Rapid Culture Confirmation of Herpes Simplex Virus by a Monoclonal Antibody-Based Enzyme Immunoassay

H. Patel, L. D. Frenkel,* M. Greenhalgh, R. Howell, and S. Patel

Department of Pediatrics, University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School, New Brunswick, New Jersey 08903-0019

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Rapid confirmation of herpes simplex virus (HSV) is essential in many clinical settings. Viral isolation in cell culture is the standard method for diagnosing HSV infection, with confirmation by specific immunological staining. The performance of the Rapid Absorbent Matrix Pad (RAMP) HSV culture confirmation test was evaluated with specimens obtained from 71 patients with suspected HSV infection and inoculated into African green monkey kidney cell lines. Forty-one culture-positive specimens were confirmed to be HSV by both the RAMP HSV test and the Bartels HSV immunoperoxidase test. Thirty immunoperoxidase-negative specimens were also negative in the RAMP HSV test. The sensitivity and specificity of the RAMP HSV test were 100%. Twelve specimens positive for cytomegalovirus, adenovirus, or enterovirus tested negative by the RAMP HSV test. Thus, the RAMP HSV test was faster than, easier to perform than, as sensitive as, and as specific as other well-documented confirmation methods.

Herpes simplex virus (HSV) is responsible for a variety of human infections, including cold sores, genital lesions, pharyngitis, ocular keratitis, encephalitis, and disseminated disease in neonates and immunocompromised patients (1, 4). Once acquired, HSV can remain latent in the regional sensory ganglia. Encephalitis and diseases in newborn or immunocompromised patients necessitate prompt therapy to limit morbidity and mortality (6, 7).

Viral isolation in cell culture is the most commonly used method for demonstration of HSV (6), one of the most common viruses isolated in many virology laboratories. Cultures that are positive for HSV usually demonstrate cytopathic effect (CPE) within 1 to 3 days (2). Cultures are generally observed for CPE for 14 days before being reported as negative. CPE-positive specimens may be confirmed by specific immunological staining procedures, such as immunofluorescence or immunoperoxidase staining (3). These methods are time consuming and require special equipment and trained personnel.

An alternative new method for the confirmation of HSV in cell culture utilizes the Rapid Absorbent Matrix Pad (RAMP) test. The RAMP test combines the specificity of monoclonal antibodies with the ease and readability of enzyme immunoassays in a 5-min visual test. The present study was done to compare the RAMP method with the Bartels immunoperoxidase (IP) staining method for culture confirmation of HSV.

All clinical specimens from oral, vulvovaginal, penile, ocular, or cutaneous vesicles or ulcers submitted to the Viral Diagnostic Laboratory for HSV isolation during a 3-month period were included in this study. The lesions were swabbed with a Dacron swab and placed in a sterile plastic screw-cap tube containing 2 ml of buffered viral transport medium (VTM). VTM was prepared by adding 16.5 ml of 35% bovine serum albumin (Sigma Chemicals, St. Louis, Mo.), 5 ml of penicillin (10,000 U/ml)-streptomycin (10,000 μg/ml)-amphotericin B (Fungizone; 25 μg/ml) solution (Whittaker M. A. Bioproducts), and 5 ml of 7.5% sodium bicarbonate (Whittaker) to 500 ml of Earle balanced salt solution (GIBCO Laboratories, Grand Island, N.Y.). VTM was then aerated at 4°C and stored at 4°C for 24 h. For processing, the swabs were expressed with sterile forceps against the sides of the transport tubes in which they arrived and then discarded. The specimen was centrifuged at 700 x g for 10 min at room temperature. The supernatant fluid was treated with 0.3 ml of penicillin-streptomycin-amphotericin B solution (concentration as noted above), and 0.3 ml of the treated supernatant was inoculated into one tube of MRC-5 fibroblasts (Ortho Diagnostic Laboratories, Raritan, N.J.), one tube of WI-38 fibroblasts (Whittaker), and two tubes of African green monkey kidney (AGMK) cells (Whittaker). The cell cultures were evaluated for CPE every 24 h for 7 days and then evaluated every other day for 7 more days.

Monoclonal antibody-based enzyme immunoassay procedure for HSV confirmation. (i) Test principle. The RAMP HSV culture confirmation test (Monoclonal Antibodies, Inc., Sunnyvale, Calif.) is an enzyme-linked immunospecific assay. The upper portion of the matrix pad contained a spot of immobilized, heat-inactivated HSV antigen. The sample from the cell culture was applied to the lower portion of the matrix pad; any HSV antigens present would be bound there. This bound antigen was then detected by an HSV-specific antibody linked to the enzyme alkaline phosphatase, the enzyme conjugate. The development of two blue spots on the matrix pad indicated that HSV was present in the cell culture.

(ii) Procedure. AGMK cell culture tubes were utilized for this assay. Positive culture tubes were tested when CPE characteristic for HSV involved 50 to 75% of the cell monolayer. Negative culture tubes, i.e., those which failed to demonstrate CPE, were tested after 14 days. The media from positive and negative AGMK cell culture tubes were discarded. Buffered sodium azide solution (1 ml) was added to the cell culture tube and vortexed for 15 s; 25 μl of this solution was applied with a micropipette to the lower portion of the RAMP pad. HSV-specific mouse monoclonal immunoglobulin G covalently linked to alkaline phosphatase was then added to the entire RAMP pad. After 3 min, the
buffered substrate solution was added, and then the stop solution (chelating agent) was added. The upper portion of the RAMP pad contained a spot of immobilized, heat-inactivated HSV antigen; therefore, for validated results the upper portion of the pad (positive control) should turn blue. Blue coloration in the lower portion indicated the presence of HSV antigen in a patient’s culture specimen. A lack of color indicated the absence of HSV in a culture specimen.

**Bartels IP staining for HSV culture confirmation.** The second AGMK cell culture tube from a given specimen was used in this assay, and the test was performed in parallel with the RAMP HSV culture confirmation test. Slides were prepared from the AGMK cell culture tubes and were fixed with acetone. These were layered with anti-HSV (rabbit antibodies in phosphate-buffered saline [PBS]) horseradish peroxidase conjugate and incubated for 30 min at 37°C. The slides were rinsed with PBS, and a chromogen-hydrogen peroxide substrate was layered onto the slides. After the slides were rinsed, Richard Allen hematoxylin counterstain was added. Slides were examined at x100 magnification. Those with two or more characteristic red viral inclusions in the fibroblast cells were judged positive, and those with fewer than two inclusions were judged negative.

Ten additional HSV isolates grown in MRC-5 cell cultures, five cytomegalovirus (CMV) isolates grown on MRC-5 cell cultures, five enterovirus isolates grown in AGMK cell cultures, two adenovirus isolates grown in MRC-5 cells, and five cell culture contaminant simian virus 40 isolates were all negative in AGMK cultures. CMV cultures were similarly assayed by both methods. CMV-characteristic CPE was confirmed by a CMV DNA probe assay (Color Gene DNA hybridization test for CMV; Enzo Diagnostics, Inc., New York, N.Y.). Enterovirus-characteristic CPE was confirmed by the New Jersey State Virology Laboratory by a neutralization method. Adenovirus was confirmed by enzyme immunoassay (Adenolclone; Cambridge Bioscience, Worcester, Mass.). Simian virus 40 isolates with foamy CPE that were negative for other viruses by the methods described above were presumed positive by exclusion of other possibilities.

As noted in Table 1, 30 culture-negative specimens were negative by both confirmation methods. The HSV culture-positive specimens grown in AGMK cells were all confirmed as positive by both the RAMP method and the IP staining method. Five simian virus 40-contaminated AGMK cell cultures, five enterovirus isolates from stool specimens grown in AGMK cell cultures, two nasopharyngeal adenovirus isolates grown in MRC-5 fibroblast cell cultures, and five urine specimens yielding cytomegalovirus isolates in MRC-5 fibroblast cell culture all tested negative in both assays. Ten genital HSV specimens grown in MRC-5 fibroblast cell cultures tested positive in both confirmation methods.

The RAMP assay and the Bartels IP staining method were found to be equally sensitive and specific for confirming HSV-positive and -negative cell cultures. As noted in Table 1, CMV, adenovirus, enterovirus, and simian virus 40 isolates were all tested to assess specificity. All of these were appropriately negative in the RAMP and IP staining assays. In addition, the HSV isolates grown in fibroblast cell cultures were confirmed as positive by both methods, thus suggesting that the RAMP assay is equally valid for HSV confirmation from fibroblast and AGMK cell cultures.

Limitations include the fact that the RAMP assay cannot distinguish HSV type 1 from HSV type 2; currently available IP kits cannot do so either. The RAMP assay has not been fully evaluated for confirmation of HSV by using specimens directly, but on several occasions our laboratory has used material from cutaneous vesicles directly for RAMP analysis with positive results. Subsequent HSV cultures were also positive. More studies are required, however, to evaluate the reliability of direct specimen testing by the RAMP assay. One false-positive result was reported from another laboratory (5) which used the RAMP assay when the viral culture was contaminated with *Staphylococcus aureus*.

The RAMP assay test pad must be read soon after completion of the test, since the blue color fades away in several hours. IP staining methods do occasionally demonstrate false-positive results with varicella-zoster virus (3), whereas the RAMP assay did not yield false-positive results (5). The major advantages of the RAMP assay are speed and ease of performance. These advantages are solidified by sensitivity and specificity equal to those of the Bartels IP staining method for confirmation of HSV cell cultures.

In immunocompromised hosts and neonates, prompt therapy of proven HSV infection can be lifesaving. On the other hand, disease in these patients thought to be caused by HSV may have other specifically treatable etiologies (8). Reliance on CPE may lead to misdiagnosis. CPE apparently characteristic for HSV could actually be due to another virus (for example, rapidly growing, high-titer CMV in a fibroblast cell line). CPE uncharacteristic of HSV (for example, very slowly developing focal CPE) may actually be a manifestation of HSV. Therefore, the ability to confirm HSV rapidly, specifically, and accurately in culture is essential.

**REFERENCES**


