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Evaluation of a New Multiplex PCR Assay for Detection of STEC from Meat Samples

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INTRODUCTION

Shiga toxin-producing *Escherichia coli* (STEC) are a group of pathogenic organisms that may cause severe disease including hemolytic uremic syndrome (HUS). STEC outbreaks have been linked to a number of food sources including meat and vegetables.

The Thermo Scientific™ SureTect™ STEC PCR Assay solution (candidate method) detects multiplex genetic targets for O157:H7 and other STEC from food and environmental samples. The SureTect STEC PCR Assay solution comprises two multiplex reactions for the simultaneous detection of the following targets:

- **Screening Assay:** O157:H7, *stx*, *eae*
- **Identification Assay:** O26, O103, O111, O145, O45, O121

This study summarizes the evaluated performance of the SureTect STEC PCR Assay solution (candidate method) for the detection of STEC from meats vs. the ISO 13136:2012 reference method[1].

METHODS

Three categories of meat samples (including beef, veal, pork and lamb) were divided into 25 g portions and artificially contaminated with a range STEC isolates from different serogroups (Table 1). The samples were then tested using the candidate method workflow (Figure 1) and associated instrumentation (Figure 2). A replicate set of samples was tested according to the ISO reference method.

Table 1. Contamination Levels

Meat Subcategory	Spiked (N)	Spike Level (CFU)	Unspiked (N)
Raw	7	0.4 – 3.6	7
Seasoned	7	0.4 – 2.2	4
Frozen	7	1.8 – 3.0	7

Post enrichment, all candidate method samples were tested and streaked onto isolation agars for confirmation including; Thermo Scientific™ Oxoid™ Chromogenic Coliform Agar and Thermo Scientific™ Oxoid™ TBX Medium.

In cases where plating direct from enrichment broth was unsuccessful, a purification step using serogroup-specific Dynabeads™ and Immunomagnetic separation (IMS) was used before plating.

MATERIALS

Figure 1. SureTect STEC PCR Assay Process Flow

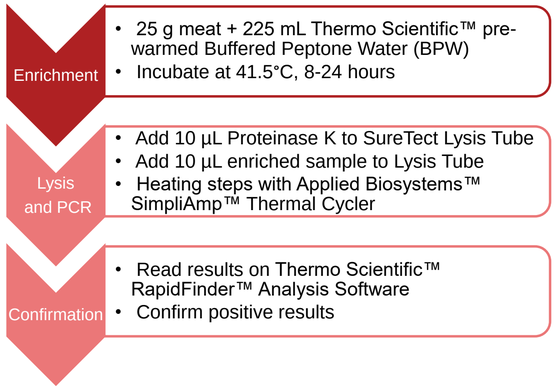


Figure 2: Applied Biosystems QuantStudio 5 Food Safety PCR System

*Applied Biosystems™ QuantStudio™ 5 Food Safety Real-Time PCR Instrument and laptop with Thermo Scientific™ RapidFinder™ Analysis software

RESULTS

Table 2. Candidate Method Performance by Meats Subcategory

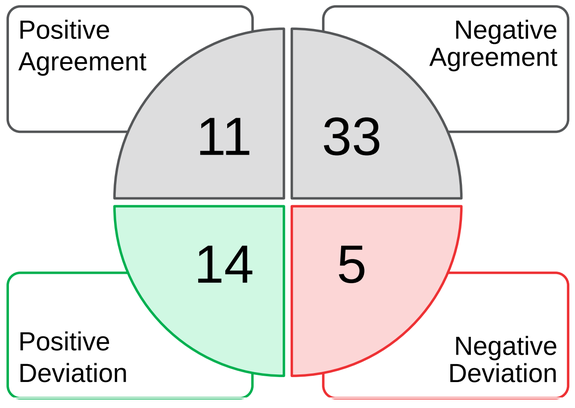
Method Performance	Raw	Seasoned	Frozen
Positive Agreement	2	3	6
Negative Agreement	11	12	10
Positive Deviation	8	4	2
Negative Deviation	0	2	3

Unpaired Study Key:

Positive Agreement = Candidate Method Positive, Reference Method Positive

Positive Deviation = Candidate Method Positive, Reference Method Negative

Figure 3. Overall Candidate Method Performance with Meats



Negative Agreement = Candidate Method Negative, Reference Method Negative

Negative Deviation = Candidate Method Negative, Reference Method Positive

RESULTS SUMMARY

The reference method returned fewer positive results from raw and seasoned meat than the candidate method. The methods performed comparably with frozen meats (Table 2).

The difference between the negative deviations and positive deviations was -9 (Figure 3), indicating that the candidate method performed better than the reference method.

CONCLUSIONS

Improved Performance

The SureTect STEC PCR Assay solution demonstrates improved performance over the ISO reference method

Rapid Time to Result

Samples are enriched for only eight hours with the SureTect method compared to the ISO reference method of 18 hours

Simple Enrichment

The SureTect workflow utilises enrichment in BPW without the need for antibiotics or proprietary media

ACKNOWLEDGEMENTS

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REFERENCES

1. ISO/TS 13136:2012 Microbiology of food and animal feed -- Real-time polymerase chain reaction (PCR)-based method for the detection of food-borne pathogens -- Horizontal method for the detection of Shiga toxin-producing Escherichia coli (STEC) and the determination of O157, O111, O26, O103 and O145 serogroups

TRADEMARKS/LICENSING

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