

COMPARISON OF DIRECT IMMUNOPEROXIDASE AND DIRECT IMMUNO-FLUORESCENCE FOR THE DETECTION OF HERPES SIMPLEX VIRUS ANTIGEN IN CELL CULTURE.

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Summary

150 specimens from suspected herpes simplex genital and skin lesions were received in virus transport medium. They were inoculated into Hep-2 cell monolayers, examined for the presence of virus by cytopathic effect (CPE), direct immunoperoxidase (DIP) and direct immunofluorescence (DIF). Of 39 (26.0%) virus-positive specimens by CPE, 37 (24.7%) were HSV-positive by DIP and 36 (24.0%) by DIF staining. DIP staining had a sensitivity of 100%, specificity of 99.1%, positive predictive value of 97.3% and negative predictive value of 100% in relation to DIF as a standard test. Of 39 specimens positive by CPE, only 25.6% were HSV-positive within 24 h post-inoculation compared to 94% HSV-positive by DIP and DIF staining at the same time.

Keywords: Herpes simplex virus, direct immunoperoxidase, direct immunofluorescence.

INTRODUCTION

Herpes simplex virus (HSV) infections are common and it is important to have good diagnostic tools, especially now, when effective therapy is available. Infections due to HSV include genital lesions, cold sores, pharyngitis, ocular keratitis, encephalitis and neonatal disease.^{1,2}

Virus isolation in cell culture is routinely used for diagnosing HSV infections with daily microscopic examination of cytopathic effect (CPE).^{3,4,5} Whilst cell culture is highly sensitive for HSV detection, it requires special facilities and 1–7 days before a result can be reported.

Immunoperoxidase (IP) staining of virus infected cell monolayer is a sensitive, specific and rapid means of detecting HSV-positive culture and stained preparations can be examined by Light microscopy and are permanent.^{3,6}

Immunofluorescence (IF) tests are useful for rapid detection of HSV antigen in a wide variety of clinical materials or in cell cultures previously inoculated with these materials.^{7,8,9} Satisfactory IF test results are directly dependent upon proper sampling and specimen preparation techniques, use of quality reagents and proper adjustment and maintenance of the fluorescence equipment.

The purpose of the present study is to evaluate the immunoperoxidase (DIP) staining procedure for the routine laboratory diagnosis of genital and non-genital HSV infections in culture-positive specimens. We report on a comparison of detection and identification

of HSV antigen in cell culture by using direct immunoperoxidase (Dako Corp) and direct immunofluorescence (Syva MicroTrak Kit).

MATERIALS AND METHODS

Patients and sources of specimens

Specimens were collected from vesicles, ulcers and crust lesions from patients attending the Klinik Kebersihan Masyarakat (Sexually Transmitted Disease Clinic), Skin Clinic and Obstetrics and Gynaecology Clinic of the General Hospital, Kuala Lumpur.

Specimens were collected by scrapping the base of the lesions with sterile swabs. The swabs were transported in Virus Transport Medium containing 1000 U penicillin, 2500 µg streptomycin, 1.0 mg kanamycin and 200 U mycostatin per milliliter. Specimens were either immediately inoculated into cell culture or frozen at -70°C for later processing.

Cell culture

Hep-2 cells were maintained in minimum essential medium. Before inoculation of the clinical specimen, the medium was removed. 0.2 ml of each clinical specimen was inoculated into two tubes of Hep-2 cell monolayer, allowed to adsorb for 1 h after which 1.0 ml maintenance medium was added and incubated in a 5% CO_2 incubator at 37°C . The cultures were examined daily and virus-positive results were reported on the basis of CPE. HSV-positive and negative controls were inoculated at the same time.

Cell monolayers were stained by DIP and DIF from the first tube 24 h after inoculation and the second tube was processed when the CPE was seen. Smears were placed onto six wells of teflon-coated slides. The cells were fixed in cold acetone for 10 minutes and stained immediately or stored in a -20°C freezer.

Direct Immunoperoxidase (DIP) Staining

HSV type 1 (Strain MacIntyre) and HSV type 2 (Strain MS) horseradish peroxidase-conjugated antisera (Dako Corp.) were used for DIP assay. The antisera were used at 1:100 dilution in Phosphate Buffered Saline (PBS) pH7.2.⁵ Titration of the antisera had been previously done using the checkerboard method. The slides were removed from the -20°C freezer and immediately placed in a 37°C incubator for 5 minutes to dry. Each well was covered with 50 μl of the working conjugate (1:100) and the slides incubated at room temperature for 1 h in a well-humidified chamber. The slides were rinsed twice with distilled water and reacted with 200 μl of the appropriately diluted substrate, 3-amino 9-ethyl carbazole (Sigma Co.), and incubated at 37°C for 45 minutes. After staining, the slides were rinsed twice with distilled water, mounted with 1 drop of glycerol (Dako Corp.) and examined under a light microscope at the magnification of $\times 100$ for infected cells. Positive HSV type 1, HSV type 2 and negative controls were processed with each batch of specimens in a similar manner. The substrate produced a reddish-brown precipitate at the site of peroxidase activity.

Direct Immunofluorescence (DIF) Staining

Fluorescein isothiocyanate-conjugated (FITC) HSV type 1 and HSV type 2 antisera were purchased from Syva Co., Palo Alto, California (Syva MicroTrak). All runs included positive and negative control slides. The wells were stained with either HSV type 1 or HSV type 2 reagents and the slides were incubated for 15 minutes at 37°C in a well-humidified chamber. The slides were rinsed with distilled water, allowed to air dry, mounted with 1 drop of mounting fluid and read using a fluorescence microscope at a magnification of $\times 400$. Positive results would show cells with fluorescent staining and the negative specimens showed reddish-brown counterstained cells.

RESULTS

A total of 150 clinical specimens from genital and skin lesions were inoculated into cell cultures and processed for the detection and identification of HSV antigen by DIP and DIF. Of 39 (26.0%) specimens positive by CPE in cell culture, 37 (24.7%) specimens were HSV-positive by DIP and 36 (24.0%) HSV-positive by DIF (Table 1).

TABLE 1
COMPARISON OF DIP AND DIF WITH CELL CULTURE FOR THE DETECTION OF HSV ANTIGEN IN CLINICAL SPECIMENS

Cell Culture	No. of specimens stained with				
	DIP		DIF		
	Pos	Neg	Pos	Neg	
Positive	39	37	2	36	3
Negative	111	0	111	0	111
Total	150	37	113	36	114

Table 2 summarizes the performance of the DIP based on DIF staining as the standard test for the detection of HSV antigen in cell culture. DIP had a sensitivity and specificity of 100% and 99.1% respectively. The positive and negative predictive values were 97.3% and 100% respectively.

TABLE 2
PERFORMANCE OF DIP USING DIF AS STANDARD TEST FOR DETECTION OF HSV ANTIGEN IN CELL CULTURE

Sensitivity	36/36	100%
Specificity	113/114	99.1%
Positive predictive value	36/37	97.3%
Negative predictive value	113/113	100%

Table 3 shows that both DIP and DIF staining could detect more than 90% of the HSV-positive cultures within 24 h post-inoculation of clinical specimens. Only 25.6% of specimens were HSV-positive by the development of CPE at the same time. The time taken for all the 39 of 150 specimens to develop CPE was 5 days.

TABLE 3
COMPARISON OF TIME TAKEN FOR DETECTION OF HSV POSITIVITY BETWEEN
DIP, DIF AND CPE IN CELL CULTURE

Method	No. detected/total no. HSV positive (%) following inoculation					No. pos/ total tested (%)
	74 h	48 h	72 h	96 h	5 days	
DIP	35/37 (94.6%)	37/37 (100%)				37/150 (24.7%)
DIF	34/36 (94.4%)	36/36 (100%)				36/150 (24.0%)
CPE	10/39 (25.6%)	20/39 (51.3%)	31/39 (79.5%)	34/39 (87.2%)	39/39 (100%)	39/150 (26.0%)

DISCUSSION

A total of 150 clinical specimens from genital and skin lesions were received for HSV culture. detection and identification of HSV antigens. Of 39 specimens which developed CPE, 37 were HSV-positive by DIP and 36 were HSV-positive by DIF. A few specimens with CPE were not identified by both staining methods. These may be due to CPE caused by viruses other than HSV or they may be caused by unhealthy cells. Our results and those of others^{3,4,6} showed that the immunoperoxidase technique is potentially useful for the detection of HSV antigen in cell culture.

This study showed that DIP could detect 94.6%, while DIF staining could detect 94.4% of virus-positive cultures within 24 h post-inoculation. Only a few specimens were not detected within that time. Using cell culture, only 10 specimens (25.6%) were positive by development of CPE at 24 h post-inoculation. Virus isolation is routinely used for the diagnosis of HSV infection.^{3,4,5} A preliminary report can be released earlier by harvesting the cells after 24 h post-inoculation and staining with DIF or/and DIP methods. Previous studies have also indicated the applicability of these methods.^{6,9}

There was a difference in the serotyping between both immunoassays. The DIF staining kit was supplied with monoclonal antibodies which reacted specifically with HSV type 1 or HSV type 2. Of the 36 HSV-positive specimens, 23 were HSV type 2 and 13 were HSV type 1. For DIP staining we used polyclonal antibodies and they reacted with both HSV type 1 and HSV type 2 which shared common antigens. Therefore we could not serotype the HSV-positive cultures with the DIP staining method.

When DIP was compared with DIF as a standard test, DIP showed a sensitivity and specificity of 100% and 99.1% respectively. The positive and negative predictive values were 97.3% and 100% respectively. There was no significant difference ($p > 0.05$) in DIP when compared with DIF as standard test for the detection of HSV antigen in cell cultures. This indicates that DIP is as good as the DIF method. Therefore, for a laboratory lacking immunofluorescent equipment, the usage of DIP staining with commercially available reagents would be highly satisfactory. Other advantages of using DIP staining are: ease in interpretation of positive and negative results, ease in performance of the technique, the requirement of simple light microscopy and permanence of staining allowing slides to be stored for later reference.

This study concludes that DIP staining is suitable for use in laboratories for a rapid diagnosis of HSV antigen in cell culture. It is reliable and comparable in terms of sensitivity and specificity to DIF staining.

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REFERENCES

1. Nahmias AJ, Roizman B. Infection with Herpes simplex virus 1 and 2. *New Engl J Med* 1973; 289: 667-74.
2. Raab B, Lorinncz AL. Genital herpes simplex concepts and treatment. *J Amer Acad Dermatol* 1981; 5: 249-62.

3. Corey L, Dragavan J, Benjamin D. Immunoperoxidase staining in the clinical virology laboratory. In: Tilton RC, ed. Rapid Methods and Automation in Microbiology. American Society for Microbiology, Washington DC, 1982: 246-53.
4. Mosely RC, Corey L, Benjamin D, Winter C, Remington ML. Comparison of viral isolation, direct immunofluorescence, and indirect immunoperoxidase technique for the detection of genital herpes simplex virus infection. *J Clin Microbiol* 1981; 13: 913-8.
5. Rawls WE. Herpes simplex virus types 1 and 2 and herpes virus simiae. In: Lenette EH, Schmidt NJ, eds. Diagnostic procedures for viral, rickettsia and chlamydial infections. 5th ed. Washington DC: American Public Health Association, 1979: 109-73.
6. Marjorie JM, Clifford LH. Rapid detection and identification of Herpes simplex virus in cell culture by direct immunoperoxidase staining procedure. *J Clin Microbiol* 1983; 18: 550-3.
7. Gardner PS, McQuillin J. Rapid Virus Diagnosis: application of immunofluorescence. Butterworths. 1974: 212-3.
8. Lyerla HC. Diagnostic application of Immunofluorescence Test in the Virology Laboratory. In: Lenette DA, Specter S, Thompson KD, eds. Diagnosis of viral infections. Baltimore: University Park Press, 1979: 103-13.
9. Marks MI. Rapid identification of Herpes simplex virus in tissue culture by direct immunofluorescence. *J Lab Clin Med* 1971; 305: 315-9.