Method Development for Enumeration of *E. coli* O157:H7 for Ground Beef, Hides, Carcasses and Feces of Cattle

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Method Development for Enumeration of *E. coli* O157:H7 for Ground Beef, Hides, Carcasses and Feces of Cattle: Project Summary

**Background**
Methodologies for the assessment of microbial pathogen load, at various steps in the beef production process, are lacking. In order to quantify the risks associated with the slaughter of animals that may harbor or shed *E. coli* O157:H7 or *Salmonella* spp., accurate estimates of the prevalence and frequency of distribution of these pathogens and their relative concentration on hides and in feces is needed. Another aspect of the need for enumeration methods is the evaluation of intervention methods. The complete picture of pathogen contamination on carcasses cannot be understood with data only relating to the presence or absence of various pathogens. Comparisons of in-plant antimicrobial interventions have traditionally been based on prevalence data alone. Yet, interventions that do not completely eliminate pathogens can still be very effective if they are significantly reducing the pathogen load on hides or carcasses. Beef processors need to know the levels of pathogens entering their plants and have the ability to quantify these levels throughout processing, in order to have greater control of their process.

At present, the majority of pathogen enumeration experiments, which are few in number, are conducted using the most probable number (MPN) dilution technique. This method is an indirect measure and provides an estimate of the number of organisms present in a sample. The method was initially developed for the purpose of determining the number of viable bacteria in a sample. One of the drawbacks encountered when this method is used for the enumeration of a particular pathogen is the competition between the target organism to be enumerated and the background microflora of the sample. If the target organism is out-competed, attempts to quantify it are inaccurate.

Enumeration methods based on direct plating have the advantage of providing a direct measure of viable bacterial counts, and can be performed on selective media, so as to decrease the presence of competing microflora.

The objective of this study was to develop a highly accurate method of enumerating *E. coli* O157:H7 at all stages of beef production.

**Methodology**
Two methods for the direct enumeration of *E. coli* O157:H7 and *Salmonella* spp were evaluated in this study. The first involves the use of the spiral plate count method (SPCM) (Gilchrist, J.E., et. al., 1973; Robinson, S.E., et. al., 2004) for the enumeration of *E. coli* O157:H7 and *Salmonella* spp. from cattle hide and fecal samples (samples expected to have high levels of the target organisms). The second method involves the use of hydrophobic grid membrane filtration (HGMF) (Sharpe & Michaud, 1975; Entis, P. et. al., 1982; Szabo, R., et. al., 1990; Blackburn & McCarthy, 2000; Sharpe, A.N., et. al., 2000; Robinson, S.E., et al., 2004) for the enumeration of *E. coli* O157:H7 and *Salmonella* spp. from carcass and ground beef samples (samples expected to have low levels of the target organisms). While these bacterial enumeration methods are themselves not novel, the application and the types of selective media used for these analyses, represent a new approach to pathogen enumeration in the beef production process.
These methods were developed and refined on a number of inoculated and naturally contaminated samples of all four types. The final methods were tested on the following four types of samples:

<table>
<thead>
<tr>
<th>Type of Sample</th>
<th>Expected Level of Pathogens</th>
<th>Enumeration Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fecal (n=3,190)</td>
<td>High</td>
<td>SPCM</td>
</tr>
<tr>
<td>Hide (n=2,280)</td>
<td>High</td>
<td>SPCM</td>
</tr>
<tr>
<td>Pre-evisceration carcass (n=760)</td>
<td>Low</td>
<td>HGMF</td>
</tr>
<tr>
<td>Ground beef (n=609)</td>
<td>Low</td>
<td>HGMF</td>
</tr>
</tbody>
</table>

All samples positive for a pathogen were naturally contaminated (i.e., none were inoculated). The hide and carcass samples came from commercial processing plants from July 2005 to January 2006.

**Findings**

In preliminary development, MPN analysis of feces, hide, carcass and ground beef samples worked well for spiked samples. Analysis of naturally contaminated samples, however, was highly inconsistent and so the use of this method was discontinued.

Spiral plate analysis for the enumeration of *E. coli* O157:H7 from naturally contaminated hide samples showed that contamination levels on hide varied by season. For Summer, 55.7% (423/760) were enrichment positive, and of those 10.4% (44/423) were enumeration positive. In the Fall, 38.7% (294/760) were enrichment positive, and 1.02% (3/294) of those were enumeration positive. In the Winter 50.4% (383/760) were enrichment positive and 7.3% (28/383) of those were enumeration positive. For *Salmonella* spp. the levels also varied by season and were slightly higher than those for *E. coli* O157:H7. *Salmonella* spp. in the Summer was 93.7% (712/760) positive for enrichment and 18.4% (131/712) of those were enumeration positive. In the Fall, 88.4% (672/760) were enrichment positive and 11.01% (74/672) of those were enumeration positive. And in Winter, 89.9% (683/760) were enrichment positive and 23.7% (162/683) of those were enumeration positive.

Spiral plate analysis of fecal samples naturally contaminated with *E. coli* O157:H7 was performed for 3,190 samples. Of these, 16.7% (532/3,190) were positive by enrichment and of those 22.9% (122/532) were positive for enumeration.

HGMF analysis of naturally contaminated pre-evisceration carcass samples for the enumeration of *E. coli* O157:H7 showed that contamination of carcasses was generally low. Of the 760 carcasses evaluated, 19.5% (148/760) were positive by enrichment and of those 16.2% (24/148) were enumeration positive. For *Salmonella* spp., levels again were higher than for *E. coli* O157:H7. Of the 760 pre-evisceration carcass samples evaluated, 55.5% (422/760) were positive by enrichment. Of those, 23.5% (99/422) were positive for enumeration.

HGMF analysis of 609 ground beef samples for the prevalence of *Salmonella* spp. showed 2.8% (17/609) to be positive by enrichment and of these, only one sample was positive for enumeration (2.0 cfu/g).
Those samples that were enrichment positive, but not enumeration positive had pathogen levels below the limit of detection.

Table 1: Summary of Enumeration Results

<table>
<thead>
<tr>
<th>Organism</th>
<th>Sample type</th>
<th>Enumeration method</th>
<th>n</th>
<th>Enrichment positive</th>
<th>Enumeration positive</th>
<th>Range cfu/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>Hide</td>
<td>SPCM</td>
<td>2280</td>
<td>1100</td>
<td>75</td>
<td>0.4-98</td>
</tr>
<tr>
<td>O157:H7</td>
<td>Carcass</td>
<td>HGMF</td>
<td>760</td>
<td>148</td>
<td>24</td>
<td>0.008-0.46</td>
</tr>
<tr>
<td></td>
<td>Feces</td>
<td>SPCM</td>
<td>3190</td>
<td>532</td>
<td>122</td>
<td>1.26x10^2-5.7x10^6</td>
</tr>
<tr>
<td><em>Salmonella</em> spp.</td>
<td>Hide</td>
<td>SPCM</td>
<td>2281</td>
<td>2067</td>
<td>367</td>
<td>0.4-343.2</td>
</tr>
<tr>
<td></td>
<td>Carcass</td>
<td>HGMF</td>
<td>760</td>
<td>422</td>
<td>99</td>
<td>0.005-3.86</td>
</tr>
<tr>
<td></td>
<td>Ground Beef</td>
<td>HGMF</td>
<td>609</td>
<td>17</td>
<td>1</td>
<td>2 cfu/g</td>
</tr>
</tbody>
</table>

Detection Limits and Cost Per Sample.

SPCM of feces or hide samples:

- **Limits of detection:**
  - Fecal = 200 CFU/g (can be modified to lower the limit to 40 cfu/g)
  - Hide = 0.4 CFU/cm² based on sampling area of 1000 cm².

- **Cost:**
  - *E. coli* O157:H7 = $0.92 per sample
  - *Salmonella* = $0.50 per sample

HGMF of ground beef samples

- **Limits of detection:**
  - At 3 ml of sample = 10 CFU/g
  - At 7 ml of sample = 2 CFU/g

- **Cost:**
  - *E. coli* O157:H7 = $2.11 per sample
  - *Salmonella* = $1.69 per sample

HGMF of carcass samples

- **Limits of Detection:**
  - At 500 µl of sample = 1 cfu/200 cm² (0.005 cfu/cm²)
  - At 300 µl of sample = 1 cfu/120 cm² (0.008 cfu/cm²)

- **Cost:**
  - *E. coli* O157:H7 = $2.11 per sample
  - *Salmonella* = $1.69 per sample
Implications
From this study, two methods for the enumeration of *E. coli* O157:H7 and *Salmonella* spp. from various types of samples were developed. The spiral plate count method is for samples (feces and hides) with high levels of pathogens and a hydrophobic grid membrane filtration method for samples (carcasses and ground beef) with low levels of pathogens, if present. These methods provide greater information about pathogen contamination, to enable processors to monitor their harvest process and ensure that it is under control. Furthermore, these methods will be extremely useful to the research community.

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