Improved Performance of Enzyme-Linked Immunosorbent Assays and the Effect of HIV Co-Infection on the Serologic Detection of Herpes Simplex Virus Type-2 in Rakai, Uganda

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Running title: Performance of Assays for Detection of HSV-2 in Uganda

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Abstract:

820 Ugandan subjects were tested by Focus HerpeSelect ELISA, Kalon HSV-2 ELISA, and BioKit rapid test, and compared to Western blot. Higher than standard index cutoff values gave optimal sensitivity and specificity. Kalon ELISA was the optimal assay when an index value of 1.5 was used (sensitivity 91.7%, specificity 92.4%).
Herpes simplex virus type 2 (HSV-2) infection is one of the most common sexually transmitted diseases, and HSV-2 is the main cause of genital ulceration worldwide (4, 5, 17). Genital HSV-2 infections are associated with an increased risk for acquiring HIV-1 (5).

Diagnosis of HSV-2 in sub-Saharan Africa and other developing countries, where the prevalence is high, has been problematic. Serologic tests for HSV-2 that use an enzyme-linked immunosorbent assay (ELISA) or dot blot are technically simple and relatively inexpensive (15), but have been complicated by a variable rate of samples with a positive HSV-2 ELISA and negative Western blot (6, 8, 10, 15). In addition, the Western blot is expensive, difficult to read, and inefficient to evaluate large numbers of samples for clinical trials (3). We and others have previously demonstrated that a higher index cutoff value is required for optimal sensitivity and specificity for the Focus HerpeSelect HSV-2 ELISA (1, 10), however, this assay still lacks specificity. Therefore, Focus HerpeSelect ELISA, Kalon HSV-2 ELISA, and BioKit rapid test, were compared to Western blot as the gold standard.

The study utilized sera from 820 subjects (273 HIV-positive and 547 HIV-negative) collected in a population-based randomized control trial of presumptive STD treatment among adults aged 15 to 19 in Rakai District, Uganda from 1994 to 1998 (7, 10). All relevant institutional review boards in Uganda, Johns Hopkins University and National Institutes of Health approved the trial. Samples were collected at the participant’s home, processed for sera, and stored at -70°C. The University of Washington HSV-2 specific Western Blot (WB) analysis was performed as previously described (2, 12). The Focus HerpeSelect HSV-2 ELISA (Focus Technologies, Cypress, CA) and Kalon ELISA (Kalon Biological Ltd, Guilford, UK) were performed according to the manufacturer’s protocol (11) with a few modifications. Samples and
controls were run in either triplicate (Focus) or duplicate (Kalon). Mean index values were used for all calculations. Any discordant results within a sample were run again. The Sure-Vue HSV-2 Rapid Test (BioKit USA Inc, Lexington, MA) test was performed according to the protocol for serum samples. HIV status was determined using two different ELISAs (Vironostika HIV-1, Organon Teknika, Charlotte, NC and Cambridge Biotech, Worcester, MA). Discordant results or new seroconversions were confirmed by HIV-1 Western Blot (Bio-Merieux-Vitek, St. Louis, MO), as previously described (7). Statistical calculations and receiver-operating characteristic curves were performed using Intercooled Stata 9.2 (StataCorp LP, College Station, TX).

To evaluate the performance of Focus HerpeSelect ELISA to detect HSV-2 seroprevalence in Sub-Saharan Africa, 820 subjects were tested. According to manufacturer’s instructions (index cutoff value 1.1), the test had a sensitivity of 99.0%, and specificity of 50.7%. An index cutoff value of 3.2 was determined to provide the optimal sensitivity (88.4%) and specificity (80.8%).

Of the 820 subjects, 538 subjects were also evaluated by Kalon ELISA. According to manufacturer’s instructions (index cutoff value 1.1), the test had a sensitivity of 95.1% and a specificity of 87.6% (Table 1). An index value cutoff of 1.5 gave the optimal sensitivity (91.7%) and specificity (92.4%) (Table 1). The receiver-operating characteristic curve demonstrates that Kalon is superior to Focus with a greater area under the curve (Figure 1).

Of the 820 subjects, 524 subjects were also evaluated by the BioKit Rapid point of care test. According to manufacturer’s instructions that indicate that low positives should be
considered positive, the sensitivity was 95.8% and the specificity was 56.1%. Adjustment of
index values was not possible for this assay.

Due to the association between HIV-1 and HSV-2 (5, 13, 14), it is important to determine
the effect of HIV-1 infection on HSV-2 antibody detection. For both ELISAs and the BioKit
assay there was a higher proportion of subjects positive for HSV-2 among the HIV-1 infected
compared to the uninfected populations (p<0.001). However, none of the assays were
significantly affected by HIV-1 status (Table 2).

This study represents the largest investigation of the performance of three different
commercial HSV-2 assays in sub-Saharan Africa. We demonstrated that Kalon HSV-2 IgG
ELISA is improved at a higher index cutoff value of 1.5 compared to subjects from the Western
hemisphere and is superior to both Focus HerpeSelect ELISA and Rapid BioKit point of care
antibody test. In addition, we found that HIV status does not significantly affect HSV-2
serologic diagnosis.

While adjusting the index values of the assays for subjects from sub-Saharan Africa
significantly improves both the sensitivity and specificity, there are still both false negatives and
false positive results. False negatives could be due to an early infection where the WB is more
sensitive. The false positives may be due to cross-reactivity with HSV-1 that is >90% prevalent
in sub-Saharan Africa (9, 16).
To create the best predictive testing strategy based on sensitivity and specificity as compared to the WB, different algorithms were analyzed. The optimal testing method with the highest combined sensitivity and specificity is to use the Focus ELISA with an index cut-off value of 1.1, and then testing all positive samples with the Kalon ELISA using an index cut-off value of 1.5. The sensitivity and specificity are then 92.0% and 92.8% respectively. While this algorithm produces the best results overall, the results were not statistically significantly different from using the Kalon test alone with a cut-off value of 1.5 (p=0.7). Overall, the quickest, most economical and accurate method for HSV-2 detection is to use the Kalon ELISA with an increased index cut-off compared to the manufacturers’ recommendation.

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1 **Abbreviations**

2 Enzyme-linked immunosorbent assay (ELISA)

3 Genital ulcer disease (GUD)

4 Herpes simplex virus type-2 (HSV-2)

5 Human immunodeficiency virus (HIV)

6 University of Washington Western blot (WB)


Figure Legends

Figure 1. Receiver-operating characteristic curves for Focus and Kalon assays. The area under the curve is 0.98 for Kalon and 0.93 for Focus.
Table 1. Performance of the Kalon HSV-2 ELISA

<table>
<thead>
<tr>
<th>Index Value</th>
<th>+/+</th>
<th>+/-</th>
<th>-/+</th>
<th>-/-</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.7</td>
<td>286</td>
<td>2</td>
<td>63</td>
<td>187</td>
<td>99.3%</td>
<td>74.8%</td>
</tr>
<tr>
<td>0.8</td>
<td>285</td>
<td>3</td>
<td>45</td>
<td>205</td>
<td>99.0%</td>
<td>98.6%</td>
</tr>
<tr>
<td>0.9</td>
<td>283</td>
<td>5</td>
<td>38</td>
<td>212</td>
<td>98.3%</td>
<td>84.8%</td>
</tr>
<tr>
<td>1.1</td>
<td>274</td>
<td>14</td>
<td>31</td>
<td>219</td>
<td>95.1%</td>
<td>87.6%</td>
</tr>
<tr>
<td>1.3</td>
<td>268</td>
<td>20</td>
<td>26</td>
<td>224</td>
<td>93.1%</td>
<td>89.6%</td>
</tr>
<tr>
<td>1.5</td>
<td>264</td>
<td>24</td>
<td>19</td>
<td>231</td>
<td>91.7%</td>
<td>92.4%</td>
</tr>
<tr>
<td>1.8</td>
<td>250</td>
<td>38</td>
<td>14</td>
<td>236</td>
<td>86.8%</td>
<td>94.4%</td>
</tr>
<tr>
<td>2.0</td>
<td>240</td>
<td>48</td>
<td>11</td>
<td>239</td>
<td>83.3%</td>
<td>95.6%</td>
</tr>
<tr>
<td>2.5</td>
<td>220</td>
<td>68</td>
<td>6</td>
<td>244</td>
<td>76.4%</td>
<td>97.6%</td>
</tr>
<tr>
<td>3.0</td>
<td>197</td>
<td>91</td>
<td>5</td>
<td>245</td>
<td>68.4%</td>
<td>98.0%</td>
</tr>
<tr>
<td>3.5</td>
<td>188</td>
<td>100</td>
<td>4</td>
<td>246</td>
<td>65.3%</td>
<td>98.4%</td>
</tr>
</tbody>
</table>

*Number of subjects positive by ELISA and WB (+/+), positive by ELISA and negative by WB (+/-), negative by ELISA and positive by WB (-/+), and negative by ELISA and WB (-/-) for HSV-2.
Table 2. Performance of HSV-2 serology assays by HIV Status

<table>
<thead>
<tr>
<th>Assay</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Concordance</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV +</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Focus</td>
<td>89.0%</td>
<td>80.6%</td>
<td>67.2%</td>
</tr>
<tr>
<td>Kalon</td>
<td>92.9%</td>
<td>86.0%</td>
<td>78.0%</td>
</tr>
<tr>
<td>BioKit</td>
<td>97.6%</td>
<td>52.1%</td>
<td>57.0%</td>
</tr>
<tr>
<td>HIV -</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Focus</td>
<td>95.9%</td>
<td>75.2%</td>
<td>73.0%</td>
</tr>
<tr>
<td>Kalon</td>
<td>92.5%</td>
<td>94.5%</td>
<td>86.5%</td>
</tr>
<tr>
<td>BioKit</td>
<td>94.4%</td>
<td>57.1%</td>
<td>49.6%</td>
</tr>
</tbody>
</table>

The samples were stratified by HIV status by test and evaluated at the adjusted cut-off values. No statistical difference between status for each test (p>0.05).