Enzyme-Linked Immunosorbent Assay Spin Amplification Technique for Herpes Simplex Virus Antigen Detection

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A comparative study of herpes simplex virus diagnosis by standard cell culture and a new hybrid test (enzyme-linked immunosorbent assay spin amplification technique) was done on 300 specimens. The new test was found to be equally sensitive and specific, much less expensive to perform, and to report all results in 48 h.

Many techniques have been developed to decrease the time necessary for the laboratory diagnosis of herpes simplex virus (HSV). Direct methods which have been tried include the enzyme-linked immunosorbent assay (ELISA) (6, 8, 11), peroxidase staining (5, 10), fluorescent-antibody staining (1, 7), and DNA probes (2, 9). None of these techniques has demonstrated acceptable sensitivity as a definitive direct test for HSV. Recent efforts have centered on developing techniques to make the standard sensitive cell culture isolation technique (CC), the current "gold standard," faster. Several techniques involve staining the inoculated monolayer after 24 to 48 h of incubation with an immunofluorescence- or peroxidase-labeled antibody (3–5). A recent development is centrifugation of the inoculum to increase the efficiency of viral infection (3). Although these techniques have the sensitivity and specificity of the traditional CC, they still require subjective interpretation of stained cells. Described here is a new test which combines the sensitivity and speed of a centrifugation-enhanced CC with the quantitative and objective results of a commercial HSV ELISA.

Three-hundred patient specimens for HSV detection, each in 2 ml of virus transport medium (Bartels Immunodiagnostics), were received at a large commercial diagnostic laboratory. About 80% of these specimens were of genital origin, with 66% of these from females. The specimens were split into two groups, one for the standard CC, and the other for the new test developed by Ortho Diagnostics, Inc., the HSV ELISA spin amplification technique (ELISA-SAT). All specimens were vortexed, and the swabs were removed and treated with 0.2 ml of a mixture of 10 ml of gentamicin (50 mg/ml) and 50 ml of amphotericin B (Fungizone) (250 μg/ml).

The ELISA-SAT was performed by inoculating samples (0.2 ml) into special flat-bottom glass culture tubes (Ortho Diagnostics) of both primary rabbit kidney cells (RK) and of human lung cells (MCR-5). The inoculated tubes were centrifuged for 30 min at 2,500 × g (the initial 100 tubes were centrifuged for 1 h) and then incubated in an upright stationary position for 44 h at 35°C. After a 0.2-ml sample was removed from each tube, pooled, and stored at −70°C, the remainder was lysed by a special lysing agent (Ortho Diagnostics), pooled, and assayed for HSV antigen by the Ortho Diagnostics HSV ELISA-SAT (cutoff, mean negative control + 0.15). Any specimen that was HSV positive by the ELISA-SAT and HSV negative by the CC was further studied. The sample frozen before the ELISA-SAT was inoculated into two standard culture tubes and screened for 5 days.

For the CC, the remainder of the original patient specimen was further treated by centrifuging at 900 × g for 20 min to reduce the amount of contamination. Then samples (0.2 ml) of the supernatant were inoculated into roller tubes of both RK (Ortho Diagnostics) and of MRC-5 (Bartels Immunodiagnostics). The tubes were incubated on a roller drum at 35°C for 5 days, with daily observation for the typical HSV cytopathic effect. The presence of HSV in the sample was confirmed and the viruses were typed by a monoclonal antibody (Syva) to HSV type 1 (HSV-1) and HSV-2 in an FA.

Selected specimens that were HSV positive by the ELISA-SAT were typed, and the results were compared with typing results obtained from corresponding specimens that were positive by the CC. Frozen antigen lysates from 24 positive specimens were thawed and centrifuged at 900 × g for 15 min. The pellet was washed in phosphate-buffered saline and centrifuged again. The resulting pellet was spotted and air dried on glass slides. After acetone fixation, standard immunofluorescence techniques with the monoclonal antibody were performed.

Of the 300 specimens, 84 (28%) were confirmed to be HSV positive. The CC detected 80 positives, and the ELISA-SAT detected 82 positives. The four specimens that were HSV positive by the ELISA-SAT and HSV negative by the CC were shown to be true HSV positives by reculture of the frozen sample and confirmation by the FA with the monoclonal antibody. The remaining 216 specimens were negative by both methods (Table 1). In detecting 82 of the 84 positives, the ELISA-SAT demonstrated 97.6% sensitivity, and the CC was 95.2% sensitive, with 80 positives detected. Both methods were 100% specific. The ELISA readings for 94% of the HSV positives were greater than 0.1 past the

<table>
<thead>
<tr>
<th>ELISA-SAT result</th>
<th>No. of specimens tested by the CC</th>
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<tbody>
<tr>
<td>+</td>
<td>78</td>
</tr>
<tr>
<td>−</td>
<td>2</td>
</tr>
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<td></td>
<td>216</td>
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* Corresponding author.
TABLE 2. Time needed to report 80 HSV-positive results by the CC

<table>
<thead>
<tr>
<th>Day</th>
<th>No. (%) of HSV-positive results by the CC</th>
<th>Cumulative %</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>22 (28)</td>
<td>28</td>
</tr>
<tr>
<td>2</td>
<td>33 (41)</td>
<td>69</td>
</tr>
<tr>
<td>3</td>
<td>10 (12)</td>
<td>81</td>
</tr>
<tr>
<td>4</td>
<td>5 (6)</td>
<td>87</td>
</tr>
<tr>
<td>5</td>
<td>10 (12)</td>
<td>100</td>
</tr>
</tbody>
</table>

endpoint: 6% of the positives were past but close to the endpoint. The reporting time for positive results by the CC is shown in Table 2. Because negative results cannot be determined until day 5 by the CC, only positives were reported on earlier days. The ELISA-SAT reported all results, both positive and negative, on day 2. Thus, the CC reported 69% of the positives by day 2, and the ELISA-SAT method reported 100%.

The ELISA-SAT by Ortho Diagnostics does not include a method to type the virus. Because the lysate used in the assay is antigenic, this material was used to prepare slides for the FA tests. Twenty-four specimens that were HSV positive by the ELISA-SAT were prepared and typed with the FA reagent. The method was easily performed and showed 100% agreement with the CC results.

The ELISA-SAT had a sensitivity and a specificity equal to or better than those of the traditional CC. In addition, all results, which were objectively determined, were available after 2 days. It was also noted that centrifugation of the specimens to avoid contamination was not required for the ELISA-SAT. Four positive cultures were missed by the CC, whereas only two were missed by the ELISA-SAT. One of the two missed had an ELISA reading that was just under the cutoff point and would probably have been repeated; and the other may have been due to a smaller antigen load or a sampling error.

Because the ELISA-SAT does not require a technologist trained to interpret virus cytopathology, our laboratory can demonstrate a significant cost improvement over the CC. By selecting only HSV-positive specimens for virus typing by the FA, both labor and reagent expenses are lower than those for tests that stain all the coverslips at 24 or 48 h. ELISA-SAT objectivity is an improvement over the interpretation-dependent FA or peroxidase staining.

In conclusion, the ELISA-SAT proved to be equal in sensitivity and specificity to the CC and reported results earlier than did the CC. The objectivity and cost savings make this test a logical choice for the reference laboratory, a great improvement on the current gold standard.

LITERATURE CITED