



CERTIFICATION

AOAC[®] Performance TestedSM

Certificate No.

061302

The AOAC Research Institute hereby certifies that the performance of the test kit known as:

Thermo Scientific[™] SureTect[™] Listeria monocytogenes PCR Assay

manufactured by

Oxoid Ltd, part of Thermo Fisher Scientific

Wade Road

Basingstoke

Hampshire, RG248PW

This method has been evaluated in the AOAC[®] *Performance Tested Methods*SM Program, and found to perform as stated by the manufacturer contingent to the comments contained in the manuscript. This certificate means that an AOAC[®] Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC *Performance Tested*SM certification mark along with the statement - "THIS METHOD'S PERFORMANCE WAS REVIEWED BY AOAC RESEARCH INSTITUTE AND WAS FOUND TO PERFORM TO THE MANUFACTURER'S SPECIFICATIONS" - on the above mentioned method for a period of one calendar year from the date of this certificate (December 12, 2018 – December 31, 2019). Renewal may be granted at the end of one year under the rules stated in the licensing agreement.

Scott Coates

Scott Coates, Senior Director
Signature for AOAC Research Institute

December 12, 2018

Date

METHOD AUTHORS

ORIGINAL VALIDATION - Jonathan Cloke, Carlos Leon-Velarde, Nathan Larson, Keron Dave, Katharine Evans, David Crabtree, Annette Hughes, Helen Simpson, Jani Holopainen, Nina Wickstrand, and Mikko Kauppinen
MODIFICATION FEBRUARY 2015 – Jonathan Cloke, Katharine Evans, David Crabtree, Annette Hughes, and Helen Simpson
MODIFICATION OCTOBER 2015 – Laura Vaahtoranta, Jukkapekka Palomaki, Paulus Artimo, Feng Haung, Maria Liikanen, and Suvi Koskela
MODIFICATION OCTOBER 2018 – Jessica Williams, Katharine Evans, David Crabtree, Annette Hughes, Charlotte Cooper, Dean Leak, Agata Dziegiel

SUBMITTING COMPANY

Oxoid Ltd., part of Thermo Fisher Scientific
 Wade Road
 Basingstroke
 Hamshire, RG24 8PW, UK

KIT NAME(S)

Thermo Scientific™ SureTect™ *Listeria monocytogenes* PCR Assay

CATALOG NUMBERS

PT0300A

INDEPENDENT LABORATORY

Original Validation
 Agriculture and Food Laboratory
 Laboratory Services Division
 University of Guelph
 95 Stone Road, West
 Guelph, Ontario, N1H 8J7
 Canada

AOAC EXPERTS AND PEER REVIEWERS

Yi Chen^{1,4}, Elliot Ryser², Mark Carter³
¹ US Food and Drug Administration, Center for Food Safety and Applied Nutrition, College Park, MD, USA
² Michigan State University, East Lansing, MI, USA
³ MC2E Inc., New Hope, PA, USA
⁴ Modifications: February 2015; October 2015; October 2018

APPLICABILITY OF METHOD

Target organism – *Listeria monocytogenes*

Matrices – Original Validation (25 g) - raw ground beef, pork frankfurters, salami, cooked sliced turkey, fresh bagged spinach, ice cream, smoked salmon, cooked prawns
 (Sponge with neutralizing broth) - stainless steel, plastic
 February 2015 Modification – (25 g) raw ground turkey, raw ground pork, pasteurized 2% milk, raw pork sausage, raw cod, pasteurized brie cheese, cooked sliced ham, and bagged lettuce
 October 2018 Modification – (25 g) – sliced deli turkey, bagged lettuce, pasteurized 2% fat milk; (1 x 1 in swab) stainless steel

REFERENCE METHOD

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)

Performance claims - Performance equivalent to the ISO Horizontal method for the detection of *Listeria monocytogenes* ISO 11290-1:1996, Amendment 1:2004 (3).

ORIGINAL CERTIFICATION DATE

June 21, 2013

CERTIFICATION RENEWAL RECORD

Renewed annually through December 2019

METHOD MODIFICATION RECORD

1. February 2015
2. October 2015
3. December 2017 Level 1
4. April 2018 Level 2
5. October 2018 Level 2

6. December 2018 Level 1

SUMMARY OF MODIFICATION

1. Matrix Extension
2. Validation of the Applied Biosystems 7500 Fast Instrument
3. Editorial changes to update company name
4. Evaluation of workflow and lyophilization steps
5. Validation of the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR (with Applied Biosystems™ RapidFinder™ Analysis Software v2.0 or greater)
6. Updated user manual to include complete AOAC workflow, update template, and minor edits

Under this AOAC® Performance TestedSM License Number, 061302 this method is distributed by:
 NONE

Under this AOAC® Performance TestedSM License Number, 061302 this method is distributed as:
 NONE

PRINCIPLE OF THE METHOD (1)

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

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

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

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

DISCUSSION OF THE VALIDATION STUDY (1)

The data presented and discussed in this report, within the statistical uncertainty of the analysis, supports the product claims of the SureTect Listeria monocytogenes assay for recovery of *L. monocytogenes* from fresh cantaloupe, salami, smoked salmon, fresh bagged spinach, cooked sliced turkey, pork frankfurters, ice cream, cooked prawns, processed cheese and raw ground beef as well as stainless steel and plastic surfaces. Additional studies conducted as part of the validation show that the assay has excellent inclusivity and is unaffected by high levels of non-target bacteria. The SureTect Listeria monocytogenes assay was also shown to have good reproducibility, and although accelerated stability testing was conducted, real-time studies are on-going and will be reported at the annual method renewal.

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

Isolate	Serotype	TCC ^a No	Source	Result
<i>Listeria monocytogenes</i>	2	867	CSF: Clinical	Positive
<i>Listeria monocytogenes</i>	7	2184	Faecal sample	Positive
<i>Listeria monocytogenes</i>	1/2a	860	Poultry	Positive
<i>Listeria monocytogenes</i>	1/2a	1215	Chorizo sausage	Positive
<i>Listeria monocytogenes</i>	1/2a	1216	Sandwich	Positive
<i>Listeria monocytogenes</i>	1/2a	1217	Carrow cheese	Positive
<i>Listeria monocytogenes</i>	1/2a	1218	Butter	Positive
<i>Listeria monocytogenes</i>	1/2a	1219	Pilau rice	Positive
<i>Listeria monocytogenes</i>	1/2a	1220	Sandwich	Positive
<i>Listeria monocytogenes</i>	1/2b	1205	Cake	Positive
<i>Listeria monocytogenes</i>	1/2b	1206	Whipped Cream	Positive
<i>Listeria monocytogenes</i>	1/2b	1207	Cheese	Positive
<i>Listeria monocytogenes</i>	1/2b	1208	Cheese	Positive
<i>Listeria monocytogenes</i>	1/2b	1209	Cream	Positive
<i>Listeria monocytogenes</i>	1/2b	1210	Cheese	Positive
<i>Listeria monocytogenes</i>	1/2c	858	Clinical sample	Positive
<i>Listeria monocytogenes</i>	1/2c	1195	Ox tongue	Positive
<i>Listeria monocytogenes</i>	1/2c	1196	Roast beef	Positive
<i>Listeria monocytogenes</i>	1/2c	1197	Topside beef	Positive
<i>Listeria monocytogenes</i>	1/2c	1198	Wiltshire ham	Positive
<i>Listeria monocytogenes</i>	1/2c	1199	Ham sandwich	Positive
<i>Listeria monocytogenes</i>	3a	812	Environmental	Positive
<i>Listeria monocytogenes</i>	3a	813	Environmental	Positive
<i>Listeria monocytogenes</i>	3a	840	Butter	Positive
<i>Listeria monocytogenes</i>	3a	870	Clinical sample	Positive
<i>Listeria monocytogenes</i>	3a	888	Food	Positive
<i>Listeria monocytogenes</i>	3a	889	Food	Positive
<i>Listeria monocytogenes</i>	3b	2179	Unknown	Positive
<i>Listeria monocytogenes</i>	3c	2180	Unknown	Positive

<i>Listeria monocytogenes</i>	4a	2181	Unknown	Positive
<i>Listeria monocytogenes</i>	4b	864	Meningitis	Positive
<i>Listeria monocytogenes</i>	4b	865	CSF: Meningitis	Positive
<i>Listeria monocytogenes</i>	4b	1224	Food- blood	Positive
<i>Listeria monocytogenes</i>	4b	1225	Chicken	Positive
<i>Listeria monocytogenes</i>	4b	1226	Dressed crab	Positive
<i>Listeria monocytogenes</i>	4b	1227	Turkey breast	Positive
<i>Listeria monocytogenes</i>	4c	2183	Bird: heart disease	Positive
<i>Listeria monocytogenes</i>	4d	863	Sheep	Positive
<i>Listeria monocytogenes</i>	4e	868	Chicken	Positive
<i>Listeria monocytogenes</i>	4e	883	Veterinary sample	Positive
<i>Listeria monocytogenes</i>	4e	884	Unknown	Positive
<i>Listeria monocytogenes</i>	4e	885	Unknown	Positive
<i>Listeria monocytogenes</i>	Untyped	874	Unknown food source-isolated by food lab	Positive
<i>Listeria monocytogenes</i>	Untyped	856	Unknown food source-isolated by food lab	Positive
<i>Listeria monocytogenes</i>	Untyped	857	Unknown food source-isolated by food lab	Positive
<i>Listeria monocytogenes</i>	Untyped	859	Tartare de Salmon	Positive
<i>Listeria monocytogenes</i>	Untyped	866	Unknown ATCC isolate	Positive
<i>Listeria monocytogenes</i>	Untyped	871	Unknown food source-isolated by food lab	Positive
<i>Listeria monocytogenes</i>	Untyped	873	Unknown food source-isolated by food lab	Positive
<i>Listeria monocytogenes</i>	Untyped	875	Unknown food source-isolated by food lab	Positive
<i>Listeria monocytogenes</i>	Untyped	881	Unknown food source-isolated by food lab	Positive
<i>Listeria monocytogenes</i>	Untyped	882	Unknown food source-isolated by food lab	Positive
<i>Listeria monocytogenes</i>	4b	1841	ATCC® 19115	Positive

®TCC. Trials Culture Collection Number - Proprietary to Thermo Fisher Scientific, Microbiology Division

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

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

^aTCC. Trials Culture Collection Number - Proprietary to Thermo Fisher Scientific, Microbiology Division, Basingstoke, UK

^bOCC. Oxoid Culture Collection - Proprietary to Thermo Fisher Scientific, Microbiology Division, Basingstoke, UK

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

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

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

^aMPN = 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.

^bN = Number of test portions

^cX = Number of positive test portions

^dPODcp = Candidate method presumptive positive outcomes divided by the total number of portions

^ePODcc = Candidate confirmation method positive outcomes divided by the total number of portions

^fdPODcp = Difference between the candidate presumptive result and the candidate method confirmed result POD values

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

^hN/A = Not applicable

ⁱIndependent laboratory study

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

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

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

^aMPN = 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.

^bN = Number of test portions

^cX = Number of positive test portions

^dPOD_{cp} = Candidate method presumptive positive outcomes divided by the total number of portions

^ePOD_{cc₂} = Reference method confirmation positive outcomes divided by the total number of portions

^fdPOD_{cp} = Difference between the candidate presumptive result and the candidate method confirmed result POD values

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

^hN/A = Not applicable

ⁱIndependent laboratory study

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

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

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

^aMPN = 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.

^bN = Number of test portions

^cX = Number of positive test portions

^dPODcc = Candidate method confirmed positive outcomes divided by the total number of portions

^ePODcc₂ = Reference method confirmation positive outcomes divided by the total number of portions

^fdPODcp = Difference between the candidate presumptive result and the candidate method confirmed result POD values

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

^hN/A = Not applicable

ⁱIndependent laboratory study

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

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

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

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

^aMPN = 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.

^bN = Number of test portions

^cX = Number of positive test portions

^dPODc = Confirmed candidate method positive outcomes divided by the total number of portions

^ePODr = Confirmed reference method positive outcomes divided by the total number of portions

^fdPODc = Difference between the candidate presumptive result and the candidate method confirmed result POD values

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

^hN/A = Not applicable

ⁱIndependent Laboratory Study

DISCUSSION OF MODIFICATION APPROVED February 2015 (8)

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

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

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

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

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

Cooked ham	<i>Listeria monocytogenes</i> (TCC 1198)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.90 (0.53, 1.48)	20	16	0.80	(0.58, 0.92)	16	0.80	(0.58, 0.92)	0.00	(-0.25, 0.25)
		2.96 (1.25, 7.00)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Bagged lettuce	<i>Listeria monocytogenes</i> (TCC 1220)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.53 (0.28, 9.66)	20	6	0.30	(0.15, 0.52)	6	0.30	(0.15, 0.52)	0.00	(-0.27, 0.27)
		1.09 (0.49, 2.46)	5	5	1.00	(0.57, 1.00)	4	0.80	(0.38, 1.00)	0.20	(-0.28, 0.62)
Raw cod ⁱ	<i>Listeria monocytogenes</i> (ATCC ^c 19115™)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.47 (0.28, 0.75)	20	9	0.45	(0.25, 0.65)	9	0.45	(0.25, 0.65)	0.00	(-0.28, 0.28)
		4.38 (1.71, 11.19)	5	5	1.00	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0.00	(-0.43, 0.43)
Brie ⁱ	<i>Listeria monocytogenes</i> (LI0512)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.46 (0.28, 0.75)	20	5	0.25	(0.11, 0.46)	5	0.25	(0.11, 0.46)	0.00	(-0.25, 0.25)
		4.38 (1.71, 11.19)	5	5	1.00	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0.00	(-0.43, 0.43)
Bagged lettuce ⁱ	<i>Listeria monocytogenes</i> (LI0549)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.37 (0.23, 0.59)	20	10	0.50	(0.29, 0.70)	10	0.50	(0.29, 0.70)	0.00	(-0.28, 0.28)
		2.19 (0.93, 5.12)	5	5	1.00	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0.00	(-0.43, 0.43)

^aMPN = 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.

^bN = Number of test portions.

^cX = Number of positive test portions.

^dPOD_{cp} = Candidate method presumptive positive outcomes divided by the total number of portions.

^ePOD_{ccz} = Candidate confirmation method positive outcomes divided by the total number of portions.

^fdPOD_{cp} = Difference between the candidate presumptive result and the candidate method confirmed result using the SureTect confirmation procedure POD values.

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

^hN/A = Not applicable.

ⁱIndependent laboratory study.

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

Matrix	Strain	MPN ^a /test portion	N ^b	SureTect Method Presumptive			Reference Confirmation (CC2)			dPOD _{cp} ^f	95% CI ^g
				X ^c	POD _{cp} ^d	95% CI	X	POD _{ccz} ^e	95% CI		
Raw ground turkey	<i>Listeria monocytogenes</i> (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	9	0.45	(0.26, 0.66)	10	0.50	(0.30, 0.70)	-0.05	(-0.33, 0.24)
		0.95 (0.47, 1.91)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Raw ground pork	<i>Listeria monocytogenes</i>	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	20	14	0.70	(0.48, 0.85)	14	0.70	(0.48, 0.85)	0.00	(-0.27, 0.27)

	(TCC 883)	(1.15, 3.79)									
		0.56 (0.23, 1.32)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Pasteurized 2% milk	<i>Listeria monocytogenes</i> (OCC 840)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.28 (0.11, 0.56)	20	4	0.20	(0.08, 0.42)	4	0.20	(0.08, 0.42)	0.00	(-0.25, 0.25)
		1.09 (0.47, 2.46)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Raw pork sausages	<i>Listeria monocytogenes</i> (TCC 867)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.01 (0.42, 1.03)	20	9	0.45	(0.26, 0.66)	10	0.50	(0.30, 0.70)	-0.05	(-0.33, 0.24)
		3.10 (1.42, 6.77)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Raw cod	<i>Listeria monocytogenes</i> (TCC 1226)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.33 (0.81, 2.30)	20	15	0.75	(0.53, 0.89)	15	0.75	(0.53, 0.89)	0.00	(-0.26, 0.26)
		4.37 (1.71, 11.19)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Brie	<i>Listeria monocytogenes</i> (TCC 1210)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.12 (0.05, 0.31)	20	5	0.25	(0.11, 0.47)	5	0.25	(0.11, 0.47)	0.00	(-0.26, 0.26)
		0.22 (0.08, 0.61)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Cooked ham	<i>Listeria monocytogenes</i> (TCC 1198)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.90 (0.53, 1.48)	20	16	0.80	(0.58, 0.92)	16	0.80	(0.58, 0.92)	0.00	(-0.25, 0.25)
		2.96 (1.25, 7.00)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Fresh bagged lettuce	<i>Listeria monocytogenes</i> (TCC 1220)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.53 (0.28, 9.66)	20	6	0.30	(0.15, 0.52)	6	0.30	(0.15, 0.52)	0.00	(-0.27, 0.27)
		1.09 (0.49, 2.46)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Raw cod ⁱ	<i>Listeria monocytogenes</i> (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	(0.26, 0.66)	9	0.45	(0.26, 0.66)	0.00	(-0.28, 0.28)
		4.38 (1.71, 11.19)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Brie ⁱ	<i>Listeria monocytogenes</i> (LI0512)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.46 (0.28, 0.75)	20	5	0.25	(0.11, 0.47)	5	0.25	(0.11, 0.47)	0.00	(-0.26, 0.26)
		4.38 (1.71, 11.19)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Bagged lettuce ⁱ	<i>Listeria monocytogenes</i> (LI0549)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.37 (0.23, 0.59)	20	10	0.50	(0.29, 0.70)	10	0.50	(0.29, 0.70)	0.00	(-0.28, 0.28)
		2.19 (0.93, 5.12)	5	5	1.00	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0.00	(-0.43, 0.43)

^aMPN = 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.

^bN = Number of test portions.

^cX = Number of positive test portions.

^dPOD_{cc} = Candidate method presumptive positive outcomes divided by the total number of portions.

^ePOD_{cc2} = Reference method confirmation positive outcomes divided by the total number of portions.

^fdPOD_{cc} = Difference between the candidate presumptive result and the candidate method confirmed result using the reference confirmation procedure POD values.

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

^hN/A = Not applicable.

ⁱIndependent laboratory study.

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

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

	<i>monocytogenes</i> (TCC 1220)	0.53 (0.28, 9.66)	20	6	0.30	(0.15, 0.52)	6	0.30	(0.15, 0.52)	0.00	(-0.27, 0.27)
		1.09 (0.49, 2.46)	5	4	0.80	(0.38, 1.00)	5	1.00	(0.57, 1.00)	-0.20	(-0.62, 0.28)
Raw cod ^d	<i>Listeria monocytogenes</i> (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	(0.26, 0.66)	9	0.45	(0.26, 0.66)	0.00	(-0.28, 0.28)
		4.38 (1.71, 11.19)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Brie ^e	<i>Listeria monocytogenes</i> (LI0512)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.46 (0.28, 0.75)	20	5	0.25	(0.11, 0.47)	5	0.25	(0.11, 0.47)	0.00	(-0.26, 0.26)
		4.38 (1.71, 11.19)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Bagged lettuce ^f	<i>Listeria monocytogenes</i> (LI0549)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.37 (0.23, 0.59)	20	10	0.50	(0.29, 0.70)	10	0.50	(0.29, 0.70)	0.00	(-0.28, 0.28)
		2.19 (0.93, 5.12)	5	5	1.00	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0.00	(-0.43, 0.43)

^aMPN = 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.

^bN = Number of test portions.

^cX = Number of positive test portions.

^dPODcc = Candidate method confirmed positive outcomes by candidate confirmation procedure divided by the total number of portions.

^ePODcc₂ = Candidate method confirmed positive outcomes by reference confirmation procedure divided by the total number of portions.

^fdPODcp = Difference between the candidate confirmation procedure confirmed result and the reference confirmation procedure confirmed result POD values.

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

^hN/A = Not applicable.

ⁱIndependent laboratory study.

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

Matrix	Strain	MPN ^a /test portion	N ^b	SureTect Method (C)			Reference Method (R)			dPOD ^c	95% CI ^g
				X ^c	POD _c ^d	95% CI	X	POD _r ^e	95% CI		
Raw ground turkey	<i>Listeria monocytogenes</i> (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	9	0.45	(0.26, 0.66)	10	0.50	(0.30, 0.70)	-0.05	(-0.33, 0.24)
		0.95 (0.47, 1.91)	5	4	0.80	(0.38, 1.00)	5	1.00	(0.57, 1.00)	-0.20	(-0.62, 0.28)
Raw ground pork	<i>Listeria monocytogenes</i> (TCC 883)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.86 (1.15, 3.79)	20	14	0.70	(0.48, 0.85)	16	0.80	(0.58, 0.92)	-0.10	(-0.35, 0.17)
		0.56 (0.23, 1.32)	5	5	1.00	(0.57, 1.00)	2	0.40	(0.12, 0.77)	0.60	(0.03, 0.88)
Pasteurized 2% milk	<i>Listeria monocytogenes</i> (TCC 840)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.28 (0.11, 0.56)	20	4	0.20	(0.08, 0.42)	5	0.25	(0.11, 0.47)	-0.05	(-0.30, 0.21)
		1.09 (0.47, 2.46)	5	5	1.00	(0.57, 1.00)	3	0.60	(0.23, 0.88)	0.40	(-0.12, 0.77)
Raw pork sausages	<i>Listeria monocytogenes</i> (TCC 867)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.01 (0.42, 1.03)	20	9	0.45	(0.26, 0.66)	10	0.50	(0.30, 0.70)	-0.05	(-0.33, 0.24)
		3.10 (1.42, 6.77)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Raw cod	<i>Listeria monocytogenes</i> (TCC 1226)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.33 (0.81, 2.30)	20	15	0.75	(0.53, 0.89)	14	0.70	(0.48, 0.85)	0.05	(-0.22, 0.31)
		4.37 (1.71, 11.19)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Brie	<i>Listeria monocytogenes</i> (TCC 1210)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.12 (0.05, 0.31)	20	5	0.25	(0.11, 0.47)	1	0.05	(0.00, 0.24)	0.20	(-0.03, 0.42)
		0.22 (0.08, 0.61)	5	4	0.80	(0.38, 1.00)	0	0.00	(0.00, 0.43)	0.80	(0.19, 1.00)
Cooked ham	<i>Listeria monocytogenes</i> (TCC 1198)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.90 (0.53, 1.48)	20	16	0.80	(0.58, 0.92)	12	0.60	(0.39, 0.78)	0.20	(-0.08, 0.44)
		2.96 (1.25, 7.00)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)

Bagged lettuce	<i>Listeria monocytogenes</i> (TCC 1220)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.53 (0.28, 9.66)	20	6	0.30	(0.15, 0.52)	9	0.45	(0.26, 0.66)	-0.15	(-0.41, 0.14)
		1.09 (0.49, 2.46)	5	4	0.80	(0.38, 1.00)	3	0.60	(0.23, 0.88)	0.20	(-0.31, 0.62)
Raw cod ⁱ	<i>Listeria monocytogenes</i> (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	20	9	0.45	(0.25, 0.65)	6	0.30	(0.14, 0.51)	0.15	(-0.14, 0.40)

		(0.28, 0.75)									
		4.38 (1.71, 11.19)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Brie ⁱ	<i>Listeria monocytogenes</i> (LI0512)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.46 (0.28, 0.75)	20	5	0.25	(0.11, 0.46)	8	0.40	(0.21, 0.61)	-0.15	(-0.40, 0.13)
		4.38 (1.71, 11.19)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Bagged lettuce ⁱ	<i>Listeria monocytogenes</i> (LI0549)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.37 (0.23, 0.59)	20	10	0.50	(0.29, 0.70)	5	0.25	(0.11, 0.46)	0.25	(-0.04, 0.49)
		2.19 (0.93, 5.12)	5	5	1.00	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0.00	(-0.43, 0.43)

^aMPN = 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.

^bN = Number of test portions.

^cX = Number of positive test portions.

^dPODc = Confirmed candidate method positive outcomes divided by the total number of portions.

^ePODr = Confirmed reference method positive outcomes divided by the total number of portions.

^fdPODc = Difference between the candidate method confirmed result and the reference method confirmed result POD values.

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

^hN/A = Not applicable.

ⁱIndependent Laboratory Study.

DISCUSSION OF MODIFICATION APPROVED OCTOBER 2015 (9)

Results from the matrices analyzed during this method modification study to validate the use of the Applied Biosystems 7500 Fast Instrument and Applied Biosystems RapidFinder Express 2.0 Software as alternative parts of the SureTect Assay workflow in comparison to the ISO reference method are summarized in Tables 3 to 6. For all three of the food matrices and the stainless steel surface analyzed, the SureTect assay returned results which were not statistically different when analyzed using POD statistical analysis compared to the reference method. Although not giving any statistically different results following the POD analysis, one of the low spiked samples for ground turkey meat and one of the low spiked samples analyzed for the stainless steel surface gave negative results with the SureTect method, while the SureTect confirmation method (Table 3) and reference method confirmation procedure (Table 4) gave positive results. For the presumptive negative sample which was confirmed as positive for raw ground turkey, the estimated plate counts from the direct plating of the 24 LEB enrichment, estimated the contamination level to be approximately 6×10^2 CFU/mL. This level of contamination is below the level of detection of the PCR assay and the false negative PCR results was most possibly due to inefficient mixing of the bulk food sample, which resulted in very low numbers of *L. monocytogenes* cells being present in the 25 g portion of the sample removed for this particular analysis. Plating methods (as used for the SureTect confirmation method) and the secondary enrichment performed for the reference method, have good sensitivity and are able in theory to detect as little as one cell.

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

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

Table 7: Inclusivity Results For The SureTect Assay (9)

Isolate	Serotype	TCC ^a No.	Source	Result
<i>Listeria monocytogenes</i>	1/2a	860	poultry	Positive
<i>Listeria monocytogenes</i>	1/2a	1215	Chorizo sausage	Positive
<i>Listeria monocytogenes</i>	1/2a	1216	sandwich	Positive
<i>Listeria monocytogenes</i>	1/2a	1217	carrow cheese	Positive
<i>Listeria monocytogenes</i>	1/2a	1218	Butter	Positive
<i>Listeria monocytogenes</i>	1/2a	1219	Pilau rice	Positive
<i>Listeria monocytogenes</i>	1/2a	1220	sandwich	Positive
<i>Listeria monocytogenes</i>	1/2b	1205	Cake	Positive
<i>Listeria monocytogenes</i>	1/2b	1206	Whipped Cream	Positive
<i>Listeria monocytogenes</i>	1/2b	1207	Cheese	Positive
<i>Listeria monocytogenes</i>	1/2b	1208	Cheese	Positive
<i>Listeria monocytogenes</i>	1/2b	1209	Cream	Positive
<i>Listeria monocytogenes</i>	1/2b	1210	Cheese	Positive
<i>Listeria monocytogenes</i>	1/2c	858	Clinical sample	Positive
<i>Listeria monocytogenes</i>	1/2c	1195	Ox tongue	Positive
<i>Listeria monocytogenes</i>	1/2c	1196	Roast beef	Positive
<i>Listeria monocytogenes</i>	1/2c	1197	Topside beef	Positive
<i>Listeria monocytogenes</i>	1/2c	1198	Wiltshire ham	Positive
<i>Listeria monocytogenes</i>	1/2c	1199	Ham sandwich	Positive
<i>Listeria monocytogenes</i>	3a	812	Environmental	Positive
<i>Listeria monocytogenes</i>	3a	813	Environmental	Positive
<i>Listeria monocytogenes</i>	3a	840	Butter	Positive
<i>Listeria monocytogenes</i>	3a	870	Clinical sample	Positive
<i>Listeria monocytogenes</i>	3a	888	Food	Positive
<i>Listeria monocytogenes</i>	3a	889	Food	Positive
<i>Listeria monocytogenes</i>	3b	2179	Unknown	Positive
<i>Listeria monocytogenes</i>	3c	2180	Unknown	Positive
<i>Listeria monocytogenes</i>	4a	2181	Unknown	Positive
<i>Listeria monocytogenes</i>	4b	864	Meningitis	Positive
<i>Listeria monocytogenes</i>	4b	865	CSF: Meningitis	Positive
<i>Listeria monocytogenes</i>	4b	1224	Food- blood	Positive
<i>Listeria monocytogenes</i>	4b	1225	Chicken	Positive

<i>Listeria monocytogenes</i>	4b	1226	Dressed crab	Positive
<i>Listeria monocytogenes</i>	4b	1227	Turkey breast	Positive
<i>Listeria monocytogenes</i>	4c	2183	Bird: heart disease	Positive
<i>Listeria monocytogenes</i>	4d	863	Sheep	Positive
<i>Listeria monocytogenes</i>	4e	868	Chicken	Positive
<i>Listeria monocytogenes</i>	4e	883	Veterinary sample	Positive
<i>Listeria grayi</i>		1172	Environmental isolate	Positive
<i>Listeria grayi</i>		1173	Butter	Positive
<i>Listeria grayi</i>		1174	Butter	Positive
<i>Listeria grayi</i>		1175	Butter	Positive
<i>Listeria grayi</i>		1176	Food	Positive
<i>Listeria innocua</i>	Unknown	1177	Chicken sandwich	Positive
<i>Listeria innocua</i>	Unknown	1178	Cooked chicken	Positive
<i>Listeria innocua</i>	Unknown	1179	Crayfish	Positive
<i>Listeria innocua</i>	Unknown	1180	Coleslaw	Positive
<i>Listeria innocua</i>	Unknown	1181	Tuna mayo sandwich	Positive
<i>Listeria ivanovii</i>	Unknown	1182	Lamb (vet sample)	Positive
<i>Listeria ivanovii</i>	Unknown	1183	Food	Positive
<i>Listeria ivanovii</i>	Unknown	1184	Food	Positive
<i>Listeria welshimeri</i>	Unknown	1185	Chicken sandwich	Positive
<i>Listeria welshimeri</i>	Unknown	1186	Food	Positive
<i>Listeria welshimeri</i>	Unknown	1187	Environmental isolate	Positive
<i>Listeria welshimeri</i>	Unknown	1188	Pastrami	Positive
<i>Listeria welshimeri</i>	Unknown	1189	Food	Positive
<i>Listeria seeligeri</i>	Unknown	1190	Cheese	Positive
<i>Listeria seeligeri</i>	Unknown	1191	Food	Positive
<i>Listeria seeligeri</i>	Unknown	1192	Environmental isolate	Positive
<i>Listeria seeligeri</i>	Unknown	1193	Cannelloni	Positive
<i>Listeria seeligeri</i>	Unknown	1194	Coleslaw	Positive
<i>L. innocua</i>	6a	862	Cow brain ATCC® 33090™	Positive
<i>Listeria innocua</i>	4ab	2185		Positive
<i>Listeria innocua</i>	6b	2187		Positive
<i>Listeria welshimeri</i>	6b	2188		Positive
<i>Listeria welshimeri</i>	4c	2189		Positive
<i>Listeria seeligeri</i>	1/2b	2190		Positive
<i>Listeria seeligeri</i>	6b	2191		Positive

®Trials Culture Collection – Proprietary.

Table 8: Exclusivity Results For The SureTect Assay (9)

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

^aTrials Culture Collection – Proprietary.

Table 9: Thermo Scientific SureTect *Listeria monocytogenes* PCR Assay Presumptive vs. SureTect Method Confirmation Procedure Confirmed Results – 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	<i>Listeria monocytogenes</i> 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	<i>Listeria monocytogenes</i> TCC 856	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.45, 0.45)
		1.26 (0.79, 2.07)	20	18	0.90	(0.70, 0.97)	18	0.90	(0.70, 0.97)	0.00	(-0.21, 0.21)
		3.00 (1.311, 6.89)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Bagged lettuce	<i>Listeria monocytogenes</i> TCC 1220	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.45, 0.45)
		1.00 (0.66, 1.55)	20	13	0.65	(0.43, 0.82)	13	0.65	(0.43, 0.82)	0.00	(-0.28, 0.28)
		1.13 (0.57, 2.25)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Stainless steel surface 4" x 4"	<i>Listeria monocytogenes</i> TCC 813 and <i>Enterococcus faecalis</i> CIP100750 X10	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.45, 0.45)
		N/A	20	6	0.30	(0.15, 0.52)	7	0.35	(0.18, 0.57)	-0.05	(-0.32, 0.23)
		N/A	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)

^aMPN= 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.

^bN=Number of test portions.

^cX=Number of positive test portions.

^dPOD_{CP}=Candidate method presumptive positive outcomes divided by the total number of portions.

^ePOD_{CC}=Candidate confirmation method positive outcomes divided by the total number of portions.

^fdPOD_{CP}=Difference between the candidate presumptive result and the candidate method confirmed result POD values.

^g95% CI=If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

^hN/A=Not applicable.

Table 10: Thermo Scientific SureTect *Listeria monocytogenes* PCR Assay Presumptive vs. Reference Method Confirmation Procedure Confirmed Results – POD Analysis (9)

Matrix	Strain	MPN ^a /test portion	N ^b	SureTect Method Presumptive			Reference Method Confirmation (RC)			dPOD _{CP} ^f	95% CI ^g
				X ^c	POD _{CP} ^d	95% CI	X	POD _{RC} ^e	95% CI		
Raw ground turkey	<i>Listeria monocytogenes</i> 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	<i>Listeria monocytogenes</i> TCC 856	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.45, 0.45)
		1.26 (0.79, 2.07)	20	18	0.90	(0.70, 0.97)	18	0.90	(0.70, 0.97)	0.00	(-0.21, 0.21)
		3.00 (1.311, 6.89)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Bagged lettuce	<i>Listeria monocytogenes</i> TCC 1220	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.45, 0.45)
		1.00 (0.66, 1.55)	20	13	0.65	(0.43, 0.82)	13	0.65	(0.43, 0.82)	0.00	(-0.28, 0.28)
		1.13 (0.57, 2.25)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Stainless steel surface 4" x 4"	<i>Listeria monocytogenes</i> TCC 813 and <i>Enterococcus faecalis</i> CIP100750 X10	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.45, 0.45)
		N/A	20	6	0.30	(0.15, 0.52)	7	0.35	(0.18, 0.57)	-0.05	(-0.32, 0.23)
		N/A	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)

^aMPN= 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.

^bN=Number of test portions.

^cX=Number of positive test portions.

^dPOD_{CP}=Candidate method presumptive positive outcomes divided by the total number of portions.

^ePOD_{RC}=Reference confirmation, positive outcomes divided by the total number of portions.

^fdPOD_{CP}=Difference between the candidate presumptive result and the reference confirmation method confirmed result POD values.

^g95% CI=If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

^hN/A=Not applicable.

Table 11: Thermo Scientific SureTect Listeria monocytogenes PCR Assay Confirmation Procedure Confirmed Results vs. Reference Method Confirmation Procedure Confirmed Results – POD Analysis (9)

Matrix	Strain	MPN ^a /test portion	N ^b	SureTect Method Confirmation (CC)			Reference Method Confirmation (RC)			dPOD _{CC} ^f	95% CI ^g
				X ^c	POD _{CC} ^d	95% CI	X	POD _{RC} ^e	95% CI		
Raw ground turkey	<i>Listeria monocytogenes</i> TCC 1272	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	<i>Listeria monocytogenes</i> TCC 856	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.45, 0.45)
		1.26 (0.79, 2.07)	20	18	0.90	(0.70, 0.97)	18	0.90	(0.70, 0.97)	0.00	(-0.21, 0.21)
		3.00 (1.311, 6.89)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Bagged lettuce	<i>Listeria monocytogenes</i> TCC 1220	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.45, 0.45)
		1.00 (0.66, 1.55)	20	13	0.65	(0.43, 0.82)	13	0.65	(0.43, 0.82)	0.00	(-0.21, 0.21)
		1.13 (0.57, 2.25)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Stainless steel surface 4" x 4"	<i>Listeria monocytogenes</i> TCC 813 and <i>Enterococcus faecalis</i> CIP100750 X10	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.45, 0.45)
		N/A	20	7	0.35	(0.18, 0.57)	7	0.35	(0.18, 0.57)	0.00	(-0.21, 0.21)
		N/A	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)

^aMPN= 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.

^bN=Number of test portions.

^cX=Number of positive test portions.

^dPOD_{CC}=Candidate method confirmed positive outcomes divided by the total number of portions.

^ePOD_{RC}=Reference confirmation, positive outcomes divided by the total number of portions.

^fdPOD_{CC}=Difference between the candidate confirmed result and the reference confirmation result POD values.

^g95% CI=If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

^hN/A=Not applicable.

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

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

^aMPN= 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.

^bN=Number of test portions.

^cX=Number of positive test portions.

^dPOD_c=Candidate method confirmed positive outcomes divided by the total number of portions.

^ePOD_R=Reference method confirmed positive outcomes divided by the total number of portions.

^fdPOD_c=Difference between the candidate confirmed result and the reference method confirmed result POD values.

^g95% CI=If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

^hN/A=Not applicable.

DISCUSSION OF MODIFICATION Approved April 2018

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 (10)

Inclusivity

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

Exclusivity

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

Matrix testing

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

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

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

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

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

Table 1. Inclusivity of the SureTect *Listeria monocytogenes* PCR Assay (10)

ID	<i>Listeria</i> species	Serotype	Source	Origin	SureTect <i>Listeria monocytogenes</i> result
812	<i>Listeria monocytogenes</i>	3a	Environmental	TCC	Positive
813	<i>Listeria monocytogenes</i>	3a	Environmental	TCC	Positive
840	<i>Listeria monocytogenes</i>	3a	Butter	TCC	Positive
856	<i>Listeria monocytogenes</i>	Untyped	Unknown food source ^a	TCC	Positive
857	<i>Listeria monocytogenes</i>	Untyped	Unknown food source	TCC	Positive
858	<i>Listeria monocytogenes</i>	1/2c	Clinical sample	TCC	Positive
859	<i>Listeria monocytogenes</i>	Untyped	Tartare de Salmon	TCC	Positive
860	<i>Listeria monocytogenes</i>	1/2a	Poultry	TCC	Positive
863	<i>Listeria monocytogenes</i>	4d	Sheep	TCC	Positive
864	<i>Listeria monocytogenes</i>	4b	Meningitis	TCC	Positive
865	<i>Listeria monocytogenes</i>	4b	CSF: Meningitis	TCC	Positive
866	<i>Listeria monocytogenes</i>	Untyped	Unknown ATCC isolate	TCC	Positive
867	<i>Listeria monocytogenes</i>	2	CSF: Clinical	TCC	Positive
868	<i>Listeria monocytogenes</i>	4e	Chicken	TCC	Positive

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

^a Unknown food source = isolated by Thermo Fisher Scientific laboratories in Basingstoke, Hampshire, UK

Table 3. Exclusivity of the SureTect Listeria monocytogenes PCR Assay (10)

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

Table 5. SureTect Listeria monocytogenes PCR Assay Results: candidate presumptive PCR result vs candidate method confirmed (via reference method) (10)

Matrix ^o	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	PODC _R ^f	95% CI		
Sliced Deli Turkey	TCC 1227 <i>L. monocytogenes</i>	N/A ⁱ	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.3	20	8	0.40	0.22, 0.61	2	0.10	0.03, 0.30	0.30	0.03, 0.53
		0.4	5	3	0.60	0.23, 0.88	0	0.00	0.00, 0.43	0.60	0.03, 0.88
Bagged Lettuce	TCC 1220 <i>L. monocytogenes</i>	N/A ⁱ	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.2	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.28, 0.28
		1.1	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
2% Pasteurized Milk	TCC 0840 <i>L. monocytogenes</i>	N/A ⁱ	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.2	20	7	0.15	0.05, 0.36	8	0.40	0.22, 0.61	-0.25	-0.48, 0.03
		0.67	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
Stainless Steel Sponge (4"x 4")	TCC 0813 <i>L. monocytogenes</i> / 10X <i>E. faecalis</i>	N/A ⁱ	10	0	0.00	0.00, 0.28	0	0.00	0.00, 0.28	0.00	-0.28, 0.28
		N/A	20	14	0.70	0.48, 0.85	14	0.70	0.48, 0.85	0.00	-0.27, 0.27
		N/A	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Stainless Steel Swab (1"x 1")	TCC 1205 <i>L. monocytogenes</i> 1/2b	N/A ⁱ	10	0	0.00	0.00, 0.28	0	0.00	0.00, 0.28	0.00	-0.28, 0.28
		N/A	20	11	0.50	0.30, 0.70	11	0.55	0.34, 0.74	-0.05	-0.33, 0.24
		N/A	5	4	0.80	0.38, 1.00	5	0.80	0.38, 1.00	0.00	-0.47, 0.47

^o 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

ⁱ N/A = Not applicable

Table 6. SureTect Listeria monocytogenes PCR Assay Results: candidate presumptive PCR result vs candidate method confirmed (via candidate method) (10)

Matrix ^g	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 1227 <i>L. monocytogenes</i>	N/A ⁱ	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.3	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.28, 0.28
		0.5	5	3	0.60	0.23, 0.88	3	0.60	0.23, 0.88	0.00	-0.46, 0.46
Bagged Lettuce	TCC 1220 <i>L. monocytogenes</i>	N/A ⁱ	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.2	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.44, 0.07
		1.1	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
2% Pasteurized Milk	TCC 0840 <i>L. monocytogenes</i>	N/A ⁱ	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.28, 0.28
		0.2	20	7	0.15	0.05, 0.36	7	0.35	0.18, 0.57	-0.20	-0.27, 0.27
		0.67	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.43, 0.43
Stainless Steel Sponge (4"x 4")	TCC 0813 <i>L. monocytogenes</i> / 10X <i>E. faecalis</i>	N/A ⁱ	10	0	0.00	0.00, 0.28	0	0.00	0.00, 0.28	0.00	-0.28, 0.28
		N/A	20	14	0.70	0.48, 0.85	14	0.70	0.48, 0.85	-0.05	-0.33, 0.24
		N/A	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Stainless Steel Swab (1"x 1")	TCC 1205 <i>L. monocytogenes</i> 1/2b	N/A ⁱ	10	0	0.00	0.00, 0.28	0	0.00	0.00, 0.28	0.00	-0.28, 0.28
		N/A	20	11	0.50	0.30, 0.70	11	0.55	0.34, 0.74	-0.05	-0.33, 0.24
		N/A	5	5	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47

^g 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

ⁱ N/A = Not applicable

Table 7. SureTect Listeria monocytogenes PCR Assay Results: candidate method confirmed (via the candidate method) vs Reference method POD summary (10)

Matrix ^g	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 1227 <i>L. monocytogenes</i>	N/A ⁱ	5	0	0.00	0.00, 0.43	1	0.20	0.00, 0.62	-0.20	-0.62, 0.28
		0.30	20	8	0.40	0.22, 0.61	9	0.45	0.26, 0.66	-0.05	-0.33, 0.24
		0.50	5	3	0.60	0.23, 0.88	1	0.20	0.00, 0.62	0.40	-0.16, 0.75
Bagged Lettuce	TCC 1220 <i>L. monocytogenes</i>	N/A ⁱ	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.20	20	10	0.50	0.30, 0.70	14	0.70	0.48, 0.85	-0.20	-0.45, 0.10
		1.10	5	4	0.80	0.38, 1.00	3	0.60	0.23, 0.88	0.20	-0.31, 0.62
2% Pasteurized Milk	TCC 0840 <i>L. monocytogenes</i>	N/A ⁱ	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.20	20	7	0.35	0.18, 0.57	3	0.35	0.18, 0.57	0.00	-0.28, 0.28
		0.67	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
Stainless Steel Sponge (4"x 4")	TCC 0813 <i>L. monocytogenes</i> / 10X <i>E. faecalis</i>	N/A ⁱ	10	0	0.00	0.00, 0.28	0	0.00	0.00, 0.28	0.00	-0.28, 0.28
		N/A	20	14	0.70	0.48, 0.85	15	0.70	0.48, 0.85	0.00	-0.27, 0.27
		N/A	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Stainless Steel Swab (1"x 1")	TCC 1205 <i>L. monocytogenes</i> 1/2b	N/A ⁱ	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

^g 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 POD_{cc} = Candidate method confirmed via the candidate method positive outcomes divided by the total number of trials

^f POD_R = Reference method divided by the total number of trials

^g dPOD_{cc} = 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

ⁱ N/A = Not applicable

Table 8. SureTect Listeria monocytogenes PCR Assay Results: candidate method confirmed (via the reference method) vs Reference method POD summary (10)

Matrix ^o	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 1227 <i>L. monocytogenes</i>	N/A ⁱ	5	0	0.00	0.00, 0.43	1	0.20	0.00, 0.62	-0.20	-0.62, 0.28
		0.3	20	2	0.10	0.03, 0.30	9	0.45	0.26, 0.66	-0.35	-0.57, -0.07
		0.5	5	0	0.00	0.00, 0.43	1	0.20	0.00, 0.62	-0.20	-0.62, 0.28
Bagged Lettuce	TCC 1220 <i>L. monocytogenes</i>	N/A ⁱ	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.2	20	10	0.50	0.30, 0.70	14	0.70	0.48, 0.85	-0.20	-0.45, 0.10
		1.1	5	4	0.80	0.38, 1.00	3	0.60	0.23, 0.88	0.20	-0.31, 0.62
2% Pasteurized Milk	TCC 0840 <i>L. monocytogenes</i>	N/A ⁱ	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.2	20	8	0.40	0.22, 0.61	7	0.35	0.18, 0.57	0.00	-0.23, 0.32
		0.67	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
Stainless Steel Sponge (4"x 4")	TCC 0813 <i>L. monocytogenes</i> / 10X <i>E. faecalis</i>	N/A ⁱ	10	0	0.00	0.00, 0.28	0	0.00	0.00, 0.28	0.00	-0.28, 0.28
		N/A	20	14	0.70	0.48, 0.85	14	0.70	0.48, 0.85	0.00	-0.27, 0.27
		N/A	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Stainless Steel Swab (1"x 1")	TCC 1205 <i>L. monocytogenes</i> 1/2b	N/A ⁱ	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

^o 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 POD_{CR} = Candidate method confirmed (via reference method) positive outcomes divided by the total number of trials

^f POD_R = Reference method positive outcomes divided by the total number of trials

^g dPOD_{CR} = 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

ⁱ N/A = Not applicable

REFERENCES CITED

1. Cloke, Jonathan, Leon-Velarde, Carlos, Larson, Nathan, Dave, Keron, Evans, Katharine, Crabtree, David, Hughes, Annette, Simpson, Helen, Holopainen, Jani, Wickstrand, Nina, and Kauppinen, Mikko., Evaluation of the Thermo Scientific™ SureTect™ Listeria monocytogenes Assay, AOAC® *Performance Tested*SM certification number 061302.
2. AOAC Research Institute Validation Outline for Thermo Scientific™ SureTect™ Listeria monocytogenes PCR Assay, Approved – June 2013.
3. 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
4. Thermo Scientific SureTect Listeria monocytogenes PCR assay. Product Insert/IFU Version: D11908_01
5. Thermo Scientific SureTect Software Manual. Version 1.1.
6. AOAC INTERNATIONAL Method Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces. 2012. AOAC International, Gaithersburg, MD, USA http://www.aoac.org/vmeth/AOAC_Validation_Guidelines_for_Food_Microbiology-Prepub_version.pdf
7. Least Cost Formulations, Ltd. , MPN Calculator-Version 1.6 <http://www.lcfltd.com/customer/LCFMPNCalculator.exe>
8. Cloke, J., Evans, K., Crabtree, D., Hughes, A., and Simpson, H., Method Modification of the Thermo Scientific SureTect Listeria monocytogenes Assay for raw meat, dairy, produce, and seafood. AOAC® *Performance Tested*SM certification number 061302 Approved February 2015.
9. Vaahtoranta, L., Palomaki, J., Artimo, P., Haug, F., Liikanen, M., and Koskela, S., Validation of the Applied Biosystems 7500 Fast Instrument for Detection of *Listeria monocytogenes* with the SureTect Listeria monocytogenes PCR Assay, AOAC® *Performance Tested*SM certification number 061302 approved October 2015.
10. Williams, J., Evans, K., Crabtree, D., Hughes, A., Cooper, C., Leak, D., Dziegiel, A., Method Modification of the Thermo Scientific SureTect Listeria species PCR Assay and the Listeria monocytogenes PCR Assay for use with the Applied Biosystems QuantStudio 5 PCR Instrument, AOAC® *Performance Tested*SM certification number 061302 approved October 2018.
11. ISO Horizontal method for the detection of *L. monocytogenes* and *L. species* in ISO 11290-1:2017