Evaluation of a New Point-of-Care Serologic Assay for Herpes Simplex Virus Type 2 Infection

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Herpes simplex virus type 2 infection is one of the most common sexually transmitted diseases. Because presentation is often atypical or subclinical, serologic testing is necessary for diagnosis, treatment, and counseling. In an urban clinic that specializes in the treatment of sexually transmitted disease, a new point-of-care rapid serologic test was compared with enzyme-linked immunosorbent assay or Western blot for the detection of herpes simplex virus type 2. With use of an enzyme-linked immunosorbent assay index cutoff value of 1.1, the rapid test was found to have a sensitivity of 97%, a specificity of 98%, a positive predictive value of 92%, and a negative predictive value of 99%. Increasing the cutoff index value to 3.5 increased the test sensitivity to 100%.

Genital herpes due to herpes simplex virus type 2 (HSV-2) infection is common and remains underdiagnosed in the United States [1]. Appropriate counseling and treatment are predicated on an accurate diagnosis of genital herpes. Because most HSV-2 infections are subclinical or unrecognized, serologic testing is often necessary to make a diagnosis; after a diagnosis is made, infected patients can be counseled that symptom awareness, condom use, and suppressive treatment may all decrease the risk of transmission to uninfected partners [2, 3].

Western blot is considered the gold standard for HSV-2 serologic testing [4], but because of its cost and limited availability, commercially available type-specific HSV-2 serologic assays are more commonly used for screening [5]. Rapid HSV-2 serologic assays that can be performed on-site with use of blood specimens obtained by fingerstick provide several potential advantages over standard laboratory-based assays. First, they allow for same-visit diagnosis and immediate counseling and treatment, if indicated. Second, they do not require additional expensive equipment, which is particularly important in areas with limited resources or infrastructure. Third, capillary blood tests are preferable to standard venipuncture because of decreased discomfort and lower risk of occupational bloodborne infection, and performance of the tests does not require a trained phlebotomist.

A new point-of-care rapid assay for the detection of HSV-2 (HerpeSelect Express; Focus Diagnostics) has recently been approved by the United States Food and Drug Administration, and preliminary studies have estimated the test to have high sensitivity (86%–100%) and specificity (97%–100%), compared with HSV-2 immunoblot and Western blot testing [6, 7]. The test requires only 2 steps but does not yet have a Clinical Laboratory Improvement Amendments waiver. To gain additional information on point-of-care test performance when the test is performed by clinic staff in an urban sexually transmitted disease clinic, we compared the results of HerpeSelect Express with our current standard HSV-2 serologic assay, the HerpeSelect HSV-2 ELISA (Focus Diagnostics), with select confirmatory Western blot testing.

Patients and methods. Patients were recruited from San Francisco's municipal sexually transmitted disease clinic during October 2007. Eligible patients included those ⩾18 years of age who were able to provide verbal consent in English, Spanish, or Russian and who were receiving a serologic test for HSV-2 infection as part of their routine clinical care. Reasons for HSV-2 testing included diagnostic workup as well as asymptomatic screening, according to California guidelines [8]. The study protocol was approved by the Committee on Human Research at the University of California, San Francisco (H9978–31317).

After informed consent was obtained by a nurse practitioner clinician, all rapid tests were performed by 1 of 3 clinic staff: a laboratory assistant, a registered nurse, and a health worker, whose duties include phlebotomy and the performance of point-of-care syphilis and HIV tests. After venipuncture, whole blood was obtained by fingerstick puncture with use of a sterile lancet (Tenderlett; International Technidyne). The first drop of whole blood was drawn up in a capillary tube provided as part of the HerpeSelect Express assay kit. The attached plunger was used to dispense the blood from the capillary tube and the blood was deposited onto the test pad of the kit. After 30

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Table 1. Results of Western blot testing on 12 specimens with discordant, equivocal, or indeterminate HerpeSelect Express test and ELISA results.

Specimen	HerpeSelect Express result	HSV-2 ELISA index value	HSV-2 ELISA interpretation	HSV-2 Western blot result	Final interpretation
1	Pos	0.2	Neg	Pos	True pos
2	Pos	0.3	Neg	Pos	True pos
3	Pos	0.9	Neg	Pos	True pos
4	Pos	0.8	Neg	Neg	False pos
5	Pos	0.6	Neg	Neg	False pos
6	Pos	0.0	Neg	Neg	False pos
7	Pos	1.0	EQ	I	NA (excluded)
8	Neg	0.9, 1.1[a]	I	Neg	True neg
9	Neg	1.0	EQ	Neg	True neg
10	Neg	1.1	Pos	Neg	True neg
11	Neg	1.2	Pos	Neg	True neg
12	Neg	2.5	Pos	Pos	False neg

NOTE. EQ, equivocal (index value, 0.9–1.1); I, indeterminate; pos, positive (index value, >1.1); neg, negative (index value, <0.9).

[a] Result could not be resolved on repeated testing.

seconds, 5 drops of the provided buffer were added to the assay, and start time was recorded in a logbook, along with the patient's assigned study identification number, patient sex, date, assay lot number, and technician initials. Result and end time were recorded after a 15–20-min interval. If the control line was not visible, the assay was considered invalid and was repeated. If a test line was visible, even if it was faint, the result was considered to be reactive and "faint marking" was noted in the logbook. Positive and negative controls (provided with the assays) were run at the start and at the midpoint of the study enrollment period. All procedures were performed in accordance with manufacturers' instructions.

Participant serum specimens were tested at the San Francisco Department of Public Health Laboratory with use of the laboratory-based HerpeSelect HSV-2 ELISA, according to the manufacturer's instructions. In contrast with the product insert, which defines any index value >1.1 as positive on the basis of data from our clinic and others [9] (J. Fuchs, personal communication), in our sexually transmitted disease clinic, results are categorized by index value: scores of 1.1–3.5 are considered to be low positive and scores >3.5 are considered to be high positive.

Study participants were not informed of results of the HerpeSelect Express assay and were instructed that the results of the HSV-2 ELISA would be available, according to normal clinic protocol. In cases in which there was a discrepancy between the HerpeSelect Express and HSV-2 ELISA results, serum specimens were sent to the laboratory at the University of Washington for Western blot analysis.

Sensitivity, specificity, positive predictive value, negative predictive value, and 95% CI (each calculated on the basis of binomial probability) were calculated using HSV-2 ELISA or Western blot results (if ELISA results were discrepant) as the standard. Analyses were stratified by the index value range of the HSV-2 ELISA result.

To achieve precise estimates, we assumed an HSV-2 ELISA positivity (index value, >1.1) of 20% on the basis of previously available clinic testing data. With 200 patients and a predicted test sensitivity of 90% and specificity of 90%, we estimated that the 95% CIs would be restricted to $\pm 10.3\%$ and $\pm 5.0\%$, respectively, on the basis of binomial distribution methods. All analyses were conducted using SAS, version 9.1 (SAS).

Results. Among study patients, the seroprevalence of HSV-2 (index value, >1.1) was 17% (33 of 199 patients) overall; seroprevalence was 18% (26 of 148 patients) among men and 12% (6 of 50 patients) among women. One additional patient with positive results for HSV-2 was transgender. With a higher cutoff (index value, >3.5), the seroprevalence decreased to 13% both overall (26 of 199 patients) and among men (19 of 148 patients) and remained 12% (6 of 50 patients) among women.

Of the 199 evaluable specimen pairs, the HerpeSelect Express and ELISA results were identical in 187 (94%). Of the 12 specimens that required Western blot testing, 7 yielded results that were concordant between the HerpeSelect Express assay and Western blot, whereas 3 were classified as false-positive HerpeSelect Express results and 1 was classified as a false-negative HerpeSelect Express test result. One specimen had indeterminate results after ELISA and Western blot testing and was not included in the analyses (table 1).

As shown in table 2, compared with HSV-2 ELISA or Western blot, the sensitivity and specificity of the HerpeSelect Express assay were 97% (95% CI, 85%–100%) and 98% (95% CI, 95%–

Table 2. HerpeSelect Express results, compared with herpes simplex virus type 2 (HSV-2) ELISA or Western blot results.

HerpeSelect Express result	No. (%) of specimens, by HSV-2 ELISA or western blot result					
	ELISA index cutoff value >1.1[a,b] (n = 198)			ELISA index cutoff value >3.5[c,d] (n = 189)		
	Positive	Negative	Total	Positive	Negative	Total
Positive	33 (17)	3 (2)	36 (18)	26 (14)	3 (2)	29 (15)
Negative	1 (1)	161 (81)	162 (82)	0 (0)	160 (85)	160 (85)
Total	34 (17)	164 (83)	198 (100)	26 (14)	163 (86)	189 (100)

NOTE. With an ELISA index value cutoff of >1.1, the HerpeSelect Express assay had a sensitivity of 97% (95% CI, 85%–100%), a specificity of 98% (95% CI, 95%–100%), a positive predictive value of 92% (95% CI, 78%–98%), and a negative predictive value of 99% (95% CI, 97%–100%). With an ELISA index value cutoff of >3.5, the HerpeSelect Express assay had a sensitivity of 100% (95% CI, 87%–100%), a specificity of 98% (95% CI, 95%–100%), a positive predictive value of 90% (95% CI, 73%–98%), and a negative predictive value of 100% (95% CI, 98%–100%).

[a] Excludes 1 specimen that yielded equivocal or inconclusive results by both ELISA and Western blot.

[b] Twelve specimens were tested by Western blot.

[c] Excludes 1 specimen that yielded equivocal or inconclusive results by both ELISA and Western blot and 9 specimens with an ELISA index value 0.9–3.5.

[d] Seven specimens were tested by Western blot.

100%), respectively. The positive predictive value was 92% (95% CI, 78%–98%), and the negative predictive value was 99% (95% CI, 97%–100%). With use of the higher cutoff (index values, >3.5) and excluding intermediate- and low-positive HSV-2 ELISA results (index value, 1.1–3.5; $n = 9$), sensitivity increased to 100% (95% CI, 87%–100%) and specificity remained 98% (95% CI, 95%–100%); the positive predictive value and negative predictive value were 90% (95% CI, 73%–98%) and 100% (95% CI, 98%–100%), respectively.

The HerpeSelect Express test was considered easy to use by the 3 clinic staff who performed the tests. However, a total of 4 (11%) of 36 positive results were noted to have a faint test line for a positive result. These 4 results were distributed between all 3 study staff. Two of these 4 results were obtained with specimens that were in the 12 specimens with discrepant or indeterminate results that were sent for Western blot analysis, and 1 result was determined to be a false-positive HerpeSelect Express test result (table 1).

Discussion. The HerpeSelect Express HSV-2 test was developed to allow health care providers access to a simple, on-site test that can be used for diagnosis of HSV-2 infection in as little as 15 min. Compared with HSV-2 ELISA at a cutoff index value of >1.1, the sensitivity and specificity of the HerpeSelect Express assay were comparable to previously reported results and support its clinical use [10]. At both cutoffs used in our study, the negative predictive values were higher than the positive predictive values, and at the relatively low overall HSV-2 seroprevalence in our participants, both the positive and negative predictive values were higher than those reported elsewhere [6].

In addition to its favorable test performance characteristics, the HerpeSelect Express assay was simple to use. Although we did not use the test results for diagnosis, patients were very willing to accept and complete testing. Our evaluation showed that this was overall an easy assay to perform and was comparable to other tests which currently have a Clinical Laboratory Improvement Amendments waiver; the test procedures did not prove burdensome in our busy municipal sexually transmitted disease clinic.

Importantly, several studies have demonstrated that serological diagnosis of HSV-2 infection (including diagnosis with a rapid test) was not associated with adverse psychological effects [11, 12]. Nonetheless, because genital herpes is a lifelong infection with implications for current and future sexual partnerships, a primary goal must be to reduce false-positive and false-negative results that might cause unnecessary distress and treatment or unwitting ongoing transmission, respectively.

Given this goal of providing the most accurate possible information to the patient, one potential drawback of a qualitative rapid test is that it does not allow for further categorization of positive results, as is the case with ELISA index values. The use of higher index cutoff values has been shown to correlate with a greater likelihood of true infection, and in multiple settings it has been demonstrated to increase specificity and positive predictive value, particularly in populations with a low prevalence of HSV-2 infection [9, 13]. Another proposed solution has been to use a 2-stage testing strategy for confirmation after HSV-2 rapid testing to increase the positive predictive value, as is the standard for HIV rapid testing [14].

There were several limitations of this study that deserve mention. Our findings among patients at a sexually transmitted disease clinic may not be generalizable to other patient populations. An additional limitation is that all specimens were not tested with both ELISA and Western blot and, of those that

were, 5 had false-positive ELISA results when compared with Western blot, whereas the HerpeSelect Express test results were correct. However, because of the cost and limited availability of Western blot assay, some studies of HSV-2 serologic tests have used alternate assays for comparison [15, 16]. Finally, because we did not disclose HerpeSelect Express results to patients, we were unable to assess the effects of rapid testing on overall counseling duration; rapid testing will likely add length to the testing visit [17].

Because the value of a screening test depends not only on its performance characteristics but also on disease prevalence, it will be important for providers to consider their local HSV-2 epidemiology to best interpret results and counsel patients, particularly patients in general populations at low risk for HSV-2 infection who may desire testing. Additionally, the most highly promising sites for widespread implementation of low-complexity rapid herpes testing may be in developing countries, many of which have a high prevalence of HSV-2 infection and have resource limitations that may preclude laboratory-based testing [13]. Additional studies will be crucial to guide the best use of all available type-specific HSV serologic tests in varied populations to most accurately diagnose genital herpes.

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