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Rapid adenovirus typing by immunoelectron microscopy

P. LUTON

From the Department of Microbiology, University College Hospital, London

SYNOPSIS A rapid method of typing adenoviruses by immunoelectron microscopy is described. This emphasizes the value of an electron microscope in diagnostic virology, especially when a rapid result is required in epidemiology.

If virological diagnosis is to be of practical value, speed in reporting is essential and methods employing electron microscopy are being investigated with this end in view. Although specific antiviral treatment is lacking for most infections at present a rapid report is of practical use in epidemiology, and also in warning the clinician that antibacterial chemotherapy is contraindicated.

The electron microscope has been used since the early 1960s for the differentiation of smallpox and chickenpox by the examination of negatively stained material from lesions. More recently, immunoelectron microscopy has been developed to make the electron microscope a more useful diagnostic tool.

Immunoelectron microscopy is a rapid, sensitive technique enabling the direct observation of virus-antibody complexes and has been used for a number of purposes. This technique enabled Best, Banatvala, Almeida, and Waterson (1967) to take the first conclusive pictures of rubella virus. Immunoelectron microscopy has been of value in the diagnosis of serum hepatitis and has also been used to study virus-antibody interactions in a number of viruses infecting human beings, including poliovirus (Hummeler, Anderson, and Brown, 1962), influenza (Lafferty and Oertelis, 1963), herpes simplex (Rigby and Johnson, 1972), and rhinovirus (Kapikian, Almeida, and Scott, 1972).

In the routine bacteriology laboratory, agglutination methods are used to type strains of different organisms, and, since using immunoelectron microscopy it is possible to visualize agglutinated viruses, it was decided to use the technique to type adenoviruses.

Adenoviruses are commonly isolated and easily distinguished in the electron microscope. A rapid typing method would be valuable, especially in the recognition of transfer of viruses between patients. No previous account in the literature of an immunoelectron microscopic typing method for this virus could be found. Most of the strains typed were isolated in our own laboratory but a number were kindly sent to us from St Mary's Hospital, Paddington.

The typings of these viruses were confirmed by neutralization in tissue culture.

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Materials and Methods

TISSUE CULTURE

The adenoviruses were propagated in HeP_2 cell monolayers in 6 in $\times \frac{5}{8}$ in glass tubes. The cells were fed on Eagles MEM plus 10% foetal calf serum, 1% glutamine, 1% glucose, and containing 500 units penicillin, 200 μg streptomycin, and 2·5 μg amphotericin B per ml.

VIRUS

The strains of adenovirus were isolated from swabs from patients with suspected virus infections and were identified by their characteristic cytopathic effect in tissue culture. The culture tubes containing the virus were frozen and thawed once, and 0·2 ml, of this suspension was inoculated into each of five other culture tubes. These tubes were incubated at 37°C and examined daily for their cytopathic effect. When there was at least 75% cytopathic effect in the tubes, the cells were scraped from the walls of three of the tubes and the cell suspensions were combined and centrifuged at 1500 rpm in an MSE bench centrifuge for 15 minutes. The supernatant medium was decanted and 0·8 ml distilled water was added to the cell pellet with shaking until lysis occurred. This suspension could then be used immediately for

immunoelectron microscopy or clarified by low-speed centrifugation before use if too much cell debris remained suspended.

The other two infected tubes were used for neutralization tests, and were frozen and thawed once. The resulting suspension diluted 1:10 with Earles B.S.S. containing lactalbumin hydrolysate (ELa) was used as the inoculum for these tests.

ANTISERA

The antisera used for immunoelectron microscopy and neutralization tests were the adenovirus neutralizing antisera supplied by the Standards Laboratory[1]. The groups I and II polyvalent pools containing antibodies to types 1, 2, 5, and 6, and types 3, 4, 7, and 14 respectively were used for group typing. Final identification was made with the individual antisera. All were used at their recommended concentrations for neutralization tests and at 10 times this concentration for immunoelectron microscopy.

NEUTRALIZATION

Equal volumes (0·2 ml) of diluted virus suspension and antiserum were mixed in 2 in $\times \frac{1}{2}$ in glass tubes and incubated for an hour at 37°C. Positive and negative (uninoculated) controls were tested in parallel with the unknown strains. After incubation, 0·2 ml aliquots from each tube were inoculated onto HeP_2 monolayers using two tubes per test. The culture tubes were then incubated at 37°C and examined daily for their cytopathic effect. A positive result was indicated by the serum which inhibited the production of a cytopathic effect for two days compared with the positive control. The virus was first tested against the two polyvalent pools, and then against the individual antisera from its particular group. Typing by this method takes on average 10-12 days and involves the use of 20 culture tubes per strain.

IMMUNOELECTRON MICROSCOPY

For immunoelectron microscopy, 0·1 ml of virus suspension obtained by lysis of the infected cells was mixed with 0·1 ml of antiserum and incubated at 37°C for one hour. A control was incubated with phosphate-buffered saline. Again the virus was tested first against the antisera pools and then against the individual antisera.

After incubation, one drop of the test suspension was mixed with one drop of phosphotungstic acid (3% aqueous, pH 6·8) on a microscope slide, and one drop of this mixture was placed onto the surface of a carbon-formvar coated 400 mesh copper grid held in fine pointed forceps. After a few seconds the excess liquid was drained off by touching a piece of filter paper to the edge of the grid. The grids were then allowed to dry and were examined in a Hitachi HS-8 electron microscope at an instrument magnification of 31 000 ×.

Typing by this method takes only four to five hours and requires only three culture tubes.

[1]Standards Laboratory, Central Public Health Laboratory, Colindale, London NW9.

Results

Electron microscopic examination of the grids prepared from controls showed normal adenovirus particles surrounded by the dark ring of phosphotungstic acid (PTA) stain (fig 1). When observed in the electron microscope a positive result was indicated by the presence of large aggregates of virus particles surrounded by a diffuse dark zone (fig 3). A negative result appeared exactly the same as the control, ie, virus particles usually singly but sometimes in groups of two or three surrounded by a definite dark ring of stain (fig 2).

It was also noted that when photographed virions in aggregates (fig 3) were less distinct in outline than individual particles in the negative tests or controls (figs 1 and 2), presumably due to the attached antibody.

Thirty-five strains isolated in our own laboratory and nine strains from St Mary's hospital were typed both by neutralization and by immunoelectron microscopy; the results of these typings are shown in the table. All typings were confirmed by neutralization tests; the strains from St Mary's hospital were

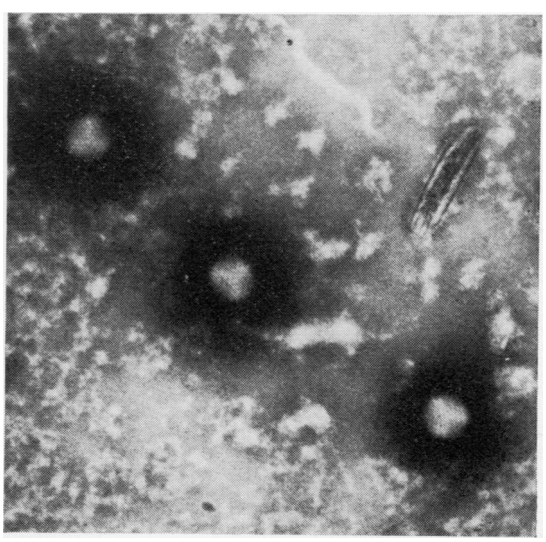

Fig 1 *Adenovirus type 3 + P.B.S. (Control) × 90 000 PTA stain*

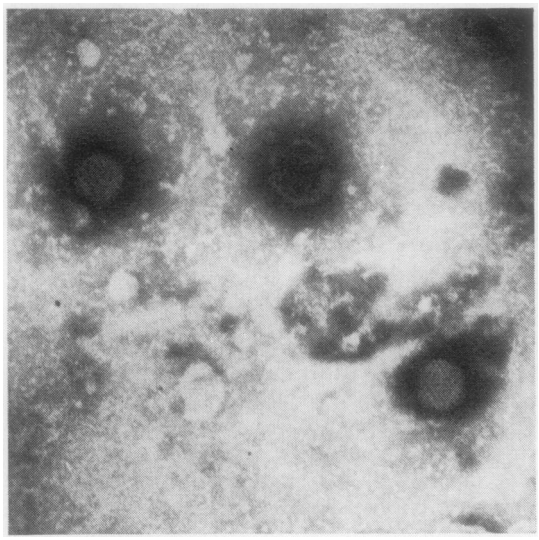

Fig 2 *Adenovirus type 3 + type 7 antiserum × 90 000 PTA stain*

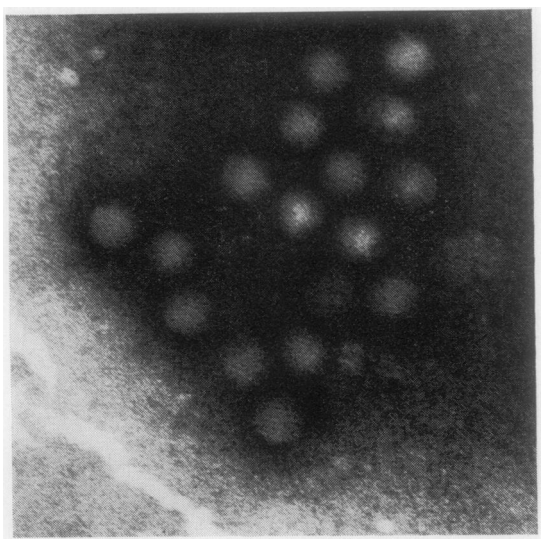

Fig 3 *Adenovirus type 3 + type 3 antiserum × 90 000 PTA stain*

No. of Strains Typed	*Group I Types*				*Group II Types*			
	1	*2*	*5*	*6*	*3*	*4*	*7*	*14*
Strains isolated at University College Hospital	—	4	3	1	24	—	3	—
Strains isolated at St Mary's	3	4	1	—	1	—	—	—

Table *Adenoviruses typed by immunoelectron microscopy*

All the strains typed by immunoelectron microscopy were confirmed by neutralization tests.

typed independently at their own virology laboratory. One of the type 7 adenoviruses required three attempts before it was satisfactorally typed by immunoelectron microscopy but even so it required only six culture tubes and took six days.

Discussion

The method of immunoelectron microscopy typing of adenoviruses described is simple and speedy, reducing the time required for typing after initial isolation from 10 to 12 days to four to five hours. The number of culture tubes required in typing each strain was reduced from 20 to three, leaving more tubes for further isolations. The results obtained by this method compare favourably with typing by neutralization as performed in our own laboratory and at St Mary's Hospital virology laboratory.

Apart from saving time and materials, another advantage is that, provided the virus is harvested when there is at least 75% cytopathic effect, there will be sufficient virus present in the fluid from the lysed cells to perform the typing without need for further concentration; occasionally the suspension may have to be diluted to give a clear result. The technique has been useful in the diagnosis of a small outbreak of adenovirus infection in the children's ward and the neonatal unit in this hospital during 1972. The infection was characterized by fever, vomiting, and diarrhoea lasting for about two days. Throat swabs and/or rectal swabs or faeces were taken from five infected children. In all cases, adenovirus was isolated. Immunoelectron microscopy showed them all to be of type 3, and the results were later confirmed by neutralization tests.

By conventional techniques, the time taken to obtain a result greatly reduces the value of diagnostic virology. This technique demonstrates the value of an electron microscope both in speed of results and in economizing on tissue culture materials.

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