Evaluation of Molecular *Salmonella* spp., *S*. Enteritidis and *S*. Typhimurium Multiplex Assay's Inclusivity and Exclusivity

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ABSTRACT

Purpose

The purpose of the study was to compare inclusivity and exclusivity of the Thermo Scientific™ RapidFinder™ Salmonella species, Typhimurium and Enteritidis Multiplex PCR Kit (RapidFinder Salmonella Multiplex Assay) against two commercially available real-time PCR assays designed to detect *Salmonella* serovar Enteritidis (Manufacturer 1) only or *Salmonella* ser. Enteritidis and *Salmonella* ser. Typhimurium (Manufacturer 2) concurrently from food samples, with a range of target and non-target microbial strains.

Methods

A total of 154 *Salmonella* isolates including 20 *Salmonella* ser. Enteritidis and 35 *Salmonella* ser. Typhimurium strains and 99 other *Salmonella* serovars plus 37 non-target strains were analyzed during the study. Samples were lyzed prior to PCR and analyzed with the RapidFinder Salmonella Multiplex Assay as detailed in the instructions for use.

The two other assays were performed on the same panel of isolates according to the manufacturers´ instructions. The same lysates were used for all assays tested.

Results

The RapidFinder Salmonella Multiplex Assay proved to be the most accurate method out of the three assays tested. All *Salmonella* target strains were correctly detected by the RapidFinder Salmonella Multiplex Assay and 98% of the non-targets tested gave correct results.

Manufacturer 1 assay also reached 100% inclusivity whereas only 92% of non-targets were returned correct negative results. Manufacturer 2 assay failed to detect some of the target strains (96%) and also gave false positive results on both targets of the assay (97% exclusivity).

INTRODUCTION

Genus *Salmonella* consists of only two species, *S. enterica* and *S. bongori.* The two species are further divided into approximately 2500 different serovars. From a food safety perspective the main serovars of *S. enterica* are *Salmonella* ser. Typhimurium and *Salmonella* ser. Enteritidis[1] in terms of cases reported annually .

Every year, approximately one million people fall ill from consumption of *Salmonella* contaminated food[2] in the US alone. Poultry and pork producers globally are under pressure from national food safety agencies, other regulatory bodies and retailers to reduce *Salmonella* rates and to monitor prevalence of specific serovars such as *Salmonella* ser. Typhimurium and *Salmonella* ser. Enteritidis.

A simple, reliable and fast multiplex PCR assay would enable producers to quickly and accurately detect these targets in a single test, allowing them to make critical decisions on raw meat batch release days before traditional culture and serological methods would allow.

MATERIALS AND METHODS

Choice of strains

One hundred and fifty-four *Salmonella* isolates and 37 non-targets were tested during the study. Majority of *Salmonella* strains were selected from same subspecies as serovars Typhimurium and Enteritidis. Non-target species were selected based on their genetic closeness to *Salmonella*.

Sample preparation

All test organisms were plated onto Tryptone Soya Agar and incubated for 20-22 hours at +37±2 °C. After incubation, the *Salmonella* strains were inoculated into Buffered Peptone Water and enriched for 18 h at +37±2 °C. Non-target strains were incubated in Brain Heart Infusion broth for 18 h at +37±2 °C.

To test for inclusivity, *Salmonella* strains were diluted into Maximum Recovery Diluent to approximately 100-500x Limit of Detection. Non-targets were tested at growth level.

Test method(s)

Incubated samples were prepared for PCR using a direct lysis protocol by pipetting 10 µl of incubated sample into pre-filled Lysis reagent 1 tubes. The Applied Biosystems™ SimpliAmp™ Thermal Cycler was used to facilitate the heating steps. After preparation, 20 ul of lysate was pipetted into PCR tubes containing RapidFinder Salmonella Multiplex Assay lyophilized PCR pellets.

PCR was conducted using the Applied Biosystems™ 7500 Fast and Applied Biosystems™ QuantStudio™ 5 Real-Time PCR instruments (Figure 1) with Applied Biosystems™ RapidFinder™ Express Software v.2.0 and RapidFinder™ Analysis Software v1.0, respectively.

The other two commercially available assays tested were performed according to the manufacturers' instructions and run on the Applied Biosystems 7500 Fast instrument and results analyzed with 7500 Fast Sequence Detection System Software v 1.4.2.1.

Data analysis

The number of positive and negative results interpreted by the RapidFinder Express Software and RapidFinder Analysis Software (Figures 2 and 3, respectively) were recorded for the RapidFinder Salmonella Multiplex Assay and compared to the results generated with the other commercially available methods.

Results for the other two commercially available assays were interpreted using manually set thresholds adjusted according to positive control reactions in the Applied Biosystems 7500 Software

Figure 1. Applied Biosystems QuantStudio 5 and Applied Biosystems 7500 Fast Real-Time PCR systems.

RESULTS

Table: RapidFinder Salmonella Multiplex Assay vs. the other two commercially available assays' results from inclusivity and exclusivity testing.

Method	Inclusivity	Exclusivity
RapidFinder Salmonella Multiplex Assay (7500 Fast and QuantStudio 5)	100%	98% [a]
Manufacturer 1	100%	92% [b]
Manufacturer 2	96% *	97% [c,d]

Key

[a] *S*. Blegdam, *S*. Moscow and *S*. Nitra returned false positives on *S*. Enteritidis target

[b] *S*. Blegdam, *S*. Moscow, *S*. Dublin, *S*. Kiel, *S*. London, *S*. Rostock, *S*. Pullorum, *S*. Gallinarum and *S*. Nitra returned false positives on *S*. Enteritidis target

[c] *S*. Blegdam, *S*. Moscow and *S*. Nitra returned false positives on *S*. Enteritidis target

[d] *S*. Tennessee returned false positive on *S*. Typhimurium target

* 2 false negatives from *S*. Enteritidis strains

The RapidFinder Salmonella Multiplex Assay correctly identified all target strains returning 100% accurate results in the inclusivity test (n=154) on both instruments (table above). Manufacturer 1 assay correctly identified all serovar Enteritidis inclusivity isolates (n=20). Manufacturer 2 assay failed to detect two *Salmonella* ser. Enteritidis strains, although all *Salmonella* ser. Typhimurium strains were correctly identified (n=55).

The RapidFinder Salmonella Multiplex Assay showed excellent results in the exclusivity test as all non-targets returned true negative result on both instruments. *Salmonella* serovars Nitra, Blegdam and Moscow however were incorrectly identified as *Salmonella* ser. Enteritidis.

The other two assays tested also returned false positive results with *Salmonella* serovars Nitra, Blegdam and Moscow as they were incorrectly identified as *Salmonella* ser. Enteritidis. Literature indicates that serotype Nitra is part of the serotype Enteritidis lineage and that serotype Blegdam is an ancestor to serotypes Enteritidis and Moscow, hence explaining the difficulties in differentiating these serotypes[3,4].

Manufacturer 1 assay also falsely detected *Salmonella* serovars Dublin, Kiel, London, Rostock, Pullorum and Gallinarum as *Salmonella* ser. Enteritidis. Of these serovars, Pullorum and Gallinarum are of major significance to Poultry industry whereas Dublin to public health[1]. Manufacturer 2 assay detected *Salmonella* ser. Tennessee falsely as *Salmonella* ser. Typhimurium.

Figure 2. Positive samples ran with RapidFinder Salmonella Multiplex Assay and analyzed with RapidFinder Express Software on 7500 Fast.

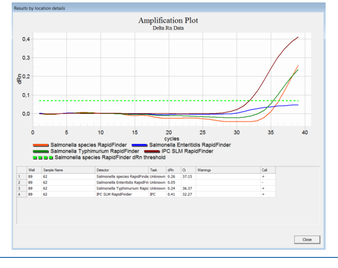
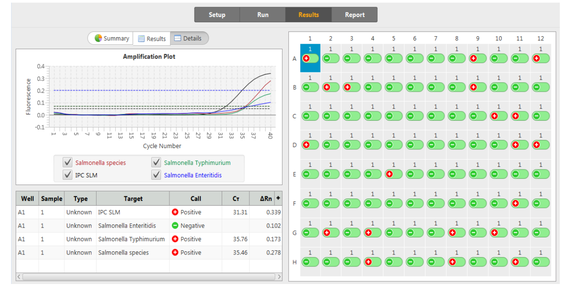


Figure 3. Positive and negative samples ran with the RapidFinder Salmonella Multiplex Assay and analyzed with RapidFinder Analysis Software on QuantStudio 5 System.



CONCLUSIONS

The study demonstrated that the RapidFinder Salmonella Multiplex Assay is more reliable for the detection and differentiation of *Salmonella* species, *Salmonella* ser. Enteritidis and *Salmonella* ser. Typhimurium than the other two commercially available real-time PCR assays.

The RapidFinder Salmonella Multiplex Assay provides ability to accurately detect and differentiate the most important serovars, including relevant variants of the target serovars like monophasic *Salmonella* ser. Typhimurium. Also, the RapidFinder Salmonella Multiplex Assay has a simple workflow, automated results interpretation and reporting.

Enabling detection of all *Salmonella* and differentiation of *Salmonella* ser. Enteritidis and *Salmonella* ser. Typhimurium, the RapidFinder Salmonella Multiplex Assay provides more confidence to sample analysis and detection of relevant serovars than the alternative products do.

REFERENCES

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TRADEMARKS

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