Superior Detection of Multiple *Salmonella* Serovars from Meat and Environmental Samples using a Multiplex PCR Method

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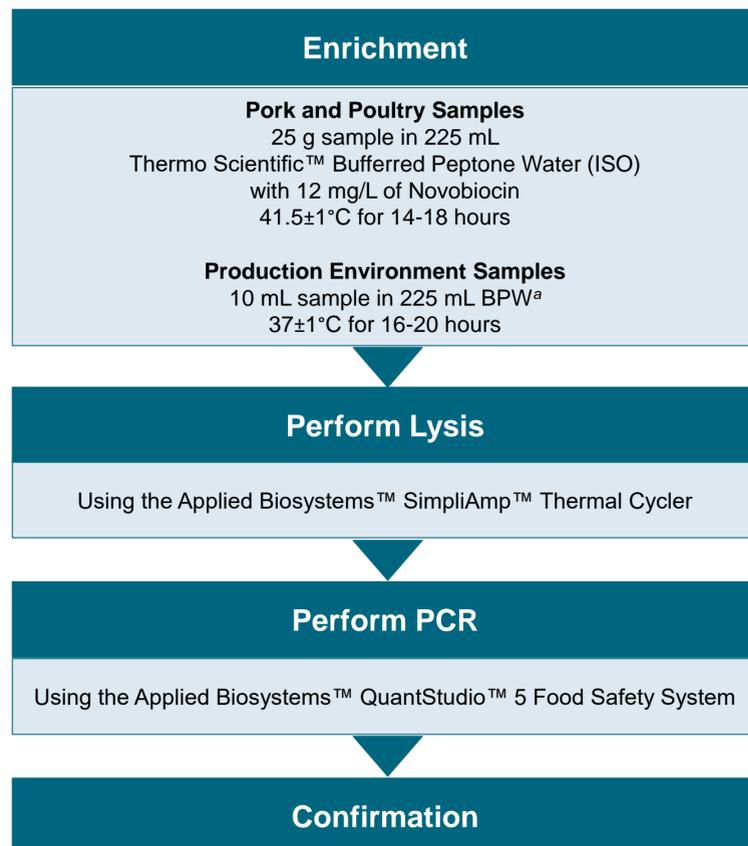
INTRODUCTION

Co-contamination of pork and poultry products often occurs with multiple serovars of *Salmonella*[1]. Limitations in international standard methods mean typically only a single isolate is identified, leading to lowered accuracy in monitoring of *Salmonella* serovar prevalence.

Studies were performed to compare the performance of the Thermo Scientific™ RapidFinder™ Salmonella species, Typhimurium and Enteritidis Multiplex PCR Kit (candidate method) to ISO 6579-1:2017 and FSIS MLG 4.09 reference methods for the detection of multiple *Salmonella* serovars from co-infected matrices. The workflow for the candidate method is detailed in Figure 1.

METHODS

Figure 1. RapidFinder Salmonella species, Typhimurium and Enteritidis workflow summary used during both studies

Enrichment
Pork and Poultry Samples 25 g sample in 225 mL Thermo Scientific™ Bufferred Peptone Water (ISO) with 12 mg/L of Novobiocin 41.5±1°C for 14-18 hours
Production Environment Samples 10 mL sample in 225 mL BPW[a] 37±1°C for 16-20 hours

Perform Lysis
Using the Applied Biosystems™ SimpliAmp™ Thermal Cycler

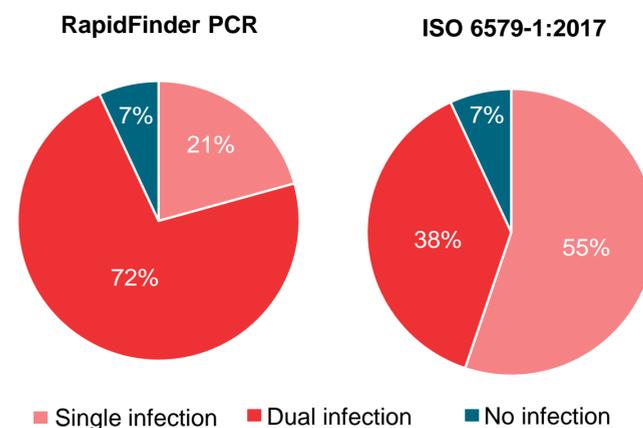| Perform PCR |
| --- |
| Using the Applied Biosystems™ QuantStudio™ 5 Food Safety System |

Confirmation

DUAL INFECTION STUDY

A total of 29 samples (meat and production environment samples) were dual-infected with *S.* species and either *S.* Typhimurium or *S.* Enteritidis at 0.6 to 4.8 CFU/25 g. Alongside 29 unspiked samples were processed using the candidate method. A replicate dataset was tested according to ISO 6579-1:2017 as an unpaired study.

Figure 2. Comparison of candidate method and ISO reference method for *Salmonella*-positive samples

Salmonella Serovars	Candidate Method		ISO 6579-1:2017
	PCR result	Confirmed result	
Single infection	6	8	16
Dual infection	21	19	11
No infection	2	2	2
Total	29	29	29

Figure 3. Percentage of positive samples for *Salmonella* in meat and production environment samples

RapidFinder PCR — Single infection 21%, Dual infection 72%, No infection 7%

ISO 6579-1:2017 — Single infection 55%, Dual infection 38%, No infection 7%

■ Single infection ■ Dual infection ■ No infection

As shown in figures 2 and 3, the candidate method detected *Salmonella* dual infection in 21 out of 29 (72%) spiked samples. The ISO method confirmed just 11 (38%) dual-infected samples.
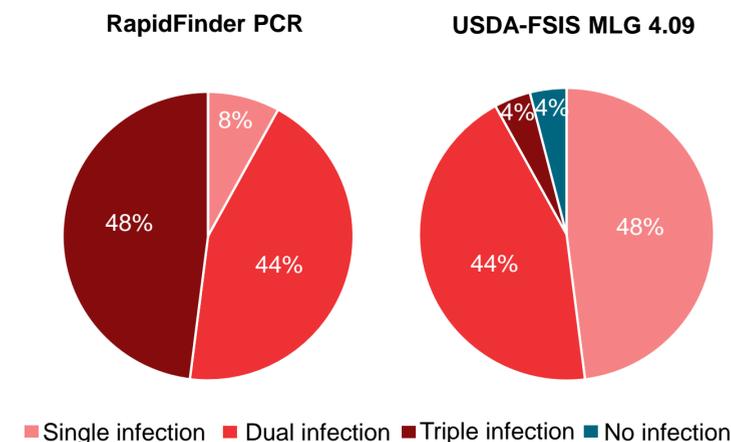
TRIPLE INFECTION STUDY

A total of 50 pork sausage samples were triple-infected with *S.* Ohio, *S.* Typhimurium and *S.* Enteritidis at 0.85 – 4.0 CFU/25 gram. Half the samples were processed according to the candidate method and the other half according to FSIS MLG 4.09. Twenty unspiked samples were tested in the same unpaired manner.

Figure 4. Comparison of candidate method and USDA-FSIS reference method for *Salmonella*-positive samples

Salmonella Serovars	Candidate Method		USDA-FSIS MLG 4.09
	PCR result	Confirmed result	
Single infection	2	7	12
Dual infection	11	11	11
Triple infection	12	7	1
No infection	0	0	1
Total	25	25	25

Figure 5. Percentage of positive samples for *Salmonella* in pork sausage samples

RapidFinder PCR — Single infection 8%, Dual infection 44%, Triple infection 48%

USDA-FSIS MLG 4.09 — Single infection 48%, Dual infection 44%, Triple infection 4%, No infection 4%

■ Single infection ■ Dual infection ■ Triple infection ■ No infection

As shown in figure 4 and 5, the candidate method detected triple infection in 12 out of 25 (48%) spiked samples. The FSIS method confirmed just one triple-infected sample.

Variation of growth rate between isolates can cause challenges in confirming co-infection as some isolate may out-grow others during enrichment and mask them on plating media. PCR methods are not limited by this variation and so can detect multiple serovars from one sample while traditional plating methods may not.

CONCLUSIONS

Superior performance

- The candidate method displayed a 34% improvement in detection of dual-infection, and 44% improvement in detection of triple-infection compared to FSIS MLG 4.09.
- The greatly increased accuracy of the candidate method in *Salmonella* serovar reporting enables potential reductions in outbreaks of *Salmonella*.

Superior detection of *Salmonella* serovars

- The candidate method detected 108 *Salmonella* isolates across both studies.
- The ISO and FSIS methods together confirmed just 75 *Salmonella* isolates (30.6 % fewer).

Improved workflow

- Candidate method workflow greatly improves the time to result, obtaining PCR data within just 16 hours

ACKNOWLEDGEMENTS

REFERENCES

1. Andino, A., & Hanning, I. (2015). Salmonella enterica: survival, colonization, and virulence differences among serovars. The Scientific World Journal, 520179.

TRADEMARKS/LICENSING

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