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Evaluation of Salmonella Spp., S. Enteritidis and S. Typhimurium Real-Time PCR Kit Performance in Co-Inoculated Poultry, Pork Meat and Environmental Surface Samples

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ABSTRACT

Purpose
The purpose of the study was to verify performance of the Thermo Scientific™ RapidFinder™ Salmonella species, Typhimurium and Enteritidis Multiplex PCR Kit (RapidFinder Salmonella Multiplex Assay) when poultry, pork and environmental surface samples were co-inoculated with low levels of two different *Salmonella* serovars. Presumptive results were compared to those obtained with the RapidFinder Salmonella Multiplex Assay's culture confirmation and the USDA-FSIS MLG 4.09[1] Isolation and Identification of *Salmonella* from Meat, Poultry, Pasteurized Egg, and Siluriformes (Fish) Products and Carcass and Environmental Sponges (reference method).

Methods
Ten poultry, pork and surface sponge samples were co-inoculated with low levels of two *Salmonella* serovars to achieve fractionally positive results in accordance with AOAC® *Performance Tested Methods*℠ (PTM). All samples analyzed with the RapidFinder Salmonella Multiplex Assay were enriched in Buffered Peptone Water (ISO formulation) + 12 mg/l novobiocin at 41.5±1 ºC for 14-16 hours, lyzed, tested and analyzed according to the protocol described in the instructions for use. Presumptive positive results were confirmed using culture and serological confirmation methods. The reference method was performed according to FSIS guidelines.

Results
The RapidFinder Salmonella Multiplex Assay proved to be an accurate method even when samples were contaminated with multiple *Salmonella* serovars. The RapidFinder Salmonella Multiplex Assay was 100% sensitive and 100% specific versus culture and serological confirmation. Detection of two serovars from a single sample was possible from poultry, pork and environmental surface sponges.

INTRODUCTION

Salmonellosis is the most frequently reported bacterial food-borne illness in the US with more than 1 million cases each year[2]. *Salmonella* serovars Typhimurium and Enteritidis are most commonly associated with disease in humans[3]; they are also included in the top 10 serovars isolated from raw meat[4]. Targets to reduce the prevalence of these two serovars in broiler chickens are being introduced by international regulators[5] thereby initiating the requirement for the food industry to prove their absence at the earliest opportunity. Rapid methods to detect *Salmonella* species are readily available, such as the Applied Biosystems™ MicroSEQ™ *Salmonella spp.* Detection Kit, however rapid methods to detect serovars Enteritidis and Typhimurium are less common.

The Thermo Scientific™ RapidFinder™ Salmonella species, Typhimurium and Enteritidis Multiplex PCR Kit provides a rapid and reliable method to detect and distinguish serovars Typhimurium and Enteritidis from *Salmonella* species in poultry, pork and environmental samples. The RapidFinder Salmonella Multiplex Assay will enable food manufacturers to simultaneously obtain a time to presumptive serovar result for *S.* Enteritidis and *S.* Typhimurium in less than 18 hours compared to lengthy serological confirmation methods.

MATERIALS AND METHODS

Sample Preparation
Twenty replicates for each matrix (raw chicken thighs with skin, raw pork sausage and stainless steel surface) were co-inoculated with low levels of 2 *Salmonella* serovars to achieve a fractionally positive dataset in accordance with AOAC guidelines[6] (Table 1). Artificially contaminated samples were acclimatized prior to testing by storing food samples at 2-8 ºC for 3 days and stainless steel at room temperature for 16-24 hours. Four replicates were left uninoculated with *Salmonella* for each sample type.

Post storage, raw meat samples were divided into 25 g portions; each stainless steel surface was sampled using sterile surface sponges pre-moistened with 10 ml Dey-Engley Neutralizing Broth.

Table 1. Inoculum information

Matrix		Raw chicken thighs with skin	Raw pork sausage	Stainless steel Surface sponges
Salmonella isolate 1	Serovar	Kentucky	Typhimurium	Poona
	Reference	RDCC[a] 3734	RDCC 3734	NCTC 4840
	Contamination level (CFU/sample)	3.0	2.0	55.0
Salmonella isolate 2	Serovar	Enteritidis	Enteritidis	Typhimurium
	Reference	NCTC 8515	NCTC 12694	RDCC 3924
	Contamination level (CFU/sample)	2.0	2.0	44.0
Background organism	Organism			*Enterococcus faecalis*
	Reference			ATCC 29212
	Contamination level (CFU/sample)			2033.0

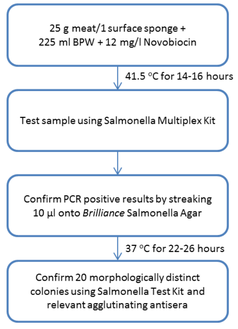
a: RDCC – Thermo Fisher Scientific in-house culture collection

Test Method
Ten inoculated and two un-inoculated replicates of each sample type were combined with 225 ml Buffered Peptone Water (ISO formulation) (BPW) supplemented with 12 mg/l novobiocin for enrichment at 41.5±1 °C for 14-16 hours (Figure 1). Post enrichment, 10 µl enriched sample was tested on the RapidFinder Salmonella Multiplex Assay; the Applied Biosystems™ SimpliAmp™ Thermal Cycler was used to facilitate an 18 minute cell lysis protocol. The 50 minutes PCR was conducted using the Applied Biosystems™ 7500 Fast Real-Time PCR Instrument with Applied Biosystems™ RapidFinder™ Express v2.0. Results were confirmed by streaking enriched samples directly onto Thermo Scientific™ Oxoid™ *Brilliance*™ Salmonella Agar before testing with Thermo Scientific™ Oxoid™ Salmonella Test Kit and relevant *Salmonella* agglutinating antisera.

Reference Method (USDA-FSIS MLG 4.09)
The remaining ten inoculated and two uninoculated replicates of each sample type were combined with BPW for enrichment: 225 ml for raw meat samples and 50 ml for environmental surface sponges. Samples were incubated at 35±2 °C for 20-24 hours according to the reference method. Enriched samples were sub-cultured in 10 ml RVS broth and TT Hajna broth which were incubated at 42±0.5 °C for 22-24 hours. Diminishing streaks were performed from each selective broth on Brilliant Green Sulfa Agar (BGS) and XLT4 Agar which were incubated at 35±2 °C for 18-24 hours. Suspect colonies were confirmed with the relevant *Salmonella* agglutinating antisera.

Data Analysis
Results for each sample type were compared using Probability of Detection (POD) analysis used during validation of alternative methods according to AOAC® PTM.

Figure 1. RapidFinder Salmonella Multiplex Assay workflow



RESULTS

Table 2. RapidFinder Salmonella Multiplex Assay vs. USDA-FSIS MLG 4.09 method with chicken thighs

Relative contamination level			Low	Un-inoculated
Replicates			10	2
Number positive results	RapidFinder Salmonella Multiplex Assay	Sp[a]	10	0
		SE[b]	7	0
	Culture confirmed	Sp	10	0
		SE	7	0
	USDA-FSIS MLG 4.09	Sp	10	0
		SE	0	0
dPOD 95% CI		Sp	0.00 (-0.28 - 0.28)	0.00 (-0.66 - 0.66)
		SE	0.70 (0.29 - 0.89)	0.00 (-0.66 - 0.66)

a: Sp – *Salmonella* species
b: SE – *Salmonella* Enteritidis

Table 3. RapidFinder Salmonella Multiplex Assay vs. USDA-FSIS MLG 4.09 method with pork sausage

Relative contamination level			Low	Un-inoculated
Replicates			10	2
Number positive results	RapidFinder Salmonella Multiplex Assay	Sp[a]	10	0
		ST[b]	6	0
		SE[c]	9	0
	Culture confirmed	Sp	10	0
		ST	6	0
		SE	9	0
	USDA-FSIS MLG 4.09	Sp	9	0
		ST	7	0
		SE	3	0
dPOD 95% CI		Sp	0.1 (-0.20 - 0.40)	0.00 (-0.66 - 0.66)
		ST	-0.1 (-0.45 - 0.28)	0.00 (-0.66 - 0.66)
		SE	0.6 (0.17 - 0.82)	0.00 (-0.66 - 0.66)

a: Sp – *Salmonella* species
b: ST – *Salmonella* Typhimurium
c: SE – *Salmonella* Enteritidis

Table 4. RapidFinder Salmonella Multiplex Assay vs. USDA-FSIS MLG 4.09 method with stainless steel surface sponges

Relative spike level			Low	Unspiked
Replicates			10	2
Number positive results	RapidFinder Salmonella Multiplex Assay	Sp[a]	7	0
		ST[b]	6	0
	Culture confirmed	Sp	7	0
		ST	6	0
	USDA-FSIS MLG 4.09	Sp	7	0
		ST	7	0
dPOD 95% CI		Sp	0.00 (-0.36 - 0.36)	0.00 (-0.66 - 0.66)
		ST	-0.10 (-0.45 - 0.28)	0.00 (-0.66 - 0.66)

a: Sp – *Salmonella* species
b: ST – *Salmonella* Typhimurium

The *Salmonella* species and *S.* Typhimurium POD analysis for all sample types indicated no significant difference between the methods tested (Tables 2, 3 and 4). The *S.* Enteritidis POD analysis revealed poor performance of the USDA-FSIS MLG method with chicken thighs and pork sausage. All *S.* Enteritidis PCR positive results were confirmed using *Brilliance* Salmonella Agar and O:9 agglutinating antisera however corresponding samples from the reference method were either completely negative (chicken thighs) or had significantly fewer positives than the RapidFinder Salmonella Multiplex Assay (pork sausage). It is possible that *S.* Enteritidis was out competed by the other Salmonella serovars used to co-infect samples during the selective secondary enrichment step of the reference method resulting in a difference in levels of growth between the two isolates. Despite testing 20 colonies from each positive sample, recovery of *S.* Enteritidis was significantly lower from poultry and pork samples from the reference method.

CONCLUSIONS

The RapidFinder Salmonella Multiplex Assay performed well in comparison to the USDA-FSIS MLG method for the detection and differentiation of *S.* Typhimurium and *S.* Enteritidis from other *Salmonella* serovars from poultry, pork and environmental surface samples.

The RapidFinder Salmonella Multiplex Assay enables the detection and distinction of *S.* Typhimurium and *S.* Enteritidis from poultry, pork and environmental surface samples. Where samples are contaminated with more than one *Salmonella* serovar, the RapidFinder Salmonella Multiplex Assay assures detection where traditional culture methods may not.

REFERENCES

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