COUNTERIMMUNOELECTROPHORESIS FOR THE DIAGNOSIS OF SMALLPOX

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SYNOPSIS

A counterimmunoelectrophoretic (CEP) technique for the laboratory diagnosis of smallpox is described. The test is four times as sensitive as agar-gel diffusion (AGD). A monospecific antibody, raised in rabbits, was used. The CEP test gave negative results with 100 normal human sera and vesicle fluid from 4 cases of chickenpox. It gave positive results with dried, 6 to 14 day-old scabs from 4 of 6 cases of clinically-frank smallpox. The same material gave negative results when tested by smear, AGD and inoculation on the chorio-allantoic membrane of the chick embryo.

INTRODUCTION

Counterimmunoelectrophoresis (CEP) makes possible the rapid detection of precipitating antigen-antibody systems. The test is more sensitive than agar-gel diffusion (AGD) because an electrical current drives all antigen towards antibody moving in the opposite direction by electroendosmotic flow. In the design of the test the mobility of the antigen is studied in immunoelectrophoresis. A suitable brand or a mixture of agars is then prepared so that when constituted in the gel the movement of the antigen and the antibody towards each other is at equal rates. This makes the test sensitive (Kelkar and Niphadkar, 1974). Much interest has been aroused of late in the possibility of using rapid simple techniques like CEP for the laboratory diagnosis of disease (Greenwood, Tugwell and Whittle, 1974). These methods are sometimes even more sensitive than methods for the isolation of organisms. To date the CEP test has been applied for detecting antigens and antibody related to the hepatitis B virus, alpha$_1$-fetoprotein (Kohn, 1970), influenza virus (Birlin and Pirojboot, 1972) malaria (Zaman *et al*, 1972), bacterial meningitis (Coonrod and Rytel, 1972), syphilis (Fujita and Takahashi, 1972), beta haemolytic streptococci (Dajani, 1973), deoxyribonucleic acid (Klayman, Farkash and Myers, 1973), dengue haemorrhagic fever (Chirdboonchart, Harisdangkal and Bhamarapravati, 1974) and hydatid disease (Kelkar and Kotwal, 1975). We describe here a CEP technique for the diagnosis of smallpox. Current laboratory methods for the diagnosis of this disease include morphology on examination of a smear, AGD and inoculation of the chorio-allantoic membrane of the chick embryo (CAM).

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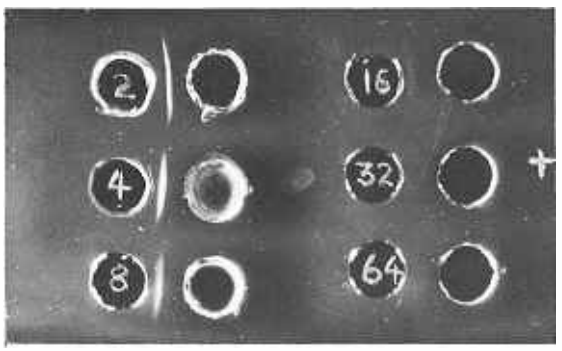
MATERIAL AND METHODS

Antibody: Antiserum was raised in rabbits using lyophilized vaccinia virus and the following method: Day 1—scarification of the shaved abdomen of the rabbit with application of the reconstituted virus. Day 7—injection of 1 ml of a 1 in 10 dilution of virus intradermally. Day 11—injection of 1 ml of a 1 in 10 dilution of virus subcutaneously. Day 18—injection of 1 ml of a 1 in 10 dilution of virus intramuscularly. Day 30—harvesting of the antiserum. This serum, when tested in immunoelectrophoresis, showed a single arc of precipitate, indicating monospecificity. Vaccinia and variola have similar antigens and this antibody served to detect both viruses.

Agar—gel diffusion test: This was done according to the method of Dumbell and Nizamuddin, 1959.

Counterimmunoelectrophoresis: The buffer used



Counterimmunoelectrophoresis for vaccinia/variola. Wells on the cathodal side filled with dilutions of standard antigen. Numbers indicate reciprocal of antigen dilution used. Antibody placed on the anodal side. A faint positive reaction is seen at an antigen dilution of 1 in 16.

was a barbital acetate buffer, pH 8.4 with the following composition: Sodium 5.5 diethyl barbiturate, 8.142 g; sodium acetate, 6.476 g; normal hydrochloric acid, 9 ml; distilled water added to 1 L volume. The buffer was used both in the tanks and in making up the gel. The gel was prepared by adding 0.85 g of Difco Noble agar to 100 ml of buffer and boiling. 1.5 ml of hot liquid gel was poured on a microscope slide. Wells were 3 mm in diameter with their edges 2 to 3 mm apart. Antibody was placed in the anodal well and antigen in the well near the cathode. Electrophoresis was carried out at room temperature for 45 minutes using a constant current of 15 mA per slide. Immune precipitates were looked for under oblique illumination. The figure illustrates positive results.

Controls: Stored frozen sera from 100 normal student volunteers were tested. Also vesicle fluid from 4 cases of chickenpox.

Cases: An outbreak of smallpox at Malkapur was the source of scabs from 6 cases of clinically-frank smallpox.

Standard antigen: Vaccinia virus was grown on CAM. The harvested virus was quantitated in terms of pocks formed on the CAM and the virus diluted to get 12 to 15 pocks per ml. This was used as standard antigen in comparing AGD and CEP.

RESULTS

Standard vaccinia antigen was diluted in doubling dilutions and tested by AGD and CEP. AGD gave positive results with a dilution of 1 in 4 while CEP gave a positive result at a dilution of 1 in 16. One hundred normal human sera and vesicle fluid from 4 cases of chickenpox were tested by CEP; none showed precipitates. Scabs from the 6 cases of smallpox were collected in dry sterile glass blubs and were received and tested 6 to 14 days after collection. The scabs from each case were emulsified in 1 ml of sterile saline containing antibiotics and tested by examination of stained smears, AGD, CEP and by inoculation of the CAM. CEP showed positive results in 4 of the cases while none of the other methods gave positive results in even a single case.

DISCUSSION

CEP is more sensitive than AGD for the detection of vaccinia/variola antigens. The test is rapid and results were obtained in 45 minutes. Scabs from the cases of smallpox were dried and were tested 6 to 14 days after collection. CEP gave positive results with material when all the other tests were negative. It appeared, therefore, to be quite sensitive in detecting traces of antigen in material exposed for several days to the high ambient temperatures of an Indian summer. There was no live virus in the dried scabs as no virus growth appeared on the CAM. Difco Noble agar was selected as the gelling agent because with this agar the movement of the gamma globulin towards the cathode by electroendosmosis was almost equal to that of viral antigen by the current towards the anode under the conditions used for the electrophoresis. The virus antigen showed a mobility similar to that of human serum beta$_1$-globulins. So far, we have tried out 3 different batches of Difco Noble agar and all of these have shown the same degree of electroendosmosis. The CEP method seems to be a simple, rapid and sensitive test for the laboratory diagnosis of smallpox.

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