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Herpes Simplex Virus Type 2 Antibody Detection Performance in Kisumu, Kenya, using the HerpeSelect ELISA, Kalon ELISA, Western Blot and Inhibition Testing

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Abstract

Background—In certain parts of Africa, type-specific HSV type-2 ELISAs may have limited specificity. To date, no study has been conducted to validate HerpeSelect and Kalon type-specific HSV-2 ELISAs using both the Western blot (WB) and Recombinant gG ELISA inhibition testing as reference standards.

Methods—A total of 120 HIV-seronegative men (aged 18-24 years) provided blood samples. HSV-2 IgG serum antibodies were detected using four different methods: i. HerpeSelect HSV-2 ELISA (n=120), ii. Kalon HSV-2 ELISA (n=120), iii. University of Washington WB (n=101), and iv. a recombinant inhibition test (n=93).

Results—HSV-2 seroprevalence differed significantly by HSV-2 detection method, ranging from 24.8% with the WB to 69.8% with the HerpeSelect ELISA. Using the WB as the reference standard, the HerpesSelect had the highest sensitivity for HSV-2 antibody detection (100%), yet lowest specificity (40%). Similar results were obtained using the inhibition test as the reference standard. The sensitivity and specificity of the Kalon test were 92% and 79%, respectively, versus

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Competing interests: WH is a full time employee of Focus Diagnostics. RAM has received research contracts, during the last 5 years from Bio-Rad Laboratories, Focus Diagnostics, and GlaxoSmithKline and speaker honoraria or consulting fees from Biokit USA and Roche Diagnostics. JSS has received consulting fees from Focus Diagnostics within the past four years. No other authors have potential competing interests to report.

the WB; and 80% and 82% versus the inhibition test. Using the inhibition test as the reference standard, the sensitivity of the WB appeared low (49%).

Conclusions—In HIV-seronegative men in western Kenya, the HerpeSelect and Kalon type-specific ELISAs had high sensitivities yet limited specificities using the WB as reference standard. Overall, the Kalon ELISA performed better than the HerpeSelect ELISA in these young men from Kisumu. Further understanding is needed for the interpretation of HSV-2 inhibition or ELISA test positive/WB seronegative results. Before HSV-2 seropositivity may be reliably reported in selected areas of Africa, performance studies of HSV-2 serological assays in individual geographical areas are recommended.

Summary—Using Western-blot as the reference standard, sensitivity and specificity were 100% and 40%, respectively for HSV-2 HerpeSelect, and 92% and 79% for HSV-2 Kalon ELISA among men from Kisumu, Kenya.

Keywords

Herpes Simplex Virus Type-2; Performance; Serology; Africa

INTRODUCTION

Infection with herpes simplex virus type-2 (HSV-2) is a key risk factor for human immunodeficiency virus-1 infection (hereafter, HIV) in sub-Saharan Africa (1). Serologic testing for HSV-2 may be clinically useful in this setting and elsewhere as an indicator of an individual's risk of HIV infection, for accurate calculation of per-contact risk of transmission of HIV, for differential diagnosis of genital ulcers, and potentially for interventions which focus on HSV-2 status to prevent acquisition or transmission of HIV(1). One of the most commonly used type-specific HSV-2 ELISAs in sub-Saharan Africa, the HerpeSelect ELISA test [(Focus Diagnostics], has been shown to have poor specificity(2), (3) when compared with the monoclonal antibody (MAb) ELISA and University of Washington Western blot (WB) gold standard in specific African populations.

A study in five African countries found that the HSV-2 HerpeSelect yielded similar results to the WB in samples from South Africa and Zimbabwe(4). However, samples from Kenya and Uganda (4) were found to have higher HSV-2 positivity detected than the WB, suggesting the possibility of false positive HerpeSelect HSV-2 results. Another possible explanation is lower sensitivity of the WB assay. As there is potential for under-detection of HSV-2 antibodies among recent seroconverters using the WB assay(5), additional performance research has incorporated HSV-2 Recombinant gG ELISA inhibition testing as an alternative reference gold-standard to the WB (4). This recombinant inhibition test measures antibody binding to multiple epitopes of HSV-2 glycoprotein G (gG2), and uses the differential absorption of type-specific antibodies to identify potential false-positive results(4).

To evaluate the performance of HSV-2 serological testing among young, HIV-seronegative men in Kisumu, Kenya, we conducted a study in the framework of a randomized controlled trial (RCT) of male circumcision for the prevention of HIV infection(6). We present here the performance of the type-specific HSV-2 HerpeSelect ELISA and Kalon HSV-2 (Kalon Biological Ltd) ELISAs, compared to two confirmatory assays of the Western blot (WB) and Recombinant gG ELISA inhibition testing.

METHODS

Study population and enrollment

Uncircumcised men aged 18 to 24 years of age in Kisumu, Kenya were invited to participate in the RCT of male circumcision. The primary aim of this RCT was to determine the effectiveness of male circumcision in reducing HIV incidence(6). Study participants were recruited from sexually transmitted infection (STI) clinics, workplaces, and community organizations. This study includes men who were initially screened to participate in the RCT who consented to serological testing. Study inclusion criteria included being uncircumcised, HIV seronegative, sexually active (defined as reporting sex within the last 12 months), and having hemoglobin 9.0 g/100 mL, as previously described (6). Data analysis inclusion required that samples be available for shipment to the University of Manitoba by early February 2002. A total of 120 men completed the initial screening.

Type-specific HSV-2 HerpeSelect and Kalon ELISA serological testing was conducted at the University Of Manitoba Department Of Medical Microbiology Laboratory in Winnipeg, Canada, per manufacturer's instructions (7;8). According to manufacturer's instructions for both assays, index values of <0.9 were classified as negative, those >1.1 as positive, and otherwise as equivocal. Due to budget constraints, a total of 101 samples were randomly chosen from the initial 120 collected for WB analyses conducted at the University of Washington. Of the 19 samples without WB results, a total of 13 (68%) were HerpeSelect seropositive, and 7 (37%) were Kalon seropositive. HSV inhibition testing, as previously described (4), was conducted on a random sample of 100 samples at Focus Diagnostics in Cypress, California. Briefly, for the latter, sera were absorbed with HSV-1 and HSV-2 antigen, and samples exhibiting 60% or greater inhibition with the HSV-2 antigen were considered positive for HSV-2 antibody.

All study protocols were reviewed and approved by the Institutional Review Boards of the University of Illinois at Chicago, University of Manitoba, University of Nairobi and the University of North Carolina, and by RTI International.

The sensitivity and specificity were calculated for the i, Kalon ELISA, ii. HerpeSelect, and iii. HerpeSelect followed by confirmation with the Kalon ELISA of HerpeSelect positive samples. Summary receiver operating characteristic (ROC) curves were also estimated (9). The ROC curves were plotted as sensitivity on the ordinate and 1-specificity (false positivity rate) on the abscissa. Explanatory power of predictive models was quantified using the C-statistic, which is equivalent to the area under the ROC curve in this context and is a commonly used measure of model predictive power (10).

RESULTS

HSV-2 seroprevalence differed substantially by HSV-2 detection method among HIV-seronegative men (aged 18-24 years), HerpeSelect and Kalon testing yielded 69.8% (83/119) and 39.0% (46/118) seropositivity, after excluding 1 and 2 equivocal samples, respectively. Of 101 WB testing results HSV-2 WB seropositivity was 24.8% (25/101), of which two samples were classified as having atypical profiles to HSV-2 proteins. Of these 101 sera, 70 were positive by HerpeSelect (30 negative, 1 equivocal), and 39 were positive by Kalon (61 were negative, 1 equivocal) (using package insert standards for positivity). Of the 19 samples without WB results, HSV-2 results were similar to those with WB results: a total of 13 (68%) were HerpeSelect seropositive, and 7 (37%) were Kalon seropositive. Inhibition testing among 100 samples yielded 45.2% seroprevalence (42/93) after excluding 7 samples with equivocal results.

As shown in Table 1, the HerpeSelect ELISA test had 100% sensitivity (25/25) but only 40% specificity (30/76) using WB as the reference standard. Kalon yielded a slightly lower sensitivity (92%, 23/25) but a substantially higher specificity (79%, 60/76) versus the WB, with the same manufacturer-specified cutoffs. In this setting, with a prevalence of approximately 25% using WB as the gold standard, the HerpeSelect ELISA had a positive predictive value (PPV) of 0.36 and negative predictive value (NPV) of1.0, and the Kalon ELISA had a PPV of 0.59 and NPV of 0.97.

When WB results were negative, HerpeSelect and Kalon yielded concordant results in 46 of 74 cases (Table 2). In the remaining 28 cases, however, the Kalon test was negative while the HerpeSelect test was positive. Among these WB-/HerpeSelect+/Kalon-discordant samples, the median index value was 1.95 (range 1.15-7.43) for HerpeSelect [(mean=2.41 (SD=1.47)), and 0.44 (range 0.19-0.89) for Kalon [(mean 0.47 (SD 0.23)). Of the 46 concordant results, 30 were dually HerpeSelect and Kalon seronegative and 16 were dually HerpeSelect and Kalon seropositive. Among these WB-/HerpeSelect+/Kalon+ samples, the median index value was 5.70 (range 2.22-7.43) for HerpeSelect [(mean=5.17 (SD=1.98)), and 2.37 (range 1.17-4.26) for Kalon [(mean= 2.38 (SD=0.93)). Among the 25 WB positive sera, HerpeSelect and Kalon ELISAs were highly concordant. The only two sera with WB+/ HerpeSelect+/Kalon- results had index values of 1.24 and 4.83 for HerpeSelect, and 0.12 and 0.29 for Kalon.

Sensitivity and specificity were calculated for a variety of additional, dichotomous cutoffs for both ELISA tests (Table 1). Using the WB as the reference gold-standard, the sensitivity of the HerpeSelect ELISA was generally higher than that of Kalon up to a cut-off of 2.0, although relative differences in sensitivity decreased with higher chosen diagnostic cut-offs for both tests. The Kalon test had consistently higher specificity than the HerpeSelect ELISA, with the exception of a chosen cut-off of 3.4, where the HerpeSelect ELISA performed at a specificity of 80%, but with a lower sensitivity of 80%. Specificity for HSV-2 detection was generally under 80% for both serological assays over a wide range of investigated cut-offs. The Kalon optimized tradeoffs at a cutoff value of 1.2 (sensitivity 92%, specificity 80%), while the HerpeSelect ELISA optimized tradeoffs between sensitivity and specificity at a dichotomous cutoff of 3.5 (sensitivity 80%, specificity 80%). The combination of HerpeSelect ELISA and Kalon did not perform markedly better than use of either test separately (Table 1).

Using the inhibition test as the reference standard and considering the manufacturer's suggested cutoffs, the HerpeSelect ELISA test had 100% sensitivity, but low specificity (54%), while the Kalon test had sensitivity 80% and specificity 82%. The HerpeSelect optimized sensitivity/specificity tradeoffs at the cutoff of 2.5 (sensitivity 74%, specificity 73%); Kalon optimized that tradeoff at a cutoff of 1.0 (81% and 82%). Again, the combination of HerpeSelect ELISA and Kalon did not improve sensitivity and specificity.

Among sera with both WB and inhibition test results (n=76), the concordance between the two tests was 74% (56/76.). Against the inhibition test, the WB had relatively lower sensitivity than inhibition testing (48.6%), with high specificity (95.1%). In contrast, the sensitivity and specificity of the inhibition test against WB were 89.5% and 68%, respectively (data not shown).

Receiver operator characteristic (ROC) curve analysis using WB as the gold standard gave a c-statistic of 0.833 for HerpeSelect, 0.869 for Kalon, and 0.880 for the two tests used together. ROC curves for all three of these results using WB as gold standard are shown in Figure 1. Using Inhibition as the gold standard yielded c-statistics of 0.806 for HerpeSelect ELISA, 0.866 for Kalon, and 0.861 for both tests together.

DISCUSSION

To our knowledge, this is the first study to compare the performance of both the HerpeSelect and Kalon HSV-2 ELISA in African sera with two confirmatory assays of the Western blot and inhibition testing. Among young men in Kisumu, HSV-2 seroprevalence varied notably by the diagnostic method choice, ranging from 25% with the Western blot to 67% with the HerpeSelect. Using the WB as the reference gold standard, the HerpeSelect and Kalon type-specific ELISAs (employed per manufacturer's instructions) had high sensitivities of over 90% (100% for HerpeSelect, 92% for Kalon). However, the specificity of the Kalon ELISA (79%) was superior to that of the Herpes-Select ELISA(40%). When inhibition testing was employed as an alternative reference standard, HSV-2 performance results were largely comparable to those based on the WB reference gold-standard, with the exception of a slightly lower sensitivity for Kalon.

These data complement previous studies of HSV-2 ELISA performance comparisons, in particular by incorporating HSV-2 inhibition testing results. All assays consistently showed low specificity of the HerpeSelect in Kisumu male sera when using the manufacturer's recommended cut-off. Among 330 samples from 4 African countries, Van Dyck et al. used the Western blot and monoclonal antibody (MAb) ELISA to test samples found to be initially discordant between the HerpeSelect ELISA and a monoclonal antibody test to identify potential HSV-2 false positive results(12). A specificity of 57% was found for the HerpeSelect among Kenyan samples. Hogrefe et al. examined 781 samples from 5 African countries using the WB and HerpeSelect ELISA. Among WB/HerpeSelect discordant sera, the inhibition assay was used and the specificity of sera from Kenya was found to be 93% versus the WB and 97% versus the inhibition assay.(4) The differences in specificity results between our data and Hogrefe et al.(4) may be related to populations studied, as no other explanatory factor has been identified. It is worth noting that although the specificity of the HerpeSelect ELISA has been found to be relatively low among populations with a low HSV-2 seroprevalence (<5%) in the United States(13), or by geographical location (e.g. Vietnam(14)), the specificity seen here in Kisumu is notably lower and may be specific to certain geographical areas of Africa.

The present study indicated that the use of a higher HerpeSelect ELISA cut-off of 3.5, rather than 1.1, would maximize overall HerpeSelect ELISA performance, but would sacrifice sensitivity, which would be reduced to 80%. Our results are consistent with results from Rakai, Uganda that found a cut-off of 3.4 for the HerpeSelect ELISA resulted in sensitivity and specificity figures both of 85% when compared with Western blot.

Unlike a previous investigation in a population of Brazilian men at high risk of both HSV-2 and HIV (15), we did not find any advantage to retesting with the (more-specific) Kalon ELISA after a positive HerpeSelect. Whether the WB or the inhibition test was chosen as the reference standard in this Kenyan population, the combination of HerpeSelect followed by Kalon retesting (according to manufacturer's instructions) resulted in HSV-2 testing results very similar to those found using a testing strategy of the Kalon ELISA alone, with about 80% test specificity. When an algorithm was chosen with a high cut-off of 3.4 for HerpeSelect ELISA, followed by retesting of positive results with the Kalon ELISA at a cut-off of 1.3, the sensitivity and specificity combination were still not higher than Kalon ELISA testing alone,

Interpretation of HSV-2 serological assays is dependent upon the choice of the reference "gold-standard". PCR is considered the true gold-standard for HSV ascertainment, but does not ascertain past exposure to HSV-2 (16). For HSV serological performance studies, the well-validated WB is considered the reference standard of choice, but requires expertise for

appropriate interpretation(17). The concordance between WB and inhibition test results of 74% was notably lower than the 95% concordance rate found in Hogrefe et al.'s study in African sera(11). Further understanding, however, is needed for the interpretation of samples in our Kenyan study that were i. WB-negative, and both HerpeSelect and Kalon ELISA seropositive (n=16), and ii. WB-negative, and HerpeSelect positive and Kalon ELISA seronegative (n=28), in order to determine whether the gold standard WB was falsely negative. It is possible that a proportion of the "false positive" samples by either ELISA may have actually been relatively recent HSV-2 seroconverters not detected by the WB. The median time of HSV-2 seroconversion following documented primary HSV-2 infection has been shown to be the shortest for the HerpeSelect test (21 days) when compared with the WB (40 days) or the Kalon ELISA (120 days)(5).

In these sera from young men in Kisumu, Kenya, the sensitivity of the WB may have been sub-optimal. Another possible explanation for the lower specificity observed with HerpeSelect is that it may have reacted to a variant strain of HSV-2 that was not detected by either the WB or Kalon assays. Of note, HSV-2 sequences in patients with HSV-2 from sub-Saharan Africa have not yet been well-studied and are not characterized in the GenBank database (18). Potential differences in HSV-2 strain or variants resulting in abnormal WB staining patterns could result in false-negative WB results. Alternatively, the HerpeSelect ELISA may be cross-reactive with an unidentified non-HSV-2 virus to a greater extent than the Kalon ELISA, although cross-reactive antibody may not necessarily have been due to another infectious agent. Both HerpeSelect and Kalon ELISAs are based on gG-2 antigens using a similar expression system, although sites of truncation of recombinant antigens used in the two ELISAs are different.

Results presented here have several key advantages: the study included men at high-risk of HSV-2 seroconversion, and laboratory testing was performed using four different type-specific serological assays. A limitation of this study is that it is based on a small sample size of young men from Kisumu, Kenya. Given that PPV and NPV values depend strongly upon prevalence, these values are not generalizable outside of this study. Further, due to cost constraints, a sub-set of sera was not tested with the Western blot, although HSV-2 ELISA seroprevalence rates were similar among sera with and without WB testing results.

In this study of young men from western Kenya, the Kalon ELISA alone performed better than the HerpeSelect ELISA, and there was no indication that any chosen algorithm for combined type-specific ELISA testing resulted in preferential test results. Further improvements in type-specific serological assays are needed for African sub-populations where currently available tests have not shown optimal sensitivity and specificity.

Key messages

HSV-2 seroprevalence ranged from 24.8% with the Western blot, 39.0% with the Kalon ELISA, to 69.8% with the HerpeSelect ELISA.

Using the Western blot as the reference standard, the HerpeSelect had the highest sensitivity for HSV-2 antibody detection (100%), yet lowest specificity (40%). The sensitivity and specificity of the Kalon test were 92% and 79%, respectively.

HerpeSelect and Kalon ELISAs had high sensitivities yet limited specificities in these young men from Kenya.

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References

1. Freeman EE, Weiss HA, Glynn JR, et al. Herpes simplex virus 2 infection increases HIV acquisition in men and women: systematic review and meta-analysis of longitudinal studies. AIDS 2006;20:73–83. [PubMed: 16327322]
2. van Dyck E, Buve A, Weiss HA, et al. Performance of commercially available enzyme immunoassays for detection of antibodies against herpes simplex virus type 2 in African populations. J Clin Microbiol 2004;42:2961–2965. [PubMed: 15243045]
3. Laeyendecker O, Henson C, Gray RH, et al. Performance of a commercial, type-specific enzyme-linked immunosorbent assay for detection of herpes simplex virus type 2-specific antibodies in Ugandans. J Clin Microbiol 2004;42:1794–1796. [PubMed: 15071053]
4. Hogrefe W, Su X, Song J, et al. Detection of herpes simplex virus type 2-specific immunoglobulin G antibodies in African sera by using recombinant gG2, Western blotting, and gG2 inhibition. J Clin Microbiol 2002;40:3635–3640. [PubMed: 12354858]
5. Ashley-Morrow R, Krantz E, Wald A. Time course of seroconversion by HerpeSelect ELISA after acquisition of genital herpes simplex virus type 1 (HSV-1) or HSV-2. Sex Transm Dis 2003;30:310–314. [PubMed: 12671550]
6. Bailey R, Moses S, Parker C, et al. Male circumcision for HIV prevention in young men in Kisumu, Kenya: a randomised controlled trial. Lancet 2007;369:643–656. [PubMed: 17321310]
7. Ashley-Morrow R, Nollkamper J, Robinson NJ, et al. Performance of focus ELISA tests for herpes simplex virus type 1 (HSV-1) and HSV-2 antibodies among women in ten diverse geographical locations. Clin Microbiol Infect 2004;10:530–536. [PubMed: 15191381]
8. van Dyck E, Buve A, Weiss HA, et al. Performance of commercially available enzyme immunoassays for detection of antibodies against herpes simplex virus type 2 in African populations. J Clin Microbiol 2004;42:2961–2965. [PubMed: 15243045]
9. Lasko T, Bhagwat J, Zou K, et al. The use of receiver operating characteristic curves in biomedical informatics. J Biomed Inform 2008;38:404–415. [PubMed: 16198999]
10. Guofen Y, Greene T. Investigating the effects of ties on measures of concordance Statistics in Medicine 2008. Statistics in Medicine. 2008 published online ahead of print. [PubMed: 18384187]
11. Hogrefe W, Su X, Song J, et al. Detection of herpes simplex virus type 2-specific immunoglobulin G antibodies in African sera by using recombinant gG2, Western blotting, and gG2 inhibition. J Clin Microbiol 2002;40:3635–3640. [PubMed: 12354858]
12. van Dycke E, Buve A, Weiss HA, et al. Performance of commercially available enzyme immunoassays for detection of antibodies against herpes simplex virus type 2 in African populations. J Clin Microbiol 2004;42:2961–2965. [PubMed: 15243045]
13. Mark H, Nanda JP, Robert J, et al. Performance of focus ELISA tests for HSV-1 and HSV-2 antibodies among university students with no history of genital herpes. Sex Transm Dis 2007;34:681–685. [PubMed: 17457239]
14. Ngo TD, Laeyendecker O, La H, et al. Use of Commercial Enzyme Immunoassays To Detect Antibodies to the Herpes Simplex Virus Type 2 Glycoprotein G in a Low-Risk Population in Hanoi, Vietnam. Clinical and Vaccine Immunology 2008;15:382–384. [PubMed: 18077617]
15. Nascimento MC, Ferreira S, Sabino E, et al. Performance of the HerpeSelect (Focus) and Kalon enzyme-linked immunosorbent 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. J Clin Microbiol 2007;45:2309–2311. [PubMed: 17507516]

16. Lorincz, A.; Smith, JS. Sexually Transmissible Viral Pathogens: The Human Papillomaviruses and Herpes Viruses. In: Lorincz, A., editor. Nucleic Acid Testing for Human Disease. 2006. p. 243-273.

17. Ashley RL. Type-specific antibodies to HSV-1 and -2: Review of methodology. Herpes 1998;5:33–38.

18. Benson JA, Karash-Mizrachi I, Lipman DJ, et al. GenBank. Nucleic Acids Res 2007;35:D21–D25. [PubMed: 17202161]

Table 1

Sensitivity, specificity, exact 95% confidence intervals, and C-statistics for HSV-2 HerpeSelect ELISA and Kalon HSV-2 ELISA tests alone and combined, compared to reference standard of Western Blot and Inhibition. Cutoffs are dichotomous (≥ cutoff, <cutoff) except for <0.9 vs. >1.1 categories (which exclude equivocal samples per manufacturer's instructions). Exclusions of equivocals lead to n=100 for WB vs. HerpeSelect and Kalon; n=92/91 for Inhibition vs. HerpeSelect/Kalon respectively.

Test assay	Cutoff	University of Washington Western Blot (n=101)			Inhibition (n=93) (excluding 7 equivocals)		
		Sensitivity % (95% CI)	Specificity % (95% CI)	Area under ROC curve	Sensitivity % (95% CI)	Specificity % (95% CI)	Area under ROC curve
HerpeSelect	<0.9 vs. >1.1	100 (86-100)	40 (29-52)	0.833	100 (92-100)	54 (39-68)	0.806
	1.0	100 (86-100)	39 (28-51)		100 (92-100)	53 (38-67)	
	1.1	100 (86-100)	41 (30-53)		100 (92-100)	55 (40-69)	
	1.5	96 (80-100)	54 (42-65)		98 (87-100)	61 (46-74)	
	2.0	88 (69-97)	61 (49-72)		86 (71-95)	67 (52-79)	
	2.5	84 (64-95)	71 (60-81)		74 (58-86)	73 (58-84)	
	3.0	80 (59-93)	75 (64-84)		67 (50-80)	75 (60-86)	
	3.4	80 (59-93)	80 (70-89)		64 (48-78)	82 (69-92)	
	3.5	80 (59-93)	80 (70-89)		64 (48-78)	82 (69-92)	
Kalon	<0.9 vs. >1.1	92 (74-99)	79 (68-87)	0.869	80 (64-91)	82 (69-92)	0.866
	0.1	100 (86-100)	16 (8-26)		100 (92-100)	25 (14-40)	
	0.2	96 (80-100)	37 (26-49)		100 (92-100)	45 (31-60)	
	0.3	92 (74-99)	51 (40-63)		98 (87-100)	63 (48-76)	
	0.5	92 (74-99)	59 (47-70)		95 (84-99)	69 (54-81)	
	1.0	92 (74-99)	78 (67-86)		81 (66-91)	82 (69-92)	
	1.1	92 (74-99)	79 (68-87)		76 (61-88)	82 (69-92)	
	1.2	92 (74-99)	80 (70-89)		74 (58-86)	82 (69-92)	
	1.3	80 (59-93)	82 (71-90)		69 (53-82)	82 (69-92)	
	1.5	80 (59-93)	82 (71-90)		67 (50-80)	82 (69-92)	
	2.0	64 (43-82)	88 (79-94)		52 (36-68)	88 (76-96)	
	2.5	48 (28-69)	91 (82-96)		40 (26-57)	90 (79-97)	
	3.0	32 (15-54)	95 (87-99)		29 (16-45)	94 (84-99)	

Test assay	Cutoff	University of Washington Western Blot (n=101)			Inhibition (n=93) (excluding 7 equivocals)		
		Sensitivity % (95% CI)	Specificity % (95% CI)	Area under ROC curve	Sensitivity % (95% CI)	Specificity % (95% CI)	Area under ROC curve
HerpeSelect (HS), then Kalon (K)	HS>1.1, K>1.1	92 (74-99)	79 (68-87)	0.880	76 (61-88)	82 (69-92)	0.861
	HS>1.1, K>1.2	92 (74-99)	80 (70-89)		74 (58-86)	82 (69-92)	
	HS>1.1, K>1.3	80 (59-93)	82 (71-90)		69 (53-82)	82 (69-92)	
	HS>1.5, K>1.2	92 (74-99)	80 (70-89)		74 (58-86)	82 (69-92)	
	HS>2.0, K>1.3	76 (55-91)	82 (71-90)		67 (50-80)	82 (69-92)	
	HS>2.5, K>1.3	76 (55-91)	84 (74-92)		64 (48-78)	84 (71-93)	
	HS>3.0, K>1.3	76 (55-91)	87 (77-94)		60 (43-74)	86 (74-94)	
	HS>3.4, K>1.3	76 (55-91)	87 (77-94)		60 (43-74)	86 (74-94)	
	HS>3.5, K>1.3	76 (55-91)	87 (77-94)		60 (43-74)	86 (74-94)	

Table 2

Herpes Simplex Virus Type-2 Seropositivity for Western blot, HerpeSelect, and Kalon ELISA assay among young men in Kisumu, Kenya

Western Blot status		Number positive by HerpeSelect and Kalon status		
		Focus HerpeSelect	Kalon	Positive
+	n=25	+	+	23
		+	−	2
		−	+	0
		−	−	0
−	n=74	+	+	16
		+	−	28
		−	+	0
		−	−	30

‡Two samples excluded for equivocal HerpeSelect or Kalon results; 99 total samples included.