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# Evaluation of the Thermo Scientific RapidFinder Salmonella species, Typhimurium and Enteritidis Multiplex PCR Kit

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## ABSTRACT

**Purpose:** To validate the Thermo Scientific™ RapidFinder™ Salmonella species, Typhimurium and Enteritidis Multiplex PCR Kit (candidate method) through the AOAC Research Institute (RI) *Performance Tested Methods*SM program.

**Methods:** The performance of the candidate method was assessed as an unpaired study in comparison to the U.S. Department of Agriculture Food Safety and Inspection Service Microbiology Laboratory Guidebook (MLG) 4.09 for food matrices[1], and the U.S. Food and Drug Administration (FDA) Bacteriological Analytical Manual (BAM) Chapter 5[2] for environmental matrices.

**Results:** The candidate method reliably detected *Salmonella* spp., *Salmonella* Enteritidis, and *Salmonella* Typhimurium in pork, poultry and environmental samples.

## INTRODUCTION

The RapidFinder Salmonella species, Typhimurium and Enteritidis Multiplex PCR Kit (candidate method) is a real-time PCR assay for the detection and differentiation of *Salmonella* species, and serotypes Typhimurium and Enteritidis from poultry, pork and environmental matrices.

## MATERIALS AND METHODS

The candidate method was validated in comparison to the MLG 4.09 and FDA BAM Chapter 5 reference methods for food samples and environmental samples respectively.

The following matrices were tested;

- Raw chicken thighs with skin
- Raw chicken wings with skin
- Cooked chicken nuggets
- Raw pork sausage
- Stainless steel environmental surface sponges.

Thermo Fisher Scientific, Basingstoke, UK, tested all matrices. In addition, two matrices (raw chicken thighs with skin and stainless steel) were analyzed independently by Q Laboratories, Inc., Ohio.

The candidate method was tested in line with the user guide[3] as summarised in Figure 2. A variety of single, dual and triple inoculations were tested; the inoculation protocols for each matrix are shown in Table 1.

**Table 1. Matrix inoculation protocols**

| Matrix | Inoculating serovar(s) |
|---|---|
| Raw chicken thighs with skin | Dual inoculation; *S.* Kentucky & *S.* Enteritidis |
| Raw chicken wings with skin | *S.* Typhimurium |
| Cooked chicken nuggets | *S.* Montevideo<br>*S.* Typhimurium |
| Raw pork sausage | Triple inoculation; *S.* Ohio, *S.* Typhimurium & *S.* Enteritidis |
| Stainless steel | Dual inoculation; *S.* Poona & *S.* Typhimurium (plus *Citrobacter freundii* at 10x concentration of *Salmonella*) |

**Figure 1. Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument**

## DATA ANALYSIS

The data collected during the study was analyzed using probability of detection (POD) analysis. Table 2 details the sets of data from each testing method and associated confirmation method that were compared. The data analysis of the methods shown in table 2 was performed separately for the results obtained using the Applied Biosystems QuantStudio 5 Real-Time PCR instrument (shown in figure 1) (using Thermo Scientific™ RapidFinder™ Analysis software v1.0) and the Applied Biosystems™ 7500 Fast Real-Time PCR instrument (using Thermo Scientific RapidFinder Express software v2.0).

**Table 2. Data sets compared using POD analysis (testing method and confirmation method combinations)**

| Method | | Method |
|---|---|---|
| **Presumptive candidate result** Unconfirmed | vs | **Confirmed candidate result** Candidate method confirmation |
| **Presumptive candidate result** Unconfirmed | vs | **Confirmed candidate result** Reference method confirmation |
| **Confirmed candidate result** Candidate method confirmation | vs | **Confirmed candidate result** Reference method confirmation |
| **Confirmed candidate result** Candidate method confirmation | vs | **MLG/BAM Reference result** Reference method confirmation |
| **Confirmed candidate result** Reference confirmation method | vs | **MLG/BAM Reference result** Reference confirmation method |

**Figure 2. Protocol for the RapidFinder Salmonella species, Typhimurium, and Enteritidis Multiplex PCR Kit**

**Day 0**

**Food samples:** Add 25 g of food sample to a 1 in 10 ratio of Buffered Peptone Water (ISO) with 12 mg/L novobiocin at room temperature

**Environmental samples:** Add sampling sponge to 225 mL Buffered Peptone Water (ISO) with 12 mg/L novobiocin at room temperature

**Day 1**

Incubate all samples for 14 h at 41.5±1 °C

Remove 1.5 mL of enrichment to a sterile sealable tube

First add 10 µL of SureTect Proteinase K to each required Lysis Tube

Second add 10 µL of enrichment to the Lysis Tube

Incubate Lysis Tubes at 37±1 °C for 10 min followed by 95±1 °C for 5 min

Allow lysates to cool at room temperature for at least 2 mins, then transfer 20 µl to RapidFinder PCR Tubes

Load into the Applied Biosystems QuantStudio 5 instrument and start PCR, review results at end of run (approx. 50 min)

Load into the Applied Biosystems 7500 Fast instrument, with a negative control tube containing nuclease free sterile water and start PCR, review results at end of run (approx. 50 min)

Confirm PCR results by culturing onto Thermo Fisher™ *Brilliance*™ Salmonella Agar (Thermo Fisher Scientific) and Tryptone Soya Agar

**Day 2**

Confirm the identification of the colonies using serological testing

## RESULTS

Very few statistically significant differences by probability of detection (POD) statistical analysis were found between the candidate or reference method for any food or environmental matrices tested. Any statistically significant differences observed were in favor of the candidate method. The candidate method showed significantly greater numbers of *S.* Enteritidis positive results on both the raw chicken thighs with skin and raw pork (dual and triple inoculation; see table 1) compared to the USDA FSIS MLG 4.09 reference method, demonstrating the candidate method was better at detecting *S.* Enteritidis from these samples than the USDA FSIS MLG 4.09 reference method. Inclusivity and exclusivity testing demonstrated that the candidate method was able to detect all the major groups of *Salmonella enterica* subspecies *enterica,* the less common subspecies of *S. enterica* and the rarely encountered *S. bongori*. No exclusivity isolates were detected. Ruggedness testing was conducted with specific method deviations which demonstrated reliable performance. Accelerated stability testing was conducted to determine a shelf-life of one year.

## CONCLUSIONS

The evaluation of the RapidFinder Salmonella species, Typhimurium and Enteritidis Multiplex PCR Kit has shown that it is an effective and reliable method for the detection and differentiation of *Salmonella* species, *Salmonella* Typhimurium, and *Salmonella* Enteritidis from the pork, poultry and environmental samples.

## REFERENCES

1. U.S. Department of Agriculture Food Safety and Inspection Service *Microbiology Laboratory Guidebook* 4.09 (2017). *Isolation and Identification of Salmonella from Meat, Poultry, Pasteurized Egg, and Siluriformes (Fish) Products and Carcass and Environmental Sponges* Revision .09.
2. U.S. Food and Drug Administration Bacteriological Analytical Manual Chapter 5: *Salmonella.* (2016) Wallace
3. Thermo Scientific RapidFinder Salmonella species, Typhimurium and Enteritidis Multiplex PCR Kit (2017) User Guide Revision A.0 (draft).

## TRADEMARKS/LICENSING



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