STUDY REPORT

Thermo Scientific SureTect Listeria species PCR Assay and Thermo Scientific SureTect Listeria monocytogenes PCR Assay method extension for use with the Applied Biosystems QuantStudio 5 Real-Time PCR Instrument AOAC-RI PTM Method Modification Validation: Inclusivity and Exclusivity

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Summary

The Thermo Scientific™ SureTect™ Listeria monocytogenes PCR Assay and Thermo Scientific™ SureTect™ Listeria species PCR Assay (candidate methods) have been certified by the AOAC Research Institute *Performance Tested Methods*℠ (PTM) Program to be used with the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument to perform PCR, and Thermo Scientific™ RapidFinder™ Analysis Software v1.0 or greater for data analysis. This study report details the inclusivity and exclusivity part of the validation.

Methodology

Choice of strains: A total of 53 *Listeria monocytogenes* isolates were analyzed by the SureTect Listeria monocytogenes PCR Assay and 68 *Listeria* species isolates were analyzed by the SureTect Listeria species PCR Assay. A total of 38 exclusivity isolates were analyzed by the SureTect Listeria monocytogenes PCR Assay and 33 exclusivity isolates were analyzed by the SureTect Listeria species PCR Assay. Isolates were obtained from national culture collections (ATCC®, USA, NCTC®, UK or Institute Pasteur, France).

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Culture enrichment

Inclusivity testing was conducted by removing isolates from storage at -80 °C and streaking onto a non-selective medium (e.g. Tryptone Soya Agar) before inoculating into 24 LEB (supplemented with 24 LEB Selective Supplement (10 mL per liter) and 24 LEB Buffer Supplement (44 mL per liter). Isolates were incubated at 37 °C for 22–26 hours before being diluted using Maximum Recovery Diluent (MRD) to a level of approximately 10^5 CFU/mL (100 times the LOD_{50}).

Exclusivity testing was conducted by removing isolates from -80 °C storage and streaking onto a non-selective medium and inoculating into Tryptone Soya Broth (TSB) and incubating for 18–24 hours at 37±1 °C. Cultured exclusivity isolates were tested undiluted at the growth level achieved in TSB using the candidate methods.

Protocol

Ten microlitres of SureTect Proteinase K Reagent and 10 µL of SureTect Lysis Reagent 2 were added to each of the required number of SureTect Lysis Tubes (supplied prefilled with Lysis Reagent 1). Twenty microlitres of the sample were added to the Lysis Tubes. The Lysis Tubes were then incubated in the Applied Biosystems™ SimpliAmp™ Thermal cycler (37±1 °C for 10 minutes, 95±1 °C for 5 minutes, and 10±1 °C for 2 minutes). Twenty microliter aliquots of the lysates were transferred to SureTect PCR Tubes containing SureTect Listeria species or SureTect Listeria monocytogenes PCR pellets. The PCR Tubes were then sealed and transferred to the QuantStudio 5 Real-Time PCR Instrument for processing.

Results

All 53 and 68 inclusivity isolates were successfully detected by the SureTect Listeria monocytogenes PCR Assay and the SureTect Listeria species PCR Assay respectively.

All 38 and 33 exclusivity isolates were correctly excluded by the SureTect Listeria monocytogenes PCR Assay and the SureTect Listeria species PCR Assay respectively.

Conclusion

The inclusivity and exclusivity data shows that the SureTect Listeria monocytogenes PCR Assay and the SureTect Listeria species PCR Assay are suitable for the detection of a range of *Listeria monocytogenes* and *Listeria* species respectively, and exclusion of non-target isolates, when using the QuantStudio 5 Real-Time PCR Instrument and associated RapidFinder Analysis Software. The AOAC-RI PTM validation certificate (License numbers: 061302 and 071304) is available from either www.thermofisher.com or the AOAC Research Institute at www.aoac.org.

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