Rapid Detection of Viral Antibody by Cellulose Acetate Electrophoresis

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An immunoelectrophoretic procedure utilizing microprecipitation and cellulose acetate electrophoresis was developed for detection of antibody to specific virus. A model system of tobacco mosaic virus and homologous rabbit antiserum is described.

The isolation and the identification of viruses and the detection of increases in antibody concentration are the main tools used in the laboratory diagnosis of viral illnesses. Standard laboratory procedures are successfully employed for the majority of viruses commonly encountered in clinical cases; however, many of these procedures are tedious and require days to weeks for completion, whereas others cannot be adapted for certain virus groups. More rapid immunological methods of detection are constantly being sought (1, 2, 4, 6).

The observations that antigen and homologous antibody form large insoluble complexes when reacted at equivalence (3) and that these complexes are electro-neutral and nonmigratory during electrophoresis (5) formed the basis of the immunoelectrophoretic procedure reported here.

The steps involved in this diagnostic procedure are shown in Fig. 1. Equal volumes of virus and serum dilutions, prepared in buffered saline (pH 7.2), were mixed in Microtiter plates (Micro-

![Flow chart illustrating steps involved in cellulose acetate electrophoretic procedure for detection of serum antibody.](http://aem.asm.org/)

1 Present address: Microbiological Associates, Inc., Bethesda, Md. 20014.

2 Present address: Microbiological Associates, Bethesda, Md.), which were then sealed with a plastic cover and floated in a water bath at 37°C for 1 hr. For determination of serum antibody, a concentrated, partially purified preparation of test (known) virus was required to obtain maximum sensitivity. After incubation, 10 μl of each virus-serum mixture were applied to the cathode end of a cellulose acetate plate [1 by 3 inches (2.54 by 7.62 cm); Helena Laboratories, Beaumont, Tex.].
soaked in buffer. The plates were electrophoresed at 220 v for 15 min in a chamber (Shandon Scientific Co., Sewickley, Pa.) containing tris-(hydroxymethyl)aminomethane-Barbital buffer (pH 8.4; ionic strength, 0.05) and subsequently fixed and stained for 15 min in Ponceau-S stain dissolved in 7.5% trichloroacetic acid. The plates were rinsed in 2% acetic acid to remove excess stain, air-dried, and then examined for elliptical precipitates at the site of sample application. A sample of virus and a sample of serum were each mixed individually with saline and tested concurrently with the virus-serum mixtures as controls. A visible precipitate, resulting from the interaction of virus and serum together with negative antigen and serum controls, indicated the presence of specific antibody.

After it was observed that concentrated tobacco mosaic virus (TMV) when reacted with TMV antiserum (rabbit) could be employed in this technique, TMV was chosen for study in subsequent experiments as a model virus-antibody system. The virus, in twofold dilutions, was reacted with twofold dilutions of either normal serum or specific antiserum, applied to cellulose acetate plates, and electrophoresed. The plates were subsequently stained and examined.

A stained plate demonstrating the results of such an experiment is shown in Fig. 2A. A triple applicator (Helena Laboratories) was used to apply simultaneously three samples to a single plate, thereby increasing the sample capacity of a given electrophoretic run. The only precipitate visible was present at the origin where the reaction mixture of TMV and antiserum was applied. No precipitate was seen when TMV was combined with either buffered saline or normal rabbit serum. Antiserum when mixed with buffered saline was similarly negative (not shown). When dilutions of antiserum were reacted with a constant amount of TMV, it was observed that the highest dilution of serum resulting in a strong visible precipitate (end-point titer) was 1:160 (Fig. 2B). A typical graded response with increasing dilutions of serum was apparent.

Employing this technique, we have recently demonstrated specific antibody to adenovirus, influenza virus, and Eastern equine encephalitis virus both in animal and human sera with highly concentrated and purified virus antigen. Current studies should provide correlation of results obtained with this test with those obtained with procedures routinely employed in the diagnosis of certain selected virus infections.

**LITERATURE CITED**