Comparative Performance of a Novel Herpes Simplex Virus Type 2-Specific Enzyme-Linked Immunosorbent Assay Using a Targeted Chain Oligopeptide, Peptide 55

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Herpes simplex virus (HSV) glycoprotein G (gG2) has been used as the basis of many serological assays for the detection of HSV type 2 (HSV-2)-specific antibodies. In the present study, an enzyme-linked immunosorbent assay (ELISA), the Pathozyme Viro HSV-2 immunoglobulin G (IgG) ELISA (Omega Diagnostics, Alva, United Kingdom), based on an immunodominant epitope of gG2 presented in a branched-chain format (peptide 55), was compared with two commercially available gG2-specific assays, the Bioelisa HSV-2 IgG assay (Biokit, S.A., Barcelona, Spain) and the HerpeSelect HSV-2 IgG assay (Focus Diagnostics, Cypress, CA). A panel of 218 well-characterized serum samples was tested. Thirty-one samples were determined to be HSV-2 IgG antibody positive and 164 samples were determined to be negative with all three kits. The levels of concordance between the tests were 95.9% between the Omega and HerpeSelect assays, 90.8% between the Omega and Bioelisa assays, and 94.5% between the HerpeSelect and Bioelisa assays. Twenty-three samples gave discordant results. Western blot results showed that of these, the results for 77% were correctly identified by the Omega assay, the results for 68% were correctly identified by the HerpeSelect assay, and the results for 13.6% were correctly identified by the Bioelisa assay. Although there was a high level of agreement between the results obtained by the three assays and no false-positive results were detected by any of the three kits, confirmation of the results for samples with discordant results by Western blotting suggested that the peptide 55-based Omega assay is the most sensitive and specific assay among the assays evaluated.

Genital herpes is one of the most common sexually transmitted infections worldwide. Historically, herpes simplex virus type 2 (HSV-2) has predominantly been associated with genital infections; however, recent reports suggest that a considerable and increasing number of genital isolates are of HSV-1 (10, 24). The clinical course of primary genital herpes infections among patients infected with HSV-1 and HSV-2 are similar; however, there are differences in epidemiology and natural history of the diseases caused by the two viral subtypes (9). HSV-2 primary infection during pregnancy has been related to spontaneous abortion, prematurity, congenital infection, and neonatal herpes. Several recent studies have reported that genital herpes may increase the susceptibility of acquiring human immunodeficiency virus by persons with high-risk behavior, because HSV-2 can cause breaks in the genital mucosal barrier (16).

HSV-1 and HSV-2 have approximately 83% nucleotide sequence similarity, and for some proteins they share more than 85% identity (4). Both serotypes therefore show extensive serological cross-reactivity. This has impeded seroepidemiologic studies of the two viruses. The major antigenic determinants are glycoprotein antigens exposed on the virion surface (22), and although HSV-1 and HSV-2 are known to express more than 11 glycoproteins, 10 of these glycoproteins express multiple epitopes common to each type. A variety of studies have concluded that only glycoprotein G (gG2) expresses epitopes specific for HSV-2 (14, 21). Consequently, this glycoprotein has been used as the basis of type-specific serological assays for the diagnosis of HSV-2 infection (1, 2, 7, 19, 20). However, epitope mapping of gG2 by Levi et al. (11) suggested that some epitopes may cross-react with HSV-1-specific antibody.

In an effort to develop even greater selectivity for HSV-2, several research groups have studied HSV-2 type-specific epitopes within gG2. Regions comprising amino acids 350 to 427 and 525 to 587 also regions comprising amino acids 625 to 641 and 676 to 699 were identified by two independent groups (6, 11), and a secreted protein of gG2 comprising amino acids 23 to 340 was described by Liljqvist et al. (13) and Gorander et al. (5). Marsden et al. (15) developed multiple antigenic peptides corresponding to residues 561 to 578 of gG2, designated peptide 55. This peptide comprises four peptide copies attached to a branched lysine core and separated from the lysine core by four glycine residues. The sensitivity of the peptide for the detection of HSV-2 antibody was evaluated by screening a panel of HSV-2-positive human serum samples previously characterized by virus isolation and typed by indirect immunofluorescence with a type-specific monoclonal antibody. Peptide 55 was found to be sensitive and specific, and no false-positive results were detected with HSV-1 antibody-positive or HSV-1 or HSV-2 antibody-negative samples. Oladepo et al. (18) compared the performance of an assay with peptide 55 with that of a commercially available HSV-2-
specific immunoglobulin G (IgG) enzyme-linked immunosorbent assay (ELISA) kit based on affinity-purified gG2 (Gull Laboratories) and found that peptide 55 had the same sensitivity; however, it was more specific than the complete protein. Nilsen et al. (17) tested the performance of an ELISA based on peptide 55 and compared it with the performance of two different assays, an ELISA developed by Ho et al. (8) based upon native gG2 selected by affinity to Helix pomatia lectin, and an ELISA based on affinity-purified gG2 (Gull Laboratories), and found that the performance of the assay with peptide 55 was better than that of the other ELISA methods.

In the present study, an ELISA (Pathoyme Viro HSV-2 ELISA IgG; Omega Diagnostics Ltd., Scotland) based on peptide 55 was compared with two other commercially available HSV-2-specific IgG ELISAs, the HerpeSelect HSV-2 ELISA (Focus Diagnostics, Cypress, CA), which uses purified recombinant type-specific gG2 antigen, and the Bioelisa HSV-2 IgG-specific assay (Biokit, S.A., Barcelona, Spain), which uses affinity-purified gG2 protein, for the abilities to detect and serologically differentiate HSV-2 antibody. The three assays are referred to in this study as the HerpeSelect, Bioelisa, and Omega assays, respectively.

MATERIALS AND METHODS

Patient sera. A total of 218 human serum samples collected between January 2006 and June 2007 were studied. The samples were collected from the serum bank of Clinical Virology, Manchester Royal Infirmary, and were stored in 100-μl aliquots at −70°C until use. The specimens were reused for this study in accordance with current Royal College of Pathologists guidelines (23) on the reuse of diagnostic specimens. The specimens and the associated clinical data were collected; the specimens were anonymously labeled by renumbering and removal of all patient identifiers from the data before use in this study. All samples had been routinely tested for the presence of HSV antibody by a common type-specific assay (DiaSorin S.p.A., Saluggia, Italy). For differentiation between HSV-1 and HSV-2 antibodies, HSV-positive samples were retested by using the Virotech HSV-1-specific gG1 IgG assay (EC103:00; Genzyme Virotech GmbH, Germany) and the Biokit HSV-2-specific gG2 IgG assay (Biokit, S.A.), respectively. Sera were grouped into one of four groups on the basis of the results of these assays: group 1 sera were HSV-1 positive and HSV-2 negative (n = 138), group 2 sera were HSV-1 negative and HSV-2 positive (n = 9), group 3 sera were HSV-1 and HSV-2 positive (n = 37), and group 4 sera were HSV-1 and HSV-2 negative (n = 34).

Test comparison. All sera were tested by each of the three HSV-2-specific assays. Testing was performed in accordance with the manufacturers’ instructions. The absorbances at 450 nm were read with an ELx808 Universal microplate reader (BioTek Instruments Inc.). The HerpeSelect and Bioelisa assays use a 1:10 dilution of test serum, whereas the Omega assay uses a 1:100 initial dilution of serum. The HerpeSelect assay uses an initial 60-min serum incubation, a 30-min incubation with assay conjugate, and 10 min of substrate development at room temperature. The Omega and Bioelisa ELISA kits require a 60-min serum incubation, a 30-min incubation with conjugate, and 15- and 30-min periods of substrate development at 37°C, respectively. In the HerpeSelect assay, an index value is obtained for each sample run; the index value is based on the absorbance of the patient sample divided by the mean absorbance of the cutoff calibrator. Index values of <0.9 and >1.1 are defined as negative and positive, respectively; a value of >0.9 but ≤1.1 is considered equivocal. For the Omega and Bioelisa assays, index values are obtained for each sample run. The index value is based on the absorbance of the patient sample divided by the mean absorbance of the cutoff (low-positive control). Samples with ratios of <0.9 and >1.1 are considered negative and positive, respectively, and ratios of ≥0.9 but ≤1.1 are considered equivocal. All samples giving discordant results by the three assays were retested in duplicate by all three ELISA kits.

Western blotting. Western blotting was performed with a Mini-Protean 3 electrophoresis cell (Bio-Rad Laboratories, Hemel Hempstead, United Kingdom), according to the manufacturer’s instructions. All reagents were purchased from either Bio-Rad or Sigma Aldrich Co. Ltd. (Gillingham, United Kingdom). The antigens were prepared from baby hamster kidney cells infected with either HSV-1 strain 17 syn+(3) or HSV-2 strain HG52 (27). Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis by using acrylamide in the stacking and the resolving gels at concentrations of 5% and 7%, respectively. Gels were made with acrylamide cross-linked with bisacrylamide (19 to 1) (catalog no. A3449; Sigma). After transfer of the proteins to a nitrocellulose membrane, the membrane was blocked with blocking buffer (4% nonfat milk, 3% bovine serum albumin in Tris-buffered saline), and then the membrane was cut into strips. The strips were incubated overnight in blocking buffer with either human serum, which were diluted 100-fold, or gG1- or gG2-specific monoclonal antibodies, which were diluted 10,000-fold and which were purchased from East Coast Bio Inc., North Berwick, ME (catalog nos. H1A020 and H2A023, respectively). These served to indicate the positions to which gG1 and gG2 migrated. The strips were washed, and then bound human antibodies were detected with peroxidase-conjugated goat anti-human antibodies, while bound gG1- and gG2-specific mouse monoclonal antibodies were detected with peroxidase-conjugated goat anti-mouse antibodies. Following visualization of the bands with a fluorometric substrate, the sera were scored as positive or negative for HSV-2 antibodies according to whether the typical band for gG2 appeared.

RESULTS

Of 218 samples, 31 (14.22%) samples were found to be positive for HSV-2 antibody and 164 (75.22%) were found to be negative for HSV-2 antibody by all three assays. The 23 remaining samples gave discordant results by the three assays. The levels of concordance between the tests were 95.9% between the Omega and HerpeSelect assays, 90.8% between the Omega and Bioelisa assays, and 94.5% between the HerpeSelect and Bioelisa assays. The sensitivity, specificity, positive predictive value, and negative predictive value were calculated for each kit by using the HerpeSelect assay as a reference standard (Table 1). Western blot analysis of the samples with discordant results showed that 21 of the samples were positive for HSV-2 and that only 1 sample was negative for HSV-2; the remaining sample showed only a very faint band typical of an HSV infection, but it was not possible to detect a band corresponding to either gG1 or gG2. This sample was classified as having an equivocal result by Western blotting. Of the 21 samples with positive results, 3 (14.3%) were detected by the Bioelisa assay, 14 (66.7%) were detected by the HerpeSelect assay, and 16 (76.2%) were detected by the Omega assay. The Western blotting results showed that of the 22 samples with discordant results, the results for 17 (77.3%) were in agreement by the Omega assay, the results for 15 (68%) were in agreement by the HerpeSelect assay, and the results for 3 (13.6%) were in agreement by the Bioelisa assay (Table 2).

| ELISA result | Omega assay | Bioelisa assay |
|--------------|-------------|----------------|
| Positive     | 40          | 33             |
| Negative     | 5           | 169            |

* A total of 218 samples were tested. Sensitivities, 91.7% and 80% for the Omega and Bioelisa assays, respectively; specificities, 97.2% and 99.4% for the Omega and Bioelisa assays, respectively; positive predictive values, 89.8% and 97.4% for the Omega and Bioelisa assays, respectively; negative predictive values, 97.8% and 94% for the Omega and Bioelisa assays, respectively.


**DISCUSSION**

The identification of HSV-2 antibody is important; it can provide an etiological diagnosis and guide antiviral therapy both in patients with symptomatic infections and in patients with asymptomatic infections; it can enable epidemiological studies to be performed to observe emerging patterns of HSV-2 infection; and perhaps most importantly, it can identify couples with discordant results for HSV infection, which may in turn help reduce the risk of transmitting HSV-2 (26). Several serological assays for the detection of HSV-2-specific IgG are widely available, and most are based on the detection of antibody to gG2. These assays vary in their sensitivities, specificities, the form of the antigen used, and the methodology used to detect HSV-2 antibody. Western blotting has been regarded as the most reliable method for the differentiation of the serological response to HSV-1 and HSV-2 infection; however, the interpretation of Western blots requires considerable skill and Western blotting is not routinely used in diagnostic laboratories.

A number of approaches have been used to improve the specificity of HSV-2-selective enzyme immunoassays, including different methods of preparation of antigen and, recently, the use of recombinant or peptide antigens. A potential difficulty in using discrete antigens and particularly subsets of these antigens produced as recombinant proteins or peptides is when mutation of the virus infecting a patient leads to the loss of an epitope represented within such assays. False-negative reactivity may therefore occur. In a recent study, Daikoku et al. (3a) identified a deletion of 63 nucleotides (gG amino acids 531 to 541) that led to a 21-amino-acid deletion in HSV-2 gG2 in 6 of 106 clinical isolates. Peptide 55, which represents residues 561 to 578 of the gG2 molecule, is removed from the reported deletion sequence of gG2 and therefore represents a robust and highly selective solution to the detection of HSV-2 antibody. As the results presented herein demonstrate, the branched peptide design utilized in the Omega assay also provides an extremely sensitive means for the detection of HSV-2 antibody.

The comparison of the results obtained by the Omega peptide 55-based assay with those obtained by two commercially available HSV-2-specific IgG ELISA kits showed that the Omega assay had better performance than either the HerpeSelect assay or the Bioelisa assay, which detected only 68% and 13.6% of the HSV-2-positive serum samples, as defined by Western blotting, among the samples with discordant results, respectively. The overall rate of concordance of the results among the assays was 89.5% (195/218 samples), and the rate of discordant results was 10.6% (23/218 samples); no false-positive test results were detected by any of the three assays. Such results reinforce the view that the region comprising peptide 55 is clearly an immunodominant epitope and that the design of anchoring multiple copies of peptide 55 within a branched lysine core allows contact with both arms of an antibody having the cognate paratope and permits the highly sensitive detection of antibodies of even low affinities.

The concordance between the individual assays for HSV-2 was relatively poor and was poor in comparison to the “gold standard,” the Western blot immunoassay. Of the three assays evaluated, the results of the Omega assay showed the greatest concordance with those of Western blotting. Such results were not unexpected. Western blotting identifies antibody reactivity with separated bands of protein. It is therefore of inherently greater sensitivity than an assay reliant upon the detection of antibody to a single protein antigen or peptide. However, as outlined above, the complexities of Western blot tests mean that they are restricted to use in research or other specialized testing facilities. In diagnostic laboratories, screening of samples for HSV antibody usually involves the use of a type-common antibody ELISA, which achieves sensitivity equivalent to that of the Western blot procedure. The differentiation of type-specific antibodies is then accomplished by type-specific antibody assays. The sensitivity of the Omega HSV-2-specific assay will ensure that very few HSV-2 antibody-positive samples will be incorrectly categorized.

Our study has shown the superior performance of the Omega assay in comparison with the performance of the Bioelisa and the HerpeSelect assays, in accordance with data provided previously (17, 18). The new Omega assay with branched peptide 55 is currently the most sensitive and specific of the commercially available assays for the detection of HSV-2 antibody.

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**TABLE 2. Discordant test results obtained by the three ELISAs retested by Western blotting**

| Western blotting result | No. of samples with the indicated result by the following assay: | Omega assay | HerpeSelect assay | Bioelisa assay |
|-------------------------|---------------------------------------------------------------|-------------|------------------|---------------|
|                         | Positive | Equivocal | Negative | Positive | Equivocal | Negative | Positive | Equivocal | Negative |
| Positive                | 16      | 0        | 4       | 14      | 0        | 10      | 3       | 0        | 18       |
| Equivocal               | 1       | 0        | 0       | 2       | 5        | 0       | 0       | 0        | 0        |
| Negative                | 0       | 0        | 1       | 0       | 0        | 1       | 0       | 1        | 0        |

* Twenty-two samples were tested.
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