Performance of the HerpeSelect (Focus) and Kalon Enzyme-Linked Immunosorbent Virus Assays for Detection of Antibodies against Herpes Simplex Virus Type 2 by Use of Monoclonal Antibody-Blocking Enzyme Immunoassay and Clinicovirological Reference Standards in Brazil

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A total of 586 serum samples were used to evaluate the performance of type-specific herpes simplex virus type 2 (HSV-2) commercial enzyme-linked immunosorbent assays (ELISAs) by using the monoclonal antibody-blocking enzyme immunoassay (MAb-EIA) and a clinicovirological panel as reference standards. The Kalon and HerpeSelect ELISAs had similar sensitivities (93.5% and 93.8% compared with the results obtained by MAb-EIA, respectively, and 100% for both ELISAs compared with the results obtained with a clinicovirological panel). The Kalon ELISA had a higher specificity (96.5% and 96.8% compared with the results obtained by MAb-EIA and with a clinicovirological panel, respectively) than the HerpeSelect ELISA (86.9% and 94% compared with the results obtained by MAb-EIA and with a clinicovirological panel, respectively). A higher cutoff significantly improved the specificity of the HerpeSelect ELISA.

The public health importance of sexually transmitted herpes simplex virus (HSV) type 2 (HSV-2) is increasingly recognized. HSV-2 is the main cause of genital ulcers worldwide (9), and its presence might facilitate human immunodeficiency virus (HIV) transmission (5). The majority of people infected with HSV-2 are unable to recognize their asymptomatic disease, which remains a threat to the control of HSV-2 transmission (4). Seroepidemiological studies have been hampered by the lack of accurate and easy-to-use tests for the detection of antibodies against HSV-2 in different populations. Western blotting (WB), a time-consuming and expensive assay, has long been used as a “gold standard” for the detection of anti-HSV type-specific antibodies (2). Another reference standard is the monoclonal antibody-blocking enzyme immunoassay (MAb-EIA) and a clinicovirological panel as reference standards. The Kalon and HerpeSelect ELISAs had similar sensitivities (93.5% and 93.8% compared with the results obtained by MAb-EIA, respectively, and 100% for both ELISAs compared with the results obtained with a clinicovirological panel). The Kalon ELISA had a higher specificity (96.5% and 96.8% compared with the results obtained by MAb-EIA and with a clinicovirological panel, respectively) than the HerpeSelect ELISA (86.9% and 94% compared with the results obtained by MAb-EIA and with a clinicovirological panel, respectively). A higher cutoff significantly improved the specificity of the HerpeSelect ELISA.

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Paulo; group 4, 29 HIV-negative men who have sex with men enrolled in a cohort study for HIV prevention in Sao Paulo; group 5, 100 college students participating in a cross-sectional HSV seroepidemiological study in Sao Paulo; and group 6, 250 children (ages, 1 to 2 years) from Sao Paulo recruited for a measles vaccine research program (Table 1). The commercial assays were performed by using the manufacturers’ instructions. Samples with optical density index values <0.9 were recorded as negative, those with index values >1.1 were recorded as positive, and those with intermediate values were recorded as equivocal. We also evaluated the performance of the HerpeSelect assay using an increased cutoff of 3.5, as suggested previously (1).

The performances of the HerpeSelect ELISA at different cutoffs and the Kalon ELISA for the detection of HSV-2 antibodies in the whole panel were determined and compared with the performances of the serological (MAb-EIA) and clinicovirological reference standard assays. The latter used group 1 as an “HSV-2-infected” reference group and group 6 as an “HSV-2-uninfected” reference group.

The McNemar chi-square test was used to estimate the statistical significance of the differences in sensitivities and specificities of the assays by comparing the number of discordant results between the Kalon and HerpeSelect assays among subjects for whom MAb-EIA results were available. Owing to a smaller sample size, statistical significance tests were not applied to estimate the different performances of these assays in comparison with that of the clinicovirological gold standard assay (groups 1 and 6).

As shown in Table 1, most patients (range, 87% to 100%) with proven HSV-2 infection (group 1) were seropositive by the MAb-EIA, Kalon ELISA, and HerpeSelect ELISA. Groups unlikely to be infected by HSV-2 (i.e., groups 5 and 6) had low seropositivity rates (range, 0.9% to 6.2%), except for those determined by the HerpeSelect ELISA by use of the manufacturer’s recommended cutoff of 1.1 (30% and 6% in groups 5 and 6, respectively). Equivocal results were found for 8/585 (1.4%) samples tested by MAb-EIA, and these were excluded from further analyses. No equivocal results were obtained by the Kalon ELISA with the 576 serum samples tested. By using the manufacturer’s recommended cutoff (>1.1) and the increased cutoff (>3.5), the HerpeSelect ELISA gave equivocal results for 18/529 (3.4%) and 81/529 (15.3%) of the samples tested, respectively. For analysis of test performance, the samples with equivocal results by the HerpeSelect ELISA were categorized as negative. As shown in Table 2, compared with the MAb-EIA as the reference standard, the sensitivity and the specificity of the Kalon ELISA were 93.5% (95% confidence interval [CI], 88% to 97%) and 96.5% (95% CI, 94% to 98%), respectively, while the sensitivity and the specificity of the HerpeSelect ELISA (cutoff >1.1) were 93.8% (95% CI: 88% to 97%) and 86.9% (95% CI, 83% to 90%), respectively, a significant difference in specificities (McNemar test, P < 0.0001). Raising the cutoff of the HerpeSelect

<table>
<thead>
<tr>
<th>Population group</th>
<th>No. of samples positive/no. tested (%)</th>
<th>Equivocal results</th>
<th>Performance indicators against clinicovirological standard (n = 280)&lt;sup&gt;c&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>MAb-EIA</td>
<td>Kalon</td>
<td>HerpeSelect (CO &gt; 1.1)</td>
</tr>
<tr>
<td></td>
<td>No. of samples positive/no. tested (%)</td>
<td>% Sensitivity (95% CI)</td>
<td>% Specificity (95% CI)</td>
</tr>
<tr>
<td></td>
<td>143/577 (24.8)</td>
<td>145/566 (25.6)</td>
<td>172/519 (33.1)</td>
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<tr>
<td></td>
<td>26/30 (87)</td>
<td>25/40 (62)</td>
<td>4/249 (1.6)</td>
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<td></td>
<td>3/29 (10)</td>
<td>12/40 (30)</td>
<td>5/29 (17)</td>
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<td></td>
<td>585</td>
<td>576</td>
<td>29</td>
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</table>

<sup>a</sup> CO, cutoff; STI, sexually transmitted infection; MSM, men who have sex with men.

<sup>b</sup> The MAb-EIA reference standard comprised 143 positive and 434 negative samples, after the exclusion of 8 samples with equivocal results.

<sup>c</sup> clinicovirological reference standard comprised samples from AIDS patients with HSV-2 ulcers (group 1) considered positive (n = 30) and children <2 years of age (group 6) considered negative (n = 250).

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**TABLE 1. Antibodies to HSV-2 detected by MAb-EIA, Kalon ELISA, and HerpeSelect ELISA, by population group, in Brazil**

**TABLE 2. Performance of HSV-2 serological assays compared to those of serological (MAb-EIA) and clinicovirological reference standards in Brazil**
ELISA to 3.5 increased the specificity (98.5%; 95% CI, 97% to 99%; McNemar test, $P < 0.0001$) but also significantly decreased the sensitivity (81.4%; 95% CI, 74% to 88%; McNemar test, $P = 0.0002$ in comparison with the results of the Kalon ELISA). Compared with the results obtained by use of the clinicovirological reference standard, the HerpeSelect ELISA (cutoff $> 1.1$) and the Kalon ELISA were 100% sensitive (95% CI, 88% to 100%), with specificities of 94% (95% CI, 90% to 97%) and 96.8% (95% CI, 94% to 99%), respectively. The sensitivity of the HerpeSelect ELISA was reduced to 89.7% (95% CI, 73% to 98%) and the specificity was increased to 99.1% (95% CI, 97% to 100%) when the cutoff was increased. The MAb-EIA was the least sensitive (86.7%; 95% CI, 69% to 96%) of all three tests, although it had a specificity comparable to those of the other assays.

The problem with the specificity of the HerpeSelect ELISA is well known. Initial studies carried out in the United States showed that in comparison with the results of WB or a combination of WB and the clinical diagnosis as the gold standard, the HerpeSelect ELISA had high sensitivities (88% and 98%, respectively) and high specificities (93.8% and 96%, respectively) (8, 10). However, discrepant results were obtained when samples from sub-Saharan African were tested (7, 14). In one study with samples from four African countries (7), the sensitivity of the HerpeSelect ELISA compared to the results of WB was 100%, while the specificities among samples from HIV-negative individuals were 70% in Uganda, 80% in Kenya, and 100% in Zimbabwe and South Africa. In a large study that evaluated 13 HSV-2 type-specific commercial assays with samples from Benin, Cameroon, Kenya, and Zambia (14), the performance of the HerpeSelect ELISA varied according to HIV infection status, with decreased specificity among HIV-positive samples (71% specificity among HIV-positive samples and 74% specificity among HIV-negative samples). It has been suggested that the generally lower specificity of the HerpeSelect assay with samples from African populations in comparison with that with samples from the United States could be due to cross-reactivity with HSV-1, which is more prevalent in African countries (14). The reason for the lower specificity of the HerpeSelect assay for the detection of HSV-2 infection in HIV-seropositive samples is unknown, but it could be related to the HIV-associated immunoglobulin G polyclonal immune stimulation that might interact with the test or to some more complex effect of HIV on the immune responses. Recognizing these limitations, investigators have recommended raising the cutoff to 3.5 to increase the specificity of the assay (1).

In contrast, with samples from the general populations of Cameroon, Kenya, and Zambia, the sensitivity of the Kalon ELISA ranged from 89% to 92% and its specificity ranged from 95% to 100% compared with the results of the MAb-EIA, with WB used as a test for resolution of discrepant results; its performance results were comparable with HIV-positive and HIV-negative samples (14). Furthermore, in comparison with WB, the Kalon assay had stable performance (sensitivity and specificity, 100%) when it was used to test samples from patients with recurrent HSV culture-positive genital ulcers in the United States, regardless of their HSV-1 seropositive status (10).

In our study, the majority of samples with equivocal results by the HerpeSelect assay belonged to groups at low risk for HSV-2 infection and gave negative results by the MAb-EIA. In a previous study, samples with equivocal results by the HerpeSelect assay were shown to be negative by WB (12). Thus, the categorization of samples with equivocal results as “negative” seems to be the most logical decision. However, it cannot be ruled out that a number of equivocal samples among the student group (group 5) represent genuine positive reactions, perhaps associated with seroconversion, as the HerpeSelect assay has been shown to detect seroconversions earlier than other tests, including WB (10). Our study design did not allow exploration of this hypothesis.

In summary, our study showed that the Kalon assay performed better than the HerpeSelect assay for the detection of HSV-2 antibodies in samples from individuals in Brazil. Raising the cutoff to 3.5, as suggested previously (1), significantly improved the specificity of the HerpeSelect assay, but this was at the expense of sensitivity.

We have no conflict of interest to declare.

REFERENCES