

Performance Comparison of Shiga Toxigenic *E. coli* (STEC) Multiplex Molecular Assays

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INTRODUCTION

Thermo Scientific™ SureTect™ *E. coli* O157:H7 and STEC Screening Assay and the Thermo Scientific™ SureTect™ STEC Identification PCR Assay (SureTect STEC Multiplex Assays; Figure 1) enable the simultaneous detection and differentiation of *E. coli* O157:H7 and the top six non-O157:H7 serogroups in two PCR reactions from raw beef, produce and dairy samples.

The purpose of the study was to compare the sensitivity and specificity of the SureTect STEC Multiplex Assays against a leading commercially available real-time PCR assay (alternative assay) designed to detect O157:H7 and the top six STEC O serogroups in raw beef samples.

Figure 1. Thermo Scientific SureTect STEC PCR System.



Left-to-right: Applied Biosystems™ SimpliAmp™ Thermal Cycler for sample prep, Applied Biosystems™ QuantStudio™ 5 Food Safety System, RapidFinder™ Analysis Software and SureTect STEC Multiplex Kits.

MATERIALS AND METHODS

For inclusivity and exclusivity testing, organism panels were prepared and tested according to Figures 2 and 3. Limit of detection (LOD) was performed according to Figure 4.

Samples were lysed prior to PCR and analyzed with SureTect STEC Multiplex Assays as detailed in the product's manual with equipment described in Figure 1. The alternative assay was performed on the same samples according to the manufacturers' instructions.

INCLUSIVITY WORKFLOW AND RESULTS

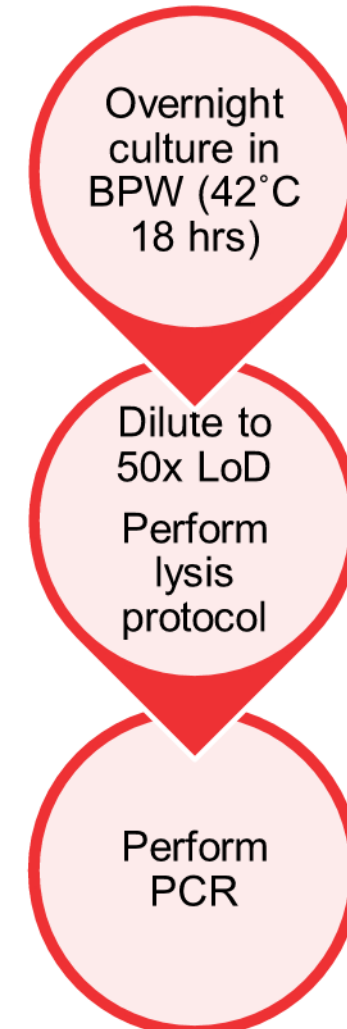


Figure 2: Inclusivity Workflow (left) and Isolate Panel (below)

<i>E. coli</i> serotype	No. strains
O103	15
O111	15
O121	13
O145	14
O157:H7	22
O26	15
O45	9
Total	103

Results Summary: Inclusivity Testing

All 103 different STEC isolates (Figure 2) were correctly identified by the SureTect STEC Multiplex Assays.

100%

The alternative assay failed to detect one isolate of O157:H7, giving a false negative result. A high number of false positive results was seen for the other serogroups for the alternative assay: O26 (11), O111 (3), O121 (7), O45 (28), O103 (17), O145 (5).

EXCLUSIVITY WORKFLOW AND RESULTS

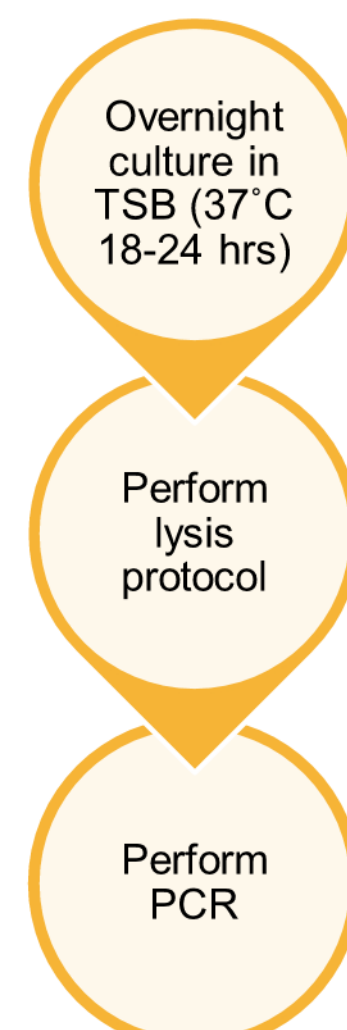


Figure 3: Exclusivity Workflow (left) and Isolate Panel (below)

Genus	No. strains
<i>Citrobacter</i>	24
<i>Enterobacter</i>	26
<i>Escherichia</i>	42
<i>Klebsiella</i>	4
<i>Salmonella</i>	3
<i>Shigella</i>	17
Total	115

Results Summary: Exclusivity Testing

The SureTect STEC Identification Assay and the alternative assay misidentified two isolates of *Enterobacter asburiae* as O45.

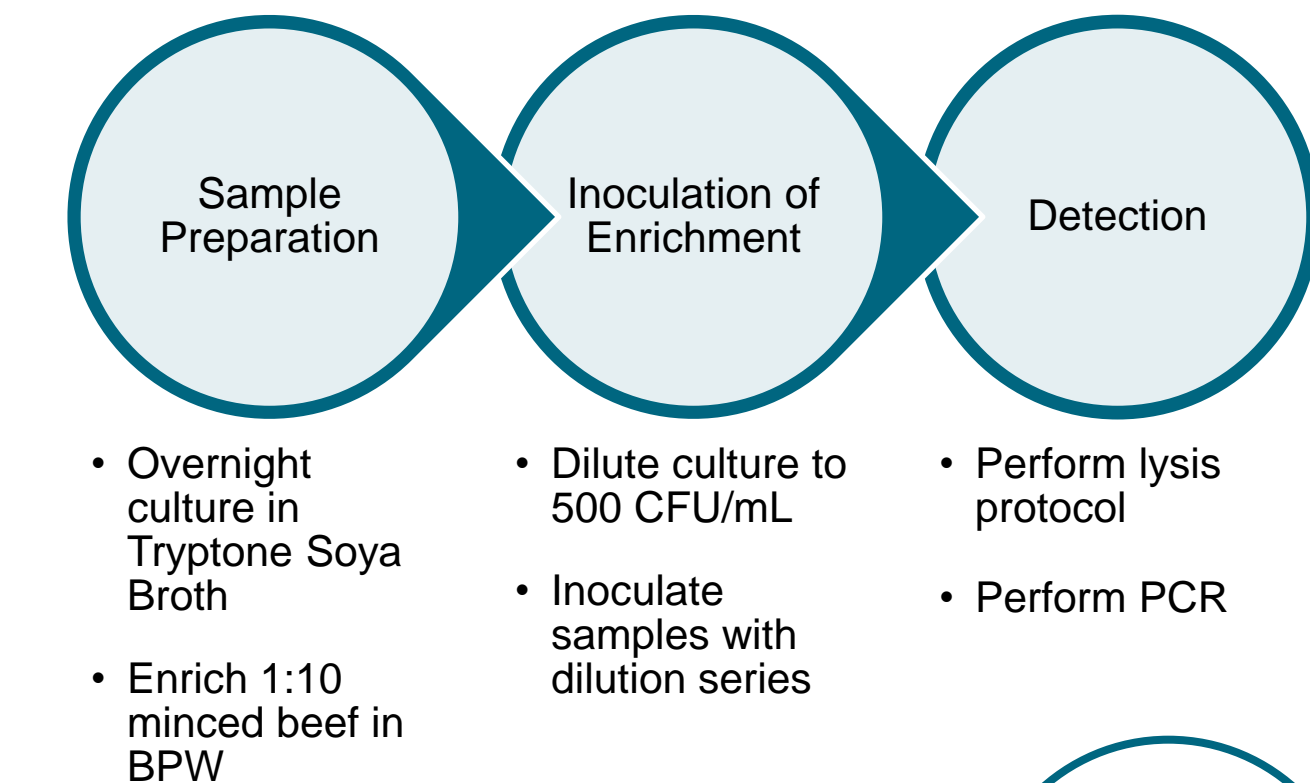
98%

The alternative assay gave a false positive O45 result for *Citrobacter werkmanii* and *Enterobacter ludwigii*.

The remaining 113 non-target strains (Figure 3) were correctly identified as negative on both methods.

LIMIT OF DETECTION WORKFLOW AND RESULTS

Figure 4: LOD Workflow



Results Summary: LOD Testing

SureTect STEC Multiplex Assays showed a LOD of $<10^4$ CFU/mL for O157:H7, O26, O111 and O121.

$<10^4$ CFU/mL

The alternative assay showed 5-10 times poorer sensitivity for O26 and O111 STEC serogroups.

CONCLUSION

The study demonstrated that the SureTect STEC Multiplex Assays are more specific and sensitive in detecting and differentiating of *E. coli* O157:H7 and the top six non-O157:H7 serogroups than the alternative assay.

TRADEMARK STATEMENT

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