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

P. A. CHAPMAN,* A. T. CERDAN MALO, C. A. SIDDONS, AND M. HARKIN

Public Health Laboratory, Sheffield S5 7BQ, United Kingdom

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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 10- to 100-fold 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 confirmed by ICS but not by IMS. Of these 20, 15 were also positive by the BPW-VCC–IMS culture system; a further 3 samples were positive by this culture system but gave a negative result in the EIA. Eight samples were negative by the BPW-VCC–IMS culture system but gave a positive result in the EIA which could not be confirmed by either confirmation system. Further examination of the eight unconfirmed EIA-positive samples yielded sorbitol-fermenting *E. coli* O157 from three samples. Of the remaining five cultures, four were positive in an EIA for verocytotoxins (VT) and two were positive in a cell culture assay for VT_1. The remaining 170 samples were negative by both EIA and BPW-VCC–IMS. The Tecra EIA and IMS are both technically simple and sensitive methods for detecting *E. coli* O157 in bovine fecal samples. There was no statistically significant difference between the numbers of positives detected by the different assays ($P = 0.29$).

Verocytotoxin-producing (VT^+) *Escherichia coli* is now recognized as a major cause of hemorrhagic colitis and the hemolytic-uremic syndrome in humans. In North America, beef, beef products, and untreated milk have been suggested as possible sources of VT^+ *E. coli* infection for humans (21, 26). In the United Kingdom, VT^+ *E. coli* O157, the most common serogroup associated with illness in humans, has been isolated from cattle (9, 11, 12), but the organism has only rarely been isolated from food (9, 11, 32), and its epidemiology therefore remains unclear. In view of the potential severity of infections caused by VT^+ *E. coli* O157, it is essential that sensitive methods be used in studies designed to elucidate further the reservoirs of the organism and routes of transmission to humans.

Most strains of VT^+ *E. coli* O157 do not ferment sorbitol whereas most other serogroups of *E. coli* do, and sorbitol MacConkey (SMAC) agar has proved useful for their isolation (20). Improvements to SMAC medium (10, 34) have resulted in increased sensitivity in isolation of *E. coli* O157 from fecal samples, and enrichment culture in buffered peptone water with vancomycin, cefsulodin, and cefixime (BPW-VCC) has been effective for isolating the organism from beef, untreated milk, farmyard slurry, and bovine and human feces (7, 9, 11, 13, 33). However, the sensitivity of such enrichment techniques has been much enhanced by use of immunomagnetic separation (IMS) of *E. coli* O157 prior to subculture (7, 11, 13, 33).

The aims of this study were to evaluate, by comparison with 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), cefixime (0.05 mg/liter), and cefsulodin (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_2HPO_4, 0.15% (wt/vol) KH_2PO_4, 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_1^- VT_2^+ phage type (PT) 2, P1642 O157:H7 VT_1^- VT_2^+ PT2, P1431 O157:H$^-$ VT_1^- VT_2^+ PT4, P1446 O157:H7 VT_1^+ VT_2^+ PT4, P1426 O157:H$^-$ VT_1^+ VT_2^+ PT8, P1506 O157:H7 VT_1^- VT_2^+ PT8, P1401 O157:H7 VT_1^+ VT_2^+ PT14, P1523 O157:H7 VT_1^- VT_2^+ PT14, P1524 O157:H7 VT_1^+ VT_2^- PT14, P1430 O157:H$^-$ VT_1^- VT_2^+ PT49; P1519 O157:H7 VT_1^- VT_2^+ PT49, and P1400 O157:H$^-$ VT_1^- VT_2^- PT RDNC.

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 ca. 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

* Corresponding author. Mailing address: Public Health Laboratory, Herries Rd., Sheffield S5 7BQ, United Kingdom. Phone: (0114) 2437749. Fax: (0114) 2421385.

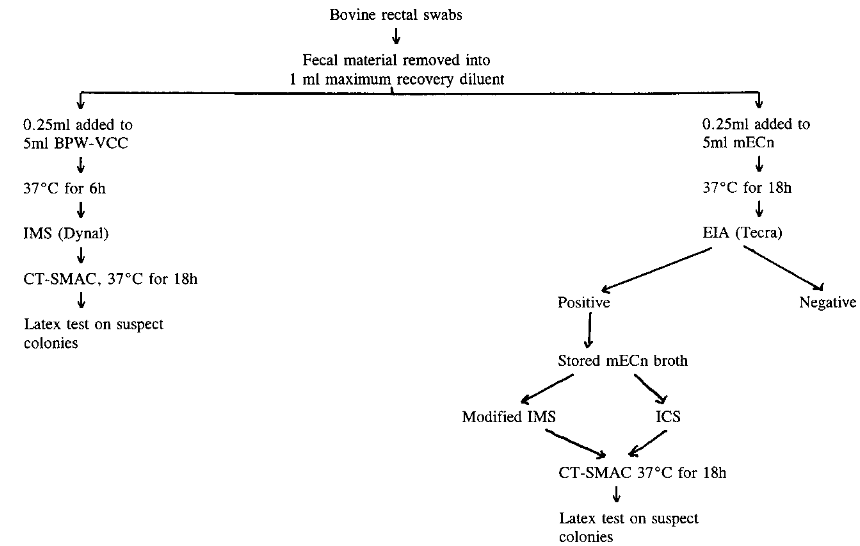

FIG. 1. Comparison of IMS/C and EIA for detection of *E. coli* O157 in bovine fecal samples, and comparison of modified IMS and ICS for confirmation of positive EIA results.

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 (DR622; 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 test samples (200 μl) were added to microwells and incubated at 37°C for 30 min. The wells were washed manually three times, after which 200 μl of antibody-enzyme conjugate was added, mixed, and incubated at 37°C for 30 min. After washing as above, 200 μl of enzyme substrate solution was added, mixed, and incubated at ambient temperature (20 to 25°C) for 20 min. After the reaction was stopped with 20 μl of stop solution, absorbances were read against an air blank in a dual-wavelength (405 and 490 nm) microplate spectrophotometer. Absorbance values provided by the manufacturer were used for interpretation of controls and test results. Enrichment cultures were stored at 4°C for up to 2 weeks for further testing; storage of broth cultures for up to 4 weeks at 4°C had previously been shown not to adversely affect recovery of *E. coli* O157 (28a).

Collection and preparation of uninoculated bovine fecal samples. A schematic outline of the comparison of IMS/C and EIA for the detection of *E. coli* O157 in uninoculated bovine fecal samples, and of the modified IMS and ICS as confirmatory tests for the EIA, is shown in Fig. 1.

Swabs (Transwabs; 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.

Confirmation of samples positive by EIA. (i) ICS. Immunocapture 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^{-4} 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 (DR622; 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

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

Strain	Limit of detection[a] (CFU/ml)			
	Direct culture	Enrichment/ subculture	EIA	IMS/C
P1394	10^3	10^3	10	1
P1400	$>10^3$	$>10^3$	10^3	10^3
P1401	$>10^3$	10^3	10^3	10^3
P1426	10^3	$>10^3$	10^3	10^3
P1430	$>10^3$	10^3	1	1
P1431	$>10^3$	10^3	10	10^2
P1446	$>10^3$	10^3	1	1
P1506	$>10^3$	10^3	10^3	$>10^3$
P1519	$>10^3$	$>10^3$	10^3	10^3
P1523	10^3	10^3	10^2	10
P1524	10^3	10^2	1	10
P1642	$>10^3$	$>10^3$	10^2	10^3

[a] 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 ($n = 200$)[a]

Detection by EIA	Confirmation by:		Detection by:		No. of samples
	ICS	IMS(mECn)	IMS/CT-SMAC	*E. coli* O157 latex test	
+	+	+	+	ND	15
+	+	+	−	ND	2
+	−	+	−	ND	2
+	+	−	−	ND	1
−	ND	ND	+	ND	2
+	−	−	−	+	3
+	−	−	−	−	5
−	ND	ND	−	ND	170

[a] 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 *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 VT_1 and VT_2. The EIA for VTs was based on capture of toxins onto EIA plates (Costar) coated with P1 glycoprotein (a natural receptor for VTs, partially purified from hydatid cyst fluid) and detection of toxin by sequential addition of rabbit antibodies to VT_1 and VT_2, 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 sample 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 VT_1 and VT_2 genes. DNAs specific for the A cistrons of the VT_1 and VT_2 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 VT_1^+, VT_2^+, 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 submerged 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, 36, 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 probabilities were calculated by using the mid-*P* method described by Lancaster (19).

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 VT_1.

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 VT^+ *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

Specimen no.	Tecra EIA				Repeat ICS result	Repeat IMS result	Toxin EIA		Vero cell assay result	Neutralization[a]
	Original		Repeat							
	OD	Result	OD	Result			OD	Result		
35	0.31	+	0.14	−	−	−	0.38	+	−	ND
49	1.13	+	0.45	+	−	−	0.22	+	−	ND
160	0.32	+	0.23	+	−	−	0.18	−	−	ND
161	0.74	+	0.63	+	−	−	1.48	+	+	VT_1 only
168	0.37	+	0.33	+	−	−	1.11	+	+	VT_1 only

[a] Antiserum neutralizing the cytotoxic effect. ND, not done.

TABLE 4. Characteristics of 25 strains of *E. coli* O157 isolated from bovine feces

Sorbitol fermentation	Result of Vero cell assay for VT	Result of DNA probe for:			H antigen[a]	Urease production	Plasmid(s) (kb)	No. of strains
		VT_1	VT_2	*eaeA*				
−	+	−	+	+	7	−	92	6
−	+	−	+	+	7	−	92, 63	2
−	+	−	+	+	7	−	92, 6.9	2
−	+	−	+	+	NM	−	92, 63	1
−	+	−	+	+	7	+	92, 63, 2	1
−	+	+	+	+	7	−	92	5
−	+	+	+	+	7	−	92, 6.9	1
−	+	+	+	+	7	−	92, 2	1
−	+	+	+	+	NM	−	92	1
−	+	+	+	+	NM	−	92, 11.5, 6.9	1
−	+	+	+	+	NM	−	92, 5.5	1
+	−	−	−	−	MNH7	−		3

[a] NM, nonmotile; MNH7, motile but not H type 7.

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 VT^+ *E. coli* O157 (15, 24), and specific methods are therefore needed. VT^+ *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 VT^+ *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 VTs, 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 VT^+ *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 Tecra 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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REFERENCES

1. **Aleksic, S., H. Karch, and J. Bockemuhl.** 1992. A biotyping scheme for Shiga-like (Vero) toxin-producing *Escherichia coli* O157 and a list of serological cross-reactions between O157 and other gram-negative bacteria. Zentralbl. Bakteriol. **276:**221–230.
2. **Beebakhee, G., M. Louie, J. De Azavedo, and J. Brunton.** Cloning and nucleotide sequence of the *eae* gene homologue from enterohaemorrhagic *Escherichia coli* serotype O157:H7. FEMS Microbiol. Lett. **91:**63–68.
3. **Borczyk, A. A., M. A. Karmali, H. Lior, and L. M. Duncan.** 1987. Bovine reservoir for verotoxin-producing *Escherichia coli* O157:H7. Lancet **i:**98.
4. **Chapman, P. A.** 1993. Purification of the verotoxins of *Escherichia coli* and production of antitoxins for use in a diagnostic test. Ph.D. thesis. University of Sheffield, Sheffield, United Kingdom.
5. **Chapman, P. A., and C. M. Daly.** 1993. An evaluation of a non-radioactive trivalent DNA probe (LTh, ST1a, ST1b) for detecting enterotoxigenic *Escherichia coli*. J. Clin. Pathol. **46:**309–312.
6. **Chapman, P. A., L. Jewes, C. A. Siddons, P. Norman, and S. L. George.** 1990.

Verotoxin-producing *Escherichia coli* infections in Sheffield during 1989. PHLS Microbiol. Digest **7:**163–166.

7. **Chapman, P. A., and C. A. Siddons.** 1996. A comparison of immunomagnetic separation and direct culture for the isolation of verocytotoxin-producing *E. coli* O157 from cases of bloody diarrhoea, non-bloody diarrhoea and asymptomatic contacts. J. Med. Microbiol. **44:**267–271.
8. **Chapman, P. A., and C. A. Siddons.** 1996. Evaluation of a commercial enzyme immunoassay for detecting *Escherichia coli* O157 in beef and beef products. Food Microbiol. **13:**175–182.
9. **Chapman, P. A., C. A. Siddons, D. J. Wright, P. Norman, J. Fox, and E. Crick.** 1993. Cattle as a possible source of verocytotoxin-producing *Escherichia coli* O157 infections in man. Epidemiol. Infect. **111:**439–447.
10. **Chapman, P. A., C. A. Siddons, P. M. Zadik, and L. Jewes.** 1991. An improved selective medium for the isolation of *Escherichia coli* O157. J. Med. Microbiol. **35:**107–110.
11. **Chapman, P. A., D. J. Wright, and R. Higgins.** 1993. Untreated milk as a source of verotoxigenic *E. coli* O157. Vet. Rec. **133:**171–172.
12. **Chapman, P. A., D. J. Wright, and P. Norman.** 1989. Verotoxin-producing *Escherichia coli* infections in Sheffield: cattle as a possible source. Epidemiol. Infect. **102:**439–445.
13. **Chapman, P. A., D. J. Wright, and C. A. Siddons.** 1994. A comparison of immunomagnetic separation and direct culture for the isolation of verocytotoxin-producing *Escherichia coli* O157 from bovine feces. J. Med. Microbiol. **40:**424–427.
14. **Clarke, R. C., S. A. McEwen, V. P. Gannon, H. Lior, and C. L. Gyles.** Isolation of verocytotoxin-producing *Escherichia coli* from milk filters in southwestern Ontario. Epidemiol. Infect. **102:**253–260.
15. **Doyle, M. P., and J. L. Schoeni.** 1984. Survival and growth characteristics of *Escherichia coli* associated with hemorrhagic colitis. Appl. Environ. Microbiol. **48:**855–856.
16. **Doyle, M. P., and J. L. Schoeni.** 1987. Isolation of *Escherichia coli* O157:H7 from retail fresh meats and poultry. Appl. Environ. Microbiol. **53:**2394–2396.
17. **Gannon, V. P. J., R. K. King, J. Y. Kim, and E. J. Golsteyn Thomas.** 1992. Rapid and sensitive method for detection of Shiga-like toxin-producing *Escherichia coli* in ground beef using the polymerase chain reaction. Appl. Environ. Microbiol. **58:**3809–3815.
18. **Johnson, R. P., R. J. Durham, S. T. Johnson, L. A. MacDonald, S. R. Jeffrey, and B. T. Butman.** 1995. Detection of *Escherichia coli* O157:H7 in meat by an enzyme-linked immunosorbent assay, EHEC-Tek. Appl. Environ. Microbiol. **61:**386–388.
19. **Lancaster, H. O.** 1961. Significance tests in discrete distributions. J. Am. Statist. Assoc. **56:**223–224.
20. **March, S. B., and S. Ratnam.** 1986. Sorbitol-MacConkey medium for the detection of *Escherichia coli* O157:H7 associated with hemorrhagic colitis. J. Clin. Microbiol. **23:**869–872.
21. **Martin, M. L., L. D. Shipman, J. G. Wells, M. E. Potter, K. Hedberg, I. K. Wachsmuth, R. V. Tauxe, J. P. Davis, J. Arnold, and J. Tilleli.** 1986. Isolation of *Escherichia coli* O157:H7 from dairy cattle associated with two cases of haemolytic-uraemic syndrome. Lancet **ii:**1043.
22. **McNemar, Q.** 1947. Note on the sampling error of the difference between correlated proportions or percentages. Psychometrika **12:**153–157.

22a. **Notley, L.** Personal communication.

23. **Ostroff, S. M., P. M. Griffin, R. V. Tauxe, L. D. Shipman, K. D. Greene, J. G. Wells, J. H. Lewis, P. A. Blake, and J. M. Kobayashi.** 1990. A statewide outbreak of *Escherichia coli* O157 infections in Washington state. Am. J. Epidemiol. **132:**239–247.
24. **Raghubeer, E. V., and J. R. Matches.** 1990. Temperature range for growth of *Escherichia coli* O157:H7 and selected coliforms in *E. coli* medium. J. Clin. Microbiol. **28:**803–805.
25. **Read, S. C., C. L. Gyles, R. C. Clarke, H. Lior, and S. McEwen.** 1990. Prevalence of verocytotoxigenic *Escherichia coli* in ground beef, pork and chicken in southwestern Ontario. Epidemiol. Infect. **105:**11–20.
26. **Riley, L. W., R. S. Remis, S. D. Helgerson, H. B. McGee, J. G. Wells, B. R. Davis, R. J. Hebert, E. S. Olcott, L. M. Johnson, N. T. Hargrett, P. A. Blake, and M. L. Cohen.** 1983. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. N. Engl. J. Med. **308:**681–685.
27. **Roberts, D., J. Thirlwell, H. Smith, and G. Willshaw.** 1992. The microbiological examination of raw beef products with particular reference to Vero cytotoxin producing *Escherichia coli*, p. 506–508. *In* Proceedings of the 3rd World Congress on Foodborne Infections and Intoxications, June 1992, Berlin, vol. 1. Institute of Veterinary Medicine, Berlin, Germany.
28. **Sernowski, L. P., and S. C. Ingham.** 1992. Frequency of false presumptive positive results obtained using a commercial ELISA kit to screen retail ground beef for *Escherichia coli* O157:H7. J. Food Prot. **55:**846.

28a. **Sheffield Public Health Laboratory.** Unpublished data.

29. **Synge, B. A., and G. F. Hopkins.** 1992. Verotoxigenic *Escherichia coli* O157 in Scottish calves. Vet. Rec. **130:**583.
30. **Szabo, R., E. Todd, J. MacKenzie, L. Parrington, and A. Armstrong.** 1990. Increased sensitivity of the rapid hydrophobic grid membrane filter enzyme-labeled antibody procedure for *Escherichia coli* O157 detection in foods and bovine feces. Appl. Environ. Microbiol. **56:**3546–3549.
31. **Wells, J. G., L. D. Shipman, K. D. Greene, E. G. Sowers, J. H. Green, D. N. Cameron, F. P. Downes, M. L. Martin, P. M. Griffin, S. M. Ostroff, M. E. Potter, R. V. Tauxe, and I. K. Wachsmuth.** 1991. Isolation of *Escherichia coli* serotype O157:H7 and other Shiga-like toxin-producing *E. coli* from dairy cattle. J. Clin. Microbiol. **29:**985–989.
32. **Willshaw, G. A., J. Thirlwell, A. P. Jones, S. Parry, R. L. Salmon, and M. Hickey.** 1994. Vero cytotoxin-producing *Escherichia coli* O157 in beefburgers linked to an outbreak of diarrhoea, haemorrhagic colitis and haemolytic uraemic syndrome in Britain. Lett. Appl. Microbiol. **19:**304–307.
33. **Wright, D. J., P. A. Chapman, and C. A. Siddons.** 1994. Immunomagnetic separation as a sensitive method for isolating *Escherichia coli* O157 from food samples. Epidemiol. Infect. **113:**31–39.
34. **Zadik, P. M., P. A. Chapman, and C. A. Siddons.** 1993. Use of tellurite for the selection of verocytotoxigenic *Escherichia coli* O157. J. Med. Microbiol. **39:**155–158.