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An evaluation of rapid methods for detecting *Escherichia coli* O157 on beef carcasses

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Abstract

Numbers of *Escherichia coli* O157 in food may be low and sensitive techniques are therefore needed for its detection. The objectives of this study were to use carcass meat samples artificially inoculated with various strains of *E. coli* O157 to compare the sensitivity of enrichment in three different media and to compare immunomagnetic separation followed by culture of magnetic beads to cefixime tellurite sorbitol MacConkey agar with three immunoassays for the detection of *E. coli* O157 in the enrichment cultures. Duplicate 250, 25 and 2–3 CFU of each of 16 strains of *E. coli* O157 added to 25-g samples of beef carcass meat were used to compare the sensitivity of (1) enrichment in supplemented tryptone soya broth (sTSB), Reveal™ 8-h and Reveal™ 20-h media, and (2) immunomagnetic separation and culture to cefixime tellurite sorbitol MacConkey agar (IMS/CT-SMAC) with Reveal™, VIP™ and STAT™ immunoassays for detecting the organism. An initial inoculum of 250 CFU/25 g meat was detected in all 32 samples by IMS/CT-SMAC performed on all enrichment media and by Reveal™ performed on Reveal™ 8-h and Reveal™ 20-h media, but in only 30, 19 and 9 samples by Reveal™, VIP™ and STAT™, respectively, performed on sTSB medium. An initial inoculum of 25 CFU/25 g meat was detected in 28, 32 and 30 of 32 samples by IMS/CT-SMAC performed on sTSB, Reveal™ 8-h and Reveal™ 20-h media, respectively, and in 32 and 30 samples by Reveal™ performed on Reveal™ 8-h and Reveal™ 20-h media, but in only 22, 11 and 2 samples by Reveal™, VIP™ and STAT™, respectively, performed on sTSB medium. An initial inoculum of 2–3 CFU/25 g meat was detected in 25, 31 and 28 of 32 samples by IMS/CT-SMAC performed on sTSB, Reveal™ 8-h and Reveal™ 20-h media, respectively, and in 25 and 23 samples by Reveal™ performed on Reveal™ 8-h and Reveal™ 20-h media, but in only 14, 1 and 0 samples by Reveal™, VIP™ and STAT™, respectively, performed on sTSB medium.

Keywords: *Escherichia coli* O157; Tryptone soya broth; Immunomagnetic separation

Verocytotoxin-producing (VT^+) *Escherichia coli* (VTEC), particularly *E. coli* O157, cause haemorrhagic colitis (HC), the haemolytic-uremic syndrome (HUS) and occasionally mild non-bloody diarrhea in man, although some infections may be asymptomatic. In the United Kingdom (UK), beef and beef products, milk and milk products, and close contact with animals have been identified as sources of human infection (Chapman et al., 1993a,b, 1997a, 2000; Willshaw et al., 1994). Numbers of *E. coli* O157 in food may be low (Bell et al., 1994; Paton et al., 1996;

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Tilden et al., 1996; Chapman et al., 2001a) and sensitive culture techniques are therefore needed for its detection. We have recently shown that enrichment in tryptone soya broth (TSB) supplemented with additional tricarboxylic acid (TCA) cycle intermediates and a phosphate buffer, with addition of selective agents after a 6-h recovery phase (sTSB), markedly improved the isolation of *E. coli* O157 from naturally contaminated raw beef, lamb and mixed meat products (Chapman et al., 2001b,c). However, although sensitive, the procedure takes a full 48 h to complete making it unsuitable for applications where a more rapid result is required.

The objectives of this study were to use carcass meat samples artificially inoculated with various strains of *E. coli* O157 to (1) compare the sensitivity of enrichment in sTSB with enrichment in two other media requiring incubation for as little as 8 h, (2) to compare immunomagnetic separation (IMS) followed by culture of magnetic beads to cefixime tellurite sorbitol MacConkey agar (Zadik et al., 1993) with Reveal™ (Neogen, Lansing, MI, USA), VIP™ (Bio-Control Systems, Bellevue, WA, USA) and STAT™ (Meridian Diagnostics, Cincinatti, OH, USA) for the detection of *E. coli* O157 in enrichment cultures, and (3) to determine the minimum levels of *E. coli* O157 needed in enrichment media to give a positive result by Reveal™.

1. Materials and methods

1.1. Bacterial strains used

Sixteen well-characterized strains of *E. coli* O157 were used for the study. All had been isolated from carcass meat samples during a previous surveillance study in the Sheffield area (Chapman et al., 2001a). All strains had been stored at −70 °C in nutrient broth (CM1, Oxoid, Basingstoke, UK) with glycerol 15% v/v since they were first isolated and should therefore have been as close to "wild-type" strains as possible. Each of the 16 strains had a different combination of phage type, toxin genotype and plasmid profile (Table 1; Chapman et al., 2001a), and had a different pulsed-field gel electrophoresis profile (data not shown). All were toxigenic and positive for the *eaeA* gene and were therefore typical of strains associated with infection in humans.

Table 1
Details of strains to be used for the evaluation

Strain number	Phage type	Toxin genotype			Plasmids (kbp)
		VT_1	VT_2	*eaeA*	
P4682	2	–	+	+	92
P4476	2	–	+	+	92, 60, 6.9
P4477	2	–	+	+	92, 60, 9.5
P4535	21/28	–	+	+	92
P4547	21/28	–	+	+	92, 2
P4470	21/28	–	+	+	92, 6.9, 2
P4469	21/28	–	+	+	92, 60, 2
P4679	32	–	+	+	92, 2
P4658	34	–	+	+	92
P3105	39	–	+	+	92
P4534	4	+	+	+	92
P4537	4	–	+	+	92, 60
P4532	8	+	+	+	92
P4468	8	+	+	+	92, 6.9
P4480	8	+	+	+	92, 60
P4670	RDNC	–	+	+	92

All strains were initially isolated from carcass meat samples and have been stored at −70 °C since first isolation (Chapman et al., 2001a).

1.2. Preparation of bacterial inoculum

Each strain of *E. coli* O157 was inoculated into nutrient broth (CM1, Oxoid), incubated at 37 °C for 18–20 h and chilled at 4–8 °C for 2 h. Serial 10-fold dilutions were prepared in maximum recovery diluent (CM733, Oxoid) and spread onto plate count agar (CM325, Oxoid) which was incubated at 37 °C for 18–20 h. Numbers of CFU/ml of *E. coli* O157 were estimated from this and appropriate dilutions of cultures in nutrient broth were prepared to inoculate meat samples with 250, 25 or 2–3 CFU.

1.3. Inoculation of carcass meat samples

Carcass meat samples were collected from a local abattoir. Trimmings from an area of approximately 100 cm^2 of the outer surface of the neck of carcasses were excised with a sterile knife. All meat samples were stored at 4–8 °C between collection and delivery to Sheffield PHL within 24 h of their collection.

For each isolation method, duplicate samples were inoculated with each dilution of each strain of *E. coli* O157 and two uninoculated samples of beef were included as negative controls with each series of dilutions. All inoculated samples were stored at 4–8 °C for 1–2 h before further processing.

1.4. Enrichment culture—Sheffield PHL method

The medium used was based on a prototype enrichment broth developed by Oxoid. This was a tryptone soya broth supplemented with additional TCA cycle intermediates, an Oxyrase™ enzyme system, a phosphate buffer and novobiocin 20 mg/l (sTSB). The 25-g carcass meat samples and the bacterial inoculum were placed in 225 ml of sTSB and blended in a stomacher (Colworth 400, Seward, London, UK) for 30 s at the medium speed setting. Suspensions were incubated at 37 °C for 6 h, after which bile salts (No. 3, Oxoid) were added to the medium to give a final concentration of 1.5 g/l and mixed well. Suspensions were then incubated at 41.5 °C for a further 18 h.

1.5. Enrichment culture—Reveal™ 8-h method

The medium was prepared fresh immediately before use by aseptically dissolving 49.6 g of sterile powdered medium in 1 l of sterile deionized water that had been preheated to 42 °C. The 25-g carcass meat samples and the bacterial inoculum were placed in 225 ml of the medium and blended in a stomacher as above. Suspensions were incubated at 42 °C for 8 h.

1.6. Enrichment culture—Reveal™ 20-h method

The medium was prepared fresh immediately before use by aseptically dissolving 37.0 g of powdered medium in 1 l of sterile deionized water. The 25-g carcass meat samples and the bacterial inoculum were placed in 225 ml of the medium and blended in a stomacher as above. Suspensions were incubated at 37 °C for 20 h.

1.7. Immunomagnetic separation (IMS)

Broth culture (1 ml) was added to 20 μl of magnetic beads coated with an antibody prepared to the lipopolysaccharide of *E. coli* O157 (Dynabeads anti-*E. coli* O157, Dynal, Oslo) in a 1.5-ml microcentrifuge tube. The beads were suspended, mixed, separated in a magnetic particle concentrator (MPC-M, Dynal) and washed twice in phosphate-buffered saline, pH 7.4, with Tween-20 0.05% v/v (PBST) as described previously (Wright et al., 1994).

1.8. Culture onto CT-SMAC

After the final wash and separation, the beads were resuspended in ca. 25 μl of nutrient broth, inoculated onto CT-SMAC medium (Zadik et al., 1993; SR172, Oxoid) and incubated overnight at 37 °C. Colonies not fermenting sorbitol from CT-SMAC were tested for agglutination with a latex test kit (DR120M, Oxoid) for detecting *E. coli* O157. Five separate colonies were tested before recording a negative result. Isolates that gave positive results were confirmed as *E. coli* by biochemical tests (Crystal ID, Becton Dickinson, Oxford, UK) and as serogroup O157 by agglutination to titer with a rabbit antiserum prepared to *E. coli* O157 (Mast Diagnostics, Liverpool, UK).

1.9. Immunoassays

Reveal™, VIP™ and STAT™ are lateral flow immunochromatographic devices designed to detect *E. coli* O157. A sample of enrichment culture is added to the sample port of the device, which initiates a lateral flow of the medium along the surface of a solid support. During this flow, any *E. coli* O157 present reacts with an antibody/chromogen complex contained in the device and forms a visible line in a viewing window. For the test to be valid a control line should form in a second viewing window.

For the Reveal™ 8-h test, a 1-ml portion of the enrichment culture was heated to 100 °C for 15 min and cooled to ambient temperature before use in the Reveal™ assay. Enrichment cultures in sTSB medium or Reveal™ 20-h medium were used directly in the assays. Reveal™ was performed on all enrichment media but VIP™ and STAT™ were performed only on sTSB medium. The assay strips were equilibrated to ambient temperature before use and 120, 100 and 100 μl of enrichment culture were added to the sample well of Reveal™, VIP™ and STAT™ assay strips,

Table 2
Overall summary of the results obtained with the three enrichment media

Inoculum used (CFU/25 g)	Number positive after:							
	Enrichment in Sheffield PHL TSB followed by:				Enrichment in Reveal™ 8-h medium followed by:		Enrichment in Reveal™ 20-h medium followed by:	
	IMS/CT-SMAC	Reveal™	VIP™	STAT™	IMS/CT-SMAC	Reveal™	IMS/CT-SMAC	Reveal™
250	32	30	19	9	32	32	32	32
25	28	22	11	2	32	32	30	31
2–3	25	14	1	0	31	25	28	23

Sixteen strains were each inoculated at each level into duplicate samples of carcass meat (32 samples at each inoculum level).

respectively. Results were recorded after 20, 10 and 10 min, respectively.

For the lowest inoculum level of each strain in each medium that was positive by Reveal™, the numbers of *E. coli* O157 per ml of enrichment culture were determined by a standard serial 10-fold dilution technique as above but with dilutions plated onto CT-SMAC.

2. Results

Results of the study are summarized in Tables 2–4. An initial inoculum of 250 CFU/25 g meat was detected in all 32 samples after IMS and CT-SMAC performed on all three enrichment media. With this inoculum, all 32 samples were positive by Reveal™ performed on Reveal™ 8-h and Reveal™ 20-h enrichment media, but only 30 (94%), 19 (59%) and 9 (28%) of 32 were positive by the Reveal™, VIP™ and STAT™ assays, respectively, when performed on the sTSB medium.

An initial inoculum of 25 CFU/25 g meat was detected in 28 (88%), 32 (100%) and 30 (94%) of 32 samples after IMS and CT-SMAC performed on sTSB, Reveal™ 8-h and Reveal™ 20-h enrichment media, respectively. With this inoculum, Reveal™ detected all 32 samples performed on Reveal™ 8-h enrichment and 30 (94%) of 32 samples performed on Reveal™ 20-h enrichment media, but only 22 (79%), 11 (39%) and 2 (7%) were positive by the Reveal™, VIP™ and STAT™ assays, respectively, when performed on the sTSB medium.

An initial inoculum of 2–3 CFU/25 g meat was detected in 25 (78%), 31 (97%) and 28 (88%) of 32 samples after IMS and CT-SMAC performed on sTSB, Reveal™ 8-h and Reveal™ 20-h enrichment media, respectively. With this inoculum, Reveal™ detected 25 (78%) and 23 (72%) of 32 samples performed on Reveal™ 8-h and Reveal™ 20-h enrichment media, respectively, but only 14 (44%), 1 (4%) and 0 (0%) of 32 were positive by the Reveal™, VIP™ and STAT™ assays, respectively, when performed on the sTSB medium.

E. coli O157 was not detected by culture or any of the immunoassays in any of the negative control samples.

The numbers of *E. coli* O157 per ml of enrichment culture from the lowest inoculum level of each strain in each medium that was positive by Reveal™ ranged from 1.3×10^4 to 1.6×10^7 (average 1.9×10^6) for sTSB, from 1.5×10^5 to 1.4×10^8 (average 2.8×10^7)

Table 3
Comparison of IMS/CT-SMAC (C) and Reveal™ assay (R) performed on the three enrichment media

Inoculum used (CFU/25 g)	Enrichment in Sheffield PHL sTSB				Enrichment in Reveal™ 8-h medium				Enrichment in Reveal™ 20-h medium			
	C+R+	C−R+	C+R−	C−R−	C+R+	C−R+	C+R−	C−R−	C+R+	C−R+	C+R−	C−R−
250	30	0	2	0	32	0	0	0	32	0	0	0
25	20	2	8	2	32	0	0	0	29	2	1	0
2–3	13	1	12	6	25	0	6	1	19	4	9	0

Sixteen strains were each inoculated at each level into duplicate samples of carcass meat (32 samples at each inoculum level). C+, positive by IMS/CT-SMAC; C−, negative by IMS/CT-SMAC; R+, positive by Reveal™; R−, negative by Reveal™.

Table 4
Comparison of the Reveal™ (R), VIP™ (V) and STAT™ (S) assays performed on Sheffield PHL sTSB

Inoculum used (CFU/25 g)	R+V+S+	R+V+S−	R+V−S−	R−V−S−	R−V+S−
250	9	10	11	2	0
25	2	9	11	10	0
2–3	0	0	14	17	1

Sixteen strains were each inoculated at each level into duplicate samples of carcass meat (32 samples at each inoculum level). R+, V+, S+, positive by Reveal™, VIP™ and STAT™ assays, respectively; R−, V−, S−, negative by Reveal™, VIP™ and STAT™ assays, respectively.

for Reveal™ 8-h medium, and from 3×10^4 to 2.9×10^8 (average 3.4×10^7) for Reveal™ 20-h medium.

3. Discussion

Beef products have been widely implicated as vehicles of *E. coli* O157 infection. Results in surveys in Europe have generally shown a very low prevalence of the organism on beef carcasses (Bonardi et al., 2001; Richards et al., 1998). We previously found *E. coli* O157 in 1.4% cattle carcasses and from 0.7% of sheep carcasses, a far higher prevalence than reported in the above studies (Chapman et al., 2001a). Although several factors may have influenced this, we used the IMS technique throughout the study and have previously shown this to be 10–100-fold more sensitive than enrichment and subculture for the isolation of *E. coli* O157 from minced beef (Wright et al., 1994). However, although IMS is simple and economical, it is laborious and time-consuming to perform and culture of the beads to CT-SMAC adds at least a further 24 h on to the time taken to obtain a result.

Enzyme immunoassays (EIAs) and immunoblot techniques have been described as rapid alternatives to culture for detecting *E. coli* O157 in enrichment cultures of food and environmental samples (Doyle and Schoeni, 1987; Padhye and Doyle, 1991; Sernowski and Ingham, 1992; Chapman and Siddons, 1996; Chapman et al., 1997b). Although sensitive, these methods may be laborious, expensive and prone to give positive results that cannot be confirmed by culture (Sernowski and Ingham, 1992; Chapman and Siddons, 1996; Chapman et al., 1997b).

In contrast to more conventional EIAs, the Reveal™ assay used in the present study was extremely rapid and easy to use. In the present study, the Reveal™ assay compared favorably with culture at inocula of 250 or 25 CFU/25 g of carcass meat (Table 2) but was less sensitive at the inoculum of 2–3 CFU/25 g, at which level it detected only 25 (81%) of 31 and 23 (82%) of 28 samples found positive by IMS and CT-SMAC using Reveal™ 8-h and Reveal™ 20-h media, respectively. The Reveal™ assay performed better than it did in a previous study in our laboratory, in which it detected *E. coli* O157 in only 62.9% of naturally contaminated meat products that were positive by IMS and culture on CT-SMAC (Chapman et al., 2001d). The most likely reason for the difference in performance in the two studies is the type of samples used. In our earlier study, raw meat samples were purchased from small butchers' shops, at which they may have been refrigerated for several days. Repeat testing in the laboratory of *E. coli* O157-positive samples required freezing and thawing prior to storage at −70 °C for up to 58 months. This almost certainly caused stress to the cells. Growth of *E. coli* O157 may be inhibited by such sublethal injury of the organism at the time of testing (Thippareddi et al., 1995) and this may have prevented recovery to levels detectable by the Reveal™ 8-h test. The majority of the samples were also processed meat samples and contained large numbers of other competing microorganisms which may have inhibited the growth of *E. coli* O157 by bacteriocin production (Hinton et al., 1991, 1992; Baylis et al., 2000) or pH reduction (Bailey et al., 1990; Bailey and Cox, 1992), both of which have an enhanced effect if the target organism is sublethally injured (Kalchayanand et al., 1992). In contrast, the present study used freshly sampled, unprocessed carcass meat inoculated with broth cultures of unstressed *E. coli* O157 cells; these conditions probably reflect more closely those that

would be encountered when examining naturally contaminated beef carcasses soon after slaughter of the animal.

The VIP™ and STAT™ assays differed widely in their performance, although both performed badly when compared to either culture or the Reveal™ assay (Tables 2 and 4). The VIP™ assay detected only 19 (51%), 11 (34.4%) and 1 (3%) of 32 samples inoculated, respectively, with 250, 25 and 2–3 CFU/25 g. The STAT™ assay detected only 9 (28.1%) and 2 (6.3%) of 32 samples inoculated, respectively, with 250 and 25 CFU/25 g and failed to detect *E. coli* O157 in any of the 32 samples inoculated with 2–3 CFU/g. Both the VIP™ and STAT™ assays performed poorly when compared to culture or Reveal™ and would be of little value for detecting the organism on carcass meat.

The numbers of *E. coli* O157 per ml of enrichment culture from the lowest inoculum level of each strain in each medium that was positive by Reveal™ ranged from 1.3×10^4 to 1.6×10^7 (average 1.9×10^6) for sTSB, from 1.5×10^5 to 1.4×10^8 (average 2.8×10^7) for Reveal™ 8-h medium, and from 3×10^4 to 2.9×10^8 (average 3.4×10^7) for Reveal™ 20-h medium. The Reveal™ assay performed least favorably with enrichment cultures in sTSB. This was probably due to the fact that fewer *E. coli* O157 per ml were present in sTSB (average 1.9×10^6) than in Reveal™ 8-h medium (average 2.8×10^7) or Reveal™ 20-h medium (average 3.4×10^7); the lower numbers may have been below the detection limit for the Reveal™ device. The performance of the sTSB medium was also disappointing with IMS and CT-SMAC in the present study, unlike a previous study (Chapman et al., 2001b) where it performed significantly better than buffered peptone water for isolation of *E. coli* O157. Again, this probably reflects differences in the sample types. The sTSB was designed specifically for the recovery of stressed *E. coli* O157 cells and may have been an inappropriate medium for the present study.

In conclusion, the Reveal™, VIP™ and STAT™ assays were all simple and rapid to use giving a result at least 24 h earlier than culture. However, although the Reveal™ assay compared favorably with culture, both VIP™ and STAT™ fell far short of the sensitivity of culture and this would seriously limit their usefulness.

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