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# Evaluation of Three Glycoprotein G2-Based Enzyme Immunoassays for Detection of Antibodies to Herpes Simplex Virus Type 2 in Human Sera

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**Three new glycoprotein G-based enzyme immunoassays (ETI-HSVK-G 2, Sorin Diagnostics Biomedica [assay A]; HSV Type 2 Specific IgG ELISA, Gull Laboratories, Inc. [assay B]; Cobas Core HSV-2 IgG EIA, Roche [assay C]) for the detection of herpes simplex virus (HSV) type 2 (HSV-2)-specific antibodies were evaluated. By testing sera from 25 individuals with culture-proven HSV-2 infection, the assays showed a sensitivity of 96%. The specificities, evaluated with sera from 70 HSV antibody-negative children, 75 HSV antibody-positive children, and 69 HSV antibody-negative adults, were 100% for assay A, 96.2% for assay B, and 97.8% for assay C, respectively. Discrepant results by any of the three assays, i.e., reactivity of a specimen in only one or two assays, occurred with similar frequencies for HSV-seronegative individuals as well as HSV-seropositive children and adults. For sera with discrepant results, the positive reactivity was mostly low. Thus, for determination of the prevalence of HSV-2 antibodies, only concordantly positive results were considered. On the basis of the results obtained with sera from 41 adults with culture-proven HSV-1 infection and from 173 HSV-antibody-positive pregnant women, the HSV-2 seroprevalence was 9.8%. The results show that the new glycoprotein G2-based enzyme immunoassays are useful tools for the detection of type-specific HSV-2 antibodies. However, if only one assay is performed, careful interpretation of the results is indicated, especially if the exhibited reactivity is low, and for determination of the definitive HSV-2 serostatus, confirmatory assays may still be necessary.**

Genital herpes, mainly caused by infection with herpes simplex virus (HSV) type 2 (HSV-2), is one of the most common sexually transmitted diseases in humans (7, 9, 11, 19, 20, 28, 29). Perinatal transmission of the virus from mothers who are shedding the virus at the time of delivery may have serious or life-threatening consequences in newborns (6, 14, 15, 22, 30, 31). Serological diagnosis of HSV-2 infection has been hampered because of the extensive cross-reactivity of the antibodies to HSV type 1 (HSV-1) (3, 4, 10). The most validated method for identifying HSV-2-specific antibodies is the Western blot assay (1, 5, 18, 24). However, Western blotting is laborious and the rate of unequivocal results depends on the investigator's expertise due to the high number of virion proteins. In recent years, HSV glycoprotein G (gG) was identified as a viral protein that specifies predominantly type-specific epitopes, and measurement of antibodies directed against HSV-2 glycoprotein G (gG2) has been reported to be useful for discrimination of HSV antibodies (12, 17, 21, 23, 25, 26, 27). Nevertheless, diagnostic assays that are based on gG have been restricted to a limited number of research laboratories (e.g., the University of Washington School of Medicine, Seattle; Stanford University School of Medicine, Stanford, Calif.; and Emory University School of Medicine, Atlanta, Ga., all in the United States) that prepare the antigen on their own, for instance, by affinity chromatography or genetic engineering. However, for widespread testing, commercially available kits are needed.

This report describes an evaluation of three newly developed, commercially available, or premarket enzyme-linked immunosorbent assays (ELISAs) based either on recombinant HSV-2 gG expressed by baculovirus-infected insect cells or on purified HSV-2 gG prepared from infected tissue cultures.

## MATERIALS AND METHODS

**Subjects and serum samples.** A total of 484 serum samples from 454 individuals were investigated. Except for the sera collected from patients with culture-proven HSV-2 infection, one serum sample per person was tested. The sera from the individuals were divided into the groups described below.

For determination of the sensitivity of the assays, 55 serum samples were obtained from 25 adults (13 men, 12 women) with culture-proven HSV-2 infection. Specimens for virus isolation were swabs from penile or preputial skin (six patients), vagina (two patients), cervix uteri (one patient), gluteal fold (three patients), gluteal skin (two patients), anal region (two patients), skin of the lower abdomen (one patient), hip (one patient), thigh (one patient), or forearm (one patient) or swabs from an unknown location (two patients). Furthermore, HSV-2 was isolated from the urine of three renal transplant patients. Virus culture was performed with Vero cells, human embryonic fibroblasts, and Graham-293 cells in tubes as described by Langenberg et al. (16). After routine culture the isolated viruses were typed with fluorescein-conjugated type-specific monoclonal antibodies (Pathfinder; Kallestad Diagnostics Inc., Sanofi Diagnostics Pasteur). Typing of the virus isolated from patient 25 was additionally performed by nested PCR by the method of Cassinotti et al. (8). Twenty-two of the HSV-2-infected individuals showed clinically manifest genital herpes; three individuals who underwent kidney transplantation shed the virus asymptomatically. All individuals were HSV immunoglobulin G (IgG) antibody positive, as determined as described below. The acute-phase serum was collected from 18 individuals on the day of swab sampling (day 0). The acute blood sample was drawn from four individuals within the week of swab sampling (day −1, day +3, day +4, day +7), and the serum was drawn from three individuals at day −32, day +21, and day +48 from the time of swab sampling. From 14 patients, 30 additional serum samples were collected between 7 months before and 25.5 months after swab sampling.

The specificities of the assays were determined by testing sera from HSV-seronegative and HSV-seropositive children and HSV-seronegative adults. The children were between 2 and 8 years old and had no clinical evidence of an acute HSV infection. These ages were chosen because maternal antibodies are lost by 2 years of age, and except for neonatal infection, HSV-2 infection is unusual at this age of life (13). One hundred twenty-four of these serum samples were

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randomly selected from children attending the university hospital. Additionally, 21 serum samples positive for HSV IgG antibodies were from children who were 5 to 8 years old. In total, 70 of the 145 children's serum samples were HSV antibody negative and 75 of them were HSV antibody positive. Thirty-eight HSV antibody-negative serum samples were obtained from 38 adults (medical staff) without a history of herpes. With the aim of demonstrating coexisting infections with HSV-1 and HSV-2, 41 serum samples were collected from 41 patients (20 males, 21 females, and 39 adults who were at least 19 years old and 2 adolescents who were 11 and 13 years old, respectively) with HSV-1 infection proven by virus isolation. Virus isolates were obtained from swabs from the lip (14 patients), throat (7 patients), tongue (1 patient), palate (1 patient), nostril (2 patients), conjunctiva (1 patient), facial skin (10 patients), and internal genitalia (1 young woman) and from a swab from an unknown location (1 adolescent). Furthermore, virus was isolated from pharyngeal wash (2 patients) and bronchial lavage (1 patient) specimens. All sera from individuals with culture-documented HSV-1 infection were HSV IgG antibody positive.

**HSV-2 seroprevalence in pregnant women.** Two hundred five serum samples from randomly selected pregnant women were analyzed for anti-gG2 antibodies. The mean age of the women was 30.6 years (age range, 16 to 43 years). One hundred seventy-three women (84.4%) were HSV IgG antibody positive, 31 (15.1%) were negative, and the specimen from 1 woman showed an equivocal level of HSV IgG antibody.

**Anti-HSV IgG antibody assays.** All sera were tested by a type-common HSV IgG enzyme immunoassay (EIA; Enzygnost Anti-HSV/IgG; Dade Behring) according to the instructions of the manufacturer. The antigen in this assay is HSV-1 McIntyre, harvested from the supernatant of infected permanent simian kidney cells.

**Anti-HSV gG2 assays.** The following enzyme immunoassays based on glycoprotein G of HSV-2 were performed: ETI-HSVK-G 2 (Sorin Diagnostics Biomedica; assay A), HSV Type 2 Specific IgG ELISA (Gull Laboratories, Inc., marketed by Fresenius; assay B), and the premarketing kit for the Cobas Core HSV-2 IgG EIA (Roche, Basel, Switzerland; assay C). Assay A is based on recombinant gG2 produced by the baculovirus system, assay B is based on gG2 of HSV-2 grown in tissue culture and affinity purified with a monoclonal antibody, and assay C is based on lectin-purified gG2. All assays were carried out according to the instructions of the manufacturers. Samples tested by assay C were processed automatically by the Cobas Core II Immunoanalyzer (Roche). Results were interpreted in accordance with the manufacturer's instructions. In assays A and C, sera with absorbance values equal to or greater than the cutoff value were considered positive, those with absorbance values ranging at most to 10% below the cutoff value were considered equivocal, and sera with absorbance values of less than 0.9 times the cutoff value were scored as negative. In assay B, samples with absorbance values of at least the absorbance value for the reference serum sample were considered positive, those with values equal to or less than 0.9 times the value for the reference serum sample were considered negative, and inclusive values between 0.91 and 0.99 times the value for the reference serum sample were scored as equivocal. For semiquantitative results, a calibration curve was established by using two calibrators and the reference serum sample with a value of 10 activity units (AcU)/ml. Sera with values of at least 10 AcU/ml were considered positive, those with values equal to or less than 0.9 times the value for the reference serum sample were considered negative, and sera with absorbance values greater than 0.9 times the value for the reference serum sample but less than the absorbance of the reference serum sample were considered equivocal. To obtain comparable values, results of assay B were additionally expressed as s/co values (optical density of the sample/optical density of the cutoff).

## RESULTS

**Sensitivities of the anti-gG2 assays.** The sensitivities of the anti-gG2 assays were evaluated by testing 55 serum samples from 25 individuals known to be infected with HSV-2 as proven by isolation of the virus. All 55 serum samples were positive by the type-common HSV IgG enzyme immunoassay. All sera were tested by assay A, and all but 1 were tested by assay B. Forty-nine serum samples obtained from 23 individuals were analyzed by assay C. Table 1 presents the results in detail.

Concordantly positive results, i.e., reactivity by all assays used, were obtained with the sera from 24 of the 25 individuals, although the quantitative correlation of the values obtained by the three assays was poor for several serum samples (e.g. sera from patients 3, 4, and 7).

The four serum samples from a 49-year-old male renal transplant recipient (patient 25), who shed the virus asymptomatically in his urine, were consistently negative.

**Specificities of the assays.** In order to evaluate the specificities of the anti-gG2 enzyme immunoassays, 70 serum samples from HSV-seronegative children, 75 HSV IgG-seropositive children, and 38 HSV-seronegative adults were investigated. All 183 serum samples were tested by assay A, all but 2 were tested by assay B, and 147 serum samples were tested by assay C. Besides one serum sample from the HSV-seronegative adult group, which was reactive in all three assays, assay B yielded four equivocal (2.2%) and four positive (2.2%) results, and assay C yielded four (2.7%) positive results. Table 2 presents the results in greater detail.

**Sera from individuals with HSV-1 infection as proven by virus isolation.** Of the 41 serum samples from as many individuals with culture-proven HSV-1 infection, 40 serum samples were analyzed by three assays. One specimen was not tested by assay C. All serum samples were positive by the type-common HSV IgG antibody assay.

Of the 40 serum samples tested by assays A, B, and C, the specimens from three adults were concordantly positive. Moreover, the specimen from one woman was positive by assays A and C but showed only equivocal levels of anti-gG2 antibodies by assay B. The sera from a male and an 11-year-old girl showed moderate reactivities in only one of the three assays (assay A or assay B). Thirty-four serum samples gave identical negative results by the three assays. The serum tested by assays A and B only was concordantly negative (Table 3).

**Seroprevalence in pregnant women.** Of the 205 serum samples from pregnant women, all of which were tested by the three assays, the 31 HSV-seronegative serum samples and the 1 serum sample with an equivocal level of type-common HSV antibodies were concordantly anti-gG2 negative. Of the 173 HSV-seropositive serum samples, 16 gave positive results by all three assays (Table 4). One further specimen was positive by assays B and C and equivocal by assay A. By calculation of the prevalence of HSV-2 infection in pregnant women on the basis of these data, a rate of 9.8% (17 of 173 HSV antibody-positive women) is indicated. Nine serum samples gave discrepant results (three specimens were reactive by assays B and C, two specimens were reactive by assay B, and four specimens were reactive by assay C). The reactivity was mostly low. However, one serum sample with a high reactivity by assay C was scored as negative by the other two assays. There was no difference in the stage of pregnancy between the group of women with discrepant anti-gG2 results and those with concordant positive or negative results. Among the women with discrepant results, three women were in the first trimester, one was in the second trimester, and five were in the third trimester. In the group of pregnant women with concordant positive results (including patient 17; Table 4) or negative results, 21.9% were in the first trimester, 24.5% were in the second trimester, and 53.6% were in the third trimester.

## DISCUSSION

The purpose of this study was to determine the diagnostic values of three newly developed gG2 enzyme immunoassays for the detection of antibodies to HSV-2. The results show that the gG2-based enzyme immunoassays tested are sensitive at detecting HSV-2 antibodies in the sera of 96% of individuals with culture-proven HSV-2 infections. Since all individuals were adults and most of them were probably coinfected with HSV-1, the assays have the potential to identify HSV-2 antibodies in the presence of HSV-1 antibodies. This was confirmed by detecting four anti-gG2-reactive adults in the group of individuals with culture-documented HSV-1 infection.

However, the sera from a male renal transplant recipient failed to react in any of the anti-gG2 antibody assays. HSV-2 was isolated from his urine 2.5 months after transplantation as

TABLE 1. Detection of antibodies to HSV gG2 by three different enzyme immunoassays in 55 serum samples from 25 patients with culture-proven HSV-2 infection[a]

| Patient | s/co for assay A[a] | Assay B[b] | | s/co for assay C[a] |
|---|---|---|---|---|
| | | AcU/ml | s/co | |
| 1 | 9.78 | 88 | 8.45 | 11.31 |
| 2 | 8.63 | 90 | NA[c] | 9.58 |
| 3 | 8.48 | 194 | NA | 13.89 |
| | 8.91 | 132 | NA | 14.11 |
| | 9.18 | 136 | NA | 13.89 |
| 4 | 7.41 | 74 | 7.55 | 14.11 |
| | 6.77 | 42 | 2.41 | 13.89 |
| 5 | 6.92 | 65 | 5.32 | 7.85 |
| 6 | 6.90 | 28 | 2.58 | 7.71 |
| | 7.90 | 40 | 3.48 | 7.66 |
| 7 | 6.85 | 40 | 3.30 | ND[d] |
| | 6.70 | 28 | 2.26 | 14.11 |
| | 9.05 | 40 | 2.39 | 13.89 |
| 8 | 6.48 | 52 | 4.28 | ND |
| | 6.21 | 56 | 3.61 | 4.50 |
| | 5.62 | 40 | 2.43 | 6.71 |
| | 6.61 | 33 | 2.17 | 5.92 |
| 9 | 6.26 | 32 | 2.63 | 8.09 |
| | 5.99 | 84 | 4.77 | 8.70 |
| | 6.64 | 60 | 5.47 | 12.05 |
| 10 | 4.75 | 23 | 1.82 | 2.35 |
| | 1.30 | 30 | 2.11 | 1.48 |
| | 2.45 | 28 | 2.54 | 3.33 |
| | 1.05 | 16 | 1.47 | 3.35 |
| 11 | 4.45 | 59 | 5.65 | 7.82 |
| 12 | 4.44 | 59 | 5.76 | 5.68 |
| 13 | 4.28 | 19 | 1.95 | 4.54 |
| | 2.69 | 32 | 2.20 | 5.83 |
| | 4.65 | 14 | 1.23 | 5.28 |
| | 10.02 | 12 | 1.07 | 5.25 |
| 14 | 3.73 | 176 | NA | 4.77 |
| | 3.31 | 68 | NA | 8.12 |
| | 3.30 | 50 | 3.95 | 6.63 |
| 15 | 3.57 | 27 | 2.21 | ND |
| 16 | 2.93 | 38 | 3.19 | ND |
| 17 | 2.39 | 19 | 1.84 | 5.14 |
| | 1.50 | 13 | 1.17 | 4.63 |
| | 1.05 | 19 | 1.50 | 3.81 |
| 18 | 2.22 | ND | ND | ND |
| | 5.73 | 19 | 1.85 | 3.85 |
| | 3.62 | 17 | 1.40 | 4.54 |
| 19 | 1.93 | 17 | 1.60 | 3.08 |
| | 2.04 | 12 | 1.16 | 4.02 |
| | 1.92 | 17 | 1.75 | 4.06 |
| 20 | 1.70 | 77 | NA | 3.17 |
| 21 | 1.64 | 13 | 1.37 | 1.73 |
| 22 | 1.54 | 17 | 1.76 | 2.28 |
| 23 | 1.44 | 34 | 2.49 | 2.10 |
| 24 | 1.35 | 12 | 1.20 | 1.87 |
| | 1.30 | 14 | 1.08 | 2.26 |
| | 1.40 | 13 | 1.06 | 1.66 |
| 25 | 0.16 | <10 | 0.80 | ND |
| | 0.25 | <10 | 0.65 | 0.41 |
| | 0.35 | <10 | 0.77 | 0.52 |
| | 0.22 | <10 | 0.49 | 0.47 |

[a] s/co of ≥1 = positive, s/co of 0.90 to 0.99 = equivocal. A total of s/co of <0.9 = negative.

[b] ≥10 AcU/ml = positive, s/co of 0.91 to 0.99 = equivocal, s/co of ≤0.9 = negative.

[c] NA, no s/co was available because serum had to be diluted for the semiquantitative test procedure.

[d] ND, not done (not enough serum).

TABLE 2. Test results for serum specimens from children and HSV antibody-negative adults for gG2 antibodies by three different gG2-based enzyme immunoassays (assays A, B, and C)

| Specimen | No. of serum samples | | |
|---|---|---|---|
| | Children[a] | | HSV IgG-negative adults ($n$ = 38) |
| | HSV IgG negative ($n$ = 70) | HSV IgG positive ($n$ = 75) | |
| Sera tested by assays A, B, and C | 60 | 61 | 26 |
| Sera negative by assays A, B, C | 58 | 57 | 23 |
| Sera with discrepant results | 2[b] | 4[c] | 3[d] |
| Sera tested by assays A and B | 10 | 14 | 10 |
| Sera negative by assays A and B | 10 | 12 | 10 |
| Sera with discrepant results | | 2[e] | |
| Sera tested by assay A | | | 2 |
| Sera negative by assay A | | | 2 |

[a] Two to 8 years of age.

[b] The two serum samples were negative by assay A (0.19 and 0.64 s/co), positive by assay B (12 and 21 AcU; 1.13 and 2.13 s/co), and negative by assay C (0.13 and 0.54 s/co).

[c] The four serum samples were negative by assay A (0.41, 0.65, 0.25, and 0.52 s/co) and positive by assay C (1.41, 1.21, 1.39, and 1.32 s/co), and by assay B the first two samples had equivocal results (<10 AcU; 0.96 and 0.92 s/co) and the third and fourth samples had negative results (<10 AcU; 0.50 and 0.48 s/co).

[d] By assay A one sample had an equivocal result (0.97 s/co) and two samples had negative results (0.24 and 0.61 s/co), by assay B the first two samples had positive results (30 AcU and 2.16 s/co; 14 AcU and 1.46 s/co) and the third sample had an equivocal result (<10 AcU and 0.98 s/co), and by assay C, the first sample had an equivocal result (0.94 s/co) and the second and third samples had negative results (0.40 and 0.29 s/co).

[e] By assay A the two samples had negative results (0.24 and 0.25 s/co), and by assay B the first sample had an equivocal result (<10 AcU and 0.97 s/co) and the second sample had a positive result (14 AcU and 1.40 s/co).

well as 1 year before and 3.5 months after transplantation (the latter data are not presented). The sera were drawn on the day of transplantation and 2.5, 5, and 12 months after transplantation. During the period from first virus isolation after transplantation to 12 months after transplantation, the patient received for the prevention of graft rejection an immunosuppressive triple therapy with 275 to 175 mg of cyclosporine, 100 to 50 mg of azathioprine, and 10 to 7.5 mg of cortisone per day. During that time, his leukocyte counts varied between 7.57 ×

TABLE 3. Test results for serum specimens from HSV IgG-positive individuals with culture-proven HSV-1 infection for gG2 antibodies by three different gG2-based enzyme immunoassays (assays A, B, and C)

| Specimen | No. of serum samples |
|---|---|
| Sera tested by assays A, B, and C | 40 |
| Sera negative by assays A, B, C | 34 |
| Sera reactive by assays A, B, C | 4[a] |
| Sera with discrepant results | 2[b] |
| Sera tested by assays A and B | 1 |
| Sera negative by assays A and B | 1 |

[a] The four serum samples were positive by assay A (2.70, 1.41, 1.39, and 1.65 s/co) and assay C (4.44, 1.29, 1.47, and 1.34 s/co). The first three samples were positive by assay B (>200 AcU but no s/co because of serum dilution, 12 AcU and 1.08 s/co, and 40 AcU and 3.29 s/co) and the fourth sample was equivocal by assay B (<10 AcU and 0.96 s/co).

[b] By assay A one sample was positive (1.12 s/co) and one sample (from an 11-year-old adolescent) was negative (0.42 s/co). By assay B the first sample was negative (<10 AcU and 0.35 s/co) and the second sample was positive (15 AcU and 1.41 s/co). Both samples were negative by assay C (0.40 and 0.72 s/co).

TABLE 4. Positive or equivocal reactivities to HSV gG2 in sera from 26 of 173 HSV-seropositive pregnant women

| Pregnant women | s/co for assay A[a] | Assay B[b] | | s/co for assay C[a] |
|---|---|---|---|---|
| | | AcU/ml | s/co | |
| 1 | 8.41 | 85 | 4.83 | 14.11 |
| 2 | 7.87 | 50 | 4.94 | 10.37 |
| 3 | 7.06 | 50 | 5.39 | 7.96 |
| 4 | 6.64 | 38 | 2.72 | 5.71 |
| 5 | 6.33 | 31 | 2.25 | 10.06 |
| 6 | 6.27 | 19 | 2.01 | 5.68 |
| 7 | 5.91 | 33 | 2.95 | 2.80 |
| 8 | 5.12 | 45 | 2.90 | 12.19 |
| 9 | 5.10 | 104 | NA[c] | 8.46 |
| 10 | 4.88 | 48 | 2.49 | 9.23 |
| 11 | 4.58 | 13 | 3.20 | 1.14 |
| 12 | 4.49 | 180 | NA | 8.37 |
| 13 | 4.19 | 42 | 2.60 | 9.13 |
| 14 | 4.07 | 17 | 2.26 | 3.30 |
| 15 | 3.33 | 17 | 1.90 | 2.00 |
| 16 | 2.68 | 24 | 1.87 | 7.82 |
| 17 | 0.90 | 27 | 2.41 | 4.79 |
| 18 | 0.59 | <10 | 0.77 | 1.70 |
| 19 | 0.58 | 11 | 1.15 | 1.41 |
| 20 | 0.56 | 32 | 2.10 | 0.55 |
| 21 | 0.48 | <10 | 0.70 | 7.52 |
| 22 | 0.45 | 19 | 1.59 | 0.64 |
| 23 | 0.45 | <10 | 0.32 | 1.02 |
| 24 | 0.42 | 18 | 1.52 | 1.20 |
| 25 | 0.41 | 13 | 1.50 | 1.30 |
| 26 | 0.34 | <10 | 0.40 | 1.23 |

[a] s/co of ≥1 = positive, s/co of 0.90 to 0.99 = equivocal, s/co of <0.9 = negative.

[b] A total of ≥10 AcU/ml = positive, s/co of 0.91 to 0.99 = equivocal, s/co of ≤0.9 = negative.

[c] NA, no s/co was available because the serum had to be diluted for the semiquantitative test procedure.

$10^3$ and $11.94 \times 10^3/\mu l$, with 72.7 to 83.6% neutrophils, 6.3 to 12.6% lymphocytes, 6.6 to 9.1% monocytes, 0.7 to 5.2% eosinophils, 0.4 to 1.7% basophils, and 1.7 to 2.0% large unstained cells. One and a half months after transplantation, the $CD4^+$:$CD8^+$ ratio was 1.17, and the absolute number of $CD3^+$ cells was 605/μl. Although the patient was immunosuppressed, the lack of seroreactivity to the particular viral antigen (gG2) was not due to a general lack of humoral immunity since the sera were clearly positive by the type-common HSV antibody test and moderately positive by the HSV Type 1 Specific IgG ELISA (Gull Laboratories, Inc., marketed by Fresenius; data not shown). Furthermore, antibodies to cytomegalovirus, Epstein-Barr virus, human herpesvirus 6, and hepatitis A virus were regularly detectable. Moreover, a total of eight other immunosuppressed individuals, i.e., four organ transplant recipients (patients 3, 6, 7, and 9), two patients with tumors of the hematopoietic system (patients 14 and 22), and two AIDS patients (patients 10 and 24) were shown to produce detectable amounts of antibodies to gG2 in our study.

The specificities of the ELISAs were evaluated with sera from HSV-seronegative children and adults (including the 32 pregnant women who did not test positive by the type-common HSV antibody assay), as well as HSV antibody-positive children. Among the HSV-seronegative adults, one serum sample obtained from a 27-year-old woman concordantly revealed a low level of reactivity by all three assays. It cannot be excluded that this serum sample contained low-titer HSV-2 antibodies and was mistyped as HSV seronegative by the type-common immunoassay with whole-virus antigen. If this serum sample is considered positive, no false-positive result was produced by assay A. The facts that six of the eight serum samples reactive by assay B and all four serum samples reactive by assay C were from HSV-seronegative or -seropositive children and that the reactivities were always low suggest that these sera may be false positive. This is supported by a large seroepidemiologic survey, in which sera from 785 children ages 1 to 14 years were tested for gG2 antigen (13), and for these children a seroprevalence of 0.25% was found. In contrast, the use of assay B or assay C to test sera from our collection of children would suggest seroprevalences in children of 4.1 and 3.3%, respectively. On the other hand, ignorance of weakly positive results by either assay B or assay C cannot be justified, since more than a third of the sera from individuals with culture-documented HSV-2 infection and with reactivity by assay B and 9% of sera with reactivity by assay C were also found to be low-level positive (s/co < 2 s/co). Therefore, calculating the specificity of the anti-gG2 assays on the basis of the data produced with sera from children and HSV-seronegative adults, thereby excluding the serum from the HSV-seronegative woman who was suspected of having low-titer gG2 antibodies and including the sera from the 32 HSV-seronegative pregnant women, assay A had a specificity of 100%, assay B had a specificity of 96.2%, and assay C had a specificity of 97.8%. (If the two serum samples with discrepant results in the group of individuals with culture-documented HSV-1 infection [Table 3] are additionally considered, the specificities for assays A and B would be 99.6 and 96.4%, respectively.)

Recently, a large premarketing evaluation of assay B was carried out by comparing this ELISA with Western blotting (2). In that study, in which 49 serum samples were used for determination of the sensitivity and 116 serum samples were used for determination of the specificity, the sensitivity and specificity (98 and 97%, respectively) were similar to those found in the present study.

Since 3 to 8% discrepant results occurred for HSV-seronegative individuals or HSV antibody-positive children because of false-positive results by assay B or C, the prevalence of HSV-2 infection was calculated for samples with concordantly reactive results by all three assays. These included 4 of 41 (9.8%) serum samples from adults with culture-proven HSV-1 infection and 17 of 173 (9.8%) serum samples from pregnant women. The frequency of discrepant reactive results was also similar for these two groups: 4.9% in the group with culture-proven HSV-1 infection and 5.2% in pregnant women. These frequencies resembled those for the groups of HSV-seronegative individuals or HSV-seropositive children.

In summary, the new commercially available gG2-based enzyme immunoassays are useful tools for the detection of type-specific HSV-2 antibodies and will clearly improve the serodiagnosis of HSV infection. However, careful interpretation of the results is indicated, especially if the exhibited reactivity is low, and for determination of the definitive HSV serostatus, confirmatory assays may still be necessary.

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