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Development of a Simple Latex Agglutination Assay for Detection of Shiga Toxin-Producing *Escherichia coli* (STEC) by Using Polyclonal Antibody against STEC▽

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Rabbit antiserum raised against the whole-cell antigen of Shiga toxin-producing *Escherichia coli* (STEC) strain VT3 (stx_1^+ stx_2^+ eae^+) was repeatedly adsorbed with heat-killed cells of different non-STEC strains and other enteric bacteria. Thus, the antiserum obtained was designated VT3 antiserum. VT3 antiserum reacted with intimin type γ. We assessed the reactivity of VT3 antiserum to whole-cell lysates of 87 strains of *E. coli* and other enteric bacteria by immunoblotting. The antiserum recognized the 97-kDa protein in whole-cell lysate from strain VT3, and 36 (83.7%) of the 43 STEC strains were positive for the STEC antigen. None of the non-STEC strains or strains of other species examined tested positive by immunoblotting. Based on this result, we developed a latex agglutination assay for the detection of STEC strains. Thirty-five (81.4%) of the 43 STEC strains tested positive for the STEC antigen by the latex agglutination assay. One (3.3%) of the 30 non-STEC strains and none of the strains of the other enteric bacteria included in this study tested positive by the latex agglutination assay. The corresponding specificity of the latex agglutination assay was approximately 98%. Results of this study showed the production of STEC antiserum and the generation of a simple, cost-effective, sensitive, and specific latex agglutination assay for establishing an etiological diagnosis of STEC.

Shiga toxin-producing *Escherichia coli* (STEC), predominantly of serotype O157:H7, is now one of the most important etiologic agents in hemorrhagic colitis and hemolytic-uremic syndrome (6, 7, 8, 12, 15, 30). The ability of STEC to cause serious disease in humans is related to the production of one or more Shiga toxins (stx_1, stx_2, or their variants), which inhibits protein synthesis of host cells, thus leading to cell death (13, 20). STEC bacteria comprise a serologically diverse group of food-borne, zoonotic pathogens, of which those of serotype O157:H7 have been epidemiologically significant worldwide because they are notoriously associated with life-threatening disease (12). However, in some geographic areas, non-O157 strains are more commonly isolated from persons with diarrhea or hemolytic-uremic syndrome than are O157 STEC strains (25, 28). Hemorrhagic colitis is caused by a number of serotypes of STEC (15). Antibodies to the O157 antigen are used in many assays to detect O157:H7 isolates in clinical and food samples. However, previous studies showed that the anti-O157 sera cross-reacted with *Citrobacter freundii* and other bacterial species (4, 24). Although detection of enteropathogenic *E. coli* (EPEC) and enterohemorrhagic *E. coli* (EHEC) by using a monoclonal antibody has been reported earlier (14), the development of monoclonal antibodies is expensive for many laboratories. Biochemical methods of identifying strains of EHEC, a subgroup of STEC, are based on biochemical markers such as sorbitol fermentation deficiency and β-D-glucuronidase nonproductivity of the O157 serotype of *E. coli* (10, 21). The existence of sorbitol-fermenting and β-D-glucuronidase-positive O157 strains reduces the reliability of these phenotypes (9). Molecular biology-based detection systems for the diagnoses of STEC (3, 5, 16, 18, 23, 26, 29) are too expensive for many laboratories. In this paper, we describe the production of antisera specific for STEC as well as their use in developing a simple assay system for the detection of STEC.

MATERIALS AND METHODS

Bacterial strains. The strains used in this experiment were *E. coli* O157:H7 strain EDL933 (STEC; stx_1^+ eae^+), VT3 (STEC; stx_1^+ stx_2^+ eae^+), enterotoxigenic *E. coli* (ETEC) (O125; stx_1 stx_2 *eae*), and 43 other strains of Shiga toxin (stx_1 or stx_2)-producing *E. coli* isolated from different sources in Kolkata, India. Shiga toxin-nonproducing *E. coli* (stx_1 stx_2 *eae*) strains DH5α, PC12 (serotype O114), PC26 (O159), PC35 (serotype not determined [ND]), PC63 (O159), and 25 other non-STEC strains (three of serotype O128, three of O114, five of O111, two of O26, three of O159, four of O antigen nontypeable, and five of undetermined serotype) were also included in the study. Other enteric bacteria (three strains of *Vibrio cholerae* [one strain each of O1 {strain NB2}, O139 {strain SG24}, and non-O1 and non-O139 {strain PC2} serotypes], three strains of *Klebsiella pneumoniae*, two strains of *Pseudomonas aeruginosa*, two strains of *Flavobacterium multivorum*, one strain of *Vibrio mimicus*, two strains of *Enterobacter agglomerans*, and one strain of *Aeromonas hydrophila*) were also used in this study. Virulence gene profiles of the strains used here are given in Results. Strains were preserved in Luria broth supplemented with 15% glycerol at −70°C and in nutrient agar stab culture at room temperature.

PCR. Amplification of the target gene was carried out by PCR assay using a bacterial cell lysate as the source of template DNA. Strains were grown on Luria agar (HiMedia) for 18 h at 37°C. Single colonies were picked from the Luria agar and then inoculated into 3 ml Luria broth (HiMedia) and incubated overnight at 37°C in a shaker. Following overnight incubation, bacterial cells from 100 μl bacterial culture were washed with normal saline by centrifugation. The cell pellet was resuspended in 1 ml of double-distilled water and boiled for 10 min. Cell debris was removed by centrifugation, and the supernatant containing the template DNA was transferred into a fresh microcentrifuge tube for PCR assay.

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TABLE 1. PCR primers and conditions used in this study

Primer	Nucleotide sequence of primer	Target	Length of PCR product (bp)	Reference(s)
EVT1	5′-CAACACTGGATGATCTCAG-3′	stx_1 family[a]	349	15, 19
EVT2	5′-CCCCCTCAACTGCTAATA-3′			
EVS1	5′-ATCAGTCGTCACTCACTGGT-3′	stx_2 family[a]	110	15, 19
EVC2	5′-CTGCTGTCACAGTGACAAA-3′			
EAE1	5′-AAACAGGTGAAACTGTTGCC-3′	EHEC *eae*[b]	350	32
EAE2	5′-CTCTGCAGATTAACCTCTGC-3′			
hlyA1	5′-GGTGCAGCAGAAAAAGTTGTAG-3′	EHEC *hlyA*[c]	1,551	25
hlyA4	5′-TCTCGCCTGATAGTGTTTGGTA-3′			

[a] PCR consists of 30 cycles, each of which consisted of three steps in the following order: denaturation at 94°C for 60 s, annealing at 55°C for 60 s, and extension at 72°C for 60 s. After 30 cycles, the final extension step of 10 min at 72°C was performed.
[b] PCR consists of 30 cycles, each of which consisted of three steps: denaturation at 94°C for 60 s, annealing at 55°C for 90 s, and extension at 72°C for 90 s.
[c] PCR consists of 30 cycles, each of which consisted of three steps: denaturation at 94°C for 30 s, annealing at 57°C for 60 s, and extension at 72°C for 90 s.

PCR amplification of the target DNA was carried out in a thermal cycler (PerkinElmer Applied Biosystems, Weiterstadt, Germany) using 200-μl PCR tubes with a reaction mixture volume of 25 μl. PCR for detecting both chromosome (stx_1, stx_2, and *eae*)- and plasmid (*hlyA*)-borne virulence genes was performed as described earlier (15, 19, 22, 25, 32). PCR products were electrophoresed through a 1.5% (wt/vol) agarose gel to resolve the amplified products, which were visualized under UV light after ethidium bromide staining. The primer sequences and conditions are given in Table 1.

Polyclonal antibody preparation. An isolated colony of STEC strain VT3 from MacConkey agar (HiMedia) was inoculated into tryptic soy broth and incubated for 18 h at 37°C with constant shaking. The cells were harvested by centrifugation and washed three times with 10 mM phosphate-buffered saline (PBS) (pH 7.4). Washed cells were suspended in PBS and heat killed by steam in an autoclave for 10 min. The bacteria were then diluted in PBS to 70% transmittance at 610 nm (17). This method was used to prepare the whole-cell antigen. On day 0, New Zealand White rabbits were immunized subcutaneously with a 2-ml emulsion comprising 1 ml whole-cell antigen and 1 ml Freund's complete adjuvant (Difco Laboratories, USA). On day 21, each of those rabbits was injected subcutaneously with a 2-ml emulsion of 1 ml whole-cell antigen and 1 ml Freund's incomplete adjuvant (Difco). On day 42, each rabbit was boosted subcutaneously with 1 ml whole-cell antigen without adjuvant. Rabbits were exsanguinated on day 49. Blood samples were allowed to clot at room temperature, and sera were collected and stored at −20°C. VT3 antibody production was determined by an agglutination assay using *E. coli* strain VT3, grown on MacConkey agar or nutrient agar, as the antigen. Sera obtained by this method were checked for cross-reactivity with other strains of *E. coli* by the slide agglutination method. Agglutination assays were performed with glass slides by mixing 20 μl of diluted antiserum (in PBS) with a loopful of bacteria.

Antiserum adsorption. Antiserum was adsorbed with heat-killed cells of *E. coli* strain DH5α, a strain of ETEC (O125), and non-STEC strains PC12 (O114), PC26 (O159), PC35 (serotype ND), and PC63 (O159) sequentially. The adsorption was repeated three times with heat-killed cells for each strain. Heat-killed bacterial cells were added to the antiserum at a ratio of 0.1 ml packed cells per ml serum, and the mixture was gently stirred at 25°C for 2 h. After centrifugation, the serum was separated. Adsorbed antiserum was stored at −20°C for later use. The cross-reactivity of the sera was tested with the cells of *E. coli* strains PC12, PC26, PC35, and PC63, ETEC, and a strain of STEC (EDL933).

Reactivity of VT3 antiserum with intimin. Plasmid pIntg934 encoding intimin type γ (*E. coli* O157:H7 intimin type) was kindly provided by J. Sinclair. This plasmid was transformed by electroporation into *E. coli* strain BL21(DE3), and cells with the plasmid were selected for ampicillin resistance. These cells produced a full-length intimin molecule of 934 amino acids of type γ in the outer membrane of the transformed bacteria (27). The reactivity of transformed and untransformed whole cells with VT3 antiserum was checked by slide agglutination.

Immunoblotting. Whole-cell bacterial lysates were prepared as follows (25). Bacteria were grown to log phase in tryptic soy broth, harvested by centrifugation, and washed three times in PBS. Cells were resuspended in 1/10 volume of PBS containing phenylmethane sulfonyl fluoride and then adjusted spectrophotometrically to a concentration of 5×10^9 cells/ml. Sodium dodecyl sulfate (SDS) sample buffer (60 mM Tris-HCl buffer, pH 6.8, 2% [wt/vol] SDS, 5% [vol/vol] 2-mercaptoethanol, 10% [vol/vol] glycerol, and 0.001% [wt/vol] bromophenol blue) was added (1:1) immediately and vortexed, and the solution was heated for 5 min at 100°C. Thirty microliters of that mixture was loaded into each well of the SDS-polyacrylamide gels. Electrophoresis was done on 12% SDS-polyacrylamide gel electrophoresis (PAGE) gels in a Mini-PROTEAN II dual slab cell (Bio-Rad Laboratories, Richmond, CA). Gels were stained with Coomassie brilliant blue to ensure even loading. Proteins separated by SDS-PAGE were blotted onto nitrocellulose membranes (0.45-μm pore size; Bio-Rad) by use of a Mini Trans-Blot electrophoretic transfer cell (Bio-Rad) in 15.6 mM Tris, 129 mM glycine, 20% methanol (pH 8.3) for 5 h at 60 V (31). After the nitrocellulose membranes were washed in 10 mM Tris-buffered saline (TBS) (pH 7.6), they were incubated in 3% bovine serum albumin (BSA) in TBS for 90 min at 37°C and then washed with 10 mM TBS containing 0.05% Tween 20 (TBS-T) to block extra binding sites. The membranes were then incubated for 1 h with a primary antibody (VT3 antiserum) diluted (1:2,000) in antibody buffer (1% BSA in TBS-T) at room temperature. After they were washed with TBS-T, primary antibody-exposed membranes were incubated with goat anti-rabbit immunoglobulin G horseradish peroxidase conjugate (Bangalore Genei, India) in antibody buffer for 1 h at room temperature. After a second thorough washing with TBS-T, the membranes were incubated with substrate (tetramethyl benzidine and 0.02% H_2O_2 in distilled water) until the color development was sufficient. The developed sheets were washed in distilled water and air dried and then scanned with a Hewlett-Packard ScanJet 2400 scanner. The image of the blot was arranged for the figure and labeled with Adobe Photoshop version 7.

Latex agglutination test. Latex beads suspended in glycine saline buffer (Bangalore Genei, India) were coated with serially diluted VT3 antiserum in glycine saline buffer. One hundred microliters of supplied beads was diluted with 200 μl of glycine saline buffer, and 300 μl of diluted antiserum was added. The solution was then incubated for 2 h at 37°C and centrifuged at 5,000 rpm for 10 min. The supernatant was carefully aspirated out. The pellet was resuspended in 1.5 ml of blocking buffer (10 mM PBS, pH 7.4, containing 3% BSA) and centrifuged. The beads were washed two more times with the blocking buffer. After the final wash, the beads were resuspended in 600 μl of blocking buffer and incubated overnight at 4°C. This method was used to coat the beads with antibody. A 20-μl volume of coated beads was mixed with one colony of live cells from either MacConkey agar or nutrient agar on a glass slide and observed for any agglutination reaction within 1 min.

RESULTS

Polyclonal antibody preparation against whole cells of STEC strain VT3. New Zealand albino rabbits were immunized with heat-killed cells of STEC strain VT3. After the third injection, antibodies were detected at high dilutions. Sera were collected and kept frozen in aliquots. On average, 8 ml of antiserum was obtained per rabbit immunized with heat-killed cells of the VT3 strain. After adsorption with the heat-killed cells of STEC strains, the cross-reactivity of the adsorbed an-

TABLE 2. Slide agglutination reactions of anti-STEC antiserum against different strains of *E. coli*

E. coli strain	Cross-reactivity[a] with:		Presence[b] of indicated gene		
	Unadsorbed sera	Adsorbed sera	stx_1^+	stx_2^+	*eae*
VT3	+++	+++	+	+	+
EDL933	+++	+++	+	−	+
ETEC	−	−	−	−	−
DH5α	−	−	−	−	−
PC12	++	−	−	−	−
PC26	++	−	−	−	−
PC35	+	−	−	−	−
PC63	++	−	−	−	−

[a] +++, strong agglutination; ++, moderate agglutination; +, agglutination; −, no agglutination.
[b] +, presence; −, absence.

TABLE 3. Virulence gene profiles obtained by PCR analysis of *E. coli* strains used in the study

Virulence gene profile[a]	No. of strains with indicated virulence gene(s)		
	Human	Cattle	Environment
stx_1^+	3	4	1
stx_2^+	4	1	0
stx_1^+ stx_2^+	5	3	1
stx_1^+ *hlyA*$^+$	0	3	1
stx_2^+ *hlyA*$^+$	0	0	0
stx_1^+ stx_2^+ *hlyA*$^+$	0	6	6
stx_1^+ *eae*$^+$	0	1	0
stx_1^+ *hlyA*$^+$ *eae*$^+$	0	3	1
stx_1 stx_2 *hlyA eae*[b]	10	2	18

[a] stx_1 (encodes Shiga toxin variant 1 [Stx1]), stx_2 (encodes Shiga toxin variant 2 [Stx2]), *hlyA* (encodes hemolysin), and *eae* (encodes intimin) gene sequences were detected by PCR (Materials and Methods).
[b] Non-STEC strains.

tiserum with the cells of other enteric bacteria (*K. pneumoniae*, *P. aeruginosa*, *V. cholerae*, *A. hydrophila*, *Klebsiella oxytoca*, *Vibrio fluvialis*, *Vibrio vulnificus*, *Enterobacter amnigenus*, *E. agglomerans*, *Flavobacterium odoratum*, *F. multivorum*, and *Serratia marcescens*) was checked. It was found that the antiserum agglutinated the cells of a strain of *K. pneumoniae* (PC47), a strain of *F. multivorum* (PC69), and a strain of *E. agglomerans* (PC56). The antiserum was then further adsorbed with the cross-reactive strains by following the method described in Materials and Methods. Thus, the antiserum specific for VT3 was obtained and designated VT3 antiserum. On average, 6 ml VT3 antiserum was obtained from 8 ml of crude sera. Slide agglutination experiments were first performed with serial dilutions of the antiserum with the live homologous *E. coli* strain VT3 cells to determine an appropriate working dilution. The reciprocal of the working dilution of the VT3 antiserum was 20. The antiserum did not agglutinate the cells of non-STEC strains (strains DH5α, PC12 [O114], PC26 [O159], PC35 [serotype ND], PC63 [O159], and ETEC [O125]), *K. pneumoniae*, *E. agglomerans*, or *F. multivorum*, in which cases the antiserum was adsorbed. It did cross-react with STEC strain EDL933 (Table 2).

PCR. PCR analysis was performed to confirm the presence of stx_1 (encodes Shiga toxin variant 1 [Stx1]), stx_2 (encodes Shiga toxin variant 2 [Stx2]), *hlyA* (encodes hemolysin), and *eae* (encodes intimin) gene sequences. STEC strains were positive either for stx_1 or stx_2 or for both stx_1 and stx_2 gene sequences. The genotypes of the strains of non-STEC and other bacterial species used in this study were stx_1 stx_2 *eae*. The virulence gene profiles of *E. coli* strains used in the present study are shown in Table 3.

Reactivity of VT3 antiserum with intimin. VT3 antiserum agglutinated the whole cells of *E. coli* strain BL21(DE3) with plasmid pIntg934 encoding intimin type γ (*E. coli* O157:H7 intimin) but did not agglutinate the untransformed cells. As the cells produced full-length intimin molecules in the outer membrane of transformed bacteria (27), the results indicated that VT3 antiserum reacted with intimin.

Immunoblotting. We assessed the reactivity of VT3 antiserum to whole-cell lysates of the strains of *E. coli* and other enteric bacteria (listed in Materials and Methods) by immunoblotting. It was found that VT3 antiserum recognized the 97-kDa protein in whole-cell lysates from strains VT3 and EDL933 and did not cross-react with the ETEC (O125) strain (Fig. 1). Immunoblot analysis was performed with the whole-cell antigens prepared from the 87 strains using VT3 antiserum. Thirty-six (83.7%) of the 43 STEC strains tested positive for the STEC antigen, and 7 (three cattle isolates and four environmental isolates) tested negative (Table 4). None of the non-STEC strains or other enteric bacteria examined here tested positive by immunoblot analysis.

Development of a simple latex agglutination method to detect STEC. Based on the results of the immunoblotting assays, a simple latex agglutination assay was developed using VT3 antiserum for the detection of STEC in the present study. The determination of a working dilution of VT3 antiserum to coat the latex beads is shown in Table 5. The results of the latex agglutination test were positive when the antiserum used in the test was at a higher dilution (between 1:1,000 and 1:2,000) than that used in the slide agglutination test (1:20). It was also calculated that 1 ml of VT3 antiserum was sufficient to perform 10,000 tests. By use of the latex agglutination assay developed in this study, it was found that 35 (81.4%) of 43 STEC strains

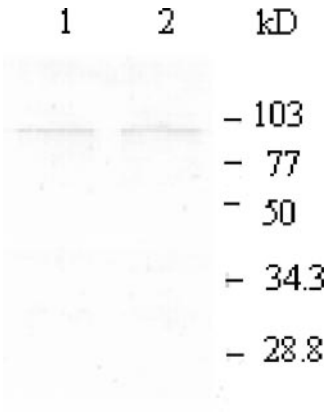

FIG. 1. Nitrocellulose immunoblot using VT3 antisera with whole-cell preparations of *E. coli* strains VT3 (O157; STEC) (lane 1), and EDL933 (O157, STEC) (lane 2). Marker sizes are in kilodaltons (phospholipase B, 103 kDa; BSA, 77 kDa; ovalbumin, 50 kDa; carbonic anhydrase, 34.3 kDa; and soybean trypsin inhibitor, 28.8 kDa).

TABLE 4. Results obtained by immunoblotting and latex agglutination assay of the STEC strains

Source	Serotype[a]	No. of positive strains (no. of strains tested) by:	
		Immunoblotting	Latex agglutination assay
Human	O29/O29:H1/O28ac:H1	12 (12)	12 (12)
Cattle	O124	4 (4)	4 (4)
	ONT	8 (10)	7 (10)
	O114	1 (1)	1 (1)
	O28ac	1 (1)	1 (1)
	O111	1 (2)	1 (2)
	O55	2 (2)	2 (2)
	O119	1 (1)	1 (1)
Environment	O111	1 (1)	1 (1)
	O136	3 (6)	3 (6)
	O125	1 (1)	1 (1)
	ONT	1 (2)	1 (2)

[a] ONT, O antigen nontypeable.

were positive for the STEC antigen and 8 (four cattle isolates and four environmental isolates) were negative (Tables 4 and 6). The corresponding sensitivity of the latex agglutination assay was 81.4%, whereas that of the immunoblot assay was 83.7%. One (3.3%) of the 30 strains of non-STEC bacteria and none of the strains of other enteric bacteria included in this study tested positive by the latex agglutination assay (Table 6). The corresponding specificity of the latex agglutination assay was approximately 98%.

DISCUSSION

The purpose of this study was the production of a polyclonal antibody specific for STEC and for use in the development of a sensitive and specific immunodiagnostic assay for the detection of STEC that would avoid expensive reagents and equipment. VT3 antiserum was specific, as shown by its reactivity in the immunoblotting assay with the STEC strains and its nonreactivity with the non-STEC strains used in the present study. A band at around 97 kDa was detected by the reaction of the whole-cell antigens of the STEC strains with VT3 antiserum. Several authors have documented that among the surface antigens on EHEC, intimin (encoded by the *eae* gene) is the most immunogenic and that its size is around 97 kDa (4, 16, 25). In the present study, it was confirmed serologically that VT3 antiserum reacted with intimin type γ. It could be indicated here that the 97-kDa protein recognized by the VT3 antiserum was intimin. Previous studies reported the existence of at least three immunologically distinct groups of intimins, i.e., those similar to intimins from either RDEC-1, EPEC E2348/69 (O127:H6), or EHEC (O157:H7); this cross-reactivity did not appear to be serogroup specific (2, 11). The primer sequences for the *eae* gene (encoding intimin) for PCR used in our study was developed based on the *eae* gene sequences of EHEC (O157:H7) (31). There might be some diversity in the *eae* gene sequences between O157 and non-O157 STEC strains, which might be the reason why *eae* gene sequences were not detected by PCR for most of the STEC strains included in the present study. However, despite the diversity in the polypeptide domain, two stretches of amino acids (WLQYGQ and WAAGA NKY) are the same in all intimins for EPEC strains (1). It was also reported earlier that a group of EPEC strains did not produce the PCR product with either Int-α or Int-β primers but was recognized poorly by both anti-intimin-α and anti-intimin-β sera (1). In the present study it was found that the strains of STEC were recognized by the VT3 antiserum and that a majority of them did not produce the PCR product with the primer for EHEC-specific *eae* sequences used in the study. An immunoblotting assay confirmed the extent of serological cross-reactivity among the STEC strains of different serotypes isolated from different sources, including humans, cattle, and the environment. The latex agglutination test could easily detect STEC organisms at concentrations between 5×10^6 and 5×10^7 CFU/ml when latex beads coated with antiserum diluted to 1:2,000 were used. Some of the evaluated STEC strains tested negative by the latex agglutination test. The strains that were negative by this assay were isolated from either cattle or the environment. This could suggest that these strains were unable to express sufficient antigen. Only 2% of non-STEC bacteria and other enteric bacteria used (see Materials and Methods) tested positive by the latex agglutination assay, supporting the specificity of the assay. Although the specificity of the immunoblotting assay was higher than that of the latex agglutination method for the detection of STEC, the latex agglutination assay avoids costly equipment and requires minimal laboratory facilities. Furthermore, the production of a polyclonal antiserum specific for STEC would be much less

TABLE 6. Analysis of 87 bacterial strains (clinical and environmental) for STEC antigen by latex agglutination and immunoblotting assays

Bacterial strain type	No. of strains tested	No (%) positive by:	
		Immunoblotting	Latex agglutination assay
STEC	43	36 (83.7)	35 (81.4)
Non-STEC	30	0 (0)	1 (3)
Other enteric bacteria[a]	14	0 (0)	0 (0)

[a] Given in Materials and Methods.

TABLE 5. Results obtained in latex agglutination test

E. coli strain	Agglutination result[a] with beads coated with VT3 antiserum at the indicated dilution (fold)								
	4	20	40	80	200	500	1,000	2,000	20,000
EDL933	+++	+++	+++	+++	+++	++	+	+	−
VT3	+++	+++	+++	+++	+++	++	+	+	−
PC12	−	−	−	−	−	−	−	−	−

[a] +++, strong agglutination; ++, moderate agglutination; +, weak agglutination; −, no agglutination.

expensive than that of a monoclonal antibody. Overall, the results of our study show the production of a highly specific polyclonal antiserum and the generation of a simple, cost-effective, sensitive, and specific latex agglutination assay for establishing an etiological diagnosis of STEC. This method may also be employed for epidemiological surveillance.

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