Detection of Herpes Simplex Virus by Using A549 Cells in Centrifugation Culture with a Rapid Membrane Enzyme Immunoassay

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The RAMP herpes simplex virus (HSV) culture confirmation test was compared with immunofluorescence (IF) staining with a specific HSV monoclonal antibody reagent for the detection of HSV in centrifugation culture. The RAMP test detected 47 of 57 IF-positive specimens (sensitivity, 88.6%) and agreed with 217 of 220 IF-negative specimens (specificity, 98.6%). The RAMP test can be performed in less than 15 min and gives an immediate visual result. However, the sensitivity and the false-positive and false-negative results need further investigation.

The use of rapid techniques for detecting herpes simplex virus (HSV) has important clinical application in both normal and immunocompromised individuals. With the current availability of effective therapy for HSV infection, the demand for accurate laboratory diagnosis of HSV has increased (2, 3).

The objective of this study was to evaluate the RAMP HSV culture confirmation test (Monoclonal Antibodies, Inc., Mountain View, Calif.) by using the centrifugation culture (shell vial) technique (7). The results from the RAMP testing were compared with those of immunofluorescence (IF) staining of coverslips from centrifugation culture by using a specific monoclonal antibody reagent and compared with standard cell culture for HSV isolation.

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MRC-5 and A549 cells (ViroMed Laboratories, Minneapolis, Minn.) were subpassed in minimal essential medium containing 10% fetal bovine serum supplemented with l-glutamine and antibiotics. Both cell lines were further subcultured into culture tubes (16 by 125 mm) and A549 cells were also seeded into 1-dram (3.5516-cm³) vials containing a 12-mm-diameter coverslip (11, 12).

Fluorescein isothiocyanate-labeled murine monoclonal antibodies (Syva Co., Palo Alto, Calif.) were used for detection of HSV in centrifugation culture and for typing of HSV isolates. For detection of HSV in shell vials, the Syva Microtrak HSV nontyping reagent (a combination of monoclonal antibodies specific for both HSV type 1 [HSV-1] and HSV-2) was used. For HSV typing, the Syva Microtrak HSV-1/HSV-2 typing kit was used.

The RAMP HSV culture confirmation test was used both for detection of HSV at 16 to 20 h following centrifugation and for confirmation of HSV isolates. The test uses a rapid absorbent matrix pad (RAMP) technology (Monoclonal Antibodies Inc., U.S. patent 3,888,629. June 1975) in an enzyme-linked immunosorbsent assay.

Clinical specimens were obtained from throats (n = 165), other oral sites (n = 50), genital lesions (n = 35), skin lesions (n = 19), and tissue sites (n = 4) of bone marrow transplant patients (prior to transplantation, all patients were screened for HSV immune status). Specimens were inoculated at a volume of 0.25 ml into two standard cell culture tubes, one containing MRC-5 cells and one containing A549 cells, and into two A549 shell vials for centrifugation culture. Standard cultures were examined daily for the presence of cytopathic effects typical for HSV. After centrifugation for 40 min at 700 × g, the vials were incubated at 36°C for 16 to 20 h, after which one cover slip was stained by IF for HSV and the additional cover slip was subjected to the RAMP test.

IF staining. Following incubation, one A549 coverslip was fixed for 10 min in cold acetone and then stained in the vial with 0.150 ml of the fluorescein isothiocyanate-conjugated nontyping reagent for 30 min at 36°C. Following staining, the monolayer was washed once with phosphate-buffered saline and once with distilled water before being mounted, cell side down, onto a glass slide and examined at a magnification of ×250 with an epifluorescence microscope.

RAMP procedure. Following incubation, the medium was removed from the vial and 1 ml of harvest buffer (included in RAMP kit) was added to the monolayer. The vial was then vortexed for 15 s, after which the 1-ml solution was transferred to a glass tube (12 by 75 mm). A volume (0.05 ml) of the solution was then spotted onto the specimen half of the matrix pad. Two drops of a specific HSV antibody-linked enzyme conjugate (reactive with both HSV-1 and HSV-2) was added, and the mixture was incubated at room temperature for 6 min. Four drops of a substrate solution was then added, and the mixture was incubated at room temperature for 3 min, after which a stop reagent was added. A blue dot represented an HSV-positive sample.

Confirmation of HSV isolates were performed by the RAMP test after 50% cytopathic effect was noted in either of the standard culture tubes. The test was performed as described above except that the time was extended to 3 min following the addition of the enzyme conjugate and 2 min following the substrate solution, according to the manufacturer instructions. For this study, all HSV isolates were subsequently stained with specific HSV monoclonal antibodies by immunofluorescence for serotype determination (6).

HSV was detected in 61 of 273 specimens (22.3%), of which 47 (77.1%) were positive by all methods (Table 1). HSV was isolated in standard culture from 57 of 273 speci-
mens (20.9%). Of the 57 HSV isolates, all were confirmed as HSV by the RAMP HSV culture confirmation test. These included 37 HSV-1, 19 HSV-2, and 1 dual isolate, as subsequently typed with monoclonal antibodies.

In centrifugation culture, 53 specimens (96.9%) were HSV positive by IF; 52 of which were isolated in standard culture. One specimen was positive by IF only. Fifty specimens were positive for HSV by the RAMP assay, of which three samples were RAMP positive only (IF and culture negative). Compared with standard culture, the RAMP assay detected 47 of 57 HSV culture-positive specimens (82.5%). Five specimens positive by standard culture were negative by both IF and RAMP. Five specimens were standard culture and IF positive but RAMP negative. These 10 RAMP-negative, standard culture-positive specimens were considered to be “low-positive” samples taking longer than 3 days (range, 4 to 6 days) before HSV cytopathic effect was observed in standard culture. HSV cytopathic effect was noted in standard culture from the other 47 isolates in a mean time of 2.1 days (range, 1 to 3 days). Additionally, the six specimens positive by IF but negative by RAMP following centrifugation culture showed 5% or less of the cell monolayer positive for HSV by IF.

The easiest means of HSV detection in the clinical laboratory is the isolation of the virus in standard cell culture. However, there is increasing demand for more rapid detection methods which can provide results within a 24-h period following collection of the specimen (2). Because of this need, the development and use of specific HSV monoclonal antibodies, DNA probes, and enzyme immunoassay techniques applied to cells taken directly from clinical specimens or following specimen inoculation into cell culture systems are becoming more routine in the clinical laboratory (11, 4, 5, 7, 8–10; A. Warford, in L. M. de la Maza and E. M. Peterson, ed., Medical Virology VIII, in press).

Compared with IF in centrifugation culture, RAMP had a sensitivity of 88.6% with a specificity of 98.6%. In regard to the six IF-positive, RAMP-negative specimens, it is possible that insufficient viral antigen was transferred from the harvested shell vial cell monolayer onto the matrix pad for a reaction to occur, since five of these six positive results were isolated in standard culture and confirmed as HSV by the RAMP test.

Compared with standard culture, the sensitivities of IF and RAMP in centrifugation culture were 91.2 and 82.5%, respectively, with specificities of 99.5 and 98.6%, respectively. Compared with standard culture, the RAMP assay had a positive predictive value of 94% and a negative predictive value of 95.5%. Of the 273 specimens, 3 were RAMP positive only. Two of these were from patients who were seronegative for HSV. The other specimen was taken from a patient who was HSV seropositive but on antiviral therapy for HSV. These positive reactions may have been the result of cellular debris being transferred from the harvested solution to the matrix pad, which may have resulted in trapping the reagent mixture and therefore causing a reaction to occur.

The RAMP assay had an apparent false-positive rate of 1.4%; that is, 3 of 273 specimens tested were RAMP positive but both IF and culture negative. Of greater concern was the false-negative rate of 17.5%; that is, 11 specimens were RAMP negative but IF and/or culture positive. The RAMP assay successfully confirmed all 57 HSV isolates positive in standard culture in this study, thus providing an accurate and reliable HSV culture confirmation assay.

The RAMP test can be performed in less than 15 min and gives an immediate visual result which compares favorably with that of IF staining in centrifugation culture from specimens containing moderate to high levels of HSV. However, further investigation or refinement of the assay is needed for detection of HSV in centrifugation culture from specimens containing low levels of virus.

LITERATURE CITED


