Modified Spin-Amplified Adsorption Procedure with Conventional Tissue Culture Tubes for Rapid Detection and Increased Recovery of Herpes Simplex Virus from Clinical Specimens

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Conventional culture tubes were used in a modification of the spin-amplified adsorption procedure for recovery of herpes simplex virus (HSV) from clinical specimens. The sensitivity of isolation of HSV from 864 specimens adsorbed by the spin-amplified method was 100% (127 of 127), compared with 88.2% (112 of 127) for stationary-phase-adsorbed specimens. Cytopathic effect developed more rapidly in 32.1% (36 of 112) of isolates adsorbed by spin amplification than in those adsorbed by stationary means. In a separate quantitative study, cultures of HSV type 1 adsorbed by spin amplification yielded higher antigen levels and greater cytopathic effect than stationary-phase-adsorbed cultures. Cells grown in conventional tissue culture tubes may be used in a spin-amplified adsorption for rapid detection and increased sensitivity of HSV isolation.

The rapid laboratory diagnosis of herpes simplex virus (HSV) infection enables proper medical management and infection control measures for patients. The specific determination of HSV infection has relied on the recovery of infectious virus in tissue cultures from patient specimens. Tissue culture cells grown in screw-cap tubes are inoculated with patient specimens, followed by a stationary phase of virus adsorption. The cultures are refed with maintenance medium and monitored for development of cytopathic effect (CPE) (10). Recently, Gleave et al. (1) reported a modification of the stationary-phase adsorption that allows decreased culture time for virus isolation from clinical specimens. These authors used low-speed centrifugation for adsorption of specimens inoculated onto cells grown on cover slips. Cytomegalovirus replication could be detected within 2 days by immunostaining with monoclonal antibody to an early antigen of cytomegalovirus. The rapid recovery of HSV has also been reported with centrifugation and immunodiagnostic detection (2, 5, 8, 9). However, all these investigators have used shell vials or flat-bottomed culture tubes for their centrifuge-mediated viral adsorptions. The current report relates a modification of the spin-amplified adsorption procedure that allows the use of conventional tissue culture tubes for enhanced and rapid recovery of HSV from clinical specimens.


Patient specimens were submitted to the clinical virology laboratory for culture and isolation of HSV. A total of 864 specimens were cultured from patients in various services, including obstetrics and gynecology, internal medicine, pediatrics, and renal transplant. Material from genital, anal, oral, and skin sites was collected by swabbing infected areas with Dacron-tipped applicator sticks. Collected specimens were transported at 4°C to the laboratory in 0.5% gelatin in Hanks balanced salt solution containing penicillin (100 U/ml), streptomycin (100 μg/ml), and fungizone (0.25 μg/ml) (GIBCO Laboratories, Grand Island, N.Y.).

For isolation of HSV in the clinical laboratory, commercially prepared cells were used. Primary rabbit kidney (PRK) cells (M.A. Bioproducts, Walkersville, Md., and ViroMed, Minneapolis, Minn.) were supplied in screw-cap test tubes (16 by 125 mm). Two culture tubes of PRK cells were inoculated with 0.2 ml of patient specimen material. One tube of rabbit kidney cells was adsorbed in the routine manner in a stationary phase at 35°C for 1 h. The other tube was processed by the modified spin-amplified procedure (see below). Following adsorption, the tubes were washed three times with 1 ml of maintenance medium consisting of Eagle minimal essential medium (EMEM; GIBCO Laboratories) containing 2% fetal bovine serum, 0.075% sodium bicarbonate, 25 mM HEPES (N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid) buffer, and antibiotics as given previously for the viral transport medium. Cells were then incubated in 1 ml of maintenance medium at 35°C and monitored daily for the extent of characteristic CPE. The degree of CPE was estimated by readings of 0, 0.5+, 1+, 2+, 3+, or 4+, correlating to 0, 12.5, 25, 50, 75, or 100% monolayer involvement, respectively.

Anal and genital specimens were monitored for 4 days, while all other cultures for HSV were monitored for 2 weeks. Data from our laboratory have previously shown that all genital HSV isolates are recovered on PRK cells within 4 days postinoculation (S. H. Loo and P. E. Oeffinger, Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, abstr. no. C-56, p. 337). One day after viral CPE was first noted, its extent was confirmed, often by a different technologist.

The modified spin-amplified procedure was used for adsorption of certain specimens after inoculation of culture tubes containing PRK or Vero cells. The culture tubes were inserted into a fixed-angle rotor (catalog no. 83; Damon/IEC, Needham Heights, Mass.) with the cell side oriented upward. The tubes were centrifuged (Damon/IEC model EXE) at room temperature (25 to 30°C) for 40 min at 1,875 rpm (750 × g). Cells were then rinsed, refed, and monitored as stated in the previous section.

A quantitative spin amplification study was undertaken with the McKrae strain of HSV type 1 (HSV-1) (kindly provided by E. C. Dunkel). The virus stock had an original titer of 4 × 10⁸ PFU/ml. The stock had been plaque purified.
three times in Vero cells prior to use. Vero cells (American Type Culture Collection, Rockville, Md.) were grown in a growth medium consisting of EMEM (Flow Laboratories, McLean, Va.) with 1% L-glutamine, penicillin (100 U/ml), streptomycin (100 μg/ml) (GIBCO Laboratories), 0.075% sodium bicarbonate (Sigma Chemical Co., St. Louis, Mo.), and 10% fetal bovine serum (KC Biologicals, Lenexa, Kan.). Cells were cultured for a maximum of 5 days until confluent at 35°C in round-bottomed, screw-top tissue culture tubes (16 by 125 mm). The McKrae strain of HSV-1 stock was serially diluted in the maintenance medium described above. Ice-chilled dilutions of 10⁻¹ to 10⁻⁹ of the virus stock were used to infect Vero cell tubes. Immediately before inoculation, the modified EMEM was decanted from the Vero cells, and 0.2 ml of diluted viral stock was added to each tube. The inoculated tubes were incubated for 1 h at 35°C for stationary adsorbed cultures or centrifuged for 40 min at 25 to 30°C for spin amplification. Mock-infected Vero cell tubes were included as negative controls. The monolayers were then washed three times, refed with maintenance medium, and incubated at 35°C. Three tubes of both stationary and spin-amplified adsorption each virus dilution were randomly harvested at 24, 48, and 72 h postinfection. Each tube was examined daily in a blinded fashion for CPE prior to harvest described above. An additional grading category of <0.5, correlating to less than 12.5% of the monolayer exhibiting CPE, was included for this comparison.

The Ortho HSV antigen enzyme-linked immunosorbent assay (ELISA; Ortho Diagnostic Systems, Inc., Raritan, N.J.) was used as per the manufacturer's instructions for the detection of HSV antigen (6; P. E. Oefinger, S. H. Loo, and J. L. Leibowitz, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, abstr. A-104, p. 18). Briefly, 0.5 to 1.0 ml of Ortho cell lysis agent was added to individual culture tubes of cells. A 0.2-ml volume of each lysed sample was transferred to microwells coated by the manufacturer with rabbit antibody to HSV-1 and HSV-2 and incubated for 2 h. All incubations occurred at 25°C throughout the procedure. The microwells were washed three times in 0.05 M phosphate-buffered saline containing 0.05% Tween. Following the washing, the kit-supplied peroxidase-conjugated rabbit anti-HSV preparation was added to each well in a volume of 0.2 ml. After incubation for 2 h, the wells were washed five times. A freshly prepared 0.2-ml volume of 4.5% ortho-phenylenediamine plus 0.6% hydrogen peroxide substrate solution was added to all wells. The samples were incubated at 25°C for 30 min, and the reaction was stopped by addition of 0.05 ml of 2 N hydrochloric acid to each well. The absorbance of each well was read at 490 nm. Serial dilutions of HSV stock in EMEM with 2% fetal bovine serum were tested by the Ortho HSV antigen ELISA to generate a standard curve. A cutoff value was designated as the negative medium control mean absorbance plus 0.15. Any specimen yielding an absorbance greater than the cutoff value was considered positive for HSV antigen.

A total of 127 of the 864 clinical samples submitted for culture were positive by CPE for recovery of HSV, yielding an isolation rate of 14.6%. All CPE-positive cultures were immunodiagnostically confirmed as HSV by the commercial ELISA. Over 94% of the specimens were found to be culture positive through detection of characteristic CPE by day 3 of culture, regardless of the adsorption procedure.

Analysis of the clinical HSV recovery data is given in Table 1. The stationary-phase and modified spin-amplified adsorptions were compared for the overall sensitivity of isolation and rapidity of progression of CPE. All 127 specimens that were positive for HSV culture were recovered from tubes that were processed by modified spin-amplified adsorption. However, only 112 of the 127 viral isolates were recovered from tubes that were processed by the stationary adsorption technique, resulting in a reduced sensitivity (88.2%) for isolation of HSV. The 15 isolates that were only recovered by the spin-amplified adsorption were obtained from 10 genital and five nongenital sites.

The two adsorption techniques were also evaluated for their effect on detection of characteristic CPE of HSV. The 112 clinical specimens that were recovered in common with both adsorption procedures were included for this consideration. Results are given in Table 2 analyzing the viral CPE development of each specimen processed by both adsorption procedures. A majority of 65.2% (73 of 112) of the specimens developed CPE at equal rates, regardless of the adsorption method. Only 2.7% (3 of 112) of the specimens processed by stationary adsorption developed CPE earlier or to a greater degree than those processed by the modified spin-amplified procedure. However, a notable proportion of 32.1% (36 of 112) of the specimens adsorbed with the spin-amplified procedure developed CPE more rapidly than the same specimens adsorbed in the stationary manner. Four of these 36 isolates were recovered from spin-amplified adsorbed cultures 1 day before the same specimens developed CPE on stationary-phase-adsorbed PRK cultures. Overall, the degree of CPE development in these 36 culture isolates recovered from spin-amplified adsorptions was 25% more advanced than in cultures processed with stationary-phase adsorptions. The data can be reconsidered with all 127 specimens that were culture positive for HSV. By this criterion, a proportion of 40.2% (51 of 127) of culture-positive specimens that were processed by the spin-amplified adsorption either had CPE present or developed it more rapidly than the same specimens adsorbed by stationary means.

The ELISA and CPE development were used to compare the sensitivity of viral isolation in the quantitative spin

### Table 1. Isolation of HSV from clinical specimens processed by both modified spin-amplified and stationary-phase adsorption

<table>
<thead>
<tr>
<th>Procedure</th>
<th>HSV recovery from specimens</th>
<th>Sensitivity [no. testing positive/positive (%)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spin amplified</td>
<td>No. positive</td>
<td>No. negative</td>
</tr>
<tr>
<td>Stationary phase</td>
<td>112</td>
<td>752</td>
</tr>
</tbody>
</table>

* Recovery of isolates was first detected by the presence of CPE, followed by confirmation of HSV by ELISA.

### Table 2. Development of CPE by 112 clinical isolates of HSV recovered mutually in PRK cells processed by modified spin-amplified and stationary-phase adsorption

<table>
<thead>
<tr>
<th>Degree of CPE after adsorption</th>
<th>No. (%) of cultures showing SA-SP relationship</th>
</tr>
</thead>
<tbody>
<tr>
<td>SA &gt; SP</td>
<td>36 (32.1)</td>
</tr>
<tr>
<td>SA &lt; SP</td>
<td>3 (2.7)</td>
</tr>
<tr>
<td>SA = SP</td>
<td>73 (65.2)</td>
</tr>
</tbody>
</table>

* The degree of CPE was determined by judging the percentage of cells exhibiting HSV-caused cytopathology. SA, Spin amplified; SP, stationary phase. The average degree of CPE in cells adsorbed by one technique was graded as greater than, less than, or equal to that of cells adsorbed by the other technique.
amplification study. A concentration of $4 \times 10^5$ PFU of the HSV-1 stock per ml was the limit of detection for the ELISA. Vero cells grown in culture tubes were inoculated with decreasing 10-fold concentrations of HSV stock and processed by either spin-amplified or stationary adsorption. On the first day postinoculation (Fig. 1A), the spin-amplified tubes with inocula of $8 \times 10^3$, $8 \times 10^2$, and $8 \times 10^1$ PFU contained greater HSV antigen levels than similarly inoculated stationary-phase tubes. Two days postinoculation (Fig. 1B), the spin-amplified technique increased the sensitivity of HSV isolation for tubes with primary inocula of $8 \times 10^3$ and $8 \times 10^1$ PFU compared with the stationary-phase adsorption tubes. The trend continued on the third day postinoculation (Fig. 1C). Undiluted samples from tubes with spin-amplified adsorbed inocula of $8 \times 10^3$ and $8 \times 10^0$ PFU yielded higher optical density readings by ELISA compared with identically inoculated tubes processed by the stationary-phase adsorption technique. A further examination of viral antigen production was undertaken with infected cells that yielded ELISA optical density readings greater than or exceeding a value of 2. ELISA values for inoculum concentrations of $8 \times 10^2$ PFU per tube on days 2 and 3 postinoculation (Fig. 1B and C) were reetermined by diluting the infected cell lysates 1:10. Again, virus adsorbed by the spin-amplified procedure yielded antigen levels, as determined by ELISA, to be 0.12 to 0.37 optical density units greater than in stationary-phase-adsorbed virus cultures (data not shown).

The progression of characteristic HSV-induced CPE was also used as a basis for comparing the stationary-phase adsorption with the spin-amplified adsorption procedure (Table 3). More advanced CPE was observed in the spin-amplified cultures than in the stationary-phase cultures. This enhancement was noted in most instances on the first day of CPE development. The trend continued during subsequent days, as viral cultures adsorbed by the spin-amplified procedure routinely contained more advanced CPE than cultures adsorbed by the stationary procedure. Notably, virus was recovered from one of three spin-amplified adsorbed tubes, each inoculated with $0.8 \times 10^{-1}$ PFU, while no virus was recovered from similar stationary-phase-adsorbed cultures.

**TABLE 3. Development of CPE by HSV-1 Mckrae strain in Vero cells processed by modified spin-amplified and stationary-phase adsorption**

<table>
<thead>
<tr>
<th>Inoculum (PFU/tube)</th>
<th>Degree of CPE (SA/SP)* at day postinoculation</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>$8 \times 10^3$</td>
<td>2+/1+</td>
</tr>
<tr>
<td>$8 \times 10^2$</td>
<td>1+/.5+</td>
</tr>
<tr>
<td>$8 \times 10^1$</td>
<td>0.5+/&lt;0.5+</td>
</tr>
<tr>
<td>$8 \times 10^0$</td>
<td>0/&lt;0.5+</td>
</tr>
<tr>
<td>$8 \times 10^{-1}$</td>
<td>0/0</td>
</tr>
</tbody>
</table>

*Average degree of HSV-caused CPE in cells from three spin-amplified (SA)-adsorbed tubes/average degree of HSV-caused CPE in cells from three stationary-phase (SP)-adsorbed tubes. The degree of CPE was determined by judging the percentage of cells exhibiting HSV-caused cytopathology. See text for explanation of grading.
Low-speed centrifugation was initially shown to enhance recovery of chlamydiae and rickettsiae when they are grown in tissue culture (3, 14). Cytomegalovirus infectivity was enhanced 10- to 100-fold when adsorption occurred with low-speed centrifugation (4, 7, 14). The mechanism of the enhancement was obscure but was hypothesized as being partially due to more efficient viral penetration of cellular membranes (4). The detection of rotavirus in concentrated, environmentally acquired sewage waste treatment samples has also been accomplished with a low-speed centrifugation procedure (12). However, the application of spin-amplified adsorption to the clinical virology laboratory was not realized until recently (1, 11). Gleave et al. (1) used a monoclonal antibody to an early antigen of cytomegalovirus together with low-speed centrifugation. These investigators detected all clinical isolates from urine within 2 days of culture compared with a maximum of 15 days by conventional culture. Swensen et al. (13) reported that clinical isolates of cytomegalovirus can also be detected immunocytochemically in conventional culture tubes within 2 days postinoculation. However, the concentration of cellular nuclei expressing early antigen was 20-fold less in conventional culture tubes than in spin-amplified shell vials. Gleave et al. (2), Pruneda and Almanza (8), and Salmon et al. (9) used a spin-amplified adsorption procedure to detect most HSV isolates within 16 to 48 h of culture initiation. Michalski et al. (5) reported a sensitivity of 97.6% for detection of HSV isolates when specimens were adsorbed onto tissue culture by the spin-amplified procedure and detected by a commercial ELISA within 2 days of specimen set-ups.

Originally, the infectivity of HSV-1 and 2 was enhanced approximately three- to sixfold when low-speed centrifugation adsorption was compared with stationary adsorption. A human cytomegalovirus laboratory strain, AD169, was found to be 30-fold enhanced in infectivity by similar centrifugal adsorption (4). These authors used petri plates for their centrifugal adsorption studies. Similarly, the clinical applications have used monolayers of cells grown on the flat surfaces of shell vials or tubes. Our data with cells grown in conventional culture tubes, that indicate adsorption mediated by low-speed centrifugation allows approximately a 10-fold increase of HSV infectivity over standard adsorption methodology, agree favorably with those of Hudson et al. (4). We have noticed that viral CPE usually starts within a small circumscribed area near the butt of the test tube. Further work is necessary to delineate the mechanism of the modified spin-amplified adsorption-mediated enhancement of HSV infectivity within culture tubes.

The development of the spin-amplified procedure has instigated a number of improvements in the clinical virology laboratory. Together with rapid diagnostic immunoassays, the time has been shortened for the final detection and reporting of viral isolates from patient specimens. From the perspective of the laboratory workload, these innovations have lessened the time necessary for final reports to be generated. The physician can realize a more timely viral diagnosis, which can then impact on several areas: appropriate initiation or duration of antiviral therapy, necessary medical management of genitally infected pregnant women, and proper infection control measures.

The data presented in this manuscript demonstrate that standard culture tubes may be used in a modified spin-amplified adsorption procedure for more sensitive and speedy recovery of clinical isolates of HSV. Prior to this report, shell vials or flat-surfaced tubes have been used in the spin-amplified procedure for enhanced viral recovery from clinical specimens. However, the innovative introduction of shell vials into the clinical virology laboratory may create workflow problems for laboratory personnel due to unfamiliarity with the vials. The shell vials also may only be used for one particular virus when cells are lysed or fixed within 4 days of culture for immunochemical detection of viral antigen, such as for HSV. A problem exists when requests are sent for both HSV and general viral culture, because additional cell types must be inoculated for proper recovery of non-HSV isolates. Reading of CPE would also be more efficient if all vessels were of only one type, such as culture tubes. Preliminary data additionally suggest that the modified-tube spin-amplified adsorption enhances recovery of cytomegalovirus (unpublished data). A savings in materials and labor may be realized if shell vials were eliminated from routine culture of HSV in the clinical virology laboratory. A more applicable spin-amplified procedure may incorporate the standard culture tubes that most clinical laboratories currently use for viral culture.

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LITERATURE CITED