CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, Feb. 2005, p. 359–360 Vol. 12, No. 2
1071-412X/05/$08.00+0 doi:10.1128/CDLI.12.2.359–360.2005

Performance of Two Commercial Enzyme-Linked Immunosorbent Assay Kits Using Recombinant Glycoprotein G2 Antigen for Detection of Herpes Simplex Virus Type 2 Specific Antibodies

Sharmila M. Reddy,[1] P. Balakrishnan,[1]* S. Uma,[1] S. P. Thyagarajan,[2] and Suniti Solomon[1]

YRG CARE, Centre for AIDS Research and Education,[1] and University of Madras,[2] Chennai, India

Received 22 July 2004/Returned for modification 15 September 2004/Accepted 17 November 2004

For 93 stored serum samples tested by HerpeSelect2 and the Euroimmun enzyme-linked immunosorbent assay for detection of herpes simplex virus type 2-specific immunoglobulin G antibodies, the concordance of positive and negative results was 100%. Moreover, all the results that were equivocal by HerpeSelect2 (negative by Euroimmun) were confirmed as being negative by a Western blot assay.

Genital herpes, which is predominantly caused by infection with herpes simplex virus type 2 (HSV-2), is one of the most common sexually transmitted diseases. Studies suggest that HSV-2 plays an important role in the transmission of human immunodeficiency virus infection in persons with high-risk behavior (1, 3–5, 9, 14). In clinically recognized disease, virus isolation or antigen detection from vesicular or ulcerative lesions is the preferred diagnostic method, but isolation may be difficult if lesions are crusted, healed, or absent (9, 13). Moreover, for community-based cross-sectional studies with asymptomatic populations, serological diagnosis is most commonly preferred (2, 3, 7, 8, 10). In recent years, HSV glycoprotein G (gG) has been identified as a viral protein that is type specific for HSV-2. Detection of antibodies produced against this gG has been proven useful for the diagnosis of primary genital herpes and as a screening test for asymptomatic pregnant women (8, 10, 13). Glycoprotein G-based enzyme-linked immunosorbent assays (ELISAs) for HSV-1 and HSV-2 are highly divergent and typically elicit very limited humoral cross-reactivity (11, 12). Therefore, the detection of gG2 antibodies is a reliable indicator of past or present HSV-2 infection. The aim of the present study was to investigate the performance of the Euroimmun kit in comparison with that of the U.S. Food and Drug Administration-approved HerpeSelect 2 kit.

A total of 93 serum samples stored at −70°C were randomly selected and tested by the HerpeSelect and Euroimmun (Lübeck, Germany) ELISA kits for HSV-2 immunoglobulin G (IgG) according to the manufacturer's instructions. The principles of HerpeSelect and Euroimmun are as follows: HerpeSelect provides a qualitative assay, and Euroimmun provides a semiquantitative or quantitative assay, for IgG class antibodies to HSV-2 in human serum. Both ELISA kits require that diluted serum samples and controls be incubated in the gG2 antigen-coated wells to allow the specific antibody present in the samples to react with the antigen. Peroxidase-conjugated anti-human IgG is added and reacts with specific IgG. An enzyme substrate and chromogen are added, and the color is allowed to develop. Color change is quantified by a spectrophotometric reading of optical density. HerpeSelect requires a 1:101 dilution of serum; high-positive, low-positive, and negative controls; and a calibrator. Euroimmun has ready-to-use (prediluted for 1:101) positive and negative controls, and calibrator 2. HerpeSelect requires a 1-h serum incubation and a 10-min substrate incubation, whereas Euroimmun requires a 30-min serum incubation and a 15-min substrate incubation. In HerpeSelect, an index value is obtained for each sample run, based on the absorbance of the patient sample divided by the mean absorbance of the cutoff calibrator; an index value of <0.9 is considered negative, a value of ≥ 0.9 but ≤ 1.1 is considered equivocal, and a value of >1.1 is considered positive. In the Euroimmun ELISA, an index value is obtained for each sample based on the absorbance of the patient sample divided by the absorbance of cutoff calibrator 2. A ratio of <1.0 is considered negative, and a ratio of ≥ 1.0 is considered positive. All the positive and negative results by the HerpeSelect ELISA were 100% in concordance with those of the Euroimmun ELISA. Of 93 randomly selected samples, 35 were positive and 35 were negative by both the kits. However, 23 samples that were equivocal by HerpeSelect were negative by the Euroimmun kit.

To confirm the results obtained by the two ELISA kits and the status of the equivocal samples, a Euroline Western blot (WB) assay was performed. The Euroline HSV-1/HSV-2 gG2 (IgG) WB kit (Euroimmun) is a commercially available kit that can differentiate between specific antibodies against HSV-1 and HSV-2. Sixteen samples positive by both ELISAs and 16 samples negative by both ELISAs were randomly chosen, as well as all 23 equivocal (by HerpeSelect) samples, and Western blotting was carried out according to the manufacturer's instructions. All 16 samples positive for HSV-2 and all 16 samples negative for HSV-2 were confirmed by the WB assay (Table 1). All 23 samples that were equivocal by HerpeSelect turned out to be negative for HSV-2 by the WB assay (Table 1), confirming the result obtained with the Euroimmun ELISA kit. Of these 23 equivocal samples, 20 were positive and 3 were negative for the presence of HSV-1 antibodies by the WB

* Corresponding author. Mailing address: YRG CARE, Centre for AIDS Research and Education, VHS, Taramani, Chennai 600 113, India. Phone: 91-44-22542929. Fax: 91-44-22542939. E-mail: bala@yrgcare.org.

TABLE 1. Results of two commercial ELISA kits and Western blotting for HSV-2 and HSV-1

ELISA result	No. of serum samples[a] with the indicated result by:		No. of samples confirmed by Western blotting	
	HerpeSelect2	Euroimmun	Positive for HSV-2	Positive for HSV-1
Positive	35	35	16	14
Negative	35	58	0	13
Equivocal	23		0	20

[a] A total of 93 serum samples were tested by the ELISAs.
[b] A total of 55 serum samples were tested by Western blotting: 16 samples positive by both ELISAs, 16 negative by both ELISAs, and 23 equivocal by the HerpeSelect2.

assay. Of 16 HSV-2-negative samples, 13 were positive for HSV-1 antibodies by the WB assay. Hence, the HerpeSelect ELISA has considerable cross-reactivity with the antigen of HSV-1.

Our study has shown that the Euroimmun ELISA for HSV-2 IgG is 100% sensitive and specific. A similar performance has been observed in a recent study (6). However, these two assays with gG2 protein disagreed for 23 (25%) of the 93 serum samples analyzed. This demonstrates the problem related to the use of a gG2 assay as the sole criterion for HSV-2 diagnosis. Therefore, an assay that measures immune responses to a single viral protein or to an individual epitope may be problematic, and an assay based on a viral antigen other than gG2, such as that described in a recent study (15), is needed. The present study was limited by the use of the WB assay kit from Euroimmun. Also, further studies with the Euroimmun ELISA are required to show the robustness and reproducibility of the results before this kit is considered for use in research and diagnosis of patients in developing countries.

We acknowledge CPC Pharmaceuticals Pvt. Ltd., Chennai, India, for funding this study.

REFERENCES

1. **Bernstein, D. I., M. A. Lovett, and Y. J. Bryson.** 1984. The effects of acyclovir on antibody response to herpes simplex virus in primary genital herpetic infections. J. Infect. Dis. **150:**7–13.
2. **Bossi, P.** 2002. Genital herpes: epidemiology, transmission, clinic, asymptomatic viral excretion, impact on other sexually transmitted diseases, prevention, and treatment. Ann. Dermatol. Venereol. **129:**477–493. (In French.)
3. **Corey, L., and H. H. Handsfield.** 2000. Genital herpes and public health: addressing a global problem. JAMA **283:**791–794.
4. **Corey, L.** 2000. Herpes simplex type 2 infection in the developing world: is it time to address this disease? Sex. Transm. Dis. **27:**30–31.
5. **Del Mar Pujades Rodriguez, M., A. Obasi, F. Mosha, J. Todd, D. Brown, J. Changalucha, D. Mabey, D. Ross, H. Grosskurth, and R. Hayes.** 2002. Herpes simplex virus type 2 infection increases HIV incidence: a prospective study in rural Tanzania. AIDS **16:**451–462.
6. **Eing, B. R., L. Lippelt, E. U. Lorentzen, W. Hafezi, W. Schlumberger, K. Steinhagen, and J. E. Kuhn.** 2002. Evaluation of confirmatory strategies for detection of type-specific antibodies against herpes simplex virus type 2. J. Clin. Microbiol. **40:**407–413.
7. **Eis-Hubinger, A. M., M. Daumer, B. Matz, and K. E. Schneweis.** 1999. Evaluation of three glycoprotein G2-based enzyme immunoassays for detection of antibodies to herpes simplex virus type 2 in human sera. J. Clin. Microbiol. **37:**1242–1246.
8. **Forsgren, M., E. Skoog, S. Jeansson, S. Olofsson, and J. Giesecke.** 1994. Prevalence of antibodies to herpes simplex virus in pregnant women in Stockholm in 1969, 1983 and 1989: implications for STD epidemiology. Int. J. STD AIDS **5:**113–116.
9. **Kinghorn, G. R.** 1994. Epidemiology of genital herpes. J. Int. Med. Res. **22**(Suppl. 1)**:**14A—23A.
10. **Lafferty, W. E.** 2002. The changing epidemiology of HSV-1 and HSV-2 and implications for serological testing. Herpes **9:**51–55.
11. **Lee, F. K., R. M. Coleman, L. Pereira, P. D. Bailey, M. Tatsuno, and A. J. Nahmais.** 1985. Detection of herpes simplex virus type 2-specific antibody with glycoprotein G. J. Clin. Microbiol. **22:**641–644.
12. **Liljeqvist, J. A., B. Svennerholm, and T. Bergstrom.** 1999. Herpes simplex virus type 2 glycoprotein G-negative clinical isolates are generated by single frameshift mutations. J. Virol. **73:**9796–9802.
13. **Martins, T. B., R. D. Woolstenhulme, T. D. Jaskowski, H. R. Hill, and C. M. Litwin.** 2001. Comparison of four enzyme immunoassays with a Western blot assay for the determination of type-specific antibodies to herpes simplex virus. Am. J. Clin. Pathol. **115:**272–277.
14. **Mbopi-Keou, F. X., N. J. Robinson, P. Mayaud, L. Belec, and D. W. Brown.** 2003. Herpes simplex virus type 2 and heterosexual spread of human immunodeficiency virus infection in developing countries: hypotheses and research priorities. Clin. Microbiol. Infect. **9:**161–171.
15. **Wales, S. Q., C. C. Smith, M. Wachsman, G. Calton, and L. Aurelian.** 2004. Performance and use of a ribonucleotide reductase herpes simplex virus type-specific serological assay. Clin. Diagn. Lab. Immunol. **11:**42–49.