STEC Detection from 25 g and 375 g Beef Samples with a New PCR Method Workflow

David Crabtree[1], Dean Leak[1], Jessica Williams[1], Ana-Maria Leonte[1], Matthew Hahs[2], Thermo Fisher Scientific, [1]Basingstoke, UK, [2]Lenexa, USA

INTRODUCTION

Cattle have been identified as a major reservoir for Shiga toxin-producing *Escherichia coli* (STEC) and may contaminate food product during slaughter and processing. Raw or under-cooked beef products pose a risk to consumers if a robust screening and identification method is not applied.

Two unpaired studies evaluated performance of the Thermo Scientific™ SureTect™ Escherichia coli O157:H7 and STEC PCR assays for screening and identification of STEC from 25-gram and 375-gram beef samples.

METHODS

Study 1: Forty-five samples of diverse matrices (Figure 3) were artificially contaminated with 1-4 CFU/sample of STEC per method. Both the alternative and ISO reference method[1] tested 25-gram samples of beef meat.

Alternative method samples were enriched for eight hours in prewarmed BPW.

Study 2: Fifty-five samples, comprising 45 diverse meat samples and ten carcass swabs, were artificially contaminated with 1-2.6 CFU/sample of STEC per method. The alternative method evaluated 375-gram meat samples whereas the USDA MLG reference method tested 25-gram samples.

Alternative method samples were enriched for 8 hours (carcass swabs) or 10 hours (375-gram beef samples) in prewarmed mTSB.

Confirmation protocol: PCR results were confirmed using plating techniques specific to each method. The alternative method workflow utilises Thermo Scientific™ Oxoid™ Chromogenic Coliform Agar (CCA) to isolate STEC from food samples (Figure 1).
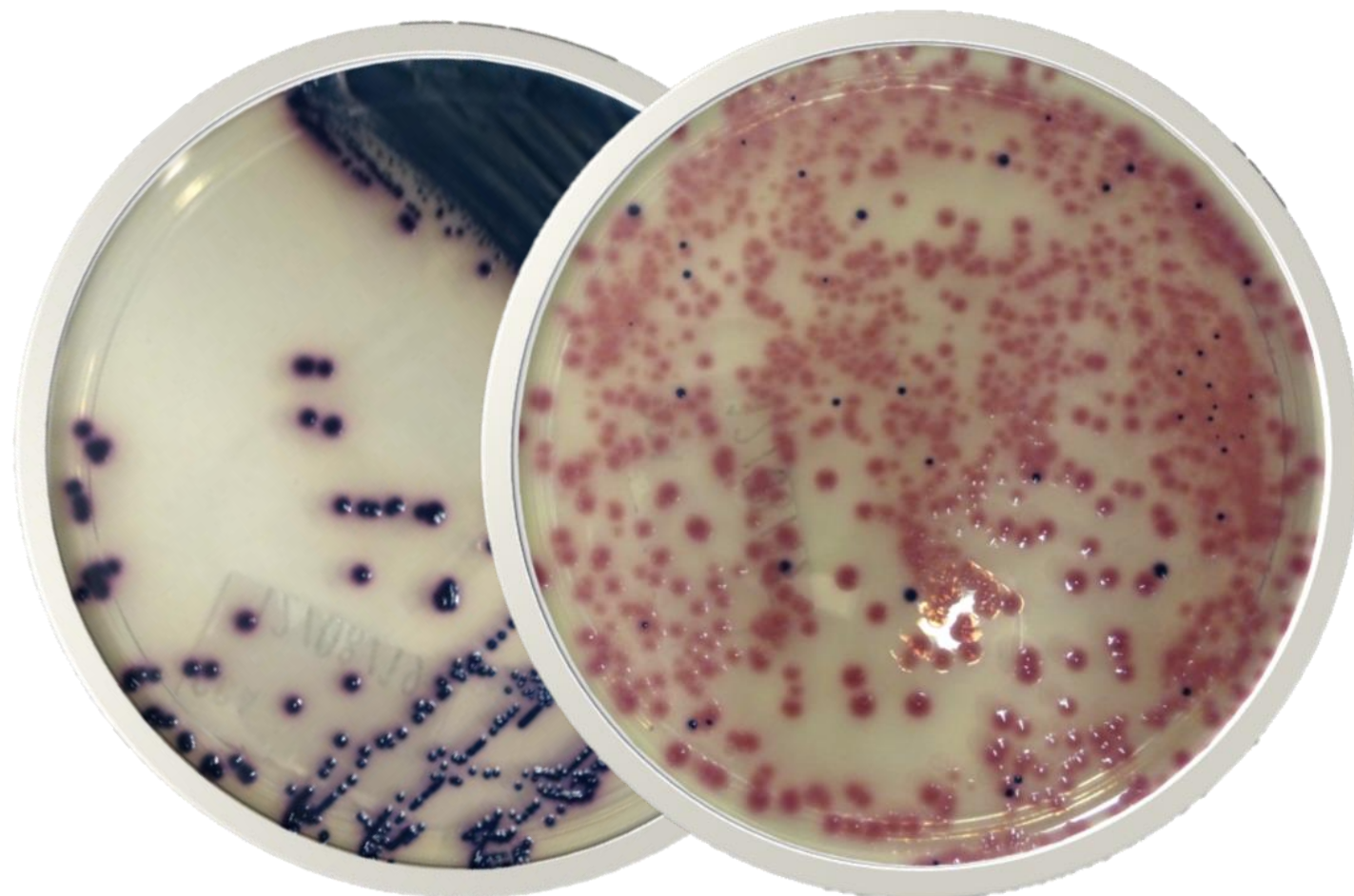
Figure 1: Typical *E. coli* growth on CCA (left) and in mixed culture with background flora (right)

CCA uses chromogenic compounds to differentiate *E. coli* (dark blue) from background flora (pink). Where background flora is present in very high numbers, immunomagnetic separation techniques are used to purify samples before plating.

RESULTS

Table 1: Method agreement between the alternative and reference methods for Studies 1 (25 g) and 2 (375 g)

	PA	NA	PD	ND
Study 1	7	24	11	3
Study 2	26	24	0	0

PA: Positive Agreement (candidate method positive, reference method positive)
NA: Negative Agreement (candidate method negative, reference method negative)
PD: Positive Deviation (candidate method positive, reference method negative)
ND: Negative Deviation (candidate method negative, reference method positive)

Study 1 demonstrated that the alternative method had superior performance for the screening and identification of *E. coli* O157:H7 and the top six non-O157:H7 serogroups from 25-gram beef samples than the ISO reference method (Figure 2). The improved performance is likely linked to the absence of novobiocin in the enrichment medium. Novobiocin is known to kill or inhibit growth of some isolates of *E. coli* which can negatively impact detection.

Study 2 demonstrated comparable performance between the alternative method for 375-gram samples and the USDA MLG reference method for 25-gram samples (Figure 2). It is not unexpected to see comparable results since the enrichment medium used in both methods for Study 2 was the same,.

Figure 3: Beef matrices tested include trim, ground, seasoned and frozen

CONCLUSIONS

Performance

- Improved performance vs. the ISO reference method
- Comparable performance with the USDA MLG reference method

Time to Result

- <10 hours for 25-gram beef samples and carcass swabs
- <12 hours for 375-gram beef samples

Simple Workflow

- No antibiotics included in enrichment
- Improved confirmation of positive samples with CCA

Harmonized Enrichment

- Enrichment conditions harmonized with the SureTect Salmonella species PCR Assay

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
2. USDA MLG 5C.00 Detection, Isolation and Identification of Top Seven Shiga Toxin-Producing Escherichia coli (STECs) from Meat Products and Carcass and Environmental Sponges

TRADEMARKS/LICENSING

ThermoFisher
SCIENTIFIC