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Improved Confirmation of STEC Contaminants Using the Thermo Scientific SureTect E. coli O157:H7 and STEC Screening and Identification PCR Assay

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INTRODUCTION

Microflora from food samples pose a challenge for shiga-toxin producing *Escherichia coli* (STEC) culture confirmation testing.

These studies assessed performance of the Thermo Scientific™ SureTect™ E. coli O157:H7 and STEC Screening and Identification PCR Workflow confirmation method using Thermo Scientific™ Ocoid™ Tryptone Bile X-Glucuronide Medium (TBX), Thermo Scientific™ Chromogenic Coliform Agar (CCA) and CHROMagar™ STEC.

MATERIALS AND METHODS

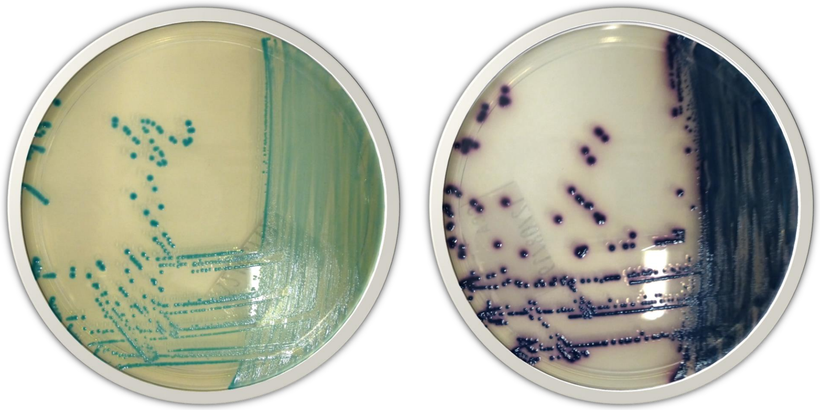
Pure Isolate Study

"Big Six" STEC pure isolates (n=38) were streaked onto CCA (Figure 1) and CHROMagar STEC and the inclusivity of both plating media compared.

Matrix Study

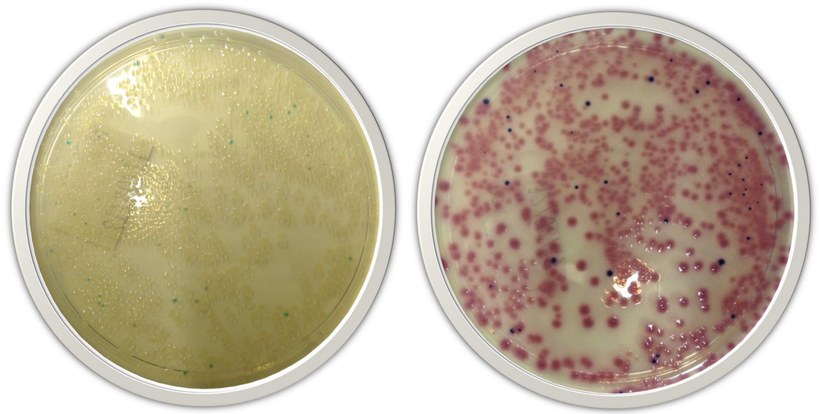
Ten vegetable samples, including sprouted seeds, were artificially contaminated with STEC at 0.67-1.79 CFU/25 g and enriched alongside unspiked samples with the same microflora. These were tested with the SureTect PCR kit and confirmed via the SureTect confirmation plating protocol (Figure 2).

Figure 1. Typical *E. coli* growth on TBX (left) and CCA (right)



TBX differentiates *E. coli* (green) from background (colourless). CCA uses chromogenic compounds to differentiate *E. coli* (dark blue) from background (pink).

Figure 2. Presumptive STEC with high levels of background flora from vegetables on TBX (left) and CCA (right)

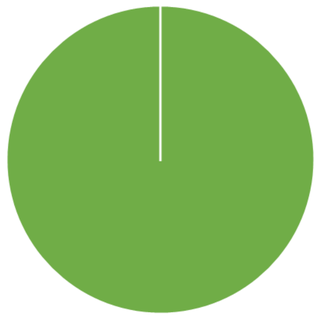


Isolating *E. coli* from CCA is simpler compared to TBX when background flora is present.

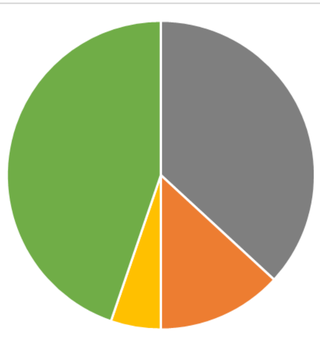
RESULTS

Figure 3. Comparative growth levels of STEC on:

a.) CCA – 38/38 isolates grew well



b.) CHROMagar –19/38 isolates had inhibited growth



Key:

- Growth to tertiary bed
- Growth to secondary bed
- Growth in primary bed only
- No growth observed

Pure Isolate Study

CCA facilitated typical morphology for 36 (94.7%) pure isolates, while CHROMagar exhibited this for 22 (57.9%). Two isolates grew strongly on CCA but had pink morphology rather than the expected blue.

CHROMagar failed to recover 14 STEC isolates and exhibited reduced growth levels for a further five (Figure 3).

Matrix Study

The SureTect method confirmed six STEC isolates despite high levels of background flora. Five positive samples were confirmed using a direct streak method on CCA while the final positive was confirmed using immunomagnetic separation and CCA. CHROMagar STEC had comparable performance to CCA.

TBX failed to confirm presence of STEC for three out of six confirmed positive samples.

CONCLUSIONS

Effective isolation of STEC relies upon the considered selection of confirmation plating media. These studies demonstrate the effectiveness of the SureTect confirmation plating method using CCA, enabling highly contaminated STEC samples to be confirmed rapidly and reliably.

TRADEMARKS/LICENSING

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