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.70 (0.39, 1.29)	20	13	0.65	(0.43, 0.82)	13	0.65	(0.43, 0.82)	0.00	(-0.28, 0.28)
		3.00 (1.25, 7.00)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Salami	<i>Listeria monocytogenes</i> (TCC 1215)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.29 (0.14, 0.51)	20	10	0.50	(0.30, 0.70)	11	0.55	(0.34, 0.74)	-0.05	(-0.33, 0.24)
		0.40 (0.23, 0.91)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Stainless steel surface (sponge)	<i>Listeria monocytogenes</i> (TCC 813) and 10x <i>Enterococcus faecalis</i> (OCC 640)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		N/A	20	11	0.55	(0.34, 0.74)	11	0.55	(0.34, 0.74)	0.00	(-0.28, 0.28)
		N/A	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Smoked salmon	<i>Listeria monocytogenes</i> (TCC 859)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.60 (0.36, 0.98)	20	7	0.35	(0.18, 0.57)	9	0.45	(0.26, 0.66)	-0.10	(-0.37, 0.19)
		1.25 (0.58, 2.69)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Spinach	<i>Listeria monocytogenes</i> (TCC 2179)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.38 (0.18, 0.66)	20	8	0.40	(0.22, 0.61)	8	0.40	(0.22, 0.61)	0.00	(-0.28, 0.28)
		0.39 (0.17, 0.84)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Cooked sliced turkey (chilled)	<i>Listeria monocytogenes</i> (TCC 1225)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.06 (0.64, 1.78)	20	19	0.95	(0.76, 1.00)	19	0.95	(0.76, 1.00)	0.00	(-0.19, 0.19)
		4.37 (1.71, 11.19)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Pork frankfurters	<i>Listeria monocytogenes</i> (TCC 884)	N/A <sup>h</sup>	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.72 (0.41, 1.19)	20	14	0.70	(0.48, 0.85)	15	0.75	(0.53, 0.89)	-0.05	(-0.31, 0.22)
		1.0 (0.46, 2.25)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)

Ice cream (vanilla)	<i>Listeria monocytogenes</i> (TCC 1206)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.24 (0.11, 0.50)	20	12	0.60	(0.39, 0.78)	12	0.60	(0.39, 0.78)	0.00	(-0.28, 0.28)
		0.64 (0.28, 1.42)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Plastic surface (sponge)	<i>Listeria monocytogenes</i> (TCC 812)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		N/A	20	16	0.80	(0.58, 0.92)	16	0.80	(0.58, 0.92)	0.00	(-0.25, 0.25)
		N/A	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Cooked prawns	<i>Listeria monocytogenes</i> (TCC 865)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.00 (0.57, 1.96)	20	15	0.75	(0.53, 0.89)	15	0.75	(0.53, 0.89)	0.00	(-0.26, 0.26)
		1.88 (0.84, 4.18)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Processed cheese	<i>Listeria monocytogenes</i> (TCC 1217)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.53 (0.28, 0.90)	20	6	0.30	(0.15, 0.52)	7	0.35	(0.18, 0.57)	-0.05	(-0.32, 0.23)
		1.48 (0.65, 3.37)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Raw ground beef (12% fat)	<i>Listeria monocytogenes</i> (TCC 1196)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.1 (0.71, 1.88)	20	13	0.65	(0.43, 0.82)	14	0.70	(0.48, 0.84)	-0.05	(-0.32, 0.23)
		1.9 (0.84, 4.18)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Fresh bagged lettuce <sup>e</sup>	<i>Listeria monocytogenes</i> (LI0549)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.37 (0.24, 0.60)	20	10	0.50	(0.29, 0.70)	10	0.50	(0.29, 0.70)	0.00	(-0.28, 0.28)
		2.19 (0.93, 5.12)	5	5	1.00	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0.00	(-0.43, 0.43)
Pork frankfurters <sup>i</sup>	<i>Listeria monocytogenes</i> (ATCC <sup>®</sup> 19115)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.58 (0.36, 0.98)	20	12	0.60	(0.38, 0.78)	12	0.60	(0.38, 0.78)	0.00	(-0.28, 0.28)
		2.96 (1.25, 7.00)	5	5	5	(0.56, 1.00)	5	5	(0.56, 1.00)	0.00	(-0.43, 0.43)
Stainless steel surface 4"x4" <sup>i</sup>	<i>Listeria monocytogenes</i> (LI7163) and 10x <i>Enterococcus faecalis</i> (ATCC <sup>®</sup> 29212)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		N/A	20	8	0.40	(0.21, 0.61)	8	0.40	(0.21, 0.61)	0.00	(-0.28, 0.28)
		N/A	5	5	1.00	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0.00	(-0.43, 0.43)

<sup>a</sup>MPN = Most Probable number is based on the POD of the reference method test portions using the Least Cost Formulations MPN calculator with 95% confidence interval.

<sup>b</sup>N = Number of test portions

<sup>c</sup>X = Number of positive test portions

<sup>d</sup>POD<sub>cp</sub> = Candidate method presumptive positive outcomes divided by the total number of portions

<sup>e</sup>POD<sub>cc2</sub> = Reference method confirmation positive outcomes divided by the total number of portions

<sup>f</sup>dPOD<sub>cp</sub> = Difference between the candidate presumptive result and the candidate method confirmed result POD values

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

<sup>h</sup>N/A = Not applicable

<sup>i</sup>Independent laboratory study

Table 5: SureTect Confirmation Method vs. Reference Confirmation-POD Analysis (1)

Matrix	Strain	MPN <sup>a</sup> /test portion	N <sup>b</sup>	SureTect Confirmation Method (CC)			Reference Method Confirmation (CC2)			dPODcc <sup>f</sup>	95% CI <sup>g</sup>
				X <sup>c</sup>	POD <sub>cc</sub> <sup>d</sup>	95% CI	X	POD <sub>cc2</sub> <sup>e</sup>	95% CI		
Cantaloupe	<i>Listeria monocytogenes</i> (TCC 2180)	N/A <sup>h</sup>	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.70 (0.39, 1.29)	20	13	0.65	(0.43, 0.82)	13	0.65	(0.43, 0.82)	0.00	(-0.28, 0.28)
		3.00 (1.25, 7.00)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Salami	<i>Listeria monocytogenes</i> (TCC 1215)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.29 (0.14, 0.51)	20	11	0.55	(0.34, 0.74)	11	0.55	(0.34, 0.74)	0.00	(-0.28, 0.28)
		0.40 (0.23, 0.91)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Stainless steel surface (sponge)	<i>Listeria monocytogenes</i> (TCC 813) and 10x <i>Enterococcus faecalis</i> (OCC 640)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		N/A	20	11	0.55	(0.34, 0.74)	11	0.55	(0.34, 0.74)	0.00	(-0.28, 0.28)
		N/A	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Smoked salmon	<i>Listeria monocytogenes</i> (TCC 859)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.60 (0.36, 0.98)	20	8	0.40	(0.22, 0.61)	9	0.45	(0.26, 0.66)	-0.05	(-0.33, 0.24)
		1.25 (0.58, 2.69)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Spinach	<i>Listeria monocytogenes</i> (TCC 2179)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.38 (0.18, 0.66)	20	8	0.40	(0.22, 0.61)	8	0.40	(0.22, 0.61)	0.00	(-0.28, 0.28)
		0.39 (0.17, 0.84)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Cooked sliced turkey (chilled)	<i>Listeria monocytogenes</i> (TCC 1225)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.06 (0.64, 1.78)	20	19	0.95	(0.76, 1.00)	19	0.95	(0.76, 1.00)	0.00	(-0.19, 0.19)
		4.37 (1.71, 11.19)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Pork frankfurters	<i>Listeria monocytogenes</i> (TCC 884)	N/A <sup>h</sup>	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.72 (0.41, 1.19)	20	14	0.70	(0.48, 0.85)	15	0.75	(0.53, 0.89)	-0.05	(-0.31, 0.22)
		1.0 (0.46, 2.25)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)

Ice cream (vanilla)	<i>Listeria monocytogenes</i> (TCC 1206)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.24 (0.11, 0.50)	20	12	0.60	(0.39, 0.78)	12	0.60	(0.39, 0.78)	0.00	(-0.28, 0.28)
		0.64 (0.28, 1.42)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Plastic surface (sponge)	<i>Listeria monocytogenes</i> (TCC 812)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		N/A	20	16	0.80	(0.58, 0.92)	16	0.80	(0.58, 0.92)	0.00	(-0.25, 0.25)
		N/A	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Cooked prawns	<i>Listeria monocytogenes</i> (TCC 865)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.00 (0.57, 1.96)	20	15	0.75	(0.53, 0.89)	15	0.75	(0.53, 0.89)	0.00	(-0.26, 0.26)
		1.88 (0.84, 4.18)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Processed cheese	<i>Listeria monocytogenes</i> (TCC 1217)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.53 (0.28, 0.90)	20	6	0.30	(0.15, 0.52)	7	0.35	(0.18, 0.57)	-0.05	(-0.32, 0.23)
		1.48 (0.65, 3.37)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Raw ground beef (12% fat)	<i>Listeria monocytogenes</i> (TCC 1196)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.1 (0.71, 1.88)	20	13	0.65	(0.43, 0.82)	14	0.70	(0.48, 0.84)	-0.05	(-0.32, 0.23)
		1.9 (0.84, 4.18)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Fresh bagged lettuce <sup>i</sup>	<i>Listeria monocytogenes</i> (LI0549)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.37 (0.24, 0.60)	20	10	0.50	(0.29, 0.70)	10	0.50	(0.29, 0.70)	0.00	(-0.28, 0.28)
		2.19 (0.93, 5.12)	5	5	1.00	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0.00	(-0.43, 0.43)
Pork frankfurters <sup>i</sup>	<i>Listeria monocytogenes</i> (ATCC <sup>®</sup> 19115)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.58 (0.36, 0.98)	20	12	0.60	(0.38, 0.78)	12	0.60	(0.38, 0.78)	0.00	(-0.28, 0.28)
		2.96 (1.25, 7.00)	5	5	5	(0.56, 1.00)	5	5	(0.56, 1.00)	0.00	(-0.43, 0.43)
Stainless steel surface 4"x4" <sup>i</sup>	<i>Listeria monocytogenes</i> (LI7163) and 10x <i>Enterococcus faecalis</i> (ATCC <sup>®</sup> 29212)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		N/A	20	8	0.40	(0.21, 0.61)	8	0.40	(0.21, 0.61)	0.00	(-0.28, 0.28)
		N/A	5	5	1.00	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0.00	(-0.43, 0.43)

<sup>a</sup>MPN = Most Probable number is based on the POD of the reference method test portions using the Least Cost Formulations MPN calculator with 95% confidence interval.

<sup>b</sup>N = Number of test portions

<sup>c</sup>X = Number of positive test portions

<sup>d</sup>POD<sub>cc</sub> = Candidate method confirmed positive outcomes divided by the total number of portions

<sup>e</sup>POD<sub>cc2</sub> = Reference method confirmation positive outcomes divided by the total number of portions

<sup>f</sup>dPOD<sub>cp</sub> = Difference between the candidate presumptive result and the candidate method confirmed result POD values

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

<sup>h</sup>N/A = Not applicable

<sup>i</sup>Independent laboratory study

**Table 6: SureTect Listeria monocytogenes assay Confirmed Results vs. Reference Method-POD Analysis (1)**

Matrix	Strain	MPN <sup>a</sup> /test portion	N <sup>b</sup>	SureTect Method (C)			Reference Method (R)			dPOD <sup>c</sup>	95% CI <sup>e</sup>
				X <sup>c</sup>	POD <sup>d</sup>	95% CI	X	POD <sup>e</sup>	95% CI		
Cantaloupe	<i>Listeria monocytogenes</i> (TCC 2180)	N/A <sup>h</sup>	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.70 (0.39, 1.29)	20	13	0.65	(0.43, 0.82)	11	0.55	(0.34, 0.74)	0.10	(-0.19, 0.37)
		3.00 (1.25, 7.00)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Salami	<i>Listeria monocytogenes</i> (TCC 1215)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.29 (0.14, 0.51)	20	11	0.55	(0.34, 0.74)	4	0.20	(0.08, 0.42)	0.35	(0.05, 0.58)
		0.40 (0.23, 0.91)	5	4	0.80	(0.38, 1.00)	3	0.60	(0.23, 0.88)	0.20	(-0.31, 0.62)
Stainless steel surface (sponge)	<i>Listeria monocytogenes</i> (TCC 813) and 10x <i>Enterococcus faecalis</i> (OCC 640)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		N/A	20	11	0.55	(0.34, 0.74)	12	0.60	(0.39, 0.78)	-0.05	(-0.33, 0.28)
		N/A	5	5	1.00	(0.57, 1.00)	4	0.80	(0.38, 1.00)	0.20	(-0.28, 0.62)
Smoked salmon	<i>Listeria monocytogenes</i> (TCC 859)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.60 (0.36, 0.98)	20	8	0.40	(0.22, 0.61)	8	0.40	(0.22, 0.61)	0.00	(-0.28, 0.28)
		1.25 (0.58, 2.69)	5	4	0.80	(0.38, 1.00)	2	0.40	(0.12, 0.77)	0.40	(-0.16, 0.75)
Spinach	<i>Listeria monocytogenes</i> (TCC 2179)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.38 (0.18, 0.66)	20	8	0.40	(0.22, 0.61)	6	0.30	(0.15, 0.52)	0.10	(-0.18, 0.36)
		0.39 (0.17, 0.84)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Cooked sliced turkey (chilled)	<i>Listeria monocytogenes</i> (TCC 1225)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.06 (0.64, 1.78)	20	19	0.95	(0.76, 1.00)	12	0.60	(0.39, 0.78)	0.35	(0.09, 0.57)
		4.37 (1.71, 11.19)	5	4	0.80	(0.38, 1.00)	5	1.00	(0.57, 1.00)	-0.20	(-0.62, 0.28)
Pork frankfurters	<i>Listeria monocytogenes</i> (TCC 884)	N/A <sup>h</sup>	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.72 (0.41, 1.19)	20	14	0.70	(0.48, 0.85)	9	0.45	(0.26, 0.66)	0.25	(-0.05, 0.50)
		1.0 (0.46, 2.25)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)

Ice cream (vanilla)	<i>Listeria monocytogenes</i> (TCC 1206)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.24 (0.11, 0.50)	20	12	0.60	(0.39, 0.78)	1	0.05	(0.00, 0.24)	0.55	(0.27, 0.74)
		0.64 (0.28, 1.42)	5	4	0.80	(0.38, 1.00)	0	0.00	(0.00, 0.43)	0.80	(0.19, 1.00)
Plastic surface (sponge)	<i>Listeria monocytogenes</i> (TCC 812)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		N/A	20	16	0.80	(0.58, 0.92)	15	0.75	(0.53, 0.89)	0.05	(-0.21, 0.30)

		N/A	5	4	0.80	(0.38, 1.00)	5	1.00	(0.57, 1.00)	-0.20	(-0.62, 0.28)
Cooked prawns	<i>Listeria monocytogenes</i> (TCC 865)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.00 (0.57, 1.96)	20	15	0.75	(0.53, 0.89)	14	0.70	(0.48, 0.85)	0.05	(-0.22, 0.31)
		1.88 (0.84, 4.18)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Processed cheese	<i>Listeria monocytogenes</i> (TCC 1217)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.53 (0.28, 0.90)	20	6	0.30	(0.15, 0.52)	9	0.45	(0.26, 0.66)	-0.15	(-0.41, 0.14)
		1.48 (0.65, 3.37)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Raw ground beef (12% fat)	<i>Listeria monocytogenes</i> (TCC 1196)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.1 (0.71, 1.88)	20	13	0.65	(0.43, 0.82)	14	0.70	(0.48, 0.84)	-0.05	(-0.32, 0.23)
		1.9 (0.84, 4.18)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Fresh bagged lettuce <sup>i</sup>	<i>Listeria monocytogenes</i> (LI0549)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.37 (0.23, 0.59)	20	10	0.50	(0.29, 0.70)	5	0.25	(0.11, 0.46)	0.25	(-0.04, 0.49)
		2.19 (0.93, 5.12)	5	5	1.00	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0.00	(-0.43, 0.43)
Pork frankfurters <sup>i</sup>	<i>Listeria monocytogenes</i> (ATCC <sup>®</sup> 19115)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.58 (0.36, 0.98)	20	12	0.60	(0.38, 0.78)	8	0.40	(0.21, 0.61)	0.20	(-0.10, 0.45)
		2.96 (1.25, 7.00)	5	5	5	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0.00	(-0.43, 0.43)
Stainless steel surface 4"x4" <sup>ii</sup>	<i>Listeria monocytogenes</i> (LI7163) and 10x <i>Enterococcus faecalis</i> (ATCC <sup>®</sup> 29212)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		N/A	20	8	0.40	(0.21, 0.61)	8	0.40	(0.21, 0.61)	0.00	(-0.28, 0.28)
		N/A	5	5	1.00	(0.56, 1.00)	4	0.80	(0.56, 1.00)	0.20	(-0.27, 0.43)

<sup>a</sup>MPN = Most Probable number is based on the POD of the reference method test portions using the Least Cost Formulations MPN calculator with 95% confidence interval.

<sup>b</sup>N = Number of test portions

<sup>c</sup>X = Number of positive test portions

<sup>d</sup>PODc = Confirmed candidate method positive outcomes divided by the total number of portions

<sup>e</sup>PODr = Confirmed reference method positive outcomes divided by the total number of portions

<sup>f</sup>dPODc = Difference between the candidate presumptive result and the candidate method confirmed result POD values

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

<sup>h</sup>N/A = Not applicable

<sup>i</sup>Independent Laboratory Study

**DISCUSSION OF MODIFICATION APPROVED FEBRUARY 2015 (8)**

The results generated during this study to extend the validated matrices of the SureTect Listeria monocytogenes assay in comparison to the ISO reference method are summarized in Tables 1 to 1. For ground raw turkey meat, pasteurized milk, raw pork sausage, raw cod fillet, cooked sliced ham, bagged lettuce and the low spiked samples of raw ground pork and brie cheese, the SureTect assay returned results that were equivalent, by POD statistical analysis at the 95% confidence interval, to the ISO reference method. The independent laboratory study, carried out on bagged lettuce, raw cod fillet and brie cheese, gave similar results to the internal method developer study and confirmed that there were no statistically significant differences between the SureTect and reference methods.

When comparing the results for the high spike level samples of raw ground pork between the SureTect and ISO reference methods, all five of these samples gave confirmed positive results with the SureTect assay, but only two of the five samples prepared and analyzed with the ISO reference method were shown to give a positive result. Similar results were seen with brie cheese, where four of the five high level spiked samples, gave a confirmed positive result with the SureTect method, compared to the ISO reference method which failed to produce any positive results with the high spiked samples, analyzed. Statistical analysis of the data for the high spiked samples of raw ground pork and brie cheese demonstrated a statistical difference by POD analysis, at the 95% confidence level between the two methods, showing that the SureTect method was able to detect a higher number of confirmed positives than the reference method.

The SureTect confirmation method and reference confirmation tests were compared by POD statistical analysis (see Table 5) to verify suitability of the SureTect confirmation procedure. Results for all food matrices analyzed during this method modification study re-confirmed that the confirmation protocol for this SureTect assay is reliable and equivalent to that of the ISO reference method, as POD statistical analysis demonstrated no statistical differences between direct plating of the 24 LEB enrichment onto *Brilliance* Listeria Agar followed by biochemical confirmation with the Microbact 12L kit and the tests detailed in the ISO reference method.

**Table 1: SureTect Listeria monocytogenes assay Presumptive vs. SureTect Confirmation Procedure Confirmed Result-POD Analysis (8)**

Matrix	Strain	MPN <sup>a</sup> /test portion	N <sup>b</sup>	SureTect Method Presumptive			SureTect Method Confirmation			dPODcp <sup>f</sup>	95% CI <sup>g</sup>
				X <sup>c</sup>	POD <sub>cp</sub> <sup>d</sup>	95% CI	X	POD <sub>cc</sub> <sup>e</sup>	95% CI		
Raw ground turkey	<i>Listeria monocytogenes</i> (TCC 1227)	N/A <sup>h</sup>	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.59 (0.31, 1.06)	20	9	0.445	(0.26, 0.66)	10	0.50	(0.30, 0.70)	-0.05	(-0.33, 0.24)
		0.95 (0.47, 1.91)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Raw ground pork	<i>Listeria monocytogenes</i> (TCC 883)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.86 (1.15, 3.79)	20	14	0.70	(0.48, 0.85)	14	0.70	(0.48, 0.85)	0.00	(-0.27, 0.27)
		0.56 (0.23, 1.32)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Pasteurized 2% milk	<i>Listeria monocytogenes</i> (TCC 840)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.28 (0.11, 0.56)	20	4	0.20	(0.08, 0.42)	4	0.20	(0.08, 0.42)	0.00	(-0.25, 0.25)
		1.09 (0.47, 2.46)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Raw pork sausages	<i>Listeria monocytogenes</i> (TCC 867)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.01 (0.42, 1.03)	20	9	0.45	(0.26, 0.66)	9	0.45	(0.26, 0.66)	0.00	(-0.28, 0.28)
		3.10 (1.42, 6.77)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Raw cod	<i>Listeria monocytogenes</i> (TCC 1226)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.33 (0.81, 2.30)	20	15	0.75	(0.53, 0.89)	15	0.75	(0.53, 0.89)	0.00	(-0.26, 0.26)
		4.37 (1.71, 11.19)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Brie	<i>Listeria monocytogenes</i> (TCC 1210)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.12 (0.05, 0.31)	20	5	0.25	(0.11, 0.47)	5	0.25	(0.11, 0.47)	0.00	(-0.26, 0.26)
		0.22 (0.08, 0.61)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Cooked ham	<i>Listeria monocytogenes</i> (TCC 1198)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.90 (0.53, 1.48)	20	16	0.80	(0.58, 0.92)	16	0.80	(0.58, 0.92)	0.00	(-0.25, 0.25)

		2.96 (1.25, 7.00)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Bagged lettuce	<i>Listeria monocytogenes</i> (TCC 1220)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.53 (0.28, 9.66)	20	6	0.30	(0.15, 0.52)	6	0.30	(0.15, 0.52)	0.00	(-0.27, 0.27)
		1.09 (0.49, 2.46)	5	5	1.00	(0.57, 1.00)	4	0.80	(0.38, 1.00)	0.20	(-0.28, 0.62)
Raw cod <sup>i</sup>	<i>Listeria monocytogenes</i> (ATCC <sup>®</sup> 19115™)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.47 (0.28, 0.75)	20	9	0.45	(0.25, 0.65)	9	0.45	(0.25, 0.65)	0.00	(-0.28, 0.28)
		4.38 (1.71, 11.19)	5	5	1.00	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0.00	(-0.43, 0.43)
Brie <sup>i</sup>	<i>Listeria monocytogenes</i> (LI0512)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.46 (0.28, 0.75)	20	5	0.25	(0.11, 0.46)	5	0.25	(0.11, 0.46)	0.00	(-0.25, 0.25)
		4.38 (1.71, 11.19)	5	5	1.00	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0.00	(-0.43, 0.43)
Bagged lettuce <sup>i</sup>	<i>Listeria monocytogenes</i> (LI0549)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.37 (0.23, 0.59)	20	10	0.50	(0.29, 0.70)	10	0.50	(0.29, 0.70)	0.00	(-0.28, 0.28)
		2.19 (0.93, 5.12)	5	5	1.00	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0.00	(-0.43, 0.43)

<sup>a</sup>MPN = Most Probable number is based on the POD of the reference method test portions using the Least Cost Formulations MPN calculator with 95% confidence interval.

<sup>b</sup>N = Number of test portions.

<sup>c</sup>X = Number of positive test portions.

<sup>d</sup>POD<sub>cp</sub> = Candidate method presumptive positive outcomes divided by the total number of portions.

<sup>e</sup>POD<sub>cc</sub> = Candidate confirmation method positive outcomes divided by the total number of portions.

<sup>f</sup>dPOD<sub>cp</sub> = Difference between the candidate presumptive result and the candidate method confirmed result using the SureTect confirmation procedure POD values.

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

<sup>h</sup>N/A = Not applicable.

<sup>i</sup>Independent laboratory study.

**Table 2: SureTect *Listeria monocytogenes* assay Presumptive vs. Reference Confirmation Procedure Confirmed Result-POD Analysis (8)**

Matrix	Strain	MPN <sup>a</sup> /test portion	N <sup>b</sup>	SureTect Method Presumptive			Reference Confirmation (CC2)			dPOD <sub>cp</sub> <sup>f</sup>	95% CI <sup>g</sup>
				X <sup>c</sup>	POD <sub>cp</sub> <sup>d</sup>	95% CI	X	POD <sub>cc</sub> <sup>e</sup>	95% CI		
Raw ground turkey	<i>Listeria monocytogenes</i> (TCC 1227 )	N/A <sup>h</sup>	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.59 (0.31, 1.06)	20	9	0.45	(0.26, 0.66)	10	0.50	(0.30, 0.70)	-0.05	(-0.33, 0.24)
		0.95 (0.47, 1.91)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Raw ground pork	<i>Listeria monocytogenes</i> (TCC 883)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.86 (1.15, 3.79)	20	14	0.70	(0.48, 0.85)	14	0.70	(0.48, 0.85)	0.00	(-0.27, 0.27)
		0.56 (0.23, 1.32)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Pasteurized 2% milk	<i>Listeria monocytogenes</i> (OCC 840)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.28 (0.11, 0.56)	20	4	0.20	(0.08, 0.42)	4	0.20	(0.08, 0.42)	0.00	(-0.25, 0.25)
		1.09	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)



		(0.47, 2.46)									
Raw pork sausages	<i>Listeria monocytogenes</i> (TCC 867)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.01 (0.42, 1.03)	20	9	0.45	(0.26, 0.66)	10	0.50	(0.30, 0.70)	-0.05	(-0.33, 0.24)
		3.10 (1.42, 6.77)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Raw cod	<i>Listeria monocytogenes</i> (TCC 1226)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.33 (0.81, 2.30)	20	15	0.75	(0.53, 0.89)	15	0.75	(0.53, 0.89)	0.00	(-0.26, 0.26)
		4.37 (1.71, 11.19)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Brie	<i>Listeria monocytogenes</i> (TCC 1210)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.12 (0.05, 0.31)	20	5	0.25	(0.11, 0.47)	5	0.25	(0.11, 0.47)	0.00	(-0.26, 0.26)
		0.22 (0.08, 0.61)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Cooked ham	<i>Listeria monocytogenes</i> (TCC 1198)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.90 (0.53, 1.48)	20	16	0.80	(0.58, 0.92)	16	0.80	(0.58, 0.92)	0.00	(-0.25, 0.25)
		2.96 (1.25, 7.00)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Fresh bagged lettuce	<i>Listeria monocytogenes</i> (TCC 1220)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.53 (0.28, 9.66)	20	6	0.30	(0.15, 0.52)	6	0.30	(0.15, 0.52)	0.00	(-0.27, 0.27)
		1.09 (0.49, 2.46)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Raw cod <sup>d</sup>	<i>Listeria monocytogenes</i> (ATCC <sup>®</sup> 19115™)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.47 (0.28, 0.75)	20	9	0.45	(0.26, 0.66)	9	0.45	(0.26, 0.66)	0.00	(-0.28, 0.28)
		4.38 (1.71, 11.19)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Brie <sup>e</sup>	<i>Listeria monocytogenes</i> (LI0512)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.46 (0.28, 0.75)	20	5	0.25	(0.11, 0.47)	5	0.25	(0.11, 0.47)	0.00	(-0.26, 0.26)
		4.38 (1.71, 11.19)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Bagged lettuce <sup>f</sup>	<i>Listeria monocytogenes</i> (LI0549)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.37 (0.23, 0.59)	20	10	0.50	(0.29, 0.70)	10	0.50	(0.29, 0.70)	0.00	(-0.28, 0.28)
		2.19 (0.93, 5.12)	5	5	1.00	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0.00	(-0.43, 0.43)

<sup>a</sup>MPN = Most Probable number is based on the POD of the reference method test portions using the Least Cost Formulations MPN calculator with 95% confidence interval.

<sup>b</sup>N = Number of test portions.

<sup>c</sup>X = Number of positive test portions.

<sup>d</sup>POD<sub>cp</sub> = Candidate method presumptive positive outcomes divided by the total number of portions.

<sup>e</sup>POD<sub>cc2</sub> = Reference method confirmation positive outcomes divided by the total number of portions.

<sup>f</sup>dPOD<sub>cp</sub> = Difference between the candidate presumptive result and the candidate method confirmed result using the reference confirmation procedure POD values.

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

<sup>h</sup>N/A = Not applicable.

<sup>i</sup>Independent laboratory study.

Table 3: SureTect Listeria monocytogenes assay: SureTect Confirmation Procedure Confirmed Result vs. Reference Confirmation Procedure Confirmed Result -POD Analysis (8)

Matrix	Strain	MPN <sup>a</sup> /test portion	N <sup>b</sup>	SureTect Confirmation Method (CC)			Reference Method Confirmation (CC2)			dPOD <sub>cc</sub> <sup>f</sup>	95% CI <sup>g</sup>
				X <sup>c</sup>	POD <sub>cc</sub> <sup>d</sup>	95% CI	X	POD <sub>cc2</sub> <sup>e</sup>	95% CI		
Raw ground turkey	<i>Listeria monocytogenes</i> (TCC 1227)	N/A <sup>h</sup>	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.59 (0.31, 1.06)	20	10	0.50	(0.30, 0.70)	10	0.50	(0.30, 0.70)	0.00	(-0.47, 0.47)
		0.95 (0.47, 1.91)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Raw ground pork	<i>Listeria monocytogenes</i> (TCC 883)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.86 (1.15, 3.79)	20	14	0.70	(0.48, 0.85)	14	0.70	(0.48, 0.85)	0.00	(-0.27, 0.27)
		0.56 (0.23, 1.32)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Pasteurized 2% milk	<i>Listeria monocytogenes</i> (TCC 840)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.28 (0.11, 0.56)	20	4	0.20	(0.08, 0.42)	4	0.20	(0.08, 0.42)	0.00	(-0.25, 0.25)
		1.09 (0.47, 2.46)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Raw pork sausages	<i>Listeria monocytogenes</i> (TCC 867)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.01 (0.42, 1.03)	20	9	0.45	(0.26, 0.66)	10	0.50	(0.30, 0.70)	-0.05	(-0.33, 0.24)
		3.10 (1.42, 6.77)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Raw cod	<i>Listeria monocytogenes</i> (TCC 1226)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.33 (0.81, 2.30)	20	15	0.75	(0.53, 0.89)	15	0.75	(0.53, 0.89)	0.00	(-0.26, 0.26)
		4.37 (1.71, 11.19)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Brie	<i>Listeria monocytogenes</i> (TCC 1210)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.12 (0.05, 0.31)	20	5	0.25	(0.11, 0.47)	5	0.25	(0.11, 0.47)	0.00	(-0.26, 0.26)
		0.22 (0.08, 0.61)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Cooked ham	<i>Listeria monocytogenes</i> (TCC 1198)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.90 (0.53, 1.48)	20	16	0.80	(0.58, 0.92)	16	0.80	(0.58, 0.92)	0.00	(-0.25, 0.25)
		2.96 (1.25, 7.00)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Bagged lettuce	<i>Listeria monocytogenes</i> (TCC 1220)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.53 (0.28, 9.66)	20	6	0.30	(0.15, 0.52)	6	0.30	(0.15, 0.52)	0.00	(-0.27, 0.27)
		1.09 (0.49, 2.46)	5	4	0.80	(0.38, 1.00)	5	1.00	(0.57, 1.00)	-0.20	(-0.62, 0.28)
Raw cod <sup>i</sup>	<i>Listeria monocytogenes</i> (ATCC <sup>®</sup> 19115 <sup>™</sup> )	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.47 (0.28, 0.75)	20	9	0.45	(0.26, 0.66)	9	0.45	(0.26, 0.66)	0.00	(-0.28, 0.28)

		4.38 (1.71, 11.19)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Brie <sup>d</sup>	<i>Listeria monocytogenes</i> (LI0512)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.46 (0.28, 0.75)	20	5	0.25	(0.11, 0.47)	5	0.25	(0.11, 0.47)	0.00	(-0.26, 0.26)
		4.38 (1.71, 11.19)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Bagged lettuce <sup>d</sup>	<i>Listeria monocytogenes</i> (LI0549)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.37 (0.23, 0.59)	20	10	0.50	(0.29, 0.70)	10	0.50	(0.29, 0.70)	0.00	(-0.28, 0.28)
		2.19 (0.93, 5.12)	5	5	1.00	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0.00	(-0.43, 0.43)

<sup>a</sup>MPN = Most Probable number is based on the POD of the reference method test portions using the Least Cost Formulations MPN calculator with 95% confidence interval.

<sup>b</sup>N = Number of test portions.

<sup>c</sup>X = Number of positive test portions.

<sup>d</sup>POD<sub>cc</sub> = Candidate method confirmed positive outcomes by candidate confirmation procedure divided by the total number of portions.

<sup>e</sup>POD<sub>cc2</sub> = Candidate method confirmed positive outcomes by reference confirmation procedure divided by the total number of portions.

<sup>f</sup>dPOD<sub>cp</sub> = Difference between the candidate confirmation procedure confirmed result and the reference confirmation procedure confirmed result POD values.

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

<sup>h</sup>N/A = Not applicable.

<sup>i</sup>Independent laboratory study.

**Table 4: SureTect Listeria monocytogenes assay Confirmed Results vs. Reference Method-POD Analysis (8)**

Matrix	Strain	MPN <sup>a</sup> /test portion	N <sup>b</sup>	SureTect Method (C)			Reference Method (R)			dPOD <sub>cp</sub> <sup>f</sup>	95% CI <sup>g</sup>
				X <sup>c</sup>	POD <sub>cc</sub> <sup>d</sup>	95% CI	X	POD <sub>cc2</sub> <sup>e</sup>	95% CI		
Raw ground turkey	<i>Listeria monocytogenes</i> (TCC 1227)	N/A <sup>h</sup>	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.59 (0.31, 1.06)	20	9	0.45	(0.26, 0.66)	10	0.50	(0.30, 0.70)	-0.05	(-0.33, 0.24)
		0.95 (0.47, 1.91)	5	4	0.80	(0.38, 1.00)	5	1.00	(0.57, 1.00)	-0.20	(-0.62, 0.28)
Raw ground pork	<i>Listeria monocytogenes</i> (TCC 883)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.86 (1.15, 3.79)	20	14	0.70	(0.48, 0.85)	16	0.80	(0.58, 0.92)	-0.10	(-0.35, 0.17)
		0.56 (0.23, 1.32)	5	5	1.00	(0.57, 1.00)	2	0.40	(0.12, 0.77)	0.60	(0.03, 0.88)
Pasteurized 2% milk	<i>Listeria monocytogenes</i> (TCC 840)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.28 (0.11, 0.56)	20	4	0.20	(0.08, 0.42)	5	0.25	(0.11, 0.47)	-0.05	(-0.30, 0.21)
		1.09 (0.47, 2.46)	5	5	1.00	(0.57, 1.00)	3	0.60	(0.23, 0.88)	0.40	(-0.12, 0.77)
Raw pork sausages	<i>Listeria monocytogenes</i> (TCC 867)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.01 (0.42, 1.03)	20	9	0.45	(0.26, 0.66)	10	0.50	(0.30, 0.70)	-0.05	(-0.33, 0.24)
		3.10 (1.42, 6.77)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Raw cod	<i>Listeria monocytogenes</i> (TCC 1226)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.33 (0.81, 2.30)	20	15	0.75	(0.53, 0.89)	14	0.70	(0.48, 0.85)	0.05	(-0.22, 0.31)

		4.37 (1.71, 11.19)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Brie	<i>Listeria monocytogenes</i> (TCC 1210)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.12 (0.05, 0.31)	20	5	0.25	(0.11, 0.47)	1	0.05	(0.00, 0.24)	0.20	(-0.03, 0.42)
		0.22 (0.08, 0.61)	5	4	0.80	(0.38, 1.00)	0	0.00	(0.00, 0.43)	0.80	(0.19, 1.00)
Cooked ham	<i>Listeria monocytogenes</i> (TCC 1198)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.90 (0.53, 1.48)	20	16	0.80	(0.58, 0.92)	12	0.60	(0.39, 0.78)	0.20	(-0.08, 0.44)
		2.96 (1.25, 7.00)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)

Bagged lettuce	<i>Listeria monocytogenes</i> (TCC 1220)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.53 (0.28, 9.66)	20	6	0.30	(0.15, 0.52)	9	0.45	(0.26, 0.66)	-0.15	(-0.41, 0.14)
		1.09 (0.49, 2.46)	5	4	0.80	(0.38, 1.00)	3	0.60	(0.23, 0.88)	0.20	(-0.31, 0.62)
Raw cod <sup>d</sup>	<i>Listeria monocytogenes</i> (ATCC <sup>®</sup> 19115 <sup>™</sup> )	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.47 (0.28, 0.75)	20	9	0.45	(0.25, 0.65)	6	0.30	(0.14, 0.51)	0.15	(-0.14, 0.40)
		4.38 (1.71, 11.19)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Brie <sup>e</sup>	<i>Listeria monocytogenes</i> (LI0512)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.46 (0.28, 0.75)	20	5	0.25	(0.11, 0.46)	8	0.40	(0.21, 0.61)	-0.15	(-0.40, 0.13)
		4.38 (1.71, 11.19)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Bagged lettuce <sup>f</sup>	<i>Listeria monocytogenes</i> (LI0549)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.37 (0.23, 0.59)	20	10	0.50	(0.29, 0.70)	5	0.25	(0.11, 0.46)	0.25	(-0.04, 0.49)
		2.19 (0.93, 5.12)	5	5	1.00	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0.00	(-0.43, 0.43)

<sup>a</sup>MPN = Most Probable number is based on the POD of the reference method test portions using the Least Cost Formulations MPN calculator with 95% confidence interval.

<sup>b</sup>N = Number of test portions.

<sup>c</sup>X = Number of positive test portions.

<sup>d</sup>PODc = Confirmed candidate method positive outcomes divided by the total number of portions.

<sup>e</sup>PODr = Confirmed reference method positive outcomes divided by the total number of portions.

<sup>f</sup>dPODc = Difference between the candidate method confirmed result and the reference method confirmed result POD values.

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

<sup>h</sup>N/A = Not applicable.

<sup>i</sup>Independent Laboratory Study.

**DISCUSSION OF MODIFICATION APPROVED OCTOBER 2015 (9)**

Results from the matrices analyzed during this method modification study to validate the use of the Applied Biosystems 7500 Fast Instrument and Applied Biosystems RapidFinder Express 2.0 Software as alternative parts of the SureTect Assay workflow in comparison to the ISO reference method.

For all three of the food matrices and the stainless steel surface analyzed, the SureTect assay returned results which were not statistically different when analyzed using POD statistical analysis compared to the reference method. Although not giving any statistically different results following the POD analysis, one of the low spiked samples for ground turkey meat and one of the low spiked samples analyzed for the stainless steel surface gave negative results with the SureTect method, while the SureTect confirmation method (Table 3) and reference method confirmation procedure gave positive results. For the presumptive negative sample which was confirmed as positive for raw ground turkey, the estimated plate counts from the direct plating of the 24 LEB enrichment, estimated the contamination level to be approximately  $6 \times 10^2$  CFU/mL. This level of contamination is below the level of detection of the PCR assay and the false negative PCR results was most possibly due to inefficient mixing of the bulk food sample, which resulted in very low numbers of *L. monocytogenes* cells being present in the 25 g portion of the sample removed for this particular analysis. Plating methods (as used for the SureTect confirmation method) and the secondary enrichment performed for the reference method, have good sensitivity and are able in theory to detect as little as one cell.

For the pasteurized milk and lettuce matrices analyzed during this study, no false results were recorded with the SureTect PCR assay and presumptive and confirmed results were in agreement between the PCR assay, candidate confirmation method and reference confirmation conducted to confirm the candidate method results. For all three food matrices and the surface samples analyzed, there was no difference according to POD statistical analysis between the SureTect PCR method and the ISO reference method (Table 6). The closest to a potential difference in performance of the two methods (in favor of the SureTect method) was for 2% fat pasteurized milk, where the confidence interval was -0.01 to 0.48 with a dPOD of 0.25. As the method was unpaired, differences in the numbers of positive results between the candidate and reference methods are inevitable, despite efforts to thoroughly mix samples and the random allocation of samples to each of the two methods from the bulk spiked sample.

The SureTect Listeria monocytogenes PCR detection method is a very simple and reliable method, which gives next day results following a simple and selective enrichment procedure. Prefilled lysis reagent tubes and the use of lyophilized PCR reagents and dedicated software to interpret results as positive or negative, all combine to reduce the user "hands on" time which is an important factor in today's busy food microbiology laboratory.

**Inclusivity Results For The SureTect Assay (9)**

Isolate	Serotype	TCC No.	Source	Sure75 LMO result
<i>Listeria monocytogenes</i>	3a	812	Environmental	+
<i>Listeria monocytogenes</i>	3a	813	Environmental	+
<i>Listeria monocytogenes</i>	3a	840	Butter	+
<i>Listeria monocytogenes</i>	Untyped	856	Unknown food source-isolated by food lab	+
<i>Listeria monocytogenes</i>	Untyped	857	Unknown food source-isolated by food lab	+
<i>Listeria monocytogenes</i>	1/2c	858	Clinical sample	+
<i>Listeria monocytogenes</i>	Untyped	859	Tartare de Salmon	+
<i>Listeria monocytogenes</i>	1/2a	860	Poultry	+
<i>Listeria monocytogenes</i>	4d	863	Sheep	+
<i>Listeria monocytogenes</i>	4b	864	Meningitis	+
<i>Listeria monocytogenes</i>	4b	865	CSF: Meningitis	+
<i>Listeria monocytogenes</i>	Untyped	866	Unknown ATCC isolate	+
<i>Listeria monocytogenes</i>	2	867	CSF: Clinical	+
<i>Listeria monocytogenes</i>	4e	868	Chicken	+
<i>Listeria monocytogenes</i>	3a	870	Clinical sample	+
<i>Listeria monocytogenes</i>	Untyped	871	Unknown food source-isolated by food lab	+
<i>Listeria monocytogenes</i>	Untyped	873	Unknown food source-isolated by food lab	+
<i>Listeria monocytogenes</i>	Untyped	874	Unknown food source-isolated by food lab	+
<i>Listeria monocytogenes</i>	Untyped	875	Unknown food source-isolated by food lab	+
<i>Listeria monocytogenes</i>	Untyped	881	Unknown food source-isolated by food lab	+
<i>Listeria monocytogenes</i>	Untyped	882	Unknown food source-isolated by food lab	+
<i>Listeria monocytogenes</i>	4e	883	Veterinary sample	+
<i>Listeria monocytogenes</i>	4e	884	Unknown	+
<i>Listeria monocytogenes</i>	4e	885	Unknown	+
<i>Listeria monocytogenes</i>	3a	888	Food	+
<i>Listeria monocytogenes</i>	3a	889	Food	+
<i>Listeria monocytogenes</i>	1/2c	1195	Ox tongue	+
<i>Listeria monocytogenes</i>	1/2c	1196	Roast beef	+
<i>Listeria monocytogenes</i>	1/2c	1197	Topside beef	+
<i>Listeria monocytogenes</i>	1/2c	1198	Wiltshire ham	+
<i>Listeria monocytogenes</i>	1/2c	1199	Ham sandwich	+
<i>Listeria monocytogenes</i>	1/2b	1205	Cake	+
<i>Listeria monocytogenes</i>	1/2b	1206	Whipped Cream	+
<i>Listeria monocytogenes</i>	1/2b	1207	Cheese	+
<i>Listeria monocytogenes</i>	1/2b	1208	Cheese	+
<i>Listeria monocytogenes</i>	1/2b	1209	Cream	+
<i>Listeria monocytogenes</i>	1/2b	1210	Cheese	+
<i>Listeria monocytogenes</i>	1/2a	1215	Chorizo sausage	+
<i>Listeria monocytogenes</i>	1/2a	1216	Sandwich	+
<i>Listeria monocytogenes</i>	1/2a	1217	Carrow cheese	+
<i>Listeria monocytogenes</i>	1/2a	1218	Butter	+

<i>Listeria monocytogenes</i>	1/2a	1219	Pilau rice	+
<i>Listeria monocytogenes</i>	1/2a	1220	Sandwich	+
<i>Listeria monocytogenes</i>	4b	1224	Food- blood	+
<i>Listeria monocytogenes</i>	4b	1225	Chicken	+
<i>Listeria monocytogenes</i>	4b	1226	Dressed crab	+
<i>Listeria monocytogenes</i>	4b	1227	Turkey breast	+
<i>Listeria monocytogenes</i>	4b	1841	ATCC 19115	+
<i>Listeria monocytogenes</i>	3b	2179	Unknown	+
<i>Listeria monocytogenes</i>	3c	2180	Unknown	+
<i>Listeria monocytogenes</i>	4a	2181	Unknown	+
<i>Listeria monocytogenes</i>	4c	2183	Bird: heart disease	+
<i>Listeria monocytogenes</i>	7	2184	Faecal sample	+

<sup>a</sup>Trials Culture Collection – Proprietary.

**Exclusivity Results For The SureTect Assay (9)**

Isolate	Source	TCC No	Sure75 LMO result
<i>Listeria ivanovii</i> subsp. <i>londoniensis</i>	NCTC	869	-
<i>Listeria grayii</i>	Unknown	872	-
<i>Listeria ivanovii</i>	Food-unknown	880	-
<i>Listeria grayii</i>	Environmental sample	1172	-
<i>Listeria grayii</i>	Butter	1174	-
<i>Listeria innocua</i>	Chicken sandwich	1177	-
<i>Listeria ivanovii</i>	Food-unknown	1183	-
<i>Listeria ivanovii</i>	Food-unknown	1184	-
<i>Listeria welshimeri</i>	Chicken sandwich	1185	-
<i>Listeria seeligeri</i>	Cheese	1190	-
<i>Listeria seeligeri</i>	Food-unknown	1191	-
<i>Proteus vulgaris</i>	Unknown	1424	-
<i>Escherichia coli</i>	NCTC	1809	-
<i>Klebsiella pneumoniae</i>	NCTC	1892	-
<i>Salmonella enterica</i> subsp. <i>enterica</i> Typhimurium	NCTC	1911	-
<i>Citrobacter freundii</i>	NCTC	1913	-
<i>Listeria welshimeri</i> 6b	NCTC	1978	-
<i>Listeria seeligeri</i> 1/2b	NCTC	1979	-
<i>Listeria innocua</i> 6a	NCTC	2186	-
<i>Listeria welshimeri</i> 6b	Institute Pasteur	2188	-
<i>Brochothrix thermosphacta</i>	Pork sausage	2192	-
<i>Kurthia gibsonii</i>	Pork sausage	2193	-
<i>Lactobacillus casei</i> subsp. <i>casei</i>	Fermented catsup	2194	-
<i>Lactobacillus delbrueckii</i> subsp. <i>lactis</i>	Emmenthal cheese	2195	-
<i>Lactobacillus plantarum</i>	Red Cheshire cheese	2196	-
<i>Enterobacter aerogenes</i>	Unknown	2200	-
<i>Staphylococcus aureus</i>	Food-unknown	2240	-
<i>Carnobacterium divergens</i>	Brie	2258	-
<i>Carnobacterium gallinarum</i>	Unknown	2259	-
<i>Carnobacterium piscicola</i>	Cooked ham	2260	-
<i>Erysipelothrix rhusiopathiae</i>	Unknown	2262	-
<i>Escherichia fergusonii</i>	Sausages	2263	-
<i>Bacillus cereus</i>	Milk	2299	-
<i>Bacillus mycoides</i>	NCTC	2300	-
<i>Propionibacterium freundenreichii</i>	Swiss cheese production	2304	-
<i>Streptococcus salivarius</i>	NCTC	2352	-
<i>Rhodococcus equi</i>	NCTC	2358	-
<i>Micrococcus luteus</i>	NCIMB	OCC 2352	-

<sup>a</sup>Trials Culture Collection – Proprietary.

**Table 7: Thermo Scientific SureTect Listeria monocytogenes PCR Assay Presumptive vs. SureTect Method Confirmation Procedure Confirmed Results – POD Analysis (9)**

Matrix	Strain	MPN <sup>a</sup> /test portion	N <sup>b</sup>	SureTect Method Presumptive (CP)			SureTect Method Confirmation (CC)			dPOD <sub>CP</sub> <sup>f</sup>	95% CI <sup>g</sup>
				X <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	X	POD <sub>CC</sub> <sup>e</sup>	95% CI		
Raw ground turkey	<i>Listeria monocytogenes</i> TCC 1227	N/A <sup>h</sup>	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.45, 0.45)
		0.69 (0.39, 1.14)	20	9	0.45	(0.26, 0.66)	10	0.50	(0.30, 0.70)	-0.05	(-0.33, 0.24)
		4.38 (0.06, 11.15)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Pasteurized 2% milk	<i>Listeria monocytogenes</i> TCC 856	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.45, 0.45)
		1.26 (0.79, 2.07)	20	18	0.90	(0.70, 0.97)	18	0.90	(0.70, 0.97)	0.00	(-0.21, 0.21)
		3.00 (1.311, 6.89)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Bagged lettuce	<i>Listeria monocytogenes</i> TCC 1220	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.45, 0.45)
		1.00 (0.66, 1.55)	20	13	0.65	(0.43, 0.82)	13	0.65	(0.43, 0.82)	0.00	(-0.28, 0.28)
		1.13 (0.57, 2.25)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Stainless steel surface 4" x 4"	<i>Listeria monocytogenes</i> TCC 813 and <i>Enterococcus faecalis</i> CIP100750 X10	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.45, 0.45)
		N/A	20	6	0.30	(0.15, 0.52)	7	0.35	(0.18, 0.57)	-0.05	(-0.32, 0.23)
		N/A	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)

<sup>a</sup>MPN= Most Probable number is based on the POD of the reference method test portions using the Least Cost Formulations MPN calculator with 95% confidence interval.

<sup>b</sup>N=Number of test portions.

<sup>c</sup>X=Number of positive test portions.

<sup>d</sup>POD<sub>CP</sub>=Candidate method presumptive positive outcomes divided by the total number of portions.

<sup>e</sup>POD<sub>CC</sub>=Candidate confirmation method positive outcomes divided by the total number of portions.

<sup>f</sup>dPOD<sub>CP</sub>=Difference between the candidate presumptive result and the candidate method confirmed result POD values.

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

<sup>h</sup>N/A=Not applicable.

**Table 8: Thermo Scientific SureTect Listeria monocytogenes PCR Assay Presumptive vs. Reference Method Confirmation Procedure Confirmed Results – POD Analysis (9)**

Matrix	Strain	MPN <sup>a</sup> /test portion	N <sup>b</sup>	SureTect Method Presumptive			Reference Method Confirmation (RC)			dPOD <sub>CP</sub> <sup>f</sup>	95% CI <sup>g</sup>
				X <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	X	POD <sub>RC</sub> <sup>e</sup>	95% CI		
Raw ground turkey	<i>Listeria monocytogenes</i> TCC 1227	N/A <sup>h</sup>	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.45, 0.45)
		0.69 (0.39, 1.14)	20	9	0.45	(0.26, 0.66)	10	0.50	(0.30, 0.70)	-0.05	(-0.33, 0.24)
		4.38 (0.06, 11.15)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Pasteurized 2% milk	<i>Listeria monocytogenes</i> TCC 856	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.45, 0.45)
		1.26 (0.79, 2.07)	20	18	0.90	(0.70, 0.97)	18	0.90	(0.70, 0.97)	0.00	(-0.21, 0.21)
		3.00 (1.311, 6.89)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Bagged lettuce	<i>Listeria monocytogenes</i> TCC 1220	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.45, 0.45)
		1.00 (0.66, 1.55)	20	13	0.65	(0.43, 0.82)	13	0.65	(0.43, 0.82)	0.00	(-0.28, 0.28)
		1.13 (0.57, 2.25)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Stainless steel surface 4" x 4"	<i>Listeria monocytogenes</i> TCC 813 and <i>Enterococcus faecalis</i> CIP100750 X10	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.45, 0.45)
		N/A	20	6	0.30	(0.15, 0.52)	7	0.35	(0.18, 0.57)	-0.05	(-0.32, 0.23)
		N/A	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)

<sup>a</sup>MPN= Most Probable number is based on the POD of the reference method test portions using the Least Cost Formulations MPN calculator with 95% confidence interval.

<sup>b</sup>N=Number of test portions.

<sup>c</sup>X=Number of positive test portions.

<sup>d</sup>POD<sub>CP</sub>=Candidate method presumptive positive outcomes divided by the total number of portions.

<sup>e</sup>POD<sub>RC</sub>=Reference confirmation, positive outcomes divided by the total number of portions.

<sup>f</sup>dPOD<sub>CP</sub>=Difference between the candidate presumptive result and the reference confirmation method confirmed result POD values.

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

<sup>h</sup>N/A=Not applicable.



**Table 9: Thermo Scientific SureTect Listeria monocytogenes PCR Assay Confirmation Procedure Confirmed Results vs. Reference Method Confirmation Procedure Confirmed Results – POD Analysis (9)**

Matrix	Strain	MPN <sup>a</sup> /test portion	N <sup>b</sup>	SureTect Method Confirmation (CC)			Reference Method Confirmation (RC)			dPOD <sub>CC</sub> <sup>f</sup>	95% CI <sup>g</sup>
				X <sup>c</sup>	POD <sub>CC</sub> <sup>d</sup>	95% CI	X	POD <sub>RC</sub> <sup>e</sup>	95% CI		
Raw ground turkey	<i>Listeria monocytogenes</i> TCC 1272	N/A <sup>h</sup>	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.45, 0.45)
		0.69 (0.39, 1.14)	20	10	0.50	(0.30, 0.70)	10	0.50	(0.30, 0.70)	0.00	(-0.28, 0.28)
		4.38 (0.06, 11.15)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Pasteurized 2% milk	<i>Listeria monocytogenes</i> TCC 856	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.45, 0.45)
		1.26 (0.79, 2.07)	20	18	0.90	(0.70, 0.97)	18	0.90	(0.70, 0.97)	0.00	(-0.21, 0.21)
		3.00 (1.311, 6.89)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Bagged lettuce	<i>Listeria monocytogenes</i> TCC 1220	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.45, 0.45)
		1.00 (0.66, 1.55)	20	13	0.65	(0.43, 0.82)	13	0.65	(0.43, 0.82)	0.00	(-0.21, 0.21)
		1.13 (0.57, 2.25)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Stainless steel surface 4" x 4"	<i>Listeria monocytogenes</i> TCC 813 and <i>Enterococcus faecalis</i> CIP100750 X10	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.45, 0.45)
		N/A	20	7	0.35	(0.18, 0.57)	7	0.35	(0.18, 0.57)	0.00	(-0.21, 0.21)
		N/A	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)

<sup>a</sup>MPN= Most Probable number is based on the POD of the reference method test portions using the Least Cost Formulations MPN calculator with 95% confidence interval.

<sup>b</sup>N=Number of test portions.

<sup>c</sup>X=Number of positive test portions.

<sup>d</sup>POD<sub>CC</sub>=Candidate method confirmed positive outcomes divided by the total number of portions.

<sup>e</sup>POD<sub>RC</sub>=Reference confirmation, positive outcomes divided by the total number of portions.

<sup>f</sup>dPOD<sub>CC</sub>=Difference between the candidate confirmed result and the reference confirmation result POD values.

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

<sup>h</sup>N/A=Not applicable.

**Table 6: Thermo Scientific SureTect Listeria monocytogenes PCR Assay Confirmed Results vs. Reference Method Results – POD Analysis (9)**

Matrix	Strain	MPN <sup>a</sup> /test portion	N <sup>b</sup>	SureTect Method Confirmed (C)			Reference Method (R)			dPOD <sub>c</sub> <sup>f</sup>	95% CI <sup>g</sup>
				X <sup>c</sup>	POD <sub>c</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI		
Raw ground turkey	<i>Listeria monocytogenes</i> TCC 1227	N/A <sup>h</sup>	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.45, 0.45)
		0.69 (0.39, 1.14)	20	9	0.45	(0.26, 0.66)	9	0.45	(0.26, 0.66)	0.00	(-0.28, 0.28)
		4.38 (0.06, 11.15)	5	4	0.80	(0.38, 1.00)	5	1.00	(0.57, 1.00)	-0.20	(-0.62, 0.28)
Pasteurized 2% milk	<i>Listeria monocytogenes</i> TCC 856	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.45, 0.45)
		1.26 (0.79, 2.07)	20	18	0.90	(0.70, 0.97)	13	0.65	(0.43, 0.82)	0.25	(-0.01, 0.48)
		3.00 (1.311, 6.89)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Bagged lettuce	<i>Listeria monocytogenes</i> TCC 1220	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.45, 0.45)
		1.00 (0.66, 1.55)	20	13	0.65	(0.43, 0.82)	13	0.65	(0.43, 0.82)	0.00	(-0.28, 0.28)
		1.13 (0.57, 2.25)	5	5	1.00	(0.57, 1.00)	4	0.80	(0.38, 1.00)	0.20	(-0.28, 0.62)
Stainless steel surface 4" x 4"	<i>Listeria monocytogenes</i> TCC 813 and <i>Enterococcus faecalis</i> CIP100750 X10	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.45, 0.45)
		N/A	20	6	0.30	(0.15, 0.52)	9	0.45	(0.26, 0.66)	-0.15	(-0.41, 0.14)
		N/A	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)

<sup>a</sup>MPN= Most Probable number is based on the POD of the reference method test portions using the Least Cost Formulations MPN calculator with 95% confidence interval.

<sup>b</sup>N=Number of test portions.

<sup>c</sup>X=Number of positive test portions.

<sup>d</sup>POD<sub>c</sub>=Candidate method confirmed positive outcomes divided by the total number of portions.

<sup>e</sup>POD<sub>R</sub>=Reference method confirmed positive outcomes divided by the total number of portions.

<sup>f</sup>dPOD<sub>c</sub>=Difference between the candidate confirmed result and the reference method confirmed result POD values.

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

<sup>h</sup>N/A=Not applicable.

**DISCUSSION OF MODIFICATION APPROVED APRIL 2018 (10)**

The reagents used in the PCR assay are provided to customers in a freeze-dried format (i.e. pellet) to improve the stability and ease-of-use of the assays. To minimize the exposure of the mixture to temperatures above freezing, the lyophilizer is cooled to -50°C prior to loading the plates in the instrument. By pre-cooling the instrument to -50°C the mixed reagents are spending ~30% less time at temperatures above freezing. The pre-cooling of the lyophilizer doesn't change the raw materials, composition or performance of the assays. The inclusivity and exclusivity of the assays remain the same as the primers and probes are not changed. Similarly, the assays' sensitivity is unaffected as the formulation of the assays is unchanged. The only effect that the change has is that it improves the stability and robustness of the assays.

When the reaction for PCR step is prepared, the user pipettes lysate on top of the freeze-dried pellet containing the PCR reagents. To date no mixing has been applied after the pipetting step. Mixing with a table-top vortex was added to ensure that the reagents are properly dissolved and the solution homogenous. The mixing step of freeze-dried reagents and the lysate doesn't change the raw materials, composition or performance of the assays. The inclusivity and exclusivity of the assays remain the same as the primers and probes are not changed. Similarly, the assays' sensitivity is unaffected as the formulation of the assays is unchanged. The only effect that the change has is that it improves the robustness of the assays.

**DISCUSSION OF MODIFICATION APPROVED OCTOBER 2018 (11)***Inclusivity*

All 53 and 68 inclusivity isolates were successfully detected by the SureTect Listeria monocytogenes PCR Assay and the SureTect Listeria species PCR Assay respectively. The results are detailed in tables 1 and 2.

*Exclusivity*

All 38 and 33 exclusivity isolates were correctly excluded by the SureTect Listeria monocytogenes PCR Assay and the SureTect Listeria species PCR Assay respectively. The results are detailed in tables 3 and 4.

*Matrix testing*

Results for both the SureTect Listeria monocytogenes and SureTect Listeria species PCR Assays using the QuantStudio 5 Real-Time PCR instrument and associated RapidFinder Analysis Software are detailed in Tables 5–8 and 9–12 respectively.

For the stainless steel surface samples, the presumptive PCR results were the same for all three PCR cyclers used for analysis, therefore the results in tables 5-12 represent the results from the QuantStudio 5 PCR Instrument, 7500 Fast PCR Instrument, and the PikoReal PCR Instrument. The original low spike testing of stainless steel sponges and swabs returned too many positive results and did not achieve fractional recovery. The stainless steel sponge and swab low spike was repeated along with an additional five unspiked samples, therefore the data presented shows a total of 10 un-spiked sample results.

The results from the bagged lettuce, 2% pasteurized milk, stainless steel swabs and sponges showed no statistically significant differences by POD analysis between the candidate methods (including presumptive results, and confirmed results via candidate and reference methods) and the reference method, or between the candidate presumptive result and the candidate method confirmed (via the candidate method and the reference method).

The sliced deli turkey samples were found to be naturally contaminated with a *L. spp.* strain; during the testing of the SureTect Listeria monocytogenes PCR Assay, the candidate method confirmed via the reference method, showed poor performance compared to the candidate presumptive PCR result and the candidate method confirmed result via the candidate method. During the reference method confirmation of the candidate method, 100 µL from the candidate enriched portions were transferred to Fraser Broth. The natural *L. spp.* contaminant overgrew the *L. monocytogenes* spike organism in the Fraser Broth. This overgrowth of *L. spp.* resulted in very few visible *L. monocytogenes* colonies (with halos) on the OCLA (ISO formulation) and therefore only two confirmed positives were observed for the low spike samples. This resulted in statistically significant differences by POD analysis in favour of the candidate method (both candidate presumptive result and candidate confirmed result via the candidate method). The results from the SureTect Listeria monocytogenes PCR Assay showed no statistically significant differences between the SureTect Listeria monocytogenes PCR Assay and the reference method for the sliced deli turkey.

The results from the SureTect Listeria species PCR Assay testing of sliced deli turkey showed that the SureTect Listeria species PCR Assay candidate method (confirmed via candidate method and reference method) had superior performance to the reference method. The 24 LEB (part of the candidate method) showed an improved recovery of heat stressed cells in comparison to the Half Fraser Broth (part of the reference method) and this resulted in a statistically significant difference by POD analysis in favor of the SureTect Listeria species PCR Assay candidate method.

**Table 1. Inclusivity of the SureTect Listeria monocytogenes PCR Assay (11)**

ID	Listeria species	Serotype	Source	Origin	SureTect Listeria monocytogenes result
812	<i>Listeria monocytogenes</i>	3a	Environmental	TCC	Positive
813	<i>Listeria monocytogenes</i>	3a	Environmental	TCC	Positive
840	<i>Listeria monocytogenes</i>	3a	Butter	TCC	Positive
856	<i>Listeria monocytogenes</i>	Untyped	Unknown food source <sup>o</sup>	TCC	Positive
857	<i>Listeria monocytogenes</i>	Untyped	Unknown food source	TCC	Positive
858	<i>Listeria monocytogenes</i>	1/2c	Clinical sample	TCC	Positive
859	<i>Listeria monocytogenes</i>	Untyped	Tartare de Salmon	TCC	Positive
860	<i>Listeria monocytogenes</i>	1/2a	Poultry	TCC	Positive
863	<i>Listeria monocytogenes</i>	4d	Sheep	TCC	Positive
864	<i>Listeria monocytogenes</i>	4b	Meningitis	TCC	Positive
865	<i>Listeria monocytogenes</i>	4b	CSF: Meningitis	TCC	Positive
866	<i>Listeria monocytogenes</i>	Untyped	Unknown ATCC isolate	TCC	Positive
867	<i>Listeria monocytogenes</i>	2	CSF: Clinical	TCC	Positive
868	<i>Listeria monocytogenes</i>	4e	Chicken	TCC	Positive
870	<i>Listeria monocytogenes</i>	3a	Clinical sample	TCC	Positive
871	<i>Listeria monocytogenes</i>	Untyped	Unknown food source	TCC	Positive
873	<i>Listeria monocytogenes</i>	Untyped	Unknown food source	TCC	Positive
874	<i>Listeria monocytogenes</i>	Untyped	Unknown food source	TCC	Positive
875	<i>Listeria monocytogenes</i>	Untyped	Unknown food source	TCC	Positive
881	<i>Listeria monocytogenes</i>	Untyped	Unknown food source	TCC	Positive
882	<i>Listeria monocytogenes</i>	Untyped	Unknown food source	TCC	Positive
883	<i>Listeria monocytogenes</i>	4e	Veterinary sample	TCC	Positive
884	<i>Listeria monocytogenes</i>	4e	Unknown	TCC	Positive
885	<i>Listeria monocytogenes</i>	4e	Unknown	TCC	Positive
888	<i>Listeria monocytogenes</i>	3a	Food	TCC	Positive
889	<i>Listeria monocytogenes</i>	3a	Food	TCC	Positive

1195	<i>Listeria monocytogenes</i>	1/2c	Ox tongue	TCC	Positive
1196	<i>Listeria monocytogenes</i>	1/2c	Roast beef	TCC	Positive
1197	<i>Listeria monocytogenes</i>	1/2c	Topside beef	TCC	Positive
1198	<i>Listeria monocytogenes</i>	1/2c	Wiltshire ham	TCC	Positive
1199	<i>Listeria monocytogenes</i>	1/2c	Ham sandwich	TCC	Positive
1205	<i>Listeria monocytogenes</i>	1/2b	Cake	TCC	Positive
1206	<i>Listeria monocytogenes</i>	1/2b	Whipped Cream	TCC	Positive
1207	<i>Listeria monocytogenes</i>	1/2b	Cheese	TCC	Positive
1208	<i>Listeria monocytogenes</i>	1/2b	Cheese	TCC	Positive
1209	<i>Listeria monocytogenes</i>	1/2b	Cream	TCC	Positive
1210	<i>Listeria monocytogenes</i>	1/2b	Cheese	TCC	Positive
1215	<i>Listeria monocytogenes</i>	1/2a	Chorizo sausage	TCC	Positive
1216	<i>Listeria monocytogenes</i>	1/2a	Sandwich	TCC	Positive
1217	<i>Listeria monocytogenes</i>	1/2a	Carrow cheese	TCC	Positive
1218	<i>Listeria monocytogenes</i>	1/2a	Butter	TCC	Positive
1219	<i>Listeria monocytogenes</i>	1/2a	Pilau rice	TCC	Positive
1220	<i>Listeria monocytogenes</i>	1/2a	Sandwich	TCC	Positive
1224	<i>Listeria monocytogenes</i>	4b	Food- blood	TCC	Positive
1225	<i>Listeria monocytogenes</i>	4b	Chicken	TCC	Positive
1226	<i>Listeria monocytogenes</i>	4b	Dressed crab	TCC	Positive
1227	<i>Listeria monocytogenes</i>	4b	Turkey breast	TCC	Positive
1841	<i>Listeria monocytogenes</i>	4b	ATCC 19115	TCC	Positive
2179	<i>Listeria monocytogenes</i>	3b	Unknown	TCC	Positive
2180	<i>Listeria monocytogenes</i>	3c	Unknown	TCC	Positive
2181	<i>Listeria monocytogenes</i>	4a	Unknown	TCC	Positive
2183	<i>Listeria monocytogenes</i>	4c	Bird: heart disease	TCC	Positive
2184	<i>Listeria monocytogenes</i>	7	Faecal sample	TCC	Positive

<sup>a</sup> Unknown food source = isolated by Thermo Fisher Scientific laboratories in Basingstoke, Hampshire, UK

**Table 3. Exclusivity of the SureTect Listeria monocytogenes PCR Assay (11)**

ID	Isolate	Source	Origin	SureTect Listeria monocytogenes result
869	<i>Listeria ivanovii</i> subsp. londoniensis	NCTC	TCC	Negative
872	<i>Listeria grayii</i>	Unknown	TCC	Negative
880	<i>Listeria ivanovii</i>	Food-unknown	TCC	Negative
1172	<i>Listeria grayii</i>	Environmental sample	TCC	Negative
1174	<i>Listeria grayii</i>	Butter	TCC	Negative
1177	<i>Listeria innocua</i>	Chicken sandwich	TCC	Negative
1183	<i>Listeria ivanovii</i>	Food-unknown	TCC	Negative
1184	<i>Listeria ivanovii</i>	Food-unknown	TCC	Negative
1185	<i>Listeria welshimeri</i>	Chicken sandwich	TCC	Negative
1190	<i>Listeria seeligeri</i>	Cheese	TCC	Negative
1191	<i>Listeria seeligeri</i>	Food-unknown	TCC	Negative
1424	<i>Proteus vulgaris</i>	Unknown	TCC	Negative
1809	<i>Escherichia coli</i>	NCTC	TCC	Negative
1892	<i>Klebsiella pneumoniae</i>	NCTC	TCC	Negative
1911	<i>Salmonella enterica</i> subsp. enterica Typhimurium	NCTC	TCC	Negative
1913	<i>Citrobacter freundii</i>	NCTC	TCC	Negative
1978	<i>Listeria welshimeri</i> 6b	NCTC	TCC	Negative
1979	<i>Listeria seeligeri</i> 1/2b	NCTC	TCC	Negative
2186	<i>Listeria innocua</i> 6a	NCTC	TCC	Negative
2188	<i>Listeria welshimeri</i> 6b	Institut Pasteur	TCC	Negative
2407	<i>Brochothrix thermosphacta</i>	Pork sausage	TCC	Negative
2193	<i>Kurthia gibsonii</i>	Pork sausage	TCC	Negative
2194	<i>Lactobacillus casei</i> subsp. casei	Fermented catsup	TCC	Negative
2195	<i>Lactobacillus delbrueckii</i> subsp. lactis	Emmenthal cheese	TCC	Negative
2196	<i>Lactobacillus plantarum</i>	Red Cheshire cheese	TCC	Negative
2200	<i>Enterobacter aerogenes</i>	Unknown	TCC	Negative
2240	<i>Staphylococcus aureus</i>	Food-unknown	TCC	Negative
2258	<i>Carnobacterium divergens</i>	Brie	TCC	Negative
2259	<i>Carnobacterium gallinarum</i>	Unknown	TCC	Negative
2260	<i>Carnobacterium piscicola</i>	Cooked ham	TCC	Negative
2262	<i>Erysipelothrix rhusiopathiae</i>	Unknown	TCC	Negative
2263	<i>Escherichia fergusonii</i>	Sausages	TCC	Negative
2299	<i>Bacillus cereus</i>	Milk	TCC	Negative
2300	<i>Bacillus mycoides</i>	NCTC	TCC	Negative
2304	<i>Propionibacterium freundenreichii</i>	Swiss cheese production	TCC	Negative
2352	<i>Streptococcus salivarius</i>	NCTC	TCC	Negative
2358	<i>Rhodococcus equi</i>	NCTC	TCC	Negative
2352	<i>Micrococcus luteus</i>	NCIMB	OCC	Negative

**Table 5. SureTect Listeria monocytogenes PCR Assay Results: candidate presumptive PCR result vs candidate method confirmed (via reference method) (11)**

Matrix <sup>a</sup>	Inoculating strain(s)	MPN <sup>b</sup> / test portion	N <sup>c</sup>	SureTect candidate Presumptive PCR result			SureTect candidate method confirmed via the reference method			dPOD <sub>CP</sub> <sup>g</sup>	95% CI <sup>h</sup>
				x <sup>d</sup>	PODC <sub>Pr</sub> <sup>e</sup>	95% CI	x	PODC <sub>R</sub> <sup>f</sup>	95% CI		
Sliced Deli Turkey	TCC 1227 <i>L. monocytogenes</i>	N/A <sup>i</sup>	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.3	20	8	0.40	0.22, 0.61	2	0.10	0.03, 0.30	0.30	0.03, 0.53
		0.4	5	3	0.60	0.23, 0.88	0	0.00	0.00, 0.43	0.60	0.03, 0.88
Bagged Lettuce	TCC 1220 <i>L. monocytogenes</i>	N/A <sup>i</sup>	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.2	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.28, 0.28
		1.1	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
2% Pasteurized Milk	TCC 0840 <i>L. monocytogenes</i>	N/A <sup>i</sup>	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.2	20	7	0.15	0.05, 0.36	8	0.40	0.22, 0.61	-0.25	-0.48, 0.03
		0.67	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
Stainless Steel Sponge (4"x 4")	TCC 0813 <i>L. monocytogenes</i> / 10X <i>E. faecalis</i>	N/A <sup>i</sup>	10	0	0.00	0.00, 0.28	0	0.00	0.00, 0.28	0.00	-0.28, 0.28
		N/A	20	14	0.70	0.48, 0.85	14	0.70	0.48, 0.85	0.00	-0.27, 0.27
		N/A	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Stainless Steel Swab (1"x 1")	TCC 1205 <i>L. monocytogenes</i> 1/2b	N/A <sup>i</sup>	10	0	0.00	0.00, 0.28	0	0.00	0.00, 0.28	0.00	-0.28, 0.28
		N/A	20	11	0.50	0.30, 0.70	11	0.55	0.34, 0.74	-0.05	-0.33, 0.24
		N/A	5	4	0.80	0.38, 1.00	5	0.80	0.38, 1.00	0.00	-0.47, 0.47

<sup>a</sup> Matrix = for the stainless steel surface matrices the data is shown combined for PikoReal, 7500 Fast and QuantStudio 5 PCR instruments

<sup>b</sup> MPN = Most Probable Number is based on the POD of reference method test portions using the Least Cost Formulations MPN calculator, with 95% confidence interval

<sup>c</sup> N = Number of test portions

<sup>d</sup> x = Number of positive test portions

<sup>e</sup> PODC<sub>Pr</sub> = Candidate presumptive PCR positive outcomes divided by the total number of trials

<sup>f</sup> PODC<sub>R</sub> = Candidate method confirmed (via reference method) positive outcomes divided by the total number of trials

<sup>g</sup> dPODC<sub>Pr</sub> = Difference between the candidate presumptive and candidate confirmed (via reference method) results

<sup>h</sup> 95% CI = If the confidence interval (CI) of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

<sup>i</sup> N/A = Not applicable

**Table 6. SureTect Listeria monocytogenes PCR Assay Results: candidate presumptive PCR result vs candidate method confirmed (via candidate method) (11)**

Matrix <sup>a</sup>	Inoculating strain(s)	MPN <sup>b</sup> / test portion	N <sup>c</sup>	SureTect candidate Presumptive PCR result			SureTect candidate method confirmed via the candidate method			dPOD <sub>CP</sub> <sup>g</sup>	95% CI <sup>h</sup>
				x <sup>d</sup>	PODC <sub>P</sub> <sup>e</sup>	95% CI	x	PODC <sub>R</sub> <sup>f</sup>	95% CI		
Sliced Deli Turkey	TCC 1227 <i>L. monocytogenes</i>	N/A <sup>i</sup>	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.3	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.28, 0.28
		0.5	5	3	0.60	0.23, 0.88	3	0.60	0.23, 0.88	0.00	-0.46, 0.46
Bagged Lettuce	TCC 1220 <i>L. monocytogenes</i>	N/A <sup>i</sup>	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.2	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.44, 0.07
		1.1	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
2% Pasteurized Milk	TCC 0840 <i>L. monocytogenes</i>	N/A <sup>i</sup>	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.28, 0.28
		0.2	20	7	0.15	0.05, 0.36	7	0.35	0.18, 0.57	-0.20	-0.27, 0.27
		0.67	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.43, 0.43
Stainless Steel Sponge (4"x 4")	TCC 0813 <i>L. monocytogenes</i> / 10X <i>E. faecalis</i>	N/A <sup>i</sup>	10	0	0.00	0.00, 0.28	0	0.00	0.00, 0.28	0.00	-0.28, 0.28
		N/A	20	14	0.70	0.48, 0.85	14	0.70	0.48, 0.85	-0.05	-0.33, 0.24
		N/A	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Stainless Steel Swab (1"x 1")	TCC 1205 <i>L. monocytogenes</i> 1/2b	N/A <sup>i</sup>	10	0	0.00	0.00, 0.28	0	0.00	0.00, 0.28	0.00	-0.28, 0.28
		N/A	20	11	0.50	0.30, 0.70	11	0.55	0.34, 0.74	-0.05	-0.33, 0.24
		N/A	5	5	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47

<sup>a</sup> Matrix = for the stainless steel surface matrices the data is shown combined for PikoReal, 7500 Fast and QuantStudio 5 PCR instruments

<sup>b</sup> MPN = Most Probable Number is based on the POD of reference method test portions using the Least Cost Formulations MPN calculator, with 95% confidence interval

<sup>c</sup> N = Number of test portions

<sup>d</sup> x = Number of positive test portions

<sup>e</sup> PODC<sub>P</sub> = Candidate presumptive PCR positive outcomes divided by the total number of trials

<sup>f</sup> PODC<sub>R</sub> = Candidate method confirmed (via candidate method) positive outcomes divided by the total number of trials

<sup>g</sup> dPODC<sub>P</sub> = Difference between the candidate presumptive and candidate confirmed (via candidate method) results

<sup>h</sup> 95% CI = If the confidence interval (CI) of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

<sup>i</sup> N/A = Not applicable

**Table 7. SureTect Listeria monocytogenes PCR Assay Results: candidate method confirmed (via the candidate method) vs Reference method POD summary (11)**

Matrix <sup>a</sup>	Inoculating strain(s)	MPN <sup>b</sup> / test portion	N <sup>c</sup>	SureTect candidate method confirmed via the candidate method result			Reference method result			dPOD <sub>cc</sub> <sup>g</sup>	95% CI <sup>h</sup>
				x <sup>d</sup>	POD <sub>cc</sub> <sup>e</sup>	95% CI	x	POD <sub>R</sub> <sup>f</sup>	95% CI		
Sliced Deli Turkey	TCC 1227 <i>L. monocytogenes</i>	N/A <sup>i</sup>	5	0	0.00	0.00, 0.43	1	0.20	0.00, 0.62	-0.20	-0.62, 0.28
		0.30	20	8	0.40	0.22, 0.61	9	0.45	0.26, 0.66	-0.05	-0.33, 0.24
		0.50	5	3	0.60	0.23, 0.88	1	0.20	0.00, 0.62	0.40	-0.16, 0.75
Bagged Lettuce	TCC 1220 <i>L. monocytogenes</i>	N/A <sup>i</sup>	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.20	20	10	0.50	0.30, 0.70	14	0.70	0.48, 0.85	-0.20	-0.45, 0.10
		1.10	5	4	0.80	0.38, 1.00	3	0.60	0.23, 0.88	0.20	-0.31, 0.62
2% Pasteurized Milk	TCC 0840 <i>L. monocytogenes</i>	N/A <sup>i</sup>	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.20	20	7	0.35	0.18, 0.57	3	0.35	0.18, 0.57	0.00	-0.28, 0.28
		0.67	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
Stainless Steel Sponge (4" x 4")	TCC 0813 <i>L. monocytogenes</i> / 10X <i>E. faecalis</i>	N/A <sup>i</sup>	10	0	0.00	0.00, 0.28	0	0.00	0.00, 0.28	0.00	-0.28, 0.28
		N/A	20	14	0.70	0.48, 0.85	15	0.70	0.48, 0.85	0.00	-0.27, 0.27
		N/A	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Stainless Steel Swab (1" x 1")	TCC 1205 <i>L. monocytogenes</i> 1/2b	N/A <sup>i</sup>	10	0	0.00	0.00, 0.28	0	0.00	0.00, 0.28	0.00	-0.28, 0.28
		N/A	20	11	0.55	0.34, 0.74	13	0.65	0.43, 0.82	-0.10	-0.37, 0.19
		N/A	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47

<sup>a</sup> Matrix = for the stainless steel surface matrices the data is shown combined for PikoReal, 7500 Fast and QuantStudio 5 PCR instruments

<sup>b</sup> MPN = Most Probable Number is based on the POD of reference method test portions using the Least Cost Formulations MPN calculator, with 95% confidence interval

<sup>c</sup> N = Number of test portions

<sup>d</sup> x = Number of positive test portions

<sup>e</sup> POD<sub>cc</sub> = Candidate method confirmed via the candidate method positive outcomes divided by the total number of trials

<sup>f</sup> POD<sub>R</sub> = Reference method divided by the total number of trials

<sup>g</sup> dPOD<sub>cc</sub> = Difference between the candidate method presumptive result and candidate method confirmed result POD values

<sup>h</sup> 95% CI = If the confidence interval (CI) of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

<sup>i</sup> N/A = Not applicable

**Table 8. SureTect Listeria monocytogenes PCR Assay Results: candidate method confirmed (via the reference method) vs Reference method POD summary (11)**

Matrix <sup>a</sup>	Inoculating strain(s)	MPN <sup>b</sup> / test portion	N <sup>c</sup>	SureTect candidate method confirmed via the reference method			Reference method result			dPOD <sub>CR</sub> <sup>g</sup>	95% CI <sup>h</sup>
				x <sup>d</sup>	POD <sub>CR</sub> <sup>e</sup>	95% CI	x	POD <sub>R</sub> <sup>f</sup>	95% CI		
Sliced Deli Turkey	TCC 1227 <i>L. monocytogenes</i>	N/A <sup>i</sup>	5	0	0.00	0.00, 0.43	1	0.20	0.00, 0.62	-0.20	-0.62, 0.28
		0.3	20	2	0.10	0.03, 0.30	9	0.45	0.26, 0.66	-0.35	-0.57, -0.07
		0.5	5	0	0.00	0.00, 0.43	1	0.20	0.00, 0.62	-0.20	-0.62, 0.28
Bagged Lettuce	TCC 1220 <i>L. monocytogenes</i>	N/A <sup>i</sup>	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.2	20	10	0.50	0.30, 0.70	14	0.70	0.48, 0.85	-0.20	-0.45, 0.10
		1.1	5	4	0.80	0.38, 1.00	3	0.60	0.23, 0.88	0.20	-0.31, 0.62
2% Pasteurized Milk	TCC 0840 <i>L. monocytogenes</i>	N/A <sup>i</sup>	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.2	20	8	0.40	0.22, 0.61	7	0.35	0.18, 0.57	0.00	-0.23, 0.32
		0.67	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
Stainless Steel Sponge (4"x 4")	TCC 0813 <i>L. monocytogenes</i> / 10X <i>E. faecalis</i>	N/A <sup>i</sup>	10	0	0.00	0.00, 0.28	0	0.00	0.00, 0.28	0.00	-0.28, 0.28
		N/A	20	14	0.70	0.48, 0.85	14	0.70	0.48, 0.85	0.00	-0.27, 0.27
		N/A	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Stainless Steel Swab (1"x 1")	TCC 1205 <i>L. monocytogenes</i> 1/2b	N/A <sup>i</sup>	10	0	0.00	0.00, 0.28	0	0.00	0.00, 0.28	0.00	-0.28, 0.28
		N/A	20	11	0.55	0.34, 0.74	13	0.65	0.43, 0.82	-0.10	-0.37, 0.19
		N/A	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47

<sup>a</sup> Matrix = for the stainless steel surface matrices the data is shown combined for PikoReal, 7500 Fast and QuantStudio 5 PCR instruments

<sup>b</sup> MPN = Most Probable Number is based on the POD of reference method test portions using the Least Cost Formulations MPN calculator, with 95% confidence interval

<sup>c</sup> N = Number of test portions

<sup>d</sup> x = Number of positive test portions

<sup>e</sup> POD<sub>CR</sub> = Candidate method confirmed (via reference method) positive outcomes divided by the total number of trials

<sup>f</sup> POD<sub>R</sub> = Reference method positive outcomes divided by the total number of trials

<sup>g</sup> dPOD<sub>CR</sub> = Difference between the candidate method confirmed via the reference method and the reference method

<sup>h</sup> 95% CI = If the confidence interval (CI) of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

<sup>i</sup> N/A = Not applicable

**DISCUSSION OF MODIFICATION APPROVED OCTOBER 2020 (13)**

*SureTect Listeria monocytogenes* PCR assay: *Inclusivity/Exclusivity*.—The reanalysis of the inclusivity data showed **4 changes from positive to warning calls**, in the PCR result comparison between RFA 1.0 and RFA 1.1 and the relevant kit files. These 4 changes are listed in the Table 31. 2 warning calls have been generated by the fact that the IPC was negative, while the target remained positive. The other 2 warning calls have been generated by the uncharacteristic shape of the PCR curve, both IPC and target are negative with new software. The exclusivity data showed **no change** in the PCR results between RFA 1.0 and RFA 1.1. Tables 15 and 16 detail the isolates used during the Inclusivity and Exclusivity studies.

*SureTect Listeria monocytogenes*: *Matrix testing*.—The reanalysis of the matrix data showed **no change** in the PCR results between RFA 1.0 and RFA 1.1 and the relevant kit files. The details are listed in Table 26.

**Table 15. Inclusivity results for the SureTect Listeria monocytogenes PCR Assay using RFA v1.0 and RFA v1.1 (13)**

ID	Isolate	Origin	Source	RFA 1.0 result	RFA 1.1 result	ID	Isolate	Origin	Source	RFA 1.0 result	RFA 1.1 result
812	<i>Listeria monocytogenes</i> 3a	Environmental	TCC <sup>a</sup>	Positive	Positive	1196	<i>Listeria monocytogenes</i> 1/2c	Roast beef	TCC	Positive	Positive
813	<i>Listeria monocytogenes</i> 3a	Environmental	TCC	Positive	Positive	1197	<i>Listeria monocytogenes</i> 1/2c	Topside beef	TCC	Positive	Positive
840	<i>Listeria monocytogenes</i> 3a	Butter	TCC	Positive	Positive	1198	<i>Listeria monocytogenes</i> 1/2c	Wiltshire ham	TCC	Positive	Positive
856	<i>Listeria monocytogenes</i>	Food	TCC	Positive	Positive	1199	<i>Listeria monocytogenes</i> 1/2c	Ham sandwich	TCC	Positive	Positive
857	<i>Listeria monocytogenes</i>	Food	TCC	Positive	Positive	1205	<i>Listeria monocytogenes</i> 1/2b	Cake	TCC	Positive	Positive
858	<i>Listeria monocytogenes</i> 1/2c	Clinical sample	TCC	Positive	Positive	1206	<i>Listeria monocytogenes</i> 1/2b	Whipped Cream	TCC	Positive	Positive
859	<i>Listeria monocytogenes</i>	Tartare de Salmon	TCC	Positive	Positive	1207	<i>Listeria monocytogenes</i> 1/2b	Cheese	TCC	Positive	Positive
860	<i>Listeria monocytogenes</i> 1/2a	Poultry	TCC	Positive	Positive	1208	<i>Listeria monocytogenes</i> 1/2b	Cheese	TCC	Positive	Warning <sup>b</sup>
863	<i>Listeria monocytogenes</i> 4d	Sheep	TCC	Positive	Positive	1209	<i>Listeria monocytogenes</i> 1/2b	Cream	TCC	Positive	Positive
864	<i>Listeria monocytogenes</i> 4b	Meningitis	TCC	Positive	Positive	1210	<i>Listeria monocytogenes</i> 1/2b	Cheese	TCC	Positive	Positive
865	<i>Listeria monocytogenes</i> 4b	CSF: Meningitis	TCC	Positive	Positive	1215	<i>Listeria monocytogenes</i> 1/2a	Chorizo sausage	TCC	Positive	Warning <sup>b</sup>
866	<i>Listeria monocytogenes</i>	Unknown ATCC isolate	TCC	Positive	Positive	1216	<i>Listeria monocytogenes</i> 1/2a	Sandwich	TCC	Positive	Positive
867	<i>Listeria monocytogenes</i> 2	CSF: Clinical	TCC	Positive	Positive	1217	<i>Listeria monocytogenes</i> 1/2a	Carrow cheese	TCC	Positive	Positive
868	<i>Listeria monocytogenes</i> 4e	Chicken	TCC	Positive	Positive	1218	<i>Listeria monocytogenes</i> 1/2a	Butter	TCC	Positive	Warning <sup>b</sup>
870	<i>Listeria monocytogenes</i> 3a	Clinical sample	TCC	Positive	Positive	1219	<i>Listeria monocytogenes</i> 1/2a	Pilau rice	TCC	Positive	Positive
871	<i>Listeria monocytogenes</i>	Food	TCC	Positive	Positive	1220	<i>Listeria monocytogenes</i> 1/2a	Sandwich	TCC	Positive	Positive
873	<i>Listeria monocytogenes</i>	Food	TCC	Positive	Positive	1224	<i>Listeria monocytogenes</i> 4b	Food- blood	TCC	Positive	Positive
874	<i>Listeria monocytogenes</i>	Food	TCC	Positive	Positive	1225	<i>Listeria monocytogenes</i> 4b	Chicken	TCC	Positive	Positive
875	<i>Listeria monocytogenes</i>	Food	TCC	Positive	Positive	1226	<i>Listeria monocytogenes</i> 4b	Dressed crab	TCC	Positive	Positive
881	<i>Listeria monocytogenes</i>	Food	TCC	Positive	Positive	1227	<i>Listeria monocytogenes</i> 4b	Turkey breast	TCC	Positive	Positive
882	<i>Listeria monocytogenes</i>	Food	TCC	Positive	Positive	1841	<i>Listeria monocytogenes</i> 4b	ATCC 19115	TCC	Positive	Positive



883	<i>Listeria monocytogenes</i> 4e	Veterinary sample	TCC	Positive	Positive	2179	<i>Listeria monocytogenes</i> 3b	Unknown	TCC	Positive	Positive
884	<i>Listeria monocytogenes</i> 4e	Unknown	TCC	Positive	Positive	2180	<i>Listeria monocytogenes</i> 3c	Unknown	TCC	Positive	Positive
885	<i>Listeria monocytogenes</i> 4e	Unknown	TCC	Positive	Positive	2181	<i>Listeria monocytogenes</i> 4a	Unknown	TCC	Positive	Positive
888	<i>Listeria monocytogenes</i> 3a	Food	TCC	Positive	Positive	2183	<i>Listeria monocytogenes</i> 4c	Bird: heart disease	TCC	Positive	Positive
889	<i>Listeria monocytogenes</i> 3a	Food	TCC	Positive	Positive	2184	<i>Listeria monocytogenes</i> 7	Faecal sample	TCC	Positive	Positive
1195	<i>Listeria monocytogenes</i> 1/2c	Ox tongue	TCC	Positive	Warning <sup>b</sup>						

<sup>a</sup>TCC = Trials Culture Collection, Thermo Fisher Scientific, UK.

<sup>b</sup>Warning result with RFA 1.1 and kit file 2.0.

**Table 16. Exclusivity results for the SureTect Listeria monocytogenes PCR Assay using RFA v1.0 and RFA v1.1 (13)**

ID	Isolate	Origin	Source	RFA 1.0 result	RFA 1.1 result	ID	Isolate	Origin	Source	RFA 1.0 result	RFA 1.1 result
869	<i>Listeria ivanovii</i> subsp. <i>londoniensis</i>	NCTC	TCC <sup>a</sup>	Negative	Negative	2188	<i>Listeria welshimeri</i> 6b	Institut Pasteur	TCC	Negative	Negative
872	<i>Listeria grayii</i>	Unknown	TCC	Negative	Negative	2407	<i>Brochothrix thermosphacta</i>	Pork sausage	TCC	Negative	Negative
880	<i>Listeria ivanovii</i>	Food	TCC	Negative	Negative	2193	<i>Kurthia gibsonii</i>	Pork sausage	TCC	Negative	Negative
1172	<i>Listeria grayii</i>	Environmental	TCC	Negative	Negative	2194	<i>Lactobacillus casei</i> subsp. <i>casei</i>	Fermented catsup	TCC	Negative	Negative
1174	<i>Listeria grayii</i>	Butter	TCC	Negative	Negative	2195	<i>Lactobacillus delbrueckii</i> subsp. <i>lactis</i>	Emmenthal cheese	TCC	Negative	Negative
1177	<i>Listeria innocua</i>	Chicken sandwich	TCC	Negative	Negative	2196	<i>Lactobacillus plantarum</i>	Red Cheshire cheese	TCC	Negative	Negative
1183	<i>Listeria ivanovii</i>	Food-unknown	TCC	Negative	Negative	2200	<i>Enterobacter aerogenes</i>	Unknown	TCC	Negative	Negative
1184	<i>Listeria ivanovii</i>	Food-unknown	TCC	Negative	Negative	2240	<i>Staphylococcus aureus</i>	Food	TCC	Negative	Negative
1185	<i>Listeria welshimeri</i>	Chicken sandwich	TCC	Negative	Negative	2258	<i>Carnobacterium divergens</i>	Brie	TCC	Negative	Negative
1190	<i>Listeria seeligeri</i>	Cheese	TCC	Negative	Negative	2259	<i>Carnobacterium gallinarum</i>	Unknown	TCC	Negative	Negative
1191	<i>Listeria seeligeri</i>	Food	TCC	Negative	Negative	2260	<i>Carnobacterium piscicola</i>	Cooked ham	TCC	Negative	Negative
1424	<i>Proteus vulgaris</i>	Unknown	TCC	Negative	Negative	2262	<i>Erysipelothrix rhusiopathiae</i>	Unknown	TCC	Negative	Negative
1809	<i>Escherichia coli</i>	NCTC	TCC	Negative	Negative	2263	<i>Escherichia fergusonii</i>	Sausages	TCC	Negative	Negative
1892	<i>Klebsiella pneumoniae</i>	NCTC	TCC	Negative	Negative	2299	<i>Bacillus cereus</i>	Milk	TCC	Negative	Negative
1911	<i>Salmonella</i> Typhimurium	NCTC	TCC	Negative	Negative	2300	<i>Bacillus mycoides</i>	NCTC	TCC	Negative	Negative
1913	<i>Citrobacter freundii</i>	NCTC	TCC	Negative	Negative	2304	<i>Propionibacterium freundenreichii</i>	Swiss cheese	TCC	Negative	Negative
1978	<i>Listeria welshimeri</i> 6b	NCTC	TCC	Negative	Negative	2352	<i>Streptococcus salivarius</i>	NCTC	TCC	Negative	Negative
1979	<i>Listeria seeligeri</i> 1/2b	NCTC	TCC	Negative	Negative	2358	<i>Rhodococcus equi</i>	Animal isolate	TCC	Negative	Negative
2186	<i>Listeria innocua</i> 6a	NCTC	TCC	Negative	Negative	2352	<i>Micrococcus luteus</i>	Unknown	OCC <sup>b</sup>	Negative	Negative

<sup>a</sup>TCC = Trials Culture Collection, Thermo Fisher Scientific, UK.

<sup>b</sup>OCC = Oxoid Culture Collection, Thermo Fisher Scientific, UK.

Table 26. Thermo Scientific SureTect Listeria monocytogenes PCR Assay result comparison for RFA v1.0 and RFA v1.1 (13)

Matrix	Inoculating Strain(s)	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	Candidate Method <sup>c</sup>		
				RFA 1.0 x <sup>d</sup>	RFA 1.0 -! <sup>e</sup>	RFA 1.1 x <sup>f</sup>
Sliced Deli Turkey	TCC 1227 <i>L. monocytogenes</i>	N/A <sup>g</sup>	5	0	N/A	0
		0.3	20	8	N/A	8
		0.5	5	3	N/A	3
Bagged Lettuce	TCC 1220 <i>L. monocytogenes</i>	N/A	5	0	N/A	0
		1.2	20	10	N/A	10
		1.1	5	4	N/A	4
2% Pasteurized Milk	TCC 0840 <i>L. monocytogenes</i>	N/A	5	0	N/A	0
		0.2	20	7	N/A	7
		0.7	5	4	N/A	4
Stainless Steel Sponge	TCC 0813 <i>L. monocytogenes</i> 10X <i>E. faecalis</i>	N/A	10	0	N/A	0
		N/A	20	14	N/A	14
		N/A	5	5	N/A	5
Stainless Steel Swab	TCC 1205 <i>L. monocytogenes</i> 1/2b	N/A	10	0	N/A	0
		N/A	20	11	N/A	11
		N/A	5	5	N/A	5

<sup>a</sup>MPN = Most Probable Number is based on the POD of reference method test portions using the Least Cost Formulations MPN calculator, with 95% confidence interval.

<sup>b</sup>N = Number of test portions, 5 unspiked, 20 low spike, 5 high spike.

<sup>c</sup>Candidate method presumptive result (PCR only).

<sup>d</sup>RFA 1.0 x = Number of positive test portions gained originally with RFA 1.0 and original kit file.

<sup>e</sup>RFA 1.0 - ! = Number of positive test portions gained originally with RFA 1.0 and original kit file (excluding samples with warning calls seen on RFA 1.1).

<sup>f</sup>RFA 1.1 x = Number of positive test portions gained originally with RFA 1.0 and original kit file.

<sup>g</sup>N/A = Not applicable.

Table 31. Thermo Scientific SureTect range warning call/changes summary using RFA 1.0 and RFA 1.1 (13)

Assay	Sample	Strain	Sample ID	RFA 1.0	RFA 1.1	Description	RFA 1.0	RFA 1.1																																										
SureTect Listeria monocytogenes	Inclusivity	TCC 1195 L. monocytogenes	27	Positive	Warning	No IPC amplification; Positive for target	<table border="1"> <thead> <tr> <th>Well</th> <th>Sample</th> <th>Type</th> <th>Target</th> <th>Call</th> <th>Cr</th> <th>ΔRn</th> </tr> </thead> <tbody> <tr> <td>A1</td> <td>27</td> <td>Unknown</td> <td>IPC LMO ST</td> <td>Positive</td> <td>33.37</td> <td>239.849.6...</td> </tr> <tr> <td>A1</td> <td>27</td> <td>Unknown</td> <td>LMO ST</td> <td>Positive</td> <td>23.40</td> <td>499.897.8...</td> </tr> </tbody> </table>	Well	Sample	Type	Target	Call	Cr	ΔRn	A1	27	Unknown	IPC LMO ST	Positive	33.37	239.849.6...	A1	27	Unknown	LMO ST	Positive	23.40	499.897.8...	<table border="1"> <thead> <tr> <th>Well</th> <th>Sample</th> <th>Type</th> <th>Target</th> <th>Call</th> <th>Cr</th> <th>ΔRn</th> </tr> </thead> <tbody> <tr> <td>A1</td> <td>27</td> <td>Unknown</td> <td>IPC LMO ST</td> <td>Negative</td> <td>27.52</td> <td>26.005.963</td> </tr> <tr> <td>A1</td> <td>27</td> <td>Unknown</td> <td>LMO ST</td> <td>Positive</td> <td>21.89</td> <td>499.897.8...</td> </tr> </tbody> </table>	Well	Sample	Type	Target	Call	Cr	ΔRn	A1	27	Unknown	IPC LMO ST	Negative	27.52	26.005.963	A1	27	Unknown	LMO ST	Positive	21.89	499.897.8...
Well	Sample	Type	Target	Call	Cr	ΔRn																																												
A1	27	Unknown	IPC LMO ST	Positive	33.37	239.849.6...																																												
A1	27	Unknown	LMO ST	Positive	23.40	499.897.8...																																												
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A1	27	Unknown	LMO ST	Positive	21.89	499.897.8...																																												
SureTect Listeria monocytogenes	Inclusivity	TCC 1208 L. monocytogenes	35	Positive	Warning	No IPC amplification; Positive for Target	<table border="1"> <thead> <tr> <th>Well</th> <th>Sample</th> <th>Type</th> <th>Target</th> <th>Call</th> <th>Cr</th> <th>ΔRn</th> </tr> </thead> <tbody> <tr> <td>C1</td> <td>35</td> <td>Unknown</td> <td>IPC LMO ST</td> <td>Positive</td> <td>23.03</td> <td>315.397.4...</td> </tr> <tr> <td>C1</td> <td>35</td> <td>Unknown</td> <td>LMO ST</td> <td>Positive</td> <td>20.12</td> <td>620.823.1...</td> </tr> </tbody> </table>	Well	Sample	Type	Target	Call	Cr	ΔRn	C1	35	Unknown	IPC LMO ST	Positive	23.03	315.397.4...	C1	35	Unknown	LMO ST	Positive	20.12	620.823.1...	<table border="1"> <thead> <tr> <th>Well</th> <th>Sample</th> <th>Type</th> <th>Target</th> <th>Call</th> <th>Cr</th> <th>ΔRn</th> </tr> </thead> <tbody> <tr> <td>C1</td> <td>35</td> <td>Unknown</td> <td>IPC LMO ST</td> <td>Negative</td> <td>38.21</td> <td>165.053.5...</td> </tr> <tr> <td>C1</td> <td>35</td> <td>Unknown</td> <td>LMO ST</td> <td>Positive</td> <td>19.04</td> <td>620.823.1...</td> </tr> </tbody> </table>	Well	Sample	Type	Target	Call	Cr	ΔRn	C1	35	Unknown	IPC LMO ST	Negative	38.21	165.053.5...	C1	35	Unknown	LMO ST	Positive	19.04	620.823.1...
Well	Sample	Type	Target	Call	Cr	ΔRn																																												
C1	35	Unknown	IPC LMO ST	Positive	23.03	315.397.4...																																												
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C1	35	Unknown	LMO ST	Positive	19.04	620.823.1...																																												
SureTect Listeria monocytogenes	Inclusivity	TCC 1215 L. monocytogenes	38	Positive	Warning	NC graph; Negative for IPC and target Correct call	<table border="1"> <thead> <tr> <th>Well</th> <th>Sample</th> <th>Type</th> <th>Target</th> <th>Call</th> <th>Cr</th> <th>ΔRn</th> </tr> </thead> <tbody> <tr> <td>G1</td> <td>38</td> <td>Unknown</td> <td>IPC LMO ST</td> <td>Negative</td> <td>40.93</td> <td>42.178.199</td> </tr> <tr> <td>G1</td> <td>38</td> <td>Unknown</td> <td>LMO ST</td> <td>Positive</td> <td>47.48</td> <td>19.931.367</td> </tr> </tbody> </table>	Well	Sample	Type	Target	Call	Cr	ΔRn	G1	38	Unknown	IPC LMO ST	Negative	40.93	42.178.199	G1	38	Unknown	LMO ST	Positive	47.48	19.931.367	<table border="1"> <thead> <tr> <th>Well</th> <th>Sample</th> <th>Type</th> <th>Target</th> <th>Call</th> <th>Cr</th> <th>ΔRn</th> </tr> </thead> <tbody> <tr> <td>G1</td> <td>38</td> <td>Unknown</td> <td>IPC LMO ST</td> <td>Negative</td> <td>41.64</td> <td>47.643.075</td> </tr> <tr> <td>G1</td> <td>38</td> <td>Unknown</td> <td>LMO ST</td> <td>Negative</td> <td>45.78</td> <td>19.931.367</td> </tr> </tbody> </table>	Well	Sample	Type	Target	Call	Cr	ΔRn	G1	38	Unknown	IPC LMO ST	Negative	41.64	47.643.075	G1	38	Unknown	LMO ST	Negative	45.78	19.931.367
Well	Sample	Type	Target	Call	Cr	ΔRn																																												
G1	38	Unknown	IPC LMO ST	Negative	40.93	42.178.199																																												
G1	38	Unknown	LMO ST	Positive	47.48	19.931.367																																												
Well	Sample	Type	Target	Call	Cr	ΔRn																																												
G1	38	Unknown	IPC LMO ST	Negative	41.64	47.643.075																																												
G1	38	Unknown	LMO ST	Negative	45.78	19.931.367																																												
SureTect Listeria monocytogenes	Inclusivity	TCC 1218 L. monocytogenes	41	Positive	Warning	NC graph; Negative for IPC and target Correct call	<table border="1"> <thead> <tr> <th>Well</th> <th>Sample</th> <th>Type</th> <th>Target</th> <th>Call</th> <th>Cr</th> <th>ΔRn</th> </tr> </thead> <tbody> <tr> <td>C2</td> <td>41</td> <td>Unknown</td> <td>IPC LMO ST</td> <td>Negative</td> <td>47.31</td> <td>23.778.765</td> </tr> <tr> <td>C2</td> <td>41</td> <td>Unknown</td> <td>LMO ST</td> <td>Positive</td> <td>25.74</td> <td>75.788.013</td> </tr> </tbody> </table>	Well	Sample	Type	Target	Call	Cr	ΔRn	C2	41	Unknown	IPC LMO ST	Negative	47.31	23.778.765	C2	41	Unknown	LMO ST	Positive	25.74	75.788.013	<table border="1"> <thead> <tr> <th>Well</th> <th>Sample</th> <th>Type</th> <th>Target</th> <th>Call</th> <th>Cr</th> <th>ΔRn</th> </tr> </thead> <tbody> <tr> <td>C2</td> <td>41</td> <td>Unknown</td> <td>IPC LMO ST</td> <td>Negative</td> <td>43.71</td> <td>29.725.198</td> </tr> <tr> <td>C2</td> <td>41</td> <td>Unknown</td> <td>LMO ST</td> <td>Negative</td> <td>11.700.831</td> <td></td> </tr> </tbody> </table>	Well	Sample	Type	Target	Call	Cr	ΔRn	C2	41	Unknown	IPC LMO ST	Negative	43.71	29.725.198	C2	41	Unknown	LMO ST	Negative	11.700.831	
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**DISCUSSION OF MODIFICATION APPROVED OCTOBER 2020 (14)**

*SureTect Listeria monocytogenes* PCR assay: *Inclusivity/Exclusivity*.—The reanalysis of the inclusivity-exclusivity data showed **no change** in the PCR results after reanalysis between the RFE kit file v1.0 and RFE kit file v2.0.

*SureTect Listeria monocytogenes*: *Matrix testing*.—The reanalysis of the matrix data showed **no change** in the PCR results after reanalysis between the RFE kit file v1.0 and RFE kit file v2.0.

**Table 17. Inclusivity results for the SureTect Listeria monocytogenes PCR Assay using the original and upgraded kit files with RFE v2.0 (14)**

ID	Isolate	Origin	Source	RFE kit file 1.0 result	RFE kit file 2.0 result	ID	Isolate	Origin	Source	RFE kit file 1.0 result	RFE kit file 2.0 result
812	<i>Listeria monocytogenes</i> 3a	Environmental	TCC <sup>a</sup>	Positive	Positive	864	<i>Listeria monocytogenes</i> 4b	Meningitis	TCC	Positive	Positive
813	<i>Listeria monocytogenes</i> 3a	Environmental	TCC	Positive	Positive	865	<i>Listeria monocytogenes</i> 4b	CSF: Meningitis	TCC	Positive	Positive
840	<i>Listeria monocytogenes</i> 3a	Butter	TCC	Positive	Positive	866	<i>Listeria monocytogenes</i>	Unknown ATCC isolate	TCC	Positive	Positive
856	<i>Listeria monocytogenes</i>	Food	TCC	Positive	Positive	867	<i>Listeria monocytogenes</i> 2	CSF: Clinical	TCC	Positive	Positive
857	<i>Listeria monocytogenes</i>	Food	TCC	Positive	Positive	868	<i>Listeria monocytogenes</i> 4e	Chicken	TCC	Positive	Positive
858	<i>Listeria monocytogenes</i> 1/2c	Clinical sample	TCC	Positive	Positive	870	<i>Listeria monocytogenes</i> 3a	Clinical sample	TCC	Positive	Positive
859	<i>Listeria monocytogenes</i>	Tartare de Salmon	TCC	Positive	Positive	871	<i>Listeria monocytogenes</i>	Food	TCC	Positive	Positive
860	<i>Listeria monocytogenes</i> 1/2a	Poultry	TCC	Positive	Positive	873	<i>Listeria monocytogenes</i>	Food	TCC	Positive	Positive
863	<i>Listeria monocytogenes</i> 4d	Sheep	TCC	Positive	Positive	874	<i>Listeria monocytogenes</i>	Food	TCC	Positive	Positive
875	<i>Listeria monocytogenes</i>	Food	TCC	Positive	Positive	1198	<i>Listeria monocytogenes</i> 1/2c	Wiltshire ham	TCC	Positive	Positive
881	<i>Listeria monocytogenes</i>	Food	TCC	Positive	Positive	1199	<i>Listeria monocytogenes</i> 1/2c	Ham sandwich	TCC	Positive	Positive
882	<i>Listeria monocytogenes</i>	Food	TCC	Positive	Positive	1205	<i>Listeria monocytogenes</i> 1/2b	Cake	TCC	Positive	Positive
883	<i>Listeria monocytogenes</i> 4e	Veterinary sample	TCC	Positive	Positive	1206	<i>Listeria monocytogenes</i> 1/2b	Whipped Cream	TCC	Positive	Positive
884	<i>Listeria monocytogenes</i> 4e	Unknown	TCC	Positive	Positive	1207	<i>Listeria monocytogenes</i> 1/2b	Cheese	TCC	Positive	Positive
885	<i>Listeria monocytogenes</i> 4e	Unknown	TCC	Positive	Positive	1208	<i>Listeria monocytogenes</i> 1/2b	Cheese	TCC	Positive	Positive
888	<i>Listeria monocytogenes</i> 3a	Food	TCC	Positive	Positive	1209	<i>Listeria monocytogenes</i> 1/2b	Cream	TCC	Positive	Positive
889	<i>Listeria monocytogenes</i> 3a	Food	TCC	Positive	Positive	1210	<i>Listeria monocytogenes</i> 1/2b	Cheese	TCC	Positive	Positive
1195	<i>Listeria monocytogenes</i> 1/2c	Ox tongue	TCC	Positive	Positive	1215	<i>Listeria monocytogenes</i> 1/2a	Chorizo sausage	TCC	Positive	Positive
1196	<i>Listeria monocytogenes</i> 1/2c	Roast beef	TCC	Positive	Positive	1216	<i>Listeria monocytogenes</i> 1/2a	Sandwich	TCC	Positive	Positive
1197	<i>Listeria</i>	Topside beef	TCC	Positive	Positive	1217	<i>Listeria</i>	Carrow cheese	TCC	Positive	Positive

	<i>monocytogenes</i> 1/2c						<i>monocytogenes</i> 1/2a				
1218	<i>Listeria monocytogenes</i> 1/2a	Butter	TCC	Positive	Positive	1841	<i>Listeria monocytogenes</i> 4b	ATCC 19115	TCC	Positive	Positive
1219	<i>Listeria monocytogenes</i> 1/2a	Pilau rice	TCC	Positive	Positive	2179	<i>Listeria monocytogenes</i> 3b	Unknown	TCC	Positive	Positive
1220	<i>Listeria monocytogenes</i> 1/2a	Sandwich	TCC	Positive	Positive	2180	<i>Listeria monocytogenes</i> 3c	Unknown	TCC	Positive	Positive
1224	<i>Listeria monocytogenes</i> 4b	Food- blood	TCC	Positive	Positive	2181	<i>Listeria monocytogenes</i> 4a	Unknown	TCC	Positive	Positive
1225	<i>Listeria monocytogenes</i> 4b	Chicken	TCC	Positive	Positive	2183	<i>Listeria monocytogenes</i> 4c	Bird: heart disease	TCC	Positive	Positive
1226	<i>Listeria monocytogenes</i> 4b	Dressed crab	TCC	Positive	Positive	2184	<i>Listeria monocytogenes</i> 7	Faecal sample	TCC	Positive	Positive
1227	<i>Listeria monocytogenes</i> 4b	Turkey breast	TCC	Positive	Positive						

<sup>a</sup>TCC = Trials Culture Collection, Thermo Fisher Scientific, UK.

**Table 18. Exclusivity results for the SureTect Listeria monocytogenes PCR Assay using the original and upgraded kit files with RFE v2.0 (14)**

ID	Isolate	Origin	Source	RFE kit file 1.0 result	RFE kit file 2.0 result	ID	Isolate	Origin	Source	RFE kit file 1.0 result	RFE kit file 2.0 result
869	<i>Listeria ivanovii</i> subsp. <i>londoniensis</i>	NCTC	TCC <sup>a</sup>	Negative	Negative	2188	<i>Listeria welshimeri</i> 6b	Institut Pasteur	TCC	Negative	Negative
872	<i>Listeria grayii</i>	Unknown	TCC	Negative	Negative	2407	<i>Brochothrix thermosphacta</i>	Pork sausage	TCC	Negative	Negative
880	<i>Listeria ivanovii</i>	Food	TCC	Negative	Negative	2193	<i>Kurthia gibsonii</i>	Pork sausage	TCC	Negative	Negative
1172	<i>Listeria grayii</i>	Environmental	TCC	Negative	Negative	2194	<i>Lactobacillus casei</i> subsp. <i>casei</i>	Fermented catsup	TCC	Negative	Negative
1174	<i>Listeria grayii</i>	Butter	TCC	Negative	Negative	2195	<i>Lactobacillus delbrueckii</i> subsp. <i>lactis</i>	Emmenthal cheese	TCC	Negative	Negative
1177	<i>Listeria innocua</i>	Chicken sandwich	TCC	Negative	Negative	2196	<i>Lactobacillus plantarum</i>	Red Cheshire cheese	TCC	Negative	Negative
1183	<i>Listeria ivanovii</i>	Food-unknown	TCC	Negative	Negative	2200	<i>Enterobacter aerogenes</i>	Unknown	TCC	Negative	Negative
1184	<i>Listeria ivanovii</i>	Food-unknown	TCC	Negative	Negative	2240	<i>Staphylococcus aureus</i>	Food	TCC	Negative	Negative
1185	<i>Listeria welshimeri</i>	Chicken sandwich	TCC	Negative	Negative	2258	<i>Carnobacterium divergens</i>	Brie	TCC	Negative	Negative
1190	<i>Listeria seeligeri</i>	Cheese	TCC	Negative	Negative	2259	<i>Carnobacterium gallinarum</i>	Unknown	TCC	Negative	Negative
1191	<i>Listeria seeligeri</i>	Food	TCC	Negative	Negative	2260	<i>Carnobacterium piscicola</i>	Cooked ham	TCC	Negative	Negative
1424	<i>Proteus vulgaris</i>	Unknown	TCC	Negative	Negative	2262	<i>Erysipelothrix rhusiopathiae</i>	Unknown	TCC	Negative	Negative
1809	<i>Escherichia coli</i>	NCTC	TCC	Negative	Negative	2263	<i>Escherichia fergusonii</i>	Sausages	TCC	Negative	Negative
1892	<i>Klebsiella pneumoniae</i>	NCTC	TCC	Negative	Negative	2299	<i>Bacillus cereus</i>	Milk	TCC	Negative	Negative
1911	<i>Salmonella</i> Typhimurium	NCTC	TCC	Negative	Negative	2300	<i>Bacillus mycoides</i>	NCTC	TCC	Negative	Negative
1913	<i>Citrobacter freundii</i>	NCTC	TCC	Negative	Negative	2304	<i>Propionibacterium freundenreichii</i>	Swiss cheese	TCC	Negative	Negative

1978	<i>Listeria welshimeri</i> 6b	NCTC	TCC	Negative	Negative	2352	<i>Streptococcus salivarius</i>	NCTC	TCC	Negative	Negative
1979	<i>Listeria seeligeri</i> 1/2b	NCTC	TCC	Negative	Negative	2358	<i>Rhodococcus equi</i>	Animal isolate	TCC	Negative	Negative
2186	<i>Listeria innocua</i> 6a	NCTC	TCC	Negative	Negative	2352	<i>Micrococcus luteus</i>	Unknown	OCC <sup>b</sup>	Negative	Negative

<sup>a</sup>TCC = Trials Culture Collection, Thermo Fisher Scientific, UK.

<sup>b</sup>OCC = Oxoid Culture Collection, Thermo Fisher Scientific, UK.

**Table 28. Thermo Scientific SureTect Listeria monocytogenes PCR Assay result comparison for the original and upgraded kit files with RFE v2.0 (14)**

Matrix	Inoculating Strain(s)	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	Candidate Method <sup>c</sup>		
				Kit file 1.0 x <sup>d</sup>	Kit file v1.0 -! <sup>e</sup>	Kit file v2.0 x' <sup>f</sup>
Raw ground turkey	<i>L. monocytogenes</i> TCC 1227	N/A <sup>g</sup>	5	0	N/A	0
		0.69	20	9	N/A	9
		4.38	5	4	N/A	4
Pasteurized 2% milk	<i>L. monocytogenes</i> TCC 856	N/A	5	0	N/A	0
		1.26	20	18	N/A	18
		3.00	5	5	N/A	5
Bagged lettuce	<i>L. monocytogenes</i> TCC 1220	N/A	5	0	N/A	0
		1.80	20	13	N/A	13
		3.20	5	5	N/A	5
Stainless steel surface 4" x 4"	<i>L. monocytogenes</i> TCC 0813 & <i>E. faecalis</i> CIP100750 X10	N/A	5	0	N/A	0
		N/A	20	6	N/A	6
		N/A	5	5	N/A	5

<sup>a</sup>MPN = Most Probable Number is based on the POD of reference method test portions using the Least Cost Formulations MPN calculator, with 95% confidence interval.

<sup>b</sup>N = Number of test portions, 5 unspiked, 20 low spike, 5 high spike.

<sup>c</sup>Candidate method presumptive result (PCR only).

<sup>d</sup>RFE 1.0 x = Number of positive test portions gained originally with RFA 1.0 and original kit file.

<sup>e</sup>RFE 1.0 - ! = Number of positive test portions gained originally with RFA 1.0 and original kit file (excluding samples with warning calls seen on RFA 1.1).

<sup>f</sup>RFE 2.0 x = Number of positive test portions gained originally with RFA 1.0 and original kit file.

<sup>g</sup>N/A = Not applicable.

**DISCUSSION OF MODIFICATION APPROVED JUNE 2023 (15)**

The results from the inclusivity/exclusivity studies confirmed that the new *Brilliance* Listeria Agar (ISO) medium can accurately detect a wide range of *L. monocytogenes* strains and inhibit the growth of non-*Listeria* bacteria. Non-*L. monocytogenes* *Listeria* species still grow on the medium however, these can be differentiated from *L. monocytogenes* from the absence of a halo around the presumptive colony. Although *L. ivanovii* can produce a halo on the medium, the halo size and quality is reduced. This has no effect on the overall performance of the method as the new *Brilliance* Listeria Agar (ISO) is part of the confirmation and not the main detection tool of the method. In addition, any colonies with a halo on the new *Brilliance* Listeria Agar (ISO) medium would be tested further with the Microbact kit.

The results from the POD studies showed a comparable performance between the candidate and reference method for all matrixes. For pork rillettes and process water, the POD results were in favor of the candidate method, while for the raw milk, smoked salmon, and ready-to-cook vegetables, the POD results favored the reference method. For the deli salad, there were no differences between the two methods. There were no statistically significant differences between candidate presumptive and confirmed results except for the smoked salmon matrix where one presumptive positive result was not confirmed.

The matrix extensions with the new *Brilliance* Listeria Agar (ISO) medium also had a lower minimum enrichment time at 20 h. Despite this 2 h reduction in the minimum enrichment time, the performance of the method was still satisfactory in comparison to the reference method.

Overall, the data from the studies supports the claims of the new *Brilliance* Listeria Agar (ISO) in the SureTect Listeria monocytogenes PCR Assay method as a reliable detection method for *L. monocytogenes* in a broad range of foods and select environmental surfaces.

**Table 2. Inclusivity: *Brilliance*™ Listeria Agar (ISO) (15)**

No.	Source ID	Origin	Strain name	Serogroup	Brilliance Listeria Agar (ISO)		
					Colony color	Halo	Result
1	Lm-P775 <sup>a</sup>	Fish	<i>L. monocytogenes</i>	Unknown	Blue/green	Yes	+
2	Lm-P777	Fish	<i>L. monocytogenes</i>	Unknown	Blue/green	Yes	+
3	Lm-P778	Fish	<i>L. monocytogenes</i>	Unknown	Blue/green	Yes	+
4	Lm-P779	Fish	<i>L. monocytogenes</i>	Unknown	Blue/green	Yes	+
5	Lm-P780	Fish	<i>L. monocytogenes</i>	Unknown	Blue/green	Yes	+
6	Lm-P781	Fish	<i>L. monocytogenes</i>	Unknown	Blue/green	Yes	+
7	Lm-P782	Salmon rillettes with salicornia	<i>L. monocytogenes</i>	Unknown	Blue/green	Yes	+
8	Lm-P783	Swab on handle of boning pliers	<i>L. monocytogenes</i>	Unknown	Blue/green	Yes	+
9	Lm-P784	Raw salmon fillet on arrival	<i>L. monocytogenes</i>	Unknown	Blue/green	Yes	+
10	Lm-P785	Raw salmon fillet on arrival	<i>L. monocytogenes</i>	Unknown	Blue/green	Yes	+
11	LM-H170	Fisherman	<i>L. monocytogenes</i>	Unknown	Blue/green	Yes	+
12	LM-H171	Bistro	<i>L. monocytogenes</i>	Unknown	Blue/green	Yes	+
13	LM-H172	Market	<i>L. monocytogenes</i>	Unknown	Blue/green	Yes	+
14	LM-H173	Norwegian	<i>L. monocytogenes</i>	Unknown	Blue/green	Yes	+
15	LM-H174	Cauliflower	<i>L. monocytogenes</i>	Unknown	Blue/green	Yes	+
16	LM-H175	Cauliflower	<i>L. monocytogenes</i>	Unknown	Blue/green	Yes	+
17	LM-I20	Pass-through No. 2 at 9.30 a.m.	<i>L. monocytogenes</i>	Unknown	Blue/green	Yes	+
18	LM-I21	Pass-through No. 3 at 6 o'clock	<i>L. monocytogenes</i>	Unknown	Blue/green	Yes	+
19	LM-I22	Soil packing	<i>L. monocytogenes</i>	Unknown	Blue/green	Yes	+
20	LM-I23	S89 tunnel front mat	<i>L. monocytogenes</i>	Unknown	Blue/green	Yes	+
21	OCC 2388 <sup>b</sup>	Unknown (ATCC <sup>c</sup> 35152)	<i>L. monocytogenes</i>	1/2a	Blue/green	Yes	+
22	TCC 860 <sup>d</sup>	Poultry	<i>L. monocytogenes</i>	1/2a	Blue/green	Yes	+
23	TCC 1205	Cake	<i>L. monocytogenes</i>	1/2b	Blue/green	Yes	+
24	TCC 1196	Roast beef	<i>L. monocytogenes</i>	1/2c	Blue/green	Yes	+
25	TCC 867	CSF	<i>L. monocytogenes</i>	2	Blue/green	Yes	+
26	TCC 812	Pie	<i>L. monocytogenes</i>	3a	Blue/green	Yes	+
27	TCC 2179	Institute Pasteur	<i>L. monocytogenes</i>	3b	Blue/green	Yes	+
28	TCC 2180	Institute Pasteur	<i>L. monocytogenes</i>	3c	Blue/green	Yes	+
29	TCC 2181	Unknown (NCTC 5214)	<i>L. monocytogenes</i>	4a	Blue/green	Yes	+
30	TCC 865	CSF: meningitis (ATCC 13932)	<i>L. monocytogenes</i>	4b	Blue/green	Yes	+
31	TCC 2183	Bird-myocardial disease (NCTC 4883 <sup>e</sup> )	<i>L. monocytogenes</i>	4c	Blue/green	Yes	+
32	TCC 863	Sheep, USA (ATCC 19117)	<i>L. monocytogenes</i>	4d	Blue/green	Yes	+
33	TCC 885	Institute Pasteur	<i>L. monocytogenes</i>	4e	Blue/green	Yes	+
34	TCC 2184	Unknown (NCTC 10890)	<i>L. monocytogenes</i>	7	Blue/green	Yes	+
35	TCC 1219	Pilau rice	<i>L. monocytogenes</i>	1/2a	Blue/green	Yes	+
36	TCC 1222	Chicken slicer – environmental	<i>L. monocytogenes</i>	1/2a	Blue/green	Yes	+
37	TCC 1216	RTE sandwich	<i>L. monocytogenes</i>	1/2a	Blue/green	Yes	+
38	TCC 1208	Cheese (ruid)	<i>L. monocytogenes</i>	1/2b	Blue/green	Yes	+
30	TCC 1211	Cheese	<i>L. monocytogenes</i>	1/2b	Blue/green	Yes	+
40	TCC 1200	Deli product	<i>L. monocytogenes</i>	1/2c	Blue/green	Yes	+
41	TCC 858	Human (ATCC 7644)	<i>L. monocytogenes</i>	1/2c	Blue/green	Yes	+
42	TCC 1204	Cooked ham	<i>L. monocytogenes</i>	1/2c	Blue/green	Yes	+
43	TCC 889	Meat isolate	<i>L. monocytogenes</i>	3a	Blue/green	Yes	+
44	TCC 840	Human sample, butter outbreak	<i>L. monocytogenes</i>	3a	Blue/green	Yes	+
45	TCC 1227	Turkey breast	<i>L. monocytogenes</i>	4b	Blue/green	Yes	+
46	TCC 864	Meningitis due to contaminated cheese (NCTCC 11994)	<i>L. monocytogenes</i>	4b	Blue/green	Yes	+

47	RDCC 3413 <sup>f</sup>	Spinach	<i>L. monocytogenes</i>	Unknown	Blue/green	Yes	+
48	RDCC 1656	Cheese manufacturer	<i>L. monocytogenes</i>	Unknown	Blue/green	Yes	+
49	RDCC 5354	Chicken thigh	<i>L. monocytogenes</i>	Unknown	Blue/green	Yes	+
50	RDCC 486	Minced beef	<i>L. monocytogenes</i>	Unknown	Blue/green	Yes	+

<sup>a</sup>*L. monocytogenes* from MicroSept culture collection (Le Lion-d' Angers, France)

<sup>b</sup>Oxoid Culture Collection (Basingstoke, UK)

<sup>c</sup>American Type Culture Collection (Manassas, VA)

<sup>d</sup>Trials Culture Collection (Basingstoke, UK)

<sup>e</sup>National Collection of Type Culture (Salisbury, UK)

<sup>f</sup>Research and Development Culture Collection (Basingstoke, UK)

**Table 3. Exclusivity: Brilliance™ Listeria Agar (ISO) (15)**

No.	Source ID <sup>a</sup>	Origin	Strain name	Brilliance Listeria Agar (ISO)		
				Colony color	Halo	Result
1	BI-R6	Egg product	<i>B. licheniformis</i>	No colonies	No	-
2	CIP 5832 <sup>b</sup>	Collection	<i>Bacillus cereus</i>	No colonies	No	-
3	CIP 6624	Collection	<i>Bacillus cereus</i>	No colonies	No	-
4	CIP 52.75T	Collection	<i>Bacillus circulans</i>	No colonies	No	-
5	CIP 88264	Collection	<i>Candida</i>	No colonies	No	-
6	IND 501	Clinical strain	<i>Enterococcus faecalis</i>	No colonies	No	-
7	IND 502	Clinical strain	<i>Enterococcus faecalis</i>	No colonies	No	-
8	ATCC 19433 <sup>c</sup>	Collection	<i>Enterococcus faecalis</i>	No colonies	No	-
9	CIP 5855	Collection	<i>Enterococcus faecium</i>	No colonies	No	-
10	IND 500	Food product	<i>Enterococcus faecium</i>	No colonies	No	-
11	Ec - U2	Camembert	<i>Escherichia coli</i>	No colonies	No	-
12	Ec - U5	Egg product	<i>Escherichia coli</i>	No colonies	No	-
13	ASEPT B 37	Egg product	<i>Escherichia coli</i>	No colonies	No	-
14	CIP 71.39	Collection	<i>Lactobacillus plantarum</i>	No colonies	No	-
15	CIP 103009 T	Collection	<i>Leuconostoc mesenteroides</i>	No colonies	No	-
16	P-P1	Egg product	<i>Pseudomonas fluorescens</i>	No colonies	No	-
17	CIP 58.69	Collection	<i>Rhodococcus equi</i>	No colonies	No	-
18	ASEPT B 38	Egg product	<i>Salmonella enteritidis</i>	No colonies	No	-
19	CIP 5710	Collection	<i>Staphylococcus aureus</i>	No colonies	No	-
20	CIP 53154	Collection	<i>Staphylococcus aureus</i>	No colonies	No	-
21	St-T2	Camembert	<i>Staphylococcus aureus</i>	No colonies	No	-
22	ATCC 25953 - St G48	Collection	<i>Staphylococcus aureus</i>	No colonies	No	-
23	20060913-	Surimi tortis	<i>Bacillus cereus</i>	No colonies	No	-
24	20060914-	Pepper pot	<i>Bacillus cereus</i>	No colonies	No	-
25	20061005-41714	Mexican tabbouleh	<i>Bacillus cereus</i>	No colonies	No	-
26	20060906-36720	Laggy milk powder	<i>Bacillus cereus</i>	No colonies	No	-
27	20060906- 36716	Niro milk powder	<i>Bacillus cereus</i>	No colonies	No	-
28	20060914- 38085	Laggy milk powder	<i>Bacillus cereus</i>	No colonies	No	-
29	20061005-41648	Niro milk powder	<i>Bacillus cereus</i>	No colonies	No	-
30	20061005-41651	Laggy milk powder	<i>Bacillus cereus</i>	No colonies	No	-
31	20060801-30997	Whole egg powder	<i>Bacillus cereus</i>	No colonies	No	-
32	20060801-30999	Whole egg powder	<i>Bacillus cereus</i>	No colonies	No	-
33	20060804-31871	Curly	<i>Pseudomonas</i>	No colonies	No	-
34	20060804-31872	Curly	<i>Pseudomonas</i>	No colonies	No	-
35	20060804-31873	Chewed up	<i>Pseudomonas</i>	No colonies	No	-
36	20060804-31874	Chewed up	<i>Pseudomonas</i>	No colonies	No	-
37	20060825-34927	Environmental gauze	<i>Pseudomonas</i>	No colonies	No	-
38	RXJ222	Fig tartlet	<i>Listeria innocua</i>	Blue	No	-
30	KUY776	Egg product environment	<i>Listeria innocua</i>	Blue	No	-
40	RYB922	Smoked sausages	<i>Listeria innocua</i>	Blue	No	-
41	TYA050	Raw tuna tataki	<i>Listeria innocua</i>	Blue	No	-
42	TQU555	Tomme with raw milk	<i>Listeria innocua</i>	Blue	No	-
43	TSA557	Pasteurized Milk Brie	<i>Listeria innocua</i>	Blue	No	-
44	TTZ273	Diced onions	<i>Listeria innocua</i>	Blue	No	-
45	TWH478	Vegetable gardener	<i>Listeria innocua</i>	Blue	No	-
46	XEN574	Salmon shell	<i>Listeria innocua</i>	Blue	No	-



47	TET819	Chicken thigh	<i>Listeria innocua</i>	Blue	No	-
48	SWZ606	Sausage patty	<i>Listeria grayi</i>	Blue	No	-
49	SWE117	Bayonne ham	<i>Listeria grayi</i>	Blue	No	-
50	RZM251	Salmon marinated in dill	<i>Listeria grayi</i>	Blue	No	-
51	RZK366	Green bean	<i>Listeria grayi</i>	Blue	No	-
52	AAZ671	Turkey cutlet scraps	<i>Listeria ivanovii</i>	Blue	Yes	+
53	APE161	Merguez	<i>Listeria ivanovii</i>	Blue	Yes	+
54	GJP629	Environment	<i>Listeria ivanovii</i>	Blue	Yes	+
55	GQD028	Environment dairy products	<i>Listeria ivanovii</i>	Blue	Yes	+
56	TXR109	Chinese noodles with vegetables	<i>Listeria welshimeri</i>	Blue	No	-
57	GLX736	Environment dairy products	<i>Listeria welshimeri</i>	Blue	No	-
58	PSX189	Environment meat products	<i>Listeria welshimeri</i>	Blue	No	-
59	TDV458	Andouille from Guéméné	<i>Listeria welshimeri</i>	Blue	No	-
60	TPR354	Toulouse sausage	<i>Listeria welshimeri</i>	Blue	No	-
61	TUH443	Smoked arctic char with 5 berries	<i>Listeria welshimeri</i>	Blue	No	-
62	TVP191	Stripped salmon	<i>Listeria welshimeri</i>	Blue	No	-
63	XCW614	Salmon shell	<i>Listeria welshimeri</i>	Blue	No	-
64	TLJ742	Hotpot	<i>Listeria welshimeri</i>	Blue	No	-
65	DSM20751	Ground	<i>Listeria seeligeri</i>	Blue	No	-
66	LHFB67	Dairy environment	<i>Listeria seeligeri</i>	Blue	No	-
67	DSM23813	Ground	<i>Listeria marthi</i>	Blue	No	-

<sup>a</sup>Unless otherwise labelled, isolates belong to MicroSepts culture collection, (France).

<sup>b</sup>Culture Collection of Pasteur Insititute, (France).

<sup>c</sup>American Type Culture Collection, (Manassas, VA).

**Table 4. Thermo Scientific SureTect Listeria monocytogenes PCR assay, 7500 Fast & Q55, presumptive vs. confirmed and candidate vs. ISO11290-1:2017 – POD results (15)**

Statistic	Matrix/Organism	Candidate presumptive (CP)			Candidate confirmed (CC) <sup>a</sup>			Candidate result (C)			Reference method (R)			C vs. R	CP vs. CC
		N <sup>b</sup>	X <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	N	X	POD <sub>CC</sub> <sup>e</sup>	N	X	POD <sub>C</sub> <sup>f</sup>	N	X	POD <sub>R</sub> <sup>g</sup>		
	Deli Salad: Piémontaise (25 g) <i>Listeria monocytogenes</i> 1/2b-3b-7 FLD375	5	0	0.00	5	0	0.00	0	0	0.00	0	0	0.00	0.00	0.00
LCL <sup>j</sup>				0.00			0.00			0.00			0.00	-0.43	-0.47
UCL <sup>k</sup>				0.43			0.43			0.43			0.43	0.43	0.47
		20	8	0.40	20	8	0.40	20	8	0.40	20	8	0.40	0.00	0.00
LCL				0.23			0.22			0.22			0.22	-0.28	-0.13
UCL				0.61			0.61			0.61			0.61	0.28	0.13
		5	4	0.80	5	4	0.80	5	4	0.80	5	5	1.00	-0.20	0.00
LCL				0.38			0.38			0.38			0.57	-0.62	-0.47
UCL			1.00			1.00			1.00			1.00	0.28	0.47	
	Rillettes (25 g) <i>Listeria monocytogenes</i> 1/2c TED200	5	0	0.00	5	0	0.00	0	0	0.00	0	0	0.00	0.00	0.00
LCL				0.00			0.00			0.00			0.00	-0.43	-0.47
UCL				0.43			0.43			0.43			0.43	0.43	0.47
		20	14	0.70	20	14	0.70	20	14	0.70	20	7	0.35	0.35	0.00
LCL				0.48			0.48			0.48			0.18	0.04	-0.13
UCL				0.86			0.86			0.86			0.57	0.58	0.13
		5	5	1.00	5	5	1.00	5	5	1.00	5	5	1.00	0.00	0.00
LCL				0.57			0.57			0.57			0.57	-0.43	-0.47
UCL			1.00			1.00			1.00			1.00	0.43	0.47	
	Raw milk (25 ml) <i>Listeria monocytogenes</i> 1/2b CLM641	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL				0.00			0.00			0.00			0.00	-0.43	-0.47
UCL				0.43			0.43			0.43			0.43	0.43	0.47
		20	11	0.55	20	11	0.55	20	11	0.55	20	12	0.60	-0.05	0.00
LCL				0.34			0.34			0.34			0.39	-0.33	-0.13
UCL				0.74			0.74			0.74			0.78	0.24	0.13
		5	5	1.00	5	5	1.00	5	5	1.00	5	5	1.00	0.00	0.00
LCL				0.57			0.57			0.57			0.57	-0.43	-0.47
UCL			1.00			1.00			1.00			1.00	0.43	0.47	
	Smoked Salmon (25 g) <i>Listeria</i>	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL				0.00			0.00			0.00			0.00	-0.43	-0.47

UCL	monocytogenes 1/2a CHT701			0.43			0.43			0.43			0.43	0.43	0.47
		20	9	0.45	20	8	0.40	20	8	0.40	20	10	0.50	-0.10	0.05
LCL				0.26			0.22			0.22			0.30	-0.37	-0.11
UCL				0.66			0.61			0.61			0.70	0.19	0.21
		5	4	0.80	5	4	0.80	5	4	0.80	5	4	0.80	0.00	0.00
LCL				0.38			0.38			0.38			0.38	-0.47	-0.47
UCL				1.00			1.00			1.00			1.00	0.47	0.47
	Ready-to-cook vegetables (25 g) <i>Listeria</i> monocytogenes 4b QDB363	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL				0.00			0.00			0.00			0.00	-0.43	-0.47
UCL				0.43			0.43			0.43			0.43	0.43	0.47
		20	12	0.60	20	12	0.60	20	12	0.60	20	13	0.65	-0.05	0.00
LCL				0.39			0.39			0.39			0.43	-0.32	-0.13
UCL				0.78			0.78			0.78			0.82	0.23	0.13
		5	4	0.80	5	4	0.80	5	4	0.80	5	4	0.80	0.00	0.00
LCL			0.38			0.38			0.38			0.38	-0.47	-0.47	
UCL			1.00			1.00			1.00			1.00	0.47	0.47	
	Process water (25 ml) <i>Listeria</i> monocytogenes 1/2c AEU531	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL				0.00			0.00			0.00			0.00	-0.43	-0.47
UCL				0.43			0.43			0.43			0.43	0.43	0.47
		20	10	0.50	20	10	0.50	20	10	0.50	20	7	0.35	0.15	0.00
LCL				0.30			0.30			0.30			0.18	-0.15	-0.13
UCL				0.70			0.70			0.70			0.57	0.41	0.13
		5	3	0.60	5	3	0.60	5	3	0.60	5	2	0.40	0.20	0.00
LCL			0.23			0.23			0.23			0.12	-0.32	-0.47	
UCL			0.88			0.88			0.88			0.77	0.60	0.47	

<sup>a</sup>Results obtained following the alternative confirmation were identical to results obtain from confirmation by ISO 11290-1:2017 reference method.

<sup>b</sup>N = Number of test portions.

<sup>c</sup>X = Number of positive test portions.

<sup>d</sup>POD<sub>CP</sub> = Candidate method presumptive positive outcomes divided by the total number of trials.

<sup>e</sup>POD<sub>CC</sub> = Candidate method confirmed positive outcomes divided by the total number of trials.

<sup>f</sup>POD<sub>C</sub> = Candidate method presumptive positive outcomes confirmed positive divided by the total number of trials.

<sup>g</sup>POD<sub>R</sub> = Reference method confirmed positive outcomes divided by the total number of trials.

<sup>h</sup>dPOD<sub>CP</sub> = Difference between the candidate method presumptive result and candidate method confirmed result POD values.

<sup>i</sup>dPOD<sub>CC</sub> = Difference between the confirmed candidate method result and reference method confirmed result POD values.

<sup>j</sup>LCL = Lower confidence limit.

<sup>k</sup>UCL = Upper confidence limit.

## DISCUSSION OF THE MODIFICATION STUDY APPROVED JANUARY 2024 (17)

The comparison study was selected to evaluate the automated procedure as it allowed for an accurate and precise comparison of the performance between the manual and automated lysis and PCR setup procedures without interference from other parts of the method, such as the enrichment. The study followed a paired study design with a post enrichment spike to assess the performance of the lysis and PCR setup procedures specifically.

Comparison studies above the LOD of the PCR assays showed that the difference in average C<sub>t</sub> values were always ±1.5 cycles when comparing the automated and manual procedures. At the LOD, the numbers of positives per dilution for each assay-matrix combination was statistically comparable when comparing the automated procedure to the manual.

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