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AOAC-RI PTM and NF Validation of the Thermo Scientific Listeria Species PCR Assay using the QuantStudio 5 Food Safety PCR Instrument

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INTRODUCTION

Studies were performed to extend the current AOAC-RI *Performance tested method*℠ (PTM) and NF VALIDATION™ by AFNOR Certification for the Thermo Scientific™ SureTect™ Listeria species PCR Assay (candidate method) to include the use of the Applied Biosystems™ QuantStudio™ 5 Real-Time Food Safety PCR Instrument with associated Applied Biosystems™ RapidFinder™ Analysis software.

Figure 1. Thermo Scientific™ SureTect™ Real-Time PCR System

Left to right:
Applied Biosystems™ SimpliAmp™ Thermal Cycler
Applied Biosystems QuantStudio 5 Real-Time PCR Instrument
Applied Biosystems RapidFinder Analysis software
Thermo Scientific™ SureTect™ Kits

The QuantStudio 5 Food Safety System uses a 6-channel, 96-well (Figure 2) cloud-enabled platform suitable for running a wide range of PCR solutions for food pathogen and authenticity testing. Instrumentation offers easy to use touch screen technology along with intuitive software to streamline the workflow.

Figure 2. Thermo Scientific SureTect PCR Tubes being loaded in the QuantStudio 5 Food Safety PCR Instrument

RESULTS

AFNOR Validation

The NF VALIDATION by AFNOR Certification extension studies were conducted in comparison to ISO 11290-1:1996 in accordance with ISO 16140-2:2016[2].

Table 1: Sensitivity, relative trueness and false positive ratio of the candidate methods

Number tested	Sensitivity of the Candidate method (%)	Sensitivity of the Reference method (%)	Relative trueness (%)	False positive ratio (%)
378	78.9	77.9	78.3	4.8

The candidate method gave equivalent or improved performance to the reference method (shown in table 1) and satisfied the requirements of ISO 16140-2:2016.

RESULTS

AOAC PTM Validation

The AOAC PTM method modification study were conducted in comparison to ISO 11290-1:1996[1] in accordance with AOAC Appendix J[3].

Table 2. POD analysis of the SureTect Listeria species PCR Assay and the ISO 11290-1:1996 Reference Method

Matrix type	Spike level	No. 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

[b]Difference in POD between the candidate and reference methods
[c]If the 95% confidence interval does not contain a zero the results are statistically significant at the 5% level
[d]Raw ground beef (9 hr and 24 hr protocols), skimmed milk powder, lettuce
[e]Plastic surface swabs (1x1") and sponges (4x4")

The Probability of Detection (POD) analysis (table 2) 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 *Listeria* spp. isolates and excluded all non-target isolates.

CONCLUSION

Superior Listeria detection

- Detects Listeria species in a broad range of foods
- Superior or equivalent performance to the ISO 11290-1:1996 reference method.

AOAC and AFNOR Validated

- Data satisfied the acceptability criteria of AOAC PTM and NF VALIDATION by AFNOR Certification

Improved workflow using the QuantStudio 5 Food Safety System

- The QuantStudio 5 Food Safety System uses a 6-channel, 96-well cloud-enabled platform
- Suitable for running PCR solutions for food pathogen and authenticity testing
- Instrumentation offers touch screen technology along with intuitive software

REFERENCES

1. 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'
2. 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
3. AOAC Appendix J:

TRADEMARKS/ LICENSING

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