

Thermo Scientific SureTect *Listeria monocytogenes* PCR Assay and SureTect *Listeria* species PCR Assay: AOAC-RI PTM and NF VALIDATION using the QuantStudio 5 PCR Instrument

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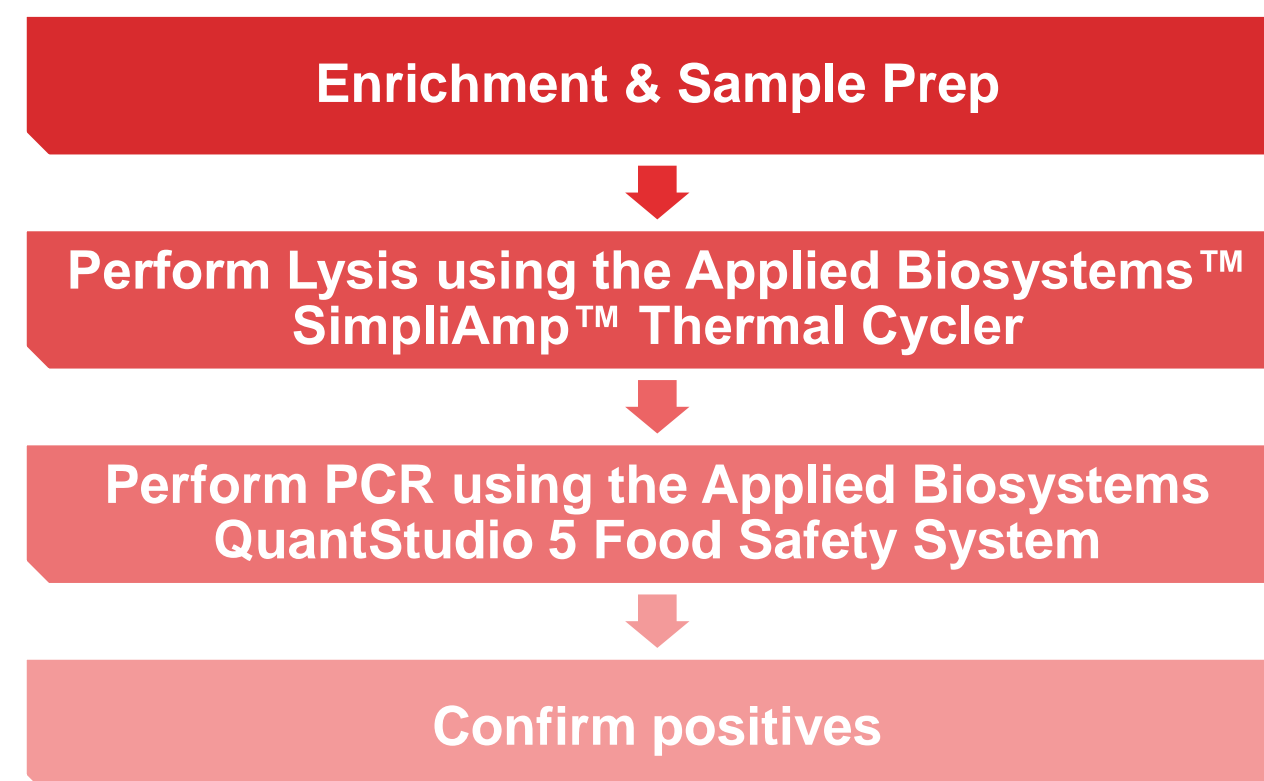
INTRODUCTION

Studies were performed to extend the current AOAC-RI Performance tested methodSM (PTM) and NF VALIDATION by AFNOR Certification claims for the Thermo ScientificTM SureTectTM *Listeria monocytogenes* PCR Assay and the Thermo ScientificTM SureTectTM *Listeria* species PCR Assay (candidate methods) to include the use of the Applied BiosystemsTM QuantStudioTM 5 Real-Time Food Safety PCR Instrument with associated Applied BiosystemsTM RapidFinderTM Analysis software.

MATERIALS AND METHODS

For the extension of the AOAC-RI PTM validation method modification studies were conducted in comparison to ISO 11290-1:1996¹. For the NF VALIDATION by AFNOR Certification extension studies were conducted in comparison to ISO 11290-1:1996 in accordance with ISO 16140-2:2016².

Figure 1: Workflow for the SureTect *Listeria monocytogenes* and SureTect *Listeria* species PCR Assays



RESULTS

AOAC PTM Validation

- The SureTect *L. monocytogenes* and SureTect *L. species* Assays returned identical results.
- The Probability of Detection (POD) analysis (table 1) demonstrated no statistically significant differences between the candidate methods and the reference method.
- Inclusivity and exclusivity testing demonstrated that the candidate methods successfully detected all target *L. monocytogenes* and *Listeria* spp. isolates and excluded all non-target isolates.

Table 1. POD analysis of the SureTect *Listeria monocytogenes* and the SureTect *Listeria* species PCR assay and the reference method

| Matrix type | Spike level | Number tested | Reference method positives | Candidate method positives ^a | dPOD ^b | 95% CI ^c |
|-----------------------------------|-------------|---------------|----------------------------|---|-------------------|---------------------|
| All food matrices ^d | n/a | 20 | 0 | 0 | 0.00 | -0.16, 0.16 |
| | Low | 80 | 46 | 38 | -0.10 | -0.25, 0.05 |
| | High | 20 | 16 | 15 | -0.05 | -0.30, 0.21 |
| All surface matrices ^e | n/a | 15 | 0 | 0 | 0.00 | -0.20, 0.20 |
| | Low | 40 | 10 | 10 | 0.00 | -0.19, 0.19 |
| | High | 10 | 8 | 8 | 0.00 | -0.34, 0.34 |

^aRepresents both SureTect *Listeria monocytogenes* and SureTect *Listeria* species PCR Assay results (results were identical)

^bDifference in POD between the candidate and reference methods

^cIf the 95% confidence interval does not contain a zero the results are statistically significant at the 5% level

^dRaw ground beef (9 hr and 24 hr protocols), skimmed milk powder, lettuce

^ePlastic surface swabs (1x1") and sponges (4x4")

AFNOR Validation

- Data was analyzed in accordance with ISO 16410-2:2016 (table 2).
- The candidate methods gave equivalent or improved performance to the reference method.

Table 2: Sensitivity, relative trueness and false positive ratio of the candidate methods

| SureTect PCR Assay | Number tested | Sensitivity of the Candidate method (%) | Sensitivity of the Reference method (%) | Relative trueness (%) | False positive ratio (%) |
|-------------------------------|---------------|---|---|-----------------------|--------------------------|
| <i>Listeria monocytogenes</i> | 387 | 80.2 | 77.8 | 81.9 | 2.7 |
| <i>Listeria</i> species | 378 | 78.9 | 77.9 | 78.3 | 4.8 |

Figure 2. Thermo Scientific SureTect Real-Time PCR System (Thermo Scientific SureTect PCR Assays, Applied Biosystems QuantStudio 5 Food Safety System and Applied Biosystems SimpliAmp Thermal Cycler)



CONCLUSION

The AOAC-RI PTM and NF VALIDATION studies demonstrated that the SureTect PCR Assays are suitable methods for the detection of *Listeria monocytogenes* and *Listeria* spp. from a variety of food and environmental surface samples when using the QuantStudio 5 Food Safety System (Figure 2).

REFERENCES

- ISO 11290-1:1996, including Amendment 1:2004 'Microbiology of the food chain -- Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. -- Part 1: Detection method'
- ISO 16140-2:2016 Microbiology of the food chain -- Method validation -- Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method

TRADEMARKS/ LICENSING

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