Evaluation Of A TaqMan Salmonella Triplex PCR Assay For *Salmonella* Spp., *S.* Enteritidis And *S.* Typhimurium Compared To ISO 6579:2002 Method In Raw Poultry, Raw Pork And Environmental Samples

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Overview

Purpose: The aim of this study was to evaluate the performance of the Thermo Scientific™ TaqMan® Salmonella Triplex Assay as a rapid PCR method for the detection and differentiation of *Salmonella* spp., *S.* Typhimurium and *S.* Enteritidis from raw poultry, raw pork and swab samples.

Methods: The TaqMan Triplex Assay was compared to the ISO 6579:2002 reference method for detection of *Salmonella* spp. from raw poultry, raw pork and environmental samples.

Results: The PCR method showed comparable performance to the traditional culture method according to AFNOR Certification and ISO 16140:2003 criteria for unpaired studies.

Introduction

Amongst serovars of *Salmonella, S.* Typhimurium and *S.* Enteritidis are the most frequently occurring cause of human illness[1]. Poultry and pork producers are under pressure from the European Food Safety Authority, and national food safety agencies within the European Union, United States Department of Agriculture Food Safety and Inspection Service and other regulatory bodies and retailers to reduce *Salmonella* rates and to monitor prevalence of specific serovars such as *S.* Typhimurium and *S.* Enteritidis. A multiplex PCR assay would enable producers to rapidly and accurately detect these three targets with a single test, allowing decisions to be made for batches of raw meats days before a culture method would allow.

Methods

Sample Preparation

Five different raw poultry and two different raw pork samples were weighed into 4 x 25 g portions. Seven swabs designed for surface testing were pre-moistened with Dey-Engley Neutralizing Broth. All samples were spiked in duplicate with <2 CFU of *S.* Typhimurium or *S.* Enteritidis. Unspiked samples were also prepared to screen for presence of *Salmonella*. All raw meat samples were stored at 2-8°C for 3 days before enrichment. All surface swabs were stored for 2 hours at room temperature before enrichment.

Samples were diluted 1:10 with Buffered Peptone Water (BPW) + 12 mg/l novobiocin for the alternative TaqMan Triplex Assay workflow and BPW for the ISO reference method. All samples were enriched for 16 hours at 37±1°C.

Test method(s)

Samples were taken from enrichment bags at 16 hours. The TaqMan Triplex Assay was performed by carrying out direct cell lysis on 10 µl of enrichment and transferring 30 µl of lysate onto TaqMan Salmonella Triplex Assay lyophilised PCR pellets. PCR was conducted using an Applied Biosystems™ 7500 Fast Real-Time PCR Instrument (Figure 1) with Applied Biosystems™ RapidFinder™ Express Software v 2.0. Culture confirmations were conducted by sub-culturing 100 µl of enrichment into 10 ml Rappaport-Vassiliadis Soya Peptone (RVS) Broth, incubating for 24±3 hours at 41.5±1°C and then performing a diminishing streak on Thermo Scientific™ Oxoid™ *Brilliance*™ Salmonella Agar which was incubated for 24 hours at 37±1°C. Reference method samples were tested following the ISO 6579:2002 method for detection of *Salmonella* spp.

Data Analysis

Method agreement was determined according to the principles of AFNOR Certification and ISO 16140:2003 criteria for unpaired studies; the number of positive and negative deviations of the alternative method from the reference method were calculated and the difference between them used to assess performance.

Figure 1. Applied Biosystems™ 7500 Fast Real-Time PCR Instrument

Results

Table 1: TaqMan Triplex Assay method vs. ISO reference method results agreement summary

Target	PA	NA	PD	ND	PPNA	Total
Salmonella spp.	10	12	4	5	0	31
S. Typhimurium	7	19	3	2	0	31
S. Enteritidis	3	24	1	1	2	31

Key

PA – positive agreement (both methods positive)
NA – negative agreement (both methods negative)
PD – positive deviation (TaqMan method positive, ISO method negative)
ND – negative deviation (TaqMan method negative, ISO method positive)
PPNA – presumptive positive, negative agreement (TaqMan method positive, TaqMan confirmation method negative, ISO method negative)

The TaqMan Triplex Assay yielded comparable results to the ISO reference method (Table 1). The difference in positive and negative deviations between the methods was +1, -1 and 0 for *Salmonella* spp., *S*. Typhimurium and *S*. Enteritidis respectively.

Conclusion

The study demonstrated that the TaqMan Salmonella Triplex Assay offers a suitable alternative workflow for the detection of *Salmonella* spp., *S*. Enteritidis and *S*. Typhimurium in raw poultry, raw pork and production environment swabs compared to the reference method detailed in ISO 6579:2002.

References

1. Hugas M., Beloeil P. A. (2014). Controling Salmonella along the food chain in the European Union - progress over the last ten years. Eurosurveillance. 19 (19): pii=20804.

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