Counterimmunoelectrophoresis Assay for Detection of Adenovirus Antigen

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A rapid, sensitive counterimmunoelectrophoresis assay was developed to detect adenovirus in stools of patients with gastroenteritis. The parameters of the assay were optimized, and its sensitivity and efficiency were examined. The assay promises to be a useful alternative to conventional techniques of cell culture isolation or electron microscopy for the detection of adenovirus in stool specimens.

Adenoviruses have been associated with infantile gastroenteritis, since they can be detected in stools of symptomatic patients by electron microscopy (EM) and cell culture isolation (3, 4, 9, 10). However, shortage of readily available EM facilities and the failure of nearly half of the adenoviruses detected by EM to grow in cell culture are drawbacks for virology laboratories (9). Adenoviruses grown in cell culture can be detected and typed by immunodiffusion and counterimmunoelectrophoresis (CIEP) (7, 13).

In this study we examined the application of CIEP to detect adenovirus in stool specimens. This approach has been reported as possible in a preliminary study (8).

Adenovirus serotypes 5 and 7 were derived from patient specimens submitted to our diagnostic laboratory for virus isolation, and they were typed by the method of Rose using antisera purchased from Microbiological Associates (12). These viruses were isolated and grown in a continuous line of human amnion cells, HAE 70 (5). Stool specimens were obtained from hospitalized gastroenteritis patients. All specimens were examined by EM, and those positive for adenovirus particles were placed in cell culture for virus isolation.

Reference adenovirus types 5 and 7 antigens were made by purifying these agents from their respective cell cultures by the method of Green and Pina (6). This purification included differential centrifugation, floc extraction, and banding on two sequential CsCl gradients in 0.01 M tris(hydroxymethyl)aminomethane (pH 8.2). Reference antibody was prepared in guinea pigs by injecting the hind muscles with 0.75 ml of each serotype, at an absorbancy of 260 nm of 1.0 to 2.0, mixed with 0.25 ml of Freund adjuvant. Ten days after the last of three weekly injections, the guinea pigs were exsanguinated, and their sera were titrated by complement fixation (CF) using the method of Bradstreet and Taylor (2).

The method of CIEP was essentially the technique used for rotavirus (8). It involved the use of a Shandon Electrophoresis apparatus model U77 with 1% agarose in 0.05 M barbital buffer (pH 8.6) on microscope slides. The antigen was placed in the cathode wells, and the antisera was added to the anode wells. After electrophoresis at 150 V for 90 min the slides were washed for 1 h in saline, stained with a solution of 1% tannic acid, and read on a Shandon dark-ground viewer.

Negative-contrast staining EM was performed according to the method described by Middleton et al. (9).

The reaction of reference adenovirus antiserum with homologous adenovirus antigen is shown in Fig. 1A. One or both of the two lines appeared midway between the wells regardless of the purity of the virus. Hence cell lysate, or pure adenovirus banding at a density of 1.34 on CsCl, produced an identical line pattern.

Similarly, the reaction of reference antiserum with an adenovirus-positive stool (Fig. 1B) produced single or double lines, although these appeared closer to the antigen well.

Reacting adenovirus type 5 antigen with homologous (type 5) or heterologous (type 7) antiserum produced identical line patterns, which indicated a group-specific reaction.

The time of electrophoresis was found to be optimal at 90 min, although lines first appeared at 60 min. A potential for artifact formation arose if the antigen was suspended in 0.01 M tris(hydroxymethyl)aminomethane (pH 8.2). This produced a fine nonspecific line between the wells. Dilution of the antigen in this buffer with 4 volumes of CIEP buffer avoided the formation of this line.

The CIEP assay was compared with other
routine systems in terms of its overall sensitivity for detecting antigen and antibody. A purified adenovirus type 5 preparation having an absorbancy at 260 nm of 1.6 was serially diluted in CIEP buffer, and the dilutions were examined by EM and CIEP. The CIEP assay showed the presence of a precipitin line up to a 1:64 dilution, and EM detected virus up to a 1:256 dilution (Table 1). The EM was thus four times as sensitive as CIEP. In similar preparations of adenovirus types 5 and 7 the EM was four to eight times as sensitive as CIEP.

Adenovirus type 5, purified from a stool of a patient by the same method used to prepare adenovirus from cell cultures, was serially diluted in CIEP buffer, and dilutions were examined by EM and CIEP. The CIEP assay showed precipitin lines detectable up to a 1:128 dilution, and EM detected virus up to a 1:1,024 dilution. Similarly, dilutions of an adenovirus patient’s stool positive by EM were examined by EM and CIEP. Virus was detected up to a 1:32 dilution by EM and a 1:8 dilution by CIEP.

Preparations of purified virus from cell culture and stool were also titrated in cell culture. The titer of the cell culture-derived virus was $10^{4.5}$ 50% tissue culture doses per ml, whereas that of the stool-derived virus was $10^{4.5}$. Thus one obtains an objective comparison of the three systems for detection of adenovirus antigen.

Serial dilutions of guinea pig and human sera were assayed for antibody to adenovirus by CF and CIEP. The guinea pig antiserum had a titer of 1:64 by both CF and CIEP. However, the human serum had a titer of 1:16 by CF and a titer of 1:64 by CIEP. This shows that the CIEP assay is at least as sensitive as CF for detection of adenovirus antibody. A more detailed study of this aspect is reported in a following paper (11).

To determine the overall efficiency of the CIEP assay in antigen detection, a small field trial was set up for detection of adenovirus in stools from gastroenteritis patients. A total of 123 stool specimens were selected, of which 35 were positive for adenovirus by EM and 32 were positive by CIEP. The remainder were negative. Hence, three specimens that were positive by EM were negative by CIEP. One specimen originally diagnosed as positive for another gastrointestinal virus by EM, but negative for adenovirus, was positive by CIEP. On reexamination, adenovirus was detected in this stool by EM, but at a low quantity. Thus the CIEP has about 90% the efficiency of EM for detection of adenovirus-positive stools. No false-positives were seen by CIEP.

Our CIEP system was evaluated in terms of its ability to identify adenovirus in cell culture lysates. Of 61 cell culture lysates positive for adenovirus by EM, 56 were positive by CIEP. In this comparison the CIEP system had 92% of the efficiency of the EM.

It is apparent from the foregoing data that CIEP is a useful, sensitive, and rapid technique for detection of adenovirus antigen both in patient stool specimens and in cell culture isolates. It also has potential for determining virus antibody titers in sera.

The reactions of antigen with our reference guinea pig antiserum produced single, or occasionally double, precipitin lines, regardless of the purity of the virus and the heterogeneity of the serum. This reflects the existence of a determinant in the virus that not only stimulated the greatest antibody production but was also most active in the precipitin line formation. Early immunodiffusion work (1, 13) has shown that there are up to three lines present on interaction of antibody with adenovirus. Of these, one line was believed to be group specific while the other two were weaker in heterologous reactions, and hence a typing system was considered possible. In subsequent developments with antisera to the

![Figure 1. CIEP of adenovirus. (A) Purified adenovirus type 5 reacted with its homologous antibody. (B) Virus-positive stool reacted with antibody to adenovirus type 5.](image-url)

**Table 1. Sensitivity of CIEP, EM, and cell culture for detection of adenovirus**

<table>
<thead>
<tr>
<th>Antigen</th>
<th>CIEP titer</th>
<th>EM titer</th>
<th>TCD$_{50}$ (per ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus type 5 from cell culture</td>
<td>1:64</td>
<td>1:256</td>
<td>$10^{4.5}$</td>
</tr>
<tr>
<td>Adenovirus type 5 from patient stool</td>
<td>1:128</td>
<td>1:1,024</td>
<td>$10^{4.5}$</td>
</tr>
<tr>
<td>Patient stool containing adenovirus as demonstrated by EM</td>
<td>1:8</td>
<td>1:32</td>
<td>—</td>
</tr>
</tbody>
</table>

* *Tissue culture doses (50%).*
penton and fiber antigen a CIEP system was produced that detected type-specific antigen in an adenovirus cell lysate (7).

When a human convalescent antiserum was employed in our CIEP, two precipitin lines were frequently produced. This is more in keeping with the above multiple-line reports. It is likely that the guinea pigs produced antibodies to mainly one component of the virus, possibly the hexon since it was stimulated with a purified antigen, whereas the human infection resulted in a broader response to multiple antigens of the virus.

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LITERATURE CITED