Counter-Immuno-electrophoresis: Rapid Method for Detecting Group-Specific Antigen and Antibodies Associated with Oncogenic Ribonucleic Acid Viruses

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Counter-immuno-electrophoresis was used for the quantitation of group-specific antigen and antibodies of C-type ribonucleic acid leukemia and sarcoma viruses.

Counter-immuno-electrophoresis (CIEP) is currently used in many laboratories to detect hepatitis-associated antigen (HAA; reference 6) and in the past has been employed to detect components of influenza virus (7), pneumococcal antigen (4), and a number of other antigens. Gel diffusion (5), complement fixation (9), immunofluorescence (8), immunoradiography (1), and hemagglutination inhibition (12) have been used to detect the group-specific (gs) antigens of avian, murine, and feline leukemia and sarcoma viruses and to detect antibody to these antigens. We report here that CIEP is also a simple, rapid, and sensitive method for detecting gs antigens and antibodies.

CIEP was performed on Kodak projection slides [3.25 by 4 inches (8.25 by 10.16 cm)] coated on one surface with 13 ml of 1% agarose in 0.02 M barbital-acetate buffer at pH 8.2. Two parallel rows of wells 3.0 mm in diameter were cut 5 mm apart (center to center). Anodal wells were filled with 10 μl of antibody, and cathodal wells were filled with 10 μl of antigen. Electrophoresis was performed at 30 mA for 60 min in an electrophoresis chamber which contained 0.04 M barbital-acetate buffer at pH 8.2. After the slides had soaked for 10 min in 0.0125% cadmium acetate in 0.85% NaCl, they were viewed by indirect illumination. Slides were washed for 24 hr in 0.85% NaCl, dried, and stained with 0.25% Amido Black 10B. Comparable results also have been obtained by using a Counterelectrophoresis Plate (Spectra Biologicals, Oxnard, Calif.), Counterimmuno-electrophoresis Plate (Abbott Laboratories, Scientific Products Division, South Pasadena, Calif.), and Cross-Over Electrophoresis Plates (Hyland, Los Angeles, Calif.).

Gel diffusion was performed in Immunoplates, pattern C (Hyland), IDF Cell I (Cordis, Miami, Fla.), and in a micro Ouchterlony test (1) with 0.5% agarose. The gel diffusion wells were filled with 10 μl of antigen or antibody, and the plates were incubated for 24 hr at room temperature and then for 24 hr at 4°C. Complement fixation was performed as described by Sever (11) with 2 units of complement.

Antisera to feline leukemia virus (FLV) were titered against FLV gs antigen [Tween-ether disrupted FLV (3)] by complement fixation, gel diffusion, and CIEP, and it was found that titers detected by CIEP were intermediate between those detected by complement fixation and those detected by gel diffusion (Table 1). In some tests, two precipitin lines formed rather than one, probably representing the detection of interspecies as well as species-specific antigen and antibodies (10). The fact that, by both CIEP and gel diffusion, murine leukemia virus (MLV) gs antibody reacted with only one line against FLV gs antigen whereas antisera to FLV reacted with two lines supports this interpretation.

CIEP was also used to detect gs antigen in tissue homogenates which had been clarified by centrifugation, but it was important that the plates be thoroughly washed in 0.04 m sodium acetate and 0.85% NaCl to remove as much of the extraneous tissue components as possible.

Wallis and Melnick (13) recently described a variation of CIEP which detected HAA, or antibodies to HAA, with a higher sensitivity. This variation did not result in a higher sensitivity of the CIEP described here for gs antigens or antibodies, probably because (i) the CIEP as used by us is also discontinuous but has only a twofold difference in ionic strength instead of a fivefold difference (13) and (ii) gs antigen is insoluble at low ionic strength.
**NOTE**

**TABLE 1. Results of gs antigen and antibody titrations by various assay methods**

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Antibody</th>
<th>CF$^a$</th>
<th>CIEP</th>
<th>Gel diffusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Hyland</td>
<td>Cordis</td>
</tr>
<tr>
<td>Feline gs</td>
<td>Guinea pig I$^b$</td>
<td>1,536$^c$</td>
<td>512</td>
<td>8</td>
</tr>
<tr>
<td>Feline gs</td>
<td>Guinea pig II</td>
<td>1,024</td>
<td>512</td>
<td>4</td>
</tr>
<tr>
<td>Feline gs</td>
<td>Guinea pig I</td>
<td>2,560</td>
<td>128</td>
<td>2</td>
</tr>
<tr>
<td>Feline gs</td>
<td>Guinea pig II</td>
<td>2,560</td>
<td>128</td>
<td>4</td>
</tr>
</tbody>
</table>

$^a$ CF, complement fixation; CIEP, counterimmunoelectrophoresis.

$^b$ Immunized with gs antigen prepared from purified virus.

$^c$ Reciprocal of highest dilution giving 50% hemolysis in complement fixation test or a definite precipitation line in CIEP or gel diffusion tests.

$^d$ Not detected.

CIEP provides a reliable, simple, and fast (60-min reaction time) method to screen various biological samples for the presence of gs antigens. For example, large numbers of fractions from gs antigen purification procedures can be easily assayed, and anticomplementary antigen or animal sera can be evaluated with this method which, although not as sensitive as the complement fixation test, is more sensitive than other gel diffusion procedures. By this technique, we could detect anti-gs antibodies in 10 of 58 marmoset sera obtained from animals bearing feline fibrosarcoma virus-induced sarcomas, whereas none of these sera was usable for CF tests because of their high anticomplementary activity.

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**LITERATURE CITED**


