STUDY REPORT

Thermo Scientific SureTect E. coli O157:H7 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™ E. coli O157:H7 species PCR Assay (candidate method) has been certified in accordance with 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 with Thermo Scientific™ RapidFinder™ Analysis Software version 1.0 or greater for data analysis. This study report details the inclusivity and exclusivity part of the validation.

Methodology

Choice of strains: A total of 52 *Escherichia coli* O157:H7 isolates, and 30 isolates closely related to *E. coli* O157:H7 were analysed by the candidate method. Isolates were obtained from national culture collections (ATCC™, USA, NCTC™, UK), the culture collection held by Q Laboratories Inc., OH, USA, and the culture collection from Michigan State University, MI, USA.

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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 Brain Heart Infusion (BHI) broth. Isolates were incubated at 35±1 °C for 20–24 hours before being diluted 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 a non-selective broth and incubating for 18–24 hours at 37±1 °C. Cultured exclusivity isolates were analysed without dilution.

Protocol

Ten microlitres of SureTect Proteinase K Reagent 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 (programmed at 37±1 °C for 10 minutes, 95±1 °C for 5 minutes, and 10±1 °C for 2 minutes, (optional holding at 4±1 °C if not processing immediately, for no longer than 72 hours)). Twenty microliter aliquots of the lysates were transferred to SureTect PCR Tubes containing SureTect E. coli O157:H7 PCR pellets. The PCR Tubes were then sealed and transferred to the QuantStudio 5 Real-Time PCR Instrument for processing.

Results

All 52 inclusivity isolates were successfully detected, and all 30 exclusivity isolates were correctly excluded by the SureTect E. coli O157:H7 species PCR Assay.

Conclusion

The inclusivity and exclusivity data shows that the SureTect E.coli O157:H7 PCR Assay is suitable for the detection of a range of *E. coli* O157:H7 isolates, 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: PTM 021501) is available from either www.thermofisher.com or the AOAC Research Institute at www.aoac.org.

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