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Premarket Evaluation of a Commercial Glycoprotein G-Based Enzyme Immunoassay for Herpes Simplex Virus Type-Specific Antibodies

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A new commercial glycoprotein G-based enzyme immunoassay (gG-EIA) was compared with Western blotting (WB) for detection of herpes simplex virus type 1 (HSV-1) or HSV-2 type-specific antibodies in 193 serum samples. Sensitivity for HSV-1 was 95%; specificity was 96%. Sensitivity for HSV-2 was 98%; specificity was 97%. Twelve of 13 serum samples with equivocal gG-EIA results were serotyped by WB.

The genital herpes epidemic continues (6, 9, 15) because most herpes simplex virus type 2 (HSV-2) infections are subclinical or undiagnosed (7, 10, 12, 20). Most persons who transmit HSV-2 to a sex partner (4, 14, 17) and most women who infect their infants at parturition (16, 22) are unaware that they have genital herpes. Since virtually all persons who are HSV-2 seropositive shed virus intermittently from anogenital sites (19), identification of subclinical infections may be important for halting transmission. In fact, once identified, more than half of "asymptomatic" patients can be taught to recognize the symptoms that accompany genital tract shedding of HSV-2 (12). Identification of pregnant women with subclinical HSV-2 and, more urgently, identification of HSV-seronegative women at risk of acquiring genital herpes close to term from an HSV-1- or HSV-2-seropositive partner could lead to counselling or antiviral interventions against neonatal herpes (5). Serology is the most effective way to diagnose subclinical HSV-2, but currently available tests are of limited value because they cannot accurately discriminate between HSV-1 and HSV-2 antibodies (1). Tests such as Western blotting (WB) can accurately identify HSV-1 and HSV-2 antibodies but are not widely available or easily adapted to commercial laboratory use (1–3). WB was used for a premarket evaluation of a rapid enzyme immunoassay (EIA) based on type-specific glycoprotein G-1 (gG-1) from HSV-1 and gG-2 from HSV-2.

Prototype 96-well plates coated with gG-1 and gG-2 were used in accordance with the manufacturer's instructions (Gull Laboratories, Salt Lake City, Utah). All incubations were for 30 min at 37°C. Test sera and a reference serum were diluted 1:21 in specimen diluent and dispensed, in duplicate, at 100 μl per well. After plates were washed, alkaline phosphatase-labeled anti-human immunoglobulin G was added. After incubation and washing, 100 μl of the substrate *p*-nitrophenyl phosphate was added. Reactions were stopped with stopping reagent, and A_{405} was determined (BioTek Instruments, Winooski, Vt.). Mean absorbance values >0.99 times that of the reference were scored as positive, those <0.91 times the reference were scored as negative, and inclusive values between 0.91 and 0.99 times the reference were scored as equivocal.

Sera submitted to the University of Washington Virology Laboratory were tested by HSV WB type-specific serology as previously described (1–3). Results were transcribed to a computerized database (Borland, Scotts Valley, Calif.), and then 193 serum samples were coded for testing by the gG-based EIA (gG-EIA). Clinical details were not available on the patients who submitted sera for HSV diagnostic testing.

Definitive typing results were obtained with both assays for 172 (89%) serum samples; 160 of these (93%) gave identical WB and gG-EIA results: 53 (33%) negative, 59 (37%) HSV-1 positive, 23 (14%) HSV-2 positive, and 25 (16%) dually positive results. Of 12 discordant results, 5 (42%) were negative by gG-EIA but positive for HSV-1 by WB; 3 of these 5 had WB profiles suggesting low antibody titers. Two serum samples were positive by gG-EIA for HSV-2 or dual antibodies but negative by WB. Four serum samples were dually positive by gG-EIA but WB positive for only HSV-1 ($n = 2$) or only HSV-2 ($n = 2$). One serum sample was HSV-1 positive by gG-EIA but dually positive by WB. The sensitivity of gG-EIA for HSV-1 was 95%, and the specificity was 96%, with positive and negative predictive values of 97 and 86%, respectively. The sensitivity of gG-EIA for HSV-2 was 98%, and the specificity was 97%, with positive and negative predictive values of 96 and 97%, respectively.

Thirteen serum samples (7%) gave equivocal gG-EIA results: five for HSV-1, six for HSV-2, and two for both viruses. Of these 13 serum samples, 5 had low titers of antibodies, as inferred from limited WB profiles. The other eight serum samples were equivocal for either HSV-1 or HSV-2 but negative for the respective antibodies by WB. These eight serum samples could be falsely negative by WB or falsely positive by gG-EIA. Thus, while five serum samples that produced equivocal gG-EIA results actually had low titers of antibodies to the correct virus type, it would not be prudent to interpret all equivocal results as indicating true positives.

While gG-EIA had high sensitivity and specificity for both HSV-1 and HSV-2, the test results may be falsely negative for sera from patients who have yet to seroconvert to gG-1 or gG-2 positivity. By WB, 5 to 10% of HSV-2 patients lack detectable antibodies to denatured gG-2 for prolonged periods after infection (13). The kinetics of seroconversion to either HSV-1 or HSV-2 positivity by gG-EIA have not been elucidated. Negative or equivocal results should be confirmed by WB, or later sera should be tested if seroconversion is suspected. Also, as

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previously described for a different gG-2-specific antibody test, the gG-EIA may be falsely negative for a substantial proportion of HIV-infected subjects (18). WB, which detects antibodies to 18 to 20 proteins, has been more sensitive in these cases (18). Further studies with clinically defined populations are needed to determine the performance of this gG-EIA with sera from immunocompromised patients.

The interpretation of any type-specific HSV serology result must incorporate history, clinical presentation, and assessment of risk for genital herpes (19). HSV-1 antibodies may be due to oral herpes or, less commonly, to genital HSV-1 infection; no serologic test can determine the site of HSV-1 infection. Conversely, essentially all HSV-2-positive antibody results derived from accurate type-specific tests are due to anogenital herpesvirus infections (11). Interpretation of HSV type-specific antibodies in pregnant women and their partners is an important issue, since serodiscordant couples are at risk of both horizontal and vertical HSV transmission. Detailed discussions of HSV serologic testing in pregnancy have been published (5, 16).

Because treatment options are available for suppression of both symptomatic and asymptomatic shedding (21), demand for type-specific serologic identification of subclinical HSV-2 infections is likely to increase (8). The gG-EIA is rapid (<1.5 h) and easy to perform manually or with an EIA processor. In our study, the gG-EIA accurately detected and typed HSV antibodies in 93% of sera (160 of 172) typed by both assays. Only 7% of sera (13 of 193) had equivocal gG-EIA results; 12 were resolved by WB. The most cost-effective and accurate approach for HSV serological testing appears to be screening by gG-EIA, followed by WB to definitively type sera with equivocal gG-EIA results.

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