Use of Commercial Enzyme Immunoassays and Immunomagnetic Separation Systems for Detecting *Escherichia coli* O157 in Bovine Fecal Samples

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A commercial enzyme immunoassay (EIA) (*E. coli* O157 Visual Immunoassay; Tecra Diagnostics) performed on enrichment cultures in modified *Escherichia coli* broth (mECn) was compared with immunomagnetic separation (IMS) (Dynabeads anti-*E. coli* O157; Dynal) performed on enrichment cultures in modified buffered peptone water (BPW-VCC) for the detection of *E. coli* O157 in bovine fecal samples. Tests on fecal suspensions inoculated with each of 12 different strains of *E. coli* O157 showed that both the EIA and IMS methods were more sensitive than direct culture or enrichment subculture methods for detection of the organism. EIA and IMS were then compared for detection of *E. coli* O157 in bovine rectal swabs. For confirmation of positive EIA tests, a commercial system (Immunocapture System [ICS]; Tecra Diagnostics) was compared with IMS; both were performed on mECn enrichment cultures. Of 200 rectal swabs examined, 17 gave positive results in the EIA which were confirmed by both confirmation systems, 2 gave positive results in the EIA which were confirmed by IMS but not by ICS, and 1 gave a positive result in the EIA which was not confirmed by either IMS method. Further examinations of samples positive in the EIA but negative by either confirmation system further confirmed the use of a previously described IMS technique (33), the use of a commercial enzyme immunoassay (Visual EIA; Tecra Diagnostics) and a commercial culture confirmation system (Immunocapture System [ICS]; Tecra Diagnostics) for detecting *E. coli* O157 in bovine fecal samples.

**MATERIALS AND METHODS**

**Media and reagents used.** Media used were the following: (i) MRD—maximum-recovery diluent (CM733; Oxoid, Basingstoke, United Kingdom); (ii) BPW-VCC (9)—buffered peptone water (CM509; Oxoid) supplemented with vancomycin (8 mg/liter), cefoxitin (0.85 mg/liter), and cefadolin (10 mg/liter); (iii) mECn—modified *E. coli* broth, consisting of 2% (wt/vol) tryptone, 0.112% (wt/vol) bile salts no. 3, 0.5% (wt/vol) lactose, 0.4% (wt/vol) *K*₂*HPO₄*, 0.15% (wt/vol) *KH*₂*PO₄*, and 0.5% (wt/vol) *NaCl* (pH 6.9); with novobiocin (20 mg/liter) added after sterilization; and (iv) CT-SMAC (34)—SMAC agar (CM813; Oxoid) supplemented with cefixime (0.05 mg/liter) and potassium tellurite (2.5 mg/liter). Magnetic beads coated with an antibody against *E. coli* O157 (Dynabeads anti-*E. coli* O157) were supplied by Dynal UK Ltd. Visual EIA kits and ICS kits were supplied by Tecra Diagnostics. The EIA uses enrichment culture in mECn prior to heat treatment and detection of O157 antigen by a standard antibody-based EIA. The ICS uses an antibody-coated polystyrene paddle, wash solution, replication medium, and selective culture on CT-SMAC.

**Comparison of sensitivity of methods.** Twelve different strains of *E. coli* O157 previously isolated from bovine feces (9, 11, 12) were each grown overnight at 37°C in nutrient broth, and CFU per milliliter were estimated by a standard serial-dilution method. The strains used were P1394 O157:H*VT*₂, P1446 O157:H*VT*₂, P1450 O157:H*VT*₂, P1456 O157:H*VT*₂, P1461 O157:H*VT*₂, P1462 O157:H*VT*₂, P1508 O157:H*VT*₂, P1512 O157:H*VT*₂, P1513 O157:H*VT*₂, P1515 O157:H*VT*₂, P1517 O157:H*VT*₂, P1519 O157:H*VT*₂, P1520 O157:H*VT*₂, and P1521 O157:H*VT*₂. The strains were grown in 2% (wt/vol) KH₂PO₄, 0.5% (wt/vol) NaCl (pH 6.9), with novobiocin (20 mg/liter), added after sterilization; and (iv) CT-SMAC (34)—SMAC agar (CM813; Oxoid) supplemented with cefixime (0.05 mg/liter) and potassium tellurite (2.5 mg/liter). Magnetic beads coated with an antibody against *E. coli* O157 (Dynabeads anti-*E. coli* O157) were supplied by Dynal UK Ltd. Visual EIA kits and ICS kits were supplied by Tecra Diagnostics. The EIA uses enrichment culture in mECn prior to heat treatment and detection of O157 antigen by a standard antibody-based EIA. The ICS uses an antibody-coated polystyrene paddle, wash solution, replication medium, and selective culture on CT-SMAC.

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Three samples of bovine feces containing large numbers of sorbitol-fermenting *E. coli*, but from which *E. coli* O157 could not be isolated, were used to make a 50% (vol/vol) suspension of feces in BPW, which was then used to prepare suspensions containing each of the above *E. coli* O157 strains at concentrations...
of ca. 10^3, 10^2, 10, 1, and 0.1 organisms per ml. All strains were tested in triplicate at each dilution.

**Direct culture.** Twenty microliters of inoculated fecal suspension was inoculated onto CT-SMAC (34) and incubated at 37°C for 18 h. Apparently non-sorbitol-fermenting colonies from CT-SMAC were tested for agglutination with a latex test kit (DRS622, Oxoid) for detecting E. coli O157. Isolates that gave positive results with this test were further characterized as described below.

**Enrichment culture and subculture to CT-SMAC.** Fecal suspension in MRD (0.25 ml) was added to 5 ml of BPW-VCC, mixed, and incubated at 37°C for 6 h. Twenty microliters was subcultured to CT-SMAC and incubated at 37°C for 18 h. Apparently non-sorbitol-fermenting colonies from CT-SMAC were tested for agglutination with a latex test kit as described above. Isolates that gave positive results with this test were further characterized as described below.

**IMS followed by culture to CT-SMAC (IMS/C) for detecting E. coli O157.** The BPW-VCC enrichment culture (1 ml) was then added to 20 μl of magnetic beads coated with an antibody against E. coli O157 (Dynabeads anti-E. coli O157) in a 1.5-ml microcentrifuge tube. The beads were suspended evenly in the broth culture by vortex mixing and were then placed in a rotating mixer so that they were mixed by inversion every 2 to 3 s for 30 min at ambient temperature. Tubes were placed in a magnetic particle concentrator (MPC-10; Dynal, Oslo, Norway), and the magnets were placed in position and left for 5 min. The culture supernatant was removed by aspiration with a Pasteur pipette, the magnetic slide was removed from the rack, the beads were washed by resuspension in 1 ml of phosphate-buffered saline (PBS) (pH 7.2) with 0.05% (vol/vol) Tween 20 (PBST), and the magnetic slide was replaced for 2 min. The beads were washed in PBST in this way once more, the magnetic slide was replaced for 2 min, the supernatant was removed, and the beads were resuspended in ca. 25 μl of nutrient broth. Beads were inoculated onto CT-SMAC and incubated overnight at 37°C. Apparently non-sorbitol-fermenting colonies were examined with a latex test kit as described above. Isolates that gave positive results with this test were further characterized as described below.

**EIA.** Fecal suspension in MRD (0.25 ml) was added to 5 ml of mECn, mixed, and incubated at 37°C for 18 h. Enrichment culture (1 ml) was then added to 50 μl of sample additive supplied with the kit, heated to 100°C for 15 min, and cooled to ambient temperature before being used in the EIA according to the manufacturer’s protocol. Positive and negative controls supplied with the kit and stored at 4°C prior to being transported to the laboratory within 24 h. Fecal material from swabs was removed into 1 ml of MRD by vigorous vortex mixing for 20 to 30 s. Samples were examined by IMS/C and EIA as described above.

**Confirmation of samples positive by EIA.** (i) ICS. Immunoassay was performed as recommended in the test protocol. Four milliliters of the mECn culture was added to the first tube of the confirmation system, and a polystyrene immunocapture paddle was inserted into the culture. After incubation at 37°C for 30 min, the paddle was washed in the tube of wash buffer provided, placed in the tube of replication medium, and incubated at 37°C for 3.5 h. The tube of replication medium with the immunocapture paddle was then vortex mixed for 20 s at medium speed. Serial 10-fold dilutions from 10^-1 to 10^-3 were made in saline, and 0.1 ml of each dilution was surface spread onto a plate of CT-SMAC and incubated overnight at 37°C. Apparently non-sorbitol-fermenting colonies were examined with a latex test kit (DRS622, Oxoid), and isolates that gave positive results with this test were further characterized as described below.

(ii) Modified IMS. The IMS technique was used on mECn cultures: 1 ml of mECn culture was added to a sterile microtube containing 20 μl of magnetic beads coated with antibody to E. coli O157 and was vortex mixed, and IMS and subculture to CT-SMAC were performed as described above. Apparently non-sorbitol-fermenting colonies were examined with a latex test kit as described above.

**Swabs (Transswabs; Medical Wire Co.) of rectal feces were taken from cattle immediately after slaughter, placed in transport medium supplied by the swab manufacturer, and stored at 4°C prior to being transported to the laboratory within 24 h. Fecal material from swabs was removed into 1 ml of MRD by vigorous vortex mixing for 20 to 30 s. Samples were examined by IMS/C and EIA as described above.**

**TABLE 1.** Comparison of direct culture, enrichment and subculture, EIA, and IMS/C for the detection of E. coli O157 in inoculated bovine fecal suspensions

<table>
<thead>
<tr>
<th>Strain</th>
<th>Direct culture</th>
<th>Enrichment/subculture</th>
<th>EIA</th>
<th>IMS/C</th>
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<tbody>
<tr>
<td>P1394</td>
<td>10^3</td>
<td>10^3</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>P1400</td>
<td>&gt;10^3</td>
<td>&gt;10^3</td>
<td>10^3</td>
<td>10^3</td>
</tr>
<tr>
<td>P1401</td>
<td>&gt;10^3</td>
<td>&gt;10^3</td>
<td>10</td>
<td>10^2</td>
</tr>
<tr>
<td>P1426</td>
<td>10^3</td>
<td>&gt;10^3</td>
<td>10^3</td>
<td>10^3</td>
</tr>
<tr>
<td>P1430</td>
<td>&gt;10^3</td>
<td>&gt;10^3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>P1431</td>
<td>&gt;10^3</td>
<td>&gt;10^3</td>
<td>10</td>
<td>10^2</td>
</tr>
<tr>
<td>P1446</td>
<td>&gt;10^3</td>
<td>&gt;10^3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>P1506</td>
<td>&gt;10^3</td>
<td>&gt;10^3</td>
<td>10</td>
<td>&gt;10^3</td>
</tr>
<tr>
<td>P1519</td>
<td>&gt;10^3</td>
<td>&gt;10^3</td>
<td>10^3</td>
<td>10^3</td>
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</tr>
<tr>
<td>P1524</td>
<td>10^3</td>
<td>10^3</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>P1642</td>
<td>&gt;10^3</td>
<td>&gt;10^3</td>
<td>10^2</td>
<td>10^3</td>
</tr>
</tbody>
</table>

*Three of three samples positive.*
TABLE 2. Comparison of EIA and ICS confirmation with IMS for the detection of E. coli O157 from bovine feces (n = 200)*

<table>
<thead>
<tr>
<th>Detection by EIA</th>
<th>Confirmation by:</th>
<th>Detection by:</th>
<th>IMSCT-SMAC</th>
<th>E. coli O157 latex test</th>
<th>No. of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ICS</td>
<td>IMS(mECn)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>15</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>ND</td>
<td>2</td>
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<tr>
<td>–</td>
<td>ND</td>
<td>ND</td>
<td>–</td>
<td>+</td>
<td>3</td>
</tr>
<tr>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>5</td>
</tr>
<tr>
<td>–</td>
<td>ND</td>
<td>ND</td>
<td>–</td>
<td>ND</td>
<td>170</td>
</tr>
</tbody>
</table>

*ICS was performed on the mECn culture followed by culture onto CT-SMAC; IMS(mECn), IMS performed on the mECn enrichment culture followed by culture to CT-SMAC; IMS/CT-SMAC, IMS performed on BPW-VCC enrichment culture followed by culture onto CT-SMAC; ND, not done.

above, and those giving a positive reaction were further characterized as described below.

Further testing of EIA-positive samples which were not confirmed by culture. Fifty apparently sorbitol-fermenting colonies from the immunocapture CT-SMAC plate were selected at random, and a latex test for E. coli O157 was performed as described above. Enrichment cultures from fecal swabs positive by EIA, but from which sorbitol-fermenting or non-sorbitol-fermenting E. coli O157 had not been isolated, had been stored at 4°C for 2 to 3 weeks after initial examinations. These were tested further as follows: (i) the ICS and IMS were repeated; and (ii) enrichment culture (0.5 ml) was centrifuged at 13,000 × g for 15 min, and the clear supernatant was used in an EIA for VTs and in a Vero cell assay. Cell culture-positive samples were tested for specific neutralization of the cytotoxic effect by antisera raised to purified VT1 and VT2. The EIA for VTs was based on capture of toxins onto EIA tested for specific neutralization of the cytotoxic effect by antisera raised to toxins in an EIA for VTs and in a Vero cell assay. Cell culture-positive samples were repeated; (ii) the ICS and IMS were repeated; and (iii) enrichment culture (0.5 ml) was centrifuged at 4°C for 2 to 3 weeks after initial examinations. These were tested further as follows: (i) the E. coli O157 EIA was repeated; (ii) the ICS and IMS were repeated; and (iii) enrichment culture (0.5 ml) was centrifuged at 13,000 × g for 15 min, and the clear supernatant was used in an EIA for VTs and in a Vero cell assay. Cell culture-positive samples were tested for specific neutralization of the cytotoxic effect by antisera raised to purified VT1 and VT2. The EIA for VTs was based on capture of toxins onto EIA plates (Costar) coated with F1 glycoprotein (a natural receptor for VTs, partially purified from hydatid cyst fluid) and detection of toxin by sequential addition of rabbit antibodies to VT1 and VT2, horseradish peroxidase-conjugated swine anti-rabbit immunoglobulins, and chromogenic substrate solution (4). Results were recorded as positive if the mean of the optical density (OD) values for the two samples wells exceeded the mean plus 3 standard deviations of the OD values for the two negative-control wells.

Characterization of isolates. Toxin type was determined by specific hybridization with DNA probes for the VT1 and VT2 genes. DNAs specific for the A cistrons of the VT1 and VT2 genes and for the eaeA gene of E. coli O157 were prepared by PCR, random-prime labelled with digoxigenin-11-DUTP, and used in colony hybridization reactions as described previously (2, 5, 9). Known VT1, VT2, and VT strains were included as controls in each batch of tests. Plasmids were extracted by an alkaline detergent method (6) and were separated by submerging gel electrophoresis in Tris-acetate-EDTA buffer with 1% agarose, stained with ethidium bromide, and visualized on a UV transilluminator. A control E. coli K-12 strain (NCTC 50192-39R861) harboring plasmids of 148, 63.4, and 6.9 kb was included with each batch of tests. For this control strain, log of plasmid size was plotted against distance migrated through the agarose gel and approximate sizes of plasmids from strains of E. coli O157 were estimated from this graph.

Statistical analysis. Results obtained in each test system were compared by McNemar’s test (22); as numbers of positives were small, the exact binomial test was used. Results obtained in each test system were compared by McNemar’s test (22); as numbers of positives were small, the exact binomial test was used.

RESULTS

With 10 of 12 strains of E. coli O157 used to inoculate bovine fecal suspensions, both EIA and IMS/C gave a 10- to 100-fold increase in sensitivity compared with direct culture or enrichment and subculture (Table 1).

Of 200 rectal swabs examined, 17 gave positive results in the EIA which were confirmed by both confirmation systems, 2 gave positive results in the EIA which were confirmed by IMS performed on the mECn enrichment culture but not by ICS, and 1 gave a positive result which was confirmed by ICS but not by IMS (Table 2). Of these 20 samples, 15 also gave positive results by the BPW-VCC–IMS–CT-SMAC culture system; a further 2 samples were positive by this culture system but gave a negative result in the EIA. Eight samples were negative by the BPW-VCC–IMS–CT-SMAC culture system but gave a positive result in the EIA which was not confirmed by either confirmation system (Table 2). The remaining 170 samples gave negative results by both EIA and BPW-VCC–IMS–CT-SMAC.

Examination by an E. coli O157 latex test of 50 sorbitol-fermenting colonies from each of the eight unconfirmed EIA-positive samples yielded sorbitol-fermenting E. coli O157 from three samples. The results of further examinations of the remaining five unconfirmed EIA-positive samples are shown in Table 3. Four of five samples again gave positive results by the Tecra EIA performed as described above, four of the five enrichment cultures gave positive results in an EIA for detecting VTs, and two of these were also positive in a Vero cell assay, the cytotoxic effect being specifically neutralized by antiserum against VT1.

All 22 strains of non-sorbitol-fermenting E. coli O157 were either H type 7 or nonmotile toxigenic strains, as determined by both Vero cell assay and DNA hybridization, and harbored the large 92-kb plasmid and the eaeA gene (Table 4). One strain produced urease, but otherwise these strains were biochemically typical of E. coli O157. The three sorbitol-fermenting strains of E. coli O157 were motile but not H type 7, were nontoxigenic, and did not harbor the 92-kb plasmid or the eaeA gene (Table 4).

There was no evidence to suggest a significant difference between the numbers of positives found in each test system (P = 0.29).

DISCUSSION

Cattle have been identified as an important reservoir of VT1 E. coli O157 in North America (3, 21, 23, 31) and the United Kingdom (9, 11, 12, 29), and food of bovine origin has recently been implicated in outbreaks of infection with this organism in the United Kingdom (11, 32). As E. coli O157 may be present

TABLE 3. Further results for EIA-positive, culture-negative mECn cultures of bovine fecal swabs

<table>
<thead>
<tr>
<th>Specimen no.</th>
<th>Teca EIA 161</th>
<th>Vero cell assay</th>
<th>Neutralization*</th>
</tr>
</thead>
<tbody>
<tr>
<td>OD</td>
<td>Result</td>
<td>OD</td>
<td>Result</td>
</tr>
<tr>
<td>35</td>
<td>0.31</td>
<td>+</td>
<td>0.14</td>
</tr>
<tr>
<td>49</td>
<td>1.13</td>
<td>+</td>
<td>0.45</td>
</tr>
<tr>
<td>160</td>
<td>0.32</td>
<td>+</td>
<td>0.23</td>
</tr>
<tr>
<td>161</td>
<td>0.74</td>
<td>+</td>
<td>0.63</td>
</tr>
<tr>
<td>168</td>
<td>0.37</td>
<td>+</td>
<td>0.33</td>
</tr>
</tbody>
</table>

* Antiserum neutralizing the cytotoxic effect: ND, not done.
in bovine fecal samples and food samples in only small numbers (13), sensitive methods are needed for its detection.

Standard methods for the isolation of E. coli, particularly those using elevated temperatures of 42 to 44°C, are ineffective for the isolation of VT1 E. coli O157 (15, 24), and specific methods are therefore needed. VT1 E. coli O157 strains do not ferment sorbitol, whereas most other E. coli strains do, and SMAC medium has become widely used for their isolation. However, SMAC medium relies entirely on differential sugar fermentation and does not select VT1 E. coli O157 from other E. coli or from non-sorbitol-fermenting genera and therefore lacks sensitivity. Improvements to the selectivity of SMAC (10, 34) have resulted in increased sensitivity in isolation of E. coli O157 from fecal samples but do not render this medium sufficiently sensitive for the isolation of small numbers of the organisms from food and environmental samples. Enrichment culture in BPW-VCC with subculture to SMAC supplemented with 0.05 mg of cefixime/liter and 0.5% (wt/vol) rhamnose has been used previously for isolating E. coli O157 from beef carcasses (9) but was of low sensitivity, detecting an initial inoculum only at the level of 2,000 CFU/10 g of beef. Inclusion of the IMS step in the isolation procedure enhanced this sensitivity at least 100-fold, to a detection limit of 2 to 20 CFU/10 g of beef (33). IMS has also been shown to markedly increase isolation rates of E. coli O157 from human and bovine fecal samples (7, 13). In this study a similar increase in sensitivity relative to direct culture, or to enrichment and subculture, was obtained by both the EIA and IMS/C methods; therefore, only EIA and IMS/C were used for the study of bovine rectal swabs.

Early EIAs to detect E. coli O157 were of low sensitivity and poor specificity (28). Inclusion of an IMS step and secondary enrichment in an EIA protocol has been shown recently to enhance both sensitivity and specificity (8, 18) but also adds significantly to the time and resources needed to perform the EIA. The EIA and confirmation system used in this study performed well, detecting about the same number of positive samples as the BPW-VCC enrichment culture, IMS, and CT-SMAC method, which has been used as the standard method in our laboratory for the past 3 years. There was no statistically significant difference between the numbers of positive results obtained in the different assays (P = 0.29). One problem encountered, as with many immunoassays, was that of positive results which could not be confirmed by culture. Eight samples gave positive EIA results which could not be confirmed by either the ICS or the IMS confirmation procedure. However, when 50 randomly selected colonies of sorbitol-fermenting E. coli per sample were screened by a latex test, three of these eight samples were shown to contain sorbitol-fermenting E. coli O157; had resources permitted screening of more than 50 colonies, then this number may have been increased further. It is therefore apparent from this study that a higher proportion of E. coli O157 strains may ferment sorbitol than had previously been realized. Although the strains of sorbitol-fermenting E. coli O157 isolated in this study were nontoxigenic and eaeA gene negative, toxigenic strains of sorbitol-fermenting E. coli O157 have been reported in clinical cases of hemorrhagic colitis and hemolytic-uremic syndrome in Germany (1), and the potential for such strains occurring elsewhere should not be overlooked. Such potential pathogens could be detected by the EIA but missed by standard culture methods based on sorbitol fermentation. When the EIA was repeated on the stored enrichment cultures from the remaining five samples which had given positive but unconfirmed results, four of five remained positive in the EIA. However, four of the five samples also gave a positive result in the EIA for VT1s, but two of these four were negative for these toxins in a Vero cell assay; the possibility of these being false-positive results in either EIA cannot, therefore, be overlooked.

Other immunological methods for the detection of E. coli O157, such as immunoblot techniques (16, 30), have been described elsewhere, but although they are sensitive, these methods can be time-consuming, technically demanding, expensive, and prone to give positive results that cannot be confirmed by culture. PCR has also been described as a sensitive method for detecting E. coli O157 in food (17), but it is technically demanding and expensive, requires confirmation by culture, and also detects VT1 E. coli of serogroups other than O157; such strains may be present in up to 17% of beef and milk samples (14, 25, 27), and their significance in relation to human illness is unclear.

In contrast, the Teca EIA and ICS and the Dynabead IMS are all technically simple and sensitive methods for the specific detection of E. coli O157 in bovine fecal samples and require a minimum of microbiological expertise for their performance. The EIA and ICS are still under development, and recent changes, particularly to the sample additive used in the EIA, may result in further improvements to the specificity of the assay (22a).

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