Heterogeneity of *Escherichia coli* Phages Encoding Vero Cytotoxins: Comparison of Cloned Sequences Determining VT1 and VT2 and Development of Specific Gene Probes

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Phages coding for production of Vero cytotoxins VT1 or VT2 in strains of *Escherichia coli* serotype O157.H7 or O157.H⁻ were morphologically indistinguishable. Their genome size and restriction enzyme digests of the phage DNA were similar. These phages were clearly different in these respects from a VT1-encoding phage isolated from a strain of *E. coli* O26.H11 (H19). However the VT1 region cloned from the phage originating in the *E. coli* O157.H7 strain was identical to the VT1 region previously cloned from the phage carried by H19. Sequences encoding VT2 that were cloned from the phage in *E. coli* O157.H⁻ have been mapped and the VT2 region identified by transposon insertion. The cloned regions coding for VT1 or VT2 production had no similarities in the presence of restriction enzyme sites over a distance of about 2 kb, and two VT1-specific probes spanning a region of about 1.4 kb did not hybridize under stringent conditions with cloned VT2 DNA. A 2 kb *HincII* fragment contained the VT2 genes but hybridized to VT1-encoding phages and recombinant plasmids via flanking phage DNA. A 0.85 kb *AvaI*–*PstI* fragment was a specific probe for VT2 sequences and did not hybridize under stringent conditions to phages or plasmid recombinants encoding VT1.

INTRODUCTION

Certain strains of *Escherichia coli* have been shown to produce a heat-labile cytotoxin active on Vero cells (Konowalchuk *et al.*, 1977). The Vero cytotoxin (VT) is clearly distinguishable from the heat-labile and heat-stable enterotoxins of *E. coli* and has been reported to be closely related to Shiga toxin produced by strains of *Shigella dysenteriae* type 1 (O'Brien *et al.*, 1982; O'Brien & LaVeck, 1983). Production of VT has been detected in certain enteropathogenic *E. coli*, particularly strains of serogroup O26, isolated from cases of diarrhoea (Wade *et al.*, 1979; Scotland *et al.*, 1980) and also in *E. coli* strains associated with cases of haemorrhagic colitis (HC) (Johnson *et al.*, 1983; Riley *et al.*, 1983) and haemolytic uraemic syndrome (HUS) (Karmali *et al.*, 1983; Gulley, 1984; Taylor *et al.*, 1986). Recent studies of *E. coli* strains belonging to serotype O157.H7 or O157.H⁻ isolated from cases in Britain of HUS or bloody diarrhoea have shown that two distinct VTs can be identified in neutralization experiments (Scotland *et al.*, 1985). These two toxins have been designated VT1 which is closely related to Shiga toxin, and VT2 identified in certain strains of O157. The terms Shiga-like toxin I (SLTI) and SLTII have also been used to describe the toxins VT1 and VT2 (Strockbine *et al.*, 1986).

The genes controlling production of VT are phage encoded in several *E. coli* strains. This was first demonstrated in the O26.H11 strain H19 isolated from a case of infantile diarrhoea (Scotland *et al.*, 1983; Smith *et al.*, 1983). VT phages have also been detected in strains of

Abbreviations: HUS, haemolytic uraemic syndrome; VT, Vero cytotoxin.

serogroup O128 and in other O26 isolates (Smith *et al.*, 1983) and in *E. coli* of serotype O157.H7 or O157.H⁻ (O'Brien *et al.*, 1984; Smith *et al.*, 1984). The VT phages from H19 (termed phage H19J) and O157.H7 strain 933 (phage 933J) had very similar morphology when examined by electron microscopy, and comparison of phage-encoded polypeptides and restriction enzyme analysis of the phage DNAs also indicated close relatedness (O'Brien *et al.*, 1984). The genes controlling production of VT have been cloned in *E. coli* K12 from phages originating in strains H19 (Willshaw *et al.*, 1985; Huang *et al.*, 1986) and 933 (Newland *et al.*, 1985). In the study of Willshaw *et al.* (1985) mapping of the cloned region from H19 led to the development of a specific DNA probe for testing strains for the presence of VT genes. Colony hybridization studies with VT⁺ strains of serogroup O157 showed that only some of these strains hybridized with the H19 probe. A second VT probe was developed from the cloned VT genes derived from a phage in strain E32511, serotype O157.H⁻, which was one of the strains that did not hybridize with the H19 probe (Willshaw *et al.*, 1985). These two types of VT genes identified by hybridization correspond to the VT1 and VT2 toxins defined by neutralization experiments (Scotland *et al.*, 1985).

In this paper we have examined VT phages from strains of serogroup O157 coding for production of either VT1 or VT2. These phages were compared to the H19 VT phage by electron microscopy and also by characterization of the cloned VT sequences using restriction enzyme analysis and DNA hybridization studies.

METHODS

Bacterial strains, phages and plasmids. The wild-type VT-producing *E. coli* strains and the VT phages are listed in Table 1. In this paper we will refer to the VT phages by the strain from which they were isolated and the type of VT they encode, e.g. ϕ 30480 (VT1), unless the VT type is clear from the text. Strain 933 was kindly provided by Dr A. D. O'Brien. Production of Vero cytotoxins VT1 and VT2 by these strains has been reported previously (Scotland *et al.*, 1985). *E. coli* K12 AB1157 (Bachmann, 1972) was used for propagation of the VT phages and AB1157 and HB101 (Maniatis *et al.*, 1982) were used as recipients in the transformation experiments. The plasmid pACYC184 (Chang & Cohen, 1978), encoding chloramphenicol and tetracycline resistance, was the vector in gene cloning experiments.

Tests for VT production. Large numbers of colonies were screened for VT production by a rapid method (Willshaw *et al.*, 1985) and VT production was confirmed using sterile culture supernatants as described previously (Scotland *et al.*, 1980).

Preparation of phages and phage DNA. Methods used for the isolation and preparation of the VT phages and phage DNA have been reported previously (Scotland *et al.*, 1983; Smith *et al.*, 1984; Willshaw *et al.*, 1985).

Electron microscopy of VT phages. Phage preparations for electron microscopy were obtained from caesium chloride gradients and contained between 10^{11} and 10^{12} p.f.u. ml⁻¹ (Willshaw *et al.*, 1985). Phage suspension was mixed with an equal volume of 0.05% bovine plasma albumin and then with a further two volumes of 3% (w/v) phosphotungstic acid pH 6.3. A drop was applied to a Formvar-carbon-coated grid and surplus liquid was removed with filter paper. Alternatively the bacteriophage suspension, mixed as before with bovine plasma albumin, was applied to a grid, excess liquid was removed and the grid was stained with 2% (w/v) aqueous uranyl acetate.

DNA techniques. Purified DNA of vector and recombinant plasmids was prepared by the method of Humphreys *et al.* (1975). For rapid screening, DNA of recombinant plasmids was extracted as described by Birnboim & Doly (1979). Restriction endonuclease treatment, recombinant DNA techniques and gel electrophoresis were as described previously (Willshaw *et al.*, 1985). Transfer of DNA fragments to nitrocellulose or nylon (Hybond-N, Amersham) membranes was by the method of Southern (1975). DNA fragments for hybridization probes were electroeluted from preparative agarose gels (1%, w/v) or acrylamide/agarose gels (3%:0.5%, each w/v) and purified using Schleicher & Schüll 'Elutips'. Fragments were labelled by nick translation using deoxyadenosine 5'- α -[³²S]thiotriphosphate (Amersham). Hybridization was done under stringent conditions as described previously (Willshaw *et al.*, 1985).

Transposon mutagenesis. Plasmid mutants that no longer coded for production of VT were obtained by insertion of Tn1000 as described by Willshaw *et al.* (1985).

RESULTS

Morphology of bacteriophages

The phages examined, their sources and the VT that they encode are shown in Table 1. As reported before (Scotland *et al.*, 1983) ϕ H19 (VT1) phage particles had elongated hexagonal

Table 1. Wild-type VT-producing *E. coli* strains

Strain	Origin	Serotype	VT(s) produced	VT phage studied
H19	Diarrhoea, England, 1965	O26. H11	VT1	ϕ H19 (VT1)*
E30480†	Bloody diarrhoea, England, 1983	O157. H7	VT1, VT2	ϕ 30480 (VT1)
E32511	HUS, England, 1983	O157. H ⁻	VT2	ϕ 32511 (VT2)
933†	Haemorrhagic colitis, USA, 1982	O157. H7	VT1, VT2	ϕ 933 (VT2)

* Evidence presented in this paper indicates that ϕ H19 is probably the same as phage H19J (O'Brien *et al.*, 1984).

† Strain E30480 was presumed to carry a second phage encoding VT2 (Scotland *et al.*, 1985) (see Discussion). Strain 933 has been shown to carry two phages termed 933J, encoding VT1, and 933W (O'Brien *et al.*, 1984; Strockbine *et al.*, 1986). The VT2 phage ϕ 933 above is probably identical to 933W.

heads and non-contractile flexible tails. In full particles (40 measured) the mean dimensions of the head were 117 nm long and 67 nm wide (ranges 111–113 nm and 61–72 nm, respectively), and of the phage tails (27 measured) were 177 nm long and 12 nm wide (ranges 166–194 nm and 11–14 nm, respectively). In contrast ϕ 30480 (VT1) and ϕ 32511 (VT2) had regular hexagonal heads and shorter, contractile tails. The ϕ 30480 (VT1) full particle heads (35 examined) measured 74 nm long (range 67–79 nm) and 74 nm wide (range 67–79 nm) with tails (15 measured) 28 nm long (range 16–33 nm) and 11 nm wide. Particles of ϕ 32511 (VT2) (17 measured) were morphologically indistinguishable from ϕ 30480 (VT1) and these phages were clearly different from ϕ H19 (VT1) (Fig. 1).

Comparison of phage DNA

DNA was prepared from phage suspensions as described in Methods and digested with restriction endonucleases. The molecular size of the phage DNA was estimated from summation of the *EcoRI* fragments. This enzyme cuts all the phage DNAs into an unambiguous number of fragments that can be sized reproducibly: ϕ 32511 (VT2) was 68 kb (mean of six determinations) and ϕ 30480 (VT1) was 73 kb (mean of five determinations). The phage DNAs were significantly larger than that of ϕ H19 (VT1) (51 kb, mean of five determinations) and were similar to that of ϕ 933 (VT2) which we have estimated to be 70 kb (mean of four determinations).

The five fragments of ϕ 32511 (VT2), ϕ 30480 (VT1) and ϕ 933 (VT2) obtained by *BamHI* digestion were indistinguishable in size (> 23, 6.30, 3.05, 0.88 and 0.39 kb) although some variation in size of the largest fragment would not have been detected on our gels. In contrast ϕ H19 (VT1) yielded seven *BamHI* fragments, none of which was the same size as those of the phages from the O157 strains. Treatment with *EcoRI* to give 10 or 11 fragments distinguished ϕ 32511 (VT2) and ϕ 30480 (VT1) from each other and from ϕ 933 (VT2) although the overall similarities of the phages were indicated by three fragments common to all the digests and a further five of similar size in at least two phages. The frequently cutting enzyme *HincII* was also useful to distinguish the phages from the O157 strains which were cleaved into at least 20 fragments.

Comparison of cloned VT sequences

The restriction maps shown in Fig. 2 represent regions containing VT sequences cloned from three phages. The cloning of VT1 from ϕ H19 (VT1) has been reported previously (Willshaw *et al.*, 1985) and the derivative NTP704 is shown here for comparative purposes (Fig. 2a). DNA of ϕ 30480 (VT1) was digested with *EcoRI* and fragments were ligated with similarly cleaved pACYC184. Of 40 transformants tested for VT production, three were VT⁺ and one carried a

Fig. 1. Electron microscopy of VT phages. Phage suspensions were stained with either 3% (w/v) phosphotungstic acid (*a*, *b*, *d*) or 2% (w/v) uranyl acetate (*c*) as described in Methods. Bar, 100 nm. (*a*) Phage from strain H19, serotype O26.H11, that codes for production of VT1. (*b*) Phage from strain E32511, serotype O157.H⁻, that codes for production of VT2. (*c*, *d*) Phage from strain E30480, serotype O157.H7, that codes for production of VT1.

recombinant plasmid (NTP708) with a molecular size of 8.4 kb whereas the others carried larger plasmids. The restriction enzyme map of the inserted DNA (4.4 kb) in NTP708 is shown in Fig. 2(*b*). We have reported that VT2 sequences could be cloned from ϕ 32511 (VT2) on a 4.7 kb *EcoRI* fragment (Willshaw *et al.*, 1985); the restriction map of this fragment carried in NTP707 has now been determined and shown in Fig. 2(*c*).

Comparison of the fragments cloned in NTP704 and NTP708 showed that central regions of these cloned DNAs, extending over about 3 kb from the leftmost *EcoRV* site, were identical in distribution of restriction sites for five restriction enzymes, namely *HincII*, *EcoRV*, *BglII*, *HindIII*, and *PvuII*. The area included the region of NTP704 that has been shown to be associated with VT1 production (Willshaw *et al.*, 1985) and which lies adjacent to the *HindIII* site within the cloned DNA. The 0.75 kb *HincII* fragment of NTP704 (Fig. 2*a*) used as a specific VT1 probe (Willshaw *et al.*, 1985) hybridized under stringent conditions with NTP708 (see below) to show the presence of a homologous 0.75 kb *HincII* fragment.

Fig. 2. Restriction enzyme maps of DNA encoding VT1 or VT2. Fragments from the phages indicated were cloned as described in the text to give the recombinant plasmids NTP704, NTP707 and NTP708. In all cases the vector, which is not shown in the figure, was pACYC184. The restriction maps of the DNA inserts of NTP704 and NTP708 have been aligned to emphasize the distribution of restriction sites. The map of NTP707 shows sites of *Tn1000* insertion as vertical arrows; from left to right, the number of independent insertion mutants obtained at each site was 2, 1, 3, 3, 1 and 1. Horizontal arrows above the maps show the restriction fragments that were used as probes in DNA hybridization. A, *Ava*I; B, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI; Ev, *Eco*RV; H, *Hind*III; Hc, *Hinc*II; P, *Pst*I; Pv, *Pvu*II. *, Extent of a *Hae*III fragment of NTP704 that is present in NTP706, the smallest cloned derivative we have that codes for VT1 production (see text and Willshaw *et al.* 1985).

The *Eco*RI fragment cloned from ϕ 32511 (VT2) was of a similar size to that in NTP708 but there appeared to be no common features in the restriction maps over the central region of about 2 kb. However, in the region to the left and right of this central area there were similarities to NTP708 in the distribution of restriction sites for *Hinc*II, *Eco*RV, *Ava*I, *Pvu*II and *Eco*RI.

Location of VT2 sequences in NTP707

Under conditions of low stringency it was found that the 0.75 kb VT1 probe derived from ϕ H19 (VT1) hybridized weakly to a 2 kb *Hinc*II fragment of ϕ 32511 (VT2) and NTP707 (Willshaw *et al.*, 1985). This result suggested the likely location of VT sequences in NTP707 (Fig. 2c) but the gene was mapped more precisely by transposon mutagenesis. A series of mutants of NTP707 carrying *Tn1000* was generated as described in Methods. Eleven strains were obtained that no longer produced VT and that carried a plasmid about 6 kb larger than NTP707 indicating that insertion of *Tn1000* had occurred. These plasmids were digested with *Eco*RI, *Bam*HI or *Xba*I and the fragments compared with those of NTP707 and with the known restriction map of *Tn1000* (Guyer, 1978). The position of these insertions of *Tn1000* and the number of independent mutants obtained are shown in Fig. 2(c).

All the *Tn1000* insertions leading to loss of VT production mapped within the right-hand half of the 2 kb *Hinc*II fragment of NTP707 that had been implicated as a possible site for VT2 sequences (Fig. 2c). Two insertion sites were immediately to the left of the *Ava*I site present in this fragment and nine lay within a 0.85 kb fragment bounded by this *Ava*I site and its adjacent *Pst*I site. This *Ava*I-*Pst*I fragment was isolated and examined as a potential probe for VT2 sequences.

Table 2. *Specificity of VT probes*

Unlabelled plasmid or phage DNA	Hybridization with labelled probe fragment*			
	VT1 probes		VT2 probes	
	0.75 kb	0.65 kb	2 kb	0.85 kb
	<i>HincII</i>	<i>HindIII-BglII</i>	<i>HincII</i>	<i>AvaI-PstI</i>
NTP704	+	ND	+	—
NTP706†	+	+	—	—
NTP708	+	+	+	—
NTP707	—	—	+	+
φH19 (VT1)	+	+	+	—
φ30480 (VT1)	+	+	+	—
φ32511 (VT2)	—	—	+	+
φ933 (VT2)	—	—	+	+

ND, Not determined.

* The extent of the probe fragments is shown in Fig. 2. Hybridization and washing were under stringent conditions (see Methods).

† NTP706 is a smaller derivative of NTP704 that codes for VT production (see Fig. 2 and Willshaw *et al.*, 1985).

Specificity of VT probes

From the appropriate digests of NTP707, the 2 kb *HincII* fragment and 0.85 kb *AvaI-PstI* fragment were purified from agarose or acrylamide/agarose gels and labelled by nick translation for use as VT2 probes. Hybridization was carried out with Southern blots of *HincII*-digested DNA of recombinant plasmids NTP704, NTP706, NTP708 and NTP707, and with *HincII*-treated phage DNAs. The recombinant plasmid NTP706, derived from φH19 (VT1), is the smallest we have that still codes for VT1 production (Willshaw *et al.*, 1985), and contains a *HaeIII* fragment of 2.5 kb indicated on Fig. 2(a).

Under stringent conditions the 2 kb *HincII* fragment hybridized to all the VT1-encoding and VT2-encoding recombinant plasmids except NTP706, which presumably contained the smallest amount of phage DNA flanking VT1-associated sequences (Table 2). In the case of NTP704 and NTP708, hybridization was to *HincII* fragments of 2 kb and 1.2 kb, respectively, which were located to the left of the 0.75 kb *HincII* VT1 probe fragment (Fig. 2a, b). The intervening *HincII* fragment of about 0.2 kb was lost from the gel used for Southern blotting. When the *AvaI-PstI* probe from NTP707 was used in hybridization no homology was detected under stringent conditions with any of the recombinant plasmids carrying VT1 sequences (Table 2). Similar results were obtained for hybridization of the probe fragments to *HincII*-digested phage DNA: the 2 kb probe detected both the VT1-encoding phages φH19 and φ30480 and the VT2 phages φ32511 and φ933, whereas the two classes were clearly differentiated by the *AvaI-PstI* probe. It appeared likely that the homology detected between the 2 kb probe and the VT1-encoding phages and recombinant plasmids was due to phage sequences present in the probe flanking the VT2 sequences.

We had previously found that some *Tn1000* insertions that led to loss of VT1 production mapped to the left of the 0.75 kb *HincII* fragment of NTP704 used as a VT1 probe (Willshaw *et al.*, 1985). These mutations lay within a *HindIII-BglII* fragment (Fig. 2a) that was encompassed by the *HincII* fragment of NTP704 that hybridized with the 2 kb VT2 probe. This *HindIII-BglII* fragment was labelled and hybridized to digested DNA of NTP708, NTP707 and the VT-encoding phages (Table 2). The probe hybridized under stringent conditions with its homologous fragment in NTP708 (Fig. 2b) but not with any fragment of NTP707. It also hybridized with φH19 (VT1) and φ30480 (VT1) but not with φ32511 (VT2) or φ933 (VT2). The hybridization of this probe therefore exactly paralleled that of the 0.75 kb VT1 probe. These results also indicated that the homology detected by the 2 kb VT2 probe in NTP704 and NTP708 was flanking phage DNA located to the left of the *BglII* site in these plasmids (Fig. 2). The total region of 1.4 kb spanned by the two specific VT1 probes had no homology under stringent conditions with VT2 sequences in NTP707 or φ32511 (VT2).

DISCUSSION

Comparison of a limited number of VT-encoding phages indicated that strains of *E. coli* O157.H7 or H⁻ contain closely related phages that may carry different VT genes. Thus ϕ 30480 and ϕ 32511, which are morphologically indistinguishable and have similar DNA restriction digest patterns, encode, respectively, VT1 and VT2. O'Brien *et al.* (1984) reported that a Verotoxin-converting phage designated 933W was spontaneously released from the O157 strain 933. Our results show that this phage, which encodes VT2 (= Shiga-like toxin II) (Strockbine *et al.*, 1986), also belongs to this family of O157 phages determining VT production. Serological evidence and DNA hybridization indicated that some strains of O157 we have examined, such as E30480, produced both VT1 and VT2 (Scotland *et al.*, 1985) (Table 1) but hybridization of a VT2-specific probe to ϕ 30480 (VT1) indicated that this phage did not carry VT2 sequences. Recent experiments in our laboratory have shown that a VT2-encoding phage is released after UV induction of E30480 (P. Rietra, personal communication) but this phage remains to be characterized. Although not reported so far, it is possible that both toxin genes could be carried on a single phage in some *E. coli* strains.

The VT-encoding phages from the O157 strains were morphologically distinct from ϕ H19 (VT1), from *E. coli* O26 strain H19, and the molecular size of the phage DNA and restriction enzyme digests also indicated that ϕ H19 (VT1) was clearly distinguishable. The morphology of ϕ H19 (VT1) resembles that of phage H19J (O'Brien *et al.*, 1984) although detailed comparison is not possible as the dimensions of H19J were not stated. The size of the ϕ H19 (VT1) genome is similar to that reported by O'Brien *et al.* (1984) for H19J (47 kb) and although our results indicate a larger number of *Bam*HI and *Eco*RI fragments for ϕ H19, it is likely that these phage isolates are the same. It has been reported by O'Brien *et al.* (1984) that the *E. coli* O157 strain 933 carries a VT1-encoding phage 933J that is identical or closely similar to H19J (= H19A) (Smith *et al.*, 1983) or ϕ H19 (VT1). So far we have not found a phage resembling ϕ H19 (VT1) in strains of *E. coli* O157. A wider study is required to survey the role of phages in determination of VT production in the *E. coli* serotypes in which this property has been found.

Several groups have now reported the cloning of the structural genes determining VT1 production from phages in strains H19 or 933 (Newland *et al.*, 1985; Willshaw *et al.*, 1985; Huang *et al.*, 1986) and the restriction maps of the cloned regions are similar. Polypeptides expressed by the cloned fragments and subclones derived from them have been studied in *E. coli* minicells (Newland *et al.*, 1985) or by *in vitro* transcription/translation (Huang *et al.*, 1986). Vero cytotoxin VT1 comprises two polypeptide subunits that correspond to the A and B subunits of Shiga toxin (Olsnes & Eiklid, 1980; O'Brien & LaVeck, 1983) and expression of these was demonstrated in the above studies. Comparison of the regions estimated to encode these subunits with the extent of the fragments we have used as probes indicates that the 0.75 kb *Hinc*II probe for VT1 contains sequences encoding the B subunit, whereas the 0.65 kb *Hind*III-*Bgl*II fragment includes A subunit sequences. It is also probable that previously obtained insertions of Tn1000 into cloned VT1 DNA that led to loss of VT production (Willshaw *et al.*, 1985) lay within both A and B subunit regions. Huang *et al.* (1986) reported that the smallest region that expressed VT1 was a 1.7 kb *Bal*I-*Bgl*II fragment from the VT phage of strain H19; this lies within a 2.5 kb *Hae*III fragment of NTP704 that was cloned in NTP706 (see above and Willshaw *et al.*, 1985) and is indicated by asterisks on Fig. 2(a). The restriction maps of the cloned fragments from ϕ H19 (VT1) and ϕ 30480 (VT1) are indistinguishable over a longer span of about 3 kb and must include flanking phage sequences particularly to the left of the VT region.

So far, genes determining VT2 production have been cloned from only one source (Willshaw *et al.*, 1985) and detailed mapping studies presented here indicate no similarities in the restriction maps of the VT1 and VT2 genes. Under stringent conditions no hybridization was detected between either the VT1 probe and NTP707 or ϕ 32511 (VT2) although at reduced stringency the 0.75 kb VT1 probe hybridized weakly to a 2 kb *Hinc*II fragment of NTP707 or ϕ 32511 (VT2) that contained part of the VT2 sequences (Willshaw *et al.*, 1985). Thus the VT genes are probably related but not closely homologous. Recently Strockbine *et al.* (1986) reported that a recombinant plasmid carrying the VT1 genes from phage 933J on a 3 kb DNA

fragment hybridized to the VT2 phage 933W. Comparison of the cloned fragment with our map shows that it is likely to contain flanking phage DNA on either side of the VT region and it is likely that the observed homology was with phage sequences. The importance of probe specificity was clear when hybridization reactions of our original 2 kb VT2 probe were compared with those of the specific *AvaI*-*PstI* fragment probe for VT2. The larger probe detected VT1-producing strains, but via homology with phage DNA and not through the VT genes themselves, and it has now been replaced by the more specific probe.

DNA probes for VT have been developed to identify Vero-cytotoxin-producing *E. coli* and to detect their presence in faecal specimens. The use of these probes in cases of HUS in Britain will be described separately (Scotland *et al.*, 1987). Although two types of VT have been identified at present, it is possible that other members of a family of VT genes will be identified, necessitating the development of further probes to study the occurrence of VT.

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