Research Note

A New Technique for *Escherichia coli* Testing of Beef and Pork Carcasses

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ABSTRACT

A novel technique has been developed to monitor *Escherichia coli* contamination on carcasses using membrane filtration and m-ColiBlue24 (mCB). mCB is a membrane filtration medium that simultaneously detects total coliforms and *E. coli* (EC) in a period of 24 ± 4 h. A study was conducted, using a sponge method to obtain samples from pork carcasses and the excision technique to remove samples from beef carcasses, that compared mCB to standard methods. On pork carcasses (n = 77), the mean values for mCB and violet red bile agar were 7.4 CFU/15 cm² and 6.1 CFU/15 cm², respectively. The paired t test (P > 0.05) indicated no significant difference between the two methods (t = 0.5; P = 0.6). Samples from beef carcasses (n = 57) were used to compare mCB to both coliform count and EC Petrifilm. Of these samples, 27 were artificially inoculated with cattle manure. The mean total coliform count was 4.2 log CFU/cm² and 4.0 log CFU/cm² on mCB and coliform count Petrifilm, respectively. The mean EC count on mCB was 4.0 log CFU/cm² and 3.5 log CFU/cm² on EC Petrifilm. When comparing mCB to both coliform count (t = 2.4; P = 0.02) and EC (t = 3.5; P < 0.01) Petrifilm, paired t tests (P ≤ 0.05) indicated significant differences.

In the food industry, the use of indicator organisms is important to maintain the quality and ensure food safety (10, 15). The term “indicator organism” is vague and can be associated with a wide variety of applications. Indicator organisms can be used to determine the amount of fecal contamination, quality of a product, and the efficacy of a process (22).

The information generated from indicator organisms can help maintain food quality by providing a better understanding of processing conditions. This information can be used by food companies to increase the efficacy of processes that increase shelf life by minimizing spoilage organisms within a product (7, 15, 28). In this sense, the indicator organism represents more of a measurement of quality, not of fecal contamination (4, 22).

Furthermore, the great number and diversity of microbes makes it difficult to test for every pathogen (10, 15). Thus, the preferred method to estimate the possibility of pathogens being present is through the detection of indicator organisms. This can be accomplished by analyzing samples for coliforms associated with fecal contamination (22). Indicator organisms, in this instance, represent a quantitative measurement of microbes of public health significance. Many times this measurement will be referred to as an index of fecal contamination or pollution (4). This index can provide a means of assessing the amount of potential human health risk associated with a product (8, 15).

Despite all efforts to assess the microbial safety of products, there continues to be an increase in the number of cases of foodborne disease associated with newly emerging and reemerging pathogens (6). This constant increase has resulted in the recent development and implementation of the Pathogen Reduction Act by the U.S. Department of Agriculture Food Safety and Inspection Service (USDA-FSIS). This program includes the use of hazard analysis critical control point (HACCP) to eliminate, reduce, and prevent hazards associated with a product. In addition to HACCP, the USDA-FSIS mandate requires *Escherichia coli* testing in federally inspected and official state inspected meat processing plants, to monitor surface contamination of beef, pork, and poultry carcasses.

A novel technique has been developed to monitor *E. coli* contamination on carcasses through membrane filtration (MF) and m-ColiBlue24 (mCB). mCB simultaneously detects *E. coli* (EC) and total coliforms (TC) in a single 24-h incubation (2). On this medium, the EC colonies appear blue, and the other TC colonies are red. Studies were conducted on pork and beef carcasses to compare mCB to violet red bile (VRB) agar and Petrifilm.

MATERIALS AND METHODS

Pork carcass samples. The samples were taken from 77 pork carcasses at commercial processing facilities in the Midwest. The sampling was carried out using sterile sponges according to the procedure described by the USDA-FSIS (30). Just prior to the
samples being placed in a stomacher (Tekmar Co., Cincinnati, Ohio) for 2 min, 20 ml of buffered peptone water was added to the samples. The homogenate (1 ml) was poured plated in VRB (Difco, Detroit, Mich.) with VRB overlay. The pour plating procedure was performed as described in Hitchins et al. (14). In addition, 1 ml of the homogenate was added to 10 ml of buffered peptone water and filtered through a 0.45-μm GN-Metricel filter (Gelman, Ann Arbor, Mich.). The filter was placed on an absorbent pad saturated in 2 ml of mCB (Hach, Loveland, Colo.) and incubated at 35°C for 24 ± 4 hr. This procedure was also performed with duplicate plates.

**Beef carcass samples.** Samples were taken from 57 beef carcasses at a commercial facility in the Southwest. A 30-cm² sample was taken from the flank of each carcass using the excision method (30). Samples were stomached (Tekmar) for 2 min with 100 ml of buffer peptone water, and the appropriate dilutions were made. To further evaluate mCB in reference to other commonly used microbiological methods, populations of coliforms and E. coli were enumerated on PetriFilm. One milliliter was placed on coliform count (CC) Petri/lm (3M Health Care, St. Paul, Minn.) and EC Petrifilm (3M Health Care) and was filtered and placed on mCB as described above. The procedure for the Petrifilm was performed according to manufacturer’s instructions. The samples run in this study were part of an in-plant study on interventions. Some of the samples (n = 27) were intentionally contaminated with cattle manure. The manure was diluted (1/4) with sterile distilled water and brushed onto the sample.

**Statistical analysis.** The statistical analysis of the pork and beef samples was performed by SAS (Statistical Analysis Systems Institute, Cary, N.C.) at Iowa State University. A paired t test was also used to compare TC and EC counts from mCB to CC and EC Petri/lm from 77 pork samples. A paired t test was used to compare TC counts between mCB and VRB from 77 pork samples. The statistical analysis of the 57 beef carcasses is shown in Table 3. The average presumptive TC count was 4.2 log CFU/cm² on mCB and 4.0 log CFU/cm² on CC Petrifilm.

**RESULTS AND DISCUSSION**

**mCB.** A new membrane filtration medium, m-ColiBlue24, simultaneously detects total coliforms and E. coli in a single 24-h incubation (2). Differentiation of colonies is based on the reduction of triphenyltetrazolium chloride by all members of the coliform group. This causes total coliform colonies to turn red. E. coli is differentiated from other coliforms through the cleavage of 5-bromo-4-chloro-3-indolyl-β-d-glucuronide (BCIG) by the glucuronidase enzyme. This enzymatic cleavage of BCIG causes E. coli colonies to turn blue (11-13). Unlike other membrane filtration media, mCB does not require the filter to be transferred to another selective medium or incubation temperature (9, 11). The differentiation of coliform colonies is based on a color reaction and does not require a UV lamp to distinguish the different colonies, like other glucuronidase assays (11-13).

mCB was designed to be used as a membrane filtration method. Membrane filtration allows larger volumes of sample to be processed, increases sensitivity (3, 21, 24, 25, 27), prevents thermal injury or death from liquid agar (19), and removes or dilutes inhibitory substances such as artificial preservatives or polyphenols (7, 26, 27).

**TABLE 1.** Statistical analysis of presumptive total coliform populations from pork carcasses

<table>
<thead>
<tr>
<th>Method</th>
<th>n</th>
<th>Mean (CFU/15 cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mCB</td>
<td>77</td>
<td>7.4</td>
</tr>
<tr>
<td>VRB</td>
<td>77</td>
<td>6.1</td>
</tr>
<tr>
<td>Comparison</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mCB vs. VRB</td>
<td>0.52</td>
<td>&gt; t</td>
</tr>
</tbody>
</table>

**TABLE 2.** Presumptive E. coli recovered from pork carcasses using mCB

<table>
<thead>
<tr>
<th>n</th>
<th>E. coli recovered (CFU/15 cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>Undetectable</td>
</tr>
<tr>
<td>7</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>21</td>
</tr>
<tr>
<td>1</td>
<td>53</td>
</tr>
</tbody>
</table>

**Pork carcass samples.** mCB has initially compared well with some of the standard methods for food analysis. In the first part of this study, mCB was compared to VRB agar for the enumeration of coliforms from pork carcasses. Table 1 shows the statistical analysis of the samples taken from 77 pork carcasses. The mean value of the TC count obtained from the carcasses was 7.4 and 6.1 CFU/15 cm² on mCB and VRB, respectively. When comparing presumptive TC counts of mCB to VRB, the paired t test value was 0.52 and the probability of a larger t (P) was 0.61. These results suggest no statistical difference between the two methods.

Table 2 represents the various levels of presumptive E. coli recovered by mCB. From the 77 samples, 60 had undetectable levels of E. coli. The other 17 samples had counts ranging from 1 to 53 CFU/15 cm². When examining the ability of mCB to detect E. coli in water, Grant found a 100% sensitivity, 97.7% specificity, 2.5% false positive error, and 0% undetected target error associated with the medium (11-13).

**Beef carcass samples.** In the second part of this study, mCB was compared to Petrifilm for the enumeration of TC and EC from beef carcasses. Petrifilm has already proven to be an effective method for coliform analysis for many types of foods. It has the approval of the Association of Analytical Chemists International and has been recognized in several countries as an effective method for coliform analysis (3). CC and EC Petrifilm has been found to compare favorably to many other methods. Coliform counts obtained by using CC and EC Petrifilm have been shown to compare favorably to VRB, VRB with 4-methylumbellifer-yl-β-d-glucuronide, and the more probable number technique for both TC and EC (5, 16-18, 20, 23).

Statistical analysis of the 57 beef carcasses is shown in Table 3. The average presumptive TC count was 4.2 log CFU/cm² on mCB and 4.0 log CFU/cm² on CC Petrifilm.
The value obtained from the paired $t$ test suggests a significant difference between mCB and CC Petrifilm when enumerating TC.

The comparison of mCB to EC Petrifilm indicated a paired $t$ test value of 3.5 ($P < 0.01$). The mean value of presumptive $E. coli$ from mCB and EC Petrifilm was 4.0 log CFU/cm$^2$ and 3.5 log CFU/cm$^2$, respectively. Presumptive $E. coli$ was recovered from 39 of the carcasses by mCB and from 31 carcasses by EC Petrifilm. Of the 57 samples, mCB detected $E. coli$ from 11 samples that were not detected by EC Petrifilm. Conversely, EC Petrifilm found $E. coli$ in five samples that were not found by mCB.

As mentioned in Materials and Methods, 27 of the beef samples were intentionally contaminated with cattle manure. It was decided that these samples would not be separated into a separate subgroup but would be analyzed as part of the noninoculated beef samples. This was done to see how both methods responded to samples with possible heavy contamination. A paired $t$ test was used to offset any variation that might occur because of this.

mCB recovered $E. coli$ from eight more carcasses than EC Petrifilm. In addition, mCB detected $E. coli$ on 11 samples in which no $E. coli$ were detected by EC Petrifilm, although the actual populations detected in these 11 samples were very low. EC Petrifilm was reported to have a greater sensitivity than the more probable number technique when counts ranged higher than 10 cells/g (16). Restaino and Lyon (23) pointed out that Petrifilm detected >40% more coliforms per gram from frozen raw ground meat than did VRB. Some of the observed differences in the present study are undoubtedly a result of the inherent differences in methodology between membrane filtration and direct plating. Future studies need to be performed to see what effect membrane filtration has on the sensitivity of mCB.

### TABLE 3. Statistical analysis of presumptive total coliform and $E. coli$ populations from beef carcasses

<table>
<thead>
<tr>
<th>Method</th>
<th>$n$</th>
<th>Mean (log CFU/cm$^2$)</th>
<th>Comparison</th>
<th>Paired $t$ test</th>
<th>Probability &gt; $t$</th>
</tr>
</thead>
<tbody>
<tr>
<td>mCB (total coliforms)</td>
<td>57</td>
<td>4.2</td>
<td>mCB vs. CC Petrifilm</td>
<td>2.4</td>
<td>0.02</td>
</tr>
<tr>
<td>CC Petrifilm (total coliforms)</td>
<td>57</td>
<td>4.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mCB ($E. coli$)</td>
<td>57</td>
<td>4.0</td>
<td>mCB vs. EC Petrifilm</td>
<td>3.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>EC Petrifilm ($E. coli$)</td>
<td>57</td>
<td>3.5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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### CONCLUSION

mCB was designed to be a straightforward method for detecting both TC and EC in a single 24-h incubation. It was originally designed for routine examination of drinking water but has initially compared well with some of the standard methods for microbial analysis of food (1). This study indicated no significant difference ($P > 0.05$) between mCB and VRB agar when testing pork carcasses. On the beef carcasses, there was a significant difference ($P \leq 0.05$) between mCB and both TC and EC Petrifilm. mCB recovered a larger number of TC and EC from the beef carcasses.

### ACKNOWLEDGMENTS

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### REFERENCES

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