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Predictors of Herpes Simplex Virus Type 2 Antibody Positivity Among Persons With No History of Genital Herpes

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Background: **The demographic, historical, and behavioral factors that predict a positive herpes simplex virus type 2 (HSV-2) antibody test in persons without a history of genital herpes have not been well-defined.**

Methods: **Individuals (age 14–30 years) without a history of genital herpes completed a questionnaire and were offered free HSV-2 antibody testing. Factors from the questionnaire were correlated with the HSV-2 antibody result.**

Results: **Univariate analysis showed that female gender was significantly associated with positive test results. In gender-specific, multiple logistic regression models, a positive HSV-2 antibody test among men was associated with older age, non-white race, and a history of sexually transmitted disease (STD). Gender-specific symptom scores from the questionnaire were not predictive in either gender, but the gender-common symptom score was marginally predictive of a positive HSV-2 antibody test in women. Among women, older age, non-white race, and STD history predicted a positive test.**

Conclusions: **Among young persons with no history of genital herpes who agreed to HSV-2 antibody testing, increasing age, non-white race, and a history of an STD were predictors of a positive test. A history of frequent pain, itching, burning, and rashes in the anogenital region was marginally associated with positive HSV-2 tests in women. These results might help guide selective use of HSV-2 antibody screening.**

GENITAL HERPES IS A HIGHLY prevalent sexually transmitted disease (STD) in the United States and worldwide. Because herpes simplex virus type 2 (HSV-2) causes most genital herpes, especially recurrent genital herpes, antibodies to HSV-2 have been used as a marker for genital herpes in a number of studies. These have included large, population-based studies such as the second and third National Health and Nutrition Examination Surveys (NHANES II[1] and NHANES III[2]) as well as a number of other studies using targeted populations such as primary care clinic attendees,[3] pregnant women,[4] STD clinic attendees,[5–7] and adolescents and young adults.[8,9] In these and other published studies, only a minority of persons with HSV-2 antibody (between approximately 10% and 30%) had a history of recognized genital herpes. However, many of these HSV-2 antibody-positive individuals actually have symptoms of genital herpes that they can be taught to recognize, and most shed virus intermittently from genital skin.[10,11]

Recently, assays for detecting antibody to the type-specific herpes simplex virus surface glycoprotein-G (gG) have become available.[12,13] Unlike earlier HSV antibody tests, these gG-based assays permit accurate detection of antibodies to HSV-1, HSV-2, or both and can determine if an individual has been exposed to either or both viruses. The performance characteristics of these newer assays are good, but their sensitivity and specificity is still somewhat less than the HSV Western blot, the research assay that is the current gold standard for serologic diagnosis of HSV infection.[14–17]

Although it is helpful to have broader access to type-specific HSV antibody tests, many clinicians are uncertain where and how to use these tests. Because of the somewhat lower specificity of the newer tests compared with Western blot, targeted use in patients with an increased probability of having a positive test would be useful. Previous studies of HSV antibody testing in different populations found that generic STD predictors such as a history of another STD and number of sex partners correlated with a positive HSV-2 antibody test.[3,6] We conducted a study of HSV-2 antibody testing in adolescents and young adults with no history of genital herpes in an effort to better define those who want to be tested and to identify predictors of a positive HSV-2 antibody test.

These results were presented in part at the International Society for Sexually Transmitted Diseases Research meeting, Ottawa, Ontario, July 27–30, 2003.

All subjects provided written informed consent for this study (parental permission also was obtained for subjects <18 years old) using documents approved by local Institutional Review Boards and by the Institutional Review Board for the Centers for Disease Control and Prevention. The protocol was conducted according to federal and local guidelines for the conduct of human research.

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Methods

Study Subjects

Subjects were recruited from 4 different sites: an STD clinic and 2 primary care medical clinics (treated as 1 site for analysis) in Indianapolis, Indiana, and an adolescent health clinic and a college student population in Cincinnati, Ohio. More detail about these study sites is contained in the accompanying manuscript.[18] In the adult sites, all subjects were between the ages of 18 and 30 at enrollment; adolescents were between the ages of 14 and 20. All subjects denied a history of genital herpes as a criterion for enrollment. All subjects provided informed consent for the study by signing a form that was approved by both the local Institutional Review Board (IRB) and the Centers for Disease Control and Prevention's IRB. Subjects under the age of 18 also required written permission of a parent for participation.

Procedures

Initially, all subjects completed a detailed, self-administered questionnaire that contained questions on demographic variables, sexual behavior, mood, medical history, and knowledge and beliefs about STDs. The study entry criteria excluded all individuals with a known history of genital herpes, but the questionnaire included queries about genital symptoms suggestive of genital herpes. After completing the questionnaire, all subjects who were sexually experienced were offered free HSV-2 antibody testing. Issues surrounding acceptance of HSV-2 antibody testing in this group is reported elsewhere.[18]

Measures

Demographic variables included self-reported age, sex, ethnic background (Hispanic or not Hispanic), and race (black, white, Asian, Native American, or Pacific Islander). Because the 2 predominant racial/ethnic groups in the target population were non-Hispanic white and black, we used a dichotomized race classification (white vs. non-white) as a control variable in the modeling of blood test results. Health-related behavioral characteristics included the number of sexual partners in the last 6 months (before enrollment) and the proportion of coital events protected by condoms in the last 3 months. We also collected clinical and health information such as the subject's self-reported history of a previous STD, symptoms that might be suggestive of genital herpes (see subsequently in this article), and the clinical site at which the subject had been enrolled. A subject was considered as having a previous STD if he or she reported a history of any of the following: chlamydia infection, gonorrhea, syphilis, trichomoniasis, cervical dysplasia, venereal warts, HIV/AIDS, pelvic inflammatory disease, or pubic lice. Symptoms assessed in both male and female subjects were pain, itching, burning, and rashes in the genital region, buttocks, or upper thighs (4 items, $\alpha = 0.68$). In addition, males were asked about getting sore spots on their genitals from catching themselves with their zipper (subsequently referred to as "zipper burn"), and females were asked about getting yeast infections and about having unexplained vaginal discharges. Response categories to all of the symptom questions were based on frequency. A 5-point scale that ranged from "almost never (less than yearly)" (value = 0) to "very often (monthly or more)" (value = 5) was used. Finally, genital herpes-related knowledge, beliefs, and attitudes were measured by summary scores of relevant items in the questionnaire. The consistency of the responses to these questions was estimated using Cronbach's α coefficient. These included knowledge (13 items, based on the 816 subjects with test results, $\alpha = 0.62$), stigma (11 items, $\alpha = 0.76$), perceived severity of genital herpes (7 items, $\alpha = 0.79$), perceived vulnerability to HSV infection (4 items, $\alpha = 0.87$), perceived benefit of getting a herpes screening test (2 items, $\alpha = 0.61$), fear of needles (3 items, $\alpha = 0.84$), and the perceived extent to which a diagnosis of genital herpes would interfere with interpersonal relationships (5 items, $\alpha = 0.84$). The knowledge and attitude measures are discussed in greater detail elsewhere.[18]

Laboratory Procedures

Blood was processed within 12 hours and serum was either assayed immediately or frozen. Serum was assayed using the HerpeSelect enzyme-linked immunoassay for HSV-2 antibody (Focus Technologies, Cypress, CA). Assays were interpreted according to the manufacturer's instructions. Positive sera were confirmed by Western blot.[19] To be considered positive for this analysis, a serum was required to have a positive HerpeSelect 2 test and a positive Western blot for HSV-2. Sera that were positive or equivocal by HerpeSelect 2 but negative by Western blot for HSV-2 were considered negative.

Statistical Methods

To assess the associations between the response variable (HSV-2 seropositivity) and the information gathered in the questionnaire, we used logistic regression analyses. We first fitted univariate logistic regression models for all of the variables listed in Table 2. This allowed us to understand the effect for each of the predictors without controlling other factors. In the analysis, if the predictor was a categorical variable with multiple response levels, a reference level was first selected (marked by asterisks in Table 2); other levels of the variable were then compared with the designated reference level. *P* values from the univariate logistic regression models are reported in the same table. We then extended the analysis to multiple logistic regression models that included relevant demographic, clinical, behavioral, and attitudinal factors as predictors. In doing so, we were able to assess the effect of each of the predictors while controlling for the effects of other covariates in the model. To determine the predictors in the final model, we used a stepwise model selection procedure to screen the variables in Table 2. A significance level of 0.10 was adopted as the criterion for a variable to enter the model and for a variable to remain in the regression model. The selected model was then confirmed by forward and backward model selection procedures. In this analysis, all 3 different model selection methods reached the same final model. To accommodate the different genital symptoms unique to each gender, we fitted separate logistic models for male and female subjects.

Results

Subject Characteristics

A total of 1199 sexually experienced subjects were enrolled in the study and completed the questionnaire. All were offered HSV-2 antibody testing and 820 subjects agreed to be tested. The data presented here refer to a group of 816 subjects because 4 subjects had test results that could not be interpreted. Because study subjects were recruited at 4 different venues with very different demographic characteristics, the age, race, and gender distributions are shown in Table 1 for each clinical site as well as the total population. Overall, more than half of the study subjects (58.8%) were women; only the STD clinic site enrolled more men than women. Nearly all subjects identified themselves as either white or black and very few considered themselves Hispanic. Because somewhat more than half of the subjects identified them-

TABLE 1. Sample Demographic Characteristics and HSV 2 Antibody Positivity by Data Collection Site

Site	N	Mean Age, Years (SD)	Male Gender No. (%)	White Race* No. (%)	Positive HSV 2 Test No. (%)
STD clinic	307	23.2 (3.64)	159 (52.0)	147 (47.9)	69 (22.5)
Medicine clinics	154	23.2 (3.64)	26 (16.9)	116 (75.3)	26 (16.9)
University students	221	22.3 (2.90)	92 (41.6)	178 (80.5)	7 (3.2)
Adolescent clinic	134	17.9 (1.57)	59 (44.0)	31 (23.1)	4 (3.0)
Total sample	816	22.1 (3.72)	336 (41.2)	472 (57.8)	106 (13.0)

*Subjects were asked to identify themselves with 1 of 5 racial categories (black, white, Asian, Native American, or Pacific Islander). Because there were few subjects in the latter 3 categories, race was dichotomized to "white" and "non-white" for analysis.
STD = sexually transmitted disease; SD = standard deviation.

selves as white, subjects were classified as white or non-white for data analysis; there were too few individuals who identified themselves as members of other races or ethnic groups to permit individual analysis.

Univariate Analysis

The factors that were considered in the univariate analysis are shown in Table 2. Among the tested variables, older age (odds ratio [OR], 1.20 for every year of increased age), non-white race (OR, 2.81), history of an STD (OR, 4.58), female gender (OR, 1.92), having more than 1 sexual partner in the last 6 months (OR, 3.86), and higher genital symptom score (OR, 1.09) were associated with HSV-2 seropositivity. Attending college (OR, 0.11) or adolescent (OR, 0.11) site compared with the STD clinic was negatively associated with a positive HSV-2 antibody test. Other significant factors included perceived vulnerability (OR, 1.10), benefit of HSV testing (OR, 1.27), and fear of needles (OR, 1.06), all of which were positively associated with a positive HSV-2 antibody test result.

Multivariate Analysis

Because the genital symptom questions included items that were unique to men or women, separate models were fitted for males and females in the multiple logistic regression analysis. The genital symptom questions were divided into those that were common in both sexes and those that were gender-specific. The results of the

TABLE 2. Univariate Predictors of HSV-2 Antibody Positivity

Variable	HSV 2 Antibody Positive (n = 106)	HSV 2 Antibody Negative (n = 710)	*P* Value
Gender			0.0042
Male	9%	91%	
Female*	16%	84%	
Age (range 14–30)	24.3 (3.6)‡	21.8 (3.6)‡	<0.0001
Race			<0.0001
White*	8%	92%	
Non-white	20%	80%	
Site			<0.0001
STD clinic*	22%	78%	
General med. clinics	17%	83%	
College campus	3%	97%	
Adolescent clinic	3%	97%	
Knowledge (0–13†)	11.2 (2.0)‡	11.5 (1.7)‡	
Severity (5–35†)	19.9 (5.3)‡	19.9 (5.7)‡	
Vulnerability (4–20†)	11.0 (4.5)‡	9.2 (4.6)‡	
Benefits (2–10†)	8.8 (1.4)‡	8.2 (1.7)‡	
Fear of Needles (3–15†)	7.7 (3.2)‡	7.1 (3.1)‡	
STD Stigma (0–11†)	1.7 (2.0)‡	2.0 (2.2)‡	
Relationship Interference (5–25†)	18.3 (3.9)‡	18.2 (4.7)‡	
Proportion of coital events protected by condoms	0.4 (0.6)‡	0.6 (1.7)‡	
Common genital symptoms** (0–20†)	2.7 (4.0)‡	1.8 (2.7)‡	
STD history			<0.0001
Yes	23%	77%	
No*	6%	94%	
No. of partners (previous 6 mo)			0.0056
0*	5%	95%	
1	11%	89%	
>1	18%	82%	

*Designated reference level for categorical variables.
†Nos. in parentheses indicate the range of possible scores for each item; all scales are constructed such that higher numbers indicate a greater effect (or perceived effect).
‡Mean and standard deviation.
**Symptoms common to both males and females.

TABLE 3. Results of the Multivariate Analysis for Male Subjects

Parameter	Odds Ratio	95% Confidence Interval	*P* Value
Age	1.331	1.182, 1.499	<0.0001
Race*	4.207	1.589, 11.138	0.0038
STD history†	5.512	2.092, 14.527	0.0006
Male symptom	1.729	0.898, 3.330	0.1016

*Comparison is between dichotomized variables, non-white to white.
†Lifetime history of any sexually transmitted disease.

multiple logistic regression models for males and females are shown in Tables 3 and 4, respectively. For males, older age (OR, 1.33), non-white race (OR, 4.21), and history of an STD (OR, 5.51) were associated with a positive HSV-2 antibody test. In other words, for the male participants, there was a 33% increase in the odds of being seropositive for each year increase in age; the odds of a positive test in a non-white male was 4.21 times that of a white male; and the odds of a positive test in a subject with an STD history was 5.51 times that of a male individual who had no known STDs in the past. The effect of the single male-specific symptom ("zipper burn") was not significantly associated with a positive HSV-2 antibody result (OR, 1.73). Finally, we noted that the reported model included clinical site as a significant control variable. For females, older age (OR, 1.11), non-white race (OR, 3.45), and history of an STD (OR, 2.27) were again associated with a positive HSV-2 antibody test. The score for symptoms common to both males and females was only marginally significantly associated with a positive test (OR, 1.074). The other parameters that were identified in the univariate analysis were not significant in the multivariate models.

Discussion

The use of antibody testing to identify herpes simplex virus infections has been unclear to clinicians for a number of years. Because of the genetic similarity of HSV-1 and HSV-2, most of the surface proteins are immunologically crossreactive. HSV antibody tests that use crude virus as the antigen in the assay cannot accurately distinguish antibody generated as a result of exposure to HSV-1 from antibody generated from exposure to HSV-2, especially in someone who was previously exposed to the heterologous HSV type (see references [12,13,20] for reviews). Nonetheless, HSV antibody test kits containing both crude HSV-1 and HSV-2 antigens continue to be marketed and offered to clinicians by diagnostic laboratories. Many of those laboratories report results from the HSV-1 and HSV-2 assays separately, giving the impression that the result is type-specific, despite ample evidence in the literature to the contrary.[21] This has given many clinicians the impression that type-specific HSV antibody testing has been available for many years. However, the only truly type-specific tests were the HSV Western blot,[22–24] the gG immunodot assay,[18,25] and a few other "homemade" assays.[26] All of these tests are research tools with only limited (if any) availability to most clinicians and none has been commercialized.

The HSV envelope protein, gG, is the only HSV surface protein that is immunologically distinct between the 2 HSV serotypes. Antibody assays for HSV-1 and HSV-2 based on gG reactivity became commercially available in 1999. Interest in using these assays has grown slowly, in part because many clinicians did not recognize the added value these tests provided compared with earlier assays. However, as interest in these type-specific assays increases, clinicians and public health authorities may need guidance on how best to use the newer tests. Although they represent a distinct improvement over previous assays, these are not perfect tests.[27] For example, the HerpeSelect enzyme immunoassay, the test used for screening in this study, has a reported sensitivity of 96% to 98% and a specificity of 95% to 98%. Although these numbers are good, when applied to a low-prevalence population, false-positive tests can be a problem. In our 2 lowest prevalence populations, the adolescent clinic attendees and the college students, we identified 11 subjects whose initial positive tests were confirmed by Western blot, but we also had 15 subjects with a positive enzyme immunoassay that failed to confirm by Western blot. These numbers are very close to those predicted for a test with 98% sensitivity and 96% specificity applied to a population with a prevalence of approximately 3% if it is assumed that those that confirmed are true-positives and those that failed to confirm are false-positive tests. Similarly, in the other 2 populations, we identified 95 confirmed HSV-2-positive subjects and 17 that failed to confirm, presumably false-positive tests. These numbers are also consistent with these specificity values in a population with a seroprevalence around 20%.

One previous study[28] attempted to develop a scoring system for predicting who was at increased risk for HSV-2 infection. This system was based on demographic parameters and behavioral risks (such as number of sexual partners). Although this system had

TABLE 4. Results of the Multivariate Analysis for Female Subjects

Parameter	Odds Ratio	95% Confidence Interval	*P* Value
Age	1.111	1.024, 1.206	0.0116
Race*	3.452	1.915, 6.224	<0.0001
STD history†	2.273	1.253, 4.124	0.0069
Common symptom score‡	1.074	0.991, 1.164	0.0823
Site§			0.0017

*Comparison is between dichotomized variables, non-white to white.
†Lifetime history of any sexually transmitted disease.
‡Score for symptoms common to both males and females.
§STD clinic was the reference site.

some use, its value varied in different clinical settings. Because previous studies showed that nearly 90% of persons who are HSV-2 antibody-positive but give no clinical history of genital herpes and who are educated about symptoms of genital herpes subsequently have genital lesions or localized genital symptoms consistent with genital herpes,[10,11] we sought to use a genital symptom questionnaire as a possible predictor of HSV-2 antibody positivity. This questionnaire asked about relatively nonspecific signs and symptoms such as pain, itching, burning, and rashes in the genital region, buttocks, or upper thighs as well as "zipper burns" in men and "yeast infections" and unexplained vaginal discharges in women. Anecdotally, these are the kinds of symptoms of genital herpes that infected persons might have but not recognize as being caused by HSV. Because genital HSV-2 infection tends to recur, we hypothesized that infected persons would experience these symptoms repeatedly, and so the questionnaire asked about the frequency of these symptoms and was scored on that basis. The symptom questionnaire scores were only marginally associated with a positive HSV-2 antibody test and only in women. It is possible that we did not ask about the most predictive symptoms or that using the frequency of the symptoms as the basis for the symptom score was not the most appropriate scoring system. It is also possible that these symptoms are too nonspecific to be helpful or that subjects without a history of genital herpes (or other STDs) pay little attention to minor symptoms. The observation that the genital symptom scores were very low for all subjects (see Table 2) is consistent with the latter possibility. We also explored the possibility that younger subjects may be less cognizant of such symptoms by analyzing the results from the medicine and STD clinics and excluding the adolescents and college students. The results of this subgroup analysis were not different from the analysis of the whole study population. Additional studies will need to be conducted to determine if a symptom score of some type can be used help determine which individuals might benefit from testing for HSV-2 antibody.

The purpose of this study was to specifically evaluate persons with no history of genital herpes for factors that may predict HSV-2 positivity. One previous study[3] evaluated adult patients in a primary care setting for HSV-2 antibody, but approximately 7% of those subjects had a history of genital herpes and the population had a mean age of 33. That study also identified older age, female gender, non-white race, and STD history as predictors of HSV-2 positivity. The previous study also identified number of sexual partners as a predictor of a positive HSV-2 antibody test. In our study, number of sexual partners was significant only in the univariate analysis. Another study[10] included a group of subjects with no history of genital herpes, but that study focused on subsequent development of symptoms rather than symptoms at baseline.

Serologic testing for HSV-2 infection can be helpful in a variety of clinical settings. Examples would include persons with a history of recurrent genital symptoms with negative HSV cultures and stable couples in which 1 partner has symptomatic genital herpes and the other partner has no history of genital herpes. This study would suggest that certain individuals such as men or women with multiple sexual partners or a history of another STD might also be candidates for HSV-2 antibody testing. However, widespread screening, especially of low-risk populations, should not be undertaken unless the performance characteristics of the assays are improved or confirmatory testing is readily available.

References

1. Johnson RE, Nahmias AJ, Magder LS, et al. A seroepidemiologic survey of the prevalence of herpes simplex virus type 2 infection in the United States. N Engl J Med 1989; 321:7–12.
2. Fleming DT, McQuillan GM, Johnson RE, et al. Herpes simplex virus type 2 in the United States, 1976 to 1994. N Engl J Med 1997; 337:1105–1111.
3. Wald A, Koutsky L, Ashley RL, et al. Genital herpes in a primary care clinic: Demographic and sexual correlates of herpes simplex type 2 infections. Sex Transm Dis 1997; 24:149–155.
4. Brown ZA, Selke S, Zeh J, et al. The acquisition of herpes simplex virus during pregnancy. N Engl J Med 1997; 337:509–515.
5. Koutsky LA, Ashley RL, Holmes KK, et al. The frequency of unrecognized type 2 herpes simplex virus infection among women. Implications for the control of genital herpes. Sex Transm Dis 1990; 17:90–94.
6. Austin H, Macaluso M, Nahmias A, et al. Correlates of herpes simplex virus seroprevalence among women attending a sexually transmitted disease clinic. Sex Transm Dis 1999; 26:329–334.
7. Whittington WLH, Celum CL, Cent A, et al. Use of a glycoprotein G-based type-specific assay to defect antibodies to herpes simplex virus type 2 among persons attending sexually transmitted disease clinics. Sex Transm Dis 2001; 28:99–104.
8. Rosenthal SL, Stanberry LR, Biro FM, et al. Seroprevalence of herpes simplex virus types 1 and 2 and cytomegalovirus in adolescents. Clin Infect Dis 1997; 24:135–139.
9. Lewis LM, Bernstein DI, Rosenthal SL, et al. Seroprevalence of herpes simplex virus-type 2 in African-American college women. J Natl Med Assoc 1999; 91:210–212.
10. Langenberg A, Benedetti J, Jenkins J, et al. Development of clinically recognizable genital lesions among women previously identified as having 'asymptomatic' herpes simplex virus type 2 infection. Ann Intern Med 1989; 110:882–887.
11. Wald A, Zeh J, Selke S, et al. Reactivation of genital herpes simplex virus type 2 infection in asymptomatic seropositive persons. N Engl J Med 2000; 342:844–850.
12. Ashley RL. Sorting out the new HSV type specific antibody tests. Sex Transm Infect 2001; 77:232–237.
13. Wald A, Ashley-Morrow R. Serological testing for herpes simplex virus (HSV)-1 and HSV-2 infection. Clin Infect Dis 2002; 35:S173–S182.
14. Ashley RL, Eagleton M. Evaluation of a novel point of care test for antibodies to herpes simplex virus type 2. Sex Transm Infect 1998; 74:228–229.
15. Ashley RL, Wu L, Pickering JW, et al. Premarket evaluation of a commercial glycoprotein G-based enzyme immunoassay for herpes simplex virus type-specific antibodies. J Clin Microbiol 1998; 36: 294–295.
16. Ashley RL, Eagleton M, Pfeiffer N. Ability of a rapid serology test to detect seroconversion to herpes simplex virus type 2 glycoprotein G soon after infection. J Clin Microbiol 1999; 37:1632–1633.
17. Ashley RL, Wald A, Eagleton M. Premarket evaluation of the POCkit HSV-2 type-specific serologic test in culture-documented cases of genital herpes simplex virus type 2. Sex Transm Dis 2000; 27:266–269.
18. Zimet GD, Rosenthal SL, Fortenberry JD, et al. Factors predicting the acceptance of herpes simplex virus type 2 antibody testing among adolescents and young adults. Sex Transm Dis 2004; 31:665–669.
19. Ashley RL, Militoni J, Lee F, et al. Comparison of western blot (immunoblot) and glycoprotein G-specific immunodot enzyme assay for detecting antibodies to herpes simplex virus types 1 and 2 in human sera. J Clin Microbiol 1988; 26:662–667.
20. Ashley RL, Wald A. Genital herpes: Review of the epidemic and potential use of type- specific serology. Clin Microbiol Rev 1999; 12:1–8.
21. Ashley R, Cent A, Maggs V, et al. Inability of enzyme immunoassays to discriminate between infections with herpes simplex virus types 1 and 2. Ann Intern Med 1991; 115:520–526.
22. Bernstein DI, Lovett MA, Bryson YJ. Serologic analysis of first-episode nonprimary genital herpes simplex virus infection. Presence of type 2 antibody in acute serum samples. Am J Med 1984; 77:1055–1060.

23. Bernstein DI, Garratty E, Lovett MA, et al. Comparison of western blot analysis to microneutralization for the detection of type-specific herpes simplex virus antibodies. J Med Virol 1985; 15:223–230.
24. Ashley R, Benedetti J, Corey L. Humoral immune response to HSV-1 and HSV-2 viral proteins in patients with primary genital herpes. J Med Virol 1985; 17:153–166.
25. Lee FK, Coleman RM, Pereira L, et al. Detection of herpes simplex virus type 2-specific antibody with glycoprotein G. J Clin Microbiol 1985; 22:641–644.
26. Slomka MJ, Ashley RL, Cowan FM, et al. Monoclonal antibody blocking tests for the detection of HSV-1- and HSV-2-specific humoral responses: comparison with Western blot assay. J Virol Methods 1995; 55:27–35.
27. Schmid DS, Brown DR, Nisenbaum R, et al. Limits in reliability of glycoprotein G-based type-specific serologic assays for herpes simplex virus types 1 and 2. J Clin Microbiol 1999; 37:376–379.
28. Copas AJ, Cowan FM, Cunningham AL, et al. An evidence based approach to testing for antibody to herpes simplex virus type 2. Sex Transm Infect 2002; 78:430–434.