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Rapid Herpes Simplex Virus Detection in Clinical Samples Submitted to a State Virology Laboratory

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Of 16,779 specimens received for herpes simplex virus (HSV) isolation since 1982, 4,465 (26.6%) were positive for HSV by either standard tissue culture or an antigen detection system (peroxidase-antiperoxidase; PAP). The overall isolation rate for genital vesicle specimens was lower (26.1%) than that for nongenital specimens (29.3%). Monthly isolation rates ranged from 19 to 32% for genital specimens and from 20 to 44% for nongenital specimens. Increasing demands for HSV isolation led to comparison of tissue culture with PAP. In the first comparison, HSV was isolated in single human fibroblast cell cultures from 1,019 of 4,261 specimens (23.9%), whereas single human fibroblast wells stained at 24 and 72 h postinoculation were PAP positive for 1,007 of 4,261 specimens (23.6%). In the second comparison, HSV was isolated from 225 of 1,026 (21.9%) specimens and duplicate human foreskin fibroblast cell wells stained at 24 and 72 h were PAP positive in 241 of 1,026 (23.5%). With the dual-well PAP system, all results were reported within 72 h, approximately 70% of positives were reported within 24 h, and considerable savings in time and materials resulted.

Increasing awareness of the frequency and spectrum of diseases caused by herpes simplex viruses (HSV) has encouraged many laboratories to begin testing for HSV and has burdened already established virology laboratories with increasing demands for services. Many different methods now exist to detect HSV in clinical specimens, with isolation in tissue culture (TC) remaining the most sensitive method and the standard by which all other methods are judged. The choice of technique differs between laboratories and depends upon many variables such as personnel, level of expertise and experience, available funds, and work load.

Primary rabbit kidney cells are generally considered to be the best for HSV isolation, with human fibroblasts being an acceptable alternative. Cell culture procedures are, however, very labor intensive, mainly because of the necessity of repeated microscopic examination of inoculated tubes over an observation period of 5 to 14 days. In addition, verification of HSV isolates is necessary, particularly if human fibroblasts are used (1, 6, 7, 9). Commercially available methods for direct detection of HSV in clinical specimens have not approached the sensitivity or specificity of cell culture (10, 15), although noncommercial methods have performed better (5, 12). Rapid detection of viral antigen after amplification in cell culture can speed up the detection of HSV, but a commercially available test has not been as sensitive as conventional cell culture procedures (2, 3, 14). Two noncommercial procedures have been shown to equal both the sensitivity and specificity of cell culture and to greatly speed up reporting of HSV results in a clinical virology laboratory. These data were based on examination of 169 and 61 specimens (8, 11).

The present report documents the experience of a state virology laboratory with almost 17,000 specimens received for HSV isolation attempts from January 1982 through July 1984. Due to the ever increasing number of specimens received, two separate studies were performed to determine whether a rapid antigen detection system (peroxidase-antiperoxidase; PAP) could replace standard TC procedures. In the first study, over 4,000 clinical specimens were examined by both the TC and PAP procedures, and it was demonstrated that PAP could totally replace TC. In the second study, in which over 1,000 specimens were examined, we slightly improved the PAP procedure; we now routinely use the improved procedure in this laboratory. The system was specifically designed for a high-volume laboratory; cell cultures maintained in 24-well culture plates are used, thereby reducing the time necessary to produce, inoculate, stain, and read the cultures.

MATERIALS AND METHODS

Specimens. All specimens were received in Virocult specimen containers (Medical Wire and Equipment, Cleveland, Ohio). Most specimens were received and processed within 1 to 3 days of collection. Minimal essential medium (5 ml) containing 2% fetal bovine serum, 50 μg of gentamicin per ml, 100 U of penicillin per ml, and 10 μg of amphotericin B per ml was added to each swab container and mixed on a vortex shaker. A 1-ml portion of specimen suspension was added to each tube and each well. The tubes were shaken slowly for 1 h at 34°C, and both the tubes and plates were incubated at 34°C. Tubes were examined daily for 11 days; plates were stained at 24 and 72 h.

Cells. Human foreskin fibroblast cells (HF) were grown and maintained in this laboratory by standard tissue explant methods. Tubes and 24-well plates (Costar) were prepared daily from HF of less than 20 passages. HF were grown in minimal essential medium containing 10% fetal calf serum and incubated in a CO_2 atmosphere at 34°C.

Stock HSV-2 control. Tenfold serial dilutions of a laboratory-acquired stock HSV-2 (verified by restriction endonuclease analysis and immunofluorescence) were run each day with the HF tubes and plates to monitor the sensitivity of the system by daily observation of the number of plaques formed in the positive control wells.

New stock virus was prepared approximately every 2

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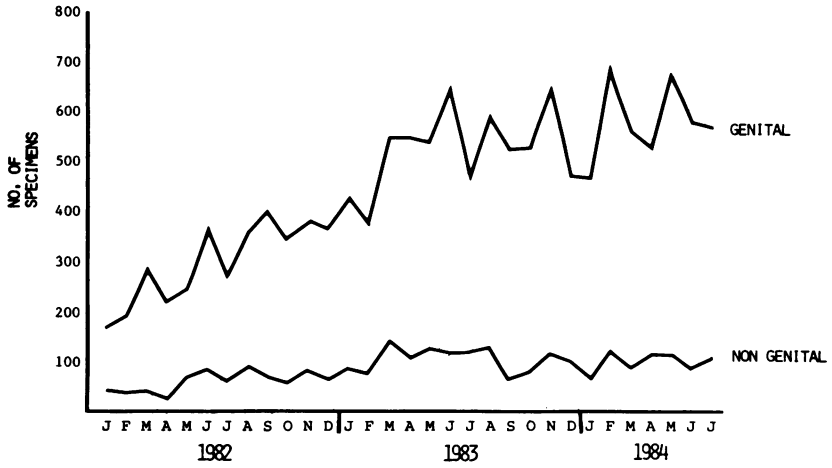

FIG. 1. Vesicle specimens submitted to the Connecticut state virology laboratory by month, 1982 through 1984.

months. Uninoculated tubes and wells (negative controls) were also used.

PAP procedure. Medium was aspirated from cells, and then the wells were rinsed once with buffer (Tris or phosphate-buffered saline). Buffered Formalin (0.5 ml; 10%) was added to each test well; wells were then incubated for 30 min at room temperature. If staining did not proceed immediately, cells were rinsed once with buffer and then left with a second rinse of buffer on them and refrigerated until staining. Formalin was removed and the cells were rinsed two times with buffer. Primary antibody (0.4 ml; HSV-2 [Accurate Chemical and Scientific Corp., New York, N.Y.] diluted 1:500 in buffer containing 1% normal swine serum) was added next. This antibody is broadly cross-reactive with HSV-1. The plates were incubated for 10 min in a 40°C water bath and then washed three times (for ~0 min, 1 min, and 3 min) with buffer. Swine anti-rabbit antibody (0.4 ml; Accurate Chemical) diluted 1:100 in buffer with 1% normal swine serum was added next, followed by incubation and washing. PAP (peroxidase-antiperoxidase; Accurate Chemical) diluted 1:200 in buffer containing 1% normal swine serum was added in a volume of 0.4 ml per well. Incubation and washing were repeated, followed by addition of 0.4 ml of substrate. Substrate (10 ml) was prepared by adding 0.5 ml of 0.4% 3-amino-9-ethyl carbazole in *N*,*N*-dimethylformamide and 0.1 ml of 3% hydrogen peroxide to 9.4 ml of 0.1 M acetate buffer (pH 5.2). Substrate was incubated on the tests for 10 min at 40°C. Plates were then rinsed two times with alkaline water (500 ml of water, 1 ml of 1 N NaOH). Mayer hematoxylin stain was added to cover wells, which were then incubated for 2 min at room temperature. Plates were then rinsed with alkaline water and air dried. Each well was read under ×40 magnification. Uninfected fibroblast cells had a light bluish color. Positive reactions appeared brick red in color in foci which appeared to run with the grain of the cells. These foci varied in size from just a few cells to an amount sufficient to completely cover the monolayer.

Contaminated or toxic specimens which destroyed the monolayer after either the 24- or 72-h incubation periods were inoculated into a tube of HF and observed for 11 days. HSV isolates obtained in this manner were confirmed by direct monoclonal fluorescent antibody staining (Syva, Palo Alto, Calif.).

The person performing the HSV PAP routine was responsible for the inoculation of any vesicle specimens into appropriate cell cultures when viruses other than herpes simplex were under consideration, i.e., enteroviruses, varicella-zoster, or other rash- or vesicle-producing viruses.

RESULTS

The number of specimens submitted to the Connecticut state virology laboratory has increased steadily since 1982. Submissions of genital vesicle swabs increased from approximately 150 to 600 per month, and submission of swabs from nongenital vesicle sources increased from about 50 to 100 per month (Fig. 1). The isolation rate for HSV (4,465 positive of 16,779 submitted) from all vesicle specimens during this time period was 26.6%. The yearly isolation rates for specimens from genital sources were consistently lower (26.8, 26.8, and 24.2%) than those from nongenital (28.0, 30.6, and 28.3%) in 1982, 1983, and 1984, respectively. Monthly isolation rates ranged from 19 to 32% for genital vesicle specimens to 20 to 44% for nongenital vesicle specimens.

Increasing demands for HSV isolation led to the first part of this study in which the HSV isolation rate in standard single HF TC tubes was compared with the isolation rate in single HF wells stained by the PAP procedure at 24 and 72 h after inoculation. All vesicle swabs submitted during this time period were included in the study. There was no significant difference between the isolation rates of HSV for the TC and the single-well PAP procedures and more than two-thirds of all positive results were detectable within 24 h (Table 1).

TABLE 1. HSV isolation: comparison of single HF tube versus single PAP wells

Test[a]	Total no. positive[b]	No. of positive results detected[c]:	
		At 24 h	At 72 h
TC	1,019 (23.9)	237 (23.3)	727 (71.3)
PAP	1,007 (23.6)	696 (69.1)	1,007 (100)

[a] A total of 4,261 specimens were tested.

[b] Percentage of specimens tested is indicated in parentheses. Number of PAP-positive, TC-negative specimens, 45; number of PAP-negative, TC-positive specimens, 57.

[c] Percentage of total positive results is indicated in parentheses.

TABLE 2. HSV isolation: comparison of single HF tube versus duplicate PAP wells

Test[a]	Total no. of positive results[b]	No. of positive results detected[c]:	
		At 24 h	At 72 h
TC	225 (21.9)	27 (12.0)	104 (46.2)
PAP	241 (23.5)	162 (67.2)	241 (100)

[a] A total of 1,026 specimens were tested.
[b] Percentage of specimens tested is indicated in parentheses. Number of PAP-positive, TC-negative specimens, 26; number of PAP-negative, TC-positive specimens, 10.
[c] Percentage of total positive results is indicated in parentheses.

In an attempt to improve the sensitivity of the PAP procedure, in the second part of the study we used two wells PAP stained at 24 h and two wells stained at 72 h in comparison with a single HF tube for the TC. Although there was still no significant difference between the number of positive results detected by each system, there were a few more positive specimens detected by PAP than were detected by TC (Table 2). The ability to report two-thirds of all positive specimens in 24 h was unchanged. During these two studies, over 5,000 vesicle swabs were examined by both PAP and TC (HF), with no virus other than HSV being found.

DISCUSSION

The routine use of the PAP system for detection of HSV antigen has significantly improved upon several aspects of the standard TC technique for HSV isolation as previously used in this laboratory. The PAP procedure was as sensitive as the TC system, and PAP results (positive and negative) were obtained within 3 days of receipt of the sample as opposed to up to 11 days with TC. Further, more than two-thirds of all positive results were detected within 24 h. Not only was the turnaround time reduced by 8 days, but the total technical time required for staining and examining PAP plates at 1 and 3 days was far less than the time required for microscopic examination of tubes daily for up to 11 days. The additional time required for verification of an HSV isolate, such as by fluorescent-antibody staining, should also be considered as a time-saving advantage of the PAP system. The PAP staining procedure was immunologically specific for HSV and did not show cross-reactivity with other viruses. Although a specific cost reduction analysis was not performed, the total technical time saved by use of the PAP procedure was approximately 50% over that required for TC. The PAP method is also flexible in that plates may be fixed at one time and stained later. The stained plates may be retained as a permanent record.

Specimens to be examined by the PAP method should be screened closely. This laboratory only uses vesicle swabs of which approximately 80% are from genital lesions. Any specimen for which a virus other than HSV is suggested by clinical history or presumptive diagnosis is inoculated into a routine set of tissue culture tubes for isolation of unknown viruses (HF, primary rhesus monkey kidney, primary cynomologous monkey kidney, A549 human lung carcinoma). Evidence that use of these guidelines (i.e., use of PAP exclusively for vesicle specimens) did not result in failure to detect viruses other than HSV is that of the more than 5,000 specimens examined in this study by both PAP and TC, no viruses other than HSV were ever isolated.

The appearance of a positive PAP test, i.e., brick-red patches on a light blue background of cells, is so morphologically distinctive that a false-positive result is unlikely to occur. Use of the double-well PAP system has allowed for detection of more HSV infections than are detected if TC alone is used. This observation has been made previously (8). The few specimens that were TC positive and PAP negative were likely to have been expected sampling variations which occur when any systems are compared. The ideal procedure would be to run both TC and PAP methods simultaneously, but the time and cost, as documented here, are prohibitive.

Even though most specimens received in this laboratory arrive through the mail between 1 and 3 days after collection, the isolation rate for HSV (26.6%) compares favorably with rates from other studies, specifically 22.4% (4), 23% (8), and 28.1% (1). Most specimens in the latter study were received and processed within 24 h of collection. The relatively high isolation rate of over 26% for the present study can be attributed in part to the use of Virocult swabs for the transport of clinical specimens. Recent studies have shown that this transport system prolongs survival times of HSV and that transport times of up to 3 days at ambient temperatures have no significant effect on HSV recovery (4, 13).

In summary, this report details how a high-volume state laboratory can accurately, rapidly, and economically detect HSV in clinical specimens by using a rapid antigen detection method (PAP).

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