An improved method for RNA extraction from carcass samples with low abundance of *Escherichia coli* O157:H7 for detection by Reverse-Transcription Polymerase Chain Reaction

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Rapid and sensitive detection of pathogenic organisms, such as *E. coli* O157:H7, is extremely important in the food safety industry. The United States Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) recently announced changes to their policy regarding *E. coli* O157:H7. These changes mean that the onus lies squarely with suppliers to the US to prove that the product is safe and free from *E. coli* O157:H7. A zero tolerance policy for the presence of the organism is currently in place (http://www.australianmeatsafety.com/newsupdates.html). To assist the livestock industries in complying with these regulations, a method for rapid detection of *E. coli* O157:H7, without culture-based pre-enrichment of carcass samples, was developed. A number of innovative PCR-based strategies to detect *E. coli* O157:H7 have been developed previously. All these methods detect genomic DNA, and PCR analysis will be positive, even if there are only dead or viable-but-non-culturable (VNBC) cells present (Botero et al. 2005). This might lead to an overestimation of the numbers of the pathogen present or false-positive results. In order to detect viable bacterial cells, McIngvale et al. (2002) and Yaron and Matthews (2002) developed reverse-transcriptase PCR (RT-PCR) assays with a detection limit of 10⁶-10⁷ CFU/mL. These methods are based on the reverse-transcription of mRNA into cDNA, which then serves as a template for the PCR reaction, thus detecting actively growing cells. However, commonly used RNA extraction procedures are limited in their ability to effectively yield RNA from cell numbers lower than 10⁶-10⁷ CFU/mL. There are commercial methods available to extract RNA from smaller numbers of cells, but the cost of these methods is prohibitive for large scale applications. We therefore developed a method to improve the recovery of RNA from samples and thus improve sensitivity of detection.

RNA was extracted from carcass liquor after swabbing a leg of lamb, purchased from a local butchery. To protect the RNA, all samples were initially preserved in 1/5 volume of 5% phenol (pH 4.3)/95% ethanol solution, after which the samples were centrifuged and the supernatant removed. RNA was extracted from the cells by resuspending the pellet in TE/1% SDS buffer, followed by the addition of an equal volume of phenol/chloroform. The cells were subjected to bead beating for 2 minutes, followed by centrifugation at 14000 g for 2 min. The aqueous phase was recovered and the RNA extracted by spin column (Qiagen, Hilden, Germany). To improve total RNA recovery from samples with low abundance of bacteria, 2x10⁸ CFU/mL non-target bacterial cells were added to the samples. In all cases a serial dilution of *E. coli* O157:H7 cells (10⁶–10⁷ CFU/mL) was added to these samples for determination of detection limits in duplicate. The quality and quantity of RNA was determined by A260/A280 readings. Reverse Transcription-PCR was performed to detect the constitutively expressed rfbE gene using the primer set described by Fortin et al. (2001). Following amplification, the RT-PCR products were visualised on a 1.5% (w/v) agarose gel with ethidium bromide.

Using conventional methods, RNA could not be successfully extracted from cell numbers below 10⁶ CFU/mL or from the carcass liquor; however the addition of non-target cells resulted in recoverable RNA without interfering with target detection using an RT-PCR assay. With this method it was possible to detect the *rfbE* gene at levels of 6.3x10⁶ and 2.6x10⁷ CFU/mL, respectively, for cultures and carcass liquor.

**Table 1.** Detection levels of *E. coli* O157:H7 in culture and in carcass liquor.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Detection level*</th>
<th>ng RNA/μL*</th>
<th>260/280*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em> O157</td>
<td>6.3x10⁶</td>
<td>568.72</td>
<td>2.17</td>
</tr>
<tr>
<td><em>E. coli</em> O157 + non-target bacteria</td>
<td>6.3x10⁶</td>
<td>369.63</td>
<td>2.17</td>
</tr>
<tr>
<td>Carcass liquor</td>
<td>No RNA</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Carcass liquor + <em>E. coli</em> O157 + non-target bacteria</td>
<td>2.6x10⁷</td>
<td>207.43</td>
<td>1.98</td>
</tr>
</tbody>
</table>

* Mean of duplicate samples  
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