British Journal of Ophthalmology, 1985, **69**, 637–639

A rapid and sensitive culture test for detecting herpes simplex virus from the eye

P WALPITA, S DAROUGAR, AND U THAKER

From the Institute of Ophthalmology, Judd Street, London WC1H 9QS

SUMMARY A rapid and sensitive culture test has been developed for detecting herpes simplex virus (HSV) in ocular infections. The virus is cultured by inoculation and centrifugation of cell monolayers grown on coverslips and the inclusions detected by an indirect immunofluorescence technique. This rapid test takes only two days to complete. By comparison, in our hands the conventional culture test, which depends on the development of cytopathic effect, took between 1 and 20 days with a mean of 4·7 days. Of the 1638 ocular clinical specimens inoculated in parallel by the two methods a total of 188 were positive for HSV. The virus was detected from 184 (97·8%) specimens by the rapid test and from 144 (76·6%) by the conventional test (McNemar's test, $U=5{\cdot}76$, $p<0{\cdot}001$).

Herpes simplex virus (HSV) is one of the commonest causes of corneal blindness in the developed countries. Some 300 000 cases of ocular herpes virus infections are diagnosed each year in the United States alone.[1]

In the early stage of primary HSV ocular infection, when typical lid lesions may be scanty, or in cases of HSV conjunctivitis or keratoconjunctivitis with no typical corneal lesions,[2,3] the differential diagnosis from other infections and acute allergic conditions is difficult on clinical grounds alone. In view of the potentially serious consequences of ocular HSV infection, and the fact that effective anti-HSV therapy is now available, rapid and sensitive laboratory diagnosis is essential for identifying all the various forms of this disease.

The traditional methods of laboratory diagnosis of viral infection, either by virus isolation in cell culture or by demonstration of rising antibody titre in paired sera, may take days or weeks to complete. Direct demonstration of the agent in exfoliated cells[4–6] has been tried but is generally less sensitive than cell culture, and it requires high-quality smears and strict criteria of identification of viral inclusions.

We describe here a rapid, simple, and sensitive cell culture method for the diagnosis of HSV ocular infections.

Correspondence to Dr P Walpita.

Materials and methods

Specimens. Conjunctival swabs were collected from patients attending the External Diseases Clinic at Moorfields Eye Hospital in London. The specimens were collected in plastic tubes containing glass beads and 2 SP transport medium with antibiotics[7] with the addition of 3% v/v fetal bovine serum. All specimens were stored in liquid nitrogen at −180°C for transport to the laboratory, where they were then stored at −70°C until cultured.

Cell culture and inoculation. HEp2 cell monolayers were obtained by seeding at a concentration of 25 000 to 30 000 cells/ml of growth medium in cell culture test tubes and in flat-bottomed plastic tubes containing 13 mm coverslips. Growth medium consisted of Eagle's minimum essential medium supplemented with vitamins, glutamine, antibiotics (vancomycin 100 µg/ml and streptomycin 50 µg/ml) and 10% fetal bovine serum. At the end of 48 hours' incubation, when the monolayers were approximately 80% confluent, growth medium was replaced with maintenance medium (same as growth medium but with only 3% fetal bovine serum).

Each specimen was whirlimixed and an equal volume inoculated into one test tube and one flat-bottomed tube containing the cell monolayers. Positive and negative controls were inoculated with each batch of tests.

Table 1 *Comparison of the steps involved in the rapid culture test and the conventional tissue culture test*

Rapid culture test	*Conventional tissue culture test*
	Initial inoculation:
1. Cell monolayer on coverslip in FBT	cell monolayers in TT
2. Inoculation	inoculation
3. Incubation 2 days	incubation 7 days
4. IF staining	examine for CPE
Course of test 2 days	1st pass:
	incubation 7 days
	examine for CPE
	2nd pass:
	incubation 7 days
	examine for CPE
	IF staining of smears from test tubes showing CPE
	Course of test up to 21 days

Conventional cell culture test. The inoculated test tubes were rolled at 35°C and examined every day for the development of cytopathic effect (CPE). Smears were made from all test tubes showing CPE and the virus identified by an indirect immunofluorescence test (IF). All negative cultures were passaged at weekly intervals before being discarded at 21 days. The individual steps involved in the test are shown in Table 1.

Rapid culture test. The inoculated flat-bottomed tubes were centrifuged at 15 000 *g* for one hour and then incubated at 35°C. After 48 hours incubation the coverslips were fixed in methanol for 10 minutes at room temperature and stained by IF. The individual steps of the rapid test are shown in Table 1.

Immunofluorescence staining method. Group specific anti-HSV serum raised in rabbits in our laboratory and anti-rabbit IgG conjugated with fluorescein isothiocyanate (Wellcome Reagents Ltd) were used for staining HSV. Optimum working dilutions were determined by previously titrating each batch of reagents.

Coverslips were mounted on a staining frame, covered with a drop of the appropriately diluted anti-HSV serum, and incubated in a humid chamber at 35°C for half an hour. The coverslips were then thoroughly washed in phosphate buffered saline at pH 7·3 for 15 minutes by means of a magnetic stirrer. They were air-dried, covered with fluorescein conjugated anti-rabbit serum, and incubated and washed in buffered saline as before. They were finally washed in distilled water for five minutes before being dried and mounted in buffered glycerol. The coverslips were examined for the presence of inclusions at a magnification of 160 times under a standard 18 UV Zeiss microscope with filter set 10. The quality of inclusions was checked under high power (×400).

Fig. 1 *Brightly fluorescing intracellular herpes simplex virus inclusions stained by an indirect immunofluorescence method. (×540).*

The criterion of positivity was the detection of one or more brightly fluorescing granular inclusions within intact cells (Fig. 1). The quality of IF reagents was controlled by including positive and negative control coverslips in each batch of tests.

Results

A total of 1683 ocular clinical specimens were inoculated in parallel by both the rapid test and the conventional cell culture test. Correlation of positivity between the two tests is shown in Table 2. HSV was detected by either or both tests in a total of 188 specimens. Of these, 184 (97·8%) were positive by the rapid test and 144 (76·6%) by the conventional test (Table 2). The difference in sensitivity between the two tests is statistically significant (McNemar's test, U=5·76, p<0·001).

Table 2 *Correlation of positivity for ocular HSV by the rapid test and conventional test*

Results of paired tests	*Number (%) of specimens positive for HSV isolation*
Nos. positive by either or both tests	188 (100)
Nos. positive by rapid test	184 (97·8)
Nos. positive by conventional test	144 (76·6)
Nos. positive by both tests	140 (74·5)
Nos. positive by rapid test only	44 (23·4)
Nos. positive by conventional test only	4 (2·1)

McNemar's test (rapid test vs. conventional test), U=5·76, p<0·001.

Table 3 *Day of first appearance of cytopathic effect in 144 ocular HSV-positive cultures by the conventional test*

Day	*Nos. showing CPE (%)*
1	26 (18·06)
2	38 (26·4)
3 to 7	57 (39·6)
8 to 14	19 (13·19)
15 to 21	4 (2·78)

Mean=4·7 days, SD 3·6 (range 1–20 days).

The number of days taken to develop CPE in the conventional test varied from 1 to 20 (Table 3). Only 44% of the HSV positive specimens showed CPE at day 2 and as many as 16% took eight days or longer.

Discussion

We have developed a rapid culture test for HSV isolation from ocular infections and found it to be considerably faster and significantly more sensitive than the conventional cell culture test. It took far fewer steps to complete and was therefore less expensive.

In this study we have used HEp2 cells which are known to be sensitive for HSV isolation.[8] The HSV isolation rate by the rapid test was 21% higher than the conventional test. This is probably due to the increased contact and penetration of the virus into cells resulting from centrifugation. In a previous study[9] we found that centrifugation at 15 000 *g* can significantly increase the sensitivity of the cell culture for HSV isolation.

In a pilot study we compared the sensitivity of the rapid test at 24, 48, and 72 hours of incubation. The sensitivity of the test was similar at 72 and 48 hours but marginally lower at 24 hours. Because of this we chose 48 hours as the optimal incubation period.

In our hands the IF staining of HSV in cell culture proved to be entirely satisfactory. The antisera we used were highly specific, and each batch was titrated for optimum working dilution. Positive and negative controls were set up with each batch of tests to control the quality of cell culture and staining. The distinction between positive and negative reaction was clear cut (Fig. 1). The positive cultures produced brightly fluorescing, granular, and intracellular inclusions. Such inclusions were not found in negative controls. Our previous work has shown that, when the strict criteria of culture staining and recognition of inclusions are adhered to, the finding of even one such inclusion is adequate for a positive diagnosis and that the chances of a false positive in the rapid test are remote (unpublished observations).

The rapid test was completed in only 48 hours, whereas the conventional test took between 1 to 20 days, with a mean of 4·7 days. It is widely believed that HSV grows rapidly in various cell lines and that most positives show CPE within 48 hours. However, there is little reported evidence to support this belief. In a study by Moore,[10] where a large proportion of the specimens were from vesicular lesions with an expectedly high virus titre, only 72% of the HSV positives were detectable at day 2. In our study HSV was detected in the conventional test in only 44% of the positive specimens within two days, and 16% took more than seven days to develop CPE (Table 3).

The results of this study show that the rapid culture test is much simpler, faster, and less expensive, and significantly more sensitive than the conventional culture test for detecting HSV from ocular infections.

We are grateful to the staff of the External Diseases Clinic and Casualty Department, Moorfields Eye Hospital, for the collection of specimens.

References

1 Pavan-Langston D. Diagnosis and management of herpes simplex ocular infections. *Int Ophthalmol Clin* 1975; **15:** 19–35.
2 Darougar S, Wishart MS, Viswalingam N. Epidemiological and clinical features of primary herpes simplex virus ocular infection. *Br J Ophthalmol* 1985; **69:** 2–6.
3 Darougar S, Hunter PA, Viswalingam N, Gibson JA, Jones BR. Acute follicular conjunctivitis and keratoconjunctivitis due to herpes simplex virus in London. *Br J Ophthalmol* 1978; **62:** 843–9.
4 Gardner PS, McGuillan J, Black MM, Richardson J. Rapid diagnosis of herpes simplex virus hominis in superficial lesions by immunofluorescent staining techniques. *Br Med J* 1968; **iv:** 89–92.
5 Chieu Liu, Llaues-Rodas R. Application of immunofluorescent staining technique to the study of pathogenesis and rapid diagnosis of viral infections. *Am J Clin Pathol* 1972; **57:** 829–34.
6 Schwartz HS, Vastine DW, Yamashioya H, West CE. Immunofluorescent detection of adenovirus antigen in epidemic keratoconjunctivitis. *Invest Ophthalmol Vis Sci* 1976; **15:** 199–207.
7 Gordon FB, Harper IA, Quan LA, Treharne JD, Dwyer RStC, Garland JA. Detections of Chlamydia (Bedsonia) in certain infections of man. I. Laboratory procedures: comparison of yolk sac and cell culture techniques for detection and isolation. *J Infect Dis* 1969; **120:** 451–62.
8 Grist NR, Bell EJ, Follett EAC, Urquhart GED. *Diagnostic methods in clinical virology*. 3rd ed. Oxford: Blackwell, 1979.
9 Darougar S, Gibson JA, Thaker U. Effects of centrifugation on herpes simplex virus isolation. *J Med virol* 1981; **8:** 231–5.
10 Moore DF. Comparison of human fibroblast cells and primary rabbit kidney cells for isolation of herpes simplex virus. *J Clin Microbiol* 1984; **19:** 548–9.