
**Sensitivity**

Assay sensitivity in multiplex format (target + amplification control) was determined by using purified DNA isolated from *E. coli* O157:H7 strain EE1293. The appropriate range for sensitivity testing was determined by running multiple PCR reactions using 10-fold dilutions of purified DNA template. (See Figure 3) Sensitivity experiments were run with 64 PCR samples at 16 copies per reaction (2 users and 4 instruments). (See Figure 4) Of the 128 samples run, 122 were detected as positive, resulting in 95.3% success. The sensitivity of the multiplex assay or limit of detection (LOD) was determined to be 16 copies of target DNA.

**Specificity Evaluation**

*E. coli* O157:H7 LT assay specificity was evaluated against panels of purified nucleic acid and bacterial cultures grown from single colony isolates. A large portion of the purified nucleic acid was generously provided by Dr. John S. Bostock of the USDA Clay Center in Nebraska. Additional nucleic acid was purified from *Escherichia coli* ATCC and STEC stock cultures. Bacterial cultures were grown and tested by Marshfield Clinic in Marshfield Wisconsin under the direction of Dr. Roy Radcliff. A summary of the specificity testing can be found in Table 1. Samples tested from overnight culture processed using the standard RAPID LT FSS protocol for cell lysis.

Inclusively testing was performed against 340 different *E. coli* O157:H7 isolates. All isolates tested positive by real-time PCR and gave melting peaks within the range of 59.5°C to 62.2°C. One additional isolate (T66) tested positive by real-time PCR but melted as an O157:non H7; Serotyping showed this was incorrectly classified and was not *O157:H7*.

Exclusivity testing was performed on 171 non (*O157:H7*) *E. coli* isolates of various serotypes (See Table 2) and 54 non *E. coli* near-neighbor isolates (See Table 3). Positive O157:H7 test results were obtained for 4 of *2 non (O157:H7) isolates originally classified as O157:H-unknown (2) and O157:non-motive (2). These results suggest that these 4 isolates are genetically O157:H7. Additional experiments including sequencing and sequencing are needed to confirm this result.

Examples of the data obtained during the specificity testing is shown in Figure 5. The sensitivity and specificity testing data is shown in Table 3.

**Background**

The RAPID LT Food Security System (FSS) is a PCR-based pathogen detection method used to detect pathogens from enriched food samples. In general, the method involves enriching a sample for a specific amount of time in commercially available media, performing mechanical cell lysis to release the DNA, denaturation of PCR reagents, DNA amplification and melting peak analysis in the R.A.P.I.D. LT instrument, and automated data and results interpretation by the R.A.P.I.D. LT software.

This study was performed to evaluate the sensitivity and specificity of the recently developed *E. coli* O157:H7 RAPID LT FSS assay.

**Methods**

**Sensitivity**

Real-time PCR curves are shown for all of the 340 nm channel and b) the 705 nm channel. Corresponding melting peaks are shown for 340 nm at 64°C and 705 nm.

PCR amplification curves and post-PCR melt analysis data is shown for an experiment in which 15 different *E. coli* isolates were tested in duplicate. a) PCR Curves. b) Melting curves. c) Melting peaks

**Conclusions**

- Sensitivity for the *E. coli* O157:H7 LT assay was determined to be 16 copies per reaction at 95.3% success.
- Exclusivity testing was performed against 171 non (*O157:H7) *E. coli* isolates of various serotypes. These data suggest that this PCR-based assay is sensitive and specific for the detection of *E. coli* O157:H7 RAPID LT FSS assay.

**Figure 1:** Multiplex PCR detection schemes.

**Figure 2:** Non-PCR melt analysis schemes.

**Figure 3:** Sensitivity dilution series testing results.

**Figure 4:** Sensitivity testing results.

**Figure 5:** Sensitivity Testing of *E. coli* O157:H7 and O157:non H7 isolates from purified nucleic acid.

**Table 1.** Sensitivity Panel Testing Composition Summary

<table>
<thead>
<tr>
<th>Organism</th>
<th>Testing Type</th>
<th>Number of isolates tested (if corrected)</th>
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<tbody>
<tr>
<td>O157:H7 isolated</td>
<td>N = 114</td>
<td>N = 114</td>
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<tr>
<td>O157:H7 isolate</td>
<td>Exclusively</td>
<td>O157:H7 (50)</td>
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<tr>
<td>O157:non H7</td>
<td>N = 104</td>
<td>N = 104</td>
</tr>
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<td>O157:non H7 isolate</td>
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**Table 2.** Exclusivity Panel Non (*O157:H7) *E. coli* Isolates of Different O Serotypes

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**Table 3.** Specificity Panel Testing Composition Summary

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<td>O157:non H7 isolate</td>
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**Figure 6:** Specificity Testing of *E. coli* O157:H7 and O157:non H7 isolates from overnight culture.

PCR amplification curves and post-PCR melt analysis data is shown for an experiment in which 15 different *E. coli* isolates were tested in duplicate. a) PCR Curves. b) Melting curves. c) Melting peaks

**Specificity Testing**

Non (*O157:H7) isolates from overnight culture processed using the standard RAPID LT FSS protocol for cell lysis.

**Figure 7:** Specificity Testing of *E. coli* O157:H7 and O157:non H7 isolates from overnight culture.

PCR amplification curves and post-PCR melt analysis data is shown for an experiment performed at Marshfield Clinic using overnight cultures of various serotypes of *E. coli*. The PCR curve and melting peak for sample T66 (Blue) is shown overlapping with T76 (Orange) (E. coli O157:H7). T66 was originally classified as *O157:H7*, but gave a negative O157:H7 test result based on melting peak analysis. Additional melting peak testing showed this isolate to be O157:H2. (a) PCR Curves b) Melting curves c) Melting peaks

**Table 4.** Specificity Testing of *E. coli* O157:H7 and O157:non H7 isolates from overnight culture.

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**Figure 8:** Specificity Testing of *E. coli* O157:H7 and O157:non H7 isolates from overnight culture.