

Detection of *Listeria monocytogenes* and *Salmonella* spp. in plant-based foods

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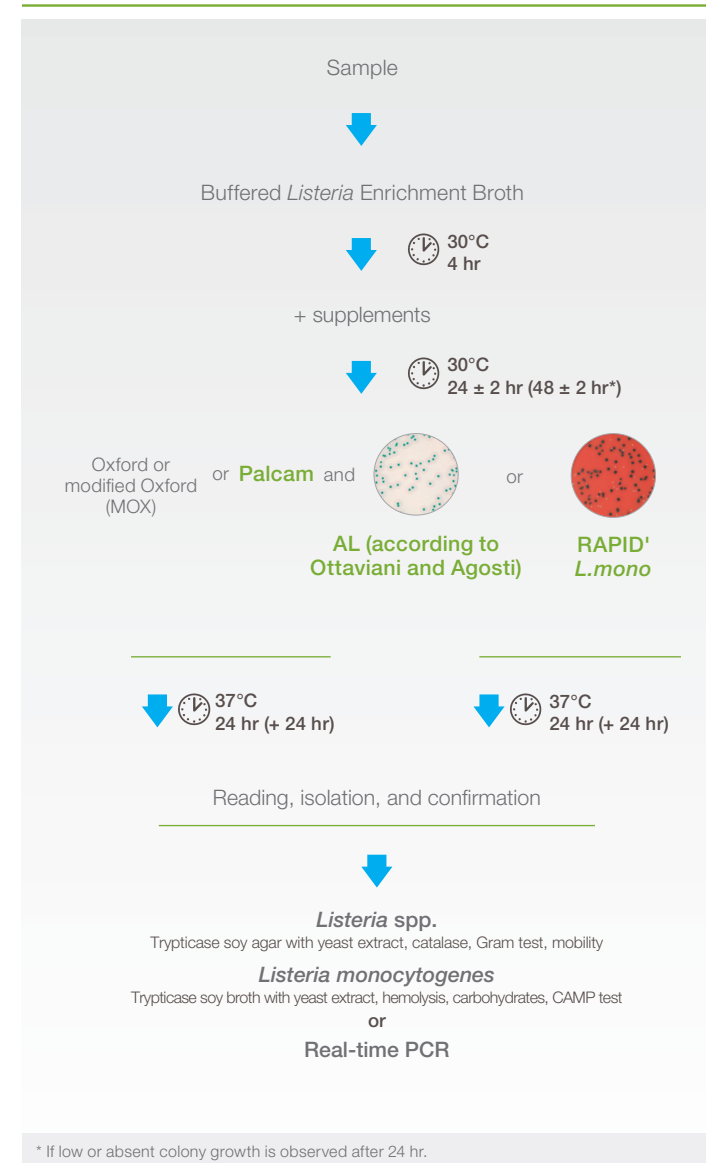
Introduction

As commercial markets grow for plant-based substitutes for foods such as meat and dairy products, producers need reliable methods for the detection of *Listeria monocytogenes* and *Salmonella* spp. with rapid and accurate results. In this study, iQ-Check *Listeria monocytogenes* II and iQ-Check *Salmonella* II methods were tested and compared to the United States Food and Drug Administration's Bacteriological Analytical Manual (FDA BAM) reference method.

Methods

A plant-based alternative to ground meat was inoculated with *L. monocytogenes* ATCC 13932 or *S. Typhimurium* ATCC 14028 from lyophilized pellets, at high and fractional inoculum levels or left uninoculated. After a food stabilization period, 25 g test portions (20 fractional, 5 high, and 5 uninoculated) were enriched at 1:10 in *Listeria* Special Broth (LSB, Bio-Rad Laboratories, catalog #3564703) for *L. monocytogenes* at 30°C for 24 hr or Buffered Peptone Water Standard (BPW, Bio-Rad Laboratories, catalog #12013260) for *Salmonella* at 37°C for 20 hr while FDA BAM reference samples (20 fractional, 5 high, and 5 uninoculated at 25 g, as well as 5 g, 10 g, and 50 g portions for MPNs) were enriched in Buffered *Listeria* Enrichment Broth (BLEB, Hardy Diagnostics for *L. monocytogenes*) or Lactose Broth (LB, Hardy Diagnostics) for *Salmonella* using FDA BAM protocols. Test method samples were analyzed using iQ-Check PCR kits and all samples were culture confirmed using FDA BAM protocols (Fig.1 and 2).

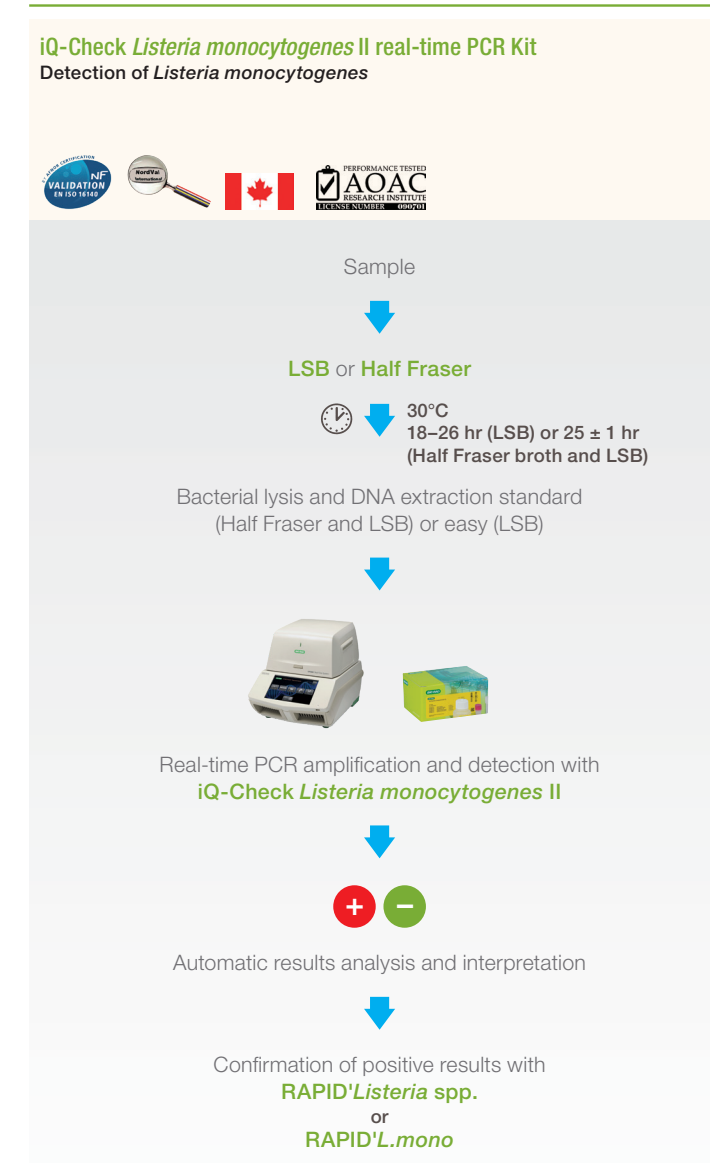
FDA BAM Protocol



A

Fig. 1. FDA BAM protocol for detection of *Listeria monocytogenes* in foods compared with iQ-Check *Listeria monocytogenes* II real-time PCR Kit protocol.

Alternative Method



B

FDA BAM Protocol



A

Fig. 2. FDA BAM protocol for detection of *Salmonella* spp. in foods compared with iQ-Check *Salmonella* II real-time PCR Kit protocol.

Results

PCR results for *L. monocytogenes* (Table 1) and *Salmonella* (Table 2) test method samples were identical to culture confirmation. When compared to the reference methods, the POD was found to be identical for the *Salmonella* test method and statistically equivalent for the *L. monocytogenes* test method.

Alternative Method



B

Table 1. iQ-Check method confirmed results compared to reference method (top) and iQ-Check method presumptive results compared to iQ-Check confirmed results (bottom) for *Listeria monocytogenes*.

Matrix	Spike level ^a	N ^b	iQ-Check Method Confirmed			Reference Method Confirmed			dPOD _{cp} ^g	95% CI ⁱ
			x ^c	POD _{cp} ^d	95% CI	x ^c	POD _{cp} ^e	95% CI		
Ground Meat Alternative	0.0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
	0.025 (0.016, 0.040)	20	12	0.60	0.39, 0.78	6	0.30	0.15, 0.52	0.30	-0.01, 0.54
	0.14 (0.067, 0.28)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	Spike level ^a	N ^b	iQ-Check Method Presumptive			iQ-Check Method Confirmed			dPOD _{cp} ^h	95% CI ⁱ
	x ^c	POD _{cp} ^f	95% CI	x ^c	POD _{cp} ^d	95% CI				
	0.0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
0.025 (0.016, 0.040)	20	12	0.60	0.39, 0.78	12	0.60	0.39, 0.78	0.00	-0.28, 0.28	
0.14 (0.067, 0.28)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43	

Table 2. iQ-Check method confirmed results compared to reference method (top) and iQ-Check method presumptive results compared to iQ-Check method confirmed results (bottom) for *Salmonella*.

Matrix	Spike level ^a	N ^b	iQ-Check Method Confirmed			Reference Method Confirmed			dPOD _{cp} ^g	95% CI ⁱ
			x ^c	POD _{cp} ^d	95% CI	x ^c	POD _{cp} ^e	95% CI		
Ground Meat Alternative	0.0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
	0.025 (0.016, 0.040)	20	12	0.60	0.39, 0.78	6	0.30	0.15, 0.52	0.30	-0.01, 0.54
	0.14 (0.067, 0.28)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	Spike level ^a	N ^b	iQ-Check Method Presumptive			iQ-Check Method Confirmed			dPOD _{cp} ^h	95% CI ⁱ
	x ^c	POD _{cp} ^f	95% CI	x ^c	POD _{cp} ^d	95% CI				
	0.0	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
0.025 (0.016, 0.040)	20	12	0.60	0.39, 0.78	12	0.60	0.39, 0.78	0.00	-0.28, 0.28	
0.14 (0.067, 0.28)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43	

^aSpike Level in cfu/g, calculated using MPN including 95% CI

^bN = Number of test portions

^cx = Number of positive test portions

^dPOD_{cp} = Candidate method confirmed positives divided by N

^ePOD_{cp} = Reference method confirmed positives divided by N

^fPOD_{cp} = Candidate method presumptive positives divided by N

^gdPOD_{cp} = Difference between the candidate method confirmed positives and reference method confirmed positives POD

^hdPOD_{cp} = Difference between the candidate method presumptive positives and candidate method confirmed positives POD

ⁱ95% CI = If the confidence interval of the dPOD includes zero, then the difference between the methods is not significant.

Significance

The results of this study demonstrated that iQ-Check real-time PCR methods are less time-consuming and perform equivalent to the FDA BAM reference method for the detection of *L. monocytogenes* and *Salmonella* in plant-based ground meat substitutes. These data have been submitted to AOAC for a method extension of validation.

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