Performance of a Commercial, Type-Specific Enzyme-Linked Immunosorbent Assay for Detection of Herpes Simplex Virus Type 2-Specific Antibodies in Ugandans

Oliver Laeyendecker,¹ Charla Henson,² Ronald H. Gray,³ Ruby H.-N. Nguyen,³
Bobbi Jo Horne,² Maria J. Wawer,⁴ David Serwadda,⁵ Noah Kiwanuka,⁶
Rhoda Ashley Morrow,⁷ Wayne Hogrefe,⁸
and Thomas C. Quinn^{1,2*}

National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda,¹ and Department of Medicine, Johns Hopkins University School of Medicine,² and Department of Population and Family Health Sciences, Bloomberg School of Public Health, Johns Hopkins University,³ Baltimore, Maryland; Heilbrun Center for Population and Family Health, Joseph L. Mailman School of Public Health, Columbia University, New York, New York⁴; Institute of Public Health, Makerere University, Kampala,⁵ and Rakai Project, Uganda Virus Research Institute, Entebbe,⁶ Uganda; Department of Laboratory Medicine, University of Washington, Seattle, Washington⁷; and Focus Technologies, Cypress, California⁸

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Two hundred forty-eight human immunodeficiency virus (HIV)-positive and 496 HIV-negative subjects in Uganda were tested by HerpeSelect herpes simplex virus type 2 enzyme-linked immunosorbent assay (ELISA) and Western blotting to optimize the ELISA for use in this population. A higher index cutoff value was required for optimal sensitivity and specificity, and overall performance of the assay was not affected by HIV status.

Infection with herpes simplex virus type 2 (HSV-2) is one of the most common sexually transmitted diseases of humans (3, 13). Infection with HSV-2 can cause genital ulcerations, and infected individuals have a lifelong risk of transmitting the viral infection to their sexual partners (2, 8). Pregnant women infected with HSV-2 can infect children during delivery (6, 12), and there is evidence of an increased risk of human immunodeficiency virus (HIV) acquisition with herpes-associated genital ulceration (4). However, studies of HSV-2 in sub-Saharan Africa, where the prevalence is high (30 to 50%), have been complicated by a variable rate of samples with a positive HSV-2 enzyme-linked immunosorbent assay (ELISA) but negative Western blot (WB) results (5). Additionally, the performance of HSV-2 ELISA in concurrently HIV type 1 (HIV-1)-infected individuals is unclear (10). In this report, the performance of the HerpeSelect HSV-2 ELISA (9) was evaluated with samples from the Rakai District in Uganda and the effect of HIV-1 infection was determined.

This study utilized stored sera from 744 subjects (248 HIV positive and 496 HIV negative) collected during a population-based randomized controlled trial of presumptive sexually transmitted disease treatment among adults aged 15 to 19 years in Rakai District, Uganda, from 1994 to 1998 (4). The HerpeSelect HSV-2 ELISA was performed according to the manufacturer's protocol (Focus Technologies, Cypress, Calif.) (9). WB analysis was performed as previously described (1) in a study that demonstrated its ability to detect 100% of sera obtained from subjects with culture-proven genital herpes infections. The sensitivity, specificity, positive predictive values

(PPV), and negative predictive values (NPV) were assessed, and a receiver operating curve (ROC) was determined (14) by using WB as the "gold standard." Samples with atypical WB results were considered negative for HSV-2 for the statistical analysis. By using index numbers as a continuous variable, the means for HIV-negative and HIV-positive groups were calculated, and a chi-square test was used to determine their differences.

The ELISA had a sensitivity of 99% and specificity of 52% compared to WB with the manufacturer's index cutoff value of 1.1 (Table 1). HSV-2 seroprevalence for this population was 62% as determined by WB and 75% as determined by ELISA at the index cutoff value of 1.1. A higher frequency (18.1%) of low-positive ELISA samples (index values between 1.1 and 3.0) was found in this population than was previously found (7%) in the United States (10). There was a higher proportion of subjects positive for HSV-2 by WB among HIV-positive subjects (71%) than among HIV-negative subjects (59%, $P < 0.001$). However, the performance of the assay was not affected by HIV serostatus. For subjects found to be positive for HSV-2 by WB, the median index value for HIV-positive subjects (6.03; interquartile range [IQR], 4.44 to 7.75; $n = 177$) did not differ significantly from that for HIV-negative subjects (6.17; IQR, 4.43 to 7.42; $n = 282$; $P = 0.89$). In addition, for subjects that were negative for HSV-2 by WB, the median index value for HIV-positive subjects (1.47; IQR, 0.65 to 2.43; $n = 71$) was not significantly different from that of the HIV-negative subjects (0.86; IQR, 0.49 to 2.12; $n = 214$; $P = 0.84$). Because HSV serology is used as a marker of herpetic infection in HIV-1 transmission studies (11), the lack of impact of HIV-1 serostatus on HSV-2 ELISA performance is significant.

The present study represents the largest investigation of the performance of an HSV-2 ELISA on sera from Uganda, a

* Corresponding author. Mailing address: Ross Building 1159, 720 Rutland Ave., Baltimore, MD 21205. Phone: (410) 955-3151. Fax: (410) 614-9775. E-mail: tqquinn@jhmi.edu.

TABLE 1. Performance of the HSV-2 ELISA with Western blot analysis

Index value	ELISA/WB ^a				Sensitivity (%)	Specificity (%)
	+/+	-/+	+/-	-/-		
1.1	459	5	136	149	98.9	52.3
1.5	456	8	111	174	98.2	61.1
2.0	444	20	86	199	95.7	69.8
2.2	441	23	73	212	95.0	74.4
2.4	429	35	64	221	92.5	77.5
2.6	421	43	60	225	90.7	79.0
2.8	414	50	58	227	89.2	79.7
3.0	406	58	53	232	87.5	81.4
3.2	404	60	48	237	87.1	83.2
3.4	394	70	45	240	84.9	84.2
3.6	380	84	41	244	81.9	85.6
3.8	370	94	40	245	79.7	86.0
4.0	366	98	37	248	78.9	87.0
5.0	317	147	28	257	68.3	90.2

^a 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.

region with previously described problematic performance of this assay (5). To optimize the ELISA for sera from Uganda, a change in the index cutoff value was necessary to more effectively differentiate positive and negative samples. Interpretation of the ROC curve demonstrated that the best index cutoff value to optimize the assay performance in this population was 3.4, with a sensitivity of 84.9% and a specificity of 84.6% (Fig. 1).

Depending on the setting where the HSV-2 ELISA is used, a different index cutoff value may need to be selected. Where WB testing is available to confirm a positive ELISA result, an

index cutoff value of 1.1 should be used to maximize sensitivity. The probability that a positive ELISA result is a false-positive result was 20% compared to that of WB as the gold standard. In the absence of a confirmatory WB test, a HerpeSelect HSV-2 ELISA index cutoff value of 2.2 is more appropriate, yielding a sensitivity of 95.0% and a specificity of 74.4%, and the probability of a false-positive result is 11%. At an index cutoff value of 2.2, with a population prevalence of 62%, the PPV would be 86% and the NPV would be 90%. The PPV and NPV have the least variation for an index cutoff value of 2.2 when population prevalence varies from 50 to 75%. For epidemiologic research in which the test is not being used for diagnostic purposes and thus the sensitivity need not be optimized at the cost of specificity, an index cutoff value of 3.4 is recommended. This index cutoff value provides a maximum sensitivity of 84.9% and a specificity of 84.2%. The probability of a false-positive result is 8%.

The possibility exists that there are antibodies in African sera that react to the gG2 protein on which the HSV-2 ELISA is based, either for genetic reasons or from reaction to other endemic infections in the region. Previously, a European study (7) demonstrated that the gG2 epitope was highly conserved. However, the Ugandan population used in the present investigation may be infected with a more heterogeneous population of viruses or have a different affinity to HSV-2 as measured by the present assay. Currently, no large-scale study has determined the diversity of these epitopes in an African population, though regional differences in assay performance have been shown (5). The potential that HSV-2 strain variation caused the discrepancy in ELISA performance in this population needs to be assessed in different developing countries.

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FIG. 1. ROC curve for 744 samples tested by ELISA and confirmed by WB. Index cutoff values of 1, 1.1, 1.5, and 2 to 4 by increments of 0.1 and 5 to 15 by whole number were plotted.

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