Technical methods

Storage of virus-infected tissue culture substrates for the detection of serum antibodies by immuno-fluorescence

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Virus-infected tissue coverslip cultures are required with increasing frequency for the determination of antibodies in patients’ sera by the technique of immunofluorescence. To avoid the necessity of preparing fresh cultures for each determination a method has been developed by which large batches of coverslip cultures may be preserved in a condition for immediate use. The coverslip cultures prepared by this technique were found after storage at room temperature to be stable for over two years without deterioration.

In the development of the method it was considered that the two important criteria to be met were (1) the minimum alteration of the viral antigen and (2) maximum storage life at room temperature.

It was suggested by Nairn (1964) that fixation with cold acetone resulted in minimum interference with antigens and effected good dehydration. A considerable number of fixatives commonly used in histology and immunofluorescence were investigated, and acetone at −60°C for 30 minutes was found to be the most satisfactory.

Batches of coverslips, carrying tissue culture monolayers infected with a variety of viruses and subsequently fixed and dried according to the method below, have been stored in small plastic boxes at room conditions for over two years without deterioration.

METHOD

All reagents used were ‘Analar’ grade.

1 Round coverslips, diameter 13 mm, were washed in hot 10% KOH for two to three minutes followed by rinsing in 1/5 NHCl and washed again three times in distilled water. After air drying the coverslips were transferred singly to a test tube 153 mm × 16 mm after which they were baked at 160°C for one and a half hours. The tubes containing coverslips were then ready for receiving 1.5 ml of cell suspension each and later, after growth of tissue, for the addition of virus at an appropriate time. The coverslips were not removed from the tubes for steps 2, 3, and 4 below.

2 The medium was discarded and the coverslips were washed with phosphate-buffered saline (Dulbecco and Vogt, 1954). The washing fluid was discarded.

3 The tubes were then very quickly rinsed twice with acetone (4°C).

4 Then the tubes were half filled with acetone (4°C) and plunged into a mixture of ethyl alcohol (95%) and solid CO₂ at −70°C for 30 minutes. It was found to be necessary to add additional solid CO₂ from time to time.

5 The acetone was discarded and the coverslips were removed from the tubes.

6 Finally the coverslips were air-dried and stored in small plastic boxes at room temperature.

Specially designed trays were used to hold the coverslips during staining, made as follows: a glass dish was filled to a height of 3 to 4 mm with molten histological paraffin wax, and while hot, glass rings were dropped into the wax.

After cooling a series of wax-bottomed glass cells were formed so that a large batch of coverslips could be stained individually at the same time, and after treatment with antiserum and subsequent washing the coverslips could be transferred to a fresh glass cell for the application of conjugate. After use, the rings were easily removed by heating the dish to ±60°C and discarding the wax. The rings were prepared for use again by boiling in soapy water which removed any traces of wax, conjugate, or serum.

After the staining procedure the coverslips were examined after mounting in PBS or buffered glycerol, and subsequently, after washing they were dehydrated in acetone at room temperature for 30 seconds. After clearing for one minute in xylene they were permanently mounted on microscopic slides (maximum thickness 1 mm).

The DPX mountant used was the tri-p-tolyl phosphate formula of Baker (1966). (DPX prepared with dibutylphthalate as plasticizer is not suitable.) Fresh mountant was prepared monthly.

Coverslips mounted in this way showed a minimum loss of fluorescence over a period of years when stored at room temperature.

REFERENCES


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