CERTIFICATION

AOAC Research Institute

Performance Tested Methods[SM]

Certificate No.

071304

The AOAC Research Institute hereby certifies the method known as:

Thermo Scientific™ SureTect™ Listeria species 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*[SM] 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*[SM] 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.

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

Issue Date	November 04, 2024
Expiration Date	December 31, 2025

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METHOD NAME
Thermo Scientific™ SureTect™ Listeria species PCR Assay

CATALOG NUMBER
A56842

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APPLICABILITY OF METHOD
Target Organism – *Listeria* species.

Matrixes – (25 g) raw ground beef, pork frankfurters, salami, cooked sliced ham, cooked sliced turkey, fresh bagged spinach, cantaloupe, processed cheese, smoked salmon, cooked prawns
(Sponge 4" x 4" enriched in 225 mL of 24 LEB supplemented with 24 LEB Selective supplement and 10 mL of LEB Buffer supplement) stainless steel, and plastic
MODIFICATION SEPTEMBER 2015 (25 g): ground pork, bagged lettuce, raw ground turkey, raw pork sausages, pasteurized 2% milk, raw cod, pasteurized brie cheese, and ice cream
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 – The study data detected no statistical difference between the Thermo Scientific™ SureTect™ Listeria species 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)

ORIGINAL CERTIFICATION DATE	CERTIFICATION RENEWAL RECORD
July 25, 2013	Renewed Annually through December 2025.

METHOD MODIFICATION RECORD	SUMMARY OF MODIFICATION
1. September 2015 Level 3	1. Matrix extension.
2. November 2015	2. Certification of ABI 7500 Fast Instrument.
3. December 2017 Level 1	3. Editorial changes to insert and labels.
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 for clarity.
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 changes to Manual resulting from approval of July 2022 Level 2 modification.
14. June 2023 Level 2	14. New formulation to the Thermo Scientific™ Oxoid™ *Brilliance*™ 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.

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NONE	NONE

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 species PCR Assay (15)

Thermo Scientific SureTect Listeria species PCR Assay – PTM 071304

Original PTM certificate issued[a]	Matrixes	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[b]
	Raw pork frankfurters	25 g	24 LEB/1-in-10	22 - 30	37±1	ISO
	Salami	25 g	24 LEB/1-in-20	22 - 30	37±1	ISO
	Cooked sliced ham	25 g	24 LEB/1-in-10	22 - 30	37±1	ISO
	Cooked sliced turkey	25 g	24 LEB/1-in-10	22 - 30	37±1	ISO
	Fresh bagged spinach	25 g	24 LEB/1-in-10	22 - 30	37±1	ISO
	Cut cantaloupe	25 g	24 LEB/1-in-10	22 - 30	37±1	ISO
	Processed cheese	25 g	24 LEB/1-in-10	22 - 30	37±1	ISO
	Smoked salmon	25 g	24 LEB/1-in-10	22 - 30	37±1	ISO
	Cooked prawns (heads off)	25 g	24 LEB/1-in-10	22 - 30	37±1	ISO
	Stainless steel (slab, brushed finish)	4”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	Matrixes	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	Matrixes	Test Portion Size	Enrichment dilution	Enrichment time (h)	Enrichment temp (°C)	Reference method
October, 2018 QS5 PCR instrument 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[c]	Matrixes	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)[d]	25 g	24 LEB/1-in-10	22 - 30	37±1	BAM[e]
	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 & ISO
	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	Matrixes	Test Portion Size	Enrichment dilution	Enrichment time (h)	Enrichment temp (°C)	Reference method
New matrices pending approval 2023, New Brilliance Listeria Agar (ISO)	Deli salad (Piemontaise)	25 g	24 LEB/1-in-10	20 - 28	37±1	ISO
	Pork 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

[a] AOAC Research Institute Certified Methods Search..

[b] EN ISO 11290-1.

[c] Matrixes approved with First Action.

[d] Matrix selected for collaborative study.

[e] BAM Chapter 10.

PRINCIPLE OF THE METHOD (1)

The Thermo Scientific™ SureTect™ Listeria species 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 the SureTect Software for the detection and identification of *Listeria* species in food and environmental samples (4, 5).

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 are found only in *Listeria*. If *Listeria* 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 species 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 coloured fluorescent dye than that in the probe used 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 the amplified 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 in 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[a]	Source	Result
Listeria monocytogenes	1/2a	860	Poultry	Positive
Listeria monocytogenes	1/2a	1215	Chorizo sausage	Positive
Listeria monocytogenes	1/2a	1216	Sandwich	Positive
Listeria monocytogenes	1/2a	1217	Carrow cheese	Positive
Listeria monocytogenes	1/2a	1218	Butter	Positive
Listeria monocytogenes	1/2a	1219	Pilau rice	Positive
Listeria monocytogenes	1/2a	1220	Sandwich	Positive
Listeria monocytogenes	1/2b	1205	Cake	Positive
Listeria monocytogenes	1/2b	1206	Whipped Cream	Positive
Listeria monocytogenes	1/2b	1207	Cheese	Positive
Listeria monocytogenes	1/2b	1208	Cheese	Positive
Listeria monocytogenes	1/2b	1209	Cream	Positive
Listeria monocytogenes	1/2b	1210	Cheese	Positive
Listeria monocytogenes	1/2c	858	Clinical sample	Positive
Listeria monocytogenes	1/2c	1195	Ox tongue	Positive
Listeria monocytogenes	1/2c	1196	Roast beef	Positive
Listeria monocytogenes	1/2c	1197	Topside beef	Positive
Listeria monocytogenes	1/2c	1198	Wiltshire ham	Positive
Listeria monocytogenes	1/2c	1199	Ham sandwich	Positive
Listeria monocytogenes	3a	812	Environmental	Positive
Listeria monocytogenes	3a	813	Environmental	Positive
Listeria monocytogenes	3a	840	Butter	Positive
Listeria monocytogenes	3a	870	Clinical sample	Positive
Listeria monocytogenes	3a	888	Food	Positive
Listeria monocytogenes	3a	889	Food	Positive
Listeria monocytogenes	3b	2179	Unknown	Positive
Listeria monocytogenes	3c	2180	Unknown	Positive
Listeria monocytogenes	4a	2181	Unknown	Positive
Listeria monocytogenes	4b	864	Meningitis	Positive
Listeria monocytogenes	4b	865	CSF: Meningitis	Positive
Listeria monocytogenes	4b	1224	Food- blood	Positive
Listeria monocytogenes	4b	1225	Chicken	Positive
Listeria monocytogenes	4b	1226	Dressed crab	Positive
Listeria monocytogenes	4b	1227	Turkey breast	Positive
Listeria monocytogenes	4c	2183	Bird: heart disease	Positive
Listeria monocytogenes	4d	863	Sheep	Positive
Listeria monocytogenes	4e	868	Chicken	Positive
Listeria monocytogenes	4e	883	Veterinary sample	Positive
Listeria grayi		1172	Environmental	Positive
Listeria grayi		1173	Butter	Positive
Listeria grayi		1174	Butter	Positive
Listeria grayi		1175	Butter	Positive

Listeria grayi		1176	Food	Positive
Listeria innocua	Unknown	1177	Chicken sandwich	Positive
Listeria innocua	Unknown	1178	Cooked chicken	Positive
Listeria innocua	Unknown	1179	Crayfish	Positive
Listeria innocua	Unknown	1180	Coleslaw	Positive
Listeria innocua	Unknown	1181	Tuna mayo sandwich	Positive
Listeria innocua	6a	862	Cow brain ATCC® 33090™	Positive
Listeria innocua	4ab	2185		Positive
Listeria innocua	6b	2187		Positive
Listeria ivanovii	Unknown	1182	Lamb (vet sample)	Positive
Listeria ivanovii	Unknown	1183	Food	Positive
Listeria ivanovii	Unknown	1184	Food	Positive
Listeria welshimeri	Unknown	1185	Chicken sandwich	Positive
Listeria welshimeri	Unknown	1186	Food	Positive
Listeria welshimeri	Unknown	1187	Environmental	Positive
Listeria welshimeri	Unknown	1188	Pastrami	Positive
Listeria welshimeri	Unknown	1189	Food	Positive
Listeria welshimeri	6b	2188		Positive
Listeria welshimeri	4c	2189		Positive
Listeria seeligeri	Unknown	1190	Cheese	Positive
Listeria seeligeri	Unknown	1191	Food	Positive
Listeria seeligeri	Unknown	1192	Environmental	Positive
Listeria seeligeri	Unknown	1193	Cannelloni	Positive
Listeria seeligeri	Unknown	1194	Coleslaw	Positive
Listeria seeligeri	1/2b	2190		Positive
Listeria seeligeri	6b	2191		Positive

[a]Trials Culture Collection Number - Proprietary to Thermo Fisher Scientific, Microbiology Division

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

Isolate	TCC [a]	Source	Result
Bacillus circulans	2303		Negative
Enterococcus faecium	598		Negative
Enterococcus faecalis	567		Negative
Leuconostoc mesenteroides subsp. *mesenteroides*	853		Negative
Pseudomonas aeruginosa	2354	Minced beef	Negative
Staphylococcus lentus	2301	Prawns	Negative
Staphylococcus schleiferi	2302	Salmon	Negative
Candida parapsilosis	1828		Negative
Lactobacillus brevis	848		Negative
Lactococcus acidophilus	2359	ATCC 4356	Negative
Bacillus mycoides	2300	Milk	Negative
Brochothrix thermosphacta	2192	Pork Sausage	Negative
Carnobacterium divergens	2257		Negative
Carnobacterium gallinarum	2259		Negative
Carnobacterium pisicola	2260	Ham	Negative
Citrobacter freundii	1911		Negative
Enterobacter aerogenes	2200		Negative
Erysipelothrix rhusiopathiae	2262		Negative
Escherichia fergusonii	2263	Sausage	Negative
Escherichia coli	1809		Negative
Klebsiella pneumoniae	1892		Negative
Kurthia gibsonii	2193	Pork sausage	Negative
Lactobacillus casei subsp. *casei*	2194	Tomato catsup	Negative
Lactobacillus delbrueckii subsp. *lactis*	2195	Emmenthal cheese production	Negative
Lactobacillus plantarum	2196	Red Cheshire cheese production	Negative
Micrococcus luteus	OCC[b] 2352		Negative
Proteus vulgaris	1424		Negative
Propionibacterium freundenreichii	2304	Swiss cheese production	Negative
Rhodococcus equi	2358	Animal isolate	Negative
Salmonella enterica subsp.Typhimurium	1911	Bovine liver	Negative
Staphylococcus aureus	2240	Food	Negative
Streptococcus salivarius	2352		Negative
Bacillus cereus	2299	Cream	Negative

[a]Trials Culture Collection - Proprietary to Thermo Fisher Scientific, Microbiology Division, Basingstoke, UK

[b]Oxoid Culture Collection - Proprietary to Thermo Fisher Scientific, Microbiology Division, Basingstoke, UK

Table 3: SureTect Listeria species Assay Presumptive vs. SureTect Confirmation Result-POD Analysis (1)

Matrix	Strain	MPN[a]/test portion	N[b]	SureTect Method Presumptive			SureTect Confirmation method result			dPODcp[f]	95% CI[g]
				X[c]	POD_{cp}[d]	95% CI	X	POD_{cc}[e]	95% CI		
Plastic	*L. monocytogenes* TCC 812	N/A[h]	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)
Stainless steel	*Listeria monocytogenes* (TCC 813) and 10x *Enterococcus faecalis* (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	10	0.50	(0.30, 0.70)	11	0.55	(0.34, 0.74)	-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	(-0.43, 0.43)
Pork frankfurters	*Listeria innocua* TCC 1177	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.74 (0.43, 1.33)	20	7	0.35	(0.18, 0.57)	7	0.35	(0.18, 0.57)	0.00	(-0.28, 0.28)
		1.18 (0.51, 2.72)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Smoked salmon	*Listeria monocytogenes* 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)
		0.6 (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)
		1.3 (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)
Processed cheese	*Listeria monocytogenes* 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, 0.96)	20	5	0.25	(0.11, 0.47)	6	0.30	(0.15, 0.52)	-0.05	(-0.31, 0.22)
		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)
Spinach	*Listeria innocua* TCC 1180	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.38 (0.83, 2.37)	20	11	0.55	(0.34, 0.74)	11	0.55	(0.34, 0.74)	0.00	(-0.28, 0.28)
		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)
Cantaloupe	*Listeria monocytogenes* TCC 1217	N/A[h]	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.7 (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 (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)
Cooked prawns (chilled)	*Listeria monocytogenes* 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)
		1.9 (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)
Cooked sliced turkey (chilled)	*Listeria innocua* TCC 1178	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.69 (0.39, 1.25)	20	9	0.45	(0.26, 0.66)	9	0.45	(0.26, 0.66)	0.00	(-0.28, 0.28)
		1.02 (0.46, 2.25)	5	5	1.00	(0.57, 1.00)	4	0.80	(0.38, 1.00)	0.20	(-0.28, 0.62)
Raw ground beef (12% fat)	*Listeria monocytogenes* 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)
		1.13 (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)
		1.88 (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)
Salami	*Listeria monocytogenes* 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	9	0.45	(0.26, 0.66)	11	0.55	(0.34, 0.74)	-0.10	(-0.37, 0.19)
		0.40 (0.23, 0.91)	5	2	0.40	(0.12, 0.77)	3	0.60	(0.23, 0.88)	-0.20	(-0.60, 0.32)
Cooked sliced ham (chilled)	*Listeria welshimeri* TCC 1188	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.02 (0.592, 2.07)	20	13	0.65	(0.43, 0.82)	14	0.70	(0.48, 0.85)	-0.05	(-0.32, 0.23)
		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 spinach[i]	*Listeria innocua* ATCC 33090	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.66 (0.37, 1.10)	20	14	0.70	(0.48, 0.85)	14	0.70	(0.48, 0.85)	0.00	(-0.26, 0.26)
		2.97 (1.25, 7.00)	5	5	1.00	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0.00	(-0.43, 0.43)
Pork frankfurters[i]	*Listeria innocua* ATCC 33091	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.38	20	12	0.60	(0.38, 0.78)	12	0.60	(0.38, 0.78)	0.00	(-0.28, 0.28)

		(0.83, 2.38)									
		2.97 (1.25, 7.00)	5	5	1.00	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0.00	(-0.43, 0.43)
Stainless steel 4"x4"[i]	*Listeria monocytogenes* LI7163 1/2a and 10x *Enterococcus faecalis* ATCC 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)

[a]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.
[b]N=Number of test portions
[c]X=Number of positive test portions
[d]PODcp=Candidate method presumptive positive outcomes divided by the total number of portions
[e]PODcc=Candidate confirmation method positive outcomes divided by the total number of portions
[f]dPODcp=Difference between the candidate presumptive result and the candidate method confirmed result POD values
[g]95% CI=If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level
[h]N/A=Not applicable
[i]Independent Laboratory Study

Table 4: SureTect Listeria species Assay Presumptive Result vs. Reference Confirmation-POD Analysis (1)

Matrix	Strain	MPN[a]/test portion	N[b]	SureTect Presumptive Result			Reference Confirmation (CC2)			dPODcp[f]	95% CI[g]
				X[c]	POD_{cp}[d]	95% CI	X	POD_{cc2}[e]	95% CI		
Plastic	*L. monocytogenes* TCC 812	N/A[h]	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)
Stainless steel	*Listeria monocytogenes* (TCC 813) and 10x *Enterococcus faecalis* (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	10	0.50	(0.30, 0.70)	11	0.55	(0.34, 0.74)	-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	(-0.43, 0.43)
Pork frankfurters	*Listeria innocua* TCC 1177	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.74 (0.43, 1.33)	20	7	0.35	(0.18, 0.57)	7	0.35	(0.18, 0.57)	0.00	(-0.28, 0.28)
		1.18 (0.51, 2.72)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Smoked salmon	*Listeria monocytogenes* 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)
		0.6 (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)
		1.3 (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)
Processed cheese	*Listeria monocytogenes* 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, 0.96)	20	5	0.25	(0.11, 0.47)	6	0.30	(0.15, 0.52)	-0.05	(-0.31, 0.22)
		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)
Spinach	*Listeria innocua* TCC 1180	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.38 (0.83, 2.37)	20	11	0.55	(0.34, 0.74)	12	0.60	(0.39, 0.78)	-0.05	(-0.33, 0.24)
		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)
Cantaloupe	*Listeria monocytogenes* TCC 1217	N/A[h]	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.7 (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 (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)

Cooked prawns (chilled)	*Listeria monocytogenes* 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)
		1.9 (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)
Cooked sliced turkey (chilled)	*Listeria innocua* TCC 1178	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.69 (0.39, 1.25)	20	9	0.45	(0.26, 0.66)	9	0.45	(0.26, 0.66)	0.00	(-0.28, 0.28)
		1.02 (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)
Raw ground beef (12% fat)	*Listeria monocytogenes* 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)
		1.13 (0.71, 1.88)	20	13	0.65	(0.43, 0.82)	14	0.70	(0.48, 0.85)	-0.05	(-0.32, 0.23)
		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)
Salami	*Listeria monocytogenes* 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	9	0.45	(0.26, 0.66)	11	0.55	(0.34, 0.74)	-0.10	(-0.37, 0.19)
		0.40 (0.23, 0.91)	5	2	0.40	(0.12, 0.77)	3	0.60	(0.23, 0.88)	-0.20	(-0.60, 0.32)
Cooked sliced ham (chilled)	*Listeria welshimeri* TCC 1188	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.02 (0.592, 2.07)	20	13	0.65	(0.43, 0.82)	14	0.70	(0.48, 0.85)	-0.05	(-0.32, 0.23)
		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 spinach[i]	*Listeria innocua* ATCC 33090	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.66 (0.37, 1.10)	20	14	0.70	(0.48, 0.85)	14	0.70	(0.48, 0.85)	0.00	(-0.26, 0.26)
		2.97 (1.25, 7.00)	5	5	1.00	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0.00	(-0.43, 0.43)
Pork frankfurters[i]	*Listeria innocua* ATCC 33091	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.38 (0.83, 2.38)	20	12	0.60	(0.38, 0.78)	12	0.60	(0.38, 0.78)	0.00	(-0.28, 0.28)
		2.97 (1.25, 7.00)	5	5	1.00	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0.00	(-0.43, 0.43)
Stainless steel 4"x4"[i]	*Listeria monocytogenes* LI7163 1/2a and 10x *Enterococcus faecalis* ATCC 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)

[a]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.
[b]N=Number of test portions
[c]X=Number of positive test portions
[d]PODcp=Candidate method presumptive positive outcomes divided by the total number of portions
[e]PODcc$_2$=Reference method confirmation positive outcomes divided by the total number of portions
[f]dPODcp=Difference between the candidate presumptive result and the candidate method confirmed result POD values
[g]95% CI=If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level
[h]N/A=Not applicable
[i]Independent Laboratory Study

Table 5 SureTect Listeria species Assay Confirmation Method (Microbact) vs. Reference Confirmation-POD Analysis (1)

Matrix	Strain	MPN[a]/test portion	N[b]	SureTect Confirmation Method (Microbact) (CC)			Reference Confirmation (CC2)			dPODcc[f]	95% CI[g]
				X[c]	POD_{cc}[d]	95% CI	X	POD_{cc2}[e]	95% CI		
Plastic	*L. monocytogenes* TCC 812	N/A[h]	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)
Stainless steel	*Listeria monocytogenes* (TCC 813) and 10x *Enterococcus faecalis* (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	(-0.43, 0.43)
		N/A	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Pork frankfurters	*Listeria innocua* TCC 1177	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.74 (0.43, 1.33)	20	7	0.35	(0.18, 0.57)	7	0.35	(0.18, 0.57)	0.00	(-0.28, 0.28)
		1.18 (0.51, 2.72)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Smoked salmon	*Listeria monocytogenes* 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)
		0.6 (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)
		1.3 (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)
Processed cheese	*Listeria monocytogenes* 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, 0.96)	20	6	0.30	(0.15, 0.52)	6	0.30	(0.15, 0.52)	0.00	(-0.47, 0.47)
		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)
Spinach	*Listeria innocua* TCC 1180	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.38 (0.83, 2.37)	20	11	0.55	(0.34, 0.74)	12	0.60	(0.39, 0.78)	-0.05	(-0.33, 0.24)
		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)
Cantaloupe melon	*Listeria monocytogenes* TCC 1217	N/A[h]	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.7 (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 (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)

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Cooked prawns (chilled)	*Listeria monocytogenes* 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)
		1.9 (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)
Cooked sliced turkey (chilled)	*Listeria innocua* TCC 1178	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.69 (0.39, 1.25)	20	9	0.45	(0.26, 0.66)	9	0.45	(0.26, 0.66)	0.00	(-0.28, 0.28)
		1.02 (0.46, 2.25)	5	4	0.80	(0.38, 1.00)	5	1.00	(0.57, 1.00)	-0.20	(-0.62, 0.28)
Raw ground beef (12% fat)	*Listeria monocytogenes* 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)
		1.13 (0.71, 1.88)	20	13	0.65	(0.43, 0.82)	14	0.70	(0.48, 0.85)	-0.05	(-0.32, 0.23)
		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)
Salami	*Listeria monocytogenes* 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	3	0.60	(0.23, 0.88)	3	0.60	(0.23, 0.88)	0.00	(-0.46, 0.46)
Cooked sliced ham (chilled)	*Listeria welshimeri* TCC 1188	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.02 (0.592, 2.07)	20	14	0.70	(0.48, 0.85)	14	0.70	(0.48, 0.85)	0.00	(-0.27, 0.27)
		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 spinach[i]	*Listeria innocua* ATCC 33090	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.66 (0.37, 1.10)	20	14	0.70	(0.48, 0.85)	14	0.70	(0.48, 0.85)	0.00	(-0.26, 0.26)
		2.97 (1.25, 7.00)	5	5	1.00	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0.00	(-0.43, 0.43)
Pork frankfurters[i]	*Listeria innocua* ATCC 33091	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.38 (0.83, 2.38)	20	12	0.60	(0.38, 0.78)	12	0.60	(0.38, 0.78)	0.00	(-0.28, 0.28)
		2.97 (1.25, 7.00)	5	5	1.00	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0.00	(-0.43, 0.43)
Stainless steel 4"x4"[i]	*Listeria monocytogenes* LI7163 1/2a and 10x *Enterococcus faecalis* ATCC 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)

[a]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.
[b]N=Number of test portions
[c]X=Number of positive test portions
[d]PODcc=Candidate method confirmed positive outcomes divided by the total number of portions
[e]$PODcc_2$=Reference method confirmation positive outcomes divided by the total number of portions
[f]dPODcp=Difference between the candidate presumptive result and the candidate method confirmed result POD values
[g]95% CI=If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level
[h]N/A=Not applicable
[i]Independent Laboratory Study

Table 6 SureTect Listeria species Assay Method Confirmed Result vs. Reference Confirmation-POD Analysis (1)

Matrix	Strain	MPN[a]/test portion	N[b]	SureTect Method Confirmed Result (C)			Reference Method (R)			dPODc[f]	95% CI[g]
				X[c]	PODc[d]	95% CI	X	PODr[e]	95% CI		
Plastic	*L. monocytogenes* TCC 812	N/A[h]	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	0.80	(0.38, 1.00)	-0.20	(-0.62, 0.28)
Stainless steel	*Listeria monocytogenes* (TCC 813) and 10x *Enterococcus faecalis* (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, 0.24)
		N/A	5	5	1.00	(0.57, 1.00)	4	0.80	(0.38, 1.00)	0.20	(-0.28, 0.62)
Pork frankfurters	*Listeria innocua* TCC 1177	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.74 (0.43, 1.33)	20	7	0.35	(0.18, 0.57)	10	0.50	(0.30, 0.70)	-0.15	(-0.41, 0.15)
		1.18 (0.51, 2.72)	5	5	1.00	(0.57, 1.00)	4	0.80	(0.38, 1.00)	0.20	(-0.28, 0.62)
Smoked salmon	*Listeria monocytogenes* 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)
		0.6 (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)
		1.3 (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)
Processed cheese	*Listeria monocytogenes* 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, 0.96)	20	6	0.30	(0.15, 0.52)	9	0.45	(0.25, 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)
Spinach	*Listeria innocua* TCC 1180	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.38 (0.83, 2.37)	20	11	0.55	(0.34, 0.74)	15	0.75	(0.53, 0.89)	-0.20	(-0.45, 0.09)
		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)
Cantaloupe	*Listeria monocytogenes* TCC 1217	N/A[h]	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.7 (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 (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)

Cooked prawns (chilled)	*Listeria monocytogenes* 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)
		1.9 (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)
Cooked sliced turkey (chilled)	*Listeria innocua* TCC 1178	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.69 (0.39, 1.25)	20	9	0.45	(0.26, 0.66)	10	0.50	(0.30, 0.70)	-0.05	(-0.33, 0.24)
		1.02 (0.46, 2.25)	5	4	0.80	(0.38, 1.00)	2	0.40	(0.12, 0.77)	0.40	(-0.16, 0.75)
Raw ground beef (12% fat)	*Listeria monocytogenes* 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)
		1.13 (0.71, 1.88)	20	13	0.65	(0.43, 0.82)	14	0.70	(0.48, 0.85)	-0.05	(-0.32, 0.23)
		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)
Salami	*Listeria monocytogenes* 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	3	0.60	(0.23, 0.88)	4	0.80	(0.38, 1.00)	-0.20	(-0.62, 0.31)
Cooked sliced ham (chilled)	*Listeria welshimeri* TCC 1188	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.02 (0.592, 2.07)	20	14	0.70	(0.48, 0.85)	15	0.75	(0.53, 0.89)	-0.05	(-0.31, 0.22)
		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 spinach[i]	*Listeria innocua* ATCC 33090	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.66 (0.37, 1.10)	20	14	0.70	(0.48, 0.85)	9	0.45	(0.25, 0.65)	0.25	(-0.05, 0.49)
		2.97 (1.25, 7.00)	5	5	1.00	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0.00	(-0.43, 0.43)
Pork frankfurters[i]	*Listeria innocua* ATCC 33091	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.38 (0.83, 2.38)	20	12	0.60	(0.38, 0.78)	15	0.75	(0.53, 0.88)	-0.15	(-0.40, 0.13)
		2.97 (1.25, 7.00)	5	5	1.00	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0.00	(-0.43, 0.43)
Stainless steel 4”x4”[i]	*Listeria monocytogenes* LI7163 1/2a and 10x *Enterococcus faecalis* ATCC 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	(-0.43, 0.43)

[a]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.
[b]N=Number of test portions
[c]X=Number of positive test portions
[d]PODc=Confirmed candidate method positive outcomes divided by the total number of portions
[e]PODr=Confirmed reference method positive outcomes divided by the total number of portions
[f]dPODc=Difference between the candidate presumptive result and the candidate method confirmed result POD values
[g]95% CI=If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level
[h]N/A=Not applicable
[i]Independent Laboratory Study

DISCUSSION OF MODIFICATION APPROVED SEPTEMBER 2015 (8)

The results from the validation study comparing the performance of the SureTect Listeria species assay and the ISO reference method for detection of *Listeria* from an extended range of food matrices are detailed in Tables 1 to 4. The SureTect PCR Assay presumptive results were compared to the SureTect recommended confirmation procedure of direct plating of the enrichment broth on *Brilliance* Listeria Agar, followed by biochemical identification using the Microbact Listeria 12L kit, and presumptive results were also compared to the ISO reference method confirmation protocol. The results from the SureTect and ISO reference confirmation procedures were also compared to each other. The presumptive SureTect results that confirmed positive were compared to the ISO reference method.

Although the SureTect method generated several PCR negative results during the initial method developer study which were confirmed as positive in ice cream, bagged lettuce, raw ground turkey, pasteurized milk and pasteurized brie cheese, POD statistical analysis (at the 95% confidence level) demonstrated no statistical significant difference between the presumptive and confirmed SureTect results for any of the matrices analysed (Tables 1 and 2). At the request of the method reviewers, a repeat analysis of three of these matrices, raw ground turkey, pasteurized milk and bagged lettuce, was conducted. Two of these three matrices gave equivalent performance during the repeat analysis. There was one false negative PCR result from a low spiked sample of bagged lettuce, which was confirmed using the SureTect confirmation protocol. The false negative results found during the initial analyses are suspected to be due to the use of chilled 24 LEB enrichment broth for the SureTect method. Investigation determined that the media had not been allowed to reach room temperature before use, likely delaying the growth of the *Listeria* and preventing it from attaining the detection level required of the SureTect assay. During the repeat testing of raw ground turkey, pasteurized milk and bagged lettuce, the enrichment broth was allowed to reach ambient temperature before being used for sample preparation. Therefore, it is important that users of this method ensure that the enrichment broth is at an appropriate temperature before use. This detail is now noted in the product's instructions for use.

When comparing the SureTect confirmation procedure to the ISO confirmation procedure, in the initial testing the SureTect procedure confirmed one more sample for ice cream, and the ISO procedure confirmed one more sample each for bagged lettuce, raw pork sausage, pasteurized brie cheese and pasteurized milk. There were no statistical differences by POD analysis. In the repeat testing for pasteurized milk, there were no differences. In the repeat testing for bagged lettuce, despite extensive subculture, it was not possible to confirm or isolate the *L. ivanovii* used in this study from the SureTect enrichments using the ISO procedure, due to the failure of this strain to grow in Fraser Broth. In this case, there was a statistically significant difference between the confirmation procedures, favoring the SureTect procedure. It is not known why this strain of *L. ivanovii* failed to grow in Fraser Broth. In previous studies and reports (7, 8), the inhibitory effect of Fraser Broth has been reported in relation to this species of *Listeria* as it can be susceptible to the antibiotics and selective agents utilized in this selective enrichment media. Despite the failure to recover any colonies from sub-culture of Fraser Broth, colonies of typical *Listeria* were obtained from the plating of the 24 LEB Broth enrichment onto *Brilliance* Listeria Agar, which were then demonstrated to be oxidase negative, catalase positive, Gram-positive short rods causing hemolysis when grown next to *Rhodococcus equi* in the CAMP test and which fermented xylose (but not rhamnose). These results are indicative for *Listeria ivanovii* (which had been spiked into the food matrix). Although not validated as an AOAC Official Method of Analysis, the Microbact 12L biochemical test kit, which is part of the SureTect Listeria Assay confirmation workflow, additionally confirmed that the presumptive positive colonies isolated on *Brilliance* Listeria Agar, were in fact colonies of *L. ivanovii* with 99.99% certainty.

With the exception of the high spiking level for raw ground pork and the low spiking level for pasteurized brie, the remaining matrices showed no statistical difference in performance between the confirmed results for the SureTect Listeria species PCR assay and the ISO reference method results (Table 4). For the high spiked level of ground pork, where all five of the samples were correctly confirmed as being positive, and the low spiked samples of brie, where eleven of the twenty samples were correctly confirmed as being positive for *Listeria*, compared to only two of the five samples for pork and four of the twenty samples for brie cheese with the reference method, the SureTect method was demonstrated to be statistically significantly better than the ISO reference method at detecting *Listeria*.

The results from the independent laboratory study separately demonstrated that there were no statistical differences between the presumptive and confirmed results obtained with the SureTect PCR assay and either of the two confirmation procedures. In addition, there were no statistical differences in the confirmed SureTect method results and those obtained from the ISO reference method, despite the small difference in actual confirmed results between these two methods, which would be expected during an unpaired study when samples of a heterogeneous nature are carried through different enrichment protocols.

Table 1. SureTect Listeria species Assay Presumptive vs. SureTect Confirmation Procedure (Microbact) Confirmed Result-POD Analysis (8)

Matrix	Strain	MPN[a]/test portion	N[b]	SureTect Method Presumptive			SureTect Confirmation Method Result			$dPOD_{cp}$[f]	95% CI[g]
				X[c]	POD_{cp}[d]	95% CI	X	POD_{cc}[e]	95% CI		
Ice cream	*L. ivanovii* TCC 1182	N/A[h]	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.37 (0.18, 0.65)	20	6	0.30	(0.15, 0.52)	7	0.35	(0.35, 0.57)	-0.05	(-0.32, 0.23)
		1.35 (0.61, 2.98)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Raw ground pork	*L. monocytogenes* 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)
Bagged lettuce	*L. ivanovii* TCC 1572	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.13 (0.717, 1.81)	20	13	0.65	(0.43, 0.82)	15	0.75	(0.53, 0.89)	-0.10	(-0.36, 0.18)

		4.73 (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)
Bagged lettuce[i]	*L. ivanovii* TCC 1572	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.43 (0.28, 0.74)	20	7	0.35	(0.18, 0.57)	8	0.40	(0.22, 0.61)	-0.05	(-0.32, 0.23)
		4.38 (1.72, 11.15)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Raw ground turkey	*L. monocytogenes* TCC 1227	N/A[h]	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	8	0.40	(0.22, 0.61)	10	0.50	(0.30, 0.70)	-0.10	(-0.37, 0.19)
		0.95 (0.47, 1.91)	5	3	0.60	(0.23, 0.88)	4	0.80	(0.38, 1.00)	-0.20	(-0.62, 0.31)
Raw ground turkey[i]	*L. monocytogenes* TCC 1227	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		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 (1.72, 11.15)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Raw pork sausage	*L. monocytogenes* 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	*L. monocytogenes* 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)
Pasteurized brie cheese	*L. seeligeri* TCC 2190	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.13 (0.02, 0.26)	20	11	0.55	(0.34, 0.74)	14	0.70	(0.48, 0.85)	-0.15	(-0.41, 0.14)
		0.33 (0.14, 0.75)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Pasteurized 2% fat milk	*L. innocua* TCC 1180	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.50 (0.90, 2.56)	20	10	0.50	(0.30, 0.70)	12	0.60	(0.39, 0.78)	-0.10	(-0.37, 0.19)
		1.88 (0.84, 4.18)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Pasteurized 2% fat milk[i]	*L. innocua* TCC 1180	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.23 (0.77, 2.00)	20	12	0.60	(0.39, 0.78)	12	0.60	(0.39, 0.78)	0.00	(-0.28, 0.28)
		1.64 (0.79, 3.39)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Raw cod[i]	*L. monocytogenes* ATCC 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)
Pasteurized brie cheese[j]	*L. seeligeri* ATCC 35967	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.54 (0.32, 0.81)	20	5	0.25	(0.11, 0.46)	5	0.25	(0.11, 0.46)	0.00	(-0.25, 0.25)
		2.97 (1.25, 7.01)	5	5	1.00	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0.00	(-0.43, 0.43)
Bagged lettuce[j]	*L. monocytogenes* (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)

[a]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.
[b]N = Number of test portions.
[c]X = Number of positive test portions.
[d]POD_{cp} = Candidate method presumptive positive outcomes divided by the total number of portions.
[e]POD_{cc} = Candidate confirmation method positive outcomes divided by the total number of portions.
[f]$dPOD_{cp}$ = Difference between the candidate presumptive result and the candidate method confirmed result POD values.
[g]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[h]N/A = Not applicable.
[i]Repeat analysis of matrix.
[j]Independent Laboratory Study.

Table 2. SureTect Listeria species Assay Presumptive Result vs. Reference Confirmation Procedure Confirmed-POD Analysis (8)

Matrix	Strain	MPN[a]/test portion	N[b]	SureTect Presumptive Result			Reference Confirmation Method			$dPOD_{cp}$[f]	95% CI[g]
				X[c]	POD_{cp}[d]	95% CI	X	POD_{cc2}[e]	95% CI		
Ice cream	*L. ivanovii* TCC 1182	N/A[h]	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.37 (0.18, 0.65)	20	6	0.30	(0.15, 0.52)	6	0.30	(0.15, 0.52)	0.00	(-0.27, 0.27)
		1.35 (0.61, 2.98)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Raw ground pork	*L. monocytogenes* 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)
Bagged lettuce	*L. ivanovii* TCC 1572	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.13 (0.717, 1.81)	20	13	0.65	(0.43, 0.82)	16	0.80	(0.58, 0.92)	-0.15	(-0.40, 0.12)
		4.73 (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)
Bagged lettuce[i]	*L. ivanovii* TCC 1572	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.43 (0.28, 0.74)	20	7	0.35	(0.18, 0.57)	0	0.00	(0.00, 0.16)	0.35	(0.12, 0.57)
		4.38 (1.72, 11.15)	5	5	1.00	(0.57, 1.00)	2	0.40	(0.12, 0.77)	0.60	(0.03, 0.88)
Raw ground turkey	*L. monocytogenes* TCC 1227	N/A[h]	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	8	0.40	(0.22, 0.61)	10	0.50	(0.30, 0.70)	-0.10	(-0.37, 0.19)
		0.95 (0.47, 1.91)	5	3	0.60	(0.23, 0.88)	4	0.80	(0.38, 1.00)	-0.20	(-0.62, 0.31)

Raw ground turkey[i]	*L. monocytogenes* TCC 1227	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		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 (1.72, 11.15)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Raw pork sausage	*L. monocytogenes* 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	*L. monocytogenes* 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)
Pasteurized brie cheese	*L. seeligeri* TCC 2190	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.13 (0.02, 0.26)	20	11	0.55	(0.34, 0.74)	15	0.75	(0.53, 0.89)	-0.20	(-0.45, 0.09)
		0.33 (0.14, 0.75)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00	0.00	(-0.47, 0.47)
Pasteurized 2% milk	*L. innocua* TCC 1180	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.50 (0.90, 2.56)	20	10	0.50	(0.30, 0.70)	13	0.65	(0.43, 0.82)	-0.15	(-0.41, 0.15)
		1.88 (0.84, 4.18)	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]	*L. innocua* TCC 1180	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.23 (0.77, 2.00)	20	12	0.60	(0.39, 0.78)	12	0.60	(0.39, 0.78)	0.00	(-0.28, 0.28)
		1.64 (0.79, 3.39)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Raw cod[j]	*L. monocytogenes* ATCC 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)
Pasteurized brie cheese[j]	*L. seeligeri* ATCC 35967	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.54 (0.32, 0.81)	20	5	0.25	(0.11, 0.46)	5	0.25	(0.11, 0.46)	0.00	(-0.25, 0.25)
		2.97 (1.25, 7.01)	5	5	1.00	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0.00	(-0.43, 0.43)
Bagged lettuce[j]	*L. monocytogenes* (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)

[a]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.
[b]N = Number of test portions.
[c]X = Number of positive test portions.
[d]POD_{cp} = Candidate method presumptive positive outcomes divided by the total number of portions.
[e]POD_{cc2} = Reference method confirmation positive outcomes divided by the total number of portions.
[f]$dPOD_{cp}$ = Difference between the candidate presumptive result and the candidate method confirmed result POD values.
[g]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[h]N/A = Not applicable.
[i]Repeat analysis of matrix.
[j]Independent Laboratory Study

Table 3. SureTect Listeria species Assay Confirmation Procedure (Microbact) vs. Reference Confirmation-POD Analysis (8)

Matrix	Strain	MPN[a]/test portion	N[b]	SureTect Confirmation Method			Reference Confirmation Method			dPOD$_c$[f]	95% CI[g]
				X[c]	POD$_{cc}$[d]	95% CI	X	POD$_{cc2}$[e]	95% CI		
Ice cream	*L. ivanovii* TCC 1182	N/A[h]	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.37 (0.18, 0.65)	20	7	0.35	(0.18, 0.57)	6	0.30	(0.15, 0.52)	0.05	(-0.23, 0.32)
		1.35 (0.61, 2.98)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Raw ground pork	*L. monocytogenes* 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)
Bagged lettuce	*L. ivanovii* TCC 1572	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.13 (0.717, 1.81)	20	15	0.75	(0.53, 0.89)	16	0.80	(0.58, 0.92)	-0.05	(-0.30, 0.21)
		4.73 (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[i]	*L. ivanovii* TCC 1572	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.43 (0.28, 0.74)	20	8	0.40	(0.22, 0.61)	0	0.00	(0.00, 0.16)	0.40	(0.16, 0.61)
		4.38 (1.72, 11.15)	5	5	1.00	(0.57, 1.00)	2	0.40	(0.12, 0.77)	0.60	(0.03, 0.88)
Raw ground turkey	*L. monocytogenes* TCC 1227	N/A[h]	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	(-0.28, 0.28)
		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 turkey[i]	*L. monocytogenes* TCC 1227	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		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 (1.72, 11.15)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Raw pork sausage	*L. monocytogenes* 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	*L. monocytogenes* 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)

Pasteurized brie cheese	*L. seeligeri* TCC 2190	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.13 (0.02, 0.26)	20	14	0.70	(0.48, 0.85)	15	0.75	(0.53, 0.89)	-0.05	(-0.31, 0.22)
		0.33 (0.14, 0.75)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Pasteurized 2% fat milk	*L. innocua* TCC 1180	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.50 (0.90, 2.56)	20	12	0.60	(0.39, 0.78)	13	0.65	(0.43, 0.82)	-0.05	(-0.32, 0.23)
		1.88 (0.84, 4.18)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Pasteurized 2% fat milk[i]	*L. innocua* TCC 1180	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.23 (0.77, 2.00)	20	12	0.60	(0.39, 0.78)	12	0.60	(0.39, 0.78)	0.00	(-0.28, 0.28)
		1.64 (0.79, 3.39)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Raw cod[j]	*L. monocytogenes* ATCC 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	5	(0.56, 1.00)	5	5	(0.56, 1.00)	0.00	(-0.43, 0.43)
Pasteurized brie cheese[j]	*L. seeligeri* ATCC 35967	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.54 (0.32, 0.81)	20	5	0.25	(0.11, 0.46)	5	0.25	(0.11, 0.46)	0.00	(-0.25, 0.25)
		2.97 (1.25, 7.01)	5	5	1.00	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0.00	(-0.43, 0.43)
Bagged lettuce[j]	*L. monocytogenes* (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)

[a]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.
[b]N = Number of test portions.
[c]X = Number of positive test portions.
[d]POD_{cc} = Candidate method confirmed positive outcomes divided by the total number of portions.
[e]POD_{cc2} = Reference confirmation method positive outcomes divided by the total number of portions.
[f]$dPOD_{cp}$ = Difference between the candidate presumptive result and the candidate method confirmed result POD values.
[g]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[h]N/A = Not applicable.
[i]Repeat analysis of matrix.
[j]Independent Laboratory Study.

Table 4. SureTect Listeria species Assay Method Confirmed Result vs. Reference Method-POD Analysis (8)

Matrix	Strain	MPN[a]/test portion	N[b]	SureTect Method			Reference Method			dPOD$_c$[f]	95% CI[g]
				X[c]	POD$_c$[d]	95% CI	X	POD$_r$[e]	95% CI		
Ice cream	*L. ivanovii* TCC 1182	N/A[h]	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.37 (0.18, 0.65)	20	6	0.30	(0.15, 0.52)	8	0.40	(0.22, 0.61)	-0.10	(-0.36, 0.18)
		1.35 (0.61, 2.98)	5	4	0.80	(0.38, 1.00)	3	0.60	(0.23, 0.88)	0.20	(-0.31, 0.62)
Raw ground pork	*L. monocytogenes* 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)
Bagged lettuce	*L. ivanovii* TCC 1572	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.13 (0.717, 1.81)	20	13	0.65	(0.43, 0.82)	14	0.70	(0.48, 0.85)	-0.05	(-0.32, 0.23)
		4.73 (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)
Bagged lettuce[i]	*L. ivanovii* TCC 1572	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.43 (0.28, 0.74)	20	8	0.40	(0.22, 0.61)	7	0.35	(0.18, 0.57)	0.05	(-0.23, 0.32)
		4.38 (1.72, 11.15)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Raw ground turkey	*L. monocytogenes* TCC 1227	N/A[h]	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	8	0.40	(0.22, 0.61)	10	0.50	(0.30, 0.70)	-0.10	(-0.37, 0.19)
		0.95 (0.47, 1.91)	5	3	0.60	(0.23, 0.88)	5	1.00	(0.57, 1.00)	-0.40	(-0.77, 0.12)
Raw ground turkey[i]	*L. monocytogenes* TCC 1227	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.69 (0.39, 1.14)	20	10	0.50	(0.30, 0.70)	9	0.45	(0.26, 0.66)	0.05	(-0.24, 0.33)
		4.38 (1.72, 11.15)	5	4	0.80	(0.38, 1.00)	5	1.00	(0.38, 1.00)	-0.20	(-0.20, 0.28)
Raw pork sausage	*L. monocytogenes* 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	*L. monocytogenes* 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)
	L. seeligeri TCC 2190	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)

Pasteurized brie cheese		0.13 (0.02, 0.26)	20	11	0.50	(0.34, 0.74)	4	0.20	(0.08, 0.42)	0.35	(0.05, 0.58)
		0.33 (0.14, 0.75)	5	4	0.80	(0.38, 1.00)	3	0.60	(0.23, 0.88)	0.20	(-0.31, 0.62)
Pasteurized 2% fat milk	*L. innocua* TCC 1180	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.50 (0.90, 2.56)	20	10	0.50	(0.30, 0.70)	15	0.75	(0.53, 0.89)	-0.25	(-0.49, 0.05)
		1.88 (0.84, 4.18)	5	5	1.00	(0.57, 1.00)	4	0.80	(0.38, 1.00)	0.20	(-0.28, 0.62)
Pasteurized 2% fat milk[i]	*L. innocua* TCC 1180	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.23 (0.77, 2.00)	20	12	0.60	(0.39, 0.78)	12	0.60	(0.39, 0.78)	0.00	(-0.28, 0.28)
		1.64 (0.79, 3.39)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.0)	0.00	(-0.47, 0.47)
Raw cod[j]	*L. monocytogenes* ATCC 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)	6	0.30	(0.14, 0.51)	0.15	(-0.14, 0.40)
		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)
Pasteurized brie[j]	*L. seeligeri* ATCC 35967	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.54 (0.32, 0.81)	20	5	0.25	(0.11, 0.46)	7	0.35	(0.18, 0.56)	-0.10	(-0.35, 0.17)
		2.97 (1.25, 7.01)	5	5	1.00	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0.00	(-0.43, 0.43)
Fresh bagged lettuce[j]	*L. monocytogenes* (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)

[a]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.
[b]N = Number of test portions.
[c]X = Number of positive test portions.
[d]POD_c = Confirmed candidate method positive outcomes divided by the total number of portions.
[e]POD_r = Confirmed reference method positive outcomes divided by the total number of portions..
[f]$dPOD_c$ = Difference between the candidate presumptive result and the candidate method confirmed result POD values
[g]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[h]N/A = Not applicable.
[i]Repeat analysis of matrix.
[j]Independent Laboratory Study.

DISCUSSION OF MODIFICATION APPROVED NOVEMBER 2015 (9):

The results for all four of the matrices analyzed during this method modification study undertaken to validate the analysis of the SureTect Listeria species PCR kit with the Applied Biosystems 7500 Fast PCR Instrument and Applied Biosystems RapidFinder Express 2.0 Software as alternative parts of the SureTect Assay workflow in comparison to the ISO reference method are summarized in Tables 3 to 6.

For the three food matrices and stainless steel surface analyzed, the SureTect method returned results which were not statistically different when analyzed using the POD statistical test compared to the ISO reference method. Although the results for the raw ground turkey and bagged lettuce, as well as the stainless steel surface did not show any statistically different results by POD analysis, one of the low spiked samples for each of ground turkey meat, bagged lettuce as well as one of the low spiked samples analyzed for the stainless steel surface gave negative results with the SureTect PCR method whereas, the SureTect confirmation method (Table 3) and reference method confirmation procedure (table 4) gave positive results. For the presumptive PCR negative samples confirmed as positive for both raw ground turkey and lettuce, plate counts made to estimate the contamination level by plating directly plating the 24 LEB enrichment, estimated the contamination level to be approximately $6x10^2$ and $1x10^2$ CFU/mL for these raw turkey and lettuce samples that gave a negative PCR result and positive culture confirmation result respectively. Levels of contamination at 10^2 CFU/mL are below the level of detection of the PCR assay and the presumptively negative PCR results seen in this study were most likely due to insufficient mixing of the bulk food samples, resulting in some of the low spiked samples receiving an inadequate number of the spiked in target cells in these 25 g portions dispensed for analysis. Plating methods, as used for the SureTect confirmation method and the secondary enrichment step, and subsequent plating in the ISO reference method have very good sensitivity, and are able in theory to detect as little as one cell present within the food matrix.

For the pasteurized milk samples analyzed during this study, no presumptively negative, culture positive results were recorded with the SureTect PCR assay and presumptive and confirmed results were both in agreement for 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, no difference was observed by POD statistical analysis between the SureTect PCR method and the ISO reference method.

The SureTect Listeria species PCR detection method is a very simple and reliable method, able to give next day results following a simple and selective enrichment procedure using 24 LEB (supplemented with both selective and buffered supplements). The prefilled lysis reagent tubes and the use of lyophilized PCR reagents combined with the automated interpretation of results as positive or negative, all combine to reduce a users “hands on” time which are key factors of importance in busy food microbiology laboratories.

Table 7: Inclusivity of the SureTect Listeria species assay (9)

Isolate	Serotype	TCC[a]	Source	Result
Listeria monocytogenes	1/2a	860	Poultry	Positive
Listeria monocytogenes	1/2a	1215	Chorizo sausage	Positive
Listeria monocytogenes	1/2a	1216	Sandwich	Positive
Listeria monocytogenes	1/2a	1217	Carrow cheese	Positive
Listeria monocytogenes	1/2a	1218	Butter	Positive
Listeria monocytogenes	1/2a	1219	Pilau rice	Positive
Listeria monocytogenes	1/2a	1220	Sandwich	Positive
Listeria monocytogenes	1/2b	1205	Cake	Positive
Listeria monocytogenes	1/2b	1206	Whipped Cream	Positive
Listeria monocytogenes	1/2b	1207	Cheese	Positive
Listeria monocytogenes	1/2b	1208	Cheese	Positive
Listeria monocytogenes	1/2b	1209	Cream	Positive
Listeria monocytogenes	1/2b	1210	Cheese	Positive
Listeria monocytogenes	1/2c	858	Clinical sample	Positive
Listeria monocytogenes	1/2c	1195	Ox tongue	Positive
Listeria monocytogenes	1/2c	1196	Roast beef	Positive
Listeria monocytogenes	1/2c	1197	Topside beef	Positive
Listeria monocytogenes	1/2c	1198	Wiltshire ham	Positive
Listeria monocytogenes	1/2c	1199	Ham sandwich	Positive
Listeria monocytogenes	3a	812	Environmental	Positive
Listeria monocytogenes	3a	813	Environmental	Positive
Listeria monocytogenes	3a	840	Butter	Positive
Listeria monocytogenes	3a	870	Clinical sample	Positive
Listeria monocytogenes	3a	888	Food	Positive
Listeria monocytogenes	3a	889	Food	Positive
Listeria monocytogenes	3b	2179	Unknown	Positive
Listeria monocytogenes	3c	2180	Unknown	Positive
Listeria monocytogenes	4a	2181	Unknown	Positive
Listeria monocytogenes	4b	864	Meningitis	Positive
Listeria monocytogenes	4b	865	CSF: Meningitis	Positive
Listeria monocytogenes	4b	1224	Food- blood	Positive
Listeria monocytogenes	4b	1225	Chicken	Positive
Listeria monocytogenes	4b	1226	Dressed crab	Positive
Listeria monocytogenes	4b	1227	Turkey breast	Positive
Listeria monocytogenes	4c	2183	Bird: heart disease	Positive
Listeria monocytogenes	4d	863	Sheep	Positive
Listeria monocytogenes	4e	868	Chicken	Positive
Listeria monocytogenes	4e	883	Veterinary sample	Positive
Listeria grayi		1172	Environmental	Positive
Listeria grayi		1173	Butter	Positive
Listeria grayi		1174	Butter	Positive
Listeria grayi		1175	Butter	Positive

Listeria grayi		1176	Food	Positive
Listeria innocua	Unknown	1177	Chicken sandwich	Positive
Listeria innocua	Unknown	1178	Cooked chicken	Positive
Listeria innocua	Unknown	1179	Crayfish	Positive
Listeria innocua	Unknown	1180	Coleslaw	Positive
Listeria innocua	Unknown	1181	Tuna mayo sandwich	Positive
Listeria innocua	6a	862	Cow brain ATCC® 33090™	Positive
Listeria innocua	4ab	2185		Positive
Listeria innocua	6b	2187		Positive
Listeria ivanovii	Unknown	1182	Lamb (vet sample)	Positive
Listeria ivanovii	Unknown	1183	Food	Positive
Listeria ivanovii	Unknown	1184	Food	Positive
Listeria welshimeri	Unknown	1185	Chicken sandwich	Positive
Listeria welshimeri	Unknown	1186	Food	Positive
Listeria welshimeri	Unknown	1187	Environmental	Positive
Listeria welshimeri	Unknown	1188	Pastrami	Positive
Listeria welshimeri	Unknown	1189	Food	Positive
Listeria welshimeri	6b	2188		Positive
Listeria welshimeri	4c	2189		Positive
Listeria seeligeri	Unknown	1190	Cheese	Positive
Listeria seeligeri	Unknown	1191	Food	Positive
Listeria seeligeri	Unknown	1192	Environmental	Positive
Listeria seeligeri	Unknown	1193	Cannelloni	Positive
Listeria seeligeri	Unknown	1194	Coleslaw	Positive
Listeria seeligeri	1/2b	2190		Positive
Listeria seeligeri	6b	2191		Positive

[a]Trials Culture Collection Number - Proprietary to Thermo Fisher Scientific, Microbiology Division, Basingstoke, UK.

Table 8: Exclusivity of the SureTect Listeria species assay (9)

Isolate	TCC[a]	Source	Result
Bacillus circulans	2303		Negative
Enterococcus faecium	598		Negative
Enterococcus faecalis	567		Negative
Leuconostoc mesenteroides subsp. *mesenteroides*	853		Negative
Pseudomonas aeruginosa	2354	Minced beef	Negative
Staphylococcus lentus	2301	Prawns	Negative
Staphylococcus schleiferi	2302	Salmon	Negative
Candida parapsilosis	1828		Negative
Lactobacillus brevis	848		Negative
Lactococcus acidophilus	2359	ATCC 4356	Negative
Bacillus mycoides	2300	Milk	Negative
Brochothrix thermosphacta	2192	Pork Sausage	Negative
Carnobacterium divergens	2257		Negative
Carnobacterium gallinarum	2259		Negative
Carnobacterium pisicola	2260	Ham	Negative
Citrobacter freundii	1911		Negative
Enterobacter aerogenes	2200		Negative
Erysipelothrix rhusiopathiae	2262		Negative
Escherichia fergusonii	2263	Sausage	Negative
Escherichia coli	1809		Negative
Klebsiella pneumoniae	1892		Negative
Kurthia gibsonii	2193	Pork sausage	Negative
Lactobacillus casei subsp. *casei*	2194	Tomato catsup	Negative
Lactobacillus delbrueckii subsp. *lactis*	2195	Emmenthal cheese production	Negative
Lactobacillus plantarum	2196	Red Cheshire cheese production	Negative
Micrococcus luteus	OCC[b] 2352		Negative
Proteus vulgaris	1424		Negative
Propionibacterium freundenreichii	2304	Swiss cheese production	Negative
Rhodococcus equi	2358	Animal isolate	Negative
Salmonella enterica subsp.Typhimurium	1913	Bovine liver	Negative
Staphylococcus aureus	2240	Food	Negative
Streptococcus salivarius	2352		Negative
Bacillus cereus	2299	Cream	Negative

[a]Trials Culture Collection - Proprietary to Thermo Fisher Scientific, Microbiology Division, Basingstoke, UK.

[b]Oxoid Culture Collection - Proprietary to Thermo Fisher Scientific, Microbiology Division, Basingstoke, UK.

Table 9. Thermo Scientific SureTect Listeria monocytogenes PCR Assay Presumptive vs. Candidate Confirmation Method Result-POD Analysis (9)

Matrix	Strain	MPN[a]/test portion	N[b]	SureTect Method Presumptive (CP)			SureTect Method Confirmation (CC)			dPOD$_{CP}$[f]	95% CI[g]
				X[c]	POD$_{CP}$[d]	95% CI	X	POD$_{CC}$[e]	95% CI		
Raw ground turkey	*Listeria monocytogenes* TCC 1227	N/A[h]	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	*Listeria innocua* TCC 1180	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.45, 0.45)
		1.23 (0.776, 2.00)	20	12	0.60	(0.39, 0.78)	12	0.60	(0.39, 0.78)	0.00	(-0.28, 0.28)
		1.64 (0.79, 3.39)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Bagged lettuce	*Listeria ivanovii* TCC 1572	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.45, 0.45)
		0.43 (0.08, 0.74)	20	7	0.35	(0.18, 0.57)	8	0.40	(0.22, 0.61)	-0.05	(-0.32, 0.23)
		4.38 (1.72, 11.12)	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"	*Listeria monocytogenes* TCC 813 and *Enterococcus faecalis* 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)

[a]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.
[b]N=Number of test portions.
[c]X=Number of positive test portions.
[d]POD$_{CP}$=Candidate method presumptive positive outcomes divided by the total number of portions.
[e]POD$_{CC}$=Candidate confirmation method positive outcomes divided by the total number of portions.
[f]dPOD$_{CP}$=Difference between the candidate presumptive result and the candidate method confirmed result POD values.
[g]95% CI=If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[h]N/A=Not applicable.

Table 10. Thermo Scientific SureTect Listeria monocytogenes PCR Assay Presumptive vs. Reference Confirmation Result-POD Analysis (9)

Matrix	Strain	MPN[a]/test portion	N[b]	SureTect PCR Presumptive Result			Reference Confirmation Method (RC)			dPOD$_{CP}$[f]	95% CI[g]
				X[c]	POD$_{CP}$[d]	95% CI	X	POD$_{RC}$[e]	95% CI		
Raw ground turkey	*Listeria monocytogenes* TCC 1227	N/A[h]	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	*Listeria innocua* TCC 1180	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.45, 0.45)
		1.23 (0.776, 2.00)	20	12	0.60	(0.39, 0.78)	12	0.60	(0.39, 0.78)	0.00	(-0.28, 0.28)
		1.64 (0.79, 3.39)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Bagged lettuce	*Listeria ivanovii* TCC 1572	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.45, 0.45)
		0.43 (0.08, 0.74)	20	7	0.35	(0.18, 0.57)	8	0.40	(0.22, 0.61)	-0.05	(-0.32, 0.23)
		4.38 (1.72, 11.12)	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”	*Listeria monocytogenes* TCC 813 and *Enterococcus faecalis* 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)

[a]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.
[b]N=Number of test portions.
[c]X=Number of positive test portions.
[d]POD$_{CP}$=Candidate method presumptive positive outcomes divided by the total number of portions.
[e]POD$_{RC}$=Reference confirmation, positive outcomes divided by the total number of portions.
[f]dPOD$_{CP}$=Difference between the candidate presumptive result and the reference confirmation method confirmed result POD values.
[g]95% CI=If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[h]N/A=Not applicable.

Table 11. Thermo Scientific SureTect Listeria monocytogenes PCR Assay Confirmation Method vs. Reference Method Confirmed Result-POD Analysis (9)

Matrix	Strain	MPN[a]/test portion	N[b]	SureTect Method Confirmed (CC)			Reference Confirmation (RC)			dPOD$_{CC}$[f]	95% CI[g]
				X[c]	POD$_{CC}$[d]	95% CI	X	POD$_{RC}$[e]	95% CI		
Raw ground turkey	*Listeria monocytogenes* TCC 1227	N/A[h]	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	*Listeria innocua* TCC 1180	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.45, 0.45)
		1.23 (0.776, 2.00)	20	12	0.60	(0.39, 0.78)	12	0.60	(0.39, 0.78)	0.00	(-0.28, 0.28)
		1.64 (0.79, 3.39)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Bagged lettuce	*Listeria ivanovii* TCC 1572	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.45, 0.45)
		0.43 (0.08, 0.74)	20	8	0.40	(0.22, 0.61)	8	0.40	(0.22, 0.61)	0.00	(-0.28, 0.28)
		4.38 (1.72, 11.12)	5	5	1.00	(0.57,1.00)	5	1.00	(0.57,1.00)	0.00	(-0.47, 0.47)
Stainless steel surface 4" x 4"	*Listeria monocytogenes* TCC 813 and *Enterococcus faecalis* 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)

[a]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.
[b]N=Number of test portions.
[c]X=Number of positive test portions.
[d]POD$_{CC}$=Candidate method confirmed positive outcomes divided by the total number of portions.
[e]POD$_{RC}$=Reference confirmation, positive outcomes divided by the total number of portions.
[f]dPOD$_{CC}$=Difference between the candidate confirmed result and the reference confirmation result POD values.
[g]95% CI=If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[h]N/A=Not applicable.

Table 6. Thermo Scientific SureTect Listeria monocytogenes PCR Assay Confirmed Result vs. Reference Method Result-POD Analysis (9)

Matrix	Strain	MPN[a]/test portion	N[b]	SureTect Method Confirmed (C)			Reference Method (R)			dPODC[f]	95% CI[g]
				X[c]	POD_C[d]	95% CI	X	POD_R[e]	95% CI		
Raw ground turkey	*Listeria monocytogenes* TCC 1227	N/A[h]	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	*Listeria innocua* TCC 1180	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.45, 0.45)
		1.23 (0.776, 2.00)	20	12	0.60	(0.39, 0.78)	12	0.60	(0.39, 0.78)	0.00	(-0.28, 0.28)
		1.64 (0.79, 3.39)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Bagged lettuce	*Listeria ivanovii* TCC 1572	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.45, 0.45)
		0.43 (0.08, 0.74)	20	7	0.35	(0.18, 0.57)	7	0.35	(0.18, 0.57)	0.00	(-0.28, 0.28)
		4.38 (1.72, 11.12)	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”	*Listeria monocytogenes* TCC 813 and *Enterococcus faecalis* 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)

[a]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.
[b]N=Number of test portions.
[c]X=Number of positive test portions.
[d]POD_c=Candidate method confirmed positive outcomes divided by the total number of portions.
[e]POD_R=Reference method confirmed positive outcomes divided by the total number of portions.
[f]$dPOD_C$=Difference between the candidate confirmed result and the reference method confirmed result POD values.
[g]95% CI=If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[h]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 2. Inclusivity of the SureTect Listeria species PCR Assay (11)

ID	Listeria species	Serotype	Source	Origin	SureTect Listeria species result
812	*Listeria monocytogenes*	3a	Environmental	TCC	Positive
813	*Listeria monocytogenes*	3a	Environmental	TCC	Positive
840	*Listeria monocytogenes*	3a	Butter	TCC	Positive
858	*Listeria monocytogenes*	1/2c	Clinical sample	TCC	Positive
860	*Listeria monocytogenes*	1/2a	Poultry	TCC	Positive
862	*Listeria. innocua*	6a	Cow brain ATCC® 33090™	TCC	Positive
863	*Listeria monocytogenes*	4d	Sheep	TCC	Positive
864	*Listeria monocytogenes*	4b	Meningitis	TCC	Positive
865	*Listeria monocytogenes*	4b	CSF: Meningitis	TCC	Positive
868	*Listeria monocytogenes*	4e	Chicken	TCC	Positive
870	*Listeria monocytogenes*	3a	Clinical sample	TCC	Positive
883	*Listeria monocytogenes*	4e	Veterinary sample	TCC	Positive
888	*Listeria monocytogenes*	3a	Food	TCC	Positive
889	*Listeria monocytogenes*	3a	Food	TCC	Positive
1172	*Listeria grayi*		Environmental	TCC	Positive
1173	*Listeria grayi*		Butter	TCC	Positive
1174	*Listeria grayi*		Butter	TCC	Positive
1175	*Listeria grayi*		Butter	TCC	Positive
1176	*Listeria grayi*		Food	TCC	Positive
1177	*Listeria innocua*	Unknown	Chicken sandwich	TCC	Positive
1178	*Listeria innocua*	Unknown	Cooked chicken	TCC	Positive
1179	*Listeria innocua*	Unknown	Crayfish	TCC	Positive
1180	*Listeria innocua*	Unknown	Coleslaw	TCC	Positive

1181	*Listeria innocua*	Unknown	Tuna mayo sandwich	TCC	Positive
1182	*Listeria ivanovii*	Unknown	Lamb (vet sample)	TCC	Positive
1183	*Listeria ivanovii*	Unknown	Food	TCC	Positive
1184	*Listeria ivanovii*	Unknown	Food	TCC	Positive
1185	*Listeria welshimeri*	Unknown	Chicken sandwich	TCC	Positive
1186	*Listeria welshimeri*	Unknown	Food	TCC	Positive
1187	*Listeria welshimeri*	Unknown	Environmental	TCC	Positive
1188	*Listeria welshimeri*	Unknown	Pastrami	TCC	Positive
1189	*Listeria welshimeri*	Unknown	Food	TCC	Positive
1190	*Listeria seeligeri*	Unknown	Cheese	TCC	Positive
1191	*Listeria seeligeri*	Unknown	Food	TCC	Positive
1192	*Listeria seeligeri*	Unknown	Environmental	TCC	Positive
1193	*Listeria seeligeri*	Unknown	Cannelloni	TCC	Positive
1194	*Listeria seeligeri*	Unknown	Coleslaw	TCC	Positive
1195	*Listeria monocytogenes*	1/2c	Ox tongue	TCC	Positive
1196	*Listeria monocytogenes*	1/2c	Roast beef	TCC	Positive
1197	*Listeria monocytogenes*	1/2c	Topside beef	TCC	Positive
1198	*Listeria monocytogenes*	1/2c	Wiltshire ham	TCC	Positive
1199	*Listeria monocytogenes*	1/2c	Ham sandwich	TCC	Positive
1205	*Listeria monocytogenes*	1/2b	Cake	TCC	Positive
1206	*Listeria monocytogenes*	1/2b	Whipped Cream	TCC	Positive
1207	*Listeria monocytogenes*	1/2b	Cheese	TCC	Positive
1208	*Listeria monocytogenes*	1/2b	Cheese	TCC	Positive
1209	*Listeria monocytogenes*	1/2b	Cream	TCC	Positive
1210	*Listeria monocytogenes*	1/2b	Cheese	TCC	Positive
1215	*Listeria monocytogenes*	1/2a	Chorizo sausage	TCC	Positive
1216	*Listeria monocytogenes*	1/2a	Sandwich	TCC	Positive
1217	*Listeria monocytogenes*	1/2a	Carrow cheese	TCC	Positive
1218	*Listeria monocytogenes*	1/2a	Butter	TCC	Positive
1219	*Listeria monocytogenes*	1/2a	Pilau rice	TCC	Positive
1220	*Listeria monocytogenes*	1/2a	Sandwich	TCC	Positive
1224	*Listeria monocytogenes*	4b	Food- blood	TCC	Positive
1225	*Listeria monocytogenes*	4b	Chicken	TCC	Positive
1226	*Listeria monocytogenes*	4b	Dressed crab	TCC	Positive
1227	*Listeria monocytogenes*	4b	Turkey breast	TCC	Positive
2179	*Listeria monocytogenes*	3b	Unknown	TCC	Positive
2180	*Listeria monocytogenes*	3c	Unknown	TCC	Positive
2181	*Listeria monocytogenes*	4a	Unknown	TCC	Positive
2183	*Listeria monocytogenes*	4c	Bird: heart disease	TCC	Positive
2185	*Listeria innocua*	4ab	Unknown	TCC	Positive
2187	*Listeria innocua*	6b	Institut Pasteur	TCC	Positive
2188	*Listeria welshimeri*	6b	Unknown	TCC	Positive
2189	*Listeria welshimeri*	4c	Institut Pasteur	TCC	Positive
2190	*Listeria seeligeri*	1/2b	Unknown	TCC	Positive
2191	*Listeria seeligeri*	6b	Unknown	TCC	Positive

Table 4. Exclusivity of the SureTect Listeria species PCR Assay (11)

ID	Isolate	Source	Origin	Listeria species result
567	*Enterococcus faecalis*	Unknown	TCC	Negative
598	*Enterococcus faecium*	Unknown	TCC	Negative
848	*Lactobacillus brevis*	Unknown	TCC	Negative
853	*Leuconostoc mesenteroides* subsp. mesenteroides	Unknown	TCC	Negative
1424	*Proteus vulgaris*	Unknown	TCC	Negative
1809	*Escherichia coli*	Unknown	TCC	Negative
1828	*Candida parapsilosis*	Unknown	TCC	Negative
1892	*Klebsiella pneumoniae*	Unknown	TCC	Negative
1911	*Salmonella enterica* subsp. enterica Typhimurium	NCTC	TCC	Negative
1913	*Citrobacter freundii*	NCTC	TCC	Negative
2407	*Brochothrix thermosphacta*	Pork Sausage	TCC	Negative
2193	*Kurthia gibsonii*	Pork sausage	TCC	Negative
2194	*Lactobacillus casei* subsp. casei	Tomato catsup	TCC	Negative
2195	*Lactobacillus delbrueckii* subsp. lactis	Emmental cheese	TCC	Negative

		production		
2196	*Lactobacillus plantarum*	Red Cheshire cheese production	TCC	Negative
2200	*Enterobacter aerogenes*	Unknown	TCC	Negative
2240	*Staphylococcus aureus*	Food	TCC	Negative
2257	*Carnobacterium divergens*	Unknown	TCC	Negative
2259	*Carnobacterium gallinarum*	Unknown	TCC	Negative
2260	*Carnobacterium pisicola*	Ham	TCC	Negative
2262	*Erysipelothrix rhusiopathiae*	Unknown	TCC	Negative
2263	*Escherichia fergusonii*	Sausage	TCC	Negative
2299	*Bacillus cereus*	Cream	TCC	Negative
2300	*Bacillus mycoides*	Milk	TCC	Negative
2301	*Staphylococcus lentus*	Prawns	TCC	Negative
2302	*Staphylococcus schleiferi*	Salmon	TCC	Negative
2303	*Bacillus circulans*	Unknown	TCC	Negative
2304	*Propionibacterium freundenreichii*	Swiss cheese production	TCC	Negative
2352	*Streptococcus salivarius*	Unknown	TCC	Negative
2354	*Pseudomonas aeruginosa*	Minced beef	TCC	Negative
2358	*Rhodococcus equi*	Animal isolate	TCC	Negative
2359	*Lactococcus acidophilus*	ATCC 4356	TCC	Negative
2352	*Micrococcus luteus*	Unknown	OCC	Negative

Table 9. SureTect Listeria species PCR Assay Results: candidate presumptive PCR result vs candidate method confirmed (via reference method) (11)

Matrix[a]	Inoculating strain(s)	MPN[b]/ test portion	N[c]	SureTect candidate Presumptive PCR result			SureTect candidate method confirmed via the reference method			dPOD$_{CPr}$[g]	95% CI[h]
				x[d]	POD$_{CPr}$[e]	95% CI	x	POD$_{CR}$[f]	95% CI		
Sliced Deli Turkey	TCC 1180 *L. innocua*	N/A[j]	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.5	20	16	0.80	0.58, 0.92	16	0.80	0.58, 0.92	0.00	-0.25, 0.25
		2.5	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Bagged Lettuce	TCC 1220 *L. monocytogenes*	N/A[j]	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 *L. monocytogenes*	N/A[j]	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.2	20	3	0.15	0.05, 0.36	7	0.35	0.18, 0.57	-0.20	-0.44, 0.07
		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 *L. monocytogenes*/ 10X *E. faecalis*	N/A[j]	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 *L. monocytogenes* 1/2b	N/A[j]	10	0	0.00	0.00, 0.28	0	0.00	0.00, 0.28	0.00	-0.28, 0.28
		N/A	20	10	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	4	0.80	0.38, 1.00	0.00	-0.47, 0.47

[a] Matrix = for the stainless steel surface matrices the data is shown combined for PikoReal, 7500 Fast and QuantStudio 5 PCR instruments
[b] 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
[c] N = Number of test portions
[d] x = Number of positive test portions
[e] PODCPr = Candidate presumptive PCR positive outcomes divided by the total number of trials
[f] PODCR = Candidate method confirmed (via reference method) positive outcomes divided by the total number of trials
[g] dPODCPr = Difference between the candidate presumptive and candidate confirmed (via reference method) results
[h] 95% CI = If the confidence interval (CI) of a dPOD does not contain zero, then the difference is statistically significant at the 5% level
[j] N/A = Not applicable

Table 10. SureTect Listeria species PCR Assay Results: candidate presumptive PCR result vs candidate method confirmed (via candidate method) (11)

Matrix[a]	Inoculating strain(s)	MPN[b]/ test portion	N[c]	SureTect candidate Presumptive PCR result			SureTect candidate method confirmed via the candidate method			$dPOD_{CPc}$[g]	95% CI[h]
				x[d]	POD_{CPc}[e]	95% CI	x	$PODC_R$[f]	95% CI		
Sliced Deli Turkey	TCC 1180 *L. innocua*	N/A[j]	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.5	20	16	0.80	0.58, 0.92	16	0.80	0.58, 0.92	0.00	-0.25, 0.25
		2.5	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Bagged Lettuce	TCC 1220 *L. monocytogenes*	N/A[j]	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 *L. monocytogenes*	N/A[j]	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.2	20	3	0.15	0.05, 0.36	7	0.35	0.18, 0.57	-0.20	-0.44, 0.07
		0.67	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.28, 0.28
Stainless Steel Sponge (4"x 4")	TCC 0813 *L. monocytogenes*/ 10X *E. faecalis*	N/A[j]	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 *L. monocytogenes* 1/2b	N/A[j]	10	0	0.00	0.00, 0.28	0	0.00	0.00, 0.28	0.00	-0.28, 0.28
		N/A	20	10	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	4	0.80	0.38, 1.00	0.00	-0.47, 0.47

[a] Matrix = for the stainless steel surface matrices the data is shown combined for PikoReal, 7500 Fast and QuantStudio 5 PCR instruments
[b] 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
[c] N = Number of test portions
[d] x = Number of positive test portions
[e] PODCPc = Candidate presumptive PCR positive outcomes divided by the total number of trials
[f] PODCR = Candidate method confirmed (via candidate method) positive outcomes divided by the total number of trials
[g] dPODCPc = Difference between the candidate presumptive and candidate confirmed (via candidate method) results
[h] 95% CI = If the confidence interval (CI) of a dPOD does not contain zero, then the difference is statistically significant at the 5% level
[j] N/A = Not applicable

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

Matrix[a]	Inoculating strain(s)	MPN[b]/ test portion	N[c]	SureTect candidate method confirmed via the candidate method result			Reference method result			dPOD$_{CC}$[g]	95% CI[h]
				x[d]	POD$_{CC}$[e]	95% CI	x	POD$_R$[f]	95% CI		
Sliced Deli Turkey	TCC 1180 *L. innocua*	N/A[j]	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.50	20	16	0.80	0.58, 0.92	6	0.30	0.15, 0.52	0.50	0.19, 0.70
		2.50	5	5	1.00	0.57, 1.00	4	0.80	0.38, 1.00	0.20	-0.28, 0.62
Bagged Lettuce	TCC 1220 *L. monocytogenes*	N/A[j]	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 *L. monocytogenes*	N/A[j]	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	7	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 *L. monocytogenes*/ 10X *E. faecalis*	N/A[j]	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 *L. monocytogenes* 1/2b	N/A[j]	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

[a] Matrix = for the stainless steel surface matrices the data is shown combined for PikoReal, 7500 Fast and QuantStudio 5 PCR instruments
[b] 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
[c] N = Number of test portions
[d] x = Number of positive test portions
[e] PODCC = Candidate method confirmed via the candidate method positive outcomes divided by the total number of trials
[f] PODR = Reference method divided by the total number of trials
[g] dPODCC = Difference between the candidate method presumptive result and candidate method confirmed result POD values
[h] 95% CI = If the confidence interval (CI) of a dPOD does not contain zero, then the difference is statistically significant at the 5% level
[j] N/A = Not applicable

Table 12. SureTect Listeria species PCR Assay Results: Candidate method confirmed (via the reference method) vs Reference method POD summary (11)

Matrix[a]	Inoculating strain(s)	MPN[b]/ test portion	N[c]	SureTect candidate method confirmed via the reference method			Reference method result			$dPOD_{CR}$[g]	95% CI[h]
				x[d]	POD_{CR}[e]	95% CI	x	POD_R[f]	95% CI		
Sliced Deli Turkey	TCC 1180 *L. innocua*	N/A[j]	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.50	20	16	0.80	0.58, 0.92	6	0.30	0.15, 0.52	0.50	0.19, 0.70
		2.50	5	5	1.00	0.57, 1.00	4	0.80	0.38, 1.00	0.20	-0.28, 0.62
Bagged Lettuce	TCC 1220 *L. monocytogenes*	N/A[j]	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 *L. monocytogenes*	N/A[j]	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	7	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 *L. monocytogenes*/ 10X *E. faecalis*	N/A[j]	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 *L. monocytogenes* 1/2b	N/A[j]	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

[a] Matrix = for the stainless steel surface matrices the data is shown combined for PikoReal, 7500 Fast and QuantStudio 5 PCR instruments
[b] 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
[c] N = Number of test portions
[d] x = Number of positive test portions
[e] PODCR = Candidate method confirmed (via reference method) positive outcomes divided by the total number of trials
[f] PODR = Reference method positive outcomes divided by the total number of trials
[g] dPODCR = Difference between the candidate method confirmed via the reference method and the reference method
[h] 95% CI = If the confidence interval (CI) of a dPOD does not contain zero, then the difference is statistically significant at the 5% level
[j] N/A = Not applicable

DISCUSSION OF MODIFICATION APPROVED OCTOBER 2020 (13)

SureTect Listeria species PCR assay: Inclusivity/Exclusivity.—The reanalysis of the inclusivity-exclusivity data showed **no change** in the PCR results between RFA 1.0 and RFA 1.1 and the relevant kit files. Tables 17 and 18 detail the isolates used during the Inclusivity and Exclusivity studies.

SureTect Listeria species: 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 27.

Table 17. Inclusivity results for the SureTect Listeria species 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	*Listeria monocytogenes* 3a	Environmental	TCC[a]	Positive	Positive	1192	*Listeria seeligeri*	Environmental	TCC	Positive	Positive
813	*Listeria monocytogenes* 3a	Environmental	TCC	Positive	Positive	1193	*Listeria seeligeri*	Cannelloni	TCC	Positive	Positive
840	*Listeria monocytogenes* 3a	Butter	TCC	Positive	Positive	1194	*Listeria seeligeri*	Coleslaw	TCC	Positive	Positive
858	*Listeria monocytogenes* 1/2c	Clinical sample	TCC	Positive	Positive	1195	*Listeria monocytogenes* 1/2c	Ox tongue	TCC	Positive	Positive
860	*Listeria monocytogenes* 1/2a	Poultry	TCC	Positive	Positive	1196	*Listeria monocytogenes* 1/2c	Roast beef	TCC	Positive	Positive
862	*Listeria innocua* 6a	Cow brain ATCC 33090	TCC	Positive	Positive	1197	*Listeria monocytogenes* 1/2c	Topside beef	TCC	Positive	Positive
863	*Listeria monocytogenes* 4d	Sheep	TCC	Positive	Positive	1198	*Listeria monocytogenes* 1/2c	Wiltshire ham	TCC	Positive	Positive
864	*Listeria monocytogenes* 4b	Meningitis	TCC	Positive	Positive	1199	*Listeria monocytogenes* 1/2c	Ham sandwich	TCC	Positive	Positive
865	*Listeria monocytogenes* 4b	CSF: Meningitis	TCC	Positive	Positive	1205	*Listeria monocytogenes* 1/2b	Cake	TCC	Positive	Positive
868	*Listeria monocytogenes* 4e	Chicken	TCC	Positive	Positive	1206	*Listeria monocytogenes* 1/2b	Whipped Cream	TCC	Positive	Positive
870	*Listeria monocytogenes* 3a	Clinical sample	TCC	Positive	Positive	1207	*Listeria monocytogenes* 1/2b	Cheese	TCC	Positive	Positive
883	*Listeria monocytogenes* 4e	Veterinary sample	TCC	Positive	Positive	1208	*Listeria monocytogenes* 1/2b	Cheese	TCC	Positive	Positive
888	*Listeria monocytogenes* 3a	Food	TCC	Positive	Positive	1209	*Listeria monocytogenes* 1/2b	Cream	TCC	Positive	Positive
889	*Listeria monocytogenes* 3a	Food	TCC	Positive	Positive	1210	*Listeria monocytogenes* 1/2b	Cheese	TCC	Positive	Positive
1172	*Listeria grayi*	Environmental	TCC	Positive	Positive	1215	*Listeria monocytogenes* 1/2a	Chorizo sausage	TCC	Positive	Positive

1173	*Listeria grayi*	Butter	TCC	Positive	Positive	1216	*Listeria monocytogenes* 1/2a	Sandwich	TCC	Positive	Positive
1174	*Listeria grayi*	Butter	TCC	Positive	Positive	1217	*Listeria monocytogenes* 1/2a	Carrow cheese	TCC	Positive	Positive
1175	*Listeria grayi*	Butter	TCC	Positive	Positive	1218	*Listeria monocytogenes* 1/2a	Butter	TCC	Positive	Positive
1176	*Listeria grayi*	Food	TCC	Positive	Positive	1219	*Listeria monocytogenes* 1/2a	Pilau rice	TCC	Positive	Positive
1177	*Listeria innocua*	Chicken sandwich	TCC	Positive	Positive	1220	*Listeria monocytogenes* 1/2a	Sandwich	TCC	Positive	Positive
1178	*Listeria innocua*	Cooked chicken	TCC	Positive	Positive	1224	*Listeria monocytogenes* 4b	Food- blood	TCC	Positive	Positive
1179	*Listeria innocua*	Crayfish	TCC	Positive	Positive	1225	*Listeria monocytogenes* 4b	Chicken	TCC	Positive	Positive
1180	*Listeria innocua*	Coleslaw	TCC	Positive	Positive	1226	*Listeria monocytogenes* 4b	Dressed crab	TCC	Positive	Positive
1181	*Listeria innocua*	Tuna mayo sandwich	TCC	Positive	Positive	1227	*Listeria monocytogenes* 4b	Turkey breast	TCC	Positive	Positive
1182	*Listeria ivanovii*	Lamb (vet sample)	TCC	Positive	Positive	2179	*Listeria monocytogenes* 3b	Unknown	TCC	Positive	Positive
1183	*Listeria ivanovii*	Food	TCC	Positive	Positive	2180	*Listeria monocytogenes* 3c	Unknown	TCC	Positive	Positive
1184	*Listeria ivanovii*	Food	TCC	Positive	Positive	2181	*Listeria monocytogenes* 4a	Unknown	TCC	Positive	Positive
1185	*Listeria welshimeri*	Chicken sandwich	TCC	Positive	Positive	2183	*Listeria monocytogenes* 4c	Bird: heart disease	TCC	Positive	Positive
1186	*Listeria welshimeri*	Food	TCC	Positive	Positive	2185	*Listeria innocua* 4ab	Unknown	TCC	Positive	Positive
1187	*Listeria welshimeri*	Environmental	TCC	Positive	Positive	2187	*Listeria innocua* 6b	Institute Pasteur	TCC	Positive	Positive
1188	*Listeria welshimeri*	Pastrami	TCC	Positive	Positive	2188	*Listeria welshimeri* 6b	Unknown	TCC	Positive	Positive
1189	*Listeria welshimeri*	Food	TCC	Positive	Positive	2189	*Listeria welshimeri* 4c	Institute Pasteur	TCC	Positive	Positive
1190	*Listeria seeligeri*	Cheese	TCC	Positive	Positive	2190	*Listeria seeligeri* 1/2b	Unknown	TCC	Positive	Positive
1191	*Listeria seeligeri*	Food	TCC	Positive	Positive	2191	*Listeria seeligeri* 6b	Unknown	TCC	Positive	Positive

[d]TCC = Trials Culture Collection, Thermo Fisher Scientific, UK.

Table 18. Exclusivity results for the SureTect Listeria species 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
567	*Enterococcus faecalis*	Unknown	TCC[a]	Negative	Negative	2257	*Carnobacterium divergens*	Unknown	TCC	Negative	Negative
598	*Enterococcus faecium*	Unknown	TCC	Negative	Negative	2259	*Carnobacterium gallinarum*	Unknown	TCC	Negative	Negative
848	*Lactobacillus brevis*	Unknown	TCC	Negative	Negative	2260	*Carnobacterium pisicola*	Ham	TCC	Negative	Negative
853	*Leuconostoc mesenteroides* subsp. mesenteroides	Unknown	TCC	Negative	Negative	2262	*Erysipelothrix rhusiopathiae*	Unknown	TCC	Negative	Negative
1424	*Proteus vulgaris*	Unknown	TCC	Negative	Negative	2263	*Escherichia fergusonii*	Sausage	TCC	Negative	Negative
1809	*Escherichia coli*	Unknown	TCC	Negative	Negative	2299	*Bacillus cereus*	Cream	TCC	Negative	Negative
1828	*Candida parapsilosis*	Unknown	TCC	Negative	Negative	2300	*Bacillus mycoides*	Milk	TCC	Negative	Negative
1892	*Klebsiella pneumoniae*	Unknown	TCC	Negative	Negative	2301	*Staphylococcus lentus*	Prawns	TCC	Negative	Negative
1911	*Salmonella* Typhimurium	NCTC	TCC	Negative	Negative	2302	*Staphylococcus schleiferi*	Salmon	TCC	Negative	Negative
1913	*Citrobacter freundii*	NCTC	TCC	Negative	Negative	2303	*Bacillus circulans*	Unknown	TCC	Negative	Negative
2407	*Brochothrix thermosphacta*	Pork Sausage	TCC	Negative	Negative	2304	*Propionibacterium freundenreichii*	Swiss cheese	TCC	Negative	Negative
2193	*Kurthia gibsonii*	Pork sausage	TCC	Negative	Negative	2352	*Streptococcus salivarius*	Unknown	TCC	Negative	Negative
2194	*Lactobacillus casei* subsp. casei	Tomato catsup	TCC	Negative	Negative	2354	*Pseudomonas aeruginosa*	Minced beef	TCC	Negative	Negative
2195	*Lactobacillus delbrueckii* subsp. lactis	Emmentha l cheese	TCC	Negative	Negative	2358	*Rhodococcus equi*	Animal isolate	TCC	Negative	Negative
2196	*Lactobacillus plantarum*	Red Cheshire cheese	TCC	Negative	Negative	2359	*Lactococcus acidophilus*	ATCC 4356	TCC	Negative	Negative
2200	*Enterobacter aerogenes*	Unknown	TCC	Negative	Negative	OCC 2352	*Micrococcus luteus*	Unknown	OCC[b]	Negative	Negative
2240	*Staphylococcus aureus*	Food	TCC	Negative	Negative						

[a]TCC = Trials Culture Collection, Thermo Fisher Scientific, UK.

[b]OCC = Oxoid Culture Collection, Thermo Fisher Scientific, UK.

Table 27. Thermo Scientific SureTect Listeria species PCR Assay result comparison for RFA v1.0 and RFA v1.1 (13)

Matrix	Inoculating Strain(s)	MPN[a]/ Test Portion	N[b]	Candidate Method[c]		
				RFA 1.0 x[d]	RFA 1.0 -!e	RFA 1.1 x[f]
Sliced Deli Turkey	TCC 1180 *L. innocua*	N/A[g]	5	0	N/A	0
		0.5	20	16	N/A	16
		2.5	5	5	N/A	5
Bagged Lettuce	TCC 1220 *L. monocytogenes*	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 *L. monocytogenes*	N/[g]	5	0	N/A	0
		0.2	20	7	N/A	7
		0.7	5	4	N/A	4
Stainless Steel Sponge	TCC 0813 *L. monocytogenes* 10X *E. faecalis*	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 *L. monocytogenes* 1/2b	N/A	10	0	N/A	0
		N/A	20	11	N/A	11
		N/A	5	5	N/A	5

[a]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.
[b]N = Number of test potions, 5 unspiked, 20 low spike, 5 high spike.
[c]Candidate method presumptive result (PCR only).
[d]RFA 1.0 x = Number of positive test portions gained originally with RFA 1.0 and original kit file.
[e]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).
[f]RFA 1.1 x = Number of positive test portions gained originally with RFA 1.0 and original kit file.
[g]N/A = Not applicable.

DISCUSSION OF MODIFICATION APPROVED OCTOBER 2020 (14)

SureTect Listeria species PCR assay: Inclusivity Exclusivity.—The reanalysis of the inclusivity-exclusivity data showed one change for one sample in the inclusivity data that changed from **a positive to a warning** as detailed in Table 33. The reason for this change was a failed amplification of the internal amplification control. Since RFE kit file v2.0 requires a positive result of the internal amplification control (IPC) for a positive sample result, the interpretation for this sample changes to 'Warning' with the new kit file.

SureTect Listeria species: 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 19. Inclusivity results for the SureTect Listeria species 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	*Listeria monocytogenes* 3a	Environmental	TCC[a]	Positive	Positive	1192	*Listeria seeligeri*	Environmental	TCC	Positive	Positive
813	*Listeria monocytogenes* 3a	Environmental	TCC	Positive	Positive	1193	*Listeria seeligeri*	Cannelloni	TCC	Positive	Positive
840	*Listeria monocytogenes* 3a	Butter	TCC	Positive	Positive	1194	*Listeria seeligeri*	Coleslaw	TCC	Positive	Positive
858	*Listeria monocytogenes* 1/2c	Clinical sample	TCC	Positive	Positive	1195	*Listeria monocytogenes* 1/2c	Ox tongue	TCC	Positive	Positive
860	*Listeria monocytogenes* 1/2a	Poultry	TCC	Positive	Positive	1196	*Listeria monocytogenes* 1/2c	Roast beef	TCC	Positive	Positive
862	*Listeria innocua* 6a	Cow brain ATCC 33090	TCC	Positive	Positive	1197	*Listeria monocytogenes* 1/2c	Topside beef	TCC	Positive	Positive
863	*Listeria monocytogenes* 4d	Sheep	TCC	Positive	Positive	1198	*Listeria monocytogenes* 1/2c	Wiltshire ham	TCC	Positive	Positive
864	*Listeria monocytogenes* 4b	Meningitis	TCC	Positive	Positive	1199	*Listeria monocytogenes* 1/2c	Ham sandwich	TCC	Positive	Positive
865	*Listeria monocytogenes* 4b	CSF: Meningitis	TCC	Positive	Positive	1205	*Listeria monocytogenes* 1/2b	Cake	TCC	Positive	Positive
868	*Listeria monocytogenes* 4e	Chicken	TCC	Positive	Positive	1206	*Listeria monocytogenes* 1/2b	Whipped Cream	TCC	Positive	Positive
870	*Listeria monocytogenes* 3a	Clinical sample	TCC	Positive	Positive	1207	*Listeria monocytogenes* 1/2b	Cheese	TCC	Positive	Positive
883	*Listeria monocytogenes* 4e	Veterinary sample	TCC	Positive	Positive	1208	*Listeria monocytogenes* 1/2b	Cheese	TCC	Positive	Positive
888	*Listeria monocytogenes* 3a	Food	TCC	Positive	Positive	1209	*Listeria monocytogenes* 1/2b	Cream	TCC	Positive	Positive
889	*Listeria monocytogenes* 3a	Food	TCC	Positive	Positive	1210	*Listeria monocytogenes* 1/2b	Cheese	TCC	Positive	Positive
1172	*Listeria grayi*	Environmental	TCC	Positive	Positive	1215	*Listeria monocytogenes* 1/2a	Chorizo sausage	TCC	Positive	Positive
1173	*Listeria grayi*	Butter	TCC	Positive	Positive	1216	*Listeria monocytogenes* 1/2a	Sandwich	TCC	Positive	Positive
1174	*Listeria grayi*	Butter	TCC	Positive	Positive	1217	*Listeria monocytogenes* 1/2a	Carrow cheese	TCC	Positive	Positive
1175	*Listeria grayi*	Butter	TCC	Positive	Positive	1218	*Listeria monocytogenes* 1/2a	Butter	TCC	Positive	Positive
1176	*Listeria grayi*	Food	TCC	Positive	Positive	1219	*Listeria monocytogenes* 1/2a	Pilau rice	TCC	Positive	Positive
1177	*Listeria innocua*	Chicken sandwich	TCC	Positive	Positive	1220	*Listeria monocytogenes* 1/2a	Sandwich	TCC	Positive	Positive
1178	*Listeria innocua*	Cooked chicken	TCC	Positive	Positive	1224	*Listeria monocytogenes* 4b	Food- blood	TCC	Positive	Positive
1179	*Listeria innocua*	Crayfish	TCC	Positive	Positive	1225	*Listeria monocytogenes* 4b	Chicken	TCC	Positive	Positive
1180	*Listeria innocua*	Coleslaw	TCC	Positive	Positive	1226	*Listeria monocytogenes* 4b	Dressed crab	TCC	Positive	Positive
1181	*Listeria innocua*	Tuna mayo sandwich	TCC	Positive	Positive	1227	*Listeria monocytogenes* 4b	Turkey breast	TCC	Positive	Positive
1182	*Listeria ivanovii*	Lamb (vet sample)	TCC	Positive	Positive	2179	*Listeria monocytogenes* 3b	Unknown	TCC	Positive	Positive
1183	*Listeria ivanovii*	Food	TCC	Positive	Warning[b]	2180	*Listeria monocytogenes* 3c	Unknown	TCC	Positive	Positive
1184	*Listeria ivanovii*	Food	TCC	Positive	Positive	2181	*Listeria monocytogenes* 4a	Unknown	TCC	Positive	Positive
1185	*Listeria welshimeri*	Chicken sandwich	TCC	Positive	Positive	2183	*Listeria monocytogenes* 4c	Bird: heart disease	TCC	Positive	Positive

1186	*Listeria welshimeri*	Food	TCC	Positive	Positive	2185	*Listeria innocua* 4ab	Unknown	TCC	Positive	Positive
1187	*Listeria welshimeri*	Environmental	TCC	Positive	Positive	2187	*Listeria innocua* 6b	Institute Pasteur	TCC	Positive	Positive
1188	*Listeria welshimeri*	Pastrami	TCC	Positive	Positive	2188	*Listeria welshimeri* 6b	Unknown	TCC	Positive	Positive
1189	*Listeria welshimeri*	Food	TCC	Positive	Positive	2189	*Listeria welshimeri* 4c	Institute Pasteur	TCC	Positive	Positive
1190	*Listeria seeligeri*	Cheese	TCC	Positive	Positive	2190	*Listeria seeligeri* 1/2b	Unknown	TCC	Positive	Positive
1191	*Listeria seeligeri*	Food	TCC	Positive	Positive	2191	*Listeria seeligeri* 6b	Unknown	TCC	Positive	Positive

[a]TCC = Trials Culture Collection, Thermo Fisher Scientific, UK.

[b]Warning result with RFE v2.0.

Table 20. Exclusivity results for the SureTect Listeria species 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
567	*Enterococcus faecalis*	Unknown	TCC[a]	Negative	Negative	2257	*Carnobacterium divergens*	Unknown	TCC	Negative	Negative
598	*Enterococcus faecium*	Unknown	TCC	Negative	Negative	2259	*Carnobacterium gallinarum*	Unknown	TCC	Negative	Negative
848	*Lactobacillus brevis*	Unknown	TCC	Negative	Negative	2260	*Carnobacterium pisicola*	Ham	TCC	Negative	Negative
853	*Leuconostoc mesenteroides* subsp. mesenteroides	Unknown	TCC	Negative	Negative	2262	*Erysipelothrix rhusiopathiae*	Unknown	TCC	Negative	Negative
1424	*Proteus vulgaris*	Unknown	TCC	Negative	Negative	2263	*Escherichia fergusonii*	Sausage	TCC	Negative	Negative
1809	*Escherichia coli*	Unknown	TCC	Negative	Negative	2299	*Bacillus cereus*	Cream	TCC	Negative	Negative
1828	*Candida parapsilosis*	Unknown	TCC	Negative	Negative	2300	*Bacillus mycoides*	Milk	TCC	Negative	Negative
1892	*Klebsiella pneumoniae*	Unknown	TCC	Negative	Negative	2301	*Staphylococcus lentus*	Prawns	TCC	Negative	Negative
1911	*Salmonella* Typhimurium	NCTC	TCC	Negative	Negative	2302	*Staphylococcus schleiferi*	Salmon	TCC	Negative	Negative
1913	*Citrobacter freundii*	NCTC	TCC	Negative	Negative	2303	*Bacillus circulans*	Unknown	TCC	Negative	Negative
2407	*Brochothrix thermosphacta*	Pork Sausage	TCC	Negative	Negative	2304	*Propionibacterium freundenreichii*	Swiss cheese	TCC	Negative	Negative
2193	*Kurthia gibsonii*	Pork sausage	TCC	Negative	Negative	2352	*Streptococcus salivarius*	Unknown	TCC	Negative	Negative
2194	*Lactobacillus casei* subsp. casei	Tomato catsup	TCC	Negative	Negative	2354	*Pseudomonas aeruginosa*	Minced beef	TCC	Negative	Negative
2195	*Lactobacillus delbrueckii* subsp. lactis	Emmenthal cheese	TCC	Negative	Negative	2358	*Rhodococcus equi*	Animal isolate	TCC	Negative	Negative
2196	*Lactobacillus plantarum*	Red Cheshire cheese	TCC	Negative	Negative	2359	*Lactococcus acidophilus*	ATCC[c] 4356	TCC	Negative	Negative
2200	*Enterobacter aerogenes*	Unknown	TCC	Negative	Negative	OCC 2352	*Micrococcus luteus*	Unknown	OCC[b]	Negative	Negative
2240	*Staphylococcus aureus*	Food	TCC	Negative	Negative						

[a]TCC = Trials Culture Collection, Thermo Fisher Scientific, UK.

[b]OCC = Oxoid Culture Collection, Thermo Fisher Scientific, UK.

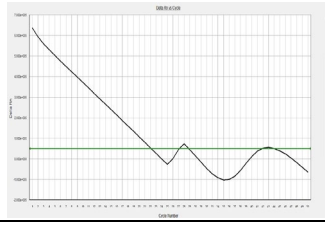
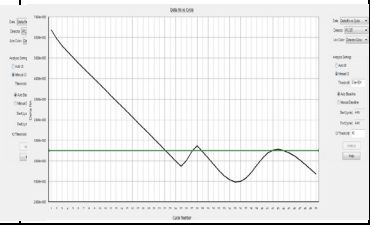
[c]ATCC = American type Culture Collection, Manassas, VA.

Table 29. Thermo Scientific SureTect Listeria species PCR Assay result comparison for the original and upgraded kit files with RFE v2.0 (14)

Matrix	Inoculating Strain(s)	MPN[a]/ Test Portion	N[b]	Candidate Method[c]		
				Kit file 1.0 x[d]	Kit file v1.0 -!e	Kit file v2.0 x[f]
Raw ground turkey	*L. monocytogenes* TCC 1227	N/A[g]	5	0	N/A	0
		0.69	20	9	N/A	9
		4.38	5	4	N/A	4
Pasteurized 2% milk	*L. innocua* TCC 1180	N/A	5	0	N/A	0
		1.23	20	12	N/A	12
		1.65	5	4	N/A	4
Bagged lettuce	*L. ivanovii* OCC 1572	N/A	5	0	N/A	0
		13.00	20	7	N/A	7
		17.90	5	4	N/A	4
Stainless steel surface 4" x 4"	*L. monocytogenes* TCC 0813 & *E. faecalis* CIP100750 X10	N/A	5	0	N/A	0
		N/A	20	6	N/A	6
		N/A	5	5	N/A	5

[a]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.
[b]N = Number of test potions, 5 unspiked, 20 low spike, 5 high spike.
[c]Candidate method presumptive result (PCR only).
[d]RFE 1.0 x = Number of positive test portions gained originally with RFA 1.0 and original kit file.
[e]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).
[f]RFE 2.0 x = Number of positive test portions gained originally with RFA 1.0 and original kit file.
[g]N/A = Not applicable.

Table 33. Thermo Scientific SureTect and RapidFinder PCR range warning call summary for the original and upgraded kit files with RFE v2.0 (14)

Assay	Sample	Strain	Sample ID	RFA 1.0	RFA 1.1	Description	RFE 1.0	RFE 2.0
SureTect Listeria species PCR Assay	Inclusivity	*Listeria ivanovii* TCC 1183	1183	Positive	Warning	No IPC amplification; Positive call for Listeria species. Well call changed to warning.		

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 *Listeria* species strains and inhibit the growth of non-*Listeria* bacteria. *L. monocytogenes* were further differentiated from *Listeria* species due to the presence of a halo around the presumptive colony. Although *L. ivanovii* can also produce a halo on the medium, the halo size and quality is reduced.

The results from the POD studies showed a comparable performance between the candidate and reference method for all matrixes. For pork rillettes and smoked salmon, the POD results were in favor of the candidate method, while for the raw milk, and ready-to-cook vegetables, the POD results favored the reference method. For the deli salad and process water, there were no differences between the two methods. There were no statistically significant differences between candidate presumptive and confirmed results.

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 species PCR Assay method as a reliable detection method for *Listeria* species L in a broad range of foods and select environmental surfaces.

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

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

40	TCC 1200	Deli product	*L. monocytogenes*	1/2c	Blue/green	Yes	+
41	TCC 858	Human (ATCC 7644)	*L. monocytogenes*	1/2c	Blue/green	Yes	+
42	TCC 1204	Cooked ham	*L. monocytogenes*	1/2c	Blue/green	Yes	+
43	TCC 889	Meat isolate	*L. monocytogenes*	3a	Blue/green	Yes	+
44	TCC 840	Human sample, butter outbreak	*L. monocytogenes*	3a	Blue/green	Yes	+
45	TCC 1227	Turkey breast	*L. monocytogenes*	4b	Blue/green	Yes	+
46	TCC 864	Meningitis due to contaminated cheese (NCTCC 11994)	*L. monocytogenes*	4b	Blue/green	Yes	+
47	RDCC 3413[f]	Spinach	*L. monocytogenes*	Unknown	Blue/green	Yes	+
48	RDCC 1656	Cheese manufacturer	*L. monocytogenes*	Unknown	Blue/green	Yes	+
49	RDCC 5354	Chicken thigh	*L. monocytogenes*	Unknown	Blue/green	Yes	+
50	RDCC 486	Minced beef	*L. monocytogenes*	Unknown	Blue/green	Yes	+
51	RXJ222	Fig tartlet	*Listeria innocua*	Not applicable	Blue	No	+
52	KUY776	Egg product environment	*Listeria innocua*	Not applicable	Blue	No	+
53	RYB922	Smoked sausages	*Listeria innocua*	Not applicable	Blue	No	+
54	TYA050	Raw tuna tataki	*Listeria innocua*	Not applicable	Blue	No	+
55	TQU555	Tomme with raw milk	*Listeria innocua*	Not applicable	Blue	No	+
56	TSA557	Pasteurized Milk Brie	*Listeria innocua*	Not applicable	Blue	No	+
57	TTZ273	Diced onions	*Listeria innocua*	Not applicable	Blue	No	+
58	TWH478	Vegetable gardener	*Listeria innocua*	Not applicable	Blue	No	+
59	XEN574	Salmon shell	*Listeria innocua*	Not applicable	Blue	No	+
60	TET819	Chicken thigh	*Listeria innocua*	Not applicable	Blue	No	+
61	SWZ606	Sausage patty	*Listeria grayi*	Not applicable	Blue	No	+
62	SWE117	Bayonne ham	*Listeria grayi*	Not applicable	Blue	No	+
63	RZM251	Salmon marinated in dill	*Listeria grayi*	Not applicable	Blue	No	+
64	RZK366	Green bean	*Listeria grayi*	Not applicable	Blue	No	+
65	AAZ671	Turkey cutlet scraps	*Listeria ivanovii*	Not applicable	Blue	Yes	+
66	APE161	Merguez	*Listeria ivanovii*	Not applicable	Blue	Yes	+
67	GJP629	Environment	*Listeria ivanovii*	Not applicable	Blue	Yes	+
68	GQD028	Environment dairy products	*Listeria ivanovii*	Not applicable	Blue	Yes	+
69	TXR109	Chinese noodles with vegetables	*Listeria welshimeri*	Not applicable	Blue	No	+
70	GLX736	Environment dairy products	*Listeria welshimeri*	Not applicable	Blue	No	+
71	PSX189	Environment meat products	*Listeria welshimeri*	Not applicable	Blue	No	+
72	TDV458	Andouille from Guéméné	*Listeria welshimeri*	Not applicable	Blue	No	+
73	TPR354	Toulouse sausage	*Listeria welshimeri*	Not applicable	Blue	No	+
74	TUH443	Smoked arctic char with 5 berries	*Listeria welshimeri*	Not applicable	Blue	No	+
75	TVP191	Stripped salmon	*Listeria welshimeri*	Not applicable	Blue	No	+
76	XCW614	Salmon shell	*Listeria welshimeri*	Not applicable	Blue	No	+
77	TLJ742	Hotpot	*Listeria welshimeri*	Not applicable	Blue	No	+
78	DSM20751	Ground	*Listeria seeligeri*	Not applicable	Blue	No	+
79	LHFB67	Dairy environment	*Listeria seeligeri*	Not applicable	Blue	No	+
80	DSM23813	Ground	*Listeria marthi*	Not applicable	Blue	No	+

[a]Unless otherwise labelled, isolates belong to MicroSept culture collection, (France).
[b]Oxoid Culture Collection (Basingstoke, UK)
[c]American Type Culture Collection (Manassas, VA)
[d]Trials Culture Collection (Basingstoke, UK)
[e]National Collection of Type Culture (Salisbury, UK)
[f]Research and Development Culture Collection (Basingstoke, UK)

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

No.	Source ID[a]	Origin	Strain name	Brilliance Listeria Aga	
				Colony color	Halo
1	BI-R6	Egg product	*B. licheniformis*	No colonies	No
2	CIP 5832[b]	Collection	*Bacillus cereus*	No colonies	No
3	CIP 6624	Collection	*Bacillus cereus*	No colonies	No
4	CIP 52.75T	Collection	*Bacillus circulans*	No colonies	No
5	CIP 88264	Collection	*Candida*	No colonies	No

6	IND 501	Clinical strain	*Enterococcus faecalis*	No colonies	
7	IND 502	Clinical strain	*Enterococcus faecalis*	No colonies	
8	ATCC 19433[c]	Collection	*Enterococcus faecalis*	No colonies	
9	CIP 5855	Collection	*Enterococcus faecium*	No colonies	
10	IND 500	Food product	*Enterococcus faecium*	No colonies	
11	Ec - U2	Camembert	*Escherichia coli*	No colonies	
12	Ec - U5	Egg product	*Escherichia coli*	No colonies	
13	ASEPT B 37	Egg product	*Escherichia coli*	No colonies	
14	CIP 71.39	Collection	*Lactobacillus plantarum*	No colonies	
15	CIP 103009 T	Collection	*Leuconostoc mesenteroïdes*	No colonies	
16	P-P1	Egg product	*Pseudomonas fluorescens*	No colonies	
17	CIP 58.69	Collection	*Rhodococcus equi*	No colonies	
18	ASEPT B 38	Egg product	*Salmonella enteritidis*	No colonies	
19	CIP 5710	Collection	*Staphylococcus aureus*	No colonies	
20	CIP 53154	Collection	*Staphylococcus aureus*	No colonies	
21	St-T2	Camembert	*Staphylococcus aureus*	No colonies	
22	ATCC 25953 - St G48	Collection	*Staphylococcus aureus*	No colonies	
23	20060913-	Surimi tortis	*Bacillus cereus*	No colonies	
24	20060914-	Pepper pot	*Bacillus cereus*	No colonies	
25	20061005-41714	Mexican tabbouleh	*Bacillus cereus*	No colonies	
26	20060906-36720	Laggy milk powder	*Bacillus cereus*	No colonies	
27	20060906- 36716	Niro milk powder	*Bacillus cereus*	No colonies	
28	20060914- 38085	Laggy milk powder	*Bacillus cereus*	No colonies	
29	20061005-41648	Niro milk powder	*Bacillus cereus*	No colonies	
30	20061005-41651	Laggy milk powder	*Bacillus cereus*	No colonies	
31	20060801-30997	Whole egg powder	*Bacillus cereus*	No colonies	
32	20060801-30999	Whole egg powder	*Bacillus cereus*	No colonies	
33	20060804-31871	Curly	*Pseudomonas*	No colonies	
34	200608040-31872	Curly	*Pseudomonas*	No colonies	
35	20060804-31873	Chewed up	*Pseudomonas*	No colonies	
36	20060804-31874	Chewed up	*Pseudomonas*	No colonies	
37	20060825-34927	Environmental gauze	*Pseudomonas*	No colonies	

[a]Unless otherwise labelled, isolates belong to MicroSepts culture collection, (France).
[b] Culture Collection of Pasteur Insititute, (France).
[c] American Type Culture Collection, (Manassas, VA).

Table 4. Thermo Scientific SureTect Listeria species PCR assay, 7500 Fast & QS5, presumptive vs. confirmed and candidate vs. ISO11290-1:2017 – POD results (15)

		Candidate presumptive (CP)			Candidate confirmed (CC)[a]			Candidate result (C)			Reference method (R)			C vs. R	CP vs.CC
Statistic	Matrix/Organism	N[b]	X[c]	POD_{CP}[d]	N	X	POD_{CC}[e]	N	X	POD_{C}[f]	N	X	POD_{R}[g]	$dPOD_{C,R}$[h]	$dPOD_{CP,CC}$[i]
	Deli Salad: Piémontaise (25 g) *Listeria monocytogenes* 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[j]				0.00			0.00			0.00			0.00	-0.43	-0.47
UCL[k]				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) *Listeria ivanovii* AAZ671	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	15	0.75	20	15	0.75	20	15	0.75	20	8	0.40	0.35	0.00
LCL				0.53			0.53			0.53			0.22	0.04	-0.13
UCL				0.89			0.89			0.89			0.61	0.58	0.13
		5	4	0.80	5	4	0.80	5	4	0.80	5	3	0.60	0.20	0.00
LCL				0.38			0.38			0.38			0.23	-0.31	-0.47
UCL				1.00			1.00			1.00			0.88	0.62	0.47
	Raw milk (25 ml) *Listeria*	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* 1/2b CLM641			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) *Listeria innocua* ABB472	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	15	0.75	20	15	0.75	20	15	0.75	20	11	0.55	0.20	0.00
LCL				053			053			053			0.34	-0.09	-0.13
UCL				0.89			0.89			0.89			0.74	0.45	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
	Ready-to-cook vegetables (25 g) *Listeria 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) *Listeria welshimeri* RVG428	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	13	0.65	20	13	0.65	20	13	0.65	20	13	0.65	0.00	0.00
LCL				0.43			0.43			0.43			0.43	-0.28	-0.13
UCL				0.82			0.82			0.82			0.82	0.28	0.13
		5	3	0.60	5	3	0.60	5	3	0.60	5	1	0.20	0.40	0.00
LCL				0.23			0.23			0.23			0.00	-0.16	-0.47
UCL				0.88			0.88			0.88			0.62	0.75	0.47

[a]Results obtained following the alternative confirmation were identical to results obtain from confirmation by ISO 11290-1:2017 reference method.
[b]N = Number of test portions.
[c]X = Number of positive test portions.
[d]POD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials.
[e]POD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials.
[f]POD_{C} = Candidate method presumptive positive outcomes confirmed positive divided by the total number of trials.
[g]POD_{R} = Reference method confirmed positive outcomes divided by the total number of trials.
[h]$dPOD_{CP}$= Difference between the candidate method presumptive result and candidate method confirmed result POD values.
[i]$dPOD_{C}$= Difference between the confirmed candidate method result and reference method confirmed result POD values.
[j]LCL = Lower confidence limit.
[k]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_t 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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