Performance of commercial enzyme-linked immunosorbent assays (ELISA) for diagnosis of HSV-1 and HSV-2 infection in a clinical setting

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Abstract

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Background

FDA-approved ELISA assays for determining type-specific herpes simplex virus (HSV) serostatus are widely used in clinics. We compared the performance of such assays with the University of Washington western blot (UW WB) in patients who sought confirmation of their HSV serostatus.

Methods

We reviewed charts of all persons evaluated at the Westover Heights Clinic (WHC) in Portland, Oregon, from July 2010 through September 2015, who had a HSV ELISA, followed by UW WB.

Results

Of 864 persons, 47% were women. The median age was 36 years (range 18-73 years). By UW WB, 286 (33%) persons were HSV-1 seropositive only, 104 (12%) were HSV-2 seropositive only, 134 (16%) were both HSV-1 and HSV-2 seropositive, 235 (27%) were HSV seronegative, and 105 (12%) were

indeterminate. Using the UW WB as reference, the ELISA was 70.2% sensitive and 91.6% specific for HSV-1, and 91.9% sensitive and 57.4% specific for HSV-2. Among 284 persons who were HSV-1 seropositive by ELISA according to manufacturer's cutoff index value ≥1.1, 255 were confirmed by the UW WB (positive predictive value, PPV=92%). Of the 412 persons that were HSV-1 seronegative by the ELISA, 304 were seronegative by UW WB (negative predictive value, NPV= 70.0%). Among 456 persons with HSV-2 ELISA seropositivity, 193 tested HSV-2 positive by the UW WB (PPV=50.7%). Of the 283 persons HSV-2 seronegative by ELISA, 17 were found UW WB positive (NPV= 93.7%). Among 261 persons with an ELISA HSV-2 index value 1.1 - 2.9, 39.8% confirmed by UW WB, compared with 78.6% of the 70 persons with an ELISA index value $\geq$3 ($p<0.0001$)). The risk of false positive HSV-2 results was not found to differ between persons with or without HSV-1 antibody (50.5% vs 47.5%, $p=0.57$).

Conclusions

FDA approved ELISAs have poor PPV for HSV-2 and poor NPV for HSV-1 in clinical practice. More accurate commercially available type-specific HSV antibody diagnostic tests are needed.

INTRODUCTION

Herpes simplex virus (HSV) infections cause significant morbidity worldwide. An estimated 3709 million people aged 0-49 years were HSV-1 infected[1], and 417 million people aged 15-49 years were HSV-2 infected in 2012[2]. HSV-2 is sexually transmitted and a common cause of genital ulcers, but an increasing proportion of first-episode genital herpes are due to HSV-1 infection[3]. Most people with genital herpes are either asymptomatic or have unrecognized disease[4]. Serological HSV testing can be used to identify asymptomatic HSV infections, confirm diagnosis in persons with atypical presentations and also to identify patients at risk for HSV acquisition[5]. Several studies have shown that patients are becoming increasingly interested in knowing their HSV serological status and that serological testing does not pose long-term psychosocial harm[6, 7].

The FDA has approved several type-specific HSV serological testing which are now commercially available to determine HSV-1 and HSV-2 serostatus. HerpeSelect and Captia are the more commonly used assays. The requirements for FDA approval of commercial assays requires limited testing of the candidate assay against an approved reference. For example, in the package insert for the Focus HerpeSelect the performance of the HerpeSelect 2 ELISA IgG assay was tested against University of Washington HSV-1 and HSV-2 western Blot from Virology laboratory and showed that the Focus HerpeSelect assay had a sensitivity of 100% and specificity of 96.1% among expectant

mothers, and a sensitivity of 96.1% and specificity of 97.0% among sexually active adults[8]. Similarly, in the package insert for the Trinity Biotech Captia assay, the performance of Trinity Biotech HSV-1 IgG ELISA was compared with a second commercially available HSV-1 IgG ELISA assay and showed that the Captia assay had 100% sensitivity and 98.1% specificity for HSV-1[9]. However, several studies aimed at further defining the accuracy of these ELISA's showed that the Focus ELISA lacks specificity especially at lower index values[10, 11]. In addition, as these tests have been used in international studies, the performance appears worse in sera from people from sub-Saharan Africa[11-13]. The reasons for worse performance of ELISA in sub-Saharan Africa are not clear, although some data suggest that HSV-1 infection is a risk factor for low specificity of HSV-2 result[14], and HSV-1 infection is universal in sub-Saharan Africa.

METHODS

Study Population and Design

This was a retrospective study of adults who presented to the Westover Heights Clinic (WHC) in Portland, Oregon between July 2010 and September 2015 for HSV testing. The WHC is a private sexually transmitted diseases (STD) clinic that has participated in several HSV research studies and clinical trials, and also provides clinical care for STD related complaints with a specific focus on genital herpes infection. HSV antibody testing was performed either at WHC or

elsewhere such as STD clinic or a commercial laboratory, and reported by the patient. The confirmatory HSV WB antibody testing was performed at the University of Washington Virology Laboratory at the request of the patient, as part of patient care at the WHC.

Laboratory testing

The western blot assay, developed at the University of Washington in the 1980s, is the gold standard type specific serologic assay for HSV-1 and HSV-2[15]. The WB results were reported as positive, negative or indeterminate separately for HSV-1 and HSV-2. Different HSV ELISA tests were used but majority of the testing, when known, were done with HerpeSelect and Captia ELISAs. Blood samples were processed according to manufacturers' specifications described elsewhere[8, 9]. The results from the ELISA were reported according to prespecified manufacturer's guidelines: <0.9 is negative, 0.9 to 1.0 is equivocal, ≥1.1 to <3.5 is low positive, and ≥3.5 is positive[8, 9]. Some of the ELISA results were self-reported by the patients, and not all index values and dates for blood draws were available in patient charts.

Study Data

Data for this analysis were extracted from WHC electronic medical records (EMR). We specifically extracted the following variables: age and sex of participants, HSV-1 and HSV-2 ELISA results, HSV-1 and HSV-2 WB results, and test dates. Only the first result of WB testing was included in the analysis. The study was approved by University of Washington Institutional Review Board

(IRB).

Statistical Analysis

We used descriptive statistics to examine the demographics and baseline characteristics of the study participants. We calculated sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the HSV ELISA assays using the WB as the reference. We also constructed receiver operating characteristic (ROC) curves for HSV-1 and HSV-2 ELISA by plotting sensitivity (true positive rate) on the vertical axis, and 1-specificity (false positive rate) on the horizontal axis. From the ROC curve, we estimated the area under the curve (AUC) or c-statistic which is a measure of the ability of the ELISA to discriminate between patients with HSV vs. those without HSV. In general, a c-statistic of 0.90-1.00 corresponds to ELISAs excellent ability to discriminate between patients with disease and those without disease. A c-statistic of 0.80-0.90 is good, 0 .70 - 0.80 is fair, 0.60 -.70 is poor, and 0.50 - 0.60 is fail[16]. A c-statistic of 0.5 means the test is no better than chance in distinguishing between persons with disease from persons without disease.

For binary data, we computed proportions and 95% confidence intervals. We used the chi square test to perform significance testing on proportions and the Wilcoxon rank-sum (Mann-Whitney) test to compare median time between blood draws for the two assays. We used a generalized linear model to determine whether HSV serostatus as determined by ELISA and the interval between blood draws for the ELISA and WB tests had significant influence on the WB result. We

used the model to calculate the prevalence ratios for WB positivity including HSV ELISA and time between blood draws as predictor variables. Next, we adjusted for age and sex in the model. All statistical tests were evaluated at significance level of 5%. Stata 14.1 (College Station, Texas) and Excel 2011 version 14.2.3. were used for all analyses.

RESULTS

Characteristics of Study Participants

In total, data on 864 persons were analyzed. Three hundred and ninety-eight (47%) were women. The median age of the participants was 36 years (range 18 – 73 years). By UW WB, 286 (33%) persons were HSV-1 seropositive only, 104 (12%) were HSV-2 seropositive only, 134 (16%) were both HSV-1 and HSV-2 seropositive, 235 (27%) were HSV seronegative, and 105 (12%) were indeterminate for either HSV-1 or HSV-2 or both (Table 1). HSV-1 seroprevalence was 55% (95% CI 52 – 59%) and HSV-2 seroprevalence was 31% (95%CI 28 – 34%).

HSV-1 ELISA Performance

Seven hundred and twenty-seven persons had been tested for HSV-1 by both ELISA and WB. Using WB as reference, the HSV-1 ELISA was found to have a sensitivity of 70% (95% CI 65 – 75%) and specificity of 92% (95% CI 88 – 95%) (Table 2). Of the 284 persons that were HSV-1 positive by ELISA; 255

(90%) were confirmed by WB, 23 (8%) were negative and 6 (2%) were indeterminate by WB yielding a PPV of 92% (95% CI 88 – 95%) (Figure 1). Of the 412 persons that screened negative by HSV-1 ELISA, 252 (61%) confirmed negative by WB, 108 (26%) were determined to be positive and 52 (13%) were indeterminate by WB yielding an NPV of 70% (95% CI 65 – 75%) (Figure 1). The ELISA test had a PPV of 75% when the index value was between 1.1 and 2.0, 87% when the index value was between 2.0 and 3.0, and 99% at index values 3.0 or above ($p<0.001$) (Figure 2). ROC analysis for HSV-1 ELISA using WB as reference yielded a c-statistic of 0.87 (95% CI 0.84 – 0.90) (Figure 3).

HSV-2 ELISA Performance

Seven hundred and eighty persons had been screened for HSV-2 by both ELISA and WB. HSV-2 ELISA had a sensitivity of 92% (95% CI 87 – 95%) and specificity of 57% (95%CI 53 – 62%) when compared to the gold standard WB (Table 2). Four hundred and fifty-six persons were HSV-2 positive by ELISA; of which 193 (42%) were confirmed by WB, 188 (41%) were negative and 75 (17%) were indeterminate by WB yielding a PPV of 51% (95% CI 46 – 56%) (Figure 3). Of the 283 persons that screened negative by HSV-2 ELISA, 17 (6%) confirmed negative by WB, 253 (89%) were determined to be positive and 13 (5%) were indeterminate by WB yielding an NPV of 94% (95% CI 90 – 96%) (Figure 4). The HSV-2 ELISA test had a PPV of 30% when the index value was between 1.1 and 2.0, 58% when the index value was between 2.0 and 3.0, and 79% at index values 3.0 or above ($p<0.001$) (Figure 5). ROC analysis for HSV-1 ELISA using WB as reference yielded a c-statistic of 0.85 (95% CI 0.82 – 0.89) (Figure 6).

Assessment of time between HSV-1 ELISA and HSV-1 WB

To determine whether ongoing seroconversion or new HSV acquisition could explain the differences between the ELISA and WB results, we assessed the influence of the interval between the two tests on WB result. Of the 727 persons that had both tests, 638 (88%) had either a positive or a negative result on both tests. Of these, 131 (21%) had discordant ELISA and WB results; 507 (79%) had concordant results; and 184 (29%) had record on the duration between blood draws for both tests. Thirty-five persons with discordant results had known duration between tests; 18 (51%) of these had blood draws for the two tests occur within 4 weeks of each other. Of 507 concordant results, 149 had duration between tests recorded. 74/149 (50%) had both tests within 4 weeks of each other; the median time between tests was 5 weeks. The median interval between ELISA and WB testing was similar for the discordant vs concordant pairs, 4 weeks vs. 5 weeks, ($p=0.67$).

We tested whether HSV-1 ELISA serostatus, duration between tests, and the interaction between these two predictor variables predicted HSV-1 serostatus as determined by WB using a generalized linear model. We also tested whether the association between HSV-1 ELISA and duration between tests modified the effect HSV-1 ELISA or time on the HSV-1 WB outcome. We found that the interaction between HSV-1 ELISA and duration between tests did not significantly impact our model. Using the reduced model, the prevalence of HSV-1 WB positivity among persons with positive HSV-1 ELISA was 3.32 times higher than prevalence among persons with negative HSV-1 ELISA ($P<0.001$). Duration

between ELISA and WB tests was not a predictor of HSV-1 WB positivity (p=0.991). Next, we included age and sex as predictor variables; and they did not significantly impact the model.

Assessment of time between HSV-2 ELISA and HSV-2 WB

Six hundred and fifty-one of the 780 persons with testing had valid results for both ELISA and WB tests. Of these, 205 (31%) had discordant ELISA and WB results; 446 (69%) had concordant results; and 206 (32%) had a record of the duration between the blood draws for these tests. Forty-three persons with discordant results had known duration between tests; 30 (70%) of these had blood draw for tests occur within 4 weeks of each other, and the median time between tests was 2 weeks. 163/446 (37%) concordant results had duration between tests recorded. Of these, 75 (46%) had both tests within 4 weeks of each other; the median time between tests was 7 weeks. Concordant ELISA and WB results seemed to be more distant chronologically than discordant ones (p=0.047).

We used a generalized linear model to test whether HSV-2 ELISA serostatus, duration between tests, and the interaction between ELISA and time between the two tests predicted HSV-2 serostatus as determined by WB. We found that the interaction between HSV-2 ELISA and duration between tests did not modify the effect of HSV-2 ELISA or time on HSV-2 WB outcome. Using a reduced generalized linear model with HSV-2 ELISA serostatus and duration between tests as predictor variables, the prevalence of HSV-2 WB positivity

among persons with positive HSV-2 ELISA was 60.97 times higher than prevalence among persons with negative HSV-2 ELISA ($p<0.001$). Duration between ELISA and WB tests was not a predictor of HSV-2 WB positivity ($p=0.342$). Age and sex of subjects also did not significantly impact model.

Assessment of HSV-1 serostatus on the performance of HSV-2 ELISA test

Using a chi-square test, we examined whether prior HSV-1 exposure increases the false positive rate of HSV-2 ELISA tests. We defined false positive as positive HSV-2 ELISA and negative HSV-2 WB. True positive was defined as both HSV-2 ELISA and HSV-2 WB positive. We compared proportion of false positives among HSV-1 WB positive persons to proportion among HSV-1 WB negative persons. The risk of false positive HSV-2 ELISA results was similar among persons with and without HSV-1 antibody (50.5% vs 47.5%, risk ratio=1.06, $p=0.57$).

DISCUSSION

Our study compared the performance of HSV-1 and HSV-2 ELISA to the gold standard, HSV WB among adults presenting to an urban STD clinic for confirmation of their HSV serostatus. Even though routine screening for HSV in the general population is not recommended, an increasing number of people are becoming interested in knowing their HSV status, and the CDC has proposed certain clinical circumstances in which serologic testing for HSV is indicated [6, 17]. An ideal test for diagnosing HSV infection should have an optimal

combination of sensitivity and specificity to correctly identify the majority of infected people. Because the commercial availability of the Western blot is limited, ELISAs are commonly used in clinical setting to detect HSV-1 and HSV-2 antibodies. Among our clinic patients who specifically presented to clinic for STD evaluation, HSV-1 seroprevalence by WB was 57% similar to the estimated seroprevalence of 53.9% in the US general population, and HSV-2 seroprevalence was 32% about twice the seroprevalence in the general US population[18]. HSV-1 ELISA had low sensitivity but high specificity, whereas HSV-2 ELISA had high sensitivity but low specificity; a general trend that has been observed in other studies[10, 12, 19].

Several concerns have been raised regarding the ability of HSV ELISAs, especially HSV-2 ELISA, to accurately identify persons with HSV infection, especially in low prevalence settings[10]. For example, in an urban US STD clinic setting where the prevalence of HSV-2 by western blot was about 13% among men, the PPV of the HSV-2 ELISA test was found to be only 84%[19]. In another US study where HSV-1 seroprevalence was 48% and HSV-2 seroprevalence was 3.4% by WB, the PPV of HSV-1 ELISA was 96.7% but that of HSV-2 ELISA was 37.5%[10]. In a different study from a wide geographical distribution, the PPV of HSV-2 ELISA ranged between 57%-95%[11]. These studies, and ours, highlight the point that over a wide range of HSV prevalences, HSV-2 ELISA have varied and of poor positive predictive values, reflecting imperfect specificity. In contrast, the HSV-1 ELISA has high positive predictive values.

The low PPV of HSV-2 ELISA means a smaller proportion of patients who

test positive on the ELISA actually have HSV-2. The false positive rate was higher in the low positive ELISA index value range, which could suggest recent infection or that ELISA is more sensitive at detecting early seroconversion than the WB[11, 20]. However it could represent a true high false positive rate, which raises concern for incorrect diagnosis of HSV-2 in uninfected patients which may negatively impact patient care.

An approach to improving PPV may be to increase increase the cut-off index value for ELISA positivity to improve on the PPV[11, 19]. In the current study, the PPV increased from 30% at index values between 1.1 and 2.0 to 79% at values 3.0 or above. However, raising the cut-off ELISA index value would decrease sensitivity of the assay, and lead to potential undiagnosed infected persons. For example, when the cut-off for positive HSV-2 ELISA was increased to 3.5, sensitivity decreased from 94% to 19%; and specificity increased from 59% to 99%.

HSV-1 results were concordant in 80% of samples tested by ELISA and WB. HSV-2 results were concordant in 70% of samples using similar testing. A study by Ashley-Morrow et al showed that the median interval from symptom onset to seroconversion was shorter for ELISA compared to WB[20]. Other studies have also shown that results of gG-based HSV tests could become negative over time[21]. In our study, discordant HSV-1 ELISA and WB results were not more distant chronologically than concordant ones, suggesting that new HSV acquisition or ongoing seroconversion did influence the observed differences in results. However, concordant HSV-2 ELISA and WB results was

more distant chronologically compared to discordant results, contrary to what we would expect if the disagreement was due to ongoing seroconversion or new HSV-2 acquisition. Time interval between the two tests did not predict HSV-1 or HSV-2 WB positivity.

Some studies have suggested that prior infection with HSV-1 results in cross-reactivity with gG-based HSV-2 tests, and therefore lowers the tests performance especially for sera from Africa populations[13, 20]. In our study, the risk of false positive HSV-2 results among persons with HSV-1 antibody was similar to that among those without HSV-1 antibody, suggesting that in our setting prior HSV-1 infection did not interfere with performance of HSV-2 ELISA.

Our study had several limitations. First, our study participants at the WHC were self-selected patients, many of whom were interested in confirming their HSV serostatus; and they may not be representative of the general population. This self-selectivity could have contributed to potential high false positive HSV rates. Secondly, some of the self-reported ELISA results may not have been correct and influenced the performance of our prediction model. Thirdly, only 20-27% of discordant results had valid duration between tests recorded. With a lot of missing data on duration between tests, the prediction of time on WB posivity is not generalizable. Some of our patients may be seeking WB confirmation of their HSV serostatus specifically because they had a low positive titer on the ELISA. Lastly our patients did not have a history of symptoms so may skew our study population toward asymptomatic which may be more likely to have unclear results.

In summary, our data and others reported elsewhere suggest that commercial ELISAs have poor positive predictive value for HSV-2 and poor negative predictive value for HSV-1 in clinical practice. Concordant HSV-2 ELISA and WB results were more chronologically distant compared to discordant results, contrary to what we would expect if the disagreement was due to ongoing seroconversion or new HSV-2 acquisition. Time interval between the two tests did not predict HSV-1 or HSV-2 WB positivity. Our data also suggest that prior HSV-1 infection does not interfere with the performance of HSV-2 ELISA. More accurate tests are needed for HSV antibody diagnostics.

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TABLES AND FIGURES

Variable	N (%)
Sex	
Men	451 (53)
Women	398 (47)
Age in years, median (range)	36 (18-73)
HSV serostatus by UW WB	864
HSV seronegative	235 (27)
HSV-1 seropositive only	286 (33)
HSV-2 seropositive only	104 (12)
HSV-1 and HSV-2 seropositive	134 (16)
Indeterminate	105 (12)

Table 1. Characteristics of study participants

	Western blot			
	HSV-1		HSV-2	
ELISA	**Positive**	**Negative**	**Positive**	**Negative**
Positive	255	23	193	188
Negative	108	252	17	253
Total	363	275	210	441

Table 2. Comparison of HSV serology results by ELISA and WB

HSV-1 ELISA Sensitivity =255/363 = 70.2% (95% CI 65.3% - 74.9%)

HSV-1 ELISA Specificity = 252/275 = 91.6% (95% CI 87.7% - 94.6%)

HSV-1 ELISA PPV = 255/278 = 91.7% (95% CI 87.8% - 94.7%)

HSV-1 ELISA NPV = 252/360 = 70.0% (95% CI 65.0% - 74.7%)

HSV-2 ELISA Sensitivity = 193/210 = 91.9% (95% CI 87.4% - 95.2%)

HSV-2 ELISA Specificity = 253/441 = 57.4% (95% CI 52.6% - 62.0%)

HSV-2 ELISA PPV = 193/381 = 50.7% (95% CI 45.7% - 55.9%)

HSV-2 ELISA NPV = 253/270 = 93.7% (95% CI 90.1% - 96.3%)

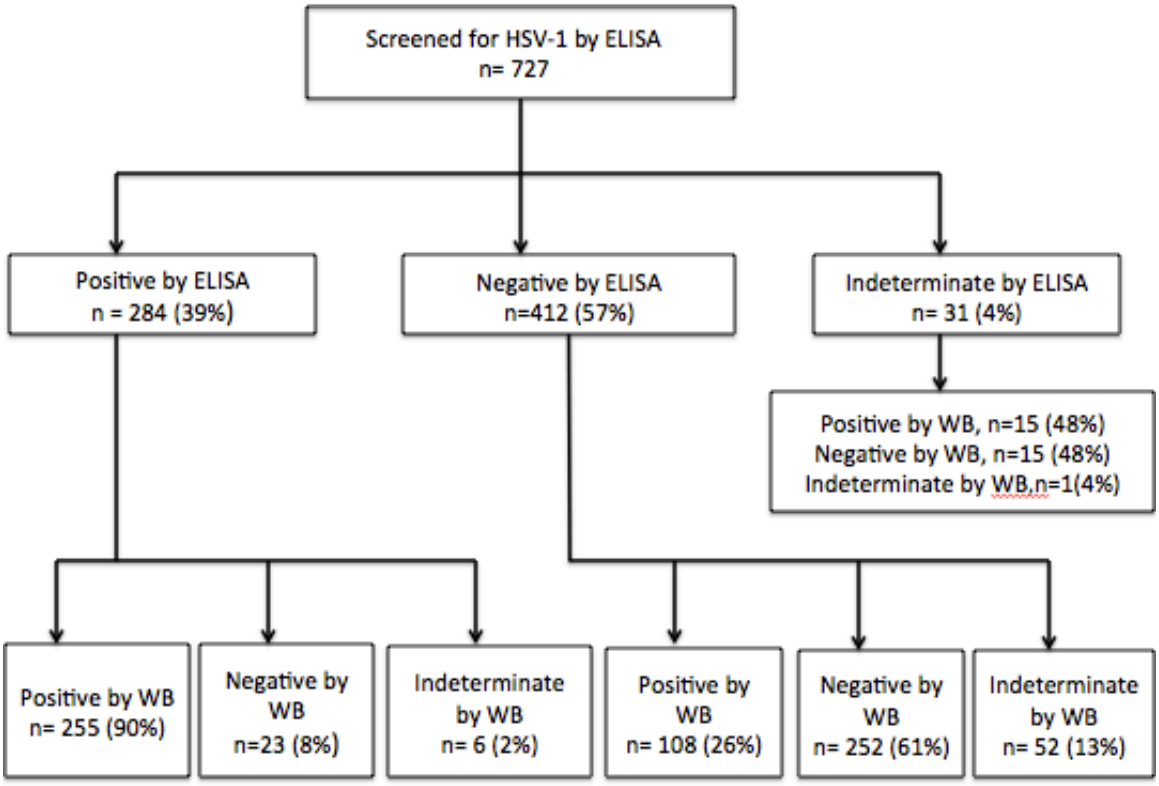

Figure 1. Performance of HSV-1 ELISA compared to western blot

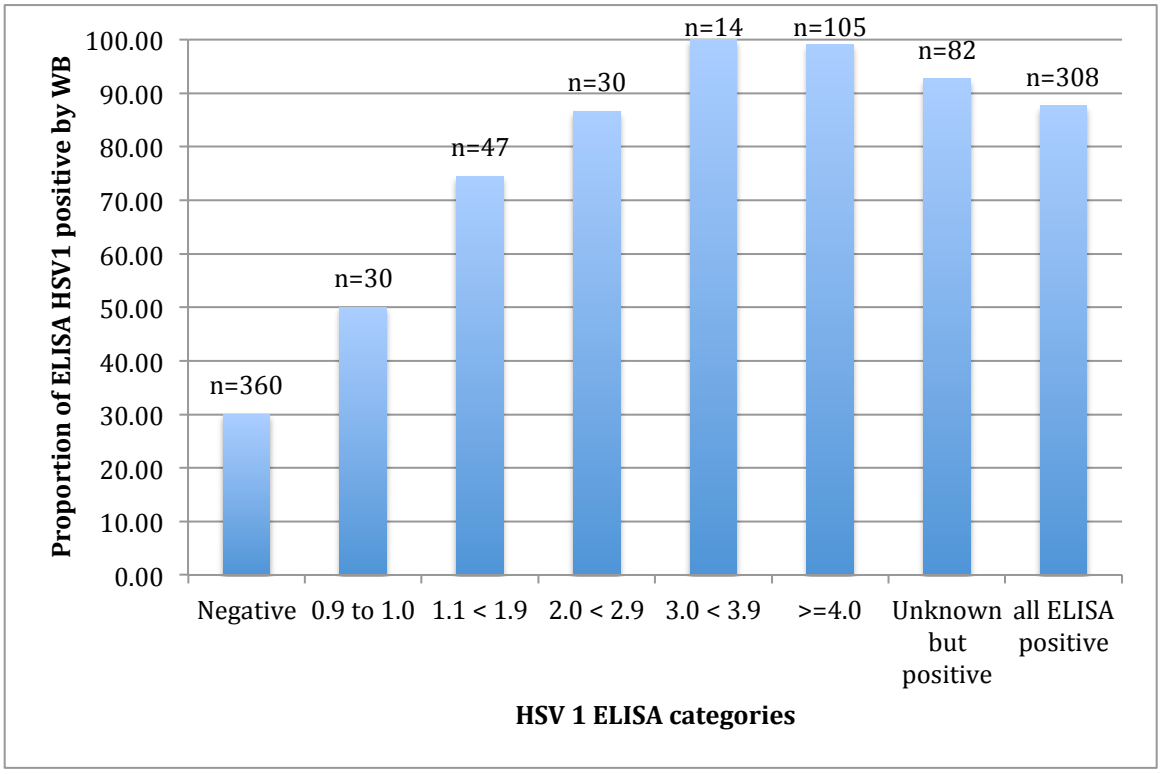

Figure 2. Proportion of HSV-1 ELISA positive results confirmed by western blot, stratified by index value category

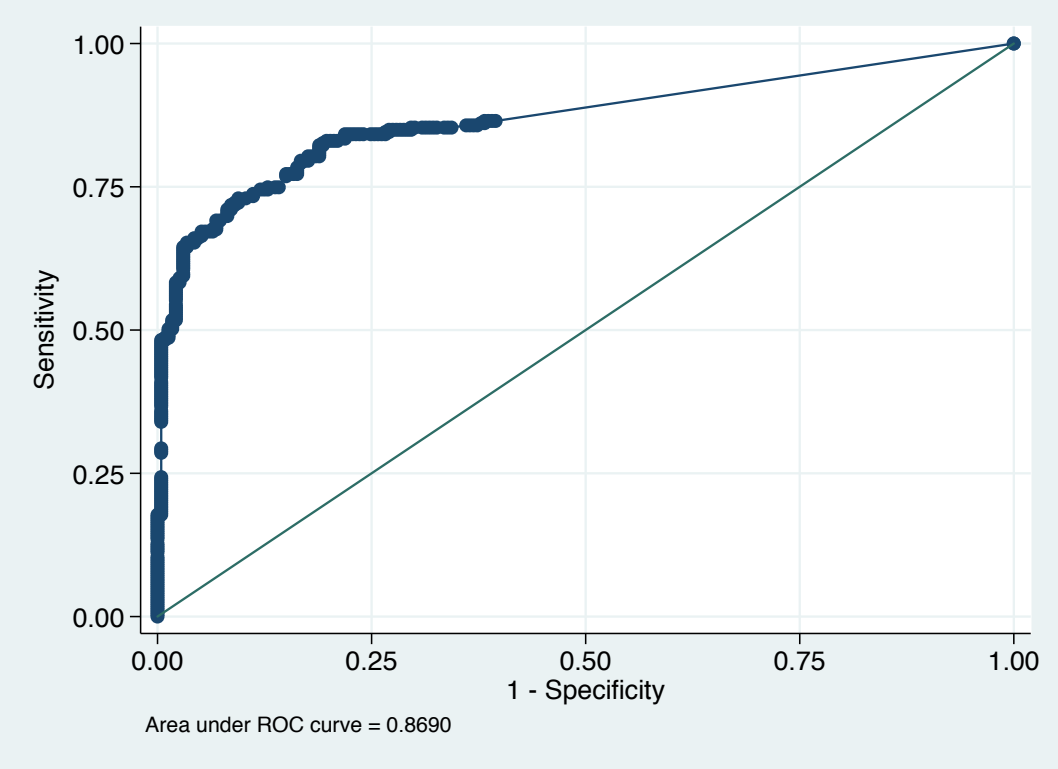

Figure 3. Receiver operating characteristic (ROC) analysis and curve for HSV-1 ELISA

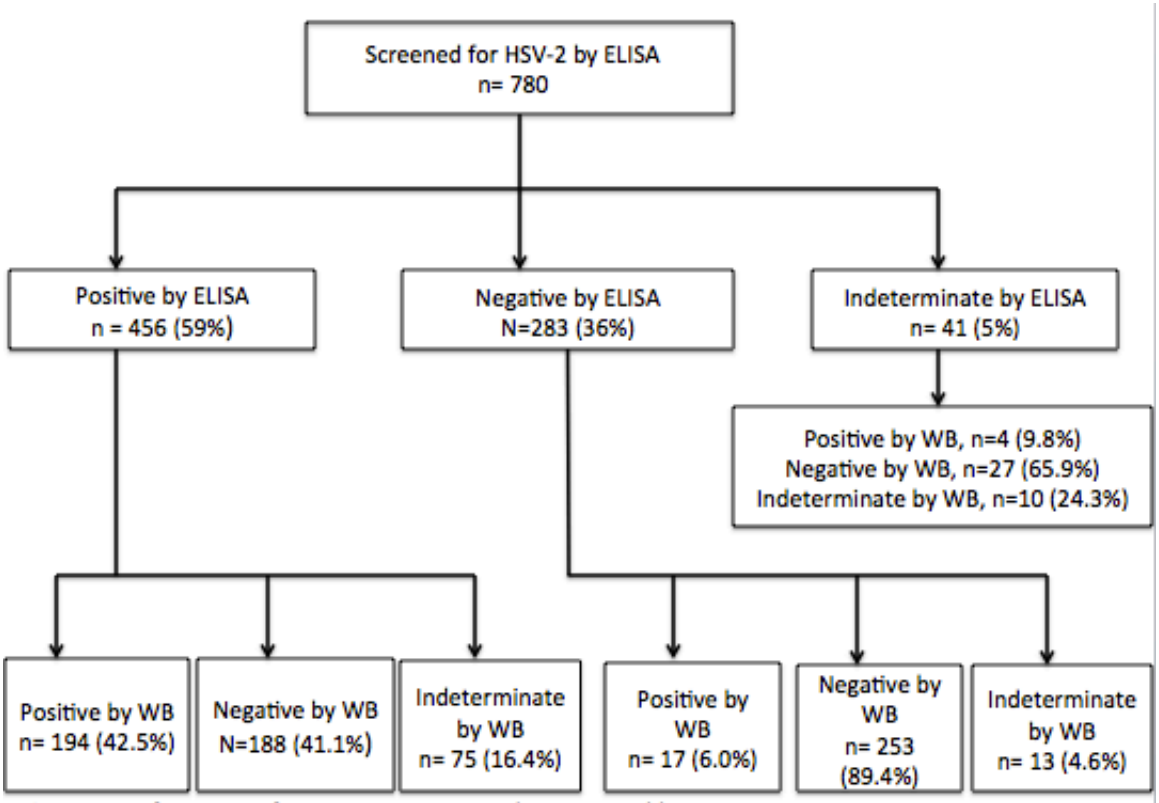

Figure 4. Performance of HSV-2 ELISA compared to western blot

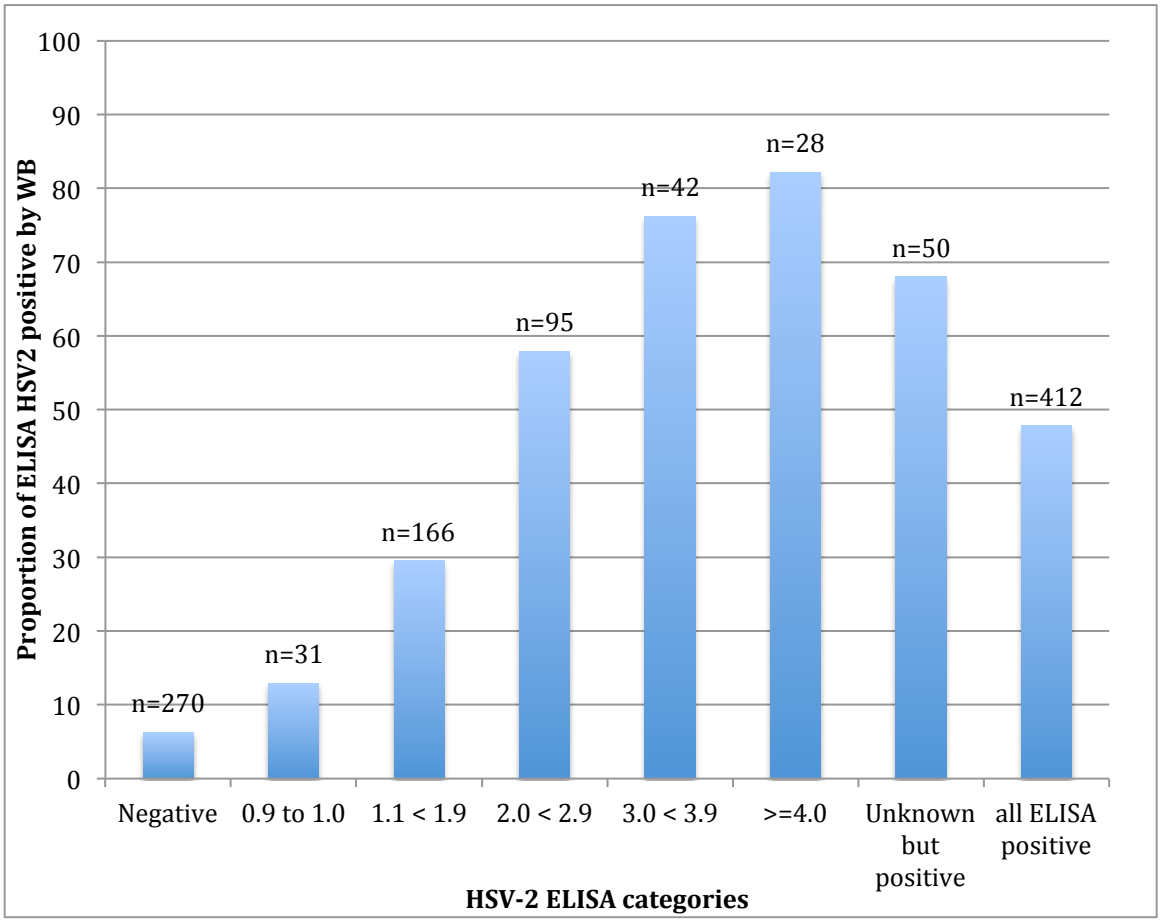

Figure 5. Proportion of HSV-2 ELISA positive results confirmed by western blot, stratified by index value category

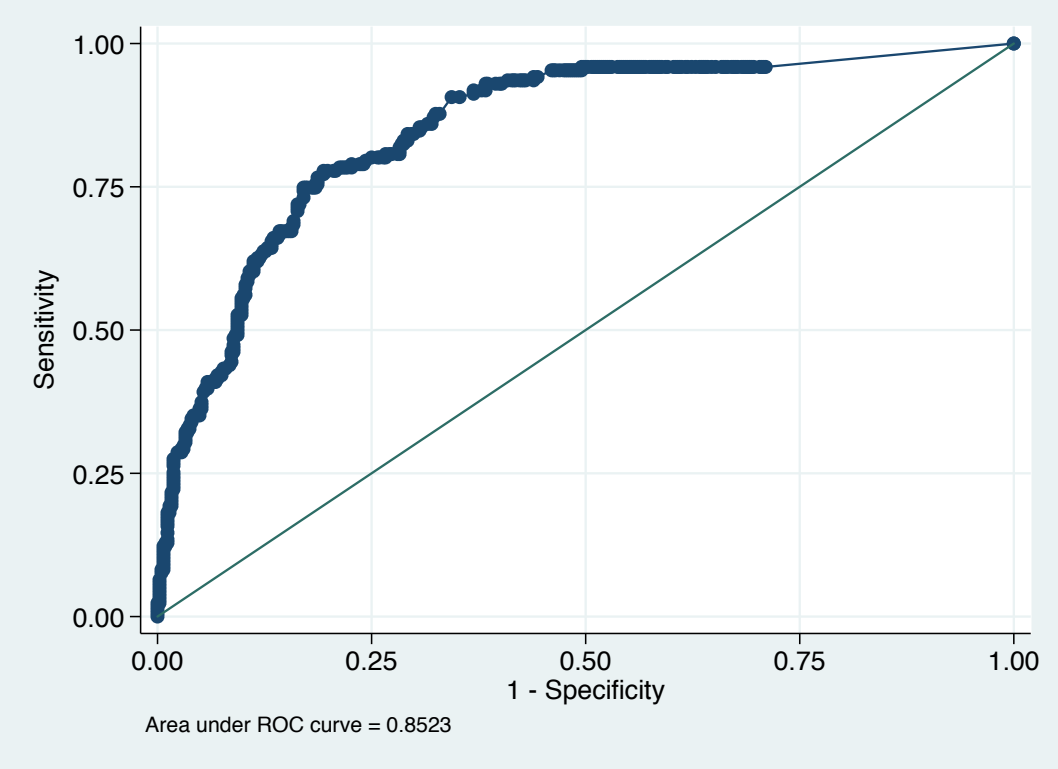

Figure 6. Receiver operating characteristic (ROC) analysis and curve for HSV-2 ELISA