



CERTIFICATION

AOAC Research Institute
***Performance Tested Methods*SM**

Certificate No.
090701

The AOAC Research Institute hereby certifies the method known as

iQ-Check *Listeria* spp. Real-Time PCR

Corporate Location
Bio-Rad Laboratories
2000 Alfred Nobel Drive
Hercules, CA 94547 USA

Manufacturing Location
Bio-Rad Laboratories
925 Alfred Nobel Drive
Hercules, CA 94547 USA

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

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

Bradley A. Stawick, AOAC Research Institute Senior Director

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MODIFICATION DECEMBER 2022: Bio-Rad – Mike Clark; ADRIA Développement – Maryse Rannou, Iorian Quero
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SUBMITTING COMPANY

Bio-Rad Laboratories
 2000 Alfred Nobel Drive
 Hercules, CA 94547
 USA

METHOD NAME

iQ-Check *Listeria* spp. Real-Time PCR

CATALOG NUMBER

357-8113

INDEPENDENT LABORATORY**Original Validation**

rtech Laboratories
 1150 West County Road F
 Arden Hills, MN 55112

GovVal Validation

Q Laboratories, Inc.
 1400 Harrison Ave.
 Cincinnati, OH 45214

Modifications - February 2016, April 2019

Q Laboratories, Inc.
 1400 Harrison Ave
 Cincinnati, OH 45214

Modification December 2022

ADRIA Développement
 Creac'h Gwen
 F.29196 Quimper Cedex, France

APPLICABILITY OF METHOD

Target organism – *Listeria* spp.

Matrixes – (sponge 4 x 4 in, swab 1 x 1 in) - stainless steel, plastic, ceramic, and sealed concrete

GovVal approved matrices (MFHPB-30) - (25 g) -liver pate, raw fermented sausage, deli ham, hot dogs, deli turkey, and stainless steel (1 x 1 in)

February 2016 Matrix Extension: (USDA/FSIS MLG 8.09) deli ham (25 g), stainless steel (swab 1 x 1 in with HiCap Neutralizing Broth), sealed concrete (sponge 4 x 4 in with Lethen Broth)

(AOAC 993.12) natural cheese (125 g)

April 2019 Modification (MLG Ch. 8.10): Stainless steel (4 x 4 in, sponge), polystyrene plastic (1 x 1 in, swab), sealed concrete (4 x 4 in, sponge)

December 2022 Modification – FDA MLG 8.13 – deli turkey (25 g)

FDA BAM – Ch. 10 – cheddar cheese (125 g), stainless steel (4 in x 4 in, sponge)

Performance claims – The study data detected no statistical difference between the iQ-Check *Listeria* spp. Real-Time PCR method and the reference methods.

REFERENCE METHODS

Microbiology Laboratory Guidebook (October 1, 2004) U.S. Department of Agriculture, Food Safety and Inspection Service, Office of Public Health Science, Chapter 8.05. (2)

Health Canada, Health Products and Food Branch (2010) *Compendium of Analytical Methods*, MFHPB – 30. (3)

United States Department of Agriculture Microbiological Laboratory Guidelines 8.09: *Isolation and Identification of Listeria monocytogenes from Red Meat, Poultry, Egg Products, and Environmental Sponges*. May 1st, 2013. (6)

AOAC Official Method 993.12 *Listeria monocytogenes* in Milk and Dairy Products: Selective Enrichment and Isolation Method, First Action 1993, Final Action 1996 (7)

U.S. Department of Agriculture Food Safety Inspection Service (2017) *Microbiological Laboratory Guidebook*, Chapter 8.10, *Isolation and Identification of Listeria monocytogenes from Red Meat, Poultry, Ready-To-Eat Siluriformes (Fish) and Egg Products, and Environmental Sponges*. (9)

Clark, M., Rannou, M., and Quero, F., Validation Study for the Bio-Rad iQ-Check *Listeria* spp. Real-Time PCR Method for the Detection of *Listeria* species in Selected Food Matrixes and Environmental Surfaces: Level 3

Modification, AOAC *Performance Tested Methods*SM certification number 090701. Approved December 21, 2022. U.S. Department of Agriculture Food Safety Inspection Service (2021) *Laboratory Guidebook*, MLG 8.13, *Isolation and Identification of Listeria monocytogenes from Red Meat, Poultry, Ready-To-Eat Siluriformes (Fish) and Egg Products, and Environmental Sponges* (11)

U.S. Food and Drug administration (2022), *FDA Bacteriological Analytical Manual*, Chapter 10, Detection of *Listeria monocytogenes* in Foods and Environmental Samples, and Enumeration of *Listeria monocytogenes* in Foods, (12)

Table 1 - Inclusivity Results (1)

Strain	Reference	Origin	iQ-Check Result
<i>Listeria monocytogenes</i>	L39	Sausage	Positive
<i>Listeria monocytogenes</i>	L119	Spinach	Positive
<i>Listeria monocytogenes</i>	L121	Neufchatel cheese	Positive
<i>Listeria monocytogenes</i>	L123	Mozzarella cheese	Positive
<i>Listeria monocytogenes</i>	L124	Perch fillet	Positive
<i>Listeria monocytogenes</i>	L130	Ground beef	Positive
<i>Listeria monocytogenes</i>	L137	Coulommier raw milk cheese	Positive
<i>Listeria monocytogenes</i>	L141	Environmental sample	Positive
<i>Listeria monocytogenes</i>	L149	Environmental sample	Positive
<i>Listeria monocytogenes</i>	L152	Environmental sample	Positive
<i>Listeria monocytogenes</i>	L156	French fries	Positive
<i>Listeria monocytogenes</i>	L176	Beef rib steak	Positive
<i>Listeria monocytogenes</i> 1/2	L20	Smoked salmon	Positive
<i>Listeria monocytogenes</i> 1/2	L25	Chicken	Positive
<i>Listeria monocytogenes</i> 1/2a	L5	Smoked salmon	Positive
<i>Listeria monocytogenes</i> 1/2a	L6	Pizza	Positive
<i>Listeria monocytogenes</i> 1/2a	L7	Munster raw milk cheese	Positive
<i>Listeria monocytogenes</i> 1/2a	L9	Munster raw milk cheese	Positive
<i>Listeria monocytogenes</i> 1/2a	L10	Potted meat	Positive
<i>Listeria monocytogenes</i> 1/2a	L11	Munster raw milk cheese	Positive
<i>Listeria monocytogenes</i> 1/2a	L12	Smoked salmon	Positive
<i>Listeria monocytogenes</i> 1/2a	L40	Munster raw milk cheese	Positive
<i>Listeria monocytogenes</i> 1/2a	L42	Chicken escalope	Positive
<i>Listeria monocytogenes</i> 1/2a	L43	Ground beef	Positive
<i>Listeria monocytogenes</i> 1/2a	L44	Sausage	Positive
<i>Listeria monocytogenes</i> 1/2a	L45	Rabbit meat	Positive
<i>Listeria monocytogenes</i> 1/2a	L47	Sauté potatoes	Positive
<i>Listeria monocytogenes</i> 1/2a	L116	Fish	Positive
<i>Listeria monocytogenes</i> 1/2a	L128	Soybean cattle cake	Positive
<i>Listeria monocytogenes</i> 1/2a	L129	Sauté potatoes	Positive
<i>Listeria monocytogenes</i> 1/2b	L13	Pig ears	Positive
<i>Listeria monocytogenes</i> 1/2b	L37	Maroille raw milk cheese	Positive
<i>Listeria monocytogenes</i> 1/2b	L48	Pig tongue	Positive
<i>Listeria monocytogenes</i> 1/2b	L49	Chicken liver	Positive
<i>Listeria monocytogenes</i> 1/2b	L51	Germain raw milk cheese	Positive
<i>Listeria monocytogenes</i> 1/2c	L14	Ground beef	Positive
<i>Listeria monocytogenes</i> 1/2c	L15	Beef	Positive
<i>Listeria monocytogenes</i> 1/2c	L16	Ground beef	Positive
<i>Listeria monocytogenes</i> 1/2c	L17	Pork breast	Positive
<i>Listeria monocytogenes</i> 1/2c	L18	Munster raw milk cheese	Positive
<i>Listeria monocytogenes</i> 1/2c	L28	Surface sponge	Positive
<i>Listeria monocytogenes</i> 1/2c	L53	Ground beef	Positive
<i>Listeria monocytogenes</i> 1/2c	L54	Beef Bourguignon	Positive
<i>Listeria monocytogenes</i> 1/2c	L117	Montbeliard sausage	Positive
<i>Listeria monocytogenes</i> 3a	L191	Fishery environment	Positive
<i>Listeria monocytogenes</i> 3a	L192	Fishery environment	Positive
<i>Listeria monocytogenes</i> 3b	L193	Fishery environment	Positive
<i>Listeria monocytogenes</i> 3b	L55	SLCC 2540 (human)	Positive
<i>Listeria monocytogenes</i> 3c	L56	SLCC 2479	Positive
<i>Listeria monocytogenes</i> 4a	L57	ATCC 19114 (ruminant brain)	Positive
<i>Listeria monocytogenes</i> 4b	L32	Munster raw milk cheese	Positive
<i>Listeria monocytogenes</i> 4b	L58	Salad	Positive
<i>Listeria monocytogenes</i> 4d	L60	ATCC 19117 (sheep)	Positive
<i>Listeria monocytogenes</i> 4d	L194	Fishery environment	Positive
<i>Listeria monocytogenes</i> 4e	L62	Reblochon raw milk cheese	Positive
<i>Listeria monocytogenes</i> 4e	L63	Munster raw milk cheese	Positive
<i>Listeria monocytogenes</i> 7	L67	SLCC 2482 (human feces)	Positive
<i>Listeria grayi</i>	L190	Frozen French fries	Positive
<i>Listeria grayi</i>	L81	ATCC 19120	Positive
<i>Listeria grayi</i>	L188	Environment	Positive
<i>Listeria grayi</i>	L143	Frozen French fries	Positive
<i>Listeria innocua</i>	L108	Gorgonzola cheese	Positive
<i>Listeria innocua</i>	L113	Smoked halibut	Positive
<i>Listeria innocua</i>	L64	Epoisses cheese	Positive

<i>Listeria innocua</i>	L65	Epoisses cheese	Positive
<i>Listeria innocua</i>	L66	Spinach	Positive
<i>Listeria innocua</i>	L72	Boulettes d'Avesnes cheese	Positive
<i>Listeria innocua</i>	L78	Cockereel	Positive
<i>Listeria innocua</i>	L175	Process water	Positive
<i>Listeria innocua</i>	L88	Pork sausage	Positive
<i>Listeria innocua 6a</i>	L77	Toulouse sausage	Positive
<i>Listeria innocua 6a</i>	L1	ATCC 33090 (cow brain)	Positive
<i>Listeria innocua 6b</i>	L2	Ground beef	Positive
<i>Listeria innocua 6b</i>	L144	Bin swab	Positive
<i>Listeria ivanovii 5</i>	L161	Ground beef	Positive
<i>Listeria ivanovii 5</i>	L153	Environment	Positive
<i>Listeria ivanovii 5</i>	L80	Collection	Positive
<i>Listeria ivanovii 5</i>	L184	Bird trap	Positive
<i>Listeria ivanovii 5</i>	L151	Ground beef	Positive
<i>Listeria ivanovii 5</i>	L179	Environmental sample	Positive
<i>Listeria ivanovii 5</i>	L154	Sausage	Positive
<i>Listeria seeligeri</i>	L115	Lake water	Positive
<i>Listeria seeligeri</i>	L140	Frozen French fries	Positive
<i>Listeria seeligeri 1/2b</i>	L84	Ground meat	Positive
<i>Listeria seeligeri 1/2b</i>	L83	Ox tongue	Positive
<i>Listeria seeligeri 1/2b</i>	L82	ATCC 35967 (soil)	Positive
<i>Listeria welshimeri</i>	L100	Pâté	Positive
<i>Listeria welshimeri</i>	L101	Ham	Positive
<i>Listeria welshimeri</i>	L155	Raw salmon fillet	Positive
<i>Listeria welshimeri</i>	L174	Spinach	Positive
<i>Listeria welshimeri</i>	L91	Dried pork sausage	Positive
<i>Listeria welshimeri 6a</i>	L89	Ground meat	Positive
<i>Listeria welshimeri 6b</i>	L90	Ground meat	Positive
<i>Listeria welshimeri 6b</i>	L86	ATCC 35897 (decaying plant)	Positive

ATCC = American Type Culture Collection, USA

L = *Listeria* culture collection, Institut Pasteur de Lille, France

SLCC = Seeliger's *Listeria* Culture Collection, Würzburg, Germany

Table 2 - Exclusivity Results (1)

Strain	Reference	Origin	iQ-Check Result
<i>Bacillus cereus</i>	IPL-BA1	Raw egg	Negative
<i>Bacillus cereus</i>	IPL-BA2	Beet root	Negative
<i>Bacillus cereus</i>	IPL-BA3	Plant	Negative
<i>Bacillus cereus</i>	IPL-BA15	Custard	Negative
<i>Bacillus cereus</i>	IPL-BA19	Environment	Negative
<i>Bacillus coagulans</i>	IPL-BA7	Milk product	Negative
<i>Bacillus mycoïdes</i>	IPL-BA6	Environment	Negative
<i>Bacillus mycoïdes</i>	IPL-BA24	Soil	Negative
<i>Bacillus pumilus</i>	IPL-BA22	Tabbouleh with poultry	Negative
<i>Brochotrix thermosphacta</i>	IPL-15	Ground meat	Negative
<i>Carnobacterium divergens</i>	IPL-46	Minced beef	Negative
<i>Carnobacterium gallinarum</i>	IPL-47	Ice slush of chicken carcasses	Negative
<i>Carnobacterium piscicola</i>	IPL-48	Raw milk	Negative
<i>Citrobacter braakii</i>	IPL-CIT86	Pork sausage	Negative
<i>Citrobacter freundii</i>	IPL-CIT24	Meat product	Negative
<i>Corynebacterium variabile</i>	IPL-COR2	ATCC 15753 (food)	Negative
<i>Enterobacter cloacae</i>	IPL-ENT76	Milk powder	Negative
<i>Enterococcus faecalis</i>	IPL-E1	Egg product	Negative
<i>Enterococcus faecalis</i>	IPL-E6	ATCC 19433	Negative
<i>Enterococcus faecium</i>	IPL-E2	ATCC 3286	Negative
<i>Enterococcus faecium</i>	IPL-E7	CIP 54.33 (Canned fish)	Negative
<i>Enterococcus faecium</i>	IPL-E9	Taramasalata	Negative
<i>Enterococcus durans</i>	IPL-E8	Meat product	Negative
<i>Enterococcus durans</i>	IPL-E10	Meat product	Negative
<i>Erysipelothrix rhusiopathiae</i>	IPL-49	Spleen of pig with endocarditis	Negative
<i>Escherichia coli</i>	IPL-EC20	Tomatoes	Negative
<i>Escherichia coli</i>	IPL-EC21	Celery with mayonnaise	Negative
<i>Escherichia coli</i>	IPL-EC35	Spinach	Negative
<i>Jonesia dentrificans</i>	IPL-L139	CIP 55134T	Negative
<i>Klebsiella pneumoniae</i>	IPL-EN63	Celery	Negative
<i>Klebsiella pneumoniae</i>	IPL-EN68	Vegetable salad	Negative
<i>Kurthia gibsonii</i>	IPL-42	Meat product	Negative
<i>Lactobacillus spp</i>	IPL-Lb11506	ATCC 11506	Negative
<i>Lactobacillus casei</i>	IPL-Lb9595	ATCC 9595	Negative
<i>Lactobacillus delbruecki ssp lactis</i>	IPL-53	Emmental cheese	Negative
<i>Lactobacillus plantarum</i>	IPL-34	Milk product	Negative
<i>Lactococcus lactis</i>	IPL-33	Milk product	Negative
<i>Micrococcus spp</i>	IPL-M1	Environment	Negative
<i>Propionibacterium freundenreichii</i>	IPL-43	Swiss cheese	Negative
<i>Proteus mirabilis</i>	IPL-EN45	Poultry	Negative
<i>Rhodococcus equi</i>	IPL-32	Meat product	Negative
<i>Rhodococcus equi</i>	IPL-R2	Lung abscess of foal	Negative
<i>Rhodotorula rubra</i>	IPL-Le1	Pastry	Negative
<i>Salmonella Brandenburg</i>	IPL-S3	Pâté	Negative
<i>Salmonella typhimurium</i>	IPL-S31	Egg product	Negative
<i>Salmonella virchow</i>	IPL-S33	Cockle	Negative
<i>Staphylococcus aureus</i>	IPL-ST17	Frozen yogurt	Negative
<i>Staphylococcus epidermidis</i>	IPL-ST20	Smoked salmon	Negative
<i>Streptococcus anginosus</i>	IPL-Str611	CIP 102921T	Negative
<i>Streptococcus anginosus</i>	IPL-Str1068	CIP 105031	Negative
<i>Streptococcus bovis</i>	IPL-Str5623	CIP 5623	Negative
<i>Streptococcus bovis</i>	IPL-StrE3	Meat product	Negative
<i>Streptococcus equinus</i>	IPL-Str1074	CIP 102504T	Negative
<i>Streptococcus intermedius</i>	IPL-Str1201	CIP 103248T	Negative
<i>Streptococcus salivarius</i>	IPL-Str1075	CIP 102505	Negative
<i>Streptococcus salivarius</i>	IPL-Str1115	CIP 53.158	Negative

ATCC = American Type Culture Collection, USA

CIP = Collection Institut Pasteur, France

IPL = Culture collection, Institut Pasteur de Lille, France

Table 4 - Method Comparison Results (1)

Surface	Level	Inoculation	# samples	iQ-Check positive	iQ-Check confirmed	Reference positive	Method Agreement	X ²
Stainless steel (internal)	Control	0	5	0	0	0	100%	-
	Low	3.1x10 ³	20	20	20	19	95%	0.00
Stainless steel (independent)	Control	0	5	0	0	0	100%	-
	Low	2.7x10 ²	20	20	20	19	95%	0.00
Plastic	Control	0	5	0	0	0	100%	-
	Low	2.3x10 ³	20	20	18	8	40%	14.4
Ceramic	Control	0	5	0	0	0	100%	-
	Low	2.3x10 ³	20	11	10	8	85%	0.40
Sealed concrete	Control	0	5	0	0	0	100%	-
	Low	3.0x10 ²	20	13	14	14	95%	0.00

DISCUSSION OF GOVVAL MODIFICATION APPROVED JULY 2011 (3)

The iQ-Check *Listeria* species kit will detect all species of *Listeria*. This proved to be problematic for the first trial of the hot dog matrix which was inoculated with *Listeria monocytogenes* at a low level as the target organism and *Listeria innocua* at a high level as the competitor organism. As expected, all samples were scored as positive by the iQ-Check method (data not shown) because all samples contained *Listeria*. A second set of hot dogs were inoculated with *Enterococcus faecalis*. Analyzing this data set gives a more accurate picture of the specificity of the kit. The second set of hot dogs resulted in fractional positive detection of target organism. The iQ-Check kit did not cross react with the competitor organism.

The iQ-Check *Listeria* species method utilizes a proprietary enrichment broth, LSB. This broth was specially formulated to be used with an easy DNA extraction protocol in combination with the iQ-Check kit. LSB combines ingredients for improved cell resuscitation and a nutritive base. The selective agents included in LSB were optimized to efficiently target competing flora without affecting *Listeria* growth. In addition, the iQ-Check *Listeria* species test is performed after a single 24 h enrichment. A secondary enrichment is not necessary due to the sensitivity of the kit. In this evaluation, the iQ-Check *Listeria monocytogenes* II kit was also tested. Since the DNA extraction is the same for all iQ-Check kits, one set of samples was processed and an aliquot tested by each PCR kit. The results from both kits had 100% agreement.

GovVal: Table 4: Method Comparison Results (3)											
Matrix	Strain	MPN ^a /25g	N ^c	iQ-Check <i>Listeria</i> spp. Method			MFHPB-30 Reference Method			dPOD ^e	95% CI ^h
				x ^d	POD ^e	95% CI	x	POD ^f	95% CI		
Liver paté	<i>L. monocytogenes</i> 1/2a	< 0.075	5	0	0	0.00, 0.44	0	0	0.00, 0.44	0	-0.44, 0.44
		0.693 (0.357-1.204)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0	-0.28, 0.28
		1.386 (0.799-2.2302)	20	15	0.75	0.53, 0.89	13	0.65	0.43, 0.82	0.10	-0.18, 0.36
Hot dogs – 2	<i>L. monocytogenes</i> 1/2b plus 10x <i>E. faecalis</i>	< 0.075	5	0	0	0.00, 0.44	0	0	0.00, 0.44	0	-0.44, 0.44
		0.511 (0.223-0.916)	20	5	0.25	0.11, 0.47	8	0.40	0.22, 0.61	-0.15	-0.40, 0.13
		0.693 (0.357-1.204)	20	11	0.55	0.34, 0.74	10	0.50	0.30, 0.70	0	-0.28, 0.28
Raw fermented sausage	<i>L. monocytogenes</i> 3b	< 0.075	5	0	0	0.00, 0.44	0	0	0.00, 0.44	0	-0.44, 0.44
		0.7975 (0.431-1.386)	20	10	0.50	0.30, 0.70	11	0.55	0.34, 0.74	-0.05	-0.33, 0.24
		1.8975 (1.095-3.288)	20	10	0.50	0.30, 0.70	15	0.75	0.53, 0.89	-0.25	-0.49, 0.05
Sliced deli turkey	<i>L. monocytogenes</i> 3c	< 0.075	5	0	0	0.00, 0.44	0	0	0.00, 0.44	0	-0.44, 0.44
		0.598 (0.288-1.050)	20	18	0.90	0.70, 0.97	15	0.75	0.53, 0.89	0.15	-0.09, 0.38
		1.610 (0.932-2.775)	20	20	1.00	0.84, 1.00	19	0.95	0.76, 1.00	0.05	-0.12, 0.24
Sliced deli ham	<i>L. monocytogenes</i> 4b	< 0.075	5	0	0	0.00, 0.44	0	0	0.00, 0.44	0	-0.44, 0.44
		1.050 (0.598-1.897)	20	13	0.65	0.43, 0.82	13	0.65	0.43, 0.82	0	-0.28, 0.28
		1.610 (0.932-2.775)	20	19	0.95	0.76, 1.00	16	0.80	0.58, 0.92	0.15	-0.07, 0.38
Stainless steel	<i>L. monocytogenes</i> 4d plus 10x <i>E. faecalis</i>	N/A ^b	5	0	0	0.00, 0.44	0	0	0.00, 0.44	0	-0.44, 0.44
		N/A	20	9	0.45	0.26, 0.66	11	0.55	0.34, 0.74	-0.10	-0.37, 0.20
		N/A	20	13	0.65	0.43, 0.82	12	0.60	0.39, 0.78	0.05	-0.23, 0.32

^aMPN = Most Probable Number is based on the POD of reference method test portions across labs using the AOAC MPN calculator, with 95% confidence interval

^bN/A = Not applicable

^cN = Number of test portions

^dx = Number of positive test portions

^ePOD_c = Confirmed candidate method positive outcomes divided by the total number of trials

^fPOD_r = Confirmed reference method positive outcomes divided by the total number of trials

^gdPOD_c = Difference between the candidate method and reference method POD values

^b95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

Table 5. Method Comparison Results – Chi Square, unpaired test portions (3)

Matrix	Strain	MPN ^a /25g	N ^c	iQ-Check <i>Listeria</i> spp. Method		MFHPB-30 Method	Chi Square ^d	Relative Sensitivity ^e
				Presumptive Pos.	Confirmed Pos.			
Liver paté	<i>L. monocytogenes</i> 1/2a	< 0.075	5	0	0	0	-	-
		0.693 (0.357-1.204)	20	10	10	10	0.00	100
		1.386 (0.799-2.2302)	20	15	15	13	0.46	115
Hot dogs – 2	<i>L. monocytogenes</i> 1/2b plus 10x <i>E. faecalis</i>	< 0.075	5	0	0	0	-	-
		0.511 (0.223-0.916)	20	5	5	8	1.00	63
		0.693 (0.357-1.204)	20	11	11	10	0.10	110
Raw fermented sausage	<i>L. monocytogenes</i> 3b	< 0.075	5	0	0	0	-	-
		0.7975 (0.431-1.386)	20	10	10	11	0.00	91
		1.8975 (1.095-3.288)	20	10	10	15	2.60	67
Sliced deli turkey	<i>L. monocytogenes</i> 3c	< 0.075	5	0	0	0	-	-
		0.598 (0.288-1.050)	20	18	18	15	1.52	120
		1.610 (0.932-2.775)	20	20	20	19	1.00	105
Sliced deli ham	<i>L. monocytogenes</i> 4b	< 0.075	5	0	0	0	-	-
		1.050 (0.598-1.897)	20	13	13	13	0.00	100
		1.610 (0.932-2.775)	20	19	19	16	2.01	119
Stainless steel	<i>L. monocytogenes</i> 4d plus 10x <i>E. faecalis</i>	N/A ^b	5	0	0	0	-	-
		N/A	20	9	9	11	0.39	82
		N/A	20	13	13	12	0.10	108

^aMPN = Most Probable Number is based on the POD of reference method test portions across labs using the AOAC MPN calculator, with 95% confidence interval

^bN/A = Not applicable

^cN = Number of test portions

^dChi Square = Mantel-Haenszel: $\chi^2 = (n-1)(ad-bc)^2 / [(a+b)(a+c)(b+d)(c+d)]$, where n = total number of samples tested by the two methods, a = number of samples positive by the test method, b = number of samples negative by the test method, c = number of samples positive by the reference method and d = number of samples negative by the reference method

^eRelative sensitivity = a/c, where a = number of samples confirmed positive by the test method and c = number of samples positive by the reference method

DISCUSSION OF MODIFICATION APPROVED FEBRUARY 2016 (5)

The iQ-Check *Listeria* spp. Kit successfully detected *Listeria* species from both food matrices and both environmental surfaces analyzed. Using POD analysis, no statistically significant differences were observed between the number of positive samples detected by the candidate method and the reference methods for both matrices and both of the environmental surfaces tested.

The iQ-Check *Listeria* spp. Kit is quick and simple to perform, providing results in about 2.5 h post incubation of the selective enrichment for 30 sample replicates. The addition of the Free DNA Removal protocol gives the added benefit of removing free DNA that may be present in the sample and giving equivalent results as compared to the reference method. The CFX Manager IDE software is user friendly with the ability to track lot information and sample identification quickly and with ease. The CFX96 software and instrument also offer the ability to utilize an open platform and set up unique runs before a run is completed. Because results are displayed in real-time, the user is able to quickly and accurately determine if results will be valid before the end of the run. The software also provides the user the option to analyze each individual Cq curves to help aid in problem solving any issues within an individual reaction. The small footprint is also a plus when working with laboratories with a limited amount of space, or a small PCR amplification room.

Table D: Summary of Results – Inclusivity Results (5)

Strain	Reference ^a	Origin	iQ-Check Result
<i>Listeria monocytogenes</i>	L39	Sausage with ham	+
<i>Listeria monocytogenes</i>	L69	Sausage	+
<i>Listeria monocytogenes</i>	L70	Smoked salmon	+
<i>Listeria monocytogenes</i>	L119	Spinach	+
<i>Listeria monocytogenes</i>	L121	Neufchatel cheese	+
<i>Listeria monocytogenes</i>	L123	Mozzarella cheese	+
<i>Listeria monocytogenes</i>	L124	Perch fillet	+
<i>Listeria monocytogenes</i>	L125	Vegetables	+
<i>Listeria monocytogenes</i>	L130	Ground beef	+
<i>Listeria monocytogenes</i>	L137	Coulommier raw milk cheese	+
<i>Listeria monocytogenes</i>	L141	Environmental sample	+
<i>Listeria monocytogenes</i> 1/2	L20	Smoked salmon	+
<i>Listeria monocytogenes</i> 1/2	L25	Chicken	+
<i>Listeria monocytogenes</i> 1/2a	L4	ATCC 35152	+
<i>Listeria monocytogenes</i> 1/2a	L5	Smoked salmon	+
<i>Listeria monocytogenes</i> 1/2a	L6	Pizza	+
<i>Listeria monocytogenes</i> 1/2a	L7	Munster raw milk cheese	+
<i>Listeria monocytogenes</i> 1/2a	L9	Munster raw milk cheese	+
<i>Listeria monocytogenes</i> 1/2a	L10	Potted meat	+
<i>Listeria monocytogenes</i> 1/2a	L11	Munster raw milk cheese	+
<i>Listeria monocytogenes</i> 1/2a	L12	Smoked salmon	+
<i>Listeria monocytogenes</i> 1/2a	L40	Munster raw milk cheese	+
<i>Listeria monocytogenes</i> 1/2a	L42	Chicken escalope	+
<i>Listeria monocytogenes</i> 1/2a	L43	Ground beef	+
<i>Listeria monocytogenes</i> 1/2a	L44	Sausage	+
<i>Listeria monocytogenes</i> 1/2a	L45	Rabbit meat	+
<i>Listeria monocytogenes</i> 1/2a	L47	Sautéed potatoes	+
<i>Listeria monocytogenes</i> 1/2a	L116	Fish	+
<i>Listeria monocytogenes</i> 1/2a	L128	Soybean cattle cake	+
<i>Listeria monocytogenes</i> 1/2a	L129	Sautéed potatoes	+
<i>Listeria monocytogenes</i> 1/2b	L13	Pig ears	+
<i>Listeria monocytogenes</i> 1/2b	L37	Maroilles raw milk	+
<i>Listeria monocytogenes</i> 1/2b	L48	Pig tongue	+
<i>Listeria monocytogenes</i> 1/2b	L49	Chicken liver	+
<i>Listeria monocytogenes</i> 1/2b	L51	Germain raw milk cheese	+
<i>Listeria monocytogenes</i> 1/2b	L52	SLCC 2755	+
<i>Listeria monocytogenes</i> 1/2c	L14	Ground beef	+
<i>Listeria monocytogenes</i> 1/2c	L15	Beef	+
<i>Listeria monocytogenes</i> 1/2c	L16	Ground beef	+
<i>Listeria monocytogenes</i> 1/2c	L17	Pork breast	+
<i>Listeria monocytogenes</i> 1/2c	L18	Munster raw milk cheese	+
<i>Listeria monocytogenes</i> 1/2c	L28	Surface sponge	+
<i>Listeria monocytogenes</i> 1/2c	L53	Ground beef	+
<i>Listeria monocytogenes</i> 1/2c	L54	Beef Bourguignon	+
<i>Listeria monocytogenes</i> 1/2c	L117	Montbéliard sausage	+
<i>Listeria monocytogenes</i> 3a	L191	Fishery environment	+
<i>Listeria monocytogenes</i> 3a	L192	Fishery environment	+
<i>Listeria monocytogenes</i> 3b	L55	SLCC 2540 (human)	+
<i>Listeria monocytogenes</i> 3c	L56	SLCC 2479	+
<i>Listeria monocytogenes</i> 3b	L193	Unknown	+
<i>Listeria monocytogenes</i> 4a	L57	ATCC 19114 (ruminant brain)	+
<i>Listeria monocytogenes</i> 4b	L32	Munster raw milk cheese	+
<i>Listeria monocytogenes</i> 4b	L 33	ATCC 19115	+
<i>Listeria monocytogenes</i> 4b	L58	Salad	+
<i>Listeria monocytogenes</i> 4d	L60	ATCC 19117 (sheep)	+
<i>Listeria monocytogenes</i> 4d	L194	Fishery environment	+
<i>Listeria monocytogenes</i> 4e	L61	ATCC 19118	+
<i>Listeria monocytogenes</i> 4e	L62	Reblochon raw milk cheese	+
<i>Listeria monocytogenes</i> 4e	L63	Munster raw milk cheese	+
<i>Listeria monocytogenes</i> 7	L67	SLCC 2482 (human feces)	+
<i>Listeria fleischmannii</i>	DSM24998	Hard cheese	+
<i>Listeria grayi</i>	L81	ATCC19120	+
<i>Listeria grayi</i>	L143	Frozen French fries	+
<i>Listeria grayi</i>	L146	CIP 103213	+

<i>Listeria grayi</i>	L147	CIP 103213/ATCC 25401	+
<i>Listeria grayi</i>	L188	Environment	+
<i>Listeria grayi</i>	L190	Frozen French fries	+
<i>Listeria innocua</i>	L3	Heifer liver	+
<i>Listeria innocua</i>	L1	ATCC 33090	+
<i>Listeria innocua</i>	L2	Ground beef	+
<i>Listeria innocua</i>	L64	Epoisses cheese	+
<i>Listeria innocua</i>	L66	Spinach	+
<i>Listeria innocua</i>	L72	Boulette d'Avesnes cheese	+
<i>Listeria innocua</i>	L78	Cockereel	+
<i>Listeria innocua</i>	L108	Gorgonzola cheese	+
<i>Listeria innocua</i>	L113	Smoked halibut	+
<i>Listeria innocua 6a</i>	L77	Toulouse sausage	+
<i>Listeria innocua 6b</i>	L76	Ground beef	+
<i>Listeria ivanovii</i>	L80	Collection	+
<i>Listeria ivanovii</i>	L151	Ground beef	+
<i>Listeria ivanovii</i>	L153	Environmental sample	+
<i>Listeria ivanovii</i>	L184	Environmental sample	+
<i>Listeria ivanovii 5</i>	L154	Sausage	+
<i>Listeria ivanovii 5</i>	L182	Environment	+
<i>Listeria marthii</i>	DSM23813	Soil	+
<i>Listeria rocourtiae</i>	DSM22097	Precut lettuce	+
<i>Listeria seeligeri</i>	L84	Ground beef	+
<i>Listeria seeligeri</i>	L115	Dirty water	+
<i>Listeria seeligeri</i>	L142	Raw milk water	+
<i>Listeria seeligeri 1/2b</i>	L82	ATCC35967	+
<i>Listeria seeligeri 1/2b</i>	L83	Ox tongue	+
<i>Listeria welhenstephanensis</i>	DSM24698	Water plant from pond	+
<i>Listeria welshimeri</i>	L87	Ground beef	+
<i>Listeria welshimeri</i>	L91	Dried pork sausage	+
<i>Listeria welshimeri</i>	L100	Pâté	+
<i>Listeria welshimeri</i>	L101	Ham	+
<i>Listeria welshimeri</i>	L155	Raw salmon fillet	+
<i>Listeria welshimeri</i>	L174	Spinach	+
<i>Listeria welshimeri 6a</i>	L89	Ground meat	+
<i>Listeria welshimeri 6b</i>	L86	ATCC 35897	+
<i>Listeria welshimeri 6b</i>	L90	Ground meat	+

^a ATCC = American Type Culture Collection, USA
 CIP = Collection Institute Pasteur, France
 L = *Listeria* culture collection, Institut Pasteur de Lille, France
 SLCC = Seeliger's *Listeria* culture collection, Wurzburg, Germany
 DSM = The Leibniz Institute DSMZ - German Collection of Microorganisms and Cell Cultures

Table 4: iQ-Check *Listeria* spp. Kit, Candidate vs. Reference – POD Results (5)

Matrix/Test Portion	Strain	MPN ^a / Test Portion	N ^b	Candidate			Reference			dPOD _c ^f	95% CI ^g
				x ^c	POD _c ^d	95% CI	X	POD _R ^e	95% CI		
Deli Ham (25 g)	<i>Listeria monocytogenes</i> ATCC 19115	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.44 (0.21, 0.76)	20	9	0.45	0.26, 0.66	6	0.30	0.15, 0.52	0.15	-0.14, 0.41
		3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Cheddar Cheese (125 g)	<i>Listeria welshimeri</i> ATCC 35897	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.55 (0.29, 0.94)	20	9	0.45	0.26, 0.66	8	0.40	0.22, 0.61	0.05	-0.24, 0.33
		3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

^aMPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval
^bN = Number of test portions
^cx = Number of positive test portions
^dPOD_c = Candidate method confirmed positive outcomes divided by the total number of trials
^ePOD_R = Reference method confirmed positive outcomes divided by the total number of trials
^fdPOD_c = Difference between the confirmed candidate method result and reference method confirmed result POD values
^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

Table 5: iQ-Check *Listeria* spp. Kit, Candidate vs. Reference – POD Results (5)

Matrix/Test Area	Strain	CFU ^a / Test Portion	N ^b	Candidate			Reference			dPOD _c ^f	95% CI ^g
				x ^c	POD _c ^d	95% CI	X	POD _R ^e	95% CI		
Stainless Steel (1" x 1" Test Area)	<i>Listeria innocua</i> ATCC 33091	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		38 & 420	20	5	0.25	0.11, 0.47	6	0.30	0.15, 0.52	-0.05	-0.31, 0.22
	<i>Enterococcus faecalis</i> ATCC 29212	200 & 2100	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Sealed Concrete (4" x 4" Test Area)	<i>Listeria monocytogenes</i> ATCC 7644	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		54	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
		170	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

^aCFU/Test Area = Results of the CFU/Test area were determined by plating the inoculum for each matrix in triplicate

^bN = Number of test portions

^cx = Number of positive test portions

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

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

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

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

Table 6: iQ-Check *Listeria* spp. Kit, Presumptive vs. Confirmed – POD Results (5)

Matrix/Test Portion	Strain	MPN ^a / Test Portion	N ^b	Presumptive			Confirmed			dPOD _{CP} ^f	95% CI ^g
				x ^c	POD _{CP} ^d	95% CI	X	POD _{CC} ^e	95% CI		
Deli Ham (25 g)	<i>Listeria monocytogenes</i> ATCC 19115	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.44 (0.21, 0.76)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.28, 0.28
		3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Cheddar Cheese (125 g)	<i>Listeria welshimeri</i> ATCC 35897	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.55 (0.29, 0.94)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.28, 0.28
		3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

^aMPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval

^bN = Number of test portions

^cx = Number of positive test portions

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

^ePOD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials

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

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

Table 7: iQ-Check *Listeria* spp. Kit, Presumptive vs. Confirmed – POD Results (5)

Matrix/Test Area	Strain	CFU ^a / Test Area	N ^b	Presumptive			Confirmed			dPOD _{CP} ^f	95% CI ^g
				x ^c	POD _{CP} ^d	95% CI	X	POD _{CC} ^e	95% CI		
Stainless Steel (1" x 1" Test Area)	<i>Listeria innocua</i> ATCC 33091	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		38 & 420	20	5	0.25	0.11, 0.47	5	0.25	0.11, 0.47	0.00	-0.26, 0.26
	<i>Enterococcus faecalis</i> ATCC 29212	200 & 2100	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Sealed Concrete (4" x 4" Test Area)	<i>Listeria monocytogenes</i> ATCC 7644	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		54	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
		170	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

^aCFU/Test Area = Results of the CFU/Test area were determined by plating the inoculum for each matrix in triplicate

^bN = Number of test portions

^cx = Number of positive test portions

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

^ePOD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials

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

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

DISCUSSION OF MODIFICATION APPROVED APRIL 2019 (8)

The iQ-Check *Listeria* spp. Test Kit successfully detected *Listeria* species on all three environmental surfaces analyzed using both Lethen and HiCap neutralizing broth. Using POD analysis, no statistically significant differences were observed between the number of positive samples detected by the candidate method and the reference method.

With the addition of the Free DNA Removal Solution, the test kit allows the user to remove free DNA from dead cells. The CFX Manager IDE is simple and easy to navigate and allows the user to view real-time results. The CFX Manager IDE provides the end user with easy to interpret results. Analysis of the curves and Cq values by a trained analyst are not required to obtain a final result.

In the method comparison study, the iQ-Check *Listeria* spp. kit demonstrated no statistically significant differences between candidate and reference method results (dPOD_{CP}), or between presumptive and confirmed results (dPOD_{CC}) for both Lethen Broth and HiCap™ Neutralizing Broth. Observed differences between the iQ-Check *Listeria* spp. kit and the reference method were due to the unpaired nature of the study. Using the LSB enrichment media paired with the iQ-Check *Listeria* spp. assay provides laboratories with a solution for next day results. The added benefit of the iQ-Check Prep automates the protocol to provide increased traceability and workflow optimization.

Table 3. iQ-Check *Listeria* spp. Results - Presumptive vs. Confirmed (8)

Surface Type and Inoculum	Neutralizing Broth	CFU ^a / Test Area	N ^b	Presumptive			Confirmed			dPOD _{CP} ^f	95% CI ^g
				x ^c	POD _{CP} ^d	95% CI	x	POD _{CC} ^e	95% CI		
Stainless Steel (4"x4", sponge) <i>L. monocytogenes</i> 1/2b UVM CWD1601 (Origin unavailable)	Lethen	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		68	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.13, 0.13
		210	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
	HiCap	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		68	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.13, 0.13
		210	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Stainless Steel (4"x4", sponge) <i>L. monocytogenes</i> 1/2b UVM CWD1584 (Origin unavailable) + <i>E. faecalis</i> ATCC 29212 (Origin human urine)	Lethen	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		51 ^h & 680 ⁱ	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.13, 0.13
		180 ^h & 3300 ⁱ	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
	HiCap	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		51 ^h & 680 ⁱ	20	6	0.30	0.15, 0.52	6	0.30	0.15, 0.52	0.00	-0.13, 0.13
		180 ^h & 3300 ⁱ	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Polystyrene Plastic (1"x1", swab) <i>L. grayi</i> ATCC 19120 (Origin animal feces)	Lethen	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		76	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.13, 0.13
	HiCap	320	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
		-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
Sealed Concrete (4"x4", sponge) <i>L. innocua</i> 4ab NCTC 10528 (Origin unavailable)	Lethen	76	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.13, 0.13
		320	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
	HiCap	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		59	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.13, 0.13
260	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47		

^aCFU/Test Area = Results of the CFU/Test area were determined by plating the inoculum for each surface in triplicate

^bN = Number of test portions

^cx = Number of positive test portions

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

^ePOD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials

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

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

^hCFU/test area of *L. monocytogenes*

ⁱCFU/test area of *E. faecalis*

Table 4. iQ-Check *Listeria* spp. Results - Candidate vs. Reference (8)

Surface Type and Inoculum	Neutralizing Broth	CFU ^a / Test Portion	N ^b	Candidate			Reference		dPOD _c ^f	95% CI ^g	
				x ^c	POD _c ^d	95% CI	X	POD _R ^e			95% CI
Stainless Steel (4"x4", sponge) <i>L. monocytogenes</i> 1/2b UVM CWD1601 (Origin unavailable)	Letheen	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		68	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.28, 0.28
		210	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	HiCap	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		68	20	7	0.35	0.18, 0.57	9	0.45	0.26, 0.66	-0.10	-0.37, 0.19
		210	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Stainless Steel (4"x4", sponge) <i>L. monocytogenes</i> 1/2b UVM CWD1584 (Origin unavailable) + <i>E. faecalis</i> ATCC 29212 (Origin human urine)	Letheen	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		51 ^h & 680 ⁱ	20	8	0.40	0.22, 0.61	10	0.50	0.30, 0.70	-0.10	-0.37, 0.19
		180 ^h & 3300 ⁱ	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	HiCap	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		51 ^h & 680 ⁱ	20	6	0.30	0.15, 0.52	10	0.50	0.30, 0.70	-0.20	-0.45, 0.10
		180 ^h & 3300 ⁱ	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Polystyrene Plastic (1"x1", swab) <i>L. grayi</i> ATCC 19120 (Origin animal feces)	Letheen	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		76	20	10	0.50	0.30, 0.70	7	0.35	0.18, 0.57	0.15	-0.15, 0.41
		320	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	HiCap	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		76	20	9	0.45	0.26, 0.66	7	0.35	0.18, 0.57	0.10	-0.19, 0.37
		320	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Sealed Concrete (4"x4", sponge) <i>L. innocua</i> 4ab NCTC 10528 (Origin unavailable)	Letheen	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		59	20	9	0.45	0.26, 0.66	7	0.35	0.18, 0.57	0.10	-0.19, 0.37
		260	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	HiCap	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		59	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
		260	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

^aCFU/Test Area = Results of the CFU/Test area were determined by plating the inoculum for each surface in triplicate

^bN = Number of test portions

^cx = Number of positive test portions

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

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

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

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

^hCFU/test area of *L. monocytogenes*

ⁱCFU/test area of *E. faecalis*

DISCUSSION OF MODIFICATION APPROVED DECEMBER 2022 (10)

One objective of this modification study was to incorporate a new enrichment (LSB II) with a reduced incubation time of 18-24 h at $37 \pm 1^\circ\text{C}$ (previously 23-25 h at $37 \pm 1^\circ\text{C}$) for deli turkey and cheddar cheese, and 16-24 h at $37 \pm 1^\circ\text{C}$ for stainless steel (previously 18-24 h at $30 \pm 1^\circ\text{C}$).

For the deli turkey matrix, one of the test portions inoculated at the high level, positive PCR result for APF Fast was observed while both confirmation procedures (alternative and traditional) concluded to the absence of the *Listeria* strain in the enriched sample. The lysate was tested twice again, and the positive PCR initially observed was confirmed. With the low inoculation levels of the bulk material, the lower limits of the kit's detection post enrichment is demonstrated, as is the sensitivity of the kit, with a Cq of 40.12 on the initial test, showing a presumptive positive without cultural confirmation. The two subsequent tests also showed high Cq levels at 37.54 and 38.45 (Table 7). The culture level would likely be at approximately 10^3 , making it difficult to see positive results with the alternative and traditional confirmation processes. With qPCR, a relative correlation can be made between Cq values and the amount of target DNA is present in the DNA extract. Cq values in the 38-40 range are equivalent to a range of 10^3 to 10^4 of the target organism.

For cheddar cheese, for one of the test portions inoculated at the low level, a negative PCR result for APF Fast was observed. The PCR was repeated twice resulting in one positive result with a late Cq value (39.2, Table 8). Note that this sample gave positive PCR results with the APF Classic. This sample was contaminated with a very low level (0.14 cfu/test portion), just at the limit of detection of the method, leading to fractional positive PCR results. At such a low level, the random distribution of microorganisms could lead to the target not being transferred from sample enrichment to lysis buffer where in a different aliquot the target organism is transferred from sample enrichment to agar plates or secondary enrichment.

For three cheddar test portions tested with the APF Classic, similar results were observed; negative PCR results were observed for the first test and upon repeat testing, positive PCR results were observed twice for two samples and one positive PCR result for one sample. For three samples which gave positive PCR results, the alternative confirmations were not able to detect the presence of *L. innocua* in the enriched test portion. Similarly, the traditional agar plates incubated for 24 h were also negative, but after 48 h, the traditional agar plates were positive. The strain was recovered when streaking the enriched LSB II broth stored for one week at 2 - 8°C. These samples were likely contaminated at a low level and the storage of the enrichment broth allow the *Listeria* strain to grow at a sufficient level during refrigeration to be recovered on the selective agar plates. Based on these results, the Classic APF is not recommended for use with cheddar cheese. For stainless steel, two test portions gave positive PCR results with the APF Fast, but there was no recovery of the *L. monocytogenes* strain in the enriched test portion. The same situation was observed for two test portions when using the APF Classic. As discussed previously with the deli turkey samples, the Cq values of these test portions were high at 38-39 (Table 9), indicating very low level of contamination. The unconfirmed positive samples observed in these could be contributed by the same random distribution of target organisms or by a high level of dead DNA generated during the acclimation period. The FDRS treatment is designed to reduce the amount of free or dead DNA in a sample, not remove it completely.

Despite the fact that some discrepancies could be observed during the trials between the two APFs tested and between the PCR results and the confirmation tests, which can be attributed to a very low level of contamination, the iQ-Check *Listeria* spp. test kit successfully detected *Listeria* species in the two food matrixes and on stainless steel using the LSB II broth, the FDRS protocol and Easy II extraction protocol with both the Classic and Fast APF PCR analysis, which were two additional objectives of this study.

The final objective, to evaluate an alternative confirmation protocol which included streaking from the test portion primary enrichment to RAPID'*L. mono*, RAPID'*Listeria* and Agar *Listeria* and API *Listeria* for confirmation of typical colonies. For cheddar cheese, the alternative confirmation protocol showed fewer confirmed positives using the three suggested agar plates as compared to the reference method confirmation protocol for the low level of cheddar cheese with RAPID'*Listeria* detecting 7 fewer confirmed positive test portions compared to the results of the reference method. For this reason, RAPID'*Listeria* is not recommended for use with cheddar cheese. Two of the agar plates showed fewer confirmed positives for deli turkey and all three agar plates showed the same number of confirmed positives for stainless steel. The discrepancies could be attributed to the enriched test portion likely at the limit of detection.

In the method comparison study, the iQ-Check *Listeria* spp. kit demonstrated no statistically significant differences between candidate and reference method results (dPOD_c), or between presumptive and confirmed results (dPOD_{cp}) for both Application Profiles tested. Observed differences between the iQ-Check *Listeria* spp. kit and the reference method were due to the unpaired nature of the study. Using the LSB II enrichment media in the iQ-Check *Listeria* spp. method allows to reduce the incubation time.

Table 4. Inclusivity results (10)

No.	Genus	Species	Molecular serotype	Source ^a	Origin	Result ^b
1	<i>Listeria</i>	<i>monocytogenes</i>	II a	Adria 1011/1410	Frozen broccoli	+
2	<i>Listeria</i>	<i>monocytogenes</i>	VI b	Adria 153	Soft cheese (Munster)	+
3	<i>Listeria</i>	<i>monocytogenes</i>	VI b	Adria1973/2400	Egg and ham pastry (Quiche Lorraine)	+
4	<i>Listeria</i>	<i>monocytogenes</i>	II a	Adria 38/181	Toulouse sausages	+
5	<i>Listeria</i>	<i>monocytogenes</i>	IV b	Adria 7111/7516	Pâté (Rillettes)	+
6	<i>Listeria</i>	<i>monocytogenes</i>	IV b	Adria913/1048	Black pudding	+
7	<i>Listeria</i>	<i>monocytogenes</i>	II a	A00C036	Poultry (guinea)	+
8	<i>Listeria</i>	<i>monocytogenes</i>	L a	A00C041	Sausage	+
9	<i>Listeria</i>	<i>monocytogenes</i>	II b	A00C044	Poultry (Duck)	+
10	<i>Listeria</i>	<i>monocytogenes</i>	II a	A00L097	Milk	+
11	<i>Listeria</i>	<i>monocytogenes</i>	II a	A00M009	Smoked salmon	+
12	<i>Listeria</i>	<i>monocytogenes</i>	II b	Ad253	Semi-hard cheese	+
13	<i>Listeria</i>	<i>monocytogenes</i>	II a	Ad266	Poultry	+
14	<i>Listeria</i>	<i>monocytogenes</i>	IV b	Ad270	Fermented sausage	+
15	<i>Listeria</i>	<i>monocytogenes</i>	II b	Ad273	Cured delicatessen	+
16	<i>Listeria</i>	<i>monocytogenes</i>	II a	Ad274	Ready-to-eat food (Asiatic meal)	+
17	<i>Listeria</i>	<i>monocytogenes</i>	II b	Ad534	Fruits	+
18	<i>Listeria</i>	<i>monocytogenes</i>	II a	Ad548	Environment (Seafood)	+
19	<i>Listeria</i>	<i>monocytogenes</i>	II b	Ad623	Bread crumbs	+
20	<i>Listeria</i>	<i>monocytogenes</i>	II a	Ad665	Raw milk	+
21	<i>Listeria</i>	<i>monocytogenes</i>	VI b	Adria 1972/2399	Puff pastry with mushrooms	+
22	<i>Listeria</i>	<i>monocytogenes</i>	IV b	Adria 2407/3139	Tripes with tomatoes	+
23	<i>Listeria</i>	<i>monocytogenes</i>	II a	Adria 2760/3145	Raw bacon	+
24	<i>Listeria</i>	<i>monocytogenes</i>	II b	Adria 32.183	Croque-Monsieur	+
25	<i>Listeria</i>	<i>monocytogenes</i>	IV b	Adria 5721/6179	Smoked bacon	+
26	<i>Listeria</i>	<i>monocytogenes</i>	II a	Adria 850/109	RTE food (deli salad with seafood)	+
27	<i>Listeria</i>	<i>monocytogenes</i>	II a	Adria 877/113	Environmental sample (pastry)	+
28	<i>Listeria</i>	<i>monocytogenes</i>	II a	A00C014	Sausage	+
29	<i>Listeria</i>	<i>monocytogenes</i>	II a	A00C022	Merguez	+
30	<i>Listeria</i>	<i>monocytogenes</i>	II a	A00C024	Sausage	+
31	<i>Listeria</i>	<i>monocytogenes</i>	II a	A00C039	Sausages	+
32	<i>Listeria</i>	<i>monocytogenes</i>	IV b	A00C040	Cooked delicatessen (Museau)	+
33	<i>Listeria</i>	<i>monocytogenes</i>	IV b	A00C042	Raw sausage	+
34	<i>Listeria</i>	<i>monocytogenes</i>	II a	A00C043	Smoked Bacon	+
35	<i>Listeria</i>	<i>monocytogenes</i>	II b	A00C052	RTE food (Osso bucco with turkey)	+
36	<i>Listeria</i>	<i>monocytogenes</i>	II a	A00C053	Gizzards	+
37	<i>Listeria</i>	<i>monocytogenes</i>	IV b	A00C054	Beef hart	+
38	<i>Listeria</i>	<i>monocytogenes</i>	II a	A00C055	Raw sausages	+
39	<i>Listeria</i>	<i>monocytogenes</i>	II a	A00E008	Environmental sample	+
40	<i>Listeria</i>	<i>monocytogenes</i>	II a	A00E049	Environmental sample (smoked salmon)	+
41	<i>Listeria</i>	<i>monocytogenes</i>	II a	A00E082	Environmental sample (smoked salmon)	+
42	<i>Listeria</i>	<i>monocytogenes</i>	IV b	A00M032	Smoked salmon	+
43	<i>Listeria</i>	<i>monocytogenes</i>	II a	A00M045	Smoked salmon	+
44	<i>Listeria</i>	<i>monocytogenes</i>	II a	A00M088	Smoked salmon	+
45	<i>Listeria</i>	<i>monocytogenes</i>	II b	Ad235	Poultry	+
46	<i>Listeria</i>	<i>monocytogenes</i>	II a	Ad260	Semi hard cheese	+
47	<i>Listeria</i>	<i>monocytogenes</i>	II b	Ad265	Tong	+
48	<i>Listeria</i>	<i>monocytogenes</i>	II b	Ad267	Dry sausage	+
49	<i>Listeria</i>	<i>monocytogenes</i>	IV b	Ad268	Cured ham	+
50	<i>Listeria</i>	<i>monocytogenes</i>	IV b	Ad272	Fermented sausage	+
51	<i>Listeria</i>	<i>grayi</i>	N/A ^c	Ad1198	Smoked salmon	+
52	<i>Listeria</i>	<i>grayi</i>	N/A	Ad1443	Pork meat sausages	+

53	<i>Listeria</i>	<i>grayi</i>	N/A	Ad1295	Spinach	+
54	<i>Listeria</i>	<i>grayi</i>	N/A	Ad1490	Salmon terrine	+
55	<i>Listeria</i>	<i>grayi</i>	N/A	Ad2148	Pork rillettes	+
56	<i>Listeria</i>	<i>innocua</i>	N/A	Ad658	Gorgonzola	+
57	<i>Listeria</i>	<i>innocua</i>	N/A	Ad660	Breadcrumbs	+
58	<i>Listeria</i>	<i>innocua</i>	N/A	Ad663	Environment (dairy industry)	+
59	<i>Listeria</i>	<i>innocua</i>	N/A	Ad671	Smoked bacon	+
60	<i>Listeria</i>	<i>innocua</i>	N/A	Ad661	Soft cheese (Pont L'Evêque)	+
61	<i>Listeria</i>	<i>ivanovii</i>	N/A	Ad466	Raw veal meat	+
62	<i>Listeria</i>	<i>ivanovii</i>	N/A	Ad1289	Raw milk cheese	+
63	<i>Listeria</i>	<i>ivanovii</i>	N/A	Ad1291	Poultry	+
64	<i>Listeria</i>	<i>ivanovii</i>	N/A	Ad1288	Ewe milk	+
65	<i>Listeria</i>	<i>ivanovii</i> subsp. <i>londoniensis</i>	N/A	CIP103466	Unknown	+
66	<i>Listeria</i>	<i>seeligeri</i>	N/A	Ad649	Cheese	+
67	<i>Listeria</i>	<i>seeligeri</i>	N/A	Ad651	Environment	+
68	<i>Listeria</i>	<i>seeligeri</i>	N/A	Ad652	Environment (dairy industry)	+
69	<i>Listeria</i>	<i>seeligeri</i>	N/A	Ad674	Soft cheese (Munster)	+
70	<i>Listeria</i>	<i>seeligeri</i>	N/A	CIP100100	Unknown	+
71	<i>Listeria</i>	<i>welshimeri</i>	N/A	Ad1276	Environment (Slaughterhouse)	+
72	<i>Listeria</i>	<i>welshimeri</i>	N/A	Ad1235	Beef meat	+
73	<i>Listeria</i>	<i>welshimeri</i>	N/A	Ad191424	Poultry	+
74	<i>Listeria</i>	<i>welshimeri</i>	N/A	Ad1175	Ready-to-eat-food	+
75	<i>Listeria</i>	<i>welshimeri</i>	N/A	Ad650	Poultry	+
76 ^d	<i>Listeria</i>	<i>fleischmanii</i>	N/A	DSM 24998	Hard Cheese	+
77 ^d	<i>Listeria</i>	<i>floridensis</i>	N/A	RDC 1330	Unknown	+
78 ^d	<i>Listeria</i>	<i>newyorkensis</i>	N/A	RDC 1329	Unknown	+
79 ^d	<i>Listeria</i>	<i>marthii</i>	N/A	DSM 23813	Soil	+
80 ^d	<i>Listeria</i>	<i>rocourtiae</i>	N/A	DSM 22097	Precut lettuce	+
81 ^d	<i>Listeria</i>	<i>grandensis</i>	N/A	RDC 1331	Unknown	+
82 ^d	<i>Listeria</i>	<i>weihenstephanensis</i>	N/A	DSM 24698	Water plant from pond	+

^a Ad, Adria, A00 = ADRIA Développement culture collection, Quimper, France; CIP = Collection de l'institut Pasteur, Paris, France, DSM = Leibniz Institute DSMZ, German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany, RDC = Bio-Rad R&D Culture Collection, Marnes-la-Couquette, France.

^b "+" indicates the target analyte was detected.

^c Not applicable.

^d Strain tested internally at Bio-Rad Laboratories, (Marnes-la-Couquette, France)

Table 5. Exclusivity results (10)

No.	Genus	Species subsp	Source ^a	Origin	Result ^b
1	<i>Bacillus</i>	<i>cereus</i>	Ad465	Salmon Terrine	-
2	<i>Bacillus</i>	<i>circulans</i>	Ad760	Vegetables	-
3	<i>Bacillus</i>	<i>coagulans</i>	Ad731	Dairy product	-
4	<i>Bacillus</i>	<i>licheniformis</i>	Ad978	Dairy product	-
5	<i>Bacillus</i>	<i>mycoides</i>	Ad762	Milk	-
6	<i>Bacillus</i>	<i>pseudomycolides</i>	Ad765	Vegetables	-
7	<i>Bacillus</i>	<i>pumilus</i>	Ad284	Ready-to-eat	-
8	<i>Bacillus</i>	<i>weihenstephanensis</i>	Ad726	Egg product	-
9	<i>Brochothrix</i>	<i>thermosphacta</i>	EN 15129	Trout	-
10	<i>Brochothrix</i>	<i>campestris</i>	CIP 102920T	Environment	-
11	<i>Carnobacterium</i>	<i>divergens</i>	CIP 101029T	Unknown	-
12	<i>Carnobacterium</i>	<i>piscicola</i>	Ad369	Raw milk	-
13	<i>Enterococcus</i>	<i>durans</i>	Ad149	Ham	-
14	<i>Enterococcus</i>	<i>faecalis</i>	Adria 89L326	Soft cheese (Vacherin)	-
15	<i>Lactobacillus</i>	<i>brevis</i>	Ad405	Ham	-
16	<i>Lactobacillus</i>	<i>curvatus</i>	Ad380	Delicatessen	-
17	<i>Lactobacillus</i>	<i>fermentum</i>	Ad482	Tomatoe juice	-
18	<i>Lactobacillus</i>	<i>sakei</i>	Ad473	Ham	-
19	<i>Lactococcus</i>	<i>lactis</i> subsp. <i>cremoris</i>	Ad137	Dairy product	-
20	<i>Leuconostoc</i>	<i>carnosum</i>	Ad411	Ham	-
21	<i>Leuconostoc</i>	<i>citreum</i>	Ad396	Ham	-

					+/ ^{c,d} - ^e
22	<i>Micrococcus</i>	<i>luteus</i>	Ad432	Cocktail (beverage)	-
23	<i>Micrococcus</i>	<i>luteus</i>	ATCC 10240	Unknown	-
24	<i>Micrococcus</i>	<i>luteus</i>	CIP A270T	Unknown	-
25	<i>Pediococcus</i>	<i>pentosaceus</i>	ATCC 33316	Unknown	-
26	<i>Propionibacterium</i>	<i>freundenreichii</i>	CNRZ 725	Dairy product	-
27	<i>Staphylococcus</i>	<i>aureus</i>	Ad165	Smoked delicatessen	-
28	<i>Staphylococcus</i>	<i>luteus</i>	Ad432	Rhum	-
29	<i>Staphylococcus</i>	<i>epidermidis</i>	Ad931	Fruits	-
30	<i>Staphylococcus</i>	<i>haemoliticus</i>	Ad989	Dairy product	-
31	<i>Streptococcus</i>	<i>bovis</i>	Adria 92L622	Dairy product	-
32	<i>Streptococcus</i>	<i>salivarius</i> subsp. <i>thermophilus</i>	Ad441	Dairy product	-

^a Ad, Adria, A00, EN = ADRIA Développement culture collection, Quimper, France; CIP = Collection de l'institut Pasteur, Paris, France; ATCC= American Type Culture Collection, Manassas, VA; CNRZ=Centre national de Recherches zootechniques, Jouy-en-Josas, France.

^b "-" indicates the non-target analyte was not detected.

^c Tested twice from culture in BHI broth.

^d "+" indicates the non-target analyte was detected.

^e Tested from culture in LSB II broth.

Table 6. iQ-Check *Listeria* spp. Results - Presumptive vs. Confirmed (10)

Matrix	Strain ^a	Lysis protocol	PCR APF ^h	CFU/ test portion or Test Area	N ^b	Presumptive			Confirmed			dPOD _{CP} ^f	95% CI ^g
						x ^c	POD _{CP} ^d	95% CI	x	POD _{CC} ^e	95% CI		
Deli turkey (25 g)	<i>Listeria monocytogenes</i> Ad2453	FDRS ⁱ + Easy II	Fast	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
				0.14 [0.03;0.33]	20	7	0.00	0.00,0.43	7	0.00	0.00,0.43	0.00	-0.13,0.13
				1.81 [0.85;4.45]	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
		FDRS + Easy II	Classic	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
				0.14 [0.03;0.33]	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.13, 0.13
				1.81 [0.85;4.45]	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
Cheddar cheese (125 g)	<i>Listeria innocua</i> Ad653	FDRS + Easy II	Fast	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
				0.14 [0.72;1.94]	20	14	0.70	0.48,0.86	15	0.75	0.53,0.89	-0.50	-0.21,0.11
				3.29 [1.63;234.2]	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
		FDRS + Easy II	Classic	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
				0.14 [0.72;1.94]	20	12	0.60	0.39,0.78	15	0.75	0.53,0.89	-0.15	-0.35,0.50
				3.29 [1.63;234.2]	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Stainless Steel (4"x4", sponge)	<i>Listeria monocytogenes</i> Ad651 + <i>Enterococcus faecalis</i> CNRZ 1307	FDRS + Easy II	Fast	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
				8.7	20	15	0.75	0.53,0.89	13	0.65	0.43,0.82	0.10	-0.08,0.28
				21.6	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
		FDRS + Easy II	Classic	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
				8.7	20	15	0.75	0.53,0.89	13	0.65	0.43,0.82	0.10	-0.08,0.28
				21.6	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47

^aAd= ADRIA Développement culture collection, Quimper, France; CNRZ= Centre National de Recherches zootechniques, Jouy-en-Josas, France.

^bN = Number of test portions.

^cx = Number of positive test portions.

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

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

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

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

^hAPF=Application Protocol File

ⁱFDRS=Free DNA Removal Solution.

Table 7. iQ-Check *Listeria* spp. Results - Candidate vs. Reference (10)

Matrix	Strain ^a	Lysis protocol	PCR APF ^h	cfu/ test portion or Test Area	N ^b	x ^c	Candidate			Reference			
							POD _c ^d	95% CI	X	POD _R ^e	95% CI	dPOD _c ^f	95% CI ^g
Deli turkey (25 g)	<i>Listeria monocytogenes</i> Ad2453	FDRS ⁱ + Easy II	Fast	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
				0.14 [0.03;0.33]	20	7	0.35	0.18,0.57	3	0.15	0.05,0.36	0.20	-0.07,0.44
				1.81 [0.85;4.45]	5	4	0.80	0.38,1.00	3	0.60	0.23,0.88	0.20	-0.31,0.62
				0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
				0.14 [0.03;0.33]	20	7	0.35	0.18,0.57	3	0.15	0.05,0.36	0.20	-0.07,0.44
		FDRS + Easy II	Classic	1.81 [0.85;4.45]	5	4	0.80	0.38,1.00	3	0.60	0.23,0.88	0.20	-0.31,0.62
				0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
				0.14 [0.03;0.33]	20	7	0.35	0.18,0.57	3	0.15	0.05,0.36	0.20	-0.07,0.44
				1.81 [0.85;4.45]	5	4	0.80	0.38,1.00	3	0.60	0.23,0.88	0.20	-0.31,0.62
				0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
Cheddar cheese (125 g)	<i>Listeria innocua</i> Ad653	FDRS + Easy II	Fast	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
				0.14 [0.72;1.94]	20	14	0.70	0.48,0.86	15	0.75	0.53,0.89	-0.05	-0.31,0.22
				3.29 [1.63;234.2]	5	5	1.00	0.57,1.00	4	0.20	0.08,0.42	0.80	-0.04,0.59
				0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
				0.14 [0.72;1.94]	20	12	0.60	0.39,0.78	15	0.75	0.53,0.89	-0.15	-0.40,0.13
		FDRS + Easy II	Classic	3.29 [1.63;234.2]	5	5	0.50	0.34,0.76	4	0.20	0.08,0.42	0.30	-0.04,0.59
				0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
				0.14 [0.72;1.94]	20	12	0.60	0.39,0.78	15	0.75	0.53,0.89	-0.15	-0.40,0.13
				3.29 [1.63;234.2]	5	5	0.50	0.34,0.76	4	0.20	0.08,0.42	0.30	-0.04,0.59
				0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
Stainless Steel (4"x4", sponge)	<i>Listeria monocytogenes</i> Ad651 + <i>Enterococcus faecalis</i> CNRZ 1307	FDRS + Easy II	Fast	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
				8.7	20	13	0.65	0.43,0.82	17	0.85	0.64,0.95	-0.20	-0.44,0.07
				21.6	5	5	1.00	0.57,1.00	4	0.80	0.38,1.00	0.20	-0.28,0.62
				0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
				8.7	20	13	0.65	0.43,0.82	17	0.85	0.64,0.95	-0.20	-0.44,0.07
		FDRS + Easy II	Classic	21.6	5	5	1.00	0.57,1.00	4	0.20	0.38,1.00	0.20	-0.28,0.62
				0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
				8.7	20	13	0.65	0.43,0.82	17	0.85	0.64,0.95	-0.20	-0.44,0.07
				21.6	5	5	1.00	0.57,1.00	4	0.20	0.38,1.00	0.20	-0.28,0.62
				0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43

^aAd= ADRIA Développement culture collection, Quimper, France ; CNRZ= Centre National de Recherches zootechniques, Jouy-en-Josas, France.

^bN = Number of test portions.

^cx = Number of positive test portions.

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

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

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

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

^hAPF=Application Protocol File

ⁱFDRS=Free DNA Removal Solution.

Table 8. Alternative Confirmation Results for the iQ-Check *Listeria* species Kit for Deli Turkey (10)

Deli Turkey (25 g)							
<i>L. monocytogenes</i> Ad2453							
Low Level							
0.14 cfu/test portion (0.03, 0.33)							
Sample #	iQ-Check <i>Listeria</i> spp.		Confirmed ^a			MLG 8.13	
	Fast	Classic	RAPID <i>L. mono</i> Agar	RAPID <i>Listeria</i> Agar	Agar <i>Listeria</i>	MOX	Final Result
1	+ ^b	+	+	+	- ^c	+	+
2	+	+	+3 ^d	-	-	+	+
3	+	+	+	+	+	+	+
4	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-
9	+	+	+	+	+	+	+
10	-	-	-	-	-	-	-
11	+	+	+	+	+	+	+
12	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-
15	+	+	+	+	+	+	+
16	-	-	-	-	-	-	-

Uninoculated								
1	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-
Total	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5

^a Agar plate results after streaking from primary enrichment

^b "+" indicates the target analyte was detected.

^c Two separate PCR tests were performed. Cq values for each test reported.

^d Three separate PCR tests were performed. Cq values for each test reported.

^e Test portion was negative after 24 h and positive after 48 h. Recommended protocol is to check the plates for typical colonies after 24 and 48 h.

^f "-" indicates the non-target analyte was not detected.

NA = not applicable

Table 10. Alternative Confirmation Results for the iQ-Check <i>Listeria</i> species Kit for Stainless Steel (10)								
Stainless Steel (4" x 4")								
<i>L. monocytogenes</i> Ad651 + <i>E. faecalis</i> CNRZ 1307								
Low Level								
8.7 cfu/test portion								
Sample #	iQ-Check <i>Listeria</i> spp		Confirmed ^a			BAM Ch 10		
	Fast	Classic	RAPID <i>L. mono</i> Agar	RAPID <i>Listeria</i> Agar	Agar <i>Listeria</i>	Agar <i>Listeria</i>	PALCAM	Final Result
1	+ ^b	+	+	+	+	+	+	+
2	- ^c	-	-	-	-	-	-	-
3	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+
6	+/ ^d 38.59/40.12	-	-	-	-	-	-	-
7	+	+	+	+	+	+	+	+
8	+	+	+	+	+	+	+	+
9	+	+	+	+	+	+	+	+
10	+/-/ ^e 39.33/NA/39.68	+/ ^d 38.59/NA	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-
12	+	+	+	+	+	+	+	+
13	-	+/ ^d 39.12/NA	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-
15	+	+	+	+	+	+	+	+
16	+	+	+	+	+	+	+	+
17	-	-	-	-	-	-	-	-
18	+	+	+	+	+	+	+	+
19	+	+	+	+	+	+	+	+
20	+	+	+	+	+	+	+	+
Total	15/20	15/20	13/20	13/20	13/20	13/20	13/20	13/20
High Level								
12.6 cfu/test portion								
1	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+
Total	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Uninoculated								
1	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-
Total	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5

^a Agar plate results after streaking from primary enrichment

^b "+" indicates the target analyte was detected.

^c "-" indicates the non-target analyte was not detected.

^d Two separate PCR tests were performed with Cq values reported.

^e Three separate PCR tests were performed with Cq values reported.

NA = not applicable

DISCUSSION OF THE MODIFICATION STUDY APPROVED JANUARY 2023 (14)

The new CFX Opus Deepwell instrument delivers the same performance as the current CFX96 Touch Deep Well instrument but with a more modern design and cloud capabilities. The improved stability of the thermal block ensures a more uniform thermal protocol. The CFX Manager Software, IDE v 3.1 brings the same performance, algorithm, and interpretation as the current CFX Manager Software, IDE v 3.0 with the only change being compatibility to both CFX96 Touch Deep Well and CFX Opus Deepwell instruments. The iQ-Check *Listeria* spp. kit gave a false negative for one sample of the deli ham. This is most likely due to the low level of target *Listeria* (0.15 MPN/25 g) in the test portions and the normal distribution of the target DNA and sampling of the test portions. No discrepancies were observed between the CFX96 Touch Deep Well and CFX Opus Deepwell instruments. In the inclusivity and exclusivity evaluations, all inclusivity organisms were correctly identified, and all exclusivity organisms were correctly excluded.

Table 29. Bio-Rad iQ-Check *Listeria* spp. Kit, Presumptive vs. Confirmed-POD Results (14)

Matrix	Strain	MPN ^a /CFU ^b Test Portion	N ^c	Presumptive			Confirmed			dPOD _{CP} ^g	95% CI ^h
				X ^d	POD _{CP} ^e	95% CI	X	POD _{CC} ^f	95% CI		
Deli ham (25 g) CFX96 Touch Deep Well	<i>Listeria monocytogenes</i> Ad 2453	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.15 (0.05, 0.35)	20	6	0.30	0.16,0.52	7	0.35	0.18,0.57	-0.05	-0.21,0.11
		1.78 (0.9, 3.5)	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
Deli ham (25 g) CFX Opus Deepwell	<i>Listeria monocytogenes</i> Ad 2453	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.15 (0.05, 0.35)	20	6	0.30	0.16,0.52	7	0.35	0.18,0.57	-0.05	-0.21,0.11
		1.78 (0.9, 3.5)	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
Stainless Steel (4"x4", sponge) CFX96 Touch Deep Well	<i>Listeria monocytogenes</i> Ad 651 + <i>Enterococcus faecalis</i> CNRZ 1307	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.7	20	8	0.40	0.22,0.61	8	0.40	0.22,0.61	0.00	-0.13,0.13
		2.7	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Stainless Steel (4"x4", sponge) CFX Opus Deepwell	<i>Listeria monocytogenes</i> Ad 651 + <i>Enterococcus faecalis</i> CNRZ 1307	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.7	20	8	0.40	0.22,0.61	8	0.40	0.22,0.61	0.00	-0.13,0.13
		2.7	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47

^aMPN = Most Probable Number is calculated using the LCF MPN calculator ver. 2.0 provided by AOAC RI, with 95% confidence interval

^bCFU = Colony Forming Units

^cN = Number of test portions

^dX = Number of positive test portions

^ePOD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials

^fPOD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials

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

^h95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

Table 30. Bio-Rad iQ-Check *Listeria* spp., Candidate vs. Reference (Unpaired) – POD Results (14)

Matrix	Strain	MPN ^a /CFU ^b Test Portion	N ^c	Candidate			Reference			dPOD _C ^g	95% CI ^h
				X ^d	POD _C ^e	95% CI	X	POD _R ^f	95% CI		
Deli ham (25 g) CFX96 Touch Deep Well	<i>Listeria monocytogenes</i> Ad 2453	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.15 (0.05, 0.35)	20	6	0.30	0.14,0.52	3	0.15	0.05,0.36	0.15	-0.11,0.39
		1.78 (0.9, 3.5)	5	4	0.80	0.38,1.00	3	0.60	0.23,0.88	0.20	-0.31,0.62
Deli ham (25 g) CFX Opus Deepwell	<i>Listeria monocytogenes</i> Ad 2453	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.15 (0.05, 0.35)	20	6	0.30	0.14,0.52	3	0.15	0.05,0.36	0.15	-0.11,0.39
		1.78 (0.9, 3.5)	5	4	0.80	0.38,1.00	3	0.60	0.23,0.88	0.20	-0.31,0.62
Stainless Steel (4"x4", sponge) CFX96 Touch Deep Well	<i>Listeria monocytogenes</i> Ad 651 + <i>Enterococcus faecalis</i> CNRZ 1307	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.7	20	8	0.40	0.22,0.61	11	0.55	0.34, 0.74	-0.15	-0.41,0.15
		2.7	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Stainless Steel (4"x4", sponge) CFX Opus Deepwell	<i>Listeria monocytogenes</i> Ad 651 + <i>Enterococcus faecalis</i> CNRZ 1307	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.7	20	8	0.40	0.22,0.61	11	0.55	0.34, 0.74	-0.15	-0.41,0.15
		2.7	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47

^aMPN = Most Probable Number is calculated using the LCF MPN calculator ver. 2.0 provided by AOAC RI, with 95% confidence interval

^bCFU = Colony Forming Units

^cN = Number of test portions

^dX = Number of positive test portions

^ePOD_C = Candidate method confirmed positive outcomes divided by the total number of trials

^fPOD_R = Reference method confirmed positive outcomes divided by the total number of trials

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

^h95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

Table 31. Bio-Rad iQ-Check *Listeria* spp. Kit, CFX Opus Deepwell vs. CFX96 Touch Deep Well–POD Results (14)

Matrix	Strain	MPN ^a /CFU ^b Test Portion	N ^c	CFX Opus Deepwell			CFX96 Touch Deep Well			dPOD _{OT} ^g	95% CI ^h
				X ^d	POD _{OC} ^e	95% CI	X	POD _{TC} ^f	95% CI		
Deli ham (25 g)	<i>Listeria monocytogenes</i> Ad 2453	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.15 (0.05, 0.35)	20	6	0.30	0.14,0.52	6	0.30	0.14,0.52	0.00	-0.13, 0.13
		1.78 (0.9, 3.5)	5	4	0.80	0.38, 1.00	4	0.80	0.38, 1.00	0.00	-0.47, 0.47
Stainless Steel (4"x4", sponge)	<i>Listeria monocytogenes</i> Ad 651 + <i>Enterococcus faecalis</i> CNRZ 1307	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.7	20	8	0.40	0.22,0.61	8	0.40	0.22,0.61	0.00	-0.13,0.13
		2.7	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47

^aMPN = Most Probable Number is calculated using the LCF MPN calculator ver. 2.0 provided by AOAC RI, with 95% confidence interval

^bCFU = Colony Forming Units

^cN = Number of test portions

^dx = Number of positive test portions

^ePOD_{OC} = CFX Opus Deepwell confirmed positive outcomes divided by the total number of trials

^fPOD_{TC} = CFX96 Touch Deep Well confirmed positive outcomes divided by the total number of trials

^gdPOD_{OT} = Difference between the CFX Opus Deepwell confirmed result and CFX96 Touch Deep Well confirmed result POD values

^h95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

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