

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

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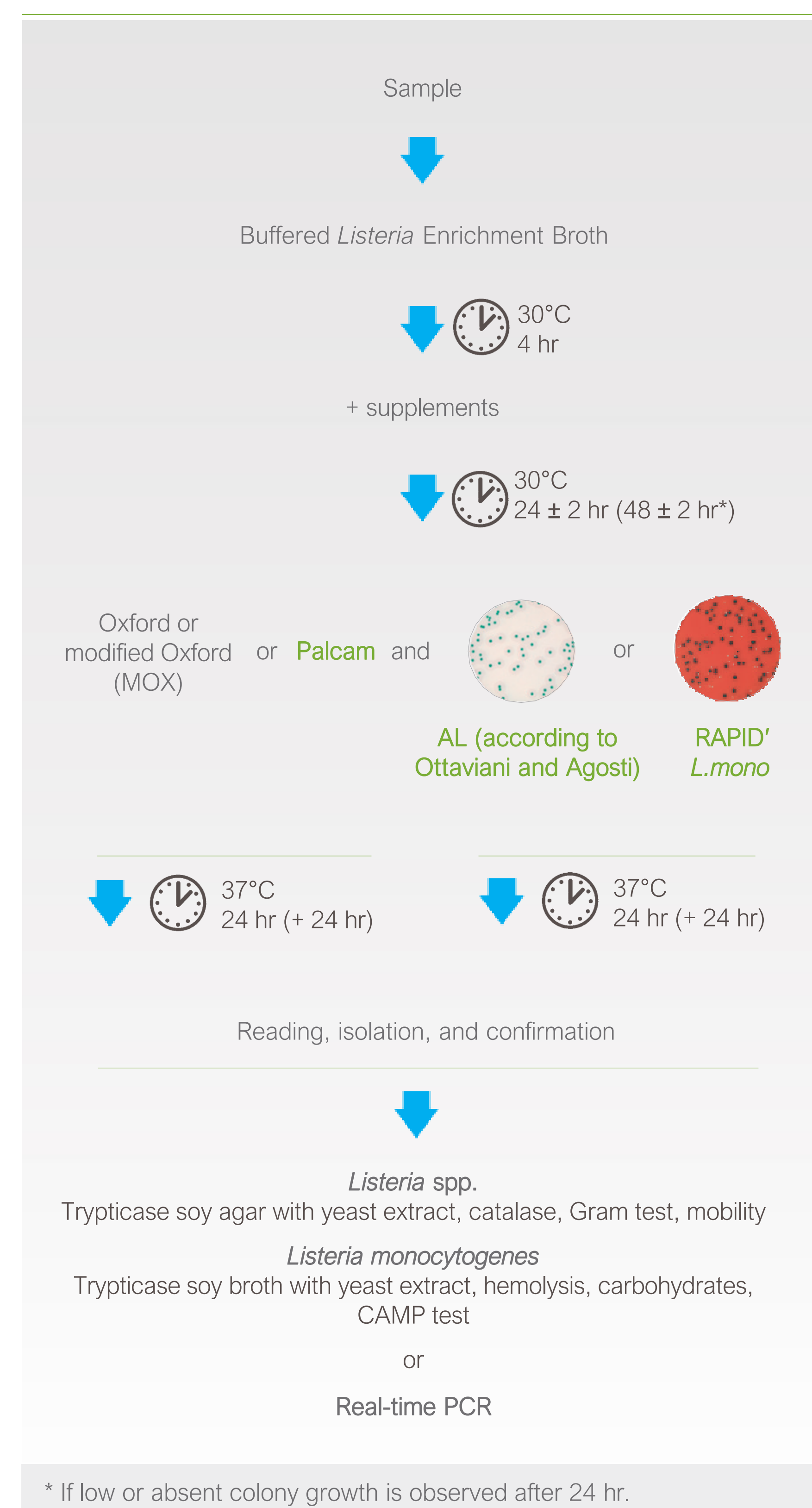
## Introduction

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



### Alternative Method

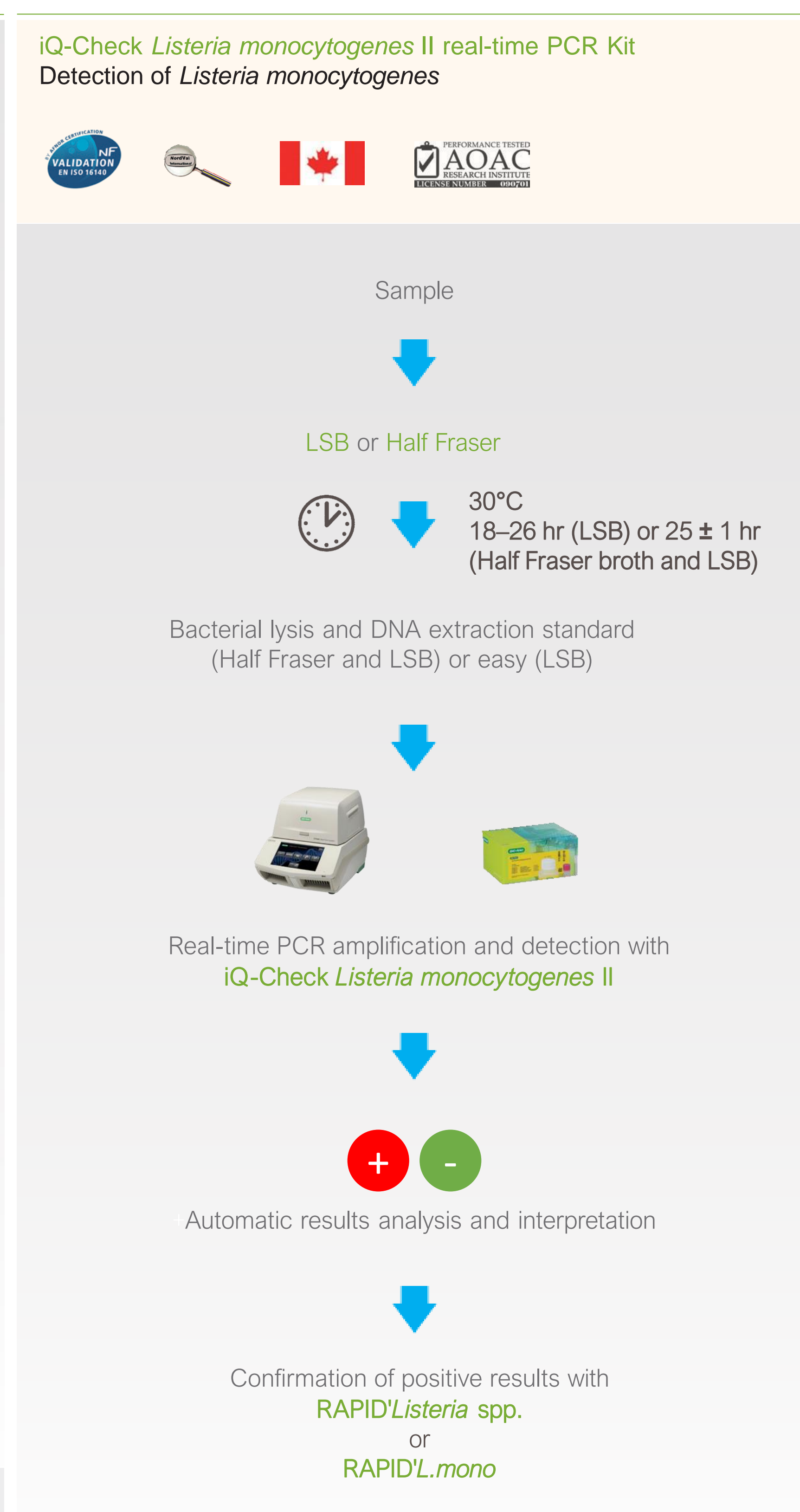
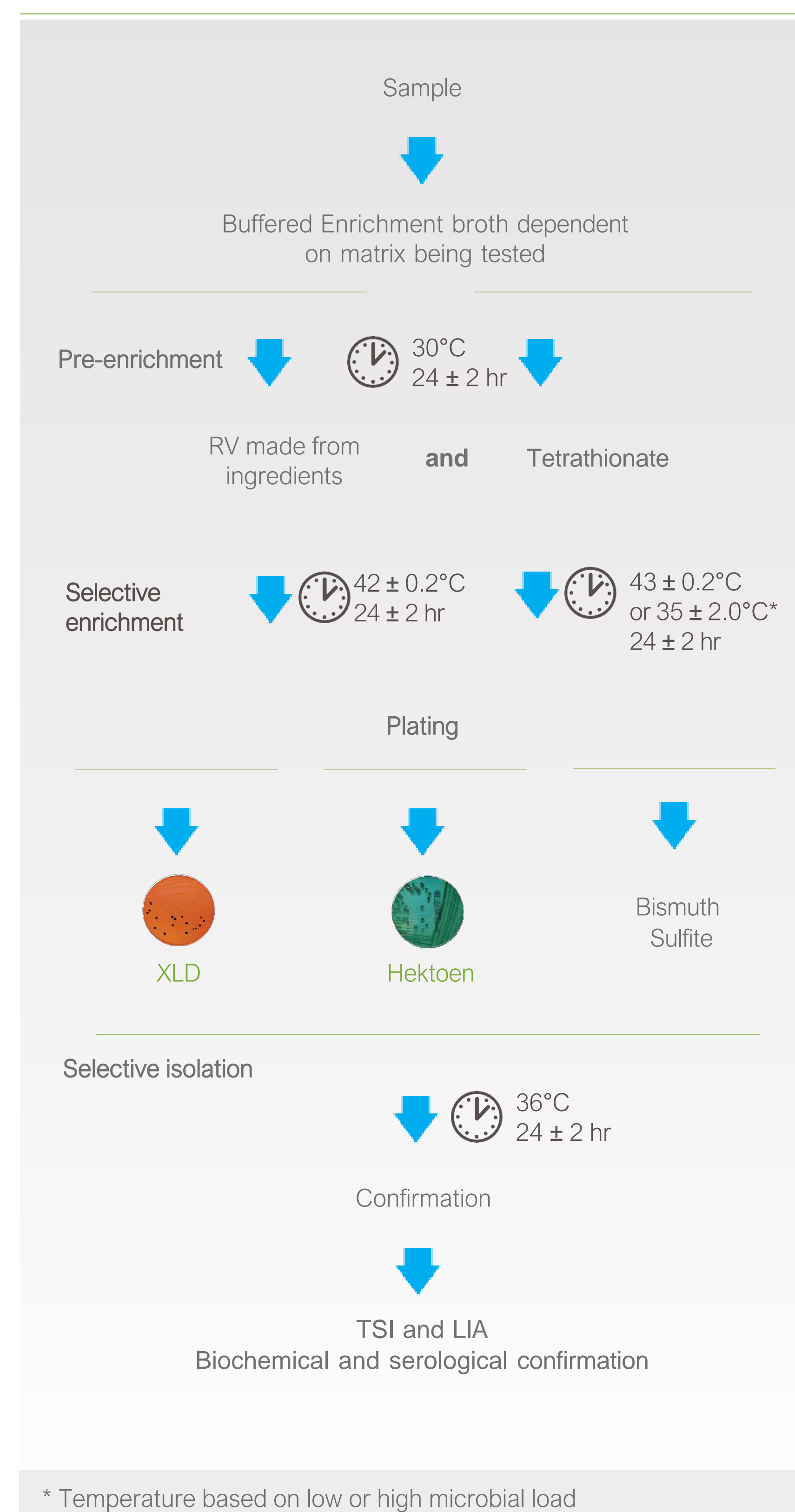


Fig. 1. FDA BAM protocol for detection of *Listeria monocytogenes* in foods compared with iQ-Check *Listeria monocytogenes* II real-time PCR Kit protocol.

| Matrix                  | Spike level <sup>a</sup> | N <sup>b</sup>                 | iQ-Check Method Confirmed   |                               |                               | Reference Method Confirmed |                               |            | dPOD <sub>cp</sub> <sup>g</sup> | 95% CI <sup>i</sup> |
|-------------------------|--------------------------|--------------------------------|-----------------------------|-------------------------------|-------------------------------|----------------------------|-------------------------------|------------|---------------------------------|---------------------|
|                         |                          |                                | x <sup>c</sup>              | POD <sub>c</sub> <sup>d</sup> | 95% CI                        | x <sup>c</sup>             | POD <sub>r</sub> <sup>e</sup> | 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 <sup>a</sup> | N <sup>b</sup>                 | iQ-Check Method Presumptive |                               |                               | iQ-Check Method Confirmed  |                               |            | dPOD <sub>cp</sub> <sup>h</sup> | 95% CI <sup>i</sup> |
|                         | x <sup>c</sup>           | POD <sub>cp</sub> <sup>f</sup> | 95% CI                      | x <sup>c</sup>                | POD <sub>c</sub> <sup>d</sup> | 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 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*.

### FDA BAM Protocol



### Alternative Method

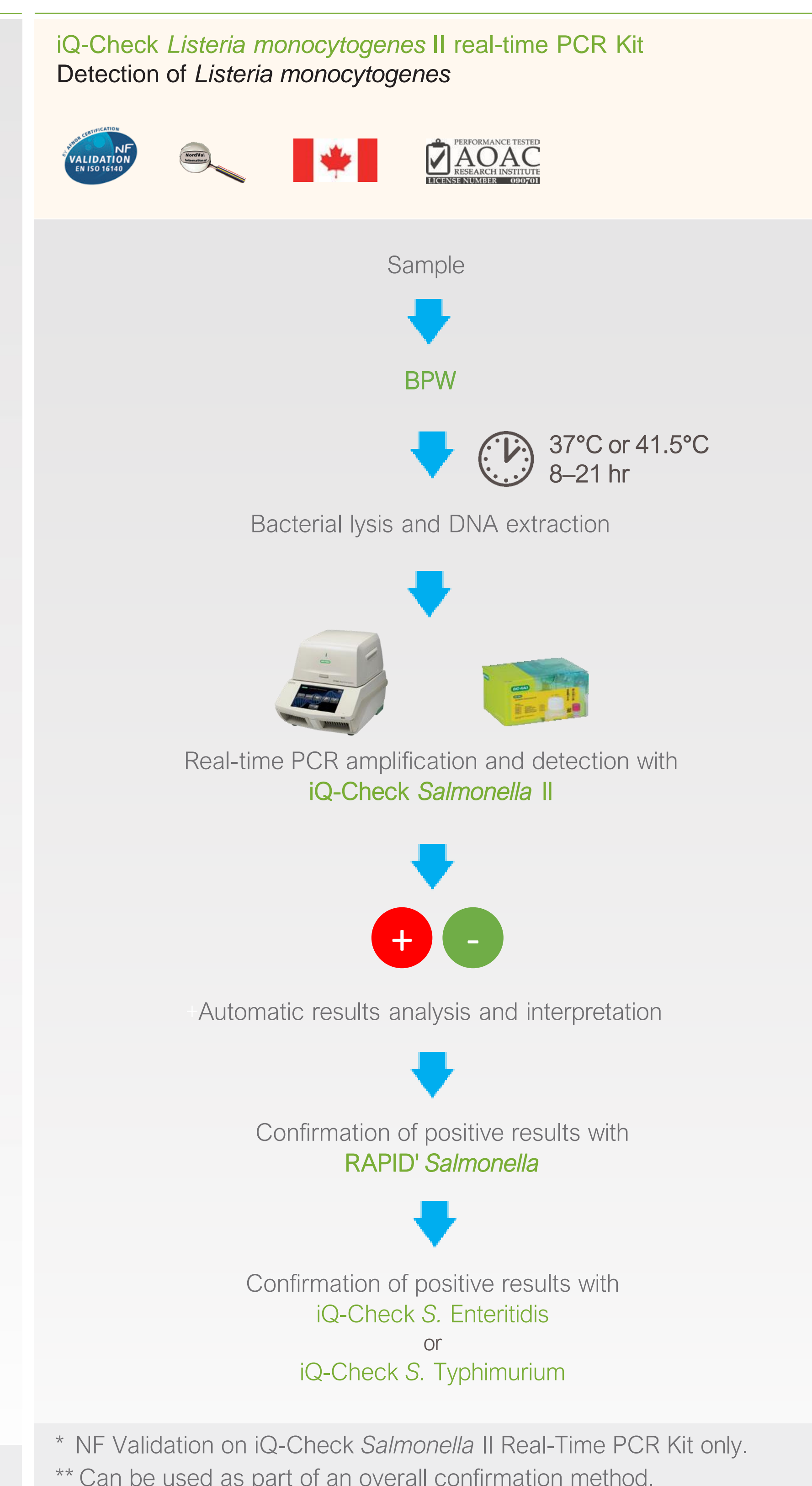


Fig. 2. FDA BAM protocol for detection of *Salmonella* spp. in foods compared with iQ-Check *Salmonella* II real-time PCR Kit protocol.

## 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.

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 <sup>a</sup> | N <sup>b</sup>                 | iQ-Check Method Confirmed   |                               |                               | Reference Method Confirmed |                               |            | dPOD <sub>c</sub> <sup>g</sup>  | 95% CI <sup>i</sup> |
|-------------------------|--------------------------|--------------------------------|-----------------------------|-------------------------------|-------------------------------|----------------------------|-------------------------------|------------|---------------------------------|---------------------|
|                         |                          |                                | x <sup>c</sup>              | POD <sub>c</sub> <sup>d</sup> | 95% CI                        | x <sup>c</sup>             | POD <sub>r</sub> <sup>e</sup> | 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 <sup>a</sup> | N <sup>b</sup>                 | iQ-Check Method Presumptive |                               |                               | iQ-Check Method Confirmed  |                               |            | dPOD <sub>cp</sub> <sup>h</sup> | 95% CI <sup>i</sup> |
|                         | x <sup>c</sup>           | POD <sub>cp</sub> <sup>f</sup> | 95% CI                      | x <sup>c</sup>                | POD <sub>c</sub> <sup>d</sup> | 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                     |                     |

<sup>a</sup> Spike Level in cfu/g, calculated using MPN including 95% CI  
<sup>b</sup> N = Number of test portions  
<sup>c</sup> x = Number of positive test portions  
<sup>d</sup> POD<sub>c</sub> = Candidate method confirmed positives divided by N  
<sup>e</sup> POD<sub>r</sub> = Reference method confirmed positives divided by N  
<sup>f</sup> POD<sub>cp</sub> = Candidate method presumptive positives divided by N  
<sup>g</sup> dPOD<sub>c</sub> = Difference between the candidate method confirmed positives and reference method confirmed positives POD  
<sup>h</sup> dPOD<sub>cp</sub> = Difference between the candidate method presumptive positives and candidate method confirmed positives POD  
<sup>i</sup> 95% 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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