Evaluation Of Real-Time PCR *Salmonella* Spp., *S*. Enteritidis And *S*. Typhimurium Assay Performance In Poultry Meat Samples

Jani Holopainen[1], Mikko Kauppinen[1], Katharine Evans[2]

[1]Thermo Fisher Scientific, Vantaa, Uusimaa, Finland; [2]Thermo Fisher Scientific, Basingstoke, Hampshire, UK

ABSTRACT

Purpose

The purpose of the study was to verify the performance of the Thermo Scientific™ TaqMan® Salmonella Triplex Assay (species, Enteritidis and Typhimurium) with direct lysis as a rapid PCR method for the detection and differentiation of *Salmonella* spp., *S*. Typhimurium and *S*. Enteritidis with a range of different poultry samples at two major poultry meat manufacturers' laboratories in Brazil.

Methods

At the first laboratory the TaqMan Salmonella Triplex Assay was compared against two other commercially available assays. However these 2 other assays do not detect *S*. Enteritidis and *S*. Typhimurium separately as they were *Salmonella* spp. specific only.

At the second laboratory no alternative method was included in the study.

Results

The TaqMan Salmonella Triplex Assay method proved to be an accurate method. Ninety-eight percent and 100% of the samples yielded expected results at laboratories 1 and 2 respectively.

INTRODUCTION

Every year, approximately one million people fall ill from consumption of *Salmonella* contaminated food[1]. 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 *S*. Typhimurium and *S*. Enteritidis.

A simple, reliable and fast multiplex PCR assay would enable producers to quickly and accurately detect these targets in a single reaction, allowing them to make critical decisions on raw meat batch release days before a culture method would allow (figure 1).

MATERIALS AND METHODS

Sample Preparation

One-hundred-and-fifty-five naturally contaminated poultry samples representing 13 different matrix types were tested at laboratory 1. Additionally, 12 poultry samples representing 2 matrix types were inoculated with *Salmonella* Enteritidis or *S*. Typhimurium at laboratory 2.

All samples were weighed and portioned in 25 g sample sizes into homogeniser bags and diluted with a 1:10 ratio of Buffered Peptone Water.

Samples were incubated at 37 °C for 18 hours.

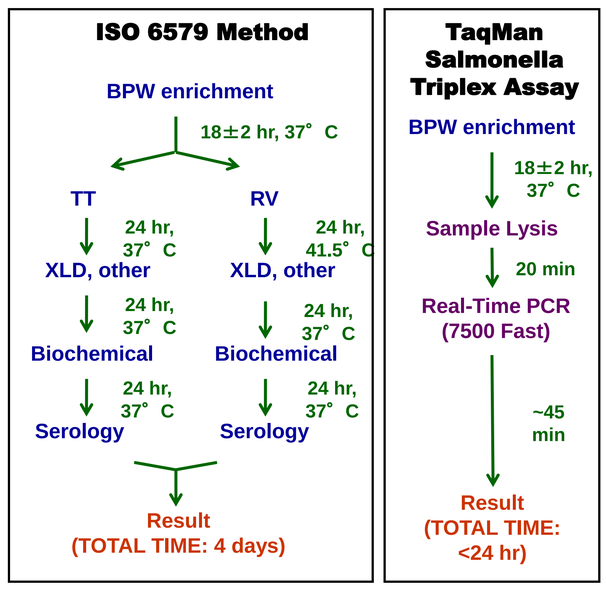
Test method(s)

Samples were removed from homogeniser bags at 18 hours. The TaqMan Salmonella Triplex Assay was performed by carrying out direct lysis on 10 µl of enrichment and transferring 30 µl of lysate onto Salmonella Triplex Assay lyophilized beads.

PCR was conducted using an Applied Biosystems™ 7500 Fast Real-Time PCR Instrument with Applied Biosystems™ RapidFinder™ Express Software v2.0 (figure 2 and 3).

At laboratory 1, the other two commercially available assays tested were performed according to the manufacturers instructions.

Figure 1. ISO reference method and TaqMan Salmonella Triplex Assay protocols



Data Analysis

The number of positive and negative presumptive results interpreted by the RapidFinder Express Software v2.0 were recorded for the TaqMan Salmonella Triplex Assay and compared to the results generated with the other commercially available methods at laboratory 1.

At laboratory 2, the results generated with the TaqMan Salmonella Triplex Assay were compared to known inoculation patterns of *S*. Typhimurium and *S*. Enteritidis.

RESULTS

Table 1. TaqMan® Salmonella Triplex Assay vs. alternative method summary from laboratory 1.

Sample	NA	PA	PD	ND	Total
Carcass	71	14	0	2	87
Parts, organs	19	0	0	0	19
Cuts	8	1	0	0	10
Seasoned	37	0	1	0	38

Key

PA – positive agreement (both methods positive)

NA – negative agreement (both methods negative)

PD – positive deviation (Triplex method positive, alternative method negative

ND – negative deviation (Triplex method negative, alternative method positive)

NaN – Not a number (negative)

Table 2. TaqMan® Salmonella Triplex Assay method from laboratory 2.

Sample Type	Inoculated organism	Salmonella Triplex Assay result		
		S. Enteritidis	*S*. Typhimurium	*S*. spp
Fresh chicken	*S*. Typhimurium	NaN	Positive	Positive
Fresh chicken	*S*. Typhimurium	NaN	Positive	Positive
Fresh chicken	*S*. Typhimurium	NaN	Positive	Positive
Fresh chicken	*S*. Typhimurium	NaN	Positive	Positive
Fresh chicken	*S*. Enteritidis	Positive	NaN	Positive
Fresh chicken	*S*. Enteritidis	Positive	NaN	Positive
Fresh chicken	*S*. Enteritidis	Positive	NaN	Positive
Breaded chicken	*S*. Typhimurium + *E. coli*	NaN	Positive	Positive
Breaded chicken	*S*. Typhimurium	NaN	Positive	Positive
Breaded chicken	*S*. Typhimurium + *E. aerogenes*	NaN	Positive	Positive
Breaded chicken	*S*. Typhimurium + *E. coli*	NaN	Positive	Positive
Breaded chicken	*S*. Typhimurium + *S*. Enteritidis	Positive	Positive	Positive

The TaqMan Salmonella Triplex Assay yielded comparable results to other commercially available assays when testing samples from different naturally contaminated chicken matrices tested by laboratory 1 (Table 1) and also accurately detected inoculated organisms when tested by laboratory 2 (Table 2).

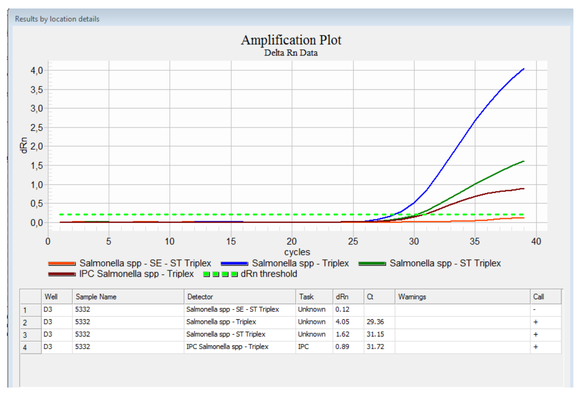
Figure 2. Applied Biosystems™ RapidFinder™ Express Software v2.0

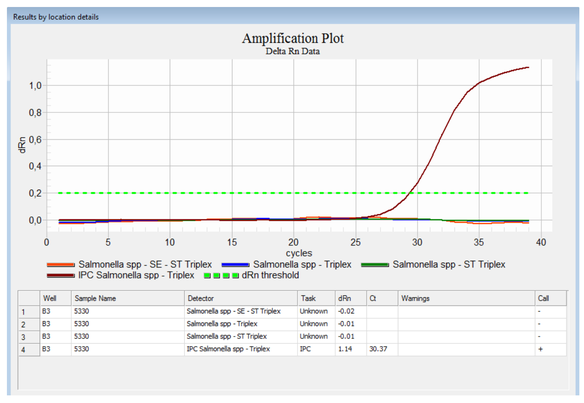
CONCLUSIONS

The study demonstrated that the TaqMan Salmonella Triplex Assay with direct lysis provides a simple, rapid and reliable method for the detection of *Salmonella* spp., *S.* Typhimurium and *S.* Enteritidis in raw and seasoned chicken and parts of chicken compared to the alternative molecular methods.

Compared to other commercially available rapid Salmonella assays, the TaqMan Salmonella Triplex Assay also provides the ability to differentiate *S*. Typhimurium and *S*. Enteritidis from the other *Salmonella* spp.

Figure 3. Positive and negative results examples for TaqMan® Salmonella Triplex Assay from the Applied Biosystems™ RapidFinder™ Express Software v2.0





REFERENCES

1. Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson M-A, Roy SL, et al. (2011) Foodborne illness acquired in the United States—major pathogens. Emerging Infectious Diseases. 2011 Jan.

TRADEMARKS

LT 2287A