Serologic Herpes Testing in the Real World

Validation of New Type-Specific Serologic Herpes Simplex Virus Tests in a Public Health Laboratory

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Background: **Serologic testing for herpes simplex virus type-2 (HSV-2) is being implemented in sexually transmitted disease (STD) clinics.**

Goal: **To determine the performance characteristics of two HSV-2 type-specific serologic assays in a public health laboratory.**

Study Design: **Sera stored from a cross-sectional study were tested with the Meridian Diagnostics and Focus Technologies HSV-2 ELISA tests and a type-specific strip immunoblot assay (Chiron Corp.) was used as the reference standard.**

Results: **Prevalence of HSV-2 infection in this sample was 44%. Compared to the reference standard, the sensitivity of the Meridian Diagnostics HSV-2 test was 95.5% (95% CI 83.3, 99.2) and specificity was 98.2% (95% CI 89.0, 99.9). The Focus Technologies test yielded 97.7% (95% CI 86.5, 99.9) sensitivity and 94.5% (95% CI 83.9, 98.6) specificity.**

Conclusions: **The performance of these HSV-2 type-specific serologic assays was adequate to support their use in high prevalence populations, such as STD clinic patients.**

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THE PREVALENCE of genital herpes due to herpes simplex virus type 2 (HSV-2) in the United States is 22% and is greater in high-risk populations.[1–4] More than 80% of those infected with HSV-2 do not report a history of symptoms and have unrecognized infection.[1] However, once HSV-2 infection is diagnosed and these individuals are taught how to recognize herpes symptoms, many come to identify their symptoms.[5–7] In addition, recent data suggest that condoms are effective in preventing genital herpes transmission from men to women and that changes in sexual behavior, such as avoiding sex when lesions are present, are also associated with reduced herpes transmission.[8]

Genital herpes prevention programs that include counseling and education about recognizing symptoms, using condoms during both asymptomatic and symptomatic periods, and avoiding sex when symptoms are present must first rely on the accurate identification of persons infected with HSV-2. Testing for HSV-2 infection with accurate type-specific serologic assays provides the opportunity to identify persons with previously unrecognized genital herpes.[9–11]

In the past, accurate serologic diagnosis of HSV-2 infection was hampered by inaccurate commercial tests that failed to distinguish between HSV type 1 (HSV-1) and HSV-2 antibodies.[12] The Western blot assay is considered the "gold standard" test to discriminate between HSV-1 and HSV-2 antibodies and is a highly sensitive and specific diagnostic tool. Although it has been available for many years, the Western blot assay is expensive and cumbersome to perform and thus not widely used.[13] Recently, type-specific serologic assays that accurately distinguish between HSV-1 and HSV-2 antibodies became commercially available and now make serologic diagnosis of herpes infection practical.[10,11] These tests are highly sensitive and specific, reasonably priced, and easy to perform.[10,11,14] The "real world" performance and practicality of these new type-specific serologic assays are only beginning to be evaluated in different populations. In the *Prevention Agenda for Genital Herpes*, the Centers for Disease Control and Prevention identified studies that describe the "real world" performance of type-specific serologic tests as a priority.[15] Such studies will help inform decisions regarding which populations and settings may be appropriate for HSV-2 testing or screening.

In 2000, the San Francisco Department of Public Health

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identified serologic HSV-2 testing as an important sexual health service for sexually transmitted disease (STD) clinic attendees and began the process of making serologic testing available. The first step in implementing serologic HSV-2 testing was to determine which new type-specific test to use. The purpose of the study was to determine the sensitivity and specificity of two HSV-2 type-specific serologic assays in a public health laboratory to help decide which test to offer to STD clinic patients.

At the time of this study (March 2000) two type-specific enzyme-linked immunosorbent assay (ELISA) serologic tests for HSV-2 infection were commercially available: the Premier Type Specific HSV-2 IgG ELISA (Meridian Diagnostics, Inc., Cincinnati, OH) and the HSV-2 IgG ELISA (recently renamed the HerpeSelect 2 ELISA; Focus Technologies [previously MRL Diagnostics], Cypress, CA). We evaluated these two tests.

Methods

Sera

We used stored (−20 °C) sera obtained from the Young Women's Survey, a population-based survey designed to measure the prevalence of HIV, STDs, and risk behaviors of young low-income women in five counties in Northern California.[3] Described in detail elsewhere, the Young Women's Study was conducted between April 1996 and January 1998, and serum samples were collected from 1635 women between ages 18 and 29 years.[3] HSV-2 prevalence in the Young Women's Survey was determined with the Chiron strip-recombinant immunoblot assay (RIBA HSV Type 1/Type 2 SIA; Chiron Corporation, Emeryville, CA). The prevalence of HSV-2 infection in this study was 35%.

For the current study, a convenience sample of 99 sera was selected from the 1635 Young Women's Survey samples.

Serologic Tests

Sera were tested for HSV-2 antibody with both the Meridian Diagnostics and Focus Technologies tests and compared with the original HSV-2 results as determined by the Chiron RIBA. Both Meridian Diagnostics and Focus Technologies use the ELISA method to identify HSV-2 antibodies. The Meridian Diagnostics test uses affinity-purified HSV-2 gG2 antigen to identify HSV-2 antibody, and the Focus Technologies test uses recombinant gG2 antigen. The Meridian Diagnostics ELISA test sensitivity was 80.5% to 98.0%, and its specificity was 96.9% to 100%.[16] Although the Meridian Diagnostics ELISA was commercially available at the time of this study, it is not currently manufactured. The Focus Technologies ELISA sensitivity and specificity were 96.1% to 100% and 96.1% to 98.7%, respectively.[17] Tests were provided by the manufacturer and performed according to the manufacturer's instructions.

The Chiron RIBA Type 1/Type 2 SIA is an immunoblot assay that differentiates between HSV-1 and HSV-2 antibodies on the basis of recombinant antigen bands for HSV-1 gG1, gB1, and HSV-2 gG2, gD2.[18] In comparison with Western blot, the Chiron RIBA was 98% sensitive and 99% specific for HSV-2.[19]

Analytic Methods

The sensitivity and specificity of the Meridian Diagnostics and Focus Technologies HSV-2 ELISAs were determined in comparison with the Chiron RIBA. We calculated 95% CIs with Epi Info 6.0.[20] We also calculated the positive and negative predictive values of these tests at different prevalence levels.

Results

Forty-four of the 99 specimens were HSV-2-positive by the Chiron RIBA, and 55 were negative. Agreement between the Chiron RIBA and the Meridian Premier HSV-2 ELISA was 96.9% (96/99). Agreement between the Chiron RIBA and the Focus Technologies HSV-2 ELISA was 95.9% (95/99).

Table 1 presents the results of the two ELISA tests. With the Meridian Premier Test, 43.4% of the results (43/99) were HSV-2-positive, yielding a sensitivity of 95.5% (95% CI, 83.3–99.2) and specificity of 98.2% (95% CI, 89.0–99.9). Forty-six of 99 (46.4%) of the Focus Technologies tests were HSV-2-positive; the sensitivity was 97.7% (95% CI, 86.5–99.9), and the specificity was 94.5% (95% CI, 83.9–98.6).

In this sample, where the HSV-2 prevalence was 44%, the positive predictive values (PPVs) for the Meridian Diagnostics and Focus Technologies tests were 97.6% and 93.5%, respectively. We also estimated the positive and negative predictive values of the two tests in a range of low-

TABLE 1. Results of the Meridian Diagnostics Premier and Focus Technologies HSV-2 Type-Specific Antibody Assays, Compared With the Chiron HSV-1/HSV-2 RIBA Immunoblot

	Chiron HSV-1/HSV-2 RIBA Immunoblot (N = 99)	
Assay	No. Positive (n = 44)	No. Negative (n = 55)
Meridian Premier		
Positive	42	1
Negative	2	54
Focus Technologies		
Positive	43	3
Negative	1	52

HSV = herpes simplex virus.

TABLE 2. Positive and Negative Predictive Values of HSV-2 Serologic Tests at Different HSV-2 Prevalence Levels

	Meridian		Focus Technologies	
True Prevalence (%)	Positive Predictive Value (%)	Negative Predictive Value (%)	Positive Predictive Value (%)	Negative Predictive Value (%)
10	85.0	99.5	66.2	99.8
20	93.2	98.9	82.0	99.3
30	95.6	98.0	88.2	99.0
40	97.2	97.0	92.2	98.4

HSV = herpes simplex virus.

to high-prevalence populations (Table 2). The PPV of the Meridian Diagnostics test ranged from 85.0% in a 10% HSV-2 prevalence population to 97.2% in a 40% prevalence population. The PPV of the Focus Technologies test ranged from 66.2% in a 10% prevalence population to 92.2% in a 40% prevalence population.

Discussion

Both the Meridian Diagnostics and Focus Technologies HSV-2 ELISA tests performed well in this public health laboratory. Sensitivity and specificity of these tests were high in this high HSV-2 prevalence sample. Our results with the Meridian Diagnostics test were consistent with the sensitivity and specificity reported by the manufacturer. In addition, our PPV calculations were similar to those estimated by Meridian Diagnostics; in their studies the PPV ranged from 85.6% in a 10% HSV-2 prevalence population to 95.8% in a population where the true prevalence was 30%.[16] In our study, the sensitivity of the Focus Technologies test was similar to the manufacturer's findings, and although the specificity was somewhat lower, the CI encompassed the manufacturer's reported specificity.

Serologic testing for HSV-2 will be an important component of any genital herpes prevention program, but recommendations for testing and screening will vary in different populations. In populations with high prevalence, such as STD clinic attendees, partners of HSV-2-infected individuals, or patients with suggestive herpes symptoms, these type-specific serologic tests will yield high positive predictive values. Conversely, in low-prevalence populations there will be higher numbers of false-positive test results, and additional testing may be appropriate. Recommendations for HSV-2 testing and screening must account for the population prevalence and the test specificity, by including additional or confirmatory testing and ensuring extensive counseling about test accuracy.

A recent study examined the use of type-specific serology in persons attending an STD clinic where the HSV-2 prevalence was 29.9% and found that the test performance was sufficient to support testing in this population without Western blot confirmatory testing.[9] Given the HSV-2 prevalence (30%) in the San Francisco municipal STD clinic, we believe that type-specific serologic testing is appropriate in this population without additional testing.

In addition to the accuracy of type-specific HSV tests, the cost of tests has been a barrier to implementing testing. Although the Western blot is highly accurate, the cost (approximately $95.00) is prohibitive for a public health laboratory. During the time of this study, the cost of both the Meridian Diagnostics and Focus Technologies tests was approximately $300 per 96-well kit, or $3.00 per test. Including labor costs, we estimate total costs are $20 per test. The lower cost of these assays may make testing in an STD clinic setting feasible.

There are several limitations to this study. First, we did not use Western blot as a confirmatory test or for the discordant results between the three assays. Although the Chiron RIBA performed very well in comparison with the Western blot,[19] the Chiron RIBA has not been approved by the U.S. Food and Drug Administration. Both Meridian Diagnostics and Focus Technologies used the Western blot as their reference test. The use of the Chiron RIBA in this study may have influenced the performance characteristics of the two ELISA tests. In addition, because of the small sample size, it was difficult to discern statistically significant differences between the tests. Last, the laboratory storage of the samples may have affected the performance of the two assays.

In summary, both the Meridian Diagnostics and the Focus Technologies HSV-2 ELISA tests performed adequately in this public health laboratory. The sensitivity and specificity observed in this study make either test appropriate for HSV-2 case identification in an STD clinic setting. The positive predictive value of these tests is adequate in high-prevalence populations without additional testing. This study indicated that the test performance of the Meridian Diagnostics and Focus Technologies HSV-2 ELISA tests in a public health laboratory should not be a deterrent to implementing HSV-2 testing in STD clinics or in other high-risk populations. Type-specific HSV-2 serologic testing has been available at the San Francisco municipal STD clinic since September 2000.

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