Evaluation of a New Multiplex PCR Assay for Detection of STEC from Meat Samples

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INTRODUCTION

Shiga toxin-producing Escherichia coli (STEC) are a group of pathogenic organisms that may cause severe disease including hemolytic uremic syndrome (HUS). STEC outbreaks have been linked to a number of food sources including meat and vegetables.

The Thermo Scientific™ SureTect™ STEC PCR Assay solution (candidate method) detects multiplex genetic targets for O157:H7 and other STEC from food and environmental samples. The SureTect STEC PCR Assay solution comprises two multiplex reactions for the simultaneous detection of the following targets:

- Screening Assay: O157:H7, stx, eae
- Identification Assay: O26, O103, O111, O145, O45, O121

This study summarizes the evaluated performance of the SureTect STEC PCR Assay solution (candidate method) for the detection of STEC from meats vs. the ISO 13136:2012 reference method.

METHODS

Three categories of meat samples (including beef, veal, pork and lamb) were divided into 25 g portions and artificially contaminated with a range STEC isolates from different serogroups (Table 1). The samples were then tested using the candidate method workflow (Figure 1) and associated instrumentation (Figure 2). A replicate set of samples was tested according to the ISO reference method.

Table 1. Contamination Levels

<table>
<thead>
<tr>
<th>Meat Subcategory</th>
<th>Spiked (N)</th>
<th>Spike Level (CFU)</th>
<th>Unspiked (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw</td>
<td>0.4 – 3.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seasoned</td>
<td>0.4 – 2.2</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Frozen</td>
<td>1.8 – 3.0</td>
<td>7</td>
<td>7</td>
</tr>
</tbody>
</table>

Table 2. Candidate Method Performance by Meats Subcategory

<table>
<thead>
<tr>
<th>Method Performance</th>
<th>Raw</th>
<th>Seasoned</th>
<th>Frozen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Agreement</td>
<td>2</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Negative Agreement</td>
<td>11</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>Positive Deviation</td>
<td>8</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Negative Deviation</td>
<td>0</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

RESULTS

The SureTect workflow utilises enrichment in BPW without the need for antibiotics or proprietary media. The SureTect method performed better than the ISO reference method (Figure 3), indicating that the candidate method performed better than the reference method.

RESULTS SUMMARY

The reference method returned fewer positive results from raw and seasoned meat than the candidate method. The methods performed comparably with frozen meats (Table 2).

The difference between the negative deviations and positive deviations was -9 (Figure 3), indicating that the candidate method performed better than the reference method.

Figure 1. SureTect STEC PCR Assay Process Flow

Figure 2: Applied Biosystems QuantStudio 5 Food Safety PCR System

Figure 3: Overall Candidate Method Performance with Meats

ACKNOWLEDGEMENTS

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REFERENCES

1. ISO/TS 13136:2012 Microbiology of food and animal feed – Real-time polymerase chain reaction (PCR) based methods for the detection of toxigenic pathogenic Escherichia coli (STEC) and the determination of O157, O111, O26, O103 and O145 serogroups

TRADEMARKS/LICENSING

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