



CERTIFICATION

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

Certificate No.
010802

The AOAC Research Institute hereby certifies the method known as

iQ-Check *Listeria monocytogenes* II 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

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

Bradley A. Stawick, AOAC Research Institute Senior Director

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SUBMITTING COMPANY

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

METHOD NAME

iQ-Check *Listeria monocytogenes* II Real-Time PCR

CATALOG NUMBER

357-8124

INDEPENDENT LABORATORY

Original Validation
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Modification 1 January 2023
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APPLICABILITY OF METHOD

Target organism – *Listeria monocytogenes*.

Matrixes – (25 g) – smoked salmon, cottage cheese, hot dogs, and deli turkey

GovVal approved Matrixes – (25 g) – liver pâté, raw fermented sausage, deli ham, and stainless steel

MODIFICATION JANUARY 2023 – FDA MLG 8.13 – deli turkey (25 g)

FDA BAM – Ch. 10 – cottage 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 monocytogenes* II Real-Time PCR method and the reference methods.

REFERENCE METHODS

AOAC International. AOAC Official Method 993.12 – *Listeria monocytogenes* in Milk and Dairy Products – Selective Enrichment and Isolation Method (Final Action 1996) (2)

United States Department of Agriculture, Food Safety and Inspection Service. Microbiology Laboratory Guidebook – Chapter 8.05. Isolation and Identification of *Listeria monocytogenes* from Red Meat, Poultry, Egg and Environmental Samples (3)

United States Food and Drug Administration, Center for Food Safety and Applied Nutrition. Bacteriological Analytical Manual - Chapter 10. *Listeria monocytogenes* (4)

GovVal Extension: Health Canada, Health Products and Food Branch (2010) *Compendium of Analytical Methods*, MFHPB – 30 (6)

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, U.S. Department of Agriculture Food Safety Inspection Service (2021) (8)

***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*, (9)**

ORIGINAL CERTIFICATION DATE	CERTIFICATION RENEWAL RECORD
January 11, 2008	Renewed through December 2026.
METHOD MODIFICATION RECORD	SUMMARY OF MODIFICATION
<ol style="list-style-type: none"> 1. December 2013 Level 2 2. GovVal Level 2 3. March 2015 Level 2 4. July 2018 Level 1 5. January 2020 Level 1 6. January 2021 Level 1 7. April 2021 Level 1 8. November 2021 Level 1 X 9. December 2022 Level 1 10. January 2023 Modification 1 Level 3 11. January 2023 Modification 2 Level 2 12. October 2023 Level 1 13. November 2024 Level 1 14. November 2025 Level 1 	<ol style="list-style-type: none"> 1. Software update 2. Matrix extension to include liver pâté, raw fermented sausage, deli ham, stainless steel 3. Manufacturing location change to Hercules, CA 4. Software update and manual edits 5. Reformatted inserts. 6. Editorial changes for clarity. 7. Software was updated from Version 3 to Version 4 allowing compatibility with Windows 10. 8. Editorial changes and addition of user information in French, German, Spanish, Portuguese, and Italian. 9. Editorial changes for clarity. 10. Modification includes: <ol style="list-style-type: none"> a. New enrichment of <i>Listeria</i> Special Broth II. b. Shorter enrichment time for Classic Application. c. Protocol File, except deli turkey. d. Optional use of Bio-Rad Free DNA Removal Solution. e. Use of <i>Listeria</i> spp Fast APF. f. Alternative confirmation method. 11. Addition of CFX Opus Deepwell, with CFX Manager Software, Industrial Diagnostic Edition version 3.1 using Free DNA Removal Solution and Fast APF protocols. 12. Editorial changes to Reference Guide and User Manual. 13. Editorial changes including software update to CFX Maestro Software, IDE v4.0. 14. Editorial changes.
<p>Under this AOAC Performance Tested MethodsSM License Number, 010802 this method is distributed by:</p> <p>NONE</p>	<p>Under this AOAC Performance Tested MethodsSM License Number, 010802 this method is distributed as:</p> <p>NONE</p>

PRINCIPLE OF THE METHOD (1)

The iQ-Check *Listeria monocytogenes* II kit is a test based on gene amplification and detection by real-time polymerase chain reaction, (RTi-PCR). Ready-to-use RTi-PCR reagents contain DNA primers and a DNA probe specific for *L. monocytogenes*, as well as DNA polymerase and nucleotides. PCR is a technique used to generate many copies of target DNA. During the PCR reaction, several cycles of heating and cooling allow DNA denaturation, by heat, followed by primers binding to the target region. The DNA polymerase then uses these primers and deoxynucleotide triphosphates (dNTPs) to extend the DNA, creating copies of the target DNA. These copies, called amplicons, are detected during the amplification by hybridizing specific oligonucleotide fluorescent probes called molecular beacons. These probes are linked to a fluorophore which fluoresces only when hybridized to the target sequence. In the iQ-Check *Listeria monocytogenes* II kits, carboxyfluorescein (FAM) is the fluorophore linked to the probe hybridizing to the *L. monocytogenes* specific DNA sequence. In the absence of target DNA, no fluorescence will be detected, and the sample determined to be negative. As the amount of amplicons increases with each round of amplification, fluorescence intensity also increases. During each PCR cycle, at the annealing step, the real-time PCR system measures this fluorescence and the associated software plots the fluorescence intensity versus number of cycles. This method allows a simple determination of the presence of *L. monocytogenes* in a sample. To monitor for a successful DNA amplification in each reaction tube, a synthetic DNA "internal control" is included in the reaction mix. This control is amplified with a specific probe at the same time as the *L. monocytogenes* target DNA sequence and detected by a second fluorophore.

DISCUSSION OF THE VALIDATION STUDY (1)

Previous studies have demonstrated the sensitivity of PCR methods used in the food safety industry (7). The use of molecular beacon probes in a PCR reaction provides increased specificity by targeting a specific sequence on the genome of the target organism. Since the assay detects specific target DNA, the growth of competitor organisms is not a major factor in the detection limit of the kit. The RTi-PCR system is able to distinguish between target and non-target DNA. A microbiology culture method based on isolation of target organisms on an agar plate can be greatly affected by the growth of competitor organisms using all the nutrients or, at the opposite, by the use of too much selective media. The iQ-Check *Listeria monocytogenes* II 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 *Listeria monocytogenes* II 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 monocytogenes* II test is performed after a single 25 h enrichment. A secondary enrichment is not necessary due to the low detection limit of the kit (LOD₅₀ ~1 cfu/25 g). Method agreement for the hot dog matrix was 85%. The iQ-Check *Listeria monocytogenes* II kit was able to identify more true positive samples than the reference method. The 3 discrepant samples detected by iQ-Check were confirmed, from the LSB culture, by standard reference method procedures. However, since the samples were unpaired, from two different enrichment broths, a direct comparison of these two results can be misleading. The same is true for the cottage cheese matrix, where there were 2 "discrepant" samples. There was no significant difference between the performance of the iQ-Check and the reference methods for any of the Matrixes tested.

Table 1 - Inclusivity Results (1)

Strain	Reference	Origin	iQ-Check Result
<i>Listeria monocytogenes</i>	IPL39	Sausage	Positive
<i>Listeria monocytogenes</i>	IPL119	Spinach	Positive
<i>Listeria monocytogenes</i>	IPL121	Neufchatel cheese	Positive
<i>Listeria monocytogenes</i>	IPL123	Mozzarella cheese	Positive
<i>Listeria monocytogenes</i>	IPL124	Perch fillet	Positive

<i>Listeria monocytogenes</i>	IPL130	Ground beef	Positive
<i>Listeria monocytogenes</i>	IPL137	Coulommier raw milk cheese	Positive
<i>Listeria monocytogenes</i>	IPL141	Environmental sample	Positive
<i>Listeria monocytogenes</i>	IPL149	Environmental sample	Positive
<i>Listeria monocytogenes</i>	IPL152	Environmental sample	Positive
<i>Listeria monocytogenes</i>	IPL156	French fries	Positive
<i>Listeria monocytogenes</i>	IPL176	Beef rib steak	Positive
<i>Listeria monocytogenes</i> 1/2	IPL20	Smoked salmon	Positive
<i>Listeria monocytogenes</i> 1/2	IPL25	Chicken	Positive
<i>Listeria monocytogenes</i> 1/2a	IPL5	Smoked salmon	Positive
<i>Listeria monocytogenes</i> 1/2a	IPL6	Pizza	Positive
<i>Listeria monocytogenes</i> 1/2a	IPL7	Munster raw milk cheese	Positive
<i>Listeria monocytogenes</i> 1/2a	IPL9	Munster raw milk cheese	Positive
<i>Listeria monocytogenes</i> 1/2a	IPL10	Potted meat	Positive
<i>Listeria monocytogenes</i> 1/2a	IPL11	Munster raw milk cheese	Positive
<i>Listeria monocytogenes</i> 1/2a	IPL12	Smoked salmon	Positive
<i>Listeria monocytogenes</i> 1/2a	IPL40	Munster raw milk cheese	Positive
<i>Listeria monocytogenes</i> 1/2a	IPL42	Chicken escalope	Positive
<i>Listeria monocytogenes</i> 1/2a	IPL43	Ground beef	Positive
<i>Listeria monocytogenes</i> 1/2a	IPL44	Sausage	Positive
<i>Listeria monocytogenes</i> 1/2a	IPL45	Rabbit meat	Positive
<i>Listeria monocytogenes</i> 1/2a	IPL47	Sauté potatoes	Positive
<i>Listeria monocytogenes</i> 1/2a	IPL116	Fish	Positive
<i>Listeria monocytogenes</i> 1/2a	IPL128	Soybean cattle cake	Positive
<i>Listeria monocytogenes</i> 1/2a	IPL129	Sauté potatoes	Positive
<i>Listeria monocytogenes</i> 1/2b	IPL13	Pig ears	Positive
<i>Listeria monocytogenes</i> 1/2b	IPL37	Maroille raw milk cheese	Positive
<i>Listeria monocytogenes</i> 1/2b	IPL48	Pig tongue	Positive
<i>Listeria monocytogenes</i> 1/2b	IPL49	Chicken liver	Positive
<i>Listeria monocytogenes</i> 1/2b	IPL50	Blood sausage	Positive
<i>Listeria monocytogenes</i> 1/2b	IPL51	Germain raw milk cheese	Positive
<i>Listeria monocytogenes</i> 1/2c	IPL14	Ground beef	Positive
<i>Listeria monocytogenes</i> 1/2c	IPL15	Beef	Positive
<i>Listeria monocytogenes</i> 1/2c	IPL16	Ground beef	Positive
<i>Listeria monocytogenes</i> 1/2c	IPL17	Pork breast	Positive
<i>Listeria monocytogenes</i> 1/2c	IPL18	Munster raw milk cheese	Positive
<i>Listeria monocytogenes</i> 1/2c	IPL28	Surface sponge	Positive
<i>Listeria monocytogenes</i> 1/2c	IPL53	Ground beef	Positive
<i>Listeria monocytogenes</i> 1/2c	IPL54	Beef Bourguignon	Positive
<i>Listeria monocytogenes</i> 1/2c	IPL117	Montbeliard sausage	Positive
<i>Listeria monocytogenes</i> 3a	IPL191	Fishery environment	Positive
<i>Listeria monocytogenes</i> 3a	IPL192	Fishery environment	Positive
<i>Listeria monocytogenes</i> 3b	IPL193	Fishery environment	Positive
<i>Listeria monocytogenes</i> 3b	IPL55	SLCC 2540 (human)	Positive
<i>Listeria monocytogenes</i> 3c	IPL56	SLCC 2479	Positive
<i>Listeria monocytogenes</i> 4b	IPL32	Munster raw milk cheese	Positive
<i>Listeria monocytogenes</i> 4b	IPL58	Salad	Positive
<i>Listeria monocytogenes</i> 4d	IPL60	ATCC 19117 (sheep)	Positive
<i>Listeria monocytogenes</i> 4d	IPL194	Fishery environment	Positive
<i>Listeria monocytogenes</i> 4e	IPL62	Reblochon raw milk cheese	Positive
<i>Listeria monocytogenes</i> 4e	IPL63	Munster raw milk cheese	Positive
<i>Listeria monocytogenes</i> 7	IPL67	SLCC 2482 (human feces)	Positive

ATCC = American Type Culture Collection, USA, IPL = *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>	IPLBA2	Beet root	Negative
<i>Bacillus coagulans</i>	IPLBA7	Milk product	Negative
<i>Bacillus mycoïdes</i>	IPLBA6	Environment	Negative
<i>Bacillus mycoïdes</i>	IPLBA24	Soil	Negative
<i>Bacillus sphaericus</i>	IPLBA5	Meat product	Negative
<i>Bacillus steaothermophilus</i>	IPLBA4	Milk product	Negative
<i>Candida albicans</i>	IPLLe3	Collection	Negative
<i>Carnobacterium divergens</i>	IPL46	Minced beef	Negative
<i>Carnobacterium gallinarum</i>	IPL47	Ice slush of chicken carcasses	Negative
<i>Carnobacterium piscicola</i>	IPL48	Raw milk	Negative
<i>Citrobacter braakii</i>	IPLCIT86	Pork sausage	Negative
<i>Citrobacter freundii</i>	IPLCIT24	Meat product	Negative
<i>Enterobacter cloacae</i>	IPLent76	Milk powder	Negative
<i>Enterococcus faecalis</i>	IPL1E1	Egg product	Negative
<i>Enterococcus faecalis</i>	IPL1E6	ATCC 19433	Negative
<i>Enterococcus faecium</i>	IPL1E2	ATCC 3286	Negative

<i>Enterococcus faecium</i>	IPL7	CIP 54.33 (Canned fish)	Negative
<i>Erysipelothrix rhusiopathiae</i>	IPL49	Spleen of pig with endocarditis	Negative
<i>Escherichia coli</i>	IPL20	Tomatoes	Negative
<i>Escherichia coli</i>	IPL35	Spinach	Negative
<i>Jonesia dentrificans</i>	IPL139	CIP 55134T	Negative
<i>Klebsiella pneumoniae</i>	IPL63	Celery	Negative
<i>Klebsiella pneumoniae</i>	IPL68	Vegetable salad	Negative
<i>Kurthia gibsonii</i>	IPL42	Meat product	Negative
<i>Lactobacillus casei</i>	IPL9595	ATCC 9595	Negative
<i>Lactobacillus delbrueckii ssp lactis</i>	IPL53	Emmental cheese	Negative
<i>Lactococcus lactis</i>	IPL33	Milk product	Negative
<i>Listeria grayi</i>	IPL190	Frozen French fries	Negative
<i>Listeria grayi</i>	IPL81	ATCC 19120	Negative
<i>Listeria innocua</i>	IPL108	Gorgonzola cheese	Negative
<i>Listeria innocua</i>	IPL113	Smoked halibut	Negative
<i>Listeria innocua</i>	IPL64	Epoisses cheese	Negative
<i>Listeria innocua</i>	IPL65	Epoisses cheese	Negative
<i>Listeria innocua</i>	IPL66	Spinach	Negative
<i>Listeria innocua</i>	IPL72	Boulettes d'Avesnes cheese	Negative
<i>Listeria innocua</i>	IPL78	Cockerel	Negative
<i>Listeria innocua 6a</i>	IPL77	Toulouse sausage	Negative
<i>Listeria innocua 6a</i>	IPL1	ATCC 33090 (cow brain)	Negative
<i>Listeria innocua 6b</i>	IPL2	Ground beef	Negative
<i>Listeria ivanovii 5</i>	IPL161	Ground beef	Negative
<i>Listeria ivanovii 5</i>	IPL153	Environment	Negative
<i>Listeria ivanovii 5</i>	IPL80	Collection	Negative
<i>Listeria ivanovii 5</i>	IPL184	Bird trap	Negative
<i>Listeria ivanovii 5</i>	IPL179	Environmental sample	Negative
<i>Listeria seeligeri</i>	IPL115	Lake water	Negative
<i>Listeria seeligeri 1/2b</i>	IPL84	Ground meat	Negative
<i>Listeria seeligeri 1/2b</i>	IPL83	Ox tongue	Negative
<i>Listeria welshimeri</i>	IPL100	Pâté	Negative
<i>Listeria welshimeri</i>	IPL101	Ham	Negative
<i>Micrococcus spp</i>	IPLM1	Environment	Negative
<i>Propionibacterium freundenreichii</i>	IPL43	Swiss cheese	Negative
<i>Proteus mirabilis</i>	IPL45	Poultry	Negative
<i>Rhodococcus equi</i>	IPL32	Meat product	Negative
<i>Rhodococcus equi</i>	IPLR2	Lung abscess of foal	Negative
<i>Rhodotorula rubra</i>	IPLLe1	Pastry	Negative
<i>Salmonella brandenburg</i>	IPLS3	Pâté	Negative
<i>Salmonella typhimurium</i>	IPLS31	Egg product	Negative
<i>Salmonella virchow</i>	IPLS33	Cockle	Negative
<i>Staphylococcus epidermidis</i>	IPLST3	Yogurt	Negative
<i>Staphylococcus epidermidis</i>	IPLST20	Smoked salmon	Negative
<i>Streptococcus bovis</i>	IPLStrE3	Meat product	Negative

ATCC = American Type Culture Collection, USA

CIP = Collection Institut Pasteur, France

IPL = Culture collection, Institut Pasteur de Lille, France

Table 3 - Method Comparison Results (1)

Matrix	Level	MPN/25 g (95%)	Samples	iQ-Check positive	LSB culture confirmed	Reference positive	Method Agreement ^a	False			False	
								X ² ^b	Sensitivity ^c	Negative ^d	Specificity ^e	Positive ^f
Smoked salmon	Control	< 0.075 (0-0.24)	5	0	0	0	100%	-	-	-	100%	0%
	Low	0.375 (0.11-1.05)	20	10	10	11	95%	0.09	91%	9%	-	-
Cottage cheese	Control	< 0.075 (0-0.24)	5	0	0	0	100%	-	-	-	100%	0%
	Low	1.075 (0.23-4.5)	20	12	12	10	90%	0.39	120%	0%	-	-
Hot dogs	Control	< 0.075 (0-0.24)	5	0	0	0	100%	-	-	-	100%	0%
	Low	1.6 (0.43-4.5)	20	10	10	7	85%	0.90	143%	0%	-	-
Deli turkey (internal)	Control	< 0.075 (0-0.24)	5	0	0	0	100%	-	-	-	100%	0%
	Low	1.075 (0.23-4.5)	20	12	13	12	95%	0.00	108%	0%	-	-
Deli turkey (independent)	Control	< 0.075 (0-0.24)	5	0	0	0	100%	-	-	-	100%	0%
	Low	0.375 (0.11-1.05)	20	8	9	8	95%	0.00	113%	0%	-	-

DISCUSSION OF THE MODIFICATION STUDY GovVal (5)

The use of DNA hybridization probes in a PCR reaction provides increased specificity by targeting a specific sequence on the genome of the target organism. Since the assay is detecting specific target DNA, the growth of competitor organisms is not a major factor in the detection limit of the kit. The PCR system is able to distinguish between target and non-target DNA. A microbiology culture method based on isolation of target organisms on an agar plate can be greatly affected by the growth of competitor organisms using all the nutrients or, at the opposite, by the use of too much selective media. The iQ-Check *Listeria monocytogenes* II 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 *Listeria monocytogenes* II 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 monocytogenes* II test is performed after a single 24 h enrichment. A secondary enrichment is not necessary due to the low detection limit of the kit. In this evaluation, the iQ-Check *Listeria* species 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.

Table 4. Method Comparison Results^v (5)

Matrix	Strain	MPN ^a /25g	N ^c	iQ-Check <i>Listeria monocytogenes</i> II Method			MFHPB-30 Reference Method			dPOD _c ^g	95% CI ^h
				x ^d	POD _c ^e	95% CI	x	POD _R ^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 – 1	<i>L. monocytogenes</i> 1/2b plus 10x <i>L. innocua</i> 4ab	< 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	10	0.50	0.30, 0.70	8	0.40	0.22, 0.61	0.10	-0.19, 0.37
		1.21 (0.693-2.302)	20	12	0.60	0.39, 0.78	14	0.70	0.48, 0.86	-0.10	-0.36, 0.18
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

^h95% 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 (5)

Matrix	Strain	MPN ^a /25g	N ^c	iQ-Check <i>Listeria monocytogenes</i> II Method		MFHPB-30 Method	Chi Square ^d	Relative Sensitivity ^e
				Presumptive Pos.	Confirmed Pos.	Positive		
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 – 1	<i>L. monocytogenes</i> 1/2b plus 10x <i>L. innocua</i> 4ab	< 0.075	5	0	0	0	-	-
		0.598 (0.288-1.050)	20	10	10	8	0.39	125
		1.21 (0.693-2.302)	20	12	12	14	0.43	86
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 confirmed 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 THE MODIFICATION STUDY APPROVED MODIFICATION 1 JANUARY 2023 (7)

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 ± 1°C for deli turkey and cottage cheese, and 16-24 h at 37 ± 1°C for stainless steel.

For the deli turkey matrix at the low level, there was one test portion that showed a negative PCR result using the Fast and Classic APFs while the traditional confirmation procedure concluded to the presence of the *L. monocytogenes* strain in the enriched sample. The lysate was retested twice using each APF, and negative PCR results were observed for both APF Fast retests and one positive PCR result (Cq: 39.49) and one negative PCR result for the APF Classic. Using the alternative confirmation protocol, only few characteristic *L. monocytogenes* colonies were observed on one of the three selective agar plates tested (RAPID'*L. mono*), indicating a probably low contamination in the enriched sample, at the limit of detection of the candidate method. A second confirmed positive sample, for the low inoculation level, gave a negative PCR result using the APF Classic, the lysate was tested three times and all the results were negative. The Cq value for the APF Fast was relatively high (39.60), once again indicating a very low level of target DNA present. Based on these results, the Classic APF is not recommended for deli turkey.

For cottage cheese, the same results were obtained with both the Fast and Classic APF protocols.

For stainless steel, one test portion for the low inoculation level gave negative a PCR result for the APF Classic, while a positive PCR was obtained with the APF Fast (Cq:38.91) with the APF Fast, the lysate was tested twice again and positive result were observed (Cq:36.29,38.24). This sample was confirmed positive.

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 monocytogenes* II Test kit successfully detected *L. monocytogenes* on the two food matrices and environmental surface analyzed using the LSB II broth, the FDRS protocol and Easy II extraction protocol whatever the Application protocol File (APF) used for PCR analysis, which were two additional objectives of this study. Using POD analysis, no statistically significant differences were observed between the number of positive samples detected by the candidate method and the reference method.

The final objective was 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 the low level cottage cheese, there were two more positive final results as compared to the number of typical colonies found on RAPID'*Listeria* agar. For this reason, RAPID'*Listeria* is not recommended for use with cottage cheese. 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 monocytogenes* II kit demonstrated no statistically significant differences between candidate and reference method results (dPOD_c), or between presumptive and confirmed results (dPOD_c) for both Application Profiles tested. Observed differences between the iQ-Check *Listeria monocytogenes* II 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 monocytogenes* II method allows to reduce the incubation time.

Table 4. Inclusivity results (7)

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	Adria 913/1048	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	+

^a Ad, Adria, A00 = ADRIA Développement culture collection, Quimper, France.

^b "+" indicates the target analyte was detected

Table 5. Exclusivity results (7)

No.	Genus	Species	Source ^a	Origin	Result ^b
1	<i>Listeria</i>	<i>grayi</i>	Ad1198	Smoked salmon	-
2	<i>Listeria</i>	<i>grayi</i>	Ad1443	Pork meat sausages	-
3	<i>Listeria</i>	<i>grayi</i>	Ad1295	Spinach	-
4	<i>Listeria</i>	<i>innocua</i>	Ad658	Gorgonzola	-
5	<i>Listeria</i>	<i>innocua</i>	Ad660	Breadcrumbs	-
6	<i>Listeria</i>	<i>innocua</i>	Ad663	Environment (dairy industry)	-
7	<i>Listeria</i>	<i>ivanovii</i>	Ad466	Raw veal meat	-
8	<i>Listeria</i>	<i>ivanovii</i>	Ad1289	Raw milk cheese	-
9	<i>Listeria</i>	<i>ivanovii</i>	Ad1291	Poultry	-

10	<i>Listeria</i>	<i>seeligeri</i>	Ad649	Cheese	-
11	<i>Listeria</i>	<i>seeligeri</i>	Ad651	Environment	-
12	<i>Listeria</i>	<i>seeligeri</i>	Ad652	Environment (dairy industry)	-
13	<i>Listeria</i>	<i>welshimeri</i>	Ad1276	Environment (Slaughterhouse)	-
14	<i>Listeria</i>	<i>welshimeri</i>	Ad1235	Beef meat	-
15	<i>Listeria</i>	<i>welshimeri</i>	Ad191424	Poultry	-
16	<i>Bacillus</i>	<i>cereus</i>	Ad465	Salmon Terrine	-
17	<i>Bacillus</i>	<i>circulans</i>	Ad760	Vegetables	-
18	<i>Bacillus</i>	<i>coagulans</i>	Ad731	Dairy product	-
19	<i>Bacillus</i>	<i>licheniformis</i>	Ad978	Dairy product	-
20	<i>Bacillus</i>	<i>pumilus</i>	Ad284	Ready-to-eat	-
21	<i>Brochotrix</i>	<i>campestris</i>	CIP 102920T	Environment	-
22	<i>Carnobacterium</i>	<i>divergens</i>	CIP 101029T	Unknown	-
23	<i>Enterococcus</i>	<i>faecalis</i>	Adria 89L326	Soft cheese (Vacherin)	-
24	<i>Lactobacillus</i>	<i>fermentum</i>	Ad482	Tomatoe juice	-
25	<i>Leuconostoc</i>	<i>citreum</i>	Ad396	Ham	-
26	<i>Micrococcus</i>	<i>luteus</i>	Ad432	Cocktail (beverage)	-
27	<i>Pediococcus</i>	<i>pentosaceus</i>	ATCC 33316	Unknown	-
28	<i>Staphylococcus</i>	<i>aureus</i>	Ad165	Smoked delicatessen	-
29	<i>Staphylococcus</i>	<i>epidermidis</i>	Ad931	Fruits	-
30	<i>Streptococcus</i>	<i>bovis</i>	Adria 92L622	Dairy product	-

^a Ad, Adria, A00 = ADRIA Développement culture collection, Quimper, France; CIP = Collection de l'institut Pasteur, Paris, France; ATCC= American Type Culture Collection, Manassas, VA.

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

Table 6. iQ-Check *Listeria monocytogenes* II Results - Presumptive vs. Confirmed (7)

Matrix	Strain ^a	Lysis protocol	PCR APF ^h	cfu/test portion or test area		Presumptive				Confirmed			
				N ^b	x ^c	POD _{cp} ^d	95% CI	x	POD _{cc} ^e	95% CI	dPOD _{cp} ^f	95% CI ^g	
Deli turkey	<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]	2	6	0.30	0.16,0.52	7	0.35	0.18,0.57	-0.05	-0.21,0.11
				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]	2	5	0.25	0.11, 0.47	7	0.35	0.18, 0.57	-0.10	-0.28, 0.08
				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
Cottage cheese	<i>Listeria monocytogenes</i> Ad618	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
				1.57 [1.01, 2.26]	2	1	0.95	0.76,1.00	1	0.95	0.76,1.00	0.00	-0.13,0.13
		FDRS + Easy II	Classic	9.26 [4.92, 234.22]	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
				0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47

Stainless Steel (4"x4" sponge)	<i>Listeria monocytogenes</i> Ad651 + <i>Enterococcus faecalis</i> CNRZ 1307	Easy II	Fast	1.57 [1.01, 2.26]	2 0	1 9	0.95	0.76,1.00	1 9	0.95	0.76,1.00	0.00	-0.13,0.13
				9.26 [4.92, 234.22]	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
				0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		Easy II	Classic	8.7	2 0	1 3	0.65	0.43,0.82	1 3	0.65	0.43,0.82	0.00	-0.13,0.13
				21.6	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
				0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
				8.7	2 0	1 2	0.60	0.39,0.78	1 3	0.65	0.43,0.82	-0.05	-0.21,0.11
				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 monocytogenes* II Results - Candidate vs. Reference (7)

Matrix	Strain ^a	Lysis protocol	PCR APF ^h	cfu/test portion or test area	Candidate				Reference				
					N ^b	x ^c	POD _{CP} ^d	95% CI	X	POD _{CC} ^e	95% CI	dPOD _{CP} ^f	95% CI ^g
Deli turkey (25g)	<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	6	0.30	0.14,0.52	3	0.15	0.05,0.36	0.15	-0.11,0.39
				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
		FDRS + Easy II	Classic	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	5	0.25	0.11,0.47	3	0.15	0.05,0.36	0.10	-0.15,0.34
				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
Cottage cheese (25g)	<i>Listeria monocytogenes</i> Ad618	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
				1.57 [1.01, 2.26]	20	19	0.95	0.76,1.00	15	0.75	0.53,0.89	0.20	-0.03,0.42
				9.26 [4.92, 234.22]	5	5	1.00	0.57,1.00	5	1.00	0.57,1.00	0.00	-0.43, 0.43
		FDRS + Easy II	Classic	0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
				1.57 [1.01, 2.26]	20	19	0.60	0.39,0.78	15	0.75	0.53,0.89	0.20	-0.03,0.42
				9.26 [4.92, 234.22]	5	5	0.50	0.34,0.76	5	1.00	0.57,1.00	0.00	-0.43, 0.43
Stainless Steel	<i>Listeria monocytogenes</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
				8.7	20	13	0.65	0.43,0.82	17	0.85	0.64,0.95	-0.20	-0.44,0.07

(4"x4" sponge)	Ad651 + <i>Enterococcus faecalis</i> CNRZ 1307	Easy II	Classic	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	12	0.65	0.43,0.82	17	0.85	0.64,0.95	-0.20	-0.49,0.03
				21.6	5	5	1.00	0.57,1.00	4	0.20	0.38,1.00	0.20	-0.28,0.62

^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 *L.monocytogenes* II Kit for Deli Turkey (7)

Deli Turkey (25 g) <i>L. monocytogenes</i> Ad2453							
Low Level 0.14 cfu/test portion (0.03, 0.33)							
Sample #	iQ-Check <i>L.monocytogenes</i> II Kit		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	+	+	+	+	+	+
2	-/-/ ^c NA/NA/NA	-/+/ ^c NA/39.49/NA	+3 ^d	-	-	+	+
3	+	+	+	+	+	+	+
4	- ^e	-	-	-	-	-	-
5	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-
9	+	+	+	+	+	+	+
10	-	-	-	-	-	-	-
11	+	-/-/ ^c NA/NA/NA	+	+	+	+	+
12	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-
15	+	+	+	+	+	+	+
16	-	-	-	-	-	-	-
17	-	-	-	-	-	-	-
18	-	-	-	-	-	-	-
19	-	-	-	-	-	-	-
20	+	+	+	+	+	+	+
Total	6/20	6/20	7/20	6/20	6/20	7/20	7/20
High Level 1.81 cfu/test portion (0.85, 4.45)							
1	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+
3	-	-	-	-	-	-	-
4	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+
Total	4/5	4/5	4/5	4/5	4/5	4/5	4/5
Uninoculated							
1	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-
Total	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 Three separate PCR tests were performed with Cq values reported.

^d Three typical colonies were found on this plate.

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

Table 9. Alternative Confirmation Results for the iQ-Check *L. monocytogenes* II Kit for Cottage Cheese (7)

Cottage Cheese (25 g)								
<i>L. monocytogenes</i> Ad618								
Low Level								
1.57 cfu/test portion (1.01, 2.26)								
Sample #	iQ-Check <i>L. monocytogenes</i> II Kit		Confirmed ^a			BAM Ch 10		
			RAPID <i>L. mono</i> Agar	RAPID <i>Listeria</i> Agar	Agar <i>Listeria</i>	Agar <i>Listeria</i>	PALCAM	Final Result
	Fast	Classic						
1	+ ^b	+	- ^c	-	-	+	+	+
2	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+
6	+	+	-	-	+	+	+	+
7	+	+	+	+	+	+	+	+
8	+	+	+	+	+	+	+	+
9	+	+	+	+	+	+	+	+
10	+	+	+	+	+	+	+	+
11	+	+	+	+	+	+	+	+
12	+	+	+	+	+	+	+	+
13	-	-	-	-	-	-	-	-
14	+	+	+	+	+	+	+	+
15	+	+	+	+	+	+	+	+
16	+	+	+	+	+	+	+	+
17	+	+	+	+	+	+	+	+
18	+	+	+	+	+	+	+	+
19	+	+	+	+	+	+	+	+
20	+	+	+	+	+	+	+	+
Total	19/20	19/20	17/20	17/20	18/20	19/20	19/20	19/20
High Level								
9.26 cfu/test portion (4.92, 234.22)								
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.

Table 10. Alternative Confirmation Results for the iQ-Check *L. monocytogenes* II Kit for Stainless Steel (7)

Stainless Steel (4" x 4")								
<i>L. monocytogenes</i> Ad651								
Low Level								
8.7 cfu/test portion								
Sample #	iQ-Check <i>L. monocytogenes</i> II Kit		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	i/+ ^c NA/35.72	+	+	+	+	+	+
2	- ^d	-	-	-	-	-	-	-
3	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+
6	-	-	-	-	-	-	-	-
7	+	+	+	+	+	+	+	+
8	+	+	+	+	+	+	+	+
9	+	+	+	+	+	+	+	+
10	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-
12	+	-/+ ^e NA/36.39/38.24	+	+	+	+	+	+
13	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-
15	+	+	+	+	+	+	+	+
16	+	+	+	+	+	+	+	+
17	-	-	-	-	-	-	-	-
18	+	+	+	+	+	+	+	+
19	+	+	+	+	+	+	+	+
20	+	+	+	+	+	+	+	+
Total	13/20	11/20	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 Two separate PCR tests were performed with Cq values reported and "i" = PCR inhibition

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

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

DISCUSSION OF THE MODIFICATION STUDY APPROVED MODIFICATION 2 JANUARY 2023 (10)

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 *L. monocytogenes* II 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. Any differences observed between the candidate and reference methods are due to tests being conducted under unpaired testing conditions. In the inclusivity and exclusivity evaluations, all inclusivity organisms were correctly identified, and all exclusivity organisms were correctly excluded.

Table 14. Inclusivity Results, iQ-Check *L. monocytogenes* II Kit (10)

No.	Genus	Species	Molecular Subtype	Source ^a	Origin	CFX96 Touch Deep Well Result ^b	CFX Opus Deepwell Result
1	<i>Listeria</i>	<i>monocytogenes</i>	VI b	Ad 153	Soft cheese (Munster)	+	+
2	<i>Listeria</i>	<i>monocytogenes</i>	II a	Ad 1011/1410	Frozen broccoli	+	+
3	<i>Listeria</i>	<i>monocytogenes</i>	VI b	Ad 1972/2399	Puff pastry with mushrooms	+	+
4	<i>Listeria</i>	<i>monocytogenes</i>	VI b	Ad 1973/2400	Puff pastry egg and ham (Quiche lorraine)	+	+
5	<i>Listeria</i>	<i>monocytogenes</i>	IV b	Ad 2407/3139	Tripes with tomatoes	+	+
6	<i>Listeria</i>	<i>monocytogenes</i>	II a	Ad 2760/3145	Raw bacon	+	+
7	<i>Listeria</i>	<i>monocytogenes</i>	II b	Ad 32.183	Croque-Monsieur	+	+
8	<i>Listeria</i>	<i>monocytogenes</i>	II a	Ad 38/181	Toulouse sausages	+	+
9	<i>Listeria</i>	<i>monocytogenes</i>	IV b	Ad 5721/6179	Smoked bacon	+	+
10	<i>Listeria</i>	<i>monocytogenes</i>	IV b	Ad 7111/7516	Pâté (Rillettes)	+	+
11	<i>Listeria</i>	<i>monocytogenes</i>	II a	Ad 850/109	RTE food (deli salad with seafood)	+	+
12	<i>Listeria</i>	<i>monocytogenes</i>	II a	Ad 877/113	Environmental sample (pastry)	+	+
13	<i>Listeria</i>	<i>monocytogenes</i>	IV b	Ad 913/1048	Black pudding	+	+
14	<i>Listeria</i>	<i>monocytogenes</i>	II a	A00C014	Sausage	+	+
15	<i>Listeria</i>	<i>monocytogenes</i>	II a	A00C022	Merguez	+	+
16	<i>Listeria</i>	<i>monocytogenes</i>	II a	A00C024	Sausage	+	+
17	<i>Listeria</i>	<i>monocytogenes</i>	II a	A00C036	Poultry (guinea)	+	+
18	<i>Listeria</i>	<i>monocytogenes</i>	II a	A00C039	Sausages	+	+
19	<i>Listeria</i>	<i>monocytogenes</i>	IV b	A00C040	Cooked delicatessen (Museau)	+	+
20	<i>Listeria</i>	<i>monocytogenes</i>	La	A00C041	Sausage	+	+
21	<i>Listeria</i>	<i>monocytogenes</i>	IV b	A00C042	Raw sausage	+	+
22	<i>Listeria</i>	<i>monocytogenes</i>	II a	A00C043	Smoked Bacon	+	+
23	<i>Listeria</i>	<i>monocytogenes</i>	II b	A00C044	Poultry (duck)	+	+
24	<i>Listeria</i>	<i>monocytogenes</i>	II b	A00C052	RTE food (Osso bucco with turkey)	+	+
25	<i>Listeria</i>	<i>monocytogenes</i>	II a	A00C053	Gizzards	+	+
26	<i>Listeria</i>	<i>monocytogenes</i>	IV b	A00C054	Beef hart	+	+
27	<i>Listeria</i>	<i>monocytogenes</i>	II a	A00C055	Raw sausages	+	+
28	<i>Listeria</i>	<i>monocytogenes</i>	II a	A00E008	Environmental sample	+	+
29	<i>Listeria</i>	<i>monocytogenes</i>	II a	A00E049	Environmental sample (smoked salmon)	+	+
30	<i>Listeria</i>	<i>monocytogenes</i>	II a	A00E082	Environmental sample (smoked salmon)	+	+
31	<i>Listeria</i>	<i>monocytogenes</i>	II a	A00L097	Milk	+	+
32	<i>Listeria</i>	<i>monocytogenes</i>	II a	A00M009	Smoked salmon	+	+
33	<i>Listeria</i>	<i>monocytogenes</i>	IV b	A00M032	Smoked salmon	+	+
34	<i>Listeria</i>	<i>monocytogenes</i>	II a	A00M045	Smoked salmon	+	+
35	<i>Listeria</i>	<i>monocytogenes</i>	II a	A00M088	Smoked salmon	+	+
36	<i>Listeria</i>	<i>monocytogenes</i>	II b	Ad 235	Poultry	+	+
37	<i>Listeria</i>	<i>monocytogenes</i>	II b	Ad 253	Hard cheese	+	+
38	<i>Listeria</i>	<i>monocytogenes</i>	II a	Ad 260	Semi hard cheese	+	+
39	<i>Listeria</i>	<i>monocytogenes</i>	II b	Ad 265	Tong	+	+
40	<i>Listeria</i>	<i>monocytogenes</i>	II a	Ad 266	Poultry	+	+
41	<i>Listeria</i>	<i>monocytogenes</i>	II b	Ad 267	Dry sausage	+	+
42	<i>Listeria</i>	<i>monocytogenes</i>	IV b	Ad 268	Cured ham	+	+
43	<i>Listeria</i>	<i>monocytogenes</i>	IV b	Ad 270	Fermented sausage	+	+
44	<i>Listeria</i>	<i>monocytogenes</i>	IV b	Ad 272	Fermented sausage	+	+
45	<i>Listeria</i>	<i>monocytogenes</i>	II b	Ad 273	Cured delicatessen	+	+
46	<i>Listeria</i>	<i>monocytogenes</i>	II a	Ad 274	Ready-to-eat food (Asiatic meal)	+	+
47	<i>Listeria</i>	<i>monocytogenes</i>	II b	Ad 534	Fruits	+	+
48	<i>Listeria</i>	<i>monocytogenes</i>	II a	Ad 548	Environment (seafood)	+	+
49	<i>Listeria</i>	<i>monocytogenes</i>	II b	Ad 623	Bread crumbs	+	+
50	<i>Listeria</i>	<i>monocytogenes</i>	II a	Ad 665	Raw milk	+	+

^a Ad, Adria, A00 = ADRIA Développement culture collection, Quimper, France

^b "+" indicates the target analyte was detected

Table 19. Exclusivity Results, iQ-Check *L. monocytogenes* II Kit (10)

No.	Genus	Species	Source	Origin	CFX96 Touch Deep Well Result ^a	CFX Opus Deepwell Result ^a
1	<i>Listeria</i>	<i>grayi</i>	Ad ^b 1198	Smoked salmon	-	-
2	<i>Listeria</i>	<i>grayi</i>	Ad 1443	Pork meat sausages	-	-
3	<i>Listeria</i>	<i>grayi</i>	Ad 1295	Spinach	-	-
4	<i>Listeria</i>	<i>innocua</i>	Ad 658	Gorgonzola	-	-
5	<i>Listeria</i>	<i>innocua</i>	Ad 660	Breadcrumbs	-	-
6	<i>Listeria</i>	<i>innocua</i>	Ad 663	Environment (dairy industry)	-	-
7	<i>Listeria</i>	<i>ivanovii</i>	Ad 466	Raw veal meat	-	-
8	<i>Listeria</i>	<i>ivanovii</i>	Ad 1289	Raw milk cheese	-	-
9	<i>Listeria</i>	<i>ivanovii</i>	Ad 1291	Poultry	-	-
10	<i>Listeria</i>	<i>seeligeri</i>	Ad 649	Cheese	-	-
11	<i>Listeria</i>	<i>seeligeri</i>	Ad 651	Environment	-	-
12	<i>Listeria</i>	<i>seeligeri</i>	Ad 652	Environment (dairy industry)	-	-
13	<i>Listeria</i>	<i>welshimeri</i>	Ad 1276	Environment (Slaughterhouse)	-	-
14	<i>Listeria</i>	<i>welshimeri</i>	Ad 1235	Beef meat	-	-
15	<i>Listeria</i>	<i>welshimeri</i>	Ad 191424	Poultry	-	-
16	<i>Bacillus</i>	<i>cereus</i>	Ad 465	Salmon Terrine	-	-
17	<i>Bacillus</i>	<i>circulans</i>	Ad 760	Vegetables	-	-
18	<i>Bacillus</i>	<i>coagulans</i>	Ad 731	Dairy product	-	-
19	<i>Bacillus</i>	<i>licheniformis</i>	Ad 978	Dairy product	-	-
20	<i>Bacillus</i>	<i>pumilus</i>	Ad 284	Ready-to-eat	-	-
21	<i>Brochotrix</i>	<i>campestris</i>	CIP ^c 102920T	Environment	-	-
22	<i>Carnobacterium</i>	<i>divergens</i>	CIP 101029T	Unknown	-	-
23	<i>Enterococcus</i>	<i>faecalis</i>	Adria ^b 89L326	Soft cheese (Vacherin)	-	-
24	<i>Lactobacillus</i>	<i>fermentum</i>	Ad 482	Tomato juice	-	-
25	<i>Leuconostoc</i>	<i>citreum</i>	Ad 396	Ham	-	-
26	<i>Micrococcus</i>	<i>luteus</i>	Ad 432	Cocktail (beverage)	-	-
27	<i>Pediococcus</i>	<i>pentosaceus</i>	ATCC ^d 33316	Unknown	-	-
28	<i>Staphylococcus</i>	<i>aureus</i>	Ad 165	Smoked delicatessen	-	-
29	<i>Staphylococcus</i>	<i>epidermidis</i>	Ad 931	Fruits	-	-
30	<i>Streptococcus</i>	<i>bovis</i>	Adria 92L622	Dairy product	-	-

^a "-" indicates the target analyte was not detected

^b Ad, Adria = ADRIA Développement culture collection, Quimper, France

^c CIP = Collection de l'institut Pasteur, Paris, France

^d ATCC = American Type Culture Collection, Manassas, VA, United States

Table 26. Bio-Rad iQ-Check *L. monocytogenes* II Kit, Presumptive vs. Confirmed-POD Results (10)

Matrix	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) 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

^aMPN = Most Probable Number is calculated using the LCF MPN calculator ver. 2.0 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 27. Bio-Rad iQ-Check *L. monocytogenes* II, Candidate vs. Reference (Unpaired) – POD Results (10)

Matrix	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) 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

^aMPN = Most Probable Number is calculated using the LCF MPN calculator ver. 2.0 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 28. Bio-Rad iQ-Check *L. monocytogenes* II Kit, CFX Opus Deepwell vs. CFX96 Touch Deep Well–POD Results (10)

Matrix	Strain	MPN ^a / Test Portion	N ^b	CFX Opus Deepwell			CFX96 Touch Deep Well			dPOD _{ot} ^f	95% CI ^g
				x ^c	POD _{oc} ^d	95% CI	X	POD _{rc} ^e	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

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

^bN = Number of test portions

^cx = Number of positive test portions

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

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

^fdPOD_{ot} = Difference between the CFX Opus Deepwell confirmed result and CFX96 Touch Deep Well 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

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