STUDY REPORT

Thermo Scientific RapidFinder Salmonella Multiplex Flex PCR Kit AOAC-RI PTM Method Modification Validation: Inclusivity and Exclusivity

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Summary

A matrix extension study was performed for AOAC Research Institute (RI) Performance Tested Method℠ certification (PTM 081701) to extend the claim for use of the Thermo Scientific™ RapidFinder™ Salmonella species, Typhimurium and Enteritidis PCR Kit, for the detection and differentiation of *Salmonella* species, *Salmonella* Typhimurium and *Salmonella* Enteritidis, in shell eggs, chicken carcass rinses and ground turkey (375 g sample size). The ground turkey samples were prepared using the Thermo Scientific™ KingFisher Flex™ Sample Purification System, therefore inclusivity and exclusivity studies were conducted using the KingFisher Flex Purification System protocol with the Thermo Scientific RapidFinder Salmonella species, Typhimurium and Enteritidis PCR Kit, combined with Applied Biosystems™ Dynabeads™ anti-*Salmonella*; known as the RapidFinder Salmonella Multiplex Flex PCR Kit (candidate method). PCR analysis was performed using the Applied Biosystems™ 7500 Fast Food Safety Real-Time PCR Instrument and associated Applied Biosystems™ RapidFinder™ Express Software (version 2.0 or greater), and the Applied Biosystems™ QuantStudio™ 5 Food Safety Real-Time PCR Instrument and associated Thermo Scientific™ RapidFinder™ Analysis Software (version 1.0 or greater) for the detection and differentiation of *Salmonella* species, *Salmonella* Typhimurium and *Salmonella* Enteritidis. Results showed that the candidate method successfully detected all *Salmonella* spp. tested with the exception of three isolates which had an O group loss mutation, and all non-*Salmonella* isolates were correctly excluded. The following is a summary of the inclusivity and exclusivity study.

Methodology

Choice of strains

A total of 200 inclusivity isolates covering all species and sub-species of *Salmonella* and 45 exclusivity isolates were analyzed during the AOAC-RI PTM validation studies. Isolates were obtained from national culture collections (ATCC® or NCTC) or other culture collections and represented a wide range of food, environmental, veterinary and clinical sources. All testing was conducted according to AOAC-RI PTM guidelines and study protocols.

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Culture enrichment

For each inclusivity isolate, one colony was inoculated into a tube containing Buffered Peptone Water (ISO) (CM1211) with 12 mg/L novobiocin and incubated for 14-18 hours at 41.5±1 °C. The broth was diluted with Maximum Recovery Diluent (MRD) to dilute the target cultures to a level of approximately 50 x 10^4 CFU/mL (50x LOD) before analyzing with the candidate method.

For each exclusivity isolate, one colony was inoculated into a tube containing Tryptone Soya Broth (TSB) and incubated for 20-24 hours at 37±2 °C. Prepared cultures were analyzed with the candidate method, at the growth level achieved during the incubation time, without being diluted.

Protocol

Firstly, the KingFisher Flex processing plates were set up as shown in the table below:

KingFisher Flex Processing Plate Set Up

Plate	Action
Tip Comb	Place a 96-well Deep Well Tip Comb in the plate
Elution Plate	Add 180 µL Lysis Reagent 1 and 10 µL Proteinase K to each sample and control well[a]
Wash Plate 1	Add 1000 µL of Wash Buffer[b] to each sample and control well
Wash Plate 2	Add 1000 µL of Wash Buffer to each sample and control well

[a](Optional) Combine Lysis Reagent 1 and Proteinase K for the required number of reactions, plus overage, then add 190 µL to each sample and control well.

[b]Wash buffer was prepared by combining 1 Oxoid™ Phosphate Buffered Saline Tablet, 50 µL of Tween™ 20, and 100 mL of Deionized water. Sterilizing by autoclaving at 115 °C for 10 minutes.

Once the processing plates were set up, 500 µL of enriched sample were added to the appropriate sample well. Similarly, 500 µL of sterile water were added to one or more wells to prepare a mock-purified (negative extraction control) sample. The prepared plates were then processed in the KingFisher Flex instrument and once complete, the elution plate, containing the sample lysate (the sealed elution plate can be stored 2-8 °C for up to 24 hours), was removed from the instrument. The prepared lysates were transferred to the 7500 Fast PCR Instrument and QuantStudio 5 PCR Instrument for processing.

Results

Out of 200 inclusivity isolates tested, 198 were correctly detected by the corresponding PCR target within the RapidFinder Salmonella Multiplex Flex PCR Kit. One *Salmonella* Enteritidis inclusivity isolate (TCC 1639) gave a positive result for the *Salmonella* species target, but gave a negative result for the *Salmonella* Enteritidis PCR target when tested using the 7500 Fast PCR Instrument and the QuantStudio 5 PCR Instrument. One *Salmonella* Enteritidis inclusivity isolate (TCC 1640) gave negative PCR results for the *Salmonella* species and the *Salmonella* Enteritidis target when tested using the QuantStudio 5 PCR Instrument only. Both isolates (TCC 1639 and TCC 1640) showed irregular (rough) colony morphologies and were confirmed by H:g,m presence as they were O:9 negative due to a mutation. The lack of O antigen can give variable results due to the cells not binding properly to the Dynabeads anti-*Salmonella* during sample lysis using the KingFisher Flex instrument, which can lead to false negative results and fractional positivity differences between instruments.

All 45 exclusivity isolates tested were correctly excluded by the RapidFinder Salmonella Multiplex Flex PCR Kit. Three exclusivity isolates (one *Citrobacter freundii* and two *Serratia marcescens*) originally gave a positive PCR result when tested using the 7500 Fast Real Time PCR Instrument, and an additional two exclusivity isolates (*Escherichia blattae* and *Proteus vulgaris*) originally gave a positive PCR result when tested using the QuantStudio 5 Real Time PCR Instrument. The five exclusivity isolates were re-incubated in the candidate enrichment broth ((BPW (ISO) with 12 mg/L novobiocin) for 20-24 hours at 41.5 ±1 °C and after reanalysis did not produce a positive PCR result on either PCR instrument.

Conclusions

Inclusivity and exclusivity testing demonstrated that the RapidFinder Salmonella Multiplex Flex PCR Kit was able to detect all the major groups of *Salmonella*, the less common subspecies of *Salmonella enterica* and the rarely encountered *Salmonella bongori* when using the candidate method using the QuantStudio 5 PCR Instrument and the 7500 Fast PCR Instrument. All exclusivity isolates were correctly excluded. The AOAC-RI PTM validation certificate (License number: 081707) is available from either **www.thermofisher.com** or the AOAC Research Institute at **www.aoac.org**.

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993-295
LT2368A
May 2018

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