

Plasma versus Serum for Detection of Herpes Simplex Virus Type 2-Specific Immunoglobulin G Antibodies with a Glycoprotein G2-Based Enzyme Immunoassay

Thomas L. Chernes, ^{1,2*} Leslie A. Meyn, ³ and Sharon L. Hillier ^{2,3}

Department of Medicine, Division of Infectious Disease,¹ Department of Obstetrics, Gynecology, and Reproductive Sciences,³ and Magee-Womens Research Institute,² University of Pittsburgh, Pittsburgh, Pennsylvania

Received 6 December 2002/Returned for modification 17 January 2003/Accepted 19 March 2003

To evaluate the consonance between plasma and serum for the detection of herpes simplex virus type 2-specific immunoglobulin G antibodies, we compared results from concurrently obtained plasma and sera in 710 sexually active women by using a glycoprotein G2-based enzyme-linked immunosorbent assay (Focus Technologies, Cypress, Calif.) and found 98.9% agreement between the two specimen types. Plasma appears to be an acceptable matrix for use with this assay.

The use of herpes simplex virus (HSV) glycoprotein G (gG)-based enzyme-linked immunosorbent assays (ELISAs) for the serologic diagnosis of HSV infections has become increasingly widespread (6). Although HSV type 1 (HSV-1) and HSV-2 exhibit a high degree of serologic cross-reactivity, an envelope glycoprotein, gG, is antigenically distinct between the two viruses (4). Therefore, detectable gG-1 and gG-2 antibodies are reliable indicators of past or present HSV-1 and HSV-2 infections, respectively. A gG-2 ELISA (HerpeSelect 2) from Focus Technologies (Cypress, Calif.) is a commercially available kit that has been approved by the U.S. Food and Drug Administration for the detection of gG-2 immunoglobulin G antibodies in the sera of sexually active adults and expectant mothers. In comparison to Western blotting, HerpeSelect 2 has a sensitivity of 97.9% and a specificity of 95.4% (3). However, it has been labeled for use only with serum.

In cross-sectional and longitudinal studies of sexually transmitted diseases, the collection and storage of blood samples from study participants are usually an integral component of the investigation. Human immunodeficiency virus (HIV) and HSV-2 are sexually transmitted diseases that are often studied in tandem, as there is increasing evidence that HSV-2 facilitates both transmission and acquisition of HIV (5). Plasma is the most appropriate matrix for HIV load determination (2), but its reliability for HSV-2 antibody detection is not known. Therefore, in any large-scale epidemiologic investigations of HIV and HSV-2, (measuring outcomes from thousands of individuals and performing multiple blood draws on each individual for extended periods), the necessity of storing both serum and plasma specimens in repository would add considerably to study costs. Furthermore, it is likely that the chance for specimen misidentification in this type of study could be reduced if it were acceptable to collect and store only one specimen type. Therefore, we sought to compare HerpeSelect 2 results from concurrently obtained plasma and sera in a

cohort of young, sexually active women in order to evaluate whether plasma was an acceptable matrix for the detection of type-specific HSV-2 antibodies.

The sera and plasma used in our investigation were collected during a prior investigation of the risk factors associated with pelvic inflammatory disease (7). Study participants were recruited from three Pittsburgh, Pennsylvania, area sites: the Allegheny County Health Department's Sexually Transmitted Diseases Clinic, the Magee-Womens Hospital Ambulatory Care Clinic, and the Family Health Council Clinic of Aliquippa. Eligible women were between 15 and 30 years of age and had either purulent cervical discharge, an untreated *Neisseria gonorrhoeae* or *Chlamydia trachomatis* infection, bacterial vaginosis, or sexual contact with a male diagnosed with gonorrhea, chlamydial, or nongonococcal urethritis. Approval for the subsequent use of the concurrently obtained plasma and sera from the pelvic inflammatory disease investigation to assess the consonance between the two specimen types for detection of HSV-2 type-specific antibodies was obtained from the institutional review board of Magee-Womens Hospital. Sera and plasma were frozen and stored at -70°C until HSV-2 antibody testing was performed.

The use of the HerpeSelect 2 ELISA to detect gG-2 antibodies in serum has been previously described (1). Laboratory personnel used only one specimen type (plasma or sera) per kit and performed all experiments blinded from the previously obtained results. Index values greater than 1.10 were considered positive, while values less than 0.90 were negative. Specimens with equivocal results (an index value between 0.90 and 1.10 inclusive) were retested. The final result for a particular specimen was considered equivocal if the repeat index value again fell between the inclusive values of 0.90 and 1.10. The concordance between individual results obtained from serum and plasma was analyzed by using Pearson's correlation coefficient.

We detected antibodies to HSV-2 in sera in 43% (314 of 730) of the women. This was similar to the 44% (315 of 724) of the women who had detectable HSV-2 plasma antibodies. The median positive index value for women with detectable HSV-2 antibodies in their plasma was 7.37, similar to the median

* Corresponding author. Mailing address: Magee-Womens Research Institute, Room 540, 204 Craft Ave., Pittsburgh, PA 15213. Phone: (412) 641-1999. Fax: (412) 641-5290. E-mail: rsite@mail.magee.edu.

positive index value of 7.55 among the HSV-2-seropositive women. There were 710 women who had both plasma and serum results available, and the comparative performances of plasma and serum for the detection of gG-2 HSV-2 antibodies are given here. There were 401 women with no detectable HSV-2 antibodies in the two specimen types, while 300 women were HSV-2 antibody positive in both. Eight women with positive index values from plasma were classified as HSV-2 antibody seronegative, and one seropositive woman had undetectable HSV-2 antibodies in her plasma. Overall, there was 98.9% agreement between the two specimen types in this cohort of young, sexually active women (Pearson correlation coefficient = 0.974; $P < 0.001$). Examination of the data identified no associations between the inconsistent results and the date on which the assay was performed or the particular laboratory personnel responsible for performing an assay. In addition, the final results were unchanged when discordant serum and plasma specimens were subsequently retested.

This study indicates that the results obtained from plasma by using the HerpeSelect 2 ELISA are comparable to those found in concurrently obtained sera. Western blot analyses could have helped clarify which specimen type is the more likely to provide accurate results, but they were not performed. Although much more extensive validation of this diagnostic assay has been performed with sera, in large clinical trials optimal sampling methods must be balanced against the projected costs incurred from the collection and storage of these samples. It is also possible that an investigator may have only plasma in repository from a previous investigation, and our findings support its use for the detection of HSV-2 antibodies with the HerpeSelect 2 ELISA. In clinical scenarios, the collection of plasma alone for HIV load measurement and HSV-2 antibody detection may limit the number of required blood draws and

facilitate specimen processing. Since the results that we obtained for sera and plasma were so highly correlated, the use of plasma with the HerpeSelect 2 ELISA to detect HSV-2 antibodies appears warranted.

The research was supported by grants U01-AI-46745 and R01-AI-41624 from the National Institute of Allergy and Infectious Diseases. Thomas L. Chernes is a scholar in the AIDS and Molecular Microbiology/Epidemiology Training Program, National Institutes of Health (T32-AI07333).

We thank Joel Lurie and Kevin Stoner for their excellent technical assistance.

REFERENCES

1. Chernes, T. L., R. L. Ashley, L. A. Meyn, and S. L. Hillier. 2003. Longitudinal reliability of the Focus glycoprotein G-based type-specific enzyme immunoassays for herpes simplex virus types 1 and 2 in women. *J. Clin. Microbiol.* **41**:671–674.
2. Holodniy, M., L. Mole, B. Yen-Lieberman, D. Margolis, C. Starkey, R. Carroll, T. Spahlinger, J. Todd, and J. Jackson. 1995. Comparative stabilities of quantitative human immunodeficiency virus RNA in plasma from samples collected in Vacutainer CPT, Vacutainer PPT, and standard Vacutainer tubes. *J. Clin. Microbiol.* **33**:1562–1566.
3. Ribes, J. A., M. Hayes, A. Smith, J. L. Winters, and D. J. Baker. 2001. Comparative performance of herpes simplex virus type 2-specific serologic assays from Meridian Diagnostics and MRL Diagnostics. *J. Clin. Microbiol.* **39**:3740–3742.
4. Roizman, B., B. Norrild, C. Chan, and L. Pereira. 1984. Identification and preliminary mapping with monoclonal antibodies of a herpes simplex virus 2 glycoprotein lacking a known type 1 counterpart. *Virology* **133**:242–247.
5. Wald, A., and K. Link. 2002. Risk of human immunodeficiency virus infection in herpes simplex virus type 2-seropositive persons: a meta-analysis. *J. Infect. Dis.* **185**:45–52.
6. Whittington, W. L., C. L. Celum, A. Cent, and R. L. Ashley. 2001. Use of a glycoprotein G-based type-specific assay to detect antibodies to herpes simplex virus type 2 among persons attending sexually transmitted diseases clinics. *Sex. Transm. Dis.* **28**:99–104.
7. Wiesenfeld, H. C., S. L. Hillier, M. A. Krohn, A. J. Amortegui, R. P. Heine, D. V. Landers, and R. L. Sweet. 2002. Lower genital tract infection and endometritis: insight into subclinical pelvic inflammatory disease. *Obstet. Gynecol.* **100**:456–463.