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# Secreted Portion of Glycoprotein G of Herpes Simplex Virus Type 2 Is a Novel Antigen for Type-Discriminating Serology

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**The secreted portion of glycoprotein G (sgG-2) of herpes simplex virus type 2 (HSV-2) was evaluated as a novel antigen in an enzyme-linked immunosorbent assay (ELISA) format for detection of type-specific immunoglobulin G (IgG) antibodies in HSV-2-infected patients. The results were compared with those obtained by a commercially available assay, the HerpeSelect 2 ELISA (the FOCUS2 assay). Five different panels of sera were analyzed: panel A consisted of 109 serum samples from patients with a culture-proven HSV-1 infection that were Western blotting (WB) negative for HSV-2; panel B consisted of 106 serum samples from patients with a culture-proven recurrent HSV-2 infection that were WB positive for HSV-2; panel C consisted of 100 serum samples with no detectable IgG antibodies against HSV-1 and HSV-2; panel D consisted of 70 HSV-2 negative "tricky" serum samples containing antinuclear IgG antibodies or IgM antibodies against other viruses or bacteria; and panel E consisted of consecutive serum samples from 21 patients presenting with a first episode of HSV-2-induced lesions. When sera in panels A to C were analyzed, the sgG-2 ELISA and the FOCUS2 assay both showed sensitivities and specificities of ≥98%. In total, among the samples in panel D, 13 serum samples (19%) were false positive by the FOCUS2 assay and 1 serum sample (1.4%) was false positive by the sgG-2 ELISA. When the sera in panel E were analyzed, the sgG-2 ELISA detected seroconversion somewhat later than WB or the FOCUS2 assay did. We conclude that sgG-2 induces an HSV-2 type-specific antibody response and can be used for type-discriminating serology.**

Herpes simplex virus (HSV) type 2 (HSV-2) infection is one of the most common sexually transmitted diseases in the world (3, 24). In the United States the prevalence of HSV-2 infection increased 30% between 1976 and 1994, and HSV-2 infection is detected in one of five persons aged 12 years or older (10). The same trend was reported among women in Sweden, where the HSV-2 antibody prevalence increased from 19 to 33% between 1969 and 1989 (11). In some African countries, the prevalence of HSV-2 has been reported to vary between 17 and nearly 70% (13). As HSV-2-induced lesions facilitate the transmission of human immunodeficiency virus (6, 32), HSV-2 infection poses an additional threat to these populations.

One obstacle to the prevention of the transmission and spread of HSV-2 is that in the majority of cases infection is transmitted without the appearance of symptoms in the newly infected host (27, 33). In this situation, detection of HSV-2 type-specific antibodies may be the only available method for establishment of a correct diagnosis. Type-specific serological assays are also essential for estimation of the seroprevalence in epidemiological studies, for counseling of patients attending a sexually transmitted disease clinic, and for HSV-2 vaccine follow-up programs. Furthermore, a type-specific assay is warranted to discriminate between primary or recurrent HSV-2 infection. This is of special importance for pregnant women, as ongoing primary HSV-2 infection during delivery represents a considerable threat to the newborn (7, 8).

Several of the viral envelope proteins of HSV-2 have been shown to be immunogenic, eliciting an antibody response in humans (2). Due to a high degree of genetic similarity between HSV-1 and HSV-2, most viral proteins induce a cross-reactive antibody response. Glycoprotein G of HSV-1 (gG-1) and HSV-2 (gG-2) is the only known viral envelope protein which elicits a type-specific antibody response. In the virus-infected cell, gG-2 is cleaved into a secreted amino-terminal portion (sgG-2) and a carboxy-terminal portion. The latter protein is further O-glycosylated, generating the cell membrane-associated mature gG-2 (mgG-2) (5, 28). The mgG-2 protein has widely been used as a prototype antigen for detection of type-specific antibodies against HSV-2 (2, 4, 12, 17).

We recently showed that monoclonal antibodies (MAbs) directed against the sgG-2 protein identified type-specific linear and nonlinear epitopes that were devoid of cross-reactivity to HSV-1 antigens. In addition, a type-specific immunoglobulin G (IgG) antibody response was detected in sera from HSV-infected patients by using sgG-2 as the antigen (18). In the present study, the performance of immunosorbent purified sgG-2 in an enzyme-linked immunosorbent assay (ELISA) format (sgG-2 ELISA) was evaluated with large panels of sera collected from patients with isolation-proven HSV-1 or HSV-2 infections. These serum panels were also analyzed by the commercially available HerpeSelect 2 ELISA (the FOCUS2 assay; Focus Technologies, Cypress, Calif.), which is approved for use by the U.S. Food and Drug Administration. In addition, consecutive serum samples derived from patients presenting with a first episode of HSV-2-induced lesions were analyzed by different gG-2 based assays.

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## MATERIALS AND METHODS

**Cells and viruses.** African green monkey kidney (GMK-AH1) cells and human epidermoid (HEp-2) cells were cultured in Eagle's minimal essential medium

supplemented with 2% calf serum and antibiotics. A local wild-type HSV-2 isolate B4327UR was used (16).

**Serum samples.** Sera were collected from clinical specimens received at the Department of Clinical Virology (Sahlgrenska University Hospital, Göteborg, Sweden). Five panels of sera were analyzed. Panel A consisted of 109 serum samples from patients with a culture-proven HSV-1 infection and with no detectable antibodies against HSV-2 by the Western blotting (WB) technique. Panel B consisted of 106 serum samples from patients with recurrent genital culture-proven HSV-2 infection that were WB positive for HSV-2. Panel C consisted of 100 serum samples which were HSV-1 and HSV-2 seronegative by ELISA with an HSV-1-derived type-common sodium deoxycholate-solubilized membrane preparation (15) and *Helix pomatia* lectin-purified mgG-2 (HPLmgG-2) as antigens. Panel D consisted of 70 "tricky" serum samples, with 19 serum samples containing IgM antibodies against *Mycoplasma pneumoniae*, 20 serum samples containing IgM antibodies against Epstein-Barr virus (EBV), 20 serum samples containing IgM antibodies against cytomegalovirus (CMV), and 11 serum samples containing antinuclear IgG antibodies (ANA). All serum samples were HSV-2 negative by WB and the HPLmgG-2 ELISA. IgM antibodies were detected by in-house assays by the immunofluorescence technique. Briefly, monolayers of P3HR1 cells infected with EBV and human embryonic cells infected with CMV were permeabilized and fixed in acetone. Serum samples were diluted in phosphate-buffered saline and incubated for 1 h at 37°C. Fluorescein isothiocyanate-labeled goat anti-human IgM (Jackson ImmunoResearch Laboratories) was used as the conjugate. Glycolipid antigens from *M. pneumoniae* were extracted with chloroform, methanol, and water and used for detection of IgM antibodies in an ELISA format. Alkaline phosphatase-conjugated anti-human IgM (DAKO) was used as the conjugate, and *p*-nitrophenyl phosphate disodium was used as the substrate. The sera were initially preadsorbed with GullSORB (Meridian Diagnostics Inc.). ANA were detected at the Department of Clinical Immunology, Göteborg, Sweden, by an indirect immunofluorescence technique with HEp-2 cells. Fluorescein isothiocyanate-labeled goat anti-human IgG (Jackson ImmunoResearch Laboratories) was used as the conjugate. Panel E consisted of serum samples from 21 patients who presented with a culture-proven first episode of HSV-2-induced lesions and whose acute-phase sera had no detectable antibodies, as determined by the HPLmgG-2 ELISA. In total, 52 consecutive serum samples were collected. The acute-phase blood samples were drawn close to the time (±7 days) or at the time (day 0) that the sample used for virus isolation was obtained. The number of serum samples drawn from each patient varied between two and five, and the time from the drawing of the first sample to the time of drawing of the last sample ranged from 2 to 545 days.

**Typing of clinical HSV-1 and HSV-2 isolates.** The isolates were cultured on GMK-AH1 cells and typed as described previously by using anti-HSV-1 and anti-HSV-2 MAbs (20).

**WB.** The WB technique is considered the "gold standard" for the detection of HSV-2-specific IgG antibodies (2). Antigens were prepared for WB by infecting HEp-2 cells with HSV-2 isolate B4327UR, as described previously (19). Briefly, the antigens were mixed with sample buffer containing sodium dodecyl sulfate and subjected to polyacrylamide gel electrophoresis under reducing conditions by using NuPAGE 7% Tris-acetate gels (Novex). The proteins were electrotransferred to an Immobilon-P transfer membrane (Millipore Corp.). The strips were incubated overnight with sera at a 1:100 dilution. A serum sample drawn from an individual with culture-confirmed HSV-2 infection and an anti-mgG-2 MAb (21) were used for correct identification of the carboxy-terminal intermediate portion of gG-2 and the mgG-2 protein. Peroxidase-labeled rabbit anti-human or rabbit anti-mouse IgG (DAKO) was used as the conjugate, and 4-chloro-1-naphthol was used as the substrate. A positive WB profile was defined as reactivity to mgG-2 (≈120 kDa) alone or in combination with reactivity to the carboxy-terminal intermediate portion of gG-2 (≈70 kDa) (21).

**HPLmgG-2 ELISA.** HPLmgG-2 was coated on Maxisorp microtiter plates, and the assay was performed as described previously (21, 29).

**sgG-2 ELISA.** Immunosorbent affinity chromatography-purified sgG-2 (0.75 mg/ml) was prepared as described earlier (18) and coated at a 1:3,000 dilution in carbonate buffer (pH 9.6) on Maxisorp microtiter plates (Nalge Nunc International). Peroxidase-conjugated goat anti-human IgG (Jackson ImmunoResearch Laboratories) was used as the conjugate at a 1:3,000 dilution, with *o*-phenylenediamine used as the substrate. Serum samples were diluted 1:100 in phosphate-buffered saline with 0.6 M NaCl, 1% skim milk, and 0.05% Tween 20 and tested in duplicate. The reaction was stopped with 1 M sulfuric acid when the optical density (OD) for a predefined HSV-2-positive serum sample reached a value of 2.0 ± 0.3. The absorbance was measured at 490 nm, and the results are given as the mean value for each duplicate. The cutoff value was defined as follows: a panel of 40 randomly selected serum samples which were negative by the ELISA with the HSV-1-derived type-common membrane antigen and the HPLmgG-2 antigen were analyzed by sgG-2 ELISA. A negative control serum sample (CS) with reactivity equal to the mean + 2 standard deviations (SDs) for the panel was included on each plate. A serum sample was considered negative if the OD value for the sample was lower than that for the CS plus 0.25 OD units. A serum sample was considered positive if the OD value for the sample was higher that that for the CS plus 0.35 OD units. Specimens with OD values greater than or equal to that for the CS plus 0.25 OD units and less than or equal to that for the CS plus 0.35 OD units were considered equivocal and were retested, and the result of the second test was used.

**Detection of HSV-2 IgM.** An in-house indirect immunofluorescence technique was used to detect IgM antibodies against HSV-2 (HSV-2 IgM), as described previously (30).

**Commercially available assays.** The serum samples in panels A to E were analyzed by an HSV-2-specific assay, the FOCUS2 assay (Focus Technologies). In addition, the sera in panel E were tested for HSV-1-specific antibodies by the HerpeSelect 1 ELISA (Focus Technologies). The assays, which are based on recombinant-produced gG-1 or gG-2 expressed in a baculovirus system, were performed according to the instructions of the manufacturer. Samples with equivocal results were retested, and the result of the second test was used.

**Statistics.** Fisher's exact test was used for determination of *P* values.

## RESULTS

**Reactivities by sgG-2 ELISA and FOCUS2 assay.** The results for sera in panels A to C obtained by the sgG-2 ELISA and the FOCUS2 assay are shown in Fig. 1A and B, respectively. All 109 serum samples in panel A, which included sera from individuals with culture-proven HSV-1 infection and no detectable HSV-2 antibodies by WB, were negative by the sgG-2 ELISA, while 2 serum samples were positive by the FOCUS2 assay. Two of 106 serum samples from patients with a culture-proven HSV-2 infection (panel B) were consistently negative by the sgG-2 ELISA. In addition, three specimens from panel B showed equivocal reactivities but became positive on retesting. Thus, 104 (of 106) serum samples were considered positive by the sgG-2 ELISA. All sera in panel B (106 of 106 serum samples) were positive by the FOCUS2 assay. One serum sample in panel C, which included 100 serum samples with no detectable antibodies against HSV-1 and HSV-2, was positive by the sgG-2 ELISA. WB confirmed that this serum sample was HSV-2 negative. None of the sera in panel C was positive by the FOCUS2 assay. The results and the distribution of the sera according to the clinical diagnosis for sera containing IgM antibodies or ANA (panel D) are shown in Fig. 2. One serum sample (of 70 tested) was false positive by the sgG-2 ELISA, while 13 of the 70 serum samples were false positive by the FOCUS2 assay. Four of these 13 serum samples presented reactivities close to the index values specified as the cutoff for positive samples.

**Sensitivities and specificities.** The sensitivities and specificities of the sgG-2 ELISA and the FOCUS2 assay, which were determined on the basis of the results for sera included in panels A to D, are summarized in Table 1. The overall sensitivity and specificity for the sgG-2 ELISA, as judged from the results presented for panels A to C, were 98 and 99.5%, respectively. The FOCUS2 assay showed a sensitivity of 100% and a specificity of 99%. The sgG-2 ELISA showed a significantly ($P = 0.001$) higher specificity (99%) than the FOCUS2 assay (81%) for the sera included in panel D (tricky sera).

**CVs of sgG-2 ELISA.** An HSV-2-positive serum sample was tested at 28 positions (wells) in the same plate. The mean ± SD OD value was 2.26 ± 0.10, giving an intra-assay coefficient of variation (CV) of 4.4%. The interassay CV was determined

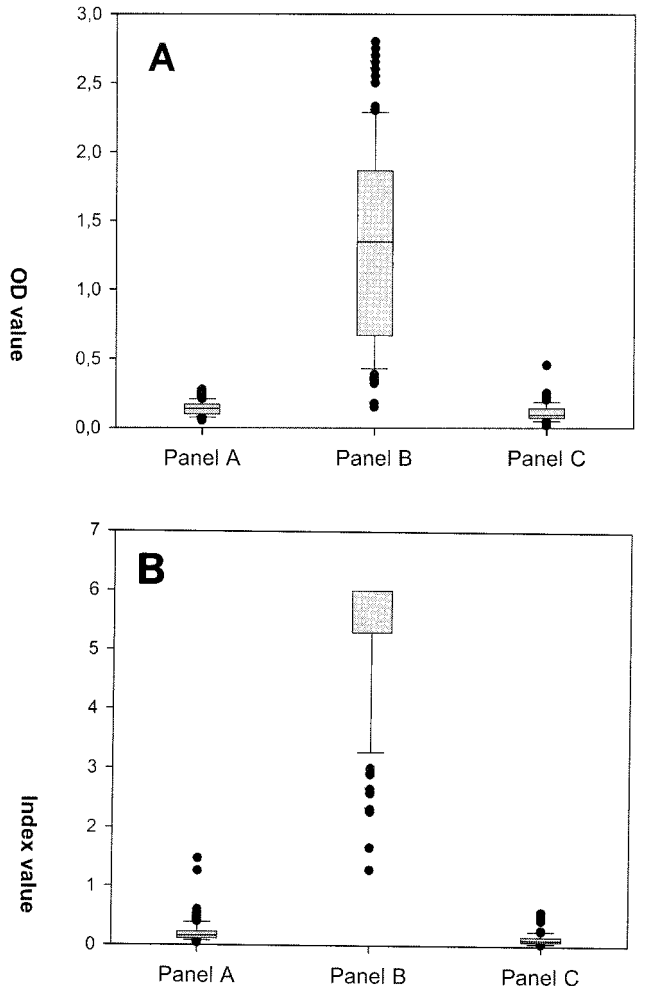


FIG. 1. Box plots showing the reactivities of sera by the sgG-2 ELISA (A) and the FOCUS2 assay (B). Panel A, HSV-1 positive and HSV-2 negative sera; panel B, HSV-2 positive sera; panel C, HSV-1- and HSV-2 negative sera. The boxes represent the 25th and 75th percentiles, and the whiskers represent the 10th and 90th percentiles. The median values are shown as horizontal lines. Dots outside the boxes indicate outliers. No upper outliers or median value are presented for the samples in panel B tested by the FOCUS2 assay, as calculated index values >6.5 were all given an index value of 6.5.

by comparing the reactivities of the serum sample on 14 different occasions. The mean ± SD OD value was 1.95 ± 0.21, giving an interassay CV of 10.8%.

**Seroconversion in patients presenting with a first episode of HSV-2-induced lesions.** Consecutive serum samples from 21 patients (panel E) were analyzed by using four different HSV-2-specific IgG assays (the sgG-2 ELISA, the FOCUS2 assay, the HPLmgG-2 ELISA, and WB) and one assay for the detection of IgM antibodies. A positive culture was obtained from HSV-2 lesions on day 0. A serum sample drawn at the same time or within 7 days prior to or after day 0 was considered an acute-phase sample. The results are shown in Fig. 3. All acute-phase serum samples were HSV-2 negative by the sgG-2 ELISA. In total, 12 patients showed seroconversion; but the serum sample from 1 additional patient (patient 21) presented with an equivocal result, even though the sample was tested twice. Acute-phase serum samples from seven patients (patients 4, 6, 11 to 13, 18, and 20) were HSV-2 positive by the FOCUS2 assay. The consecutive serum samples from one patient (patient 4) were negative. The consecutive serum samples from 14 patients showed seroconversion. Thus, HSV-2 infection was detected by the FOCUS2 assay in all 21 patients. Fifteen patients showed seroconversion by the HPLmgG-2 ELISA. When sera were assayed by the WB technique, the acute-phase sera of 3 patients (patients 7, 12, and 20) were HSV-2 positive and 16 patients showed seroconversion in consecutive serum samples. Thus, HSV-2 infection was detected in 19 patients by the WB technique. IgM antibodies were detected in acute-phase serum samples or consecutive serum samples in 16 patients. Three patients were infected with HSV-1, as judged by the reactivity to the gG-1 antigen (Fig. 3).

TABLE 1. Performance of the sgG-2 ELISA and the FOCUS2 assay

| Serum sample panel | % Sensitivity | | % Specificity | |
|---|---|---|---|---|
| | sgG-2 ELISA | FOCUS2 assay | sgG-2 ELISA | FOCUS2 assay |
| A | —[a] | — | 100 (109/109) | 98 (107/109) |
| B | 98 (104/106) | 100 (106/106) | — | — |
| C | — | — | 99 (99/100) | 100 (100/100) |
| D | — | — | 99 (69/70) | 81 (57/70) |

[a] —, not applicable.

## DISCUSSION

Most of the envelope glycoproteins of HSV-1 and HSV-2 have extensive genetic similarities and induce a cross-reactive antibody response. Commercially available assays based on crude antigen preparations therefore present inaccurate results with low specificities (1). The purpose of the present study was to evaluate sgG-2 as a novel antigen in an ELISA format

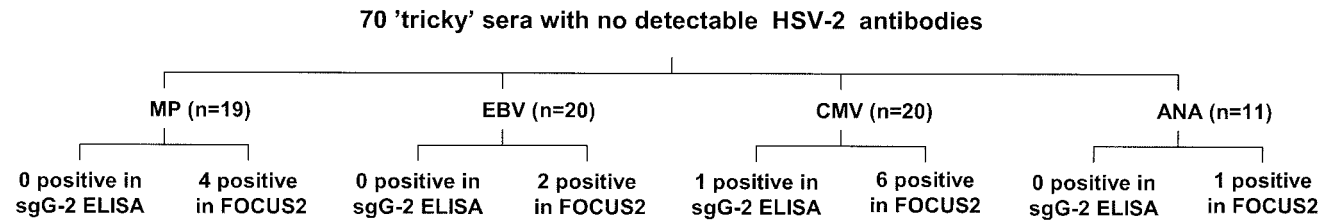


FIG. 2. Outcomes of the sgG-2 ELISA and the FOCUS2 assay for 70 HSV-2-negative serum samples containing IgM antibodies against CMV, EBV, or *M. pneumoniae* or for sera with ANA.

| | no. 1 | | | | | no. 2* | | | | no. 3 | | | no. 4 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Serum collected at day:** | 0 | 10 | 40 | 103 | 199 | −7 | 185 | 192 | 236 | 0 | 43 | 90 | 0 | 228 |
| WB | − | + | + | + | + | − | + | + | + | − | + | + | − | − |
| HPLmgG−2 ELISA | − | − | − | − | − | − | + | + | + | − | − | + | − | − |
| sgG−2 ELISA | − | − | − | − | − | − | + | + | + | − | − | + | − | − |
| FOCUS2 | − | + | + | + | + | − | + | + | + | − | + | + | + | − |
| HSV-2 IgM | 160 | 160 | 160 | 20 | 10 | − | 80 | 40 | 20 | − | − | − | 80 | − |

| | no. 5 | | | no. 6 | | | no. 7* | | | no. 8 | | no. 9 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Serum collected at day:** | 0 | 75 | 330 | 0 | 4 | 44 | 0 | 38 | 300 | 0 | 139 | 0 | 545 |
| WB | − | + | + | − | + | + | + | + | + | − | − | − | + |
| HPLmgG−2 ELISA | − | + | + | − | − | + | − | − | + | − | − | − | + |
| sgG−2 ELISA | − | ± | + | − | − | + | − | − | − | − | + | − | + |
| FOCUS2 | − | + | + | + | + | + | − | + | + | − | + | − | + |
| HSV-2 IgM | − | − | − | − | 160 | 160 | − | − | − | 160 | 160 | 10 | − |

| | no. 10 | | | no. 11 | | no. 12 | | no. 13 | | no. 14 | | no. 15 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Serum collected at day:** | 0 | 22 | 236 | 0 | 24 | 1 | 120 | 0 | 2 | 0 | 38 | 0 | 14 |
| WB | − | + | + | − | + | + | + | − | + | − | + | − | + |
| HPLmgG−2 ELISA | − | − | + | − | + | − | + | − | − | − | + | − | + |
| sgG−2 ELISA | − | + | + | − | + | − | − | − | − | − | + | − | − |
| FOCUS2 | − | + | + | + | + | + | + | + | + | − | + | − | + |
| HSV-2 IgM | − | 64 | 80 | NA | − | − | 40 | 160 | 160 | − | 160 | 20 | − |

| | no. 16 | | no. 17 | | no. 18* | | no. 19 | | no. 20 | | no. 21 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Serum collected at day:** | 0 | 94 | 0 | 14 | 0 | 26 | 0 | 17 | 0 | 20 | 6 | 22 |
| WB | − | + | − | + | − | + | − | + | + | + | − | + |
| HPLmgG−2 ELISA | − | + | − | + | − | − | − | + | − | + | − | − |
| sgG−2 ELISA | − | + | − | + | − | − | − | − | − | + | − | ± |
| FOCUS2 | − | + | − | + | + | + | − | + | + | + | − | + |
| HSV-2 IgM | − | − | 20 | 160 | − | 160 | − | 80 | 40 | − | − | 64 |

FIG. 3. Times to seroconversion for 21 patients (patients 1 to 21) presenting with a first episode of HSV-2-induced lesions and with no detectable IgG antibodies against HSV-2 in the acute-phase sera by the HPLmgG-2 ELISA. HSV-2 isolation was performed with samples obtained on day 0. For all patients except patients 2, 12, and 21, acute-phase sera were drawn at day 0. Sera were also assayed for HSV-2 IgG antibodies by WB, the sgG-2 ELISA, and the FOCUS2 assay. HSV-1 antibody status was evaluated by using a commercially available assay (HerpeSelect 1 ELISA). Patients with IgG antibodies against HSV-1 are indicated by asterisks. Results are denoted as positive (+) or negative (−). Equivocal results are denoted ±. IgM antibodies against HSV-2 (HSV-2 IgM) were detected by an indirect immunofluorescence technique and are presented as titer values. NA, sample not available.

for type-specific serology and to compare the performance of the sgG-2 ELISA with that of a well-documented and commercially available assay based on recombinant-produced gG-2 (the FOCUS2 assay). We showed here that among sera collected from patients with culture-proven recurrent HSV-2-induced lesions (panel B), the sgG-2 ELISA had a sensitivity of 98%, results similar to those of the FOCUS2 assay (100%). The latter assay has previously been shown to be very sensitive compared to the results of WB (25). The specificities of the sgG-2 ELISA and the FOCUS2 assay were also high (>99%) for the sera included in panels A and C. In contrast, when tricky sera (panel D) were tested, the sgG-2 ELISA had a significantly lower number of false-positive results compared with the number obtained by the FOCUS2 assay. When the

protocol for the sgG-2 ELISA was evaluated, we included 0.6 M sodium chloride in the incubation buffer to avoid nonspecific reactivity. This finding may be due to the fact that sgG-2 is a highly positively charged protein (pI $\approx$ 10.3). The recombinant-produced gG-2 antigen used in the FOCUS2 assay contains a truncated protein of 661 amino acids, including sgG-2. According to information provided by the manufacturer, the incubation buffer does not contain additional sodium chloride, which may explain the nonspecific reactivity for sera containing IgM antibodies. It is notable that nine of the samples with false-positive results by the FOCUS2 assay showed reactivities at the same levels as the low-positive control and would thereby be judged to be clearly positive. The other four serum samples showed relatively low levels of reactivity, with index values close to the cutoff value. A positive result judged on the basis of the results of the FOCUS2 assay should perhaps be interpreted with caution when sera from patients with acute viral or bacterial infections are analyzed.

Among the samples from patients presenting with a first episode of HSV-2-induced lesions, acute-phase sera from eight patients were HSV-2 positive by either the WB technique or the FOCUS2 assay. IgM antibodies are frequently detected after a primary HSV-2 infection (12, 31). Six of the eight patients developed IgM antibodies, a finding which suggests that the positive IgG reactivities of the acute-phase sera reflected an early seroconversion. However, it has been described previously that patients presenting with a first episode of HSV-2-induced lesions indeed have recurrent infections (22). Some of these eight patients may therefore represent recurrently infected individuals. The acute-phase serum sample from one patient (patient 4) was positive by the FOCUS2 assay, but a consecutive serum sample was negative. In other studies, this phenomenon has been described as "seroreversion" (14, 26). Sporadic reversal from positive reactivity to negative reactivity has been reported by use of the Gull gG-based HSV-2 ELISA. However, this was not related to a loss of WB profiles and most likely represents a fluctuation in the test itself (1). Nevertheless, among HSV-2-positive pregnant women, the loss of reactivity to mgG-2 by ELISA is a common finding during the end of pregnancy (9).

An aspect of relevance to the performance of a type-specific assay is the period of time from the time of transmission of HSV-2 to the time when antibodies are initially detected in the host. Although few serum samples were included in panel E, our results suggest that after HSV-2 infection antibodies against mgG-2 are detected earlier than antibodies against sgG-2. The anti-mgG-2 antibodies recognize linear epitopes of the protein to a high degree (21). WB can therefore be used as a sensitive and specific method for confirmation of the presence of type-specific anti-gG-2 antibodies. In contrast, anti-sgG-2 antibodies identify mostly nonlinear epitopes, which by WB have no reactivity to virus-infected cell lysates or purified sgG-2 antigen prepared under reducing conditions (18). This finding makes WB unsuitable as an alternative and sensitive method for the detection of anti-sgG-2 antibodies. The observed delayed antibody response to sgG-2 may therefore be explained by a lower sensitivity of the sgG-2 ELISA compared to those of assays based on mgG-2 or whole gG-2 antigens. One limitation of the present study is that all HSV-2-positive serum samples examined were drawn from patients presenting with HSV-2-induced lesions. Thus, sgG-2 must be further evaluated in clinical settings with sera from patients with asymptomatic HSV-2 infection.

It is crucial that an assay have a high degree of specificity to obtain a high predictive value of positive results. False-positive results lead to the provision of incorrect information to individuals and couples, which may cause psychological distress (23). In clinical settings, one strategy to increase the accuracy of an HSV-2-specific assay would be to confirm the results for positive samples by another assay. The WB technique is not suitable for routine diagnostic purposes, as the method is laborious and complex to perform and its results are difficult to interpret. The sgG-2 protein represents a novel antigen which, when used in an ELISA format, offers a high degree of specificity. sgG-2 can therefore be used as an additional antigen for the detection of type-specific IgG antibodies against HSV-2.

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