Evaluation of an enzyme-linked immunoassay employing a covalently bound capture antibody for direct detection of herpes simplex virus.

C A Needham and P Hurlbert

Evaluation of an Enzyme-Linked Immunoassay Employing a Covalently Bound Capture Antibody for Direct Detection of Herpes Simplex Virus

CYNTHIA A. NEEDHAM* AND PAMELA HURLBERT

Department of Laboratory Medicine, Lahey Clinic Medical Center, 41 Mall Road, Burlington, Massachusetts 01805

Received 8 August 1991/Accepted 5 November 1991

The FDL enzyme-linked immunosorbent assay (ELISA; Fairleigh Dickinson Laboratories, Inc., Abilene, Tex.) for the detection of herpes simplex virus (HSV) makes use of a covalently attached antibody. This assay was compared with viral isolation and with the Ortho HSV Antigen Detection ELISA (Ortho Diagnostic Systems, Inc., Raritan, N.J.). One hundred forty-eight specimens were tested. The FDL ELISA identified 66 of 184 specimens from which HSV was isolated, yielding a sensitivity of 63% and a specificity of 95%. These results compared favorably with those obtained by using the Ortho ELISA. The total test time was shorter and the washing step was simpler than that with the Ortho assay, making the FDL assay an attractive alternative to similar methodologies.

The enzyme-linked immunosorbent assay (ELISA) is a commonly used method for detecting viral antigens in clinical material. These assays rely on a double antibody, or "sandwich," technique to capture and subsequently identify the target antigen. The capture antibody is typically immobilized on the plastic surface of a microwell plate by means of overnight incubation at 4°C at an alkaline pH (5). This process is believed to contribute to problems with the reproducibility of test results when low levels of antigen are assayed. The extensive washing required in ELISAs may remove some of the primary reagent, permitting nonspecific attachment of secondary reagents to the solid phase.

In the FDL ELISA (Fairleigh Dickinson Laboratories, Inc., Abilene, Tex.), the primary reagent is covalently bound to a chemically reactive substance that coats the plastic surface of the microwells provided in the kit (1). This procedure is reputed to bind five times more of the primary reagent (capture antibody) and to prevent its inadvertent loss during washing. In this study we compared the FDL ELISA with viral isolation in cell culture and with the Ortho HSV Antigen Detection ELISA (Ortho Diagnostic Systems, Inc., Raritan, N.J.) using direct testing of patient specimens. The Ortho assay uses a traditional method for the immobilization of capture antibodies and has been well characterized (2-4, 6).

Specimens were collected by using two sterile Dacron swabs with plastic shafts. The swabs were placed in a transporter containing Amies transport medium and were delivered immediately to the laboratory on wet ice. On receipt, both swabs were transferred to 2.0 ml of viral transport medium (Hanks balanced salt solution with 0.5% gelatin, 20 U penicillin per ml, 20 μg of streptomycin per ml, and 0.5 μg of amphotericin B per ml; all products were from GIBCO Laboratories, Grand Island, N.Y.). The swabs were placed at 4°C and processed for cell culture within 24 h. The specimens were subjected to vortexing, the swabs were extracted, and the samples were centrifuged for 5 min at 2,000 rpm (900 × g). Two primary rabbit kidney cell culture tubes (Viromed Laboratories, Minnetonka, Minn.) from which the maintenance medium had been decanted, were each inoculated with 0.25 ml of the specimen supernatant. The remainder of the original specimen was frozen at −70°C for later testing or was analyzed immediately by ELISA.

Inoculated cell cultures were incubated at 36°C for 1 h, the supernatant was decanted, and the maintenance medium was added. The cultures were incubated in a stationary rack at 36°C and were examined daily for 7 days to detect evidence of a typical cytopathic effect. Cells from cultures with a typical herpes simplex virus (HSV) cytopathic effect were confirmed and typed by direct fluorescent-antibody staining (HSV1/HSV2 Culture Identification Kit; Syva Co., Palo Alto, Calif.).

All 148 patient samples were tested by FDL ELISA. Seventy specimens were assayed before they were frozen at −70°C. The remaining 78 specimens were thawed and subjected to vortexing before they were assayed. Samples (100 μl) of each specimen, along with duplicate samples of positive and negative control specimens, were transferred to wells coated by the manufacturer with covalently bound, mixed-polyclonal rabbit anti-HSV type 1 and type 2 capture antibody (7). The assay was performed according to the specifications of the manufacturer.

Visual interpretation was performed immediately after the last incubation by examining the microwells against a white background for the presence of a blue color. Results were interpreted as positive if the color of the test sample was more intense than that of the negative control. Results were interpreted as negative when the color intensity was equal to or less than that of the negative control. The wells were subsequently evaluated by using a microtiter plate reader (Molecular Devices Corp., Menlo Park, Calif.) at 630 nm.

The Ortho HSV Antigen Detection ELISA was used to assay 66 of the 148 patient specimens. Fifty-six specimens were assayed as fresh samples at the time of cell culture, and 10 samples were from previously frozen specimens. A 200-μl sample of each specimen and duplicate 200-μl samples of positive and negative control specimens were pipetted into microwells to which mixed polyclonal rabbit anti-HSV type 1 and type 2 had been immobilized by the manufacturer. The assay was performed according to the specifications of the

* Corresponding author.
TABLE 1. Comparison of FDL ELISA with primary rabbit kidney cell culture for detection of HSV

<table>
<thead>
<tr>
<th>Specimen and FDL ELISA result</th>
<th>No. of specimens with the following cell culture isolation results*:</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>HSV type 1</td>
<td>Negative</td>
<td>HSV type 2</td>
</tr>
<tr>
<td>Genital</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>15</td>
<td>21</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>5</td>
<td>13</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Nongenital</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>23</td>
<td>7</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>13</td>
<td>7</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>38</td>
<td>28</td>
<td>66</td>
<td></td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>20</td>
<td>38</td>
<td></td>
</tr>
</tbody>
</table>

*: The sensitivity was 63%, and the specificity was 95%.

The specimens assayed by the FDL ELISA were obtained from 81 genital and 67 nongenital sites. Specimens from the nongenital sites included oral, dermal, and ocular lesions as well as specimens from the lower respiratory tract. HSV was isolated in cell cultures from 104 of the specimens. The FDL ELISA detected 66 of these, yielding a sensitivity of 63% (Table 1). Two of the 44 culture-negative specimens were found to be antigen positive by ELISA, giving the test a specificity of 95%. No significant difference in the diagnostic accuracy of the FDL test was found between specimens obtained from genital and nongenital sites, between specimens assayed fresh and those assayed after freezing, or between specimens from which HSV type 1 was recovered and those from which HSV type 2 was recovered.

Visual interpretation of results diminished the sensitivity of the assay to some degree. Of the 66 true-positive specimens detected by using spectrophotometric reading, 6 were interpreted as negative by visual reading, reducing the assay sensitivity by 10%.

A comparison of results obtained by the FDL ELISA and Ortho HSV Antigen Detection ELISA is presented in Table 2. Specimens testing positive and negative by the ELISAs were the same, with the exception of the single specimen that yielded the falsely positive Ortho ELISA result. Sensitivity and specificity were virtually identical for both assay methods. The results indicate that covalent bonding for attachment of the capture antibody to the plastic surface of the assay wells used in the FDL assay does not enhance the diagnostic performance of the ELISA when routine clinical specimens are used.

However, the technical methodology of the FDL ELISA offers some advantage over the ELISA methods typified by the Ortho test. The total test time for the FDL assay was approximately 1 h and 15 min, in comparison with 2 h and 30 min for the Ortho assay. The FDL assay uses a single wash step, which is accomplished by simple rapid manual manipulation. The wells are washed vigorously by forcibly directing a stream of wash buffer into each well. This maneuver is repeated three times. All reagents are supplied by the manufacturer in dropper bottles, and all reagents are stable when they are stored according to the recommendation of the manufacturer. The FDL assay can be interpreted without the aid of a spectrophotometer. However, in our experience the sensitivity of the assay diminished when the results were read manually.

In contrast, the Ortho test uses two separate wash steps that must be performed with care by using a microwell strip washer. This maneuver is repeated twice during the first wash step and five times during the second one. The substrate solution is prepared each time the assay is performed. All reagents are pipetted, and three additional steps are needed for which pipetting is necessary.

We conclude that the FDL ELISA compares favorably in sensitivity and specificity with the Ortho assay, a similar antigen detection method. The FDL assay should prove useful, particularly when large volumes of specimens are tested, in view of the ease with which it can be performed. The specificity of the FDL assay should allow its use for both direct specimen testing and culture confirmation. However, its sensitivity is not sufficient to replace the culture method for patient specimens that test negative by this assay. Because the FDL ELISA is performed with specimens that are preserved in standard viral transport media, it has the virtue of preserving virus viability until direct test results are available.

This study was funded in part by a grant from Cambridge Medical Technology, Burlington, Mass.

REFERENCES