

The use of sorbitol-MacConkey agar in conjunction with a specific antiserum for the detection of Vero cytotoxin-producing strains of *Escherichia coli* O 157

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SUMMARY

Using DNA probes specific for the genes encoding Vero cytotoxins 1 and 2 in hybridization experiments on faecal samples, Vero cytotoxin-producing *Escherichia coli* (VTEC) of serogroup O 157 were detected in 21 of 63 cases of haemorrhagic colitis, 9 of 31 cases of non-bloody diarrhoea and 14 of 68 cases of haemolytic uraemic syndrome. Compared with these results sorbitol-MacConkey agar in conjunction with a specific O 157 antiserum gave a sensitivity of 62% in haemorrhagic colitis, 56% in non-bloody diarrhoea and 57% in haemolytic uraemic syndrome. The specificity of this method was 100% in all three groups. This demonstrates that sorbitol-MacConkey agar is a useful screening method for the detection of VTEC of serogroup O 157 when used in conjunction with a specific homologous antiserum. However, this method does not detect VTEC belonging to other serogroups and such strains were found, particularly in cases of haemolytic uraemic syndrome.

INTRODUCTION

Konowalchuk, Speirs & Stavric (1977) described strains of *Escherichia coli* that produced a cytotoxin active on Vero cells and which was termed Vero cytotoxin (VT). The toxin was distinct from the heat-labile (LT) and heat-stable (ST) enterotoxins of *E. coli*. Subsequently two distinct Vero cytotoxins, designated VT1 and VT2, were identified by toxin neutralization experiments (Scotland, Smith & Rowe, 1985; Karmali *et al.* 1986). VT1 has been shown to be very closely related to Shiga toxin and to be neutralized by rabbit antiserum prepared against Shiga toxin (O'Brien & Holmes, 1987). In contrast, anti-Shiga toxin did not neutralize VT2 produced by *E. coli* strain E32511 of serotype O 157.H⁻ (Scotland, Smith & Rowe, 1985). The production of VT1 and VT2 has been shown to be bacteriophage-determined in some strains of *E. coli* (Scotland *et al.* 1983; Smith, Green & Parsell, 1983). Specific DNA probes for VT1 and VT2 have been developed for use in hybridization experiments for the detection of Vero cytotoxin-producing *E. coli* (VTEC) (Newland *et al.* 1985; Willshaw *et al.* 1985, 1987).

VTEC have been implicated in the aetiology of haemorrhagic colitis (HC) and

haemolytic uraemic syndrome (HUS). HC comprises an initial watery diarrhoea followed by gross blood in the stools accompanied by severe abdominal pain and typically with no fever. The disease has been shown to affect all age groups. Outbreaks of HC have been reported in the US and Canada and the vehicles of infection included undercooked hamburger meat and milk (Wells *et al.* 1983; Riley *et al.* 1983; Borczyk *et al.* 1987). Additionally sporadic cases have occurred over wide areas of North America. HUS is characterized by microangiopathic haemolytic anaemia, thrombocytopenia and acute renal failure (Von Gasser *et al.* 1955). The syndrome often follows a gastrointestinal prodromal illness characterized by bloody diarrhoea. Most cases occur in the summer months affecting infants and young children, although adult cases also occur. Although most HUS cases appear to be sporadic, outbreaks have been reported in several countries. A variety of bacteria and viruses have been associated with HUS, but VTEC strains have recently been recognized as important pathogens in this disease (Karmali *et al.* 1983, 1985; Gransden *et al.* 1986).

In July 1985 there was an outbreak of HC in East Anglia (Morgan *et al.* 1988) and in 1983 an outbreak of HUS occurred in the West Midlands (Taylor *et al.* 1986). These events prompted prospective studies to evaluate the role of VTEC in HC and HUS. In a study from October 1985 to October 1986 VTEC were identified in 39% of faecal samples from cases of HC in England and Wales (Smith *et al.* 1987). Thirty of the 32 VTEC strains isolated belonged to serogroup O 157. In a study of children with HUS in the UK there was evidence of VTEC infection in 33% of cases (Scotland *et al.* 1988). VTEC were isolated from 19 cases and in 15 they belonged to serogroup O 157. In the remaining cases the VTEC strains belonged to O serogroups 26, 104, 153 and 163. VTEC of serogroups other than O 157 have also been isolated from patients with HUS and HC in North America (Karmali *et al.* 1985; Bopp *et al.* 1987).

The accumulation of evidence linking VTEC of serogroup O 157 with HC and HUS has required the development of a rapid screening method for this organism in routine stool culture. It has been reported that *E. coli* O 157 VT+ strains are non-sorbitol fermenters (NSF) and this has permitted the development of a MacConkey agar medium containing sorbitol (SMAC agar) instead of lactose in order to differentiate visually the NSF colonies (Farmer & Davis, 1985). A slide agglutination test with a specific O 157 antiserum enabled presumptive identification of the O 157 serogroup (Harris *et al.* 1985; March & Ratnam, 1986; Walker, Upson & Warren, 1988).

In the present study we have evaluated the use of SMAC agar in conjunction with a specific O 157 antiserum as a means of screening for VTEC of serogroup O 157 in faecal samples from cases of HC, non-bloody diarrhoea, (NBD) and HUS. Specific DNA probes provide a sensitive method for the detection of VTEC in faecal samples (Smith *et al.* 1987; Scotland *et al.* 1988) and this has been used as a standard method to assess the sensitivity of SMAC agar plus antiserum for *E. coli* O 157.

MATERIALS AND METHODS

Specimens

Faecal specimens from cases of HC, NBD and HUS were received from clinical laboratories in the UK from April 1986 to April 1987. For the purpose of this study HC cases were defined as patients with an acute episode of diarrhoea associated with frank blood in the stools and from whom no *Salmonella*, *Shigella* or *Campylobacter* sp. was isolated. They also had no recent history of foreign travel or of antibiotic treatment. The HUS cases were diagnosed by the local clinicians and usually had a prodromal bloody diarrhoea. During the study 65 samples from cases of HC, 36 from cases of NBD and 73 from cases of HUS were tested using sorbitol-MacConkey agar plus O 157 antiserum and DNA hybridization techniques. This permitted the determination of the sensitivity and specificity of the SMAC medium used together with the specific antiserum for the detection of the O 157 serogroup. Sensitivity was defined as the proportion of specimens with a positive DNA hybridization result that also gave a positive result using the SMAC agar and O 157 antiserum technique. Specificity was defined as the proportion of specimens with a negative DNA hybridization result that also gave a negative result using SMAC agar and O 157 antiserum (Fletcher, Fletcher & Wagner, 1982).

Preparation of slide agglutinating antiserum

An O 157 rabbit antiserum was prepared by standard methods using as a vaccine a heated suspension (100 °C, 2.5 h) of *E. coli* O 157.H19, strain Abbotstown A2 (Gross & Rowe, 1985). A strain of *E. coli* O 157.H⁻, strain E32511, agglutinated rapidly with the O 157 antiserum and was used as a control.

Testing of faecal specimens for non-sorbitol fermenting organisms

Each faecal specimen was suspended in an equal volume of phosphate-buffered saline and a sterile swab was used to inoculate a sorbitol-MacConkey agar plate (SMAC agar; Oxoid MacConkey agar no. 3 and 1% sorbitol). The inoculum was streaked for single colonies and incubated for 18 h at 37 °C. Where possible five NSF colonies (appearing colourless on the medium) were picked onto MacConkey agar plates (Oxoid CM7) and tested by slide agglutination with the O 157 antiserum. Any agglutinating colonies were subsequently identified biochemically (Cowan, 1974) and their serotype confirmed by tube agglutination using a heated suspension (Gross & Rowe, 1985).

DNA hybridization tests

Serial tenfold dilutions (0.1 ml) of the faecal suspensions were spread on MacConkey agar plates for use in DNA hybridization tests (Willshaw *et al.* 1985). Colonies from dilutions of the faecal samples were transferred onto nylon filters (Hybond-N, Amersham) by replica plating or cultures were spotted onto filters on MacConkey agar plates and incubated for 5–6 h at 37 °C. The filters were then processed for colony hybridization (Maniatis, Fritsch and Sambrook, 1982) and the bacterial DNA fixed by exposure to u.v. for 4 min. Specific radio-labelled DNA probes for VT1 and VT2 (Willshaw *et al.* 1987) were used to test for the presence

of genes determining VT production by colony hybridization with subsequent detection by autoradiography (Willshaw *et al.* 1985).

VT production

VT production was confirmed by testing filtered supernatants of strains for a cytotoxic effect on Vero cells which could be neutralized by antisera raised in rabbits against VT1 and VT2 (Scotland, Day & Rowe, 1980; Scotland *et al.*, 1988).

RESULTS

Haemorrhagic colitis and non-bloody diarrhoea surveys

The ages of the patients ranged from 2 yr 6 months to 86 yr (mean 36 yr) for the HC survey, and from 1 yr to 86 yr (mean 30 yr) for the NBD survey.

A total of 63 of the 65 faecal samples from cases of haemorrhagic colitis gave growth on MacConkey and SMAC agar, non-sorbitol fermenters being identified in 36. Of these, 13 were confirmed as O 157 using the O 157 antiserum (Table 1). When tested by hybridization with specific VT probes, 23 of the 63 (37%) were found to contain strains hybridizing either with one or both of the probes. All 23 strains produced VT as demonstrated on Vero cells, and 21 belonged to the O 157 serogroup (19 were O 157.H⁷ and 2 were O 157.H⁻). The remaining two non O 157 VTEC were of serotypes O 91.H⁻ and O ?H8. These two strains were sorbitol fermenters and therefore not detected on SMAC agar. All *E. coli* O 157 detected by use of SMAC agar plus O 157 antiserum were also detected by probe. Of the eight O 157 VTEC that were missed on SMAC agar, six were present at a frequency of 1% or less, one of 2% and one of 8% as judged by DNA hybridization.

NSF were seen in 16 of the 31 faecal samples from cases of NBD that gave growth on both MacConkey and SMAC agar. Of the 16, 5 were confirmed as O 157 using the O 157 antiserum (Table 1). When tested with the VT probes, *E. coli* O 157 were found in 9 (29%), 8 being serotyped as O 157.H⁷ and one as O 157.H⁻; all produced VT in tissue culture. All *E. coli* O 157 detected by use of SMAC agar and O 157 antiserum were also detected by probe. Of the 4 which were missed with the SMAC medium, 3 had less than 1% VT+ colonies and one had 5%.

In evaluating the use of SMAC agar for the detection of O 157 strains, it was necessary to determine the frequency of NSF organisms other than *E. coli* O 157 in the specimens. Of 63 cases of HC, 36 (57%) grew NSF organisms. Testing of single colonies from each of these samples showed that 23 grew only NSF organisms other than *E. coli* O 157, 8 grew NSF organisms of which all those tested were *E. coli* O 157 and 5 grew a mixed population of NSF organisms including *E. coli* O 157. Of the NSF colonies tested from the latter group, the number of *E. coli* O 157 colonies present ranged from 1 to 4 out of 5 colonies. NSF organisms other than *E. coli* O 157 were therefore present in 28 of the specimens, a frequency of 44%.

Of the faecal specimens from 31 cases of NBD, 16 (52%) grew NSF organisms. In 11 of these none of the NSF colonies agglutinated with the *E. coli* O 157 antiserum, while in 3 all the NSF colonies tested were *E. coli* O 157 and in 2 there was a mixed population of NSF organisms including *E. coli* O 157 (1 of 5 and 3 of

Table 1. Detection of VTEC by the use of SMAC agar and O 157 antiserum compared with VT probes in cases of haemorrhagic colitis and non-bloody diarrhoea

	No. of samples	Non-sorbitol fermenters		VTEC (DNA probes)	O 157 VT+ (DNA probes)
		O 157 + ve	O 157 - ve		
Haemorrhagic colitis (HC)	63 (100)	13 (21)	23 (37)	23 (37)	21 (33)
Non-bloody diarrhoea (NBD)	31 (100)	5 (16)	11 (36)	9(29)	9 (29)

Figures in parentheses are percentages.

5 NSF colonies respectively). NSF organisms other than *E. coli* O 157 were present in 13 of the specimens, a frequency of 42%.

Haemolytic uraemic syndrome survey

The ages of the patients ranged from 1 month to 80 yr (mean 6 yr). Sixty-eight yielded coliform growth on MacConkey agar but only 65 on SMAC agar. *E. coli* O 157 was detected in 8 (12%) of these 65 specimens when tested with the specific O 157 antiserum. When all 68 specimens were hybridized with the specific VT probes, VTEC were detected in 20 (29%) (Table 2). Fourteen were serotyped as O 157.H7 and included the 8 detected by use of SMAC agar plus O 157 antiserum; the remaining 6 were serotyped as O 26.H11, O 55.H16, O 105ac.H18, O 145.H25, O 163.H19 and O 165.H19.

Of the 14 HUS cases positive for *E. coli* O 157 by probe, 11 (79%) had prodromal bloody diarrhoea and 3 (21%) had non-bloody diarrhoea. In those 6 cases where *E. coli* O 157 was detected by probe but not by SMAC agar plus O 157 antiserum, one specimen yielded no growth on SMAC agar, 4 specimens grew 1% or fewer VT+ colonies and a sixth sample grew 7%. From the 65 specimens that grew on SMAC agar, 34 (52%) grew NSF organisms. In 26 none of the NSF colonies agglutinated with the O 157 antiserum, while in 5 all the NSF colonies tested were *E. coli* O 157 and in 3 there was a mixed population of NSF organisms including *E. coli* O 157. Of the NSF colonies tested from the latter group, the number of *E. coli* O 157 colonies present ranged from 1 out of 4 to 4 out of 5 colonies. NSF organisms other than *E. coli* O 157 were present in 29 of the specimens, a frequency of 45%.

Specificity and sensitivity using SMAC agar and *E. coli* O 157 antiserum

SMAC agar detected NSF organisms which were not *E. coli* O 157 but belonged to the genera *Proteus*, *Enterobacter*, *Hafnia* and *Pseudomonas* as well as *E. coli* belonging to serogroups O 8, O 18ac and O 33. None of these NSF strains agglutinated with the O 157 antiserum. Because of the large number of NSF organisms that were not *E. coli* O 157, the specificity of the SMAC medium alone

Table 2. *Detection of VTEC by the use of SMAC agar and O 157 antiserum compared with VT probes in cases of haemolytic uraemic syndrome*

Prodrome	No. of samples	Non-sorbitol fermenters		VTEC (DNA probes)	O 157 VT+ (DNA probes)
		O 157 +ve	O 157 -ve		
Bloody diarrhoea syndrome	47 (69)	7	17	15	11
Non-bloody diarrhoea	14 (21)	1	6	4	3
No diarrhoea	7 (10)	0	3	1	0
Total	*68 (100)	8 (12)	26 (38)	20 (29)	14 (21)

Figures in parentheses are percentages.

* Only 65 samples gave growth on SMAC agar.

Table 3. *Sensitivity and specificity of SMAC agar plus antiserum for E. coli serogroup O 157*

	Sensitivity	Specificity	
		SMAC alone	SMAC plus antiserum
HC	13/21 (62)	19/42 (45)	42/42 (100)
NBD	5/9 (56)	11/22 (50)	22/22 (100)
HUS	8/14 (57)	28/54 (52)	54/54 (100)

Figures in brackets are percentages.

for the detection of the O 157 serogroup was between 45 and 52% for all surveys. In conjunction with a specific O 157 antiserum the specificity was 100% in all surveys (Table 3).

E. coli O 157 was found in 13 (21%) of 63 HC cases using the SMAC agar and O 157 antiserum, while VT probes indicated that 21 (33%) were positive for O 157. This gave a sensitivity of 62% for the SMAC medium plus the O 157 antiserum. The accuracy of the test, defined as the proportion of all test results, both positive and negative, which were correct in both the DNA hybridization method and by the use of SMAC agar plus antiserum was 87% for the HC survey. Similarly, in both other surveys the probe method detected *E. coli* O 157 in a greater proportion of samples giving a sensitivity of 56% at an accuracy of 87% for the NBD survey and a sensitivity of 57% at an accuracy of 91% for the HUS survey.

DISCUSSION

The principal VTEC serogroup found to be associated with HC and HUS is *E. coli* O 157. The failure of VTEC strains of this serogroup to ferment sorbitol within 24 h provides a simple but relatively non-specific screening test. SMAC agar used in conjunction with *E. coli* O 157 antiserum allows a presumptive identification of *E. coli* O 157 upon primary isolation from stool cultures. It has been noted that

growth in the presence of sorbitol can induce sorbitol fermenting mutants (March & Ratnam, 1986) and therefore care must be taken on repeat testing.

The specificity of the SMAC medium alone for *E. coli* O 157 serogroup was only 45–52% in the NBD, HC and HUS surveys, due to the presence of non-sorbitol fermenting organisms other than *E. coli* O 157. However, in conjunction with a specific O 157 antiserum, false-positive identifications were not made, giving a specificity of 100%. Non-sorbitol fermenting strains of *Escherichia hermanni*, *Brucella abortus*, *Yersinia enterocolitica* serogroup O 9 and some serotypes belonging to *Salmonella* group N have been shown to give cross-reactions with a specific antiserum for *E. coli* O 157 (Lior & Borczyk 1987; Notenboom *et al.* 1987). Such cross-reactions indicate the necessity for the biochemical identification of presumptive positive organisms (Haldane *et al.* 1986).

Taking the sensitivity of the specific VT DNA probes as 100% the sensitivity of the SMAC medium plus O 157 antiserum for the detection of *E. coli* O 157 in the HC, NBD and HUS surveys was 62, 56 and 57% respectively. The failure to detect some VT+ O 157 by SMAC agar and antiserum could be explained by at least two observations. Firstly, in some cases the percentage of *E. coli* O 157 in the faecal samples, as indicated by the DNA probe tests, was low and NSF colonies were not visible on SMAC agar. Secondly there was a high frequency of other non-sorbitol fermenters, and since only five NSF colonies from each SMAC plate were tested with the O 157 antiserum, the likelihood of detecting an O 157 was significantly reduced. The specific VT probes were able to detect VT+ colonies in samples where they represented less than 1% of all colonies, whereas the use of the screening medium and antiserum failed to detect VT+ *E. coli* O 157 present at frequencies of 8% or less.

Although *E. coli* O 157 is the serogroup most commonly associated with HC and HUS, VTEC of many other serogroups have been identified (Karmali *et al.* 1985; Bopp *et al.* 1987; Smith *et al.* 1987; Scotland *et al.* 1988) but all have been shown to ferment sorbitol. The use of SMAC medium together with a specific O 157 antiserum therefore allows a rapid and useful screening method for the detection of VT+ *E. coli* O 157 on primary culture in the clinical laboratory. The use of such a screening medium together with a specific antiserum was more successful in the study of HC where serogroups other than the O 157 were rarely isolated. In contrast 30% of the VTEC associated HUS cases studied can be attributed to these non O 157 serogroups.

This study clearly shows that the frequent presence of NSF other than *E. coli* O 157, necessitates the use of a specific O antiserum to support SMAC for the detection of VTEC of serogroup O 157.

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