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Research article

Comparison of the sensitivity of a 24 h-shell vial assay, and conventional tube culture, in the isolation of Herpes simplex virus – 1 from corneal scrapings

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Abstract

Background: Herpes simplex keratitis is a sight threatening ocular infection. A rapid and specific diagnosis is essential for the institution of specific antiviral therapy and to avoid complications that can arise from misdiagnosis and inappropriate treatment. Though a variety of techniques are available, isolation of Herpes simplex virus 1 (HSV-1) in culture provides the most reliable and specific method, and is considered as the gold standard in laboratory diagnosis of herpes simplex keratitis. We report a comparative study of the sensitivity of a 24 h-shell vial assay and conventional tube culture in the isolation of HSV-1 from corneal scrapings.

Methods: A total of 74 corneal scrapings obtained from 74 patients with a clinical suspicion of herpes simplex keratitis submitted for the isolation of HSV-1, were simultaneously inoculated into shell vial and tube cultures employing the vero cell line. Shell vial and tube cultures were terminated at 24 h and fifth day respectively. Isolation of HSV-1 was confirmed employing an indirect immunofluorescence assay.

Results: HSV-1 was isolated from 24/74 (32.4%) specimens employing both the methods. Sensitivity of both the techniques were found to be similar (20/24, 83.3%) (P = 1.0).

Conclusion: A 24 h-shell vial assay is a rapid alternative technique in comparison to the time consuming conventional tube cultures for the isolation of HSV-1, especially from corneal scrapings for the laboratory diagnosis of herpes simplex keratitis.

Background

Herpes simplex keratitis (HSK) is a sight threatening ocular infection, which is a leading cause of corneal blindness and occurs worldwide [1]. HSK can present both in its typical and atypical forms. HSK is one of the most challenging ocular viral infection confronting the clinician, both from a diagnostic and therapeutic perspective [2], especially when it occurs in its atypical form. It is essential that a rapid and specific diagnosis is offered under such circumstances, for the institution of specific antiviral therapy

and to avoid complications that can arise from misdiagnosis and inappropriate treatment. A number of methods have been used for the rapid diagnosis of HSK [3–5]. However, isolation of HSV-1 in culture is considered as the "Gold Standard" in the laboratory diagnosis of HSK. HSV-1 can be isolated in culture by various techniques including conventional tube cultures (TC), centrifugation enhancement (spin amplification) of HSV replication referred to as shell vial assay (SV), high speed rolling technique and the suspension – infection culture [6].

SV is a commonly used technique for the detection of Cytomegalovirus [7]. It has been successfully adapted for the detection of HSV from clinical specimens [8,9]. However, tube cultures are often infected in parallel to detect the low viral load specimens, which may not be detected by SV [6]. The outcome of these techniques may also depend on the nature of specimen processed and the viral load. Corneal scraping is the most common specimen obtained from patients for the laboratory diagnosis of infectious keratitis due to viral and non-viral agents. There are no reports comparing the performance characteristics of a 24 h SV and TC in the isolation of HSV especially from corneal scrapings, for the laboratory diagnosis of HSK. Therefore, we report here our results of a comparison of the sensitivities of these two techniques using corneal scraping as the specimen.

Methods

Determination of sample size

To determine the sample size, 11 patients clinically diagnosed as cases of HSK (either dendritic or geographic ulcers) were included in a preliminary study. Corneal scrapings were collected from these patients for: a) Papanicolaou (PAP) stain for the detection of multinucleated giant cells, b) detection of viral antigen and c) shell vial and tube cultures for the isolation of HSV-1. Multinucleated giant cells were observed in 2 specimens while viral antigen could be detected in all the 11 specimens. HSV-1 could be isolated in 6/11 specimens (54.5%). TC yielded virus in all the 6/11 (54.5%) specimens while SV was positive only in 3/11 (27.2%) specimens. Based on these results, the sample size was determined to be 74 (alpha risk = 5%, power 90%, 95% CI) using a computer assisted statistical programme (Epi Info, Version 6.04b, CDC, U. S. A).

Specimens

Corneal scrapings were obtained after consent from a total of 74 patients with a clinical suspicion of HSK. Corneal scraping was collected using the slit lamp or operating microscope after the instillation of topical anaesthetic (4% Lignocaine hydrochloride or 0.5% Proparacaine hydrochloride). Specimens were transferred to a vial containing 1 ml of viral transport medium (VTM) and transported to the virology laboratory immediately. Additionally, scrapings were collected from all the patients and transferred onto sterile glass slides for other virological investigations like a) detection of viral antigen and b) PAP stain for the detection of multinucleated giant cells. A confirmed laboratory diagnosis was offered to our clinicians when HSV-1 was isolated in culture and/or viral antigen was detected in a given specimen. PAP stain served as a valuable adjunct in the laboratory diagnosis of HSK. HSV-1 was isolated employing a shell vial assay and conventional tube cultures using vero cells (Fig. 1) (National Facility for Animal Tissue and Cell Cultures, Pune, Maharashtra, India). Shell vials and conventional tube cultures were prepared as per standard procedures [10]. Specimens collected in VTM were vortexed vigorously for 30 seconds and an equal volume (0.5 ml) of the sample was inoculated into a shell vial and a tube culture in parallel.

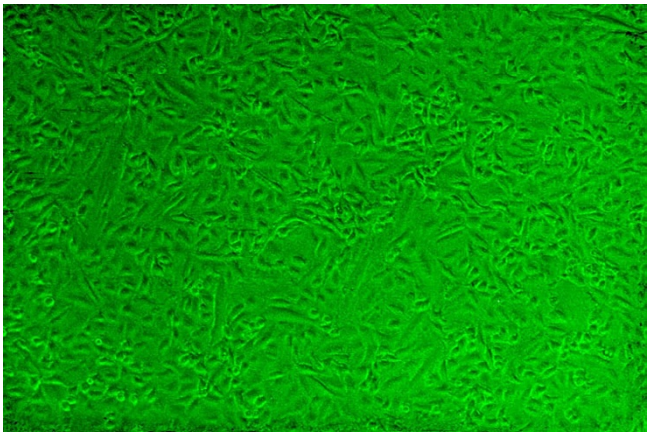

Figure 1
Shell vial culture/Tube culture. Uninfected monolayer of vero cells. Phase contrast microscopy, × 40.

Shell vial cultures

Following inoculation of the specimen, the shell vial was centrifuged at 700 × g at room temperature followed by incubation at 36°C for 1 h for adsorption. The inoculum was discarded and 1 ml of maintenance medium (MEM with 1 % foetal bovine serum) was added. The vial was incubated for 24 hrs at 36°C in a CO_2 incubator. The coverslip was removed, fixed in cold acetone for 30 minutes at -70°C and stained by an indirect immunofluorescence assay (IFA) using a polyclonal antibody to HSV-1 (Dako, Carpinteria, LA).

Tube cultures

Following inoculation of the specimen into a tube culture, the culture was incubated for 1 h at 36°C for adsorption, the inoculum was removed and 1.5 ml of maintenance medium (MEM with 1% fetal bovine serum) was added. Cultures were incubated at 36°C in a CO_2 incubator for

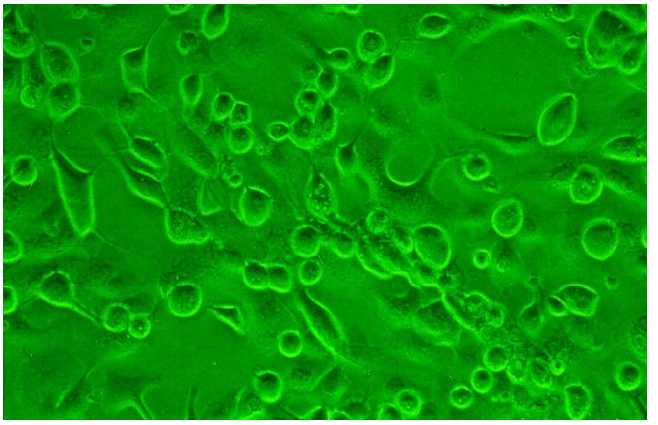

Figure 2
Tube culture inoculated with a clinical specimen. Note the presence of typical cytopathic effect (CPE) caused by HSV-1. Phase contrast microscopy, × 200.

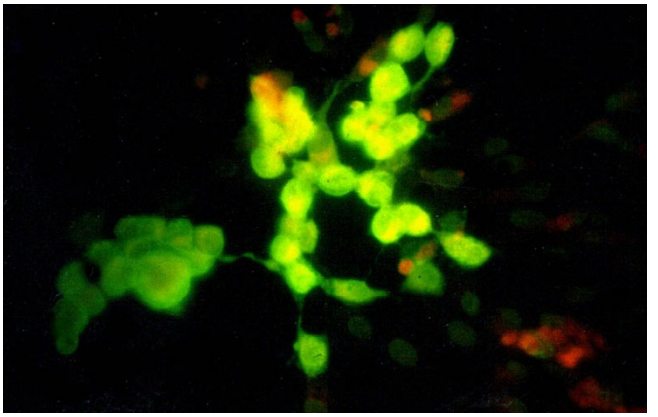

Figure 3
Shell vial culture positive for HSV-1. Note the presence of brightly fluorescing (apple green) HSV-1 infected cells. Indirect immunofluorescence assay, × 250

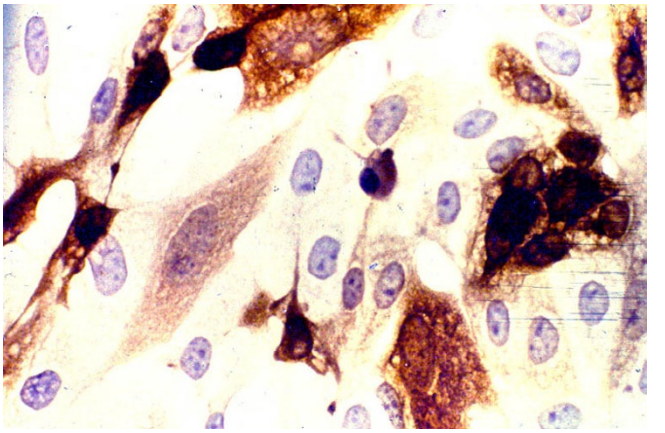

Figure 4
Shell vial culture positive for HSV-1. Note the presence of HSV-1 infected cells (stained dark brown). Uninfected cells appear bluish purple due to counterstaining with haematoxylin. Indirect immunoperoxidase assay, × 500.

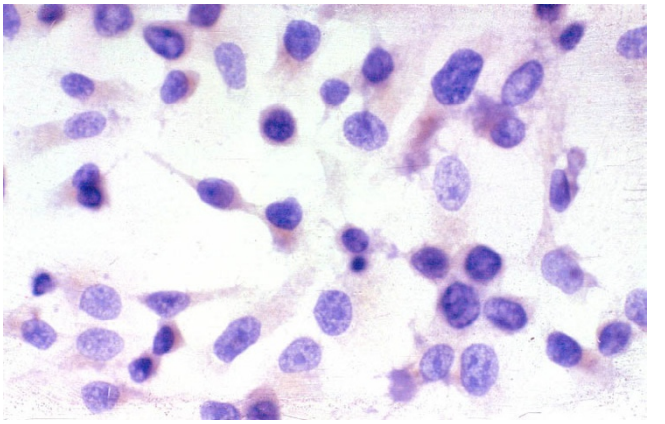

Figure 5
Shell vial culture negative for HSV-1. Note the absence of any staining of the cells. Indirect immunoperoxidase assay, × 500.

five days and observed for the presence of cytopathic effect (CPE) everyday. Cultures were terminated on the fifth day or as soon as CPE was observed (Fig. 2), whichever was earlier. Cells were scraped from the tube, washed in PBS, pH 7.2 and spotted onto a sterile glass slide. Smears were air dried, fixed in cold acetone for 30 minutes at -70°C and stained by an indirect immunofluorescence assay (IFA) using a polyclonal antibody to HSV-1 (Dako, Carpinteria, LA).

Statistical analysis

Statistical analysis was performed on results using a computer assisted statistical program (Epi Info, Version 6.04b, CDC, U. S. A.). Chi square test for proportions (with Yates correction when necessary) was used. *P* value was considered significant if less than 0.05.

Results

A total of 74 specimens were inoculated in parallel into SV and TC. HSV-1 was isolated by either or both the techniques in 24/74 (32.4%) specimens (Figs. 3,4,5,6). Viral antigen was detected in 54/74 (72.9%) and multinucleated giant cells were seen in 12/74 (16.2%) specimens. A confirmed laboratory diagnosis could be offered in 60/74 (81%) patients based on the criteria mentioned earlier (Table 1).

Correlation of positivity between the two tests (SV versus TC) is summarized in Table 2. The rate of isolation of

Table 1: Results of virological investigations performed on corneal scrapings (n = 74)

Investigations	No. of specimens positive
Culture	6(2)
Antigen detection	36(8)
Culture + Antigen detection	18(2)
Total	60 (12)

Figures in parentheses indicate the number of specimens positive for multinucleated giant cells in PAP stained corneal scrapings.

HSV-1 by either of the techniques was similar (20/24, 83.3%) (Table 2). The difference in sensitivity between the two techniques was not statistically significant (P = 1.00).

Table 2: Comparison of the sensitivity of SV and TC (n = 24)

Technique (SV, TC)	No. of specimens positive for HSV-1 (%)
HSV-1 isolated by either or both techniques	24(100)
HSV-1 isolated by SV	20(83.3)
HSV-1 isolated by TC	20(83.3)
HSV-1 isolated by SV and TC	16(66.6)
HSV-1 isolated by SV only	4(16.6)
HSV-1 isolated by TC only	4(16.6)

A majority of the positive specimens (16/20) showed CPE by the end of 5 days in the TC while it was observed only in 1/20 positive specimens inoculated into SV. Though CPE was not evident in 4/20 specimens in TC, the IFA revealed the presence of infected cells. CPE was seen only after 48 h in all these TC.

Discussion

Our results show that the sensitivities of the SV and TC techniques are comparable (83.3%) and suitable for the isolation of HSV-1 using corneal scrapings for the laboratory diagnosis of HSK. Sensitivity of SV has been reported to be in the range of 70–98% in comparison with TC in the isolation of HSV [6]. We found no difference in the sensitivity of these two techniques, suggesting that both techniques can be used for the laboratory diagnosis of HSK using corneal scrapings.

There are a number of studies comparing these two techniques and other techniques as well for the detection of

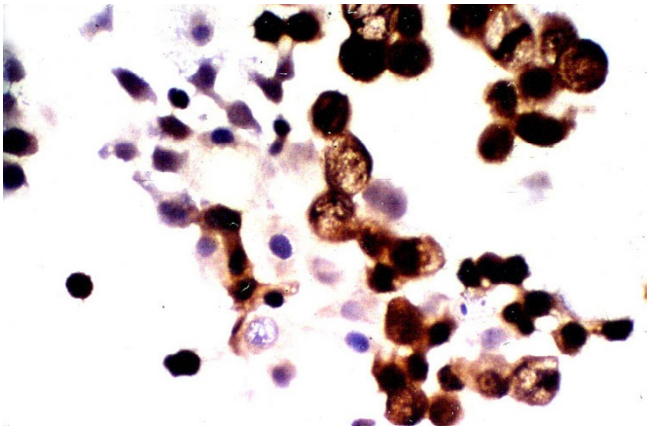

Figure 6
Tube culture positive for HSV-1. Note the presence of HSV-1 infected cells (stained dark brown). Uninfected cells appear bluish purple due to counterstaining with haematoxylin. Indirect immunoperoxidase assay, × 500.

HSV infections [6,8,11,12] with varied results. This may be attributed to the variety of specimens processed, time of collection of specimens, sample collection method, disease pathogenesis and the cell line employed. To the best of our knowledge based on a MEDLINE search, there are no reports comparing the sensitivities of a 24 h SV and the TC, especially for the diagnosis of HSK using corneal scrapings.

As mentioned earlier, HSK is a potentially blinding ocular infection warranting a prompt antiviral therapy. Towards this end, we chose a 24 h SV, since a confirmatory report can be provided the next day following the day of specimen collection (approximately within 3 Oh). Tube cultures were terminated the fifth day since our earlier observations (unpublished data) showed that more than 95% of our virus strains were isolated from cases of HSK in less than five days in TC using either vero/A549/HEp2 or BHK 21 cells. We preferred to use vero cells since our experience suggested that this cell line performed better than the others we had used (over a two-year period) for the isolation of HSV-1 from cases of HSK.

A study by Walpita et al. showed that the shell vial assay was more sensitive than the conventional tube culture for the detection of HSV from ocular infections [8] using conjunctival swabs. The details of various ocular infections (Keratitis, conjunctivitis, keratouveitis etc.) they have included in their study have not been provided. These authors have considered the results of a 48 h SV and the TC were processed for 21 days. Our results cannot be directly compared with that of these authors. Nevertheless, both the studies suggest that SV is a suitable alternative to TC for the isolation of HSV. Further, our study confirms that

SV can be employed for the diagnosis of HSK, especially using corneal scrapings. We believe that corneal scraping is a more suitable specimen than conjunctival swab for the laboratory diagnosis of HSK, since a large number of cells can be collected by scraping.

The rate of isolation of HSV-1 in our study was only 32.3% while the viral antigen detection assay was more sensitive (72.9%). However, this technique has its own disadvantages including false positivity. In general, the rates of isolation of HSV-1 in cultures from corneal specimens have been low [13], irrespective of the cell line used. A recent study has reported that isolates from herpetic keratitis grow better in corneal epithelial cells and rabbit corneal epithelial cells may be more suitable for isolating HSV from the cornea [14]. Employing such cell lines of corneal origin may prove beneficial in improving the rates of HSV-1 isolation for the laboratory diagnosis of HSK. Such studies are being done in our laboratory using a recently described immortalized human corneal epithelial cell line employing the shell vial assay [15].

In conclusion, our data suggest that in comparison to TC which is cumbersome, expensive and time consuming, SV is a rapid culture assay, is much simpler, easy to perform and economical for the isolation of HSV-1 from corneal scrapings, for a confirmatory laboratory diagnosis of HSK.

Competing Interests
None declared

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