Performance of the Focus and Kalon Enzyme-Linked Immunosorbent Assays for Antibodies to Herpes Simplex Virus Type 2 Glycoprotein G in Culture-Documented Cases of Genital Herpes

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The Centers for Disease Control and Prevention’s Sexually Transmitted Disease Treatment Guidelines 2002 (6) recommends that tests based on glycoprotein G-2 (gG-2) be used for diagnosis of genital herpes. Recently only three such products on the market are cleared by the Food and Drug Administration to diagnose HSV-2: HerpeSelect HSV-2 ELISA and HerpeSelect Immunoblot IgG (both from Focus Technologies, Cypress, Calif.) and POCkit-HSV-2 (Diagnology, Belfast, Northern Ireland [ceased production in September 2003]) (1). The Focus HSV-2 enzyme-linked immunosorbent assay (ELISA) is a standard, automatable immunoassay that is cost-effective for most laboratories. However, concerns have been raised over the specificity of Focus HSV-2 ELISA for sera from African countries (E. Van Dyck, A. Buve, D. Brown, and M. Laga, 17th INSTI, abstr. T079 ISSTDR, 2001).

A recombinant gG-2-based herpes simplex virus type 2 (HSV-2) enzyme-linked immunosorbent assay from Focus and Kalon were performed with specimens from 118 patients with culture-documented genital herpes episodes, and their results were compared. Sensitivity was 52% by Kalon and 86% by Focus for first HSV-2 episodes and 100% (for each of the two tests) for recurrent HSV-2. Median times to seroconversion were 120 days by the Kalon assay, 21 days by the Focus assay, and 68 days by Western blotting assay. Values for specificity were 100% (Kalon) and 93% (Focus).

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only, and 2 failed to seroconvert in any of the three tests. The final subject was negative by Kalon and WB but equivocal by Focus in the last serum.

Overall sensitivity for seroconversion (Groups 1 and 2) was 15 of 29 (52%) for Kalon, 24 of 28 (86%) for Focus, and 26 of 30 (87%) for WB. Median follow-up time for Kalon nonseroconverters was 86 days (range, 58 to 139), while median follow-up time for Kalon seroconverters was over 3 months (median, 105 days; range, 47 to 193). Sensitivity for all three tests was lower in nonprimary than in primary patients (Table 1) in contrast to findings with other gG-2 assays (3, 8), possibly due to the fact that follow-up time was shorter for nonprimary subjects (median, 81 days) than for primary patients (median, 104 days).

Thirty subjects from Group 3 had their sera drawn within 6 weeks of a culture-documented recurrence of HSV-2 and were HSV-2 seropositive by WB. Twenty-four had a history of when their first episode occurred; their length of time with HSV-2 infection was a median of 7.5 years (range, 1 to 31 years). All 30 (100%) were positive by the Kalon and Focus tests, suggesting that both are sensitive for antibodies in patients with established infection.

![FIG. 1. Time to seroconversion following onset of genital infection in HSV-1-seronegative, HSV-2-seronegative patients (primary episode). Note that censored data (the day from onset that a subject remained seronegative when he/she left the study) affect the curves. Thus, seven subjects failed to seroconvert by Kalon at their latest serum postonset (days 66 to 136) and were dropped from further consideration. Of the remaining subjects, the final one seroconverted by Kalon at day 168. Only one subject who was still eligible failed to seroconvert by day 117 by Focus and was censored at the point where the curve is truncated.](http://jcm.asm.org/)

### TABLE 1. Sensitivity and specificity of Kalon and Focus ELISAs for sera from culture-documented and WB-confirmed genital herpes episodes

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Patient status at onset</th>
<th>No. of patients</th>
<th>Median no. of days postonset (range)</th>
<th>Kalon Sensitivity (%)</th>
<th>Kalon Specificity (%)</th>
<th>Focus Sensitivity (%)</th>
<th>Focus Specificity (%)</th>
<th>WB sensitivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>First HSV-2 episode; HSV-1 and HSV-2 seronegative</td>
<td>16</td>
<td>104 (66–168)</td>
<td>9/15 (60%)</td>
<td>NA</td>
<td>14/15 (93%)</td>
<td>NA</td>
<td>16/16 (100%)</td>
</tr>
<tr>
<td>2</td>
<td>First HSV-2 episode; HSV-1 seropositive, HSV-2 seronegative</td>
<td>14</td>
<td>81 (47–193)</td>
<td>6/14 (43%)</td>
<td>NA</td>
<td>10/13 (77%)</td>
<td>NA</td>
<td>10/14 (71%)</td>
</tr>
<tr>
<td>3</td>
<td>Recurrent HSV-2 episode; HSV-1 seronegative, HSV-2 seropositive</td>
<td>30</td>
<td>68 (11–150)</td>
<td>30/30 (100%)</td>
<td>NA</td>
<td>30/30 (100%)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>4</td>
<td>Recurrent HSV-1 episode; HSV-1 seropositive, HSV-2 seronegative</td>
<td>58</td>
<td>67 (0–541)</td>
<td>NA</td>
<td>58/58 (100%)</td>
<td>NA</td>
<td>54/58 (93%)</td>
<td>NA</td>
</tr>
</tbody>
</table>

*Genital herpes status was determined by monoclonal antibody typing of the HSV isolate cultured from the presenting lesion. Seropositivity for HSV-1 or HSV-2 was determined at the onset of the lesion by WB. All Group 1 and 2 subjects eventually seroconverted to HSV-2 by WB.*

*Days from presenting episode to seroconversion for patients who became positive or last available sample for those who remained seronegative.*

*Sensitivity percent was 100 times the number of positive results for infected patients divided by the sum of the number of positive results for infected patients plus the number of negative results for infected patients. Culture status was used as the gold standard for infected persons. All infected persons had HSV-2 cultured from lesions. Sera with equivocal test results were excluded.*

*Specificity percent was 100 times the number of negative results for uninfected patients divided by the sum of the number of negative results for uninfected patients plus the number of positive results for uninfected patients. HSV-2-uninfected status was determined by culture (HSV-1 only was isolated from the lesion) and WB (HSV-1 positive but HSV-2 negative).*

*WB sensitivity was determined only for Groups 1 and 2 in sera drawn after the first episode. Groups 3 and 4 were preselected, in part, by WB results.*

*NA, not available.*
Of 58 sera from Group 4 patients with culture-documented recurrent HSV-1 episodes and only HSV-1 antibodies by WB, all were negative for HSV-2 antibodies by Kalon, for a specificity of 100% (Table 1). Fifty-four were negative for HSV-2 by the Focus test, giving a specificity (93%) slightly lower than that previously published in a study using WB as a comparator test (2). The four Focus positive sera from Group 4 had index values barely above the cutoff of 1.10 in the Focus test (median, 1.28; range, 1.21 to 1.81). These four sera could represent false-positive Focus test results or inaccurate culture typing. Alternatively, the patients could have been seroconverting to HSV-2. Previous studies have shown that patients can become positive by Focus before they seroconvert by WB (4). Further testing by more-refined methods might help resolve this discordance (9).

In summary, while both Kalon and Focus were accurate in detecting established HSV-2 infection, the Kalon gG-based HSV-2 serology was very insensitive for the detection of antibody to early HSV-2 infection. One-half did not seroconvert by 4 months or more, and only 5 of 30 had seroconverted by 2 months after infection, which is far slower than Focus, WB (4), or POCKit (3). If possible, Focus or POCKit should be used to test patients who may have been recently infected; if tested and found negative by Kalon, such patients should be retested by one of these gG-based tests (1).

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REFERENCES