

# Thermo Scientific SureTect Salmonella species 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™ Salmonella species PCR Assay (candidate method) has been certified in accordance with the AOAC Research Institute *Performance Tested Methods*<sup>SM</sup> (PTM) Program to be used with the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument to perform PCR, and the 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 115 *Salmonella* species isolates, and 36 non-*Salmonella* isolates were analyzed by the candidate method. Isolates were obtained from national culture collections (ATCC™, USA, NCTC™, UK or Institute Pasteur, France).

## 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 Buffered Peptone Water (BPW) ISO. Isolates were incubated at 37±1 °C for 18–20 hours before being diluted using Maximum Recovery Diluent to a level of approximately 10<sup>5</sup> CFU/mL (100 times the LOD<sub>50</sub>).

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 at 37±1 °C for 18–24 hours. 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 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 Salmonella species PCR pellets. The PCR Tubes were then sealed and transferred to the QuantStudio 5 Real-Time PCR Instrument for processing.

## Results

All 115 inclusivity isolates were successfully detected, and all 36 exclusivity isolates were correctly excluded by the SureTect Salmonella species PCR Assay.

## Conclusions

The inclusivity and exclusivity data show that the SureTect Salmonella species PCR Assay is suitable for the detection of a range of *Salmonella* species 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: [051303](#)) is available from either [www.thermofisher.com](http://www.thermofisher.com) or the AOAC Research Institute at [www.aoac.org](http://www.aoac.org).

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