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 *RapidFinder*® *Salmonella* Multiplex Assay tests against two commercially available time PCR assays designed to detect *Salmonella* serovars 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-relevant 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 typed prior to PCR and analyzed with the *RapidFinder* Salmonella Multiplex Assay as detailed in the instructions for use.

The two assay sets 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. *Salmonella* target strains were correctly detected by *RapidFinder* Salmonella Multiplex Assay and 98% of the non-target 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 *Salmonella* strains (98%) 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 species of *S. enterica* are *Salmonella* ser. Typhimurium and *Salmonella* ser. Enteritidis. Every year, approximately one million people fall ill from consumption of *Salmonella* contaminated food 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 reliable, rapid and versatile PCR multiplex assay would enable producers to quickly and accurately detect these targets in a single test, allowing them to make critical decisions on new meat/batch release delays before traditional culture and serogrouping methods would show.

**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 subspces as serious *Typhimurium* and *Enteritidis*. Non-target species were selected based on their genetic closeness to *Salmonella*.

Sample preparation

All test organisms were plated onto TCBS Soyas 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 ± 1t at +37±2°C. Non-target strains were inoculated in Brain Heart Infusion broth for 18 ± 1t at +37±2°C. To test for inclusivity, *Salmonella* strains were diluted into Maximum Recovery Diluent to approximately 100-500 Limit of Detection. Non-targets were tested at growth level.

Test method(s)

Incubated samples were prepared for PCR using a direct lyse protocol by pipetting 10 µl of inoculated sample into pre-filled Lyse Reactant 1 tubes. The Applied Biosystems™ SimpliAmp™ Thermal Cycler was used to facilitate the heating steps. After preheating, 40 µl of lyse was pipetted into PCR tubes containing *RapidFinder* Salmonella Multiplex Assay (cycling parameters are detailed in the instructions for use).

PCR was conducted using the Applied Biosystems™ 7500 Fast and Applied Biosystems™ QuantStudio™ 3 Real-Time PCR instruments. Figure 1 with Applied Biosystems™ RapidFinder™ Express Software v2.0 and Applied Biosystems™ RapidFinder™ Analysis Software v2.0, respectively.

The other two commercially available assays were performed according to the manufacturers’ instructions and run on the Applied Biosystems™ 7500 Fast instrument and results analyzed with 7500 Fast System Detection Software System v1 4.3.2.

**Data analysis**

The number of positive and negative results interpreted by the *RapidFinder* Express Software and *RapidFinder* Analysis Software (Figure 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.

**RESULTS**

<table>
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<tr>
<th>Table: RapidFinder Salmonella Multiplex Assay vs. the other two commercially available assay results from inclusivity and exclusivity testing.</th>
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<tr>
<td>Method</td>
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<td><em>RapidFinder</em> Salmonella Multiplex Assay (7500 Fast and QuantStudio 3)</td>
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<td>Manufacturer 1</td>
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<td>Manufacturer 2</td>
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**Key**

- S. Blegdam, S. Moscov and S. Nitra returned false positive on S. Enteritidis target
- S. Blegdam, S. Moscov and S. Nitra returned false results on S. Enteritidis target
- S. Typhimurium returned false positive on S. Typhimurium target
- 2 false negatives from S. Enteritidis strain
- The *RapidFinder* Salmonella Multiplex Assay correctly identified all target strains returning 100% accurate results in the inclusivity test (n=154).

**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. The *RapidFinder* Salmonella Multiplex Assay provides more confidence to sample analysis and detection of relevant serovars than the alternative products do.

**REFERENCES**


**TRADEMARKS**

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