



# CERTIFICATION

**AOAC<sup>®</sup> Performance Tested<sup>SM</sup>**

Certificate No.

**071304**

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

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

manufactured by

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

This method has been evaluated in the AOAC<sup>®</sup> *Performance Tested Methods<sup>SM</sup>* Program, and found to perform as stated by the manufacturer contingent to the comments contained in the manuscript. This certificate means that an AOAC<sup>®</sup> Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC *Performance Tested<sup>SM</sup>* 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.

A handwritten signature in black ink that reads "Scott Coates".

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Scott Coates, Senior Director  
Signature for AOAC Research Institute

December 12, 2018

\_\_\_\_\_  
Date

**METHOD AUTHORS**

**ORIGINAL VALIDATION:** Jonathan Cloke, Katharine Evans, David Crabtree, Annette Hughes, Helen Simpson, Jani Holopainen, Nina Wickstrand, Mikko Kauppinen, Carlos Leon-Velarde, Nathan Larson, Keron Dave

**MODIFICATION SEPTEMBER 2015:** Jonathan Cloke, Katharine Evans, David Crabtree, Annette Hughes, Helen Simpson, Jani Holopainen, Nina Wickstrand, Mikko Kauppinen, Carlos Leon-Velarde, Nathan Larson, Keron Dave

**MODIFICATION NOVEMBER 2015:** Jonathan Cloke, Julia Arizanova, David Crabtree, Helen Simpson, Katharine Evans, Laura Vaahoranta, Jukkapekka Palomaki, Paulus Artimo, Feng Huang, Maria Liikanen, and Suvi Koskela

**MODIFICATION OCTOBER 2018** – Jessica Williams, Katharine Evans, David Crabtree, Annette Hughes, Charlotte Cooper, Dean Leak, Agata Dziejgiel

**SUBMITTING COMPANY**

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

**KIT NAME(S)**

Thermo Scientific™ SureTect™ Listeria species PCR Assay

**CATALOG NUMBERS**

PT0200A

**INDEPENDENT LABORATORY****Original Validation and September 2015 Modification:**

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<sup>1,4</sup>, Elliot Ryser<sup>2</sup>, Mark Carter<sup>3</sup>

<sup>1</sup> US FDA CFSAN, College Park, MD, USA

<sup>2</sup> Michigan State University, East Lansing, MI, USA

<sup>3</sup> MC2E Inc., New Hope, PA, USA

<sup>4</sup> Modification: September 2015; November 2015; October 2018

**APPLICABILITY OF METHOD**

Target organism – *Listeria* species

Matrices – (25 g) raw ground beef, pork frankfurters, salami, cooked sliced ham, cooked sliced turkey, fresh bagged spinach, cantaloupe, processed cheese, smoked salmon, cooked prawns (sponge 4" x 4" enriched in 225 mL of 24 LEB supplemented with 24 LEB Selective supplement and 10 mL of LEB Buffer supplement) stainless steel, and plastic

**MODIFICATION SEPTEMBER 2015 (25 g):** ground pork, bagged lettuce, raw ground turkey, raw pork sausages, pasteurized 2% milk, raw cod, pasteurized brie cheese, and ice cream

Performance claims - Performance equivalent to the reference method.

**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)

**ORIGINAL CERTIFICATION DATE**

July 25, 2013

**CERTIFICATION RENEWAL RECORD**

Renewed Annually through December 2018

**METHOD MODIFICATION RECORD**

1. September 2015
2. November 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 approved
2. Certification of ABI 7500 Fast Instrument
3. Editorial changes to insert and labels
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 Tested<sup>SM</sup> License Number, 071304 this method is distributed by:

NONE

Under this AOAC® Performance Tested<sup>SM</sup> License Number, 071304 this method is distributed as:

NONE

#### PRINCIPLE OF THE METHOD (1)

The Thermo Scientific™ SureTect™ *Listeria* species PCR assay is a Real-Time Polymerase Chain Reaction (PCR) test intended to be used in conjunction with the Thermo Scientific PikoReal™ Real-Time PCR Instrument and the SureTect Software for the detection and identification of *Listeria* species in food and environmental samples (4, 5).

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

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

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

The data presented and discussed in this report, within the statistical uncertainty of the analysis, supports the product claims of the SureTect *Listeria monocytogenes* assay for recovery of *L. monocytogenes* from fresh cantaloupe, salami, smoked salmon, fresh bagged spinach, cooked sliced turkey, pork frankfurters, ice cream, cooked prawns, processed cheese and raw ground beef as well as stainless steel and plastic surfaces. Additional studies conducted as part of the validation show that the assay has excellent inclusivity and is unaffected by high levels of non-target bacteria. 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 <sup>a</sup>	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	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 innocua</i>	6a	862	Cow brain ATCC® 33090™	Positive
<i>Listeria innocua</i>	4ab	2185		Positive

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<i>Listeria innocua</i>	6b	2187		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	Positive
<i>Listeria welshimeri</i>	Unknown	1188	Pastrami	Positive
<i>Listeria welshimeri</i>	Unknown	1189	Food	Positive
<i>Listeria welshimeri</i>	6b	2188		Positive
<i>Listeria welshimeri</i>	4c	2189		Positive
<i>Listeria seeligeri</i>	Unknown	1190	Cheese	Positive
<i>Listeria seeligeri</i>	Unknown	1191	Food	Positive
<i>Listeria seeligeri</i>	Unknown	1192	Environmental	Positive
<i>Listeria seeligeri</i>	Unknown	1193	Cannelloni	Positive
<i>Listeria seeligeri</i>	Unknown	1194	Coleslaw	Positive
<i>Listeria seeligeri</i>	1/2b	2190		Positive
<i>Listeria seeligeri</i>	6b	2191		Positive

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

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

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

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

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

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

Matrix	Strain	MPN <sup>a</sup> /test portion	N <sup>b</sup>	SureTect Method Presumptive			SureTect Confirmation method result			dPODcp <sup>f</sup>	95% CI <sup>g</sup>
				χ <sup>c</sup>	POD <sub>cp</sub> <sup>d</sup>	95% CI	X	POD <sub>cc</sub> <sup>e</sup>	95% CI		
Plastic	<i>L. monocytogenes</i> TCC 812	N/A <sup>h</sup>	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		N/A	20	16	0.80	(0.58, 0.92)	16	0.80	(0.58, 0.92)	0.00	(-0.25, 0.25)
		N/A	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Stainless steel	<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	10	0.50	(0.30, 0.70)	11	0.55	(0.34, 0.74)	-0.05	(-0.33, 0.24)
		N/A	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Pork frankfurters	<i>Listeria innocua</i> TCC 1177	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.74 (0.43, 1.33)	20	7	0.35	(0.18, 0.57)	7	0.35	(0.18, 0.57)	0.00	(-0.28, 0.28)
		1.18 (0.51, 2.72)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Smoked salmon	<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)
		0.6 (0.36, 0.98)	20	7	0.35	(0.18, 0.57)	8	0.40	(0.22, 0.61)	-0.05	(-0.32, 0.23)
		1.3 (0.58, 2.69)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Processed cheese	<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, 0.96)	20	5	0.25	(0.11, 0.47)	6	0.30	(0.15, 0.52)	-0.05	(-0.31, 0.22)
		1.48 (0.65, 3.37)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Spinach	<i>Listeria innocua</i> TCC 1180	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.38 (0.83, 2.37)	20	11	0.55	(0.34, 0.74)	11	0.55	(0.34, 0.74)	0.00	(-0.28, 0.28)
		4.37 (1.71, 11.19)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Cantaloupe	<i>Listeria monocytogenes</i> TCC 1217	N/A <sup>h</sup>	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.7 (0.39, 1.29)	20	13	0.65	(0.43, 0.82)	13	0.65	(0.43, 0.82)	0.00	(-0.28, 0.28)
		3 (1.25, 7.00)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)

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Cooked prawns (chilled)	<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)
		1.9 (0.84, 4.18)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Cooked sliced turkey (chilled)	<i>Listeria innocua</i> TCC 1178	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.69 (0.39, 1.25)	20	9	0.45	(0.26, 0.66)	9	0.45	(0.26, 0.66)	0.00	(-0.28, 0.28)
		1.02 (0.46, 2.25)	5	5	1.00	(0.57, 1.00)	4	0.80	(0.38, 1.00)	0.20	(-0.28, 0.62)
Raw ground beef (12% fat)	<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)
		1.13 (0.71, 1.88)	20	13	0.65	(0.43, 0.82)	13	0.65	(0.43, 0.82)	0.00	(-0.28, 0.28)
		1.88 (0.84, 4.18)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Salami	<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	9	0.45	(0.26, 0.66)	11	0.55	(0.34, 0.74)	-0.10	(-0.37, 0.19)
		0.40 (0.23, 0.91)	5	2	0.40	(0.12, 0.77)	3	0.60	(0.23, 0.88)	-0.20	(-0.60, 0.32)
Cooked sliced ham (chilled)	<i>Listeria welshimeri</i> TCC 1188	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.02 (0.592, 2.07)	20	13	0.65	(0.43, 0.82)	14	0.70	(0.48, 0.85)	-0.05	(-0.32, 0.23)
		2.96 (1.25, 7.00)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Bagged spinach <sup>i</sup>	<i>Listeria innocua</i> ATCC 33090	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.66 (0.37, 1.10)	20	14	0.70	(0.48, 0.85)	14	0.70	(0.48, 0.85)	0.00	(-0.26, 0.26)
		2.97 (1.25, 7.00)	5	5	1.00	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0.00	(-0.43, 0.43)
Pork frankfurters <sup>i</sup>	<i>Listeria innocua</i> ATCC 33091	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.38 (0.83, 2.38)	20	12	0.60	(0.38, 0.78)	12	0.60	(0.38, 0.78)	0.00	(-0.28, 0.28)
		2.97 (1.25, 7.00)	5	5	1.00	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0.00	(-0.43, 0.43)
Stainless steel 4"x4" <sup>i</sup>	<i>Listeria monocytogenes</i> LI7163 1/2a 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)

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

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

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

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

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

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

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

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

<sup>i</sup>Independent Laboratory Study



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

Matrix	Strain	MPN <sup>a</sup> /test portion	N <sup>b</sup>	SureTect Presumptive Result			Reference Confirmation (CC2)			dPOD <sub>cp</sub> <sup>f</sup>	95% CI <sup>g</sup>
				X <sup>c</sup>	POD <sub>cp</sub> <sup>d</sup>	95% CI	X	POD <sub>cc2</sub> <sup>e</sup>	95% CI		
Plastic	<i>L. monocytogenes</i> TCC 812	N/A <sup>h</sup>	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		N/A	20	16	0.80	(0.58, 0.92)	16	0.80	(0.58, 0.92)	0.00	(-0.25, 0.25)
		N/A	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Stainless steel	<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	10	0.50	(0.30, 0.70)	11	0.55	(0.34, 0.74)	-0.05	(-0.33, 0.24)
		N/A	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Pork frankfurters	<i>Listeria innocua</i> TCC 1177	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.74 (0.43, 1.33)	20	7	0.35	(0.18, 0.57)	7	0.35	(0.18, 0.57)	0.00	(-0.28, 0.28)
		1.18 (0.51, 2.72)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Smoked salmon	<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)
		0.6 (0.36, 0.98)	20	7	0.35	(0.18, 0.57)	9	0.45	(0.26, 0.66)	-0.10	(-0.37, 0.19)
		1.3 (0.58, 2.69)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Processed cheese	<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, 0.96)	20	5	0.25	(0.11, 0.47)	6	0.30	(0.15, 0.52)	-0.05	(-0.31, 0.22)
		1.48 (0.65, 3.37)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Spinach	<i>Listeria innocua</i> TCC 1180	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.38 (0.83, 2.37)	20	11	0.55	(0.34, 0.74)	12	0.60	(0.39, 0.78)	-0.05	(-0.33, 0.24)
		4.37 (1.71, 11.19)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Cantaloupe	<i>Listeria monocytogenes</i> TCC 1217	N/A <sup>h</sup>	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.7 (0.39, 1.29)	20	13	0.65	(0.43, 0.82)	13	0.65	(0.43, 0.82)	0.00	(-0.28, 0.28)
		3 (1.25, 7.00)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)

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Cooked prawns (chilled)	<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)
		1.9 (0.84, 4.18)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Cooked sliced turkey (chilled)	<i>Listeria innocua</i> TCC 1178	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.69 (0.39, 1.25)	20	9	0.45	(0.26, 0.66)	9	0.45	(0.26, 0.66)	0.00	(-0.28, 0.28)
		1.02 (0.46, 2.25)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Raw ground beef (12% fat)	<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)
		1.13 (0.71, 1.88)	20	13	0.65	(0.43, 0.82)	14	0.70	(0.48, 0.85)	-0.05	(-0.32, 0.23)
		1.88 (0.84, 4.18)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Salami	<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	9	0.45	(0.26, 0.66)	11	0.55	(0.34, 0.74)	-0.10	(-0.37, 0.19)
		0.40 (0.23, 0.91)	5	2	0.40	(0.12, 0.77)	3	0.60	(0.23, 0.88)	-0.20	(-0.60, 0.32)
Cooked sliced ham (chilled)	<i>Listeria welshimeri</i> TCC 1188	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.02 (0.592, 2.07)	20	13	0.65	(0.43, 0.82)	14	0.70	(0.48, 0.85)	-0.05	(-0.32, 0.23)
		2.96 (1.25, 7.00)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Bagged spinach <sup>i</sup>	<i>Listeria innocua</i> ATCC 33090	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.66 (0.37, 1.10)	20	14	0.70	(0.48, 0.85)	14	0.70	(0.48, 0.85)	0.00	(-0.26, 0.26)
		2.97 (1.25, 7.00)	5	5	1.00	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0.00	(-0.43, 0.43)
Pork frankfurters <sup>j</sup>	<i>Listeria innocua</i> ATCC 33091	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.38 (0.83, 2.38)	20	12	0.60	(0.38, 0.78)	12	0.60	(0.38, 0.78)	0.00	(-0.28, 0.28)
		2.97 (1.25, 7.00)	5	5	1.00	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0.00	(-0.43, 0.43)
Stainless steel 4"x4" <sup>i</sup>	<i>Listeria monocytogenes</i> L17163 1/2a 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)

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

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

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

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

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

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

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

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

<sup>i</sup>Independent Laboratory Study

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

Matrix	Strain	MPN <sup>h</sup> /test portion	N <sup>b</sup>	SureTect Confirmation Method (Microbact) (CC)			Reference Confirmation (CC2)			dPODcc <sup>f</sup>	95% CI <sup>g</sup>
				X <sup>c</sup>	POD <sub>cc</sub> <sup>d</sup>	95% CI	X	POD <sub>cc2</sub> <sup>e</sup>	95% CI		
Plastic	<i>L. monocytogenes</i> TCC 812	N/A <sup>h</sup>	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		N/A	20	16	0.80	(0.58, 0.92)	16	0.80	(0.58, 0.92)	0.00	(-0.25, 0.25)
		N/A	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Stainless steel	<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	(-0.43, 0.43)
		N/A	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Pork frankfurters	<i>Listeria innocua</i> TCC 1177	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.74 (0.43, 1.33)	20	7	0.35	(0.18, 0.57)	7	0.35	(0.18, 0.57)	0.00	(-0.28, 0.28)
		1.18 (0.51, 2.72)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Smoked salmon	<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)
		0.6 (0.36, 0.98)	20	8	0.40	(0.22, 0.61)	9	0.45	(0.26, 0.66)	-0.05	(-0.33, 0.24)
		1.3 (0.58, 2.69)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Processed cheese	<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, 0.96)	20	6	0.30	(0.15, 0.52)	6	0.30	(0.15, 0.52)	0.00	(-0.47, 0.47)
		1.48 (0.65, 3.37)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Spinach	<i>Listeria innocua</i> TCC 1180	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.38 (0.83, 2.37)	20	11	0.55	(0.34, 0.74)	12	0.60	(0.39, 0.78)	-0.05	(-0.33, 0.24)
		4.37 (1.71, 11.19)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Cantaloupe melon	<i>Listeria monocytogenes</i> TCC 1217	N/A <sup>h</sup>	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.7 (0.39, 1.29)	20	13	0.65	(0.43, 0.82)	13	0.65	(0.43, 0.82)	0.00	(-0.28, 0.28)
		3 (1.25, 7.00)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)

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Cooked prawns (chilled)	<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)
		1.9 (0.84, 4.18)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Cooked sliced turkey (chilled)	<i>Listeria innocua</i> TCC 1178	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.69 (0.39, 1.25)	20	9	0.45	(0.26, 0.66)	9	0.45	(0.26, 0.66)	0.00	(-0.28, 0.28)
		1.02 (0.46, 2.25)	5	4	0.80	(0.38, 1.00)	5	1.00	(0.57, 1.00)	-0.20	(-0.62, 0.28)
Raw ground beef (12% fat)	<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)
		1.13 (0.71, 1.88)	20	13	0.65	(0.43, 0.82)	14	0.70	(0.48, 0.85)	-0.05	(-0.32, 0.23)
		1.88 (0.84, 4.18)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Salami	<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	3	0.60	(0.23, 0.88)	3	0.60	(0.23, 0.88)	0.00	(-0.46, 0.46)
Cooked sliced ham (chilled)	<i>Listeria welshimeri</i> TCC 1188	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.02 (0.592, 2.07)	20	14	0.70	(0.48, 0.85)	14	0.70	(0.48, 0.85)	0.00	(-0.27, 0.27)
		2.96 (1.25, 7.00)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Bagged spinach <sup>i</sup>	<i>Listeria innocua</i> ATCC 33090	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.66 (0.37, 1.10)	20	14	0.70	(0.48, 0.85)	14	0.70	(0.48, 0.85)	0.00	(-0.26, 0.26)
		2.97 (1.25, 7.00)	5	5	1.00	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0.00	(-0.43, 0.43)
Pork frankfurters <sup>i</sup>	<i>Listeria innocua</i> ATCC 33091	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.38 (0.83, 2.38)	20	12	0.60	(0.38, 0.78)	12	0.60	(0.38, 0.78)	0.00	(-0.28, 0.28)
		2.97 (1.25, 7.00)	5	5	1.00	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0.00	(-0.43, 0.43)
Stainless steel 4"x4" <sup>ii</sup>	<i>Listeria monocytogenes</i> LI7163 1/2a 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)

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

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

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

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

<sup>e</sup>PODcc<sub>2</sub>=Reference method confirmation positive outcomes divided by the total number of portions

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

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

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

<sup>i</sup>Independent Laboratory Study

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

Matrix	Strain	MPN <sup>a</sup> /test portion	N <sup>b</sup>	SureTect Method Confirmed Result (C)			Reference Method (R)			dPOD <sup>c</sup>	95% CI <sup>e</sup>
				X <sup>c</sup>	POD <sup>d</sup>	95% CI	X	POD <sup>e</sup>	95% CI		
Plastic	<i>L. monocytogenes</i> TCC 812	N/A <sup>h</sup>	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		N/A	20	16	0.80	(0.58, 0.92)	15	0.75	(0.53, 0.89)	0.05	(-0.21, 0.30)
		N/A	5	4	0.80	(0.38, 1.00)	5	0.80	(0.38, 1.00)	-0.20	(-0.62, 0.28)
Stainless steel	<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, 0.24)
		N/A	5	5	1.00	(0.57, 1.00)	4	0.80	(0.38, 1.00)	0.20	(-0.28, 0.62)
Pork frankfurters	<i>Listeria innocua</i> TCC 1177	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.74 (0.43, 1.33)	20	7	0.35	(0.18, 0.57)	10	0.50	(0.30, 0.70)	-0.15	(-0.41, 0.15)
		1.18 (0.51, 2.72)	5	5	1.00	(0.57, 1.00)	4	0.80	(0.38, 1.00)	0.20	(-0.28, 0.62)
Smoked salmon	<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)
		0.6 (0.36, 0.98)	20	8	0.40	(0.22, 0.61)	8	0.40	(0.22, 0.61)	0.00	(-0.28, 0.28)
		1.3 (0.58, 2.69)	5	4	0.80	(0.38, 1.00)	2	0.40	(0.12, 0.77)	0.40	(-0.16, 0.75)
Processed cheese	<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, 0.96)	20	6	0.30	(0.15, 0.52)	9	0.45	(0.25, 0.66)	-0.15	(-0.41, 0.14)
		1.48 (0.65, 3.37)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Spinach	<i>Listeria innocua</i> TCC 1180	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.38 (0.83, 2.37)	20	11	0.55	(0.34, 0.74)	15	0.75	(0.53, 0.89)	-0.20	(-0.45, 0.09)
		4.37 (1.71, 11.19)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Cantaloupe	<i>Listeria monocytogenes</i> TCC 1217	N/A <sup>h</sup>	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.7 (0.39, 1.29)	20	13	0.65	(0.43, 0.82)	11	0.55	(0.34, 0.74)	0.10	(-0.19, 0.37)
		3 (1.25, 7.00)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)

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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)	14	0.70	(0.48, 0.85)	0.05	(-0.22, 0.31)
		1.9 (0.84, 4.18)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Cooked sliced turkey (chilled)	<i>Listeria innocua</i> TCC 1178	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.69 (0.39, 1.25)	20	9	0.45	(0.26, 0.66)	10	0.50	(0.30, 0.70)	-0.05	(-0.33, 0.24)
		1.02 (0.46, 2.25)	5	4	0.80	(0.38, 1.00)	2	0.40	(0.12, 0.77)	0.40	(-0.16, 0.75)
Raw ground beef (12% fat)	<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)
		1.13 (0.71, 1.88)	20	13	0.65	(0.43, 0.82)	14	0.70	(0.48, 0.85)	-0.05	(-0.32, 0.23)
		1.88 (0.84, 4.18)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Salami	<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	3	0.60	(0.23, 0.88)	4	0.80	(0.38, 1.00)	-0.20	(-0.62, 0.31)
Cooked sliced ham (chilled)	<i>Listeria welshimeri</i> TCC 1188	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.02 (0.592, 2.07)	20	14	0.70	(0.48, 0.85)	15	0.75	(0.53, 0.89)	-0.05	(-0.31, 0.22)
		2.96 (1.25, 7.00)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Bagged spinach <sup>i</sup>	<i>Listeria innocua</i> ATCC 33090	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.66 (0.37, 1.10)	20	14	0.70	(0.48, 0.85)	9	0.45	(0.25, 0.65)	0.25	(-0.05, 0.49)
		2.97 (1.25, 7.00)	5	5	1.00	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0.00	(-0.43, 0.43)
Pork frankfurters <sup>i</sup>	<i>Listeria innocua</i> ATCC 33091	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.38 (0.83, 2.38)	20	12	0.60	(0.38, 0.78)	15	0.75	(0.53, 0.88)	-0.15	(-0.40, 0.13)
		2.97 (1.25, 7.00)	5	5	1.00	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0.00	(-0.43, 0.43)
Stainless steel 4"x4" <sup>ii</sup>	<i>Listeria monocytogenes</i> LI7163 1/2a 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	(-0.43, 0.43)

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

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

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

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

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

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

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

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

<sup>i</sup>Independent Laboratory Study

**DISCUSSION OF MODIFICATION APPROVED SEPTEMBER 2015 (8):**

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

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

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

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

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

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

Matrix	Strain	MPN <sup>a</sup> /test portion	N <sup>b</sup>	SureTect Method Presumptive			SureTect Confirmation Method Result			dPOD <sub>cp</sub> <sup>f</sup>	95% CI <sup>g</sup>
				X <sup>c</sup>	POD <sub>cp</sub> <sup>d</sup>	95% CI	X	POD <sub>cc</sub> <sup>e</sup>	95% CI		
Ice cream	<i>L. ivanovii</i> TCC 1182	N/A <sup>h</sup>	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.37 (0.18, 0.65)	20	6	0.30	(0.15, 0.52)	7	0.35	(0.35, 0.57)	-0.05	(-0.32, 0.23)
		1.35 (0.61, 2.98)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Raw ground pork	<i>L. 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)
Bagged lettuce	<i>L. ivanovii</i> TCC 1572	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.13 (0.717, 1.81)	20	13	0.65	(0.43, 0.82)	15	0.75	(0.53, 0.89)	-0.10	(-0.36, 0.18)
		4.73 (1.71, 11.19)	5	4	0.80	(0.38, 1.00)	5	1.00	(0.57, 1.00)	-0.20	(-0.62, 0.28)
Bagged lettuce <sup>i</sup>	<i>L. ivanovii</i> TCC 1572	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.43 (0.28, 0.74)	20	7	0.35	(0.18, 0.57)	8	0.40	(0.22, 0.61)	-0.05	(-0.32, 0.23)
		4.38 (1.72, 11.15)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Raw ground turkey	<i>L. monocytogenes</i> TCC 1227	N/A <sup>h</sup>	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.59 (0.31, 1.06)	20	8	0.40	(0.22, 0.61)	10	0.50	(0.30, 0.70)	-0.10	(-0.37, 0.19)
		0.95 (0.47, 1.91)	5	3	0.60	(0.23, 0.88)	4	0.80	(0.38, 1.00)	-0.20	(-0.62, 0.31)
Raw ground turkey <sup>i</sup>	<i>L. monocytogenes</i> TCC 1227	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.69 (0.39, 1.14)	20	10	0.50	(0.30, 0.70)	10	0.50	(0.30, 0.70)	0.00	(-0.28, 0.28)
		4.38 (1.72, 11.15)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Raw pork sausage	<i>L. 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>L. 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)
Pasteurized brie cheese	<i>L. seeligeri</i> TCC 2190	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.13	20	11	0.55	(0.34, 0.74)	14	0.70	(0.48, 0.85)	-0.15	(-0.41, 0.14)



		(0.02, 0.26)									
		0.33 (0.14, 0.75)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Pasteurized 2% fat milk	<i>L. innocua</i> TCC 1180	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.50 (0.90, 2.56)	20	10	0.50	(0.30, 0.70)	12	0.60	(0.39, 0.78)	-0.10	(-0.37, 0.19)
		1.88 (0.84, 4.18)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Pasteurized 2% fat milk <sup>i</sup>	<i>L. innocua</i> TCC 1180	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.23 (0.77, 2.00)	20	12	0.60	(0.39, 0.78)	12	0.60	(0.39, 0.78)	0.00	(-0.28, 0.28)
		1.64 (0.79, 3.39)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Raw cod <sup>j</sup>	<i>L. 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	(0.25, 0.65)	9	0.45	(0.25, 0.65)	0.00	(-0.28, 0.28)
		4.38 (1.71, 11.19)	5	5	1.00	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0.00	(-0.43, 0.43)
Pasteurized brie cheese <sup>l</sup>	<i>L. seeligeri</i> ATCC 35967	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.54 (0.32, 0.81)	20	5	0.25	(0.11, 0.46)	5	0.25	(0.11, 0.46)	0.00	(-0.25, 0.25)
		2.97 (1.25, 7.01)	5	5	1.00	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0.00	(-0.43, 0.43)
Bagged lettuce <sup>l</sup>	<i>L. monocytogenes</i> (LI0549)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)

		0.37 (0.23, 0.59)	20	10	0.50	(0.29, 0.70)	10	0.50	(0.29, 0.70)	0.00	(-0.28, 0.28)
		2.19 (0.93, 5.12)	5	5	1.00	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0.00	(-0.43, 0.43)

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

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

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

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

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

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

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

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

<sup>i</sup>Repeat analysis of matrix.

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

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

Matrix	Strain	MPN <sup>a</sup> /test portion	N <sup>b</sup>	SureTect Presumptive Result			Reference Confirmation Method			dPOD <sub>cp</sub> <sup>f</sup>	95% CI <sup>g</sup>
				X <sup>c</sup>	POD <sub>cp</sub> <sup>d</sup>	95% CI	X	POD <sub>cc</sub> <sup>e</sup>	95% CI		
Ice cream	<i>L. ivanovii</i> TCC 1182	N/A <sup>h</sup>	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.37 (0.18, 0.65)	20	6	0.30	(0.15, 0.52)	6	0.30	(0.15, 0.52)	0.00	(-0.27, 0.27)
		1.35 (0.61, 2.98)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Raw ground pork	<i>L. 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)

Bagged lettuce	<i>L. ivanovii</i> TCC 1572	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.13 (0.717, 1.81)	20	13	0.65	(0.43, 0.82)	16	0.80	(0.58, 0.92)	-0.15	(-0.40, 0.12)
		4.73 (1.71, 11.19)	5	4	0.80	(0.38, 1.00)	5	1.00	(0.57, 1.00)	-0.20	(-0.62, 0.28)
Bagged lettuce <sup>i</sup>	<i>L. ivanovii</i> TCC 1572	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.43 (0.28, 0.74)	20	7	0.35	(0.18, 0.57)	0	0.00	(0.00, 0.16)	0.35	(0.12, 0.57)
		4.38 (1.72, 11.15)	5	5	1.00	(0.57, 1.00)	2	0.40	(0.12, 0.77)	0.60	(0.03, 0.88)
Raw ground turkey	<i>L. monocytogenes</i> TCC 1227	N/A <sup>h</sup>	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.59 (0.31, 1.06)	20	8	0.40	(0.22, 0.61)	10	0.50	(0.30, 0.70)	-0.10	(-0.37, 0.19)
		0.95 (0.47, 1.91)	5	3	0.60	(0.23, 0.88)	4	0.80	(0.38, 1.00)	-0.20	(-0.62, 0.31)
Raw ground turkey <sup>i</sup>	<i>L. monocytogenes</i> TCC 1227	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.69 (0.39, 1.14)	20	10	0.50	(0.30, 0.70)	10	0.50	(0.30, 0.70)	0.00	(-0.28, 0.28)
		4.38 (1.72, 11.15)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Raw pork sausage	<i>L. 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>L. 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)
Pasteurized brie cheese	<i>L. seeligeri</i> TCC 2190	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.13 (0.02, 0.26)	20	11	0.55	(0.34, 0.74)	15	0.75	(0.53, 0.89)	-0.20	(-0.45, 0.09)

		0.33 (0.14, 0.75)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Pasteurized 2% milk	<i>L. innocua</i> TCC 1180	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.50 (0.90, 2.56)	20	10	0.50	(0.30, 0.70)	13	0.65	(0.43, 0.82)	-0.15	(-0.41, 0.15)
		1.88 (0.84, 4.18)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Pasteurized 2% milk <sup>j</sup>	<i>L. innocua</i> TCC 1180	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.23 (0.77, 2.00)	20	12	0.60	(0.39, 0.78)	12	0.60	(0.39, 0.78)	0.00	(-0.28, 0.28)
		1.64 (0.79, 3.39)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Raw cod <sup>j</sup>	<i>L. 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	(0.25, 0.65)	9	0.45	(0.25, 0.65)	0.00	(-0.28, 0.28)
		4.38 (1.71, 11.19)	5	5	1.00	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0.00	(-0.43, 0.43)
Pasteurized brie cheese <sup>j</sup>	<i>L. seeligeri</i> ATCC 35967	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.54 (0.32, 0.81)	20	5	0.25	(0.11, 0.46)	5	0.25	(0.11, 0.46)	0.00	(-0.25, 0.25)
		2.97 (1.25, 7.01)	5	5	1.00	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0.00	(-0.43, 0.43)
Bagged lettuce <sup>j</sup>	<i>L. 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	20	10	0.50	(0.29, 0.70)	10	0.50	(0.29, 0.70)	0.00	(-0.28, 0.28)

		(0.23, 0.59)									
		2.19	5	5	1.00	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0.00	(-0.43, 0.43)
		(0.93, 5.12)									

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

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

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

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

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

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

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

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

<sup>i</sup>Repeat analysis of matrix.

<sup>j</sup>Independent Laboratory Study

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

Matrix	Strain	MPN <sup>a</sup> /test portion	N <sup>b</sup>	SureTect Confirmation Method			Reference Confirmation Method			dPOD <sub>c</sub> <sup>f</sup>	95% CI <sup>g</sup>
				X <sup>c</sup>	POD <sub>cc</sub> <sup>d</sup>	95% CI	X	POD <sub>cc2</sub> <sup>e</sup>	95% CI		
Ice cream	<i>L. ivanovii</i> TCC 1182	N/A <sup>h</sup>	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.37 (0.18, 0.65)	20	7	0.35	(0.18, 0.57)	6	0.30	(0.15, 0.52)	0.05	(-0.23, 0.32)
		1.35 (0.61, 2.98)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Raw ground pork	<i>L. 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)
Bagged lettuce	<i>L. ivanovii</i> TCC 1572	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)

		1.13 (0.717, 1.81)	20	15	0.75	(0.53, 0.89)	16	0.80	(0.58, 0.92)	-0.05	(-0.30, 0.21)
		4.73 (1.71, 11.19)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Bagged lettuce <sup>i</sup>	<i>L. ivanovii</i> TCC 1572	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.43 (0.28, 0.74)	20	8	0.40	(0.22, 0.61)	0	0.00	(0.00, 0.16)	0.40	(0.16, 0.61)
		4.38 (1.72, 11.15)	5	5	1.00	(0.57, 1.00)	2	0.40	(0.12, 0.77)	0.60	(0.03, 0.88)
Raw ground turkey	<i>L. monocytogenes</i> TCC 1227	N/A <sup>h</sup>	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.59 (0.31, 1.06)	20	10	0.50	(0.30, 0.70)	10	0.50	(0.30, 0.70)	0.00	(-0.28, 0.28)
		0.95 (0.47, 1.91)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Raw ground turkey <sup>i</sup>	<i>L. monocytogenes</i> TCC 1227	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.69 (0.39, 1.14)	20	10	0.50	(0.30, 0.70)	10	0.50	(0.30, 0.70)	0.00	(-0.28, 0.28)
		4.38 (1.72, 11.15)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Raw pork sausage	<i>L. 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>L. 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)
Pasteurized brie cheese	<i>L. seeligeri</i> TCC 2190	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.13 (0.02, 0.26)	20	14	0.70	(0.48, 0.85)	15	0.75	(0.53, 0.89)	-0.05	(-0.31, 0.22)
		0.33 (0.14, 0.75)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)

Pasteurized 2% fat milk	<i>L. innocua</i> TCC 1180	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.50 (0.90, 2.56)	20	12	0.60	(0.39, 0.78)	13	0.65	(0.43, 0.82)	-0.05	(-0.32, 0.23)
		1.88 (0.84, 4.18)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Pasteurized 2% fat milk <sup>i</sup>	<i>L. innocua</i> TCC 1180	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.23 (0.77, 2.00)	20	12	0.60	(0.39, 0.78)	12	0.60	(0.39, 0.78)	0.00	(-0.28, 0.28)
		1.64 (0.79, 3.39)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Raw cod <sup>j</sup>	<i>L. 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	(0.25, 0.65)	9	0.45	(0.25, 0.65)	0.00	(-0.28, 0.28)
		4.38 (1.71, 11.19)	5	5	5	(0.56, 1.00)	5	5	(0.56, 1.00)	0.00	(-0.43, 0.43)
Pasteurized brie cheese <sup>k</sup>	<i>L. seeligeri</i> ATCC 35967	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.54 (0.32, 0.81)	20	5	0.25	(0.11, 0.46)	5	0.25	(0.11, 0.46)	0.00	(-0.25, 0.25)
		2.97 (1.25, 7.01)	5	5	1.00	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0.00	(-0.43, 0.43)
Bagged lettuce <sup>l</sup>	<i>L. 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	5	5	1.00	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0.00	(-0.43, 0.43)

		(0.93, 5.12)									
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<sup>a</sup>MPN = Most Probable number is based on the POD of the reference method test portions using the Least Cost Formulations MPN calculator with 95% confidence interval.

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

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

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

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

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

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

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

<sup>i</sup>Repeat analysis of matrix.

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

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

Matrix	Strain	MPN <sup>a</sup> /test portion	N <sup>b</sup>	SureTect Method			Reference Method			dPOD <sub>c</sub> <sup>f</sup>	95% CI <sup>g</sup>
				X <sup>c</sup>	POD <sub>c</sub> <sup>d</sup>	95% CI	X	POD <sub>c</sub> <sup>e</sup>	95% CI		
Ice cream	<i>L. ivanovii</i> TCC 1182	N/A <sup>h</sup>	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.37 (0.18, 0.65)	20	6	0.30	(0.15, 0.52)	8	0.40	(0.22, 0.61)	-0.10	(-0.36, 0.18)
		1.35 (0.61, 2.98)	5	4	0.80	(0.38, 1.00)	3	0.60	(0.23, 0.88)	0.20	(-0.31, 0.62)
Raw ground pork	<i>L. 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)
Bagged lettuce	<i>L. ivanovii</i> TCC 1572	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.13 (0.717, 1.81)	20	13	0.65	(0.43, 0.82)	14	0.70	(0.48, 0.85)	-0.05	(-0.32, 0.23)
		4.73 (1.71, 11.19)	5	4	0.80	(0.38, 1.00)	5	1.00	(0.57, 1.00)	-0.20	(-0.62, 0.28)
Bagged lettuce <sup>i</sup>	<i>L. ivanovii</i> TCC 1572	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.43 (0.28, 0.74)	20	8	0.40	(0.22, 0.61)	7	0.35	(0.18, 0.57)	0.05	(-0.23, 0.32)



		4.38 (1.72, 11.15)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Raw ground turkey	<i>L. monocytogenes</i> TCC 1227	N/A <sup>n</sup>	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.59 (0.31, 1.06)	20	8	0.40	(0.22, 0.61)	10	0.50	(0.30, 0.70)	-0.10	(-0.37, 0.19)
		0.95 (0.47, 1.91)	5	3	0.60	(0.23, 0.88)	5	1.00	(0.57, 1.00)	-0.40	(-0.77, 0.12)
Raw ground turkey <sup>i</sup>	<i>L. monocytogenes</i> TCC 1227	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.69 (0.39, 1.14)	20	10	0.50	(0.30, 0.70)	9	0.45	(0.26, 0.66)	0.05	(-0.24, 0.33)
		4.38 (1.72, 11.15)	5	4	0.80	(0.38, 1.00)	5	1.00	(0.38, 1.00)	-0.20	(-0.20, 0.28)
Raw pork sausage	<i>L. 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>L. 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)
Pasteurized brie cheese	<i>L. seeligeri</i> TCC 2190	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.13 (0.02, 0.26)	20	11	0.50	(0.34, 0.74)	4	0.20	(0.08, 0.42)	0.35	(0.05, 0.58)
		0.33 (0.14, 0.75)	5	4	0.80	(0.38, 1.00)	3	0.60	(0.23, 0.88)	0.20	(-0.31, 0.62)
Pasteurized 2% fat milk	<i>L. innocua</i> TCC 1180	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		1.50 (0.90, 2.56)	20	10	0.50	(0.30, 0.70)	15	0.75	(0.53, 0.89)	-0.25	(-0.49, 0.05)
		1.88 (0.84, 4.18)	5	5	1.00	(0.57, 1.00)	4	0.80	(0.38, 1.00)	0.20	(-0.28, 0.62)
Pasteurized 2%	<i>L. innocua</i> TCC 1180	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)

fat milk <sup>f</sup>		1.23 (0.77, 2.00)	20	12	0.60	(0.39, 0.78)	12	0.60	(0.39, 0.78)	0.00	(-0.28, 0.28)
		1.64 (0.79, 3.39)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.0)	0.00	(-0.47, 0.47)
Raw cod <sup>f</sup>	<i>L. 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	(0.25, 0.65)	6	0.30	(0.14, 0.51)	0.15	(-0.14, 0.40)
		4.38 (1.71, 11.19)	5	5	1.00	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0.00	(-0.43, 0.43)
Pasteurized brie <sup>f</sup>	<i>L. seeligeri</i> ATCC 35967	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.54 (0.32, 0.81)	20	5	0.25	(0.11, 0.46)	7	0.35	(0.18, 0.56)	-0.10	(-0.35, 0.17)
		2.97 (1.25, 7.01)	5	5	1.00	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0.00	(-0.43, 0.43)
Fresh bagged lettuce <sup>f</sup>	<i>L. monocytogenes</i> (LI0549)	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
		0.37 (0.23, 0.59)	20	10	0.50	(0.29, 0.70)	5	0.25	(0.11, 0.46)	0.25	(-0.04, 0.49)
		2.19 (0.93, 5.12)	5	5	1.00	(0.56, 1.00)	5	1.00	(0.56, 1.00)	0.00	(-0.43, 0.43)

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

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

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

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

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

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

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

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

<sup>i</sup>Repeat analysis of matrix.

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

**DISCUSSION OF MODIFICATION APPROVED NOVEMBER 2015 (9):**

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

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

For the pasteurized milk samples analyzed during this study, no presumptively negative, culture positive results were recorded with the SureTect PCR assay and presumptive and confirmed results were both in agreement for the PCR assay, candidate confirmation method and reference confirmation conducted to confirm the candidate method results.

For all three food matrices and the surface samples analyzed, no difference was observed by POD statistical analysis between the SureTect PCR method and the ISO reference method (Table 6).

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

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

Isolate	Serotype	TCC <sup>a</sup>	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	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 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 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	Positive
<i>Listeria welshimeri</i>	Unknown	1188	Pastrami	Positive
<i>Listeria welshimeri</i>	Unknown	1189	Food	Positive
<i>Listeria welshimeri</i>	6b	2188		Positive
<i>Listeria welshimeri</i>	4c	2189		Positive
<i>Listeria seeligeri</i>	Unknown	1190	Cheese	Positive
<i>Listeria seeligeri</i>	Unknown	1191	Food	Positive
<i>Listeria seeligeri</i>	Unknown	1192	Environmental	Positive
<i>Listeria seeligeri</i>	Unknown	1193	Cannelloni	Positive
<i>Listeria seeligeri</i>	Unknown	1194	Coleslaw	Positive
<i>Listeria seeligeri</i>	1/2b	2190		Positive
<i>Listeria seeligeri</i>	6b	2191		Positive

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

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

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

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

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

Matrix	Strain	MPN <sup>a</sup> /test portion	N <sup>b</sup>	SureTect Method Presumptive (CP)			SureTect Method Confirmation (CC)			dPOD <sub>CP</sub> <sup>f</sup>	95% CI <sup>g</sup>
				X <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	X	POD <sub>CC</sub> <sup>e</sup>	95% CI		
Raw ground turkey	<i>Listeria monocytogenes</i> TCC 1227	N/A <sup>h</sup>	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.45, 0.45)
		0.69 (0.39, 1.14)	20	9	0.45	(0.26, 0.66)	10	0.50	(0.30, 0.70)	-0.05	(-0.33, 0.24)
		4.38 (0.06, 11.15)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Pasteurized 2% milk	<i>Listeria innocua</i> TCC 1180	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.45, 0.45)
		1.23 (0.776, 2.00)	20	12	0.60	(0.39, 0.78)	12	0.60	(0.39, 0.78)	0.00	(-0.28, 0.28)
		1.64 (0.79, 3.39)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Bagged lettuce	<i>Listeria ivanovii</i> TCC 1572	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.45, 0.45)
		0.43 (0.08, 0.74)	20	7	0.35	(0.18, 0.57)	8	0.40	(0.22, 0.61)	-0.05	(-0.32, 0.23)
		4.38 (1.72, 11.12)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Stainless steel surface 4" x 4"	<i>Listeria monocytogenes</i> TCC 813 and <i>Enterococcus faecalis</i> CIP100750 X10	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.45, 0.45)
		N/A	20	6	0.30	(0.15, 0.52)	7	0.35	(0.18, 0.57)	-0.05	(-0.32, 0.23)
		N/A	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)

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

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

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

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

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

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

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

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

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

Matrix	Strain	MPN <sup>a</sup> /test portion	N <sup>b</sup>	SureTect PCR Presumptive Result			Reference Confirmation Method (RC)			dPOD <sub>CP</sub> <sup>f</sup>	95% CI <sup>g</sup>
				X <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	X	POD <sub>RC</sub> <sup>e</sup>	95% CI		
Raw ground turkey	<i>Listeria monocytogenes</i> TCC 1227	N/A <sup>h</sup>	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.45, 0.45)
		0.69 (0.39, 1.14)	20	9	0.45	(0.26, 0.66)	10	0.50	(0.30, 0.70)	-0.05	(-0.33, 0.24)
		4.38 (0.06, 11.15)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Pasteurized 2% milk	<i>Listeria innocua</i> TCC 1180	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.45, 0.45)
		1.23 (0.776, 2.00)	20	12	0.60	(0.39, 0.78)	12	0.60	(0.39, 0.78)	0.00	(-0.28, 0.28)
		1.64 (0.79, 3.39)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Bagged lettuce	<i>Listeria ivanovii</i> TCC 1572	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.45, 0.45)
		0.43 (0.08, 0.74)	20	7	0.35	(0.18, 0.57)	8	0.40	(0.22, 0.61)	-0.05	(-0.32, 0.23)
		4.38 (1.72, 11.12)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Stainless steel surface 4" x 4"	<i>Listeria monocytogenes</i> TCC 813 and <i>Enterococcus faecalis</i> CIP100750 X10	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.45, 0.45)
		N/A	20	6	0.30	(0.15, 0.52)	7	0.35	(0.18, 0.57)	-0.05	(-0.32, 0.23)
		N/A	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)

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

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

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

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

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

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

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

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

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

Matrix	Strain	MPN <sup>a</sup> /test portion	N <sup>b</sup>	SureTect Method Confirmed (CC)			Reference Confirmation (RC)			dPOD <sub>CC</sub> <sup>f</sup>	95% CI <sup>g</sup>
				X <sup>c</sup>	POD <sub>CC</sub> <sup>d</sup>	95% CI	X	POD <sub>RC</sub> <sup>e</sup>	95% CI		
Raw ground turkey	<i>Listeria monocytogenes</i> TCC 1227	N/A <sup>h</sup>	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.45, 0.45)
		0.69 (0.39, 1.14)	20	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 innocua</i> TCC 1180	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.45, 0.45)
		1.23 (0.776, 2.00)	20	12	0.60	(0.39, 0.78)	12	0.60	(0.39, 0.78)	0.00	(-0.28, 0.28)
		1.64 (0.79, 3.39)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Bagged lettuce	<i>Listeria ivanovii</i> TCC 1572	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.45, 0.45)
		0.43 (0.08, 0.74)	20	8	0.40	(0.22, 0.61)	8	0.40	(0.22, 0.61)	0.00	(-0.28, 0.28)
		4.38 (1.72, 11.12)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.47, 0.47)
Stainless steel surface 4" x 4"	<i>Listeria monocytogenes</i> TCC 813 and <i>Enterococcus faecalis</i> CIP100750 X10	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.45, 0.45)
		N/A	20	7	0.35	(0.18, 0.57)	7	0.35	(0.18, 0.57)	0.00	(-0.21, 0.21)
		N/A	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)

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

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

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

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

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

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

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

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



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

Matrix	Strain	MPN <sup>a</sup> /test portion	N <sup>b</sup>	SureTect Method Confirmed (C)			Reference Method (R)			dPODC <sup>f</sup>	95% CI <sup>g</sup>
				X <sup>c</sup>	POD <sub>c</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI		
Raw ground turkey	<i>Listeria monocytogenes</i> TCC 1227	N/A <sup>h</sup>	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.45, 0.45)
		0.69 (0.39, 1.14)	20	9	0.45	(0.26, 0.66)	9	0.45	(0.26, 0.66)	0.00	(-0.28, 0.28)
		4.38 (0.06, 11.15)	5	4	0.80	(0.38, 1.00)	5	1.00	(0.57, 1.00)	-0.20	(-0.62, 0.28)
Pasteurized 2% milk	<i>Listeria innocua</i> TCC 1180	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.45, 0.45)
		1.23 (0.776, 2.00)	20	12	0.60	(0.39, 0.78)	12	0.60	(0.39, 0.78)	0.00	(-0.28, 0.28)
		1.64 (0.79, 3.39)	5	4	0.80	(0.38, 1.00)	4	0.80	(0.38, 1.00)	0.00	(-0.47, 0.47)
Bagged lettuce	<i>Listeria ivanovii</i> TCC 1572	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.45, 0.45)
		0.43 (0.08, 0.74)	20	7	0.35	(0.18, 0.57)	7	0.35	(0.18, 0.57)	0.00	(-0.28, 0.28)
		4.38 (1.72, 11.12)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Stainless steel surface 4" x 4"	<i>Listeria monocytogenes</i> TCC 813 and <i>Enterococcus faecalis</i> CIP100750 X10	N/A	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.45, 0.45)
		N/A	20	6	0.30	(0.15, 0.52)	9	0.45	(0.26, 0.66)	-0.15	(-0.41, 0.14)
		N/A	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)

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

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

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

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

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

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

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

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

## DISCUSSION OF MODIFICATION Approved April 2018

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 2. Inclusivity of the SureTect *Listeria species* PCR Assay (10)**

ID	<i>Listeria species</i>	Serotype	Source	Origin	SureTect <i>Listeria species</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
858	<i>Listeria monocytogenes</i>	1/2c	Clinical sample	TCC	Positive
860	<i>Listeria monocytogenes</i>	1/2a	Poultry	TCC	Positive
862	<i>Listeria innocua</i>	6a	Cow brain ATCC® 33090™	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
868	<i>Listeria monocytogenes</i>	4e	Chicken	TCC	Positive
870	<i>Listeria monocytogenes</i>	3a	Clinical sample	TCC	Positive
883	<i>Listeria monocytogenes</i>	4e	Veterinary sample	TCC	Positive
888	<i>Listeria monocytogenes</i>	3a	Food	TCC	Positive
889	<i>Listeria monocytogenes</i>	3a	Food	TCC	Positive
1172	<i>Listeria grayi</i>		Environmental	TCC	Positive

1173	<i>Listeria grayi</i>		Butter	TCC	Positive
1174	<i>Listeria grayi</i>		Butter	TCC	Positive
1175	<i>Listeria grayi</i>		Butter	TCC	Positive
1176	<i>Listeria grayi</i>		Food	TCC	Positive
1177	<i>Listeria innocua</i>	Unknown	Chicken sandwich	TCC	Positive
1178	<i>Listeria innocua</i>	Unknown	Cooked chicken	TCC	Positive
1179	<i>Listeria innocua</i>	Unknown	Crayfish	TCC	Positive
1180	<i>Listeria innocua</i>	Unknown	Coleslaw	TCC	Positive
1181	<i>Listeria innocua</i>	Unknown	Tuna mayo sandwich	TCC	Positive
1182	<i>Listeria ivanovii</i>	Unknown	Lamb (vet sample)	TCC	Positive
1183	<i>Listeria ivanovii</i>	Unknown	Food	TCC	Positive
1184	<i>Listeria ivanovii</i>	Unknown	Food	TCC	Positive
1185	<i>Listeria welshimeri</i>	Unknown	Chicken sandwich	TCC	Positive
1186	<i>Listeria welshimeri</i>	Unknown	Food	TCC	Positive
1187	<i>Listeria welshimeri</i>	Unknown	Environmental	TCC	Positive
1188	<i>Listeria welshimeri</i>	Unknown	Pastrami	TCC	Positive
1189	<i>Listeria welshimeri</i>	Unknown	Food	TCC	Positive
1190	<i>Listeria seeligeri</i>	Unknown	Cheese	TCC	Positive
1191	<i>Listeria seeligeri</i>	Unknown	Food	TCC	Positive
1192	<i>Listeria seeligeri</i>	Unknown	Environmental	TCC	Positive
1193	<i>Listeria seeligeri</i>	Unknown	Cannelloni	TCC	Positive
1194	<i>Listeria seeligeri</i>	Unknown	Coleslaw	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
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
2185	<i>Listeria innocua</i>	4ab	Unknown	TCC	Positive
2187	<i>Listeria innocua</i>	6b	Institut Pasteur	TCC	Positive
2188	<i>Listeria welshimeri</i>	6b	Unknown	TCC	Positive
2189	<i>Listeria welshimeri</i>	4c	Institut Pasteur	TCC	Positive
2190	<i>Listeria seeligeri</i>	1/2b	Unknown	TCC	Positive
2191	<i>Listeria seeligeri</i>	6b	Unknown	TCC	Positive

Table 4. Exclusivity of the SureTect *Listeria* species PCR Assay (10)

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

**Table 9. SureTect Listeria species PCR Assay Results: candidate presumptive PCR result vs candidate method confirmed (via reference method) (10)**

Matrix <sup>a</sup>	Inoculating strain(s)	MPN <sup>b</sup> / test portion	N <sup>c</sup>	SureTect candidate Presumptive PCR result			SureTect candidate method confirmed via the reference method			dPOD <sub>CPr</sub> <sup>g</sup>	95% CI <sup>h</sup>
				x <sup>d</sup>	POD <sub>CPr</sub> <sup>e</sup>	95% CI	x	PODC <sub>R</sub> <sup>f</sup>	95% CI		
Sliced Deli Turkey	TCC 1180 <i>L. innocua</i>	N/A <sup>j</sup>	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.5	20	16	0.80	0.58, 0.92	16	0.80	0.58, 0.92	0.00	-0.25, 0.25
		2.5	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Bagged Lettuce	TCC 1220 <i>L. monocytogenes</i>	N/A <sup>j</sup>	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.2	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.28, 0.28
		1.1	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
2% Pasteurized Milk	TCC 0840 <i>L. monocytogenes</i>	N/A <sup>j</sup>	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.2	20	3	0.15	0.05, 0.36	7	0.35	0.18, 0.57	-0.20	-0.44, 0.07
		0.67	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
Stainless Steel Sponge (4" x 4")	TCC 0813 <i>L. monocytogenes</i> / 10X <i>E. faecalis</i>	N/A <sup>j</sup>	10	0	0.00	0.00, 0.28	0	0.00	0.00, 0.28	0.00	-0.28, 0.28
		N/A	20	14	0.70	0.48, 0.85	14	0.70	0.48, 0.85	0.00	-0.27, 0.27
		N/A	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Stainless Steel Swab (1" x 1")	TCC 1205 <i>L. monocytogenes</i> 1/2b	N/A <sup>j</sup>	10	0	0.00	0.00, 0.28	0	0.00	0.00, 0.28	0.00	-0.28, 0.28
		N/A	20	10	0.50	0.30, 0.70	11	0.55	0.34, 0.74	-0.05	-0.33, 0.24
		N/A	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47

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

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

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

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

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

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

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

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

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

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

Matrix <sup>d</sup>	Inoculating strain(s)	MPN <sup>b</sup> / test portion	N <sup>c</sup>	SureTect candidate Presumptive PCR result			SureTect candidate method confirmed via the candidate method			dPOD <sub>CPc</sub> <sup>g</sup>	95% CI <sup>h</sup>
				x <sup>d</sup>	POD <sub>CPc</sub> <sup>e</sup>	95% CI	x	PODC <sub>R</sub> <sup>f</sup>	95% CI		
Sliced Deli Turkey	TCC 1180 <i>L. innocua</i>	N/A <sup>i</sup>	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.5	20	16	0.80	0.58, 0.92	16	0.80	0.58, 0.92	0.00	-0.25, 0.25
		2.5	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Bagged Lettuce	TCC 1220 <i>L. monocytogenes</i>	N/A <sup>i</sup>	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.2	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.28, 0.28
		1.1	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
2% Pasteurized Milk	TCC 0840 <i>L. monocytogenes</i>	N/A <sup>i</sup>	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.2	20	3	0.15	0.05, 0.36	7	0.35	0.18, 0.57	-0.20	-0.44, 0.07
		0.67	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.28, 0.28
Stainless Steel Sponge (4" x 4")	TCC 0813 <i>L. monocytogenes</i> / 10X <i>E. faecalis</i>	N/A <sup>i</sup>	10	0	0.00	0.00, 0.28	0	0.00	0.00, 0.28	0.00	-0.28, 0.28
		N/A	20	14	0.70	0.48, 0.85	14	0.70	0.48, 0.85	0.00	-0.27, 0.27
		N/A	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Stainless Steel Swab (1" x 1")	TCC 1205 <i>L. monocytogenes</i> 1/2b	N/A <sup>i</sup>	10	0	0.00	0.00, 0.28	0	0.00	0.00, 0.28	0.00	-0.28, 0.28
		N/A	20	10	0.50	0.30, 0.70	11	0.55	0.34, 0.74	-0.05	-0.33, 0.24
		N/A	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47

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

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

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

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

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

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

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

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

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

**Table 11. SureTect Listeria species PCR Assay Results: candidate method confirmed (via the candidate method) vs Reference method POD summary (10)**

Matrix <sup>a</sup>	Inoculating strain(s)	MPN <sup>b</sup> / test portion	N <sup>c</sup>	SureTect candidate method confirmed via the candidate method result			Reference method result			dPOD <sub>cc</sub> <sup>g</sup>	95% CI <sup>h</sup>
				x <sup>d</sup>	POD <sub>cc</sub> <sup>e</sup>	95% CI	x	POD <sub>R</sub> <sup>f</sup>	95% CI		
Sliced Deli Turkey	TCC 1180 <i>L. innocua</i>	N/A <sup>i</sup>	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.50	20	16	0.80	0.58, 0.92	6	0.30	0.15, 0.52	0.50	0.19, 0.70
		2.50	5	5	1.00	0.57, 1.00	4	0.80	0.38, 1.00	0.20	-0.28, 0.62
Bagged Lettuce	TCC 1220 <i>L. monocytogenes</i>	N/A <sup>i</sup>	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.20	20	10	0.50	0.30, 0.70	14	0.70	0.48, 0.85	-0.20	-0.45, 0.10
		1.10	5	4	0.80	0.38, 1.00	3	0.60	0.23, 0.88	0.20	-0.31, 0.62
2% Pasteurized Milk	TCC 0840 <i>L. monocytogenes</i>	N/A <sup>i</sup>	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.20	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
		0.67	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
Stainless Steel Sponge (4" x 4")	TCC 0813 <i>L. monocytogenes</i> / 10X <i>E. faecalis</i>	N/A <sup>i</sup>	10	0	0.00	0.00, 0.28	0	0.00	0.00, 0.28	0.00	-0.28, 0.28
		N/A	20	14	0.70	0.48, 0.85	14	0.70	0.48, 0.85	0.00	-0.27, 0.27
		N/A	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Stainless Steel Swab (1" x 1")	TCC 1205 <i>L. monocytogenes</i> 1/2b	N/A <sup>i</sup>	10	0	0.00	0.00, 0.28	0	0.00	0.00, 0.28	0.00	-0.28, 0.28
		N/A	20	11	0.55	0.34, 0.74	13	0.65	0.43, 0.82	-0.10	-0.37, 0.19
		N/A	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47

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

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

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

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

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

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

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

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

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

**Table 12. SureTect Listeria species PCR Assay Results: Candidate method confirmed (via the reference method) vs Reference method POD summary (10)**

Matrix <sup>a</sup>	Inoculating strain(s)	MPN <sup>b</sup> / test portion	N <sup>c</sup>	SureTect candidate method confirmed via the reference method			Reference method result			dPOD <sub>CR</sub> <sup>g</sup>	95% CI <sup>h</sup>
				x <sup>d</sup>	POD <sub>CR</sub> <sup>e</sup>	95% CI	x	POD <sub>R</sub> <sup>f</sup>	95% CI		
Sliced Deli Turkey	TCC 1180 <i>L. innocua</i>	N/A <sup>i</sup>	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.50	20	16	0.80	0.58, 0.92	6	0.30	0.15, 0.52	0.50	0.19, 0.70
		2.50	5	5	1.00	0.57, 1.00	4	0.80	0.38, 1.00	0.20	-0.28, 0.62
Bagged Lettuce	TCC 1220 <i>L. monocytogenes</i>	N/A <sup>i</sup>	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		1.20	20	10	0.50	0.30, 0.70	14	0.70	0.48, 0.85	-0.20	-0.45, 0.10
		1.10	5	4	0.80	0.38, 1.00	3	0.60	0.23, 0.88	0.20	-0.31, 0.62
2% Pasteurized Milk	TCC 0840 <i>L. monocytogenes</i>	N/A <sup>i</sup>	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.20	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
		0.67	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
Stainless Steel Sponge (4" x 4")	TCC 0813 <i>L. monocytogenes</i> / 10X <i>E. faecalis</i>	N/A <sup>i</sup>	10	0	0.00	0.00, 0.28	0	0.00	0.00, 0.28	0.00	-0.28, 0.28
		N/A	20	14	0.70	0.48, 0.85	14	0.70	0.48, 0.85	0.00	-0.27, 0.27
		N/A	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Stainless Steel Swab (1" x 1")	TCC 1205 <i>L. monocytogenes</i> 1/2b	N/A <sup>i</sup>	10	0	0.00	0.00, 0.28	0	0.00	0.00, 0.28	0.00	-0.28, 0.28
		N/A	20	11	0.55	0.34, 0.74	13	0.65	0.43, 0.82	-0.10	-0.37, 0.19
		N/A	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47

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

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

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

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

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

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

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

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

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



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