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Rapid diagnosis of genital herpes by detecting cells infected with virus in smears with fluorescent monoclonal antibodies

M BOTCHERBY,* C GILCHRIST,*† J BREMNER,* M A BYRNE,*† J R W HARRIS,* D TAYLOR-ROBINSON*† *From the *Jefferiss Research Wing, Praed Street Clinic, St. Mary's Hospital, London, and the †Division of Sexually Transmitted Diseases, Clinical Research Centre, Harrow, Middlesex*

Herpes simplex virus (HSV) is a common cause of infection, and while the importance of technical help in a clinically difficult diagnosis is accepted, the importance of HSV typing is less well known. Knowledge of the virus type can, however, be useful as the rate of recurrence of a genital HSV-1 infection is considerably less than that of a HSV-2 infection.¹ Furthermore, certain antiviral drugs are more effective against one or other type of virus^{2 3}; typing is also useful epidemiologically to determine the association of the virus with other conditions.

The traditional method of detecting HSV is isolation in cell culture, which usually takes two to three days, and longer if subculture is required. Typing can then be undertaken by immunological or restriction endonuclease analysis, or by monoclonal antibodies in cell cultures. A means of diagnosing and typing HSV rapidly, would therefore be valuable in the management and treatment of herpetic infections. In this study cells infected with virus from presumptive herpetic lesions were sought using the MicroTrak HSV-1/HSV-2 Direct Specimen Identification/Typing reagents produced by Syva, and the results were compared with those obtained from isolating and typing the virus in cell culture.

Material and methods

COLLECTION OF SPECIMENS

The cap of a vesicle was lifted with a sterile needle

and, in the case of ulcers, pus removed with a sterile swab. The base of the lesion was then wiped firmly with a swab moistened with sterile water. Specimens were not taken from crusted lesions, because this would have interfered with healing. The swab was rolled over a two-well slide and the smear air dried and fixed in acetone for 10 minutes. Slides were stored at -20°C. Another specimen was taken in viral transport medium and stored in liquid nitrogen until virus isolation in cell culture was attempted.

ISOLATION AND TYPING OF VIRUS IN CELL CULTURE

Confluent monolayers of Vero cells (Flow laboratories) were inoculated with 0.1 ml of the specimen (two tubes per specimen) and incubated at 33°C. The cultures were examined daily for the development of a cytopathic effect. When half the cells in a culture were affected (++) they were all harvested by scraping, and transferred to a two-well slide. They were fixed, as described above, and the virus typed using the MicroTrak Culture Confirmation reagents according to the manufacturer's specifications. The time taken for all the cells in a culture to become affected (++++) was recorded.

DETECTION AND TYPING OF CELLS INFECTED WITH HSV IN LESIONS (DIRECT TEST)

A 30 µl volume of the fluorescent monoclonal antibody to HSV-1 (MicroTrak Direct Specimen Identification/Typing Reagent; Syva) was added to the well on the left of the slide containing the fixed smears, and 30 µl of the HSV-2 reagent to the well on the right. The slides were incubated at 37°C for 15 minutes in a moist chamber, rinsed with distilled water (care being taken to avoid cross contamination of the wells), air dried and mounted with the mounting medium provided (buffered glycerol, pH 9.4). They were examined at a magnification of 200 with an Olympus microscope fitted with an epifluorescence system and a halogen light source. A positive result was recorded when at least one fluorescing cell was found, and a specimen was regarded as negative when no fluorescing cells were found among at least 20 counterstained basal or parabasal epithelial cells. Smears containing fewer cells were rejected. Slides were read blind by one observer (MB) as culture results were not available until the next day at the earliest and clinical details were not disclosed until the end of the study.

Results

Samples were taken from 58 patients considered by

the clinician to have genital herpes. A single specimen was taken from each patient, except for two from whom two specimens were obtained, giving a total of 60 specimens. HSV was isolated in cell culture from 36 of the specimens (34 patients). Twenty five of the specimens were adequate for the direct test and 22 were positive; 18 of the latter were positive also in culture (table 1). Of the four specimens that were not positive on culture, three were bacterially contaminated. The typing by the direct test duplicated the confirmation typing on culture. There was only one adequate specimen in the direct test that was positive and yet negative on culture (a false positive); a second specimen from this patient proved to be positive on culture. There were no specimens that were negative in the direct test but positive on culture (false negative). Thirty five specimens, however, were submitted for the direct test but were deemed inadequate; of these, 18 were positive on culture.

The correlation between the speed with which a cytopathic effect developed in cell culture and the number of fluorescing cells identified in the direct test on individual lesions was assessed. The speed of the cytopathic effect was regarded as an indirect measure of the virus titre, rapid development being associated with a high virus titre. All but one of the specimens adequate for direct testing were associated with high virus titres, and no specimen taking more than three days to produce + + + + produced a positive result in the direct test (table 2). This indicated that a large number of infected cells were required for this technique to be successful.

An attempt was made to determine whether the kind of lesion (vesicular or ulcerated) had more influence on the direct test than the age of the lesion. Table 3 shows that inadequate specimens were

Table 2 Correlation between speed of cytopathic effect and No of infected cells in direct test

No of days to reach + + + +	No of specimens	Average No of infected cells in direct test	% of total No of cells fluorescing in direct test
2	12	21	41.5
3	6	4	24.5
4	5	0	0
5	3	0	0

derived as often from ulcers of a few days' duration as from older ulcers. Fewer inadequate specimens came from vesicular lesions (three of 11) than from ulcerated lesions (32 of 51). The three vesicles providing inadequate specimens were very small, two being seen only on colposcopy. It seems that the state of the lesion rather than its age is the critical factor in the success of this technique.

Discussion

The obvious difficulty encountered in the direct immunofluorescence test for diagnosing herpetic infections is that of obtaining sufficient infected basal epithelial cells. We found that the age of the lesion was not as critical as its stage of development in producing a positive result. This confirms reports that vesicular lesions are more suitable for immunofluorescence techniques than older, ulcerated lesions.^{4,5} Our results indicate that early lesions contain more infectious virus than later lesions, and if the lesion is still blistered the infected basal cells will be numerous and moist, making collection easier. The

Table 1 Results of testing specimens from patients suspected of having genital herpes for HSV in cell culture and for cells infected with virus by fluorescent monoclonal antibodies

No of specimens	Result of virus detection	
	In cell culture	By direct test
18*†	+	+
3	—	—
0	+	—
1†	—	+
3	Bacterially contaminated	+
18	+	Inadequate specimen
16	—	Inadequate specimen
1	Bacterially contaminated	Inadequate specimen

*Two specimens were taken from each of two patients in this group.

†Virus isolated from a second specimen nine days later.

‡Same HSV type indicated by both methods in all 18 cases.

Table 3 Distribution of inadequate and adequate specimens for direct test in relation to age of herpetic lesions

Age of lesion (days)	Specimens from ulcerated (U) or vesicular (V) lesions of culture positive patients that were:	
	Inadequate	Adequate
1	V*	V V U U
2	V† U U U U U U U U	V V V V U U U
3	V† U U U	V U U U
4	U U U U U	U U U
5	U U U U	V U
6	U U U	U U
7	U U U U U U	U U
8	U	
9		U
10	U	
14	U	

*Small lesion less than 24 hours old. †Small vesicles yielding inadequate specimens identified only by colposcopy.

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approach, however, should be to collect epithelial cells without excess vesicle fluid, because this may contain viral antigens that react with the monoclonal antibodies and produce a green haze over the slide, making it difficult to interpret. On the other hand, once the lesion is ulcerated, infected cells are less numerous, and numbers sufficient to provide an adequate sample difficult to obtain, their collection proving painful for the patient. Despite these problems, however, the value of the direct test seems obvious. Apart from its ability to give a result within 30 minutes of the specimen being taken, it was found to be sensitive if an adequate specimen was obtained; and there was only one occasion on which the direct test was positive but HSV was not isolated in culture. HSV was isolated, however, from this patient on a second sampling. The typing of HSV by the direct test was also specific. We do not foresee this technique replacing cell culture procedures, but it seems logical to use the two in combination; a sample taken for examination in cell culture at the same time as one for the direct test could be discarded if the latter specimen was adequate, which our results indicate would occur in about 40% of cases.

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Requests for reprints to: Dr D Taylor-Robinson, Division of Sexually Transmitted Diseases, Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ, England.

Rapid immunofluorescence staining of human renal biopsy specimens using microwave irradiation

K Y CHIU, K W CHAN *From the Histopathology Unit, Department of Pathology, University of Hong Kong, Queen Mary Hospital Compound, Hong Kong*

Immunological studies of renal tissue are indispensable both in clinical practice and in experimental glomerulonephritis. Immunofluorescence is the usual technique used to measure concentrations of immunoglobulins and complements when diagnosing renal disease, especially IgA nephropathy and membranous glomerulonephritis.¹⁻⁴ Immunofluorescence is performed on fresh frozen sections,⁵ and detachment of the section from the glass slide is a common problem during incubation and washing, especially when the sections have been incompletely dried. To ensure firm adherence the cryocut sections are usually dried for about 30 minutes at room temperature and then incubated with the fluorescein conjugated antibodies for another 30 minutes.⁵ We describe a method in which microwave irradiation is used for the

rapid drying of frozen sections and incubation of fluorescein conjugated antisera.

Material and methods

Human renal tissue obtained by percutaneous needle biopsy was divided into three portions for light microscopy, electron microscopy, and immunofluorescence staining. For immunofluorescence, fresh renal tissue was embedded in ornithine carbonyl transferase medium and snap frozen in liquid nitrogen. Cryostat sections were cut at 6 μ m, and spare sections for this study were stored at -30°C before immunostaining. We selected 12 cases for study after the definitive diagnoses had been made and the formal pathology reports had been issued; six showed IgA nephropathy, four active diffuse proliferative lupus nephritis, and two showed idiopathic membranous glomerulonephritis. Before immunostaining, the frozen sections were placed horizontally on the rim of the carousel turntable in a domestic microwave oven (Sharp Model R-7200, Japan) and irradiated for (12, 36, 48) seconds and (one, two, five, 10, 20) minutes at the medium to low (defrost) setting for drying. The oven operated at 2450 MHz and had a maximum output of 600 W. After microwave defrosting the sections were washed in two changes of phosphate buffered saline (PBS) pH 7.4 for two