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Performance of Focus ELISA Tests for HSV-1 and HSV-2 Antibodies Among University Students With No History of Genital Herpes

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Abstract

Objectives—To define the performance characteristics of the Focus ELISA HSV-1 and HSV-2 assay among 100 university students.

Study Design—HSV-1 and HSV-2 Focus ELISA and Western Blot assays were performed on sera from university students who reported no history of genital herpes.

Results—HSV-2 and HSV-1 seroprevalence by Western Blot were 3.4% and 48%, respectively. In this population, the positive predictive value of the Focus HSV-2 ELISA was 37.5%, the sensitivity was 100%, and specificity was 94.1%. The PPV of the Focus HSV-1 ELISA was 96.7%, the sensitivity was 69.0%, and the specificity was 97.8%.

Conclusions—In this low-prevalence population, the positive predictive value of the Focus HSV-2 ELISA test was low. This finding, together with those reported elsewhere, indicates that caution is warranted when recommending HSV screening in low-prevalence or heterogeneous populations. Consideration should be given to raising the cutoff index value for defining a positive test result.

INFECTIONS CAUSED BY THE HERPES simplex virus types 1 (HSV-1) and 2 (HSV-2) are highly prevalent.[1] They are associated with substantial morbidity and transmission and acquisition of the human immunodeficiency virus (HIV).[2] In the reported literature, approximately 17% of adults in the United States have antibodies to HSV-2 and 58% have antibodies to HSV-1.[1] Over two-thirds are unaware of their infections, and the majority of infections are transmitted by these individuals.[3] While HSV-1 is primarily the cause oral-labial herpes and HSV-2 causes genital infection, HSV-1 accounts for increasing proportions of newly diagnosed primary genital herpes.[4-7]

In the last decade, type-specific serological assays that detect HSV-1 and HSV-2 antibodies have become commercially available, prompting debate about their use as a screening test. Some experts argue that identifying people with unrecognized HSV-2 may result in a decreased risk of transmission from such persons to others.[8-10] Others express concerns about the accuracy of the tests, the burden on healthcare practitioners to provide counseling, and the

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psychosocial distress that may accompany a diagnosis.[11-13] Because of the low associated morbidity and lack of associated social stigma, until recently serological testing for HSV-1 had not been considered valuable by most experts and had not been requested by patients. However, interest in HSV-1 testing has increased concomitantly with the awareness of HSV-1 as a cause of genital herpes and the affect of HSV type on the prognosis and subsequent counseling related to an infection. In addition, experts have recently suggested that an awareness of HSV-1 antibody status can assist in the interpretation of a serological diagnosis of HSV-2.[14]

In the *Prevention Agenda for Genital Herpes*, the Centers for Disease Control and Prevention called for studies that describe the "real world" performance of type-specific assays for herpes virus infections.[15] HerpeSelect HSV-1 and HerpeSelect HSV-2 enzyme-linked immunosorbent assays (ELISA) (Focus Diagnostics, Cypress, CA) are among the most commonly used tests for serodiagnosis of HSV infection[16] and have previously been shown to be sensitive and specific for the diagnosis of HSV-2 in high-prevalence populations.[17-19] However, the prevalence of a disease in a population affects screening test performance. Positive predictive value (PPV) refers to the proportion of patients with positive test results who actually have the disease (i.e., true positives), and in low-prevalence settings, even tests with high sensitivity and specificity can have poor positive predictive values. Several recent studies found that false-positive HSV results are not unlikely in select patient groups tested by ELISAs.[14,20] We embarked on a study of university students with no history of genital herpes to develop appropriate counseling strategies for HSV testing and identified problems with the positive predictive value of certain tests.

Methods

Study Subjects and Procedures

Participants from 1 urban university were recruited by flyers and announcements in classrooms as part of a broader study examining the impact of serological testing on students without a history of genital herpes or genital sores. Eligible subjects were: (a) 18-40 years of age; (b) full- or part-time students; (c) sexually active (i.e., self-reported having oral, anal, or vaginal sex in the past 6 months) and (d) without a known history of genital sores or genital herpes.

After obtaining written informed consent, data collection consisted of self-administered questionnaires that assessed demographic characteristics, herpes knowledge, depression, anxiety, and sexual behavior. Blood samples were collected and participants were provided with HSV test results by phone approximately 2 weeks after enrollment. Participants were compensated $20.00 for questionnaire completion. Subjects who tested HSV-2 positive were asked to return to the clinic within 1 week to meet with the study staff to ensure that all questions were answered. In addition, all subjects were offered free counseling from the school clinic and extra meetings with the study staff to review results and answer questions. The research protocol was reviewed and approved by the University Institutional Review Board.

Laboratory Methods

Samples were initially tested by HerpeSelect HSV-1 ELISA IgG and by HerpeSelect HSV-2 ELISA IgG (Focus Diagnostics) at the Zenilman laboratory and then stored. ELISA tests were performed according to the manufacturer's instructions. The results of Herpe Select HSV-1 and HSV-2 ELISAs were defined as follows: index values <0.9, negative; >1.1, positive; and inclusive for values between 0.9 and 1.1.

In response to a test result with a low index value, subsequent difficulty with interpretation, and new concerns about the PPV of HSV ELISA tests, Western blot (WB) testing was added

to the protocol for all collected samples. All samples were retested for HSV-1 and HSV-2 by WB at the University of Washington Diagnostic Virology Laboratory (UWDVL).

After study enrollment was nearly complete, Focus Diagnostics released a second generation HerpeSelect ELISA test. All samples which had positive first generation HSV-2 Focus ELISA results were reassessed with the second-generation test (n = 8). To further evaluate the samples with discordant results, second-generation HSV-2 Focus ELISA results that were discordant with WB results were sent to Focus Diagnostics for retesting using the HSV-2 inhibition assay as described by Hoegrefe et al.[21] (N = 3). Briefly, the patient's serum was diluted in diluents, 1 containing purified HSV-1 viral lysate and the second containing purified HSV-2 viral lysate. The samples were then tested per the Focus HSV-2 procedure using the HerpeSelect gG-2 coated microtiter plates. The absorbance of the HSV-2 lysate sample was divided by the absorbance HSV-1 lysate sample to calculate the percent inhibition caused by the addition of HSV-2 lysate. A percent inhibition of 60% or greater confirmed the presence of HSV-2 antibodies. The HSV-1 lysate diluent served as a control for any effects of nongG2 material present in the viral lysate.

Statistical Methods

Sensitivity, specificity, positive predictive value, and Fisher exact methods for 95% CI of the Focus-HSV tests were calculated using the University of Washington WB results as the reference standard. Frequency distributions of selected demographic and behavioral measures were computed using SPSS.

Results

Participant Demographics

Study participants were, on average, 24.5 years old (range 18-39). Almost two-thirds were female (64%) and more than two-thirds (69%) were white, reflecting the ethnic composition of university student body. Two out of five participants were enrolled in graduate programs (Table 1).

Sexual Orientation, Behavior, and Condom Use

The majority of the participants were self-reported heterosexuals (86%). Three-quarters of the participants reported 1 main sex partner in the past 3 months and one quarter reported at least 1 casual sex partner during the same time period. Almost half (47%) of the participants reported using a condom most of the time or always with a main sex partner during the past 3 months. Among those who reported having casual sex partners, 45% reported using condoms always during sex and 12% reported never using condoms during sex with their casual partners (Table 1).

HSV-2 gG ELISA Performance

One hundred subjects enrolled in the study. Because WB testing was initiated after the start of the project, several of the first samples (n = 8) were not sent for WB testing and were excluded from analysis. In addition, 4 inconclusive results for HSV-1 and 3 inconclusive results for HSV-2 were excluded from the analysis.

Eighty-nine samples were tested for HSV-2 by Focus ELISA and WB. Three of the 89 samples were HSV-2 positive by WB (3.4% prevalence). Eight of the 89 samples were HSV-2 positive by the Focus ELISA tests (9% prevalence). Compared with HSV-2 Western blot analysis, sensitivity of the HSV-2 Focus ELISA was 100% (95% CI 30.9-100%), and specificity was 94.1% (95% CI 86.3-97.8). Positive and negative predictive values (PPV, NPV) of the Focus ELISA were 37.5% (CI 10.2-74.1) and 100.0% (CI 94.3-100.0), respectively (Table 2).

Because we tested only the 8 HSV-2 positive samples with the second-generation Focus ELISA test, we were unable to accurately calculate the sensitivity, specificity, and predictive value of the new kit. However, of the 8 participants who had a positive HSV-2 result with the first generation Focus ELISA test, 6 tested positive when the second-generation test was used. Three of these six participants also tested positive by WB. Two participants who tested negative on the second-generation Focus ELISA test were also found to be negative by WB (Table 3).

As mentioned previously, the 3 samples that had discordant second generation Focus ELISA and WB results were sent to Focus Diagnostics for inhibition assays. Index values were reassessed there before inhibition assays were run. One sample had a negative index value when tested by Focus Diagnostics, and therefore, an inhibition test was not completed on this sample. The 2 remaining samples had inhibition assays >90% and were interpreted as positive for HSV-2 by the inhibition assay criteria.

HSV-1 gG ELISA Performance

Eighty-eight samples were tested for HSV-1 by Focus ELISA and WB. By WB analysis, HSV-1 prevalence was 48%. Concordant results for HSV-1 were obtained for all but 11 specimens. With the exception of one sample, all of the HSV-1 discordant samples were Focus negative, but WB positive. Compared with HSV-1 WB, sensitivity of the HSV-1 Focus ELISA was 69.0% (95% CI 52.7-81.9%), and specificity was 97.8% (95% CI 87.0-99.9). Positive and negative predictive values of the Focus ELISA were 96.7% (CI 80.9-99.8) and 77.6% (CI 64.4-87.1), respectively (Table 2).

Discussion

Concern about the performance of commercially available type-specific serological testing for HSV-2 has increased the need for studies in low-prevalence populations. In our study, among university students with a 3.4% seroprevalence of HSV-2 by WB, the positive predictive value of the Focus HSV-2 ELISA test was 37.5% (95% CI 10.2-74.1). Even though our sample size was small, this rate is unlikely to be acceptable to clinicians or patients.

Other recent investigations have raised concerns about the PPV of ELISA tests for HSV-2 in low-prevalence populations. In a recent analysis of sera from 108 men seeking clinical care at the University of Washington STD clinic, a subset of a group with a 13% prevalence of HSV-2 by ELISA, Golden et al. report a PPV of 85%.[14] In an analysis of 1238 sera from non-HIV infected Vietnamese housewives with a seroprevalence of 8.7% by ELISA, only 25 out of 108 samples that were positive by ELISA were confirmed positive by WB.[22] This corresponds with a PPV of 23.1%. Other investigators have used data from higher prevalence populations to estimate the PPV in lower-prevalence groups. Ashley-Morrow and colleagues estimated a PPV of 61.9% in a 10% seroprevalence population using data from 2 populations in the United States.[23] Turner and colleagues estimated a PPV of 66% in a 10% prevalence population of low-income women in California.[19]

This and other studies highlight a major issue interpreting positive HSV-2 results in low-prevalence populations. Low positive predictive values reflect frequent positive results in individuals who do not actually have the disease (i.e., false positives). Although most guidelines do not recommend HSV-2 serological screening in the general population or in pregnant women,[24-26] there are growing calls from experts to expand screening programs to these populations.[27,28] As illustrated in this study, false positives are not unlikely in low-prevalence populations. Incorrectly labeling people as infected with HSV may have significant consequences for their well-being and perceived health.

In an effort to improve the accuracy of the ELISA tests for HSV, some authors have suggested selecting a higher index cutoff value to define positivity.[14,21,29] We found that discordant ELISA and Western Blot results in this study were more common in samples with low index values. In the current study, increasing the cut off index value defining positivity to 3.0 would have increased the PPV to 75.0%. Clinicians using this test in low-prevalence populations should consider using a higher index value to define positivity.

This study also illustrates current challenges in determining the true HSV-2 status of an individual using a serological test. The Western Blot is currently considered the gold standard for detecting HSV, and it is the test by which the performance characteristics of most HSV ELISAs are determined. However, in a recent study of patients with culture-documented first episodes of genital herpes, HSV seroconversion was detected significantly faster by HerpeSelect than by WB.[30] In our study, 2 of the samples that tested HSV-2 positive by WB tested HSV-2 negative by both Focus ELISA and inhibition assays. We did not collect data on exposure to HSV, nor on the development of symptoms after testing, but it is possible that these 2 results reveal early HSV-2 seroconversions and the positive Focus ELISA result reflects the actual disease status, rather than the negative WB. Test accuracy may vary by prevalence of the disease, timing of the test, and the presence or absence of symptoms. More studies are needed to evaluate the limitations of serological tests for HSV.

HSV-1 results were concordant in 86% of the samples tested by both ELISA HSV-1 and WB. All but 1 of the discordant results were ELISA HSV-1 negative but WB positive. This rate corresponds to a PPV of 96.7%, but a sensitivity of only 69%. Ashley-Morrow and colleagues recently presented an analysis of the performance characteristics of Focus-HSV-1 in 969 sera from 8 countries and found a sensitivity of 99% and specificity of 78%.[20] Performance characteristics varied by country and suggested a geographic difference in test performance. Significant differences in the sites and HSV-1 prevalence (93-99% in the study of Ashley-Morrow et al., 48% in our study) might explain the different performance characteristics in these 2 studies. Further investigation is warranted to determine the performance characteristics of the Focus HSV-1 tests.

There are several limitations to this study. First this was a small sample which was not sufficient to narrowly define the sensitivity and specificity of the tests. More studies are clearly needed to determine the performance characteristics of these tests in low-prevalence populations. However, our findings are in range of what others have found in populations with a low HSV-2 prevalence. Second, as mentioned previously, Focus Diagnostics has released a second-generation HerpeSelect ELISA. We evaluated the 8 HSV-2 positive samples with this new test, and of the 5 samples that had discordant ELISA and WB HSV-2 results using the first generation test, 2 were no longer discordant when using the second-generation kit. Thus, it appears that the second-generation Focus ELISA may be somewhat more specific than the first-generation test. Finally, we used a convenient sample of students at 1 university. These findings can not necessarily be generalized to other populations or even students at other universities.

In summary, these data and those reported elsewhere suggest that the ELISA may have an unacceptably low PPV among populations with a relatively low prevalence of HSV-2. Clinicians using the test among low-prevalence populations should be prepared to inform patients of the possibility of false-positive results and should consider using a higher index value to define positivity. Rates of HSV vary considerably by age, gender, race, and other risk factors. Serological testing for HSV may play an important role in prevention programs in high-risk target populations, but these results emphasize the risks of using 1 currently available test to screen for HSV-2 in low-prevalence populations.

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TABLE 1

Sociodemographic and Sexual Behavior Characteristics of Study Participants (N = 100)

Characteristics	Number	Percent
Age		
18-22 y	37	37.0
23-25 y	29	29.0
26-30 y	24	24.0
31-39 y	10	10.0
Mean age		
24.5 ± 4.4 y		
Gender		
Female	64	64.0
Male	36	36.0
Race/Ethnicity		
Asian/Pacific Islander	12	12.0
Latino	2	2.0
AA/Black	6	6.0
White	69	69.0
Caribbean/West Indian	2	2.0
Multiracial	7	7.0
Other	2	2.0
Sexual orientation		
Heterosexual	86	86.0
Homosexual	5	5.0
Bisexual	6	6.0
Unknown	3	3.0
School year		
Freshman	4	4.0
Sophomore	5	5.0
Junior	14	14.0
Senior	22	22.0
Graduate student	41	41.0
Special student	14	14.0
University division		
Arts and sciences	35	35.0
Engineering	14	14.0
Medicine	9	9.0
Nursing	29	29.0
Public health	14	14.0
Number of main sexual partners in past 3 mo		
None	14	14.0
1	75	75.0
2 or more	8	8.0
Unknown	3	3.0

Characteristics	Number	Percent
Frequency of condom use during intercourse with main partner		
Never	20	24.1
Less than half of the time	14	16.9
Half or most of the time	12	14.4
Always	26	31.3
Unknown	11	13.3
Not applicable	17	—
Number of casual sexual partners in past 3 mo		
None	73	73.0
1	14	14.0
2 or more	10	10.0
Unknown	3	3.0
Frequency of condom use during intercourse with casual partner		
Never	3	12.5
Less than half of the time	2	8.3
Half-most of the time	1	4.2
Always	11	45.8
Unknown	7	29.2
Not applicable	76	—

TABLE 2

Serology Results by HerpeSelect HSV ELISA (Focus) and Western Blot (WB)

	Western Blot			
	HSV-2		HSV-1	
ELISA	**Positive**	**Negative**	**Positive**	**Negative**
Positive	3	5	29	1
Negative	0	81	13	45
Total	3	86	42	46

Sensitivity: 100.0% and 69.0%, respectively, for HSV-2 and HSV-1.

Specificity: 94.1% and 94.1%, respectively, for HSV-2 and HSV-1.

PPV: 37.5% and 96.7%, respectively, for HSV-2 and HSV-1.

NPV: 100% and 77.6%, respectively, for HSV-2 and HSV-1.

TABLE 3

Results of Testing for HSV-2 Antibodies by Focus ELISA, Western Blot, and Inhibition Assays

Specimen	HSV-1 ELISA KIT 1 Result	HSV-2 ELISA GEN 1 KIT Index Value	HSV-2 ELISA GEN 2 KIT Result	HSV-2 ELISA GEN 2 KIT Index Value	HSV-2 WB Result	Focus Diagnostics Index Value	Inhibition Assay
1	-	1.19	+	1.3O	-	0.68	*a*
2	+	3.46	+	2.43	-	2.3	>90%
3	+	2.40	-	0.36	-		
4	-	6.46	+	3.20	+		
5	-	9.32	+	6.86	+		
6	+	1.76	+	2.34	-	2.34	>90%
7	-	1.81	-	0.61	-		
8	+	5.16	+	4.79	+		

a, testing by Focus Technologies resulted in a negative index value (<0.90) and sample was therefore excluded from inhibition assays.

HSV-1, herpes simplex virus type 1; HSV-2, herpes simplex virus type 2; ELISA, Focus Technologies enzyme-linked immunosorbent assay; GEN 1, first generation; Focus ELISA kit GEN 2, second-generation Focus ELISA kit; WB, Western blot.