

ABSTRACT

Introduction: Shiga toxin-producing *Escherichia coli* (STEC) are adulterants in raw, non-intact beef according to FSIS and the FDA 2020 Leafy Greens Action Plan provides guidance to reduce STEC outbreaks across the USA. Beef products and produce require reliable screening techniques supportive of sampling strategies in these industries.

Purpose: This study tested 375 g test portions with an alternative method (Thermo Scientific™ SureTect™) for beef and produce compared to reference methodology.

Methods: Ground, frozen, and seasoned beef, beef carcass sponges (Micro Tally Swab), lettuce, frozen vegetables and cut cantaloupe were tested in 375 g portions. The alternative method was compared to 25 g reference methodology. Per matrix, 12-14 unpaired samples were artificially contaminated with 1-2 CFU STEC/test portion. All samples were confirmed with a modified version of the USDA/FSIS method (beef and swabs), and FDA/BAM method (produce). Presumptive colonies were isolated on chromogenic agar and tested directly with the PCR assay.

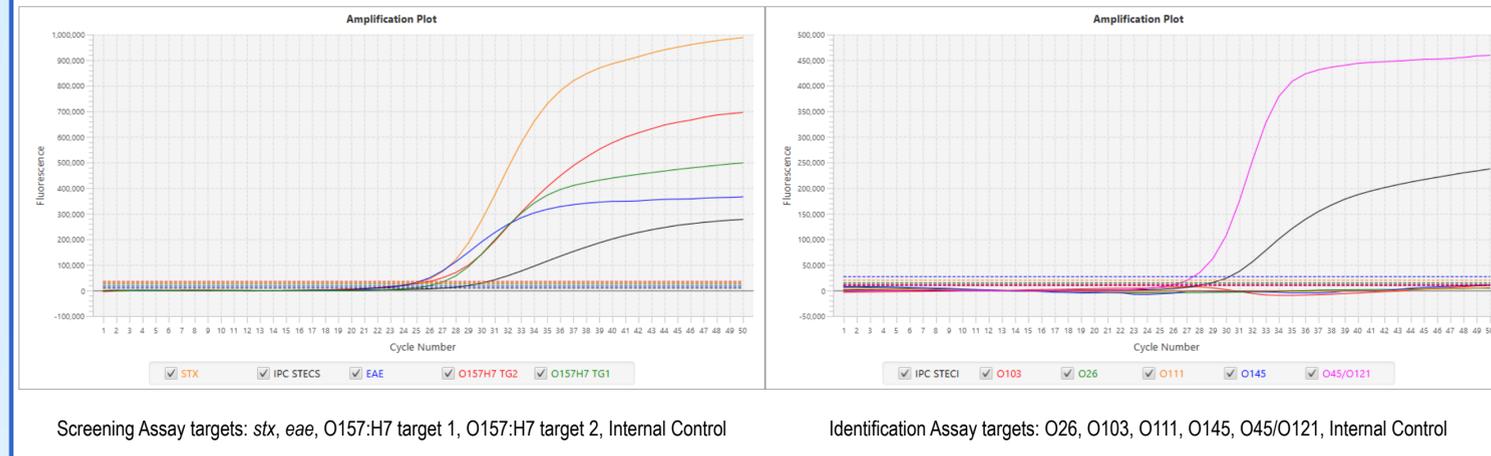
Results: The alternative method correctly detected *E. coli* O157:H7 and non-O157 STEC from all matrices after 8 hours incubation except for fresh raw ground beef which was successful after 10 hours incubation.

Significance: The alternative method for STEC detection is a suitable substitute for reference methodology for large samples of beef and produce. The workflow is simple and easy to conduct while increasing laboratory efficiency when compared to conducting the reference methods.



The alternative method SureTect kit contains pre-filled reagent tubes for 96 samples in one box with all components stored at 4-8°C. The PCR assays were run on the Applied Biosystems™ QuantStudio 5 thermal cycler using the Thermo Scientific™ RapidFinder™ Analysis Software.

RESULTS



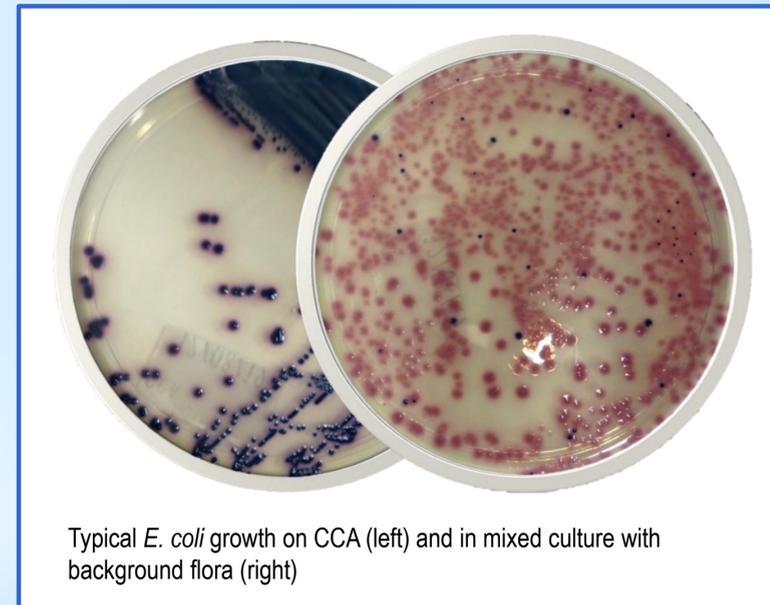
Matrix	PA	NA	PD	ND
Raw ground beef (8 h)	5	7	0	2
Raw ground beef (10 h)	7	7	0	0
Frozen beef	6	6	0	0
Seasoned beef	6	6	0	0
Beef carcass wipes	7	5	0	0
Iceberg lettuce	8	4	0	0
Mixed frozen vegetables	9	5	0	0
Cantaloupe	8	4	0	0

Method agreement data presented for 8 hour enrichments unless stated.

PA: Positive Agreement (alternative and reference method positive)
NA: Negative Agreement (alternative and reference method negative)

PD: Positive Deviation (alternative method positive, reference method negative)
ND: Negative Deviation (alternative method negative, reference method positive)

The confirmation workflow of the alternative method utilized Thermo Scientific™ Oxoid™ Chromogenic Coliform Agar (CCA) to differentiate *E. coli* (dark blue) from background flora (pink).



DISCUSSION

Deviations: The negative deviations for raw ground beef were attributed to the identification PCR assay only. Standard kit troubleshooting steps require a re-incubation and repeat test which corrected these deviations at 10 hours total enrichment.

Confirmation: Inclusion of chromogenic media correctly confirmed all PCR positive samples. Further confirmation was also applied to individual colonies by picking from plates applying the same PCR workflow.

CONCLUSIONS

Time to Result: The alternative method detected and identified STEC after 8 hours enrichment for all matrices (except raw ground beef).

Large Sample Testing: The alternative method for 375 g samples performed comparably to the reference method.

Simplicity: The alternative method workflow was simple and easy to conduct compared to the reference and other rapid methods; the workflow benefits from minimal handling steps, and harmonized lysis and PCR cycling parameters between screening and identification kits.