Detection of Verotoxin in Stool Specimens

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A total of 132 fecal specimens containing verotoxin (VT) were subjected to counter-current immunoelectrophoresis (CIE). Of these, 113 (85.6%) were found to be positive by CIE. Another 71 stool specimens containing E. coli serogroup O157 but with flagellar antigens other than H7 were tested for verotoxin by CIE. These stool specimens were negative for VT on Vero cell monolayers. Of these 71 stool specimens, 6 (8.5%) gave positive tests for verotoxin by CIE. Forty stool specimen filtrates which were negative for VT (negative controls) were also subjected to CIE. One of these stool specimen filtrates gave a line of precipitation by CIE. The specificity of the CIE test was 93.7%, and the sensitivity was 85.6%. False-positive results may have been due to an antibody component against the somatic antigen (O157) in the antitoxin used; this is a limitation of the CIE test.

In a related evaluation, 302 stool specimen filtrates containing VT were retested with Vero cell suspension cultures in microdilution plates. Of these, 281 stool specimen filtrates showed cytotoxic effects within 24 h, while the remaining 21 filtrates showed the effects within 48 h. The use of Vero cell suspension culture is as reliable as the use of Vero cell monolayers and provides detection of verotoxin 24 to 48 h sooner.

Verotoxin (VT) produced by several serotypes of Escherichia coli, including O157:H7, causes hemorrhagic colitis (2, 8) and hemolytic uremic syndrome in humans (3, 5). VT produces irreversible cytopathic effects in Vero cell monolayers in 24 to 72 h (3, 4). Karmali et al. (4) demonstrated fecal VT in 40 stool specimens obtained from patients suffering from hemolytic uremic syndrome and from family contacts of index hemolytic uremic syndrome patients.

We report here the results of testing stool specimens for the presence of VT by using (i) the counter-current immunoelectrophoresis (CIE) method and (ii) either Vero cell suspensions (African green monkey kidney cell line) in microdilution plates or Vero cell monolayers.

Vero cells were grown in polystyrene 150-cm² tissue culture flasks as described previously (6, 7). Stool specimen filtrates were prepared by emulsifying specimens in a 1:2 dilution with phosphate-buffered saline at pH 7.2 and then centrifuging them for 10 min at 2,500 × g. Supernatants were filtered through membranes (pore size, 0.45 µm; Millipore Ltd., Mississauga, Ontario, Canada) and either tested on the same day or kept at −70°C if a delay was anticipated. The stool specimen filtrates were used for detecting VT either by CIE or by Vero cell culture.

A total of 132 stool specimen filtrates previously tested on Vero cells and known to contain VT (stored at −70°C) were used for the CIE experiment. These stool specimen filtrates were subjected to CIE with anti-VT serum produced in rabbits. (Anti-VT serum was kindly supplied by H. Lior, Laboratory Centre for Disease Control, Ottawa, Ontario, Canada.) The CIE method used was the one described by Hammond et al. (1) and Spence et al. (9) with modifications as described below. Three milliliters of 1.0% agarose (agarose B from Pharmacia Fine Chemicals, Dorval, Quebec, Canada) dissolved in 0.06 M barbitial buffer, pH 8.8 (Gelman Sciences Inc., Ann Arbor, Mich.), was used per microscopic slide. The barbitial buffer contained 0.05% (wt/vol) sodium azide and was filtered through a membrane filter (pore size, 0.22 µm; Sartarius Co., obtained from British Drug Houses, Toronto, Ontario, Canada). With a stainless steel punch, two 3-mm-diameter wells were punched about 3 cm apart in the agarose gel on a glass slide. The specimen to be tested (antigen) was placed in the well on the cathode side (negative pole), and the antiserum was placed in the well on the anode side (positive pole). The agarose slides were then subjected to CIE with 300 ml of 0.06 M barbitial buffer, pH 8.8, in each side of the electrophoresis tank. Filter paper (Whatman no. 1) strips were used as wicks. The wicks were attached on both sides of the agarose slide after they were moistened in the barbitial buffer. Air bubbles which formed between the wick and the agarose surface were removed by pressing the wicks gently. The agarose slides so prepared were subjected to 4 × 6mA of current per slide for 60 min. In each experiment, a filtrate of a broth culture of E. coli serotype O157:H7 known to produce VT was used as a positive control. A negative control of uninoculated broth was also included. A fine line of precipitation in the gel between the two wells was considered a positive result for VT.

Of 132 stool specimen filtrates containing VT, only 113 (85.6%) filtrates were found to be positive by the CIE method. Of 40 stool specimen filtrates negative for VT in Vero cells (negative controls) when subjected to CIE, 1 gave a positive line of precipitation. Seventy-one stool specimens containing E. coli serotype O157 but flagellar antigen other than H7 were tested for VT by CIE. Six (8.5%) of these stool specimens were found to be positive by CIE, though no VT was detected in these 71 stool specimens (Table 1). The specificity of the CIE test was 93.7%, and the sensitivity was 85.6%.

Three hundred and two stool specimen filtrates previously tested on Vero cell monolayers and known to contain VT (stored at −70°C) were retested on Vero cell suspensions in microdilution plates as described previously (6). Vero cell suspensions were inoculated with these stool specimen filtrates on the same day that the Vero cells were trypsinized. The same 302 stool specimen filtrates were also retested in Vero cell monolayers grown for 24 h in microdilution plates. VT (positive control) and all the filtrates were neutralized in separate test tubes by adding equal volumes of anti-VT antibody. The mixture was incubated at 37°C for 30
TABLE 1. VT detection by CIE

<table>
<thead>
<tr>
<th>Type (no.) of stool specimen filtrates (1:2 in PBS)</th>
<th>No. of CIE results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive (132)*</td>
<td>Positive</td>
</tr>
<tr>
<td>113</td>
<td></td>
</tr>
<tr>
<td>Control (40)</td>
<td>1</td>
</tr>
<tr>
<td>E. coli O157 positive (71)*</td>
<td>6</td>
</tr>
</tbody>
</table>

* PBS, Phosphate-buffered saline.
* Sensitivity, 85.6%; specificity, 93.7%.
* Stool specimens positive for VT in Vero cells.
* Control group; stool specimens negative for VT and Clostridium difficile toxin.

Stool specimens negative for VT but containing E. coli with somatic antigen O157 and negative for flagellar antigen H7.

Four wells were used per specimen in the microdilution plate containing Vero cell suspensions. Two wells received 20 μl of filtrate or VT (positive control), and the other two wells received 40 μl of the neutralized mixture. A saline control and a negative control were also included. The cytotoxic effect of VT was characterized by the rounding up of Vero cells initially within 48 h and the subsequent destruction of the Vero cell monolayers with cell detachment in 72 h (described by Mohamed Karmali [Clin. Microbiol. News. 9:65–70, 1987]).

Of 302 stool specimen filtrates tested in Vero cell suspensions, 281 (93%) gave positive results in 24 h, and all of the 302 specimens (100%) were positive in 48 h. These 302 stool specimen filtrates were retested in Vero cell monolayers; only 3 (1.0%) of them gave positive results in 24 h, and 17 (5.6%) more were positive in 48 h. It took 72 h for the remaining 282 specimens (93.4%) to give positive results. An additional 113 stool specimen filtrates were tested for VT in Vero cell monolayers and Vero cell suspensions and were found to be negative by both systems.

The CIE method was easy to perform, and it took only 2 h to complete the test; however, it gave 19 (14.4%) false-negative results and 7 (6.3%) false-positive results. From three stool specimens which were negative for VT, three strains of E. coli containing somatic antigen O157 were isolated in culture. Both filtrates of these cultures were negative for VT in Vero cell monolayers but were positive by CIE. The Laboratory Centre for Disease Control in Ottawa confirmed that these cultures did not contain flagellar antigen H7 and were negative for VT. This would explain the false-positive results, as they may have been due to the antibody content against a somatic antigen (O157) in the antitoxin used in the CIE method. The false-negative results suggested that the minimum concentration of VT required to produce a positive result in the Vero cell system is insufficient to form a line of precipitation by the CIE test.

By using Vero cell suspensions instead of Vero cell monolayers, we were able to detect VT in 93% of the positive stool specimens in 24 h. During the same period of 24 h we detected VT in only three stool specimens (1.0%) by the conventional method of using Vero cell monolayers. Within 48 h, however, all the stool specimens (100%) gave positive findings in Vero cell suspensions, while only 20 stool specimens (6.6%) were positive during the same period in Vero cell monolayers. The use of Vero cell suspensions was equally reliable and provided results 24 to 48 h sooner.

LITERATURE CITED


