The preparation of virus-infected cells for use in immunofluorescence techniques

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SYNOPSIS This paper describes the preparation of virus-infected cells for the detection of serum antibodies by the use of immunofluorescence techniques. The variations in virus growth in cells in relation to the length of incubation of cell monolayers and polio virus using two different fluorescein isothiocyanate conjugates are discussed. It is shown that there is a critical time after virus infection when a definite positive immunofluorescent reaction is given. Furthermore, a method is described enabling the clinical pathologist to prepare infected cells with the use of an automatic tube inoculator at any time during 24 hours.

There is a need for a ready supply of cells infected with specific viruses in order to detect antibody in clinical specimens. Such cells, fixed and stored, would be useful in the rapid detection of rubella antibody in the blood of a pregnant woman who had recently been in contact with German measles. Sander (1969) has already described the method of fixing cells grown on coverslips so that they may be stored for long periods at room temperature. It is important, however, to use preparations at a time when the virus may be detected most easily. Thus the critical point at which the number of virus particles is at a maximum before too much damage is done to the cell must be determined for each system of virus and cell.

Darnell, Levintlow, Thoren, and Hooper (1961) and Mayor (1961) have already shown, using other systems, that there is no linear relationship between the time after infection and ability to detect virus.

The purpose of this report is to investigate the optimal time for staining the cells and to describe an automatic tube inoculation apparatus in order to arrange that the critical time of harvest is convenient for the clinical pathologist.

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Materials and Methods

PREPARATION OF INFECTED CELLS

Standard test tubes each containing a 13 mm diameter round coverslip were sterilized at 160°C for 90 minutes. These sterile tubes were then seeded with 1.5 ml of a suspension of monkey kidney cells (10^5 particles/ml) in Parker 199 medium containing 5% calf serum and incubated so that the cover glass was horizontal. The cells grew to a confluent sheet in about four days at which time the medium was changed and inoculated with 10^5 particles of poliomyelitis virus. After further incubation at 37°C the cells on each of two cover glasses were fixed at a time interval each hour from one to 24 hours after virus infection.

FIXATION OF CELLS

The medium in each tube was discarded and acetone at 4°C used as a rinse before replacing with 5 ml of fresh cold acetone. The tubes were then placed in a bath of industrial spirit and solid CO₂ (-60°C) for 30 minutes. After fixation the coverslips were removed and

Fig. 1a Apparatus for tube inoculation (front)
1 Weighted bar (recessed for plunger tops)
2 1 ml syringe
3 Rubber bung with needle for injection and plugged air vent
4 Test tube
5 Solenoid
6 Tissue culture rack

Fig. 1b Apparatus for tube inoculation (back)
7 Switch activating rod (sprung)
8 Micro switch
9 Bar release trigger
10 Release arms
11 Distance bar

Fig. 1c Electrical circuit
TS Time switch
S Solenoid
RY Relay 24V DC
HC Hour counter
+ See text (micro switch no. 8)

Conjugate	Polio Virus Type	Time after Virus Infection (in Hours) ¹										
		1	2	3	4	5	6	7	8	9	10	11
A	I	—	(+)²	+	+	+	(+)	(+)	(+)	++	+(+)	++
	II	—	—	—	+	+	++	(+)	+	++	++	++
	III	+	(+)	++	+	+	(+)	+	+	(+)	+	(+)
B	I	—	+	+	+(+)	+(+)	+(+)	(+)	+(+)	+	+	+
	II	—	—	—	—	+	++	+	+	+++	++(+)	++(+)
	III	(+)	—	(+)	(+)	(+)	(+)	(+)	+	+(+)	++	++

Table Results for each of two conjugates, A and B

¹ — = no fluorescence, + = weak fluorescence, ++ = good fluorescence, +++ = strong fluorescence.

dried in the air and stored at room temperature. These cells are quite stable and may be stored indefinitely.

STAINING AND MOUNTING CELLS

Each monolayer of cells was stained by conjugate by the sandwich technique (Sander, 1969), using type-specific antiserum. Control cells were taken and fixed each time infected cells were sampled in order that the immunofluorescence given by conjugate alone on infected and normal cells, as well as the immunofluorescence given by non-infected cells, with the complete staining technique could be checked.

After staining and dehydration the coverslips were mounted in freshly made DPX prepared by dissolving 18.75 ml of tri-p-tolyl phosphate and 25 g of distrene 80 in 100 ml of xylene (Baker, 1966).

ILLUMINATION SYSTEM

A monocular microscope was used fitted with HBO 200 mercury vapour lamp in a Zeiss housing, 3 mm Ug 1 filter Schott Jena, surface aluminized mirror, Zeiss cardioid dark-field condenser (water immersed), and a 5 mm GG 13 barrier filter Schott Jena (colourless).

APPARATUS FOR TUBE INOCULATION

A standard tissue culture rack (6) was fitted with two uprights, each 330 × 40 mm, which were grooved at the top to a depth of 65 mm (Figs. 1a and 1b). To the centre back of the rack a 24V DC solenoid (5) was fitted and this was also attached to two release arms (10) which in turn were connected to a bar release trigger (9).

The electrical circuit (Fig. 1c) consisted of a time switch, TS, having open contacts in the normal position, a microswitch (8) with the NO contact to the solenoid (5) and a 24V DC relay, the NO contact of which was connected to the hour counter HC 240V AC.

In operation the test tube (4) containing coverslip and medium was placed in the back row of the rack; the syringe assembly, filled with virus suspension and kept at 37°C to prevent

leakage, was inserted in the test tube and the plunger raised to fit into the recess of the weighted bar (1) which rested on the bar release trigger (9).

After a preset time, the time switch activated the solenoid which released the trigger to allow the bar to drop, simultaneously injecting the virus suspension into the test tube. At the end of the travel the dropping bar contacted the microswitch via rod (7) which deactivated the solenoid and activated the hour counter giving the time of incubation with virus. Although the apparatus was constructed for eight test tubes it could be made larger, bearing in mind the need to fit the apparatus into an incubator.

Results

The results of intensity of illumination of cells shown in arbitrary degrees, indicated by — to + + +, with (+) indicating an intermediate value, for each of two conjugates, A and B, are shown in the Table. These results are reproducible and have remained constant for four years.

It is clear that the optimum time after virus infection for fixing the cells (++ or greater) differs for each of the three types of polio-myelitis virus. It is also of interest to note that this time is dependent also upon the conjugate used. With type III virus, for example, good staining can be seen in three hours with conjugate A whereas virus growth for 10 hours is required with conjugate B.

Discussion

It is well recognized that the replication of viruses in cell cultures goes through an eclipse phase, during which virus cannot be detected, to a phase of complete virus production. If this relationship held when virus was detected by immunofluorescent techniques then it would be rational to leave the cell cultures for a given time after virus infection and thereafter virus would always be detectable. This indeed would

Time after Virus Infection (in Hours)

12	13	14	15	16	17	18	19	20	21	22	23	24
++	+(+)	+(+)	(+)	—	—	+	+	(+)	+	+	(+)	(+)
++(+)	—	(+)	(+)	+	(+)	—	+	+(+)	(+)	+	+(+)	+(+)
+	++	++(+)	+++	+	+	+	++	++	+++	++(+)	+(+)	++
++	+++	+++	++	+	+	—	—	—	—	(+)	++	+++
+++	+++	++	++(+)	++	(+)	(+)	(+)	(+)	(+)	—	(+)	(+)
++	—	+	+	+(+)	+(+)	+(+)	(+)	+(+)	—	+(+)	+(+)	+(+)

Table—continued

*Symbols in brackets signify intermediate values.

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be satisfactory if it were not critical to make an early diagnosis of virus infection or of the presence of antibody. It is clear from these data, however, that there is a critical time, after virus infection, at which virus is present in sufficient quantity to give a definite positive reaction easily distinguishable from the autoimmune fluorescence seen to some degree with all cells and all conjugates.

The differences in the time at which virus may be detected by different conjugates are surprising but it must be emphasized that these results are reproducible, and they underline the need to investigate the optimum time for each virus, each cell substrate, and each conjugate. When the optimal time for each system has been

established, however, the results are reproducible.

These data will enable the clinical pathologist to prepare infected cells using the automatic tube inoculator at any time during the 24-hour daily period so that the cells will be at the optimum time for virus detection at any convenient moment to him.

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