Novel Approach for Specific Detection of Herpes Simplex Virus Type 1 and 2 Antibodies and Immunoglobulin G and M Antibodies

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Recently a few new herpes simplex virus (HSV) type-specific serological diagnostic tests have been introduced to the commercial market, but these tests have some limitations. Moreover, it is not yet clear which commercial test can be regarded as a “gold standard” for the serodiagnosis of HSV infections. In order to improve the clinical diagnostic value of serological tests for the detection of HSV infections, we developed novel, competition-based enzyme-linked immunosorbent assays for the specific determination of HSV type 2 antibodies (SeroHSV2) and HSV type 1 antibodies (SeroHSV1) and two complementary tests for the detection of HSV immunoglobulin M (IgM) and IgG antibodies (SeroHSV IgM and SeroHSV IgG). These four new kits were evaluated in comparison with some commercial kits for the detection of HSV antibodies that are commonly used at present in Israeli clinical laboratories. The results indicate that SeroHSV2 is highly sensitive (>92%) and highly specific (>94%). SeroHSV2 does not cross-react with other alphaherpesvirus antibodies. SeroHSV1 is highly sensitive (>94%) and specific (>91%) compared to four commercial available kits. SeroHSV IgM is highly specific (>92%) in comparison with other commercial HSV IgM tests. The sensitivity of SeroHSV IgM ranges between 50 and 70% compared to these tests. Further investigation of the discrepant results obtained by using in-house competition tests indicated that SeroHSV IgM is more sensitive. SeroHSV IgG was also found to be highly sensitive (>94%) and highly specific (>92%) compared to the other commercial HSV IgG tests.

Herpes simplex virus (HSV) is one of the most widespread viruses known to cause acute and recurrent infections in humans. Once acquired, HSV infects the sensory nerve cells innervating the mucosal areas involved in the acute infection, migrates to the regional sensory ganglion, and remains latent. When active, the virus may cause the development of vesicles and ulcers (4, 6).

There are two types of HSV. HSV type 1 (HSV-1, also known as herpescivirus labialis) most commonly infects the oral region, causing “cold sores.” HSV-2 (commonly known as herpesvirus genitalis) most often infects the genital and perianal regions. Each of the two types can cause infections in the sites with which the other is more commonly associated and can also infect other organs such as the skin, the eyes, and the brain (6). Most HSV-infected persons may shed the virus periodically even in the absence of clinical manifestations (1, 13).

Seroepidemiologic studies have shown that about 80% of adults worldwide have antibodies to HSV-1. Serological assays however, do not indicate the site of infection. Therefore, it is difficult to define what proportion of HSV-1-seropositive individuals has genital HSV-1 or orolabial infections. Several studies suggest that the rate of genital HSV-1 infection, like the rate of HSV-2 infection, is increasing (10).

In recent years there has been a tremendous increase in the reported incidences of genital herpes; HSV-2 seroprevalence in the United States is estimated between 20 and 25% by the age of 40 years. Genital herpes is more prevalent among women than among men. In patients with sexually transmitted diseases, the seroprevalence may reach 50% (5, 8). Approximately 85% of women, and nearly all men, with symptomatic acquisition of genital HSV-2 infection will have acute recurrent episodes within the first year at an average rate of four to five times per year (2). The tendency toward recurrent episodes is greater with genital HSV-2 infection than with HSV-1 (13).

The chronic nature of this disease results in persistent psychosocial or psychosexual distress for many patients. Patients undergoing chemotherapy, organ transplant recipients, and patients with human immunodeficiency virus (HIV) infection who suffer from episodes of HSV-1 or -2 can develop severe and extensive lesions (1).

The most severely affected population is neonates, who acquire HSV infection after exposure to the virus during delivery (3).

Numerous studies have revealed that the majority of HSV-2 infections are undiagnosed. It is also estimated that about 20% of patients with HSV-2 antibodies are truly asymptomatic or have lesions only in locations such as the cervix, which are difficult to observe (5).

Definitive diagnosis of genital herpes infections is fundamental to the management of patients and the development of strategies to prevent transmission to partners and neonates. Clinical presentation alone is often insufficient to diagnose genital herpes. Detection of type-specific antibodies is an important and essential part of accurate diagnosis, even in silent carriers of HSV-2 infections.

Recently, a few new type-specific serodiagnostic tests have been introduced to the market (1, 7, 9). In order to improve the clinical diagnostic value of the serodiagnosis of HSV infections, we have developed a highly specific and sensitive
screening tool for the determination of HSV-1 and -2 antibodies as well as complementary tests for the detection of immunoglobulin M (IgM) and IgG antibodies.

MATERIALS AND METHODS

SeroHSV diagnostic kits. (i) SeroHSV1 and SeroHSV2. SeroHSV1 and SeroHSV2 (Savyon Diagnostics Ltd., Ashdod, Israel) are semiquantitative competitive binding enzyme-linked immunosorbent assays (ELISA) intended for the specific detection and determination of HSV-1 and HSV-2 antibodies in human serum samples. SeroHSV1 and SeroHSV2 microtiter plates are coated with partially purified virus proteins from HSV1 (MacIntyre strain) and HSV2 (G strain).

The principle of the test is as follows. The serum to be tested is diluted with serum diluent containing either HSV-1 or HSV-2–specific monoclonal antibodies (MAbs) raised against either HSV-1 glycoprotein G (gG1) or gG2 major immunodominant epitopes and incubated with either SeroHSV1 or SeroHSV2 plates. If human HSV-1 or HSV-2 antibodies are present in the sample, they will compete with the respective specific MAbs. If, however, there are no specific HSV-1 or HSV-2 antibodies in the human sample, only HSV1- or HSV2-Mabs will be bound on the respective plate. Ready-to-use calibrator solutions containing predetermined MAbs specific to either HSV-1 or HSV-2, with (calibrators P00 and P35) or without (P100) different amounts of either HSV-1- or HSV-2–positive human serum, were used in the test. The signals obtained with different calibrators are inversely proportional to the amount of either HSV-1 or HSV-2 human antibodies. The P100 calibrator is defined as 100% of the signal, the P00 calibrator is defined as 80% of the signal, and the P35 calibrator is defined as 35% of the signal.

Tests were performed as described in the manufacturer’s instruction manuals. Human serum samples, positive controls, and negative controls were prediluted 1:4 with a ready-to-use serum diluent containing either HSV-1– or HSV-2–specific mouse antibodies. Each serum sample was measured against all three calibrators. Fifty microliters of a calibrator and a diluted serum sample or control was added to the corresponding microtiter well and incubated 1 h at 37°C. Microtiter wells were washed three times with wash buffer. Then, 50 μl of 1:100-diluted anti–mouse antibodies conjugated to horseradish peroxidase (HRP) was added and incubated 1 h at 37°C. Microtiter wells were washed three times with wash buffer, and then 100 μl of TMB substrate was added and incubated 15 min at room temperature. Subsequently, 100 μl of stop solution was added and absorbance (expressed as optical density [OD]) was read at 450 and 620 nm. Test results were calculated and interpreted according to the instruction manual as follows. The OD values of the three calibrators (P100, P00, and P35) were plotted on the y axis against their respective concentrations expressed as percentages of signal on the x axis. Then the best-fitted linear curve was drawn and was used for the interpolation of the concentration (expressed as a percentage of signal) of each of the sample sera. SeroHSV1 and SeroHSV2 results that demonstrated OD of >90% were considered negative, and those that gave a signal of <75% were considered positive. Signals between 75 and 80% were considered borderline.

(ii) SeroHSV IgM. SeroHSV IgM (Savyon Diagnostics Ltd.) is a qualitative ELISA intended for the specific detection of IgM antibodies to HSV-1 and -2 viruses in human serum. SeroHSV IgM microtiter plates are coated with partially purified virus proteins from HSV-1 and HSV-2. The test was performed as described in the manufacturer’s instruction manual. Human serum samples, positive controls, and negative controls were prediluted 1:105 with a ready-to-use serum diluent containing a stripping solution that eliminates nonspecific binding of antibodies with binding capacities of 10, 50, or 100 arbitrary binding units [BU/ml, referred to, respectively, as P10, P50, and P100 calibrators], diluted serum samples and controls were added to HSV-1 and HSV-2–precoated microtiter plates, and incubated 1 h at 37°C. Microtiter wells were washed three times with wash buffer. Then 50 μl of 1:300-diluted anti–human IgM antibodies conjugated to HRP was added and incubated 1 h at 37°C. Microtiter wells were washed three times with wash buffer, and then 100 μl of TMB substrate was added and incubated 15 min at room temperature. Subsequently, 100 μl of stop solution was added and the absorbance (OD) was determined at 450 and 620 nm. The results were calculated and interpreted according to the instruction manual. The OD values of the three calibrators (P100, P50, and P10) were plotted on the y axis against their respective concentrations (100 BU/ml for P100, 50 BU/ml for P50, and 10 BU/ml for P10) on the x axis. Then the best-fitted linear curve was drawn and was used for the interpolation of the concentration (in arbitrary binding units per milliliter) of each of the tested serum samples. Results below 10 BU/ml were considered negative, and those above 10 BU/ml were considered positive.

Results. Serum samples were obtained from four different Israeli clinical laboratories. Each of the laboratories precharacterized and preselected the samples utilizing the routinely used commercial serodiagnostic HSV kits. The samples were sent to Savyon Diagnostics and were Tested blindly by the SeroHSV diagnostic tests. Sensitivity and specificity were determined relative to the results obtained by the different commercial kits described below.

The HSV Type 2–Specific IgG ELISA (Gull Laboratories Inc.) is a quantitative ELISA intended for the specific detection of HSV-2–specific IgG antibodies in human serum samples. Purified HSV-2–glycoprotein gG2 is used as the antigen in this test.

The HSV Type 1–Specific IgG ELISA (Gull Laboratories Inc.) is a qualitative ELISA intended for the specific detection of HSV-1–specific IgG antibodies in human serum samples. Affinity-purified HSV-1–glycoprotein G (gG1, MS strain) is used as the antigen in this test.

The BioELISA HSV-2 IgG (Biokit, S.A., Barcelona, Spain) is a qualitative ELISA intended to detect HSV-2–specific IgG antibodies in human serum samples. Purified HSV-1 glycoprotein gG1 is used as the antigen in this test.

The HSV-1 and HSV-2 Differentiation Immunoblot IgG (MRL Diagnostics) is intended for use on serum samples previously determined to be positive for HSV antibodies. The MRL immunoblot test utilizes purified HSV and recombinant antigens (gG1 and gG2) immobilized on a nitrocellulose membrane.

The HSV-1 IgG ELISA (Eliza Tests, Gilching, Germany) is an ELISA for the quantitative determination of human IgG antibodies to HSV-1 in human serum samples. Serum samples for the detection of IgM antibodies to HSV-1 and -2 were tested in human serum samples. In this test, partially purified HSV (MacIntyre and St. Joer strains) antigens are used.

The Herpes Simplex Virus I ELISA (Genzyme Virotech GmbH, Rüsselsheim, Germany) is an ELISA for the quantitative determination of IgG and IgM antibodies to HSV-1 and HSV-2. Serum samples were pretreated by RF-SorboTech for the removal of excessive IgG levels.

The Herpes Simplex Virus II ELISA (Genzyme Virotech GmbH) is an ELISA for semiquantitative and qualitative detection of IgG and IgM antibodies to HSV-2 in human serum samples. Serum samples for the detection of IgM antibodies to HSV-1 and -2 were tested in human serum samples. In this test, HSV-1 and HSV-2 antigens were used.

Relative sensitivity and specificity of SeroHSV2. Four sets of precharacterized serum samples derived from four different clinical laboratories and evaluated by four different commercial tests were tested blindly on the Savyon SeroHSV2 kit.

The four different commercial tests detect specific human IgG antibodies to HSV-2 and utilize either purified or recombinant glycoprotein gG2 as the antigen.

The following serum samples were tested with the SeroHSV2 kit: (i) 244 serum samples precharacterized by the HSV Type 2–Specific IgG ELISA (Gull Laboratories Inc.), by which 71 samples were interpreted as positive and 173 as negative; (ii) 99 samples precharacterized by the ETI-HSVK-G2 (Sorin BioMedicina Diagnostics S.P.A., Saluggia, Italy) is an ELISA for the detection of IgM antibodies to HSV-1 and -2 in human serum. Serum samples for the detection of IgM antibodies to HSV-1 and -2 were tested in human serum samples. In this test, HSV-1 and HSV-2 antigens were used.

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Relative sensitivity and specificity of SeroHSV2. Four sets of precharacterized serum samples derived from four different clinical laboratories and evaluated by four different commercial tests were tested blindly on the Savyon SeroHSV2 kit.

The four different commercial tests detect specific human IgG antibodies to HSV-2 and utilize either purified or recombinant glycoprotein gG2 as the antigen.

The following serum samples were tested with the SeroHSV2 kit: (i) 244 serum samples precharacterized by the HSV Type 2–Specific IgG ELISA (Gull Laboratories Inc.), by which 71 samples were interpreted as positive and 173 as negative; (ii) 99 samples precharacterized by the ETI-HSVK-G2 (Sorin BioMedicina Diagnostics S.P.A., Saluggia, Italy) is an ELISA for the detection of IgM antibodies to HSV-1 and -2 in human serum. Serum samples for the detection of IgM antibodies to HSV-1 and -2 were tested in human serum samples. In this test, HSV-1 and HSV-2 antigens were used.

Relative sensitivity and specificity of SeroHSV2. Four sets of precharacterized serum samples derived from four different clinical laboratories and evaluated by four different commercial tests were tested blindly on the Savyon SeroHSV2 kit.
Relative sensitivity and specificity of SeroHSV1. Four sets of precharacterized serum samples derived from four different clinical laboratories and evaluated by four different commercial tests were tested blindly with the Savyon SeroHSV1 kit. Three of the four different commercial tests (from Gull Laboratories Inc., Sorin Biomedica Diagnostics S.P.A., and MRL Diagnostics) detect specific human IgG antibodies to HSV-1 and utilize either purified or recombinant glycoprotein gG1 as the antigen. The fourth test detects specific IgG antibodies to HSV (Dade Behring). Most of the serum samples tested with this kit originated from patients at high risk of infection with HSV-1.

The following serum samples were tested with the SeroHSV1 kit: (i) 113 samples precharacterized by the HSV Type 1-Specific IgG ELISA (Gull Laboratories Inc.), by which 69 samples were interpreted as positive and 44 as negative; (ii) 53 samples precharacterized by the ETI-HSVK-G1 (Sorin Biomedica Diagnostics S.P.A.), by which 38 samples were interpreted as positive and 15 as negative; (iii) 98 samples precharacterized by the HSV-1 and HSV-2 Differentiation Immunoblot IgG (MRL Diagnostics), by which 77 samples were interpreted as HSV-1 IgG positive and 21 were interpreted as HSV-1 IgG negative; and (iv) 147 samples precharacterized by the Enzygnost Anti-HSV IgG (Dade Behring Marburg GmbH), by which 98 samples were interpreted as positive and 49 as negative.

Relative sensitivity and specificity of SeroHSV IgM. To evaluate the relative sensitivity and specificity of SeroHSV IgM, four sets of serum samples precharacterized by four different commercial tests and derived from four different clinical laboratories were tested blindly with the Savyon SeroHSV IgM kit. The following serum samples were tested with the SeroHSV IgM kit: (i) 61 samples precharacterized by the HSV 1-2 IgM ELISA (Gull Laboratories Inc.), by which 26 samples were interpreted as HSV IgM positive and 35 were interpreted as negative; (ii) 30 samples precharacterized by the Herpes Simplex Virus II ELISA (Enzyme Virotech GmbH), by which 4 samples were interpreted as HSV IgM positive and 26 were interpreted as HSV IgM negative; (iii) 155 samples precharacterized by the Enzygnost Anti-HSV IgM (Dade Behring Marburg GmbH), by which 10 samples were HSV IgM positive and 144 were HSV IgM negative; and (iv) 244 samples precharacterized by the Anti-HSV-1 and -2 IgM Enzyme Immunoassay Kit (Diaisorin, Sorin Diagnostics s.r.l.), by which 16 samples were HSV IgM positive and 228 were HSV IgM negative.

Evaluation of discrepancy IgM results by competition assay. The SeroHSV IgM test was performed as described in the manufacturer’s instruction manual with one alteration: the serum samples were prediluted 1:105 with or without the presence of either HSV-1 and -2 antigens or Mycoplasma pneumoniae antigens at a concentration 10 times higher than that of the HSV antigens with which the microtiter plates were coated. With true-positive results, the excess of free HSV antigens will compete with the immobilized HSV antigens on the specific HSV antibodies. If, however, the results are false positives, no competition should be observed. M. pneumoniae antigens served as a control to show that the competition was due to the specific antigen and was not due to any excess antigen.

Relative sensitivity and specificity of SeroHSV IgG. To evaluate the sensitivity and specificity of SeroHSV IgG, four sets of serum samples derived from four different clinical laboratories were precharacterized by nine different commercial kits and were tested blindly with the Savyon SeroHSV IgG kit.

The following serum samples were tested with the SeroHSV IgG kit: (i) 120 samples precharacterized by the HSV Type 2-Specific IgG ELISA and the HSV Type 1-Specific IgG ELISA (Gull Laboratories Inc.), by which 81 samples were interpreted as HSV positive and 39 were interpreted as negative; (ii) 51 samples precharacterized by the BioELISA HSV-2 IgG and the BioELISA HSV-1 IgG (Biokit, S.A.), by which 38 samples were interpreted as HSV positive and 13 were interpreted as HSV negative; (iii) 39 samples precharacterized by the HSV-1 and HSV-2 Differentiation Immunoblot IgG (MRL Diagnostics), by which 32 samples were interpreted as HSV positive and 7 were interpreted as HSV negative; (iv) 150 samples precharacterized by the Enzygnost Anti-HSV/IgG (Dade Behring Marburg GmbH), by which 100 samples were HSV IgG positive and 50 were HSV IgG negative; and (v) 138 samples precharacterized by the SeroHSV1 & SeroHSV2 tests (Savyon Diagnostics Ltd.), by which 93 samples were interpreted as HSV positive and 43 were interpreted as negative.

### RESULTS

Relative sensitivity and specificity of SeroHSV2. In an attempt to determine the sensitivity and specificity of any kit, one needs to first define the “gold standard.” Since there is no agreement yet regarding which test is to be considered the “gold standard” for the specific detection of HSV-2 antibodies, we have evaluated the sensitivity and specificity of SeroHSV2 in comparison to results obtained with the kits routinely used in Israeli clinical laboratories. The relative sensitivity and specificity of SeroHSV2 in comparison with each of the commercial kits are presented in Table 1.

SeroHSV2 is highly sensitive (>92%) and highly specific (>94%) compared to the commercially available HSV-2 IgG tests used (Table 1). The lower sensitivity (81%) that was calculated when SeroHSV2 results were compared to those of the MRL test probably does not reflect the real sensitivity of SeroHSV2. As noted in the footnote to Table 1, the three serum samples that were positive by the MRL test and negative by SeroHSV2 were also negative by the HSV Type 2-Specific IgG ELISA (Gull Laboratories Inc.)

### Table 1. Relative sensitivity and specificity of SeroHSV2 on the basis of results with different commercial kits

| Commercial kit (manufacturer’s name) | Sensitivity (no. positive by SeroHSV2/no. positive by other test [%]) | Specificity (no. negative by SeroHSV2/no. negative by other test [%]) |
|-------------------------------------|---------------------------------------------------------------------|---------------------------------------------------------------------|
| Gull                                | 71/77 (92.2)                                                         | 162/173 (93.6)                                                       |
| Sorin                               | 7/7 (100)                                                           | 92/92 (100)                                                         |
| MRL                                 | 13/16 (81.25)*                                                      | 53/53 (100)                                                         |
| Biokit                              | 6/6 (100)                                                           | 75/76 (98.7)                                                        |

*The three serum samples that were positive by the MRL test and negative by SeroHSV2 were also negative by the HSV Type 2-Specific IgG ELISA (Gull Laboratories Inc.).

### Table 2. Specificity of SeroHSV2

| Sample type (n) | SeroHSV2 result | No. positive | No. negative | Specificity (%) |
|----------------|-----------------|--------------|--------------|----------------|
| HSV-1 IgG positive | 2               | 66           |              | 97.05          |
| HSV-2 negative (68) | 7               | 79           |              | 92             |
| VZV IgG positive (86) | 1               | 16           |              | 94             |

* Determined by other tests as described in the text.
ranging between 91 and 93%. Of the four serum samples that were SeroHSV1 positive and Gull HSV-1 IgG negative, one in fact was HSV IgM positive by the SeroHSV IgM test.

Relative sensitivity and specificity of SeroHSV IgM compared to different commercial kits. In the absence of agreement as to which commercial test can be regarded as the "gold standard" for the detection of HSV IgM antibodies, the sensitivity and specificity of SeroHSV IgM was determined in comparison with the results obtained from tests currently used in several Israeli clinical laboratories (Table 4).

SeroHSV IgM is highly specific (>92%) compared to three different commercial kits (Gull, Virotech, and Dade Behring) (Table 4). The lowest specificity (65%) was observed when SeroHSV IgM results were compared to Diasorin results. Of the 80 serum samples positive by SeroHSV IgM and negative by the Diasorin test, 11 were also tested by the Gull IgM test and found positive, and seven were also tested by the Dade Behring test and found positive. These results may indicate a lower sensitivity of the Diasorin test. The sensitivity of SeroHSV IgM ranged between 50 and 70% relative to the test to which it was compared. The 12 serum samples that were negative by SeroHSV IgM and positive by the Gull test were also negative by the Diasorin test. The three serum samples which were negative by SeroHSV IgM and positive by the Dade Behring test actually yielded very low positive results by the Dade Behring test and borderline results by the SeroHSV IgM test. The eight serum samples which were positive by the Diasorin test and negative by SeroHSV IgM were also negative by the Gull test. Therefore, these findings might suggest that the Diasorin IgM test is less specific than SeroHSV IgM and the Gull test.

To determine whether the 11 serum samples found positive by SeroHSV IgM and negative by the Dade Behring HSV IgM test were false positives of the SeroHSV IgM or false negatives of the Dade Behring test, we ran a competition assay on these samples (see Materials and Methods). The competition assay results are summarized in Table 5.

The competition assay yielded the following results (Table 5). All the serum samples which were positive by SeroHSV IgM and negative by the Dade Behring test competed specifically with the mixture of HSV-1 and -2 antigens and not with the M. pneumoniae antigen. There were no significant differences between the COI values obtained without any free antigen and those obtained with free M. pneumoniae antigen. However, the COI values were reduced dramatically when free HSV antigen was added. These results (Tables 4 and 5) suggest that SeroHSV IgM is highly specific and highly sensitive compared to the Dade Behring and other commercial tests.

Relative sensitivity and specificity of SeroHSV IgG. To evaluate the sensitivity and specificity of the SeroHSV IgG kit, we decided to compare the results obtained by SeroHSV IgG with results obtained with other commercial kits that are in routine use in Israeli clinical laboratories.

Based on the results obtained with SeroHSV2 on the above four sets of serum samples, the relative sensitivity and specificity of SeroHSV IgG were calculated in comparison either to combinations of results obtained with commercial HSV-1- and HSV-2-specific serological kits (Gull Laboratories Inc., Biokit, S.A., MRL, and Savyon Diagnostics Ltd.) or to results obtained with one test that detected HSV IgG antibodies (Dade Behring), as summarized in Table 6.

SeroHSV IgG is highly sensitive (>94%) and highly specific (>92%) compared to the available commercial kits (Table 6). The highest sensitivity and specificity for SeroHSV IgG were obtained when its results were compared to either the combined results of SeroHSV1 and SeroHSV2, the combined results of the Biokit, S.A., kits, or the results of the non-type-specific Dade Behring test.

### Table 3: Relative sensitivity and specificity of SeroHSV1 on the basis of results with different commercial kits

| Commercial kit (manufacturer’s name) | Sensitivity (no. positive by SeroHSV1/no. positive by other test [%]) | Specificity (no. negative by SeroHSV1/no. negative by other test [%]) |
|--------------------------------------|-------------------------------------------------------------|-------------------------------------------------------------|
| Gull Laboratories Inc.               | 66/69 (95.6)                                                | 40/44 (90.9)                                                |
| Biokit, S.A.                         | 38/38 (100)                                                 | 14/15 (92.3)                                                |
| MRL                                 | 72/77 (93.5)                                                | 19/21 (92.5)                                                |
| Dade Behring                         | 96/98 (97.9)                                                | 45/49 (91.8)                                                |

### Table 4: Relative sensitivity and specificity of SeroHSV IgM on the basis of results with different commercial kits

| Commercial kit (manufacturer’s name) | Sensitivity (no. positive by SeroHSV1 IgM/no. positive by other test [%]) | Specificity (no. negative by SeroHSV1 IgM/no. negative by other test [%]) |
|--------------------------------------|-------------------------------------------------------------|-------------------------------------------------------------|
| Gull                                 | 14/26 (53.8)                                                | 34/35 (97.1)                                                |
| Virotech                             | 2/4 (50)                                                    | 26/26 (100)                                                 |
| Dade Behring                         | 7/10 (70)                                                   | 133/144 (92.3)*                                            |
| Diasorin                             | 8/16 (50)                                                   | 148/228 (64.9)                                              |

* Another six samples showed borderline results by the SeroHSV IgM test.

### Table 5: SeroHSV IgM competition assay

| Serum sample no. | No competition | Competition | Control |
|------------------|----------------|-------------|---------|
| 107              | 2.07           | 0.75        | 1.73    |
| 113              | 1.54           | 0.52        | 1.30    |
| 138              | 1.55           | 0.10        | 1.54    |
| 141              | 1.53           | 0.50        | 1.37    |
| 143              | 3.35           | 0.43        | 3.04    |
| 144              | 1.42           | 0.53        | 1.26    |
| 205              | 2.12           | 0.41        | 1.96    |
| 244              | 1.48           | 0.42        | 1.27    |
| 308              | 1.48           | 0.29        | 1.27    |
| 324              | 1.84           | 0.92        | 1.75    |
| 347              | 1.40           | 0.50        | 1.30    |

* Determined as described for SeroHSV IgM in Materials and Methods.

* Addition of free HSV-1 plus HSV-2 antigens at a concentration 10 times higher than that of the bound antigen.

* Addition of free M. pneumoniae antigen at a 10-fold-higher concentration relative to the bound antigen.

### Table 6: Relative sensitivity and specificity of SeroHSV IgG on the basis of results with different commercial tests

| Commercial kits* (by manufacturer) | Sensitivity (no. positive by SeroHSV IgG/no. positive by other test [%]) | Specificity (no. negative by SeroHSV IgG/no. negative by other test [%]) |
|------------------------------------|-------------------------------------------------------------|-------------------------------------------------------------|
| Gull Laboratories Inc.             | 78/81 (96.3)                                                | 36/39 (92.3)                                                |
| Biokit, S.A.                       | 37/38 (97.4)                                                | 13/13 (100)                                                 |
| MRL (immuno blot)                  | 30/32 (93.8)                                                | 7/7 (100)                                                   |
| Savyon Diagnostics Ltd.            | 93/95 (97.8)                                                | 42/43 (97.7)                                                |
| Dade Behring                       | 97/100 (97)                                                 | 49/50 (98)                                                  |

* Combined results of for HSV-1 and HSV-2 tests are shown.
DISCUSSION

Most of the new HSV IgG type-specific tests are based on either specific recombinant or purified native gG1 and gG2 proteins. In spite of the high specificities achieved with these tests, cross-reactions might be observed. Homology analysis reveals a 38% sequence identity between gG1 and gG2 proteins. Moreover, the purity level of the recombinant and the purification of gG proteins might affect the specificity. Sensitivity might also be lower either due to the lack of posttranslational epitopes in recombinant proteins or due to the late appearance of HSV IgG antibodies (2 to 3 months postinfection) (1). Detection of IgM antibodies might shorten the time needed to detect seroconversion.

Using a competition-based ELISA such as SeroHSV1 or SeroHSV2 minimizes these problems. Both tests are highly specific, since these tests are based on the ability of human antibodies to compete with MAbs against highly specific major immunodominant epitopes. No adverse impact on sensitivity was observed as determined by comparison to the other HSV IgG type-specific commercial tests. Higher sensitivities (>94 and >97%) and specificities (>91 and >94%) were observed for SeroHSV1 and SeroHSV2, respectively, relative to the other commercial kits. The detection of total antibodies instead of only one type of antibody (IgG or IgM) probably leads to better evaluation of the actual prevalence rates of HSV infections. Indeed, one serum sample that was found positive by SeroHSV1 and negative by the Gull HSV1 IgG was IgM positive. Due to the use of the competition technique, SeroHSV2 and SeroHSV1 might also serve as screening and confirmatory tools in large epidemiological studies for diagnosis of HSV-1 and -2 infections. Competition-based enzyme immunoassays have been successfully used previously for the identification of HSV-1 and -2 antibodies (11, 12).

Real confusion exists regarding the quality of the results obtained by the kits that are currently commercially available for the detection of HSV IgM antibodies. Many of the results obtained by the different tests cannot be confirmed by using a second test (Table 3). In an attempt to improve the current clinical diagnostic value of HSV IgM antibody testing, Savyon Diagnostics has developed the SeroHSV IgM test. We have optimized the removal of rheumatoid factor in order to eliminate IgG interference without lowering the sensitivity of the test. SeroHSV IgM is highly specific (>92%) compared to three different commercial kits (Gull, Virotech, and Dade Behring). The sensitivity of SeroHSV IgM ranges between 50 and 70% relative to the tests it is compared to. The lowest correlation was found with the Diasorin IgM test.

The best agreement was observed when SeroHSV IgM was compared to the Dade Behring test: a sensitivity of 70% and a specificity of 92%. A competition assay was run in order to clarify the discrepant results, and it clearly supports the SeroHSV IgM results rather than the Dade Behring HSV IgM test results.

SeroHSV IgG is highly sensitive (>94%) and highly specific (>92%) compared to the available commercial kits. The best relative sensitivity and specificity was obtained with the Biokit, Dade Behring, and Savyon Diagnostics (combined SeroHSV1 and SeroHSV2) tests. Both SeroHSV IgG and the Dade Behring IgG test can be used as screening tools for the detection of HSV IgG antibodies. The intended use of the MRL immunoassay is to distinguish HSV-1 from HSV-2 antibodies in prescreened samples. Each sample is tested in a single mode, and therefore this test is not appropriate for mass screening. SeroHSV kits are ELISA based, automated, and user-friendly, and therefore they are appropriate for screening.

We believe that the major advantage in specific typing of HSVs lies in improved diagnosis of HSV-2 and the consequent prevention of the spread of HSV infection to neonates and sexual partners. The majority of the population may be asymptomatic and may show signs of carrying the virus, which may lead to active maternal infection during pregnancy and damage to the newborn at the time of delivery (14). Although specific antiviral therapy is not yet used in pregnancies, it will not be long before such therapy is advocated, mainly for HSV-2-seropositive women. HSV-1 infection through sexual contact has increased significantly during the past few years. The prognosis of HSV-1 infection is much better than that of HSV-2; therefore, it is recommended that genital herpes be detected by a specific test for HSV antibodies and confirmed by culture and typing of the isolates. Indications for using nonspecific HSV-1 and -2 IgG and IgM tests include conditions such as rashes in immunocompromised patients, suspected HSV encephalitis, and other severe or complicated HSV infections.

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REFERENCES

1. Ashley, R. L., and A. Wald. 1999. Genital herpes: review of the epidemic and potential use of type-specific serology. Clin. Microbiol. Rev. 12:1–8.
2. Benedetti, J., I. Corey, and R. Ashley. 1994. Recurrence rates of genital herpes after symptomatic first-episode infection. Ann. Intern. Med. 121:847–854.
3. Brown, Z. A., S. Selke, J. Zeh, J. Kopelman, A. Maslow, R. L. Ashley, D. H. Watts, S. Berry, M. Herd, and L. Corey. 1997. The acquisition of herpes simplex virus during pregnancy. N. Engl. J. Med. 337:509–515.
4. Corey, L. 1990. Genital herpes, p. 391–413. In K. Holmes, P. A. Mardh, P. F. Sparling, and P. J. Wiesner (ed.), Sexually transmitted diseases, 2nd ed. McGraw-Hill Book Co., New York, N. Y.
5. Corey, L. 1994. The current trend in genital herpes. Progress in prevention. Sex. Transm. Dis. 21(Suppl. 2):S38–S44.
6. Corey, L., and P. G. Spear. 1986. Infections with herpes simplex viruses. N. Engl. J. Med. 314:686–691.
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. Fleming, D. T., G. M. McQuillan, R. J. Johnson, A. J. Nahmins, S. O. Aral, F. K. Lee, and M. E. St. Louis. 1997. Herpes simplex virus type 2 in the United States, 1976 to 1994. N. Engl. J. Med. 337:1105–1111.
9. Groen, J., G. Van Dijk, H. G. M. Nieters, W. I. Van der Meijden, and A. D. M. E. Osterhaus. 1998. Comparison of two enzyme-linked immunosorbent assays and one rapid immunoblot assay for detection of herpes simplex virus type 2-specific antibodies in serum. J. Clin. Microbiol. 36:845–847.
10. Ross, J. D. C., I. W. Smith, and R. A. Elton. 1993. The epidemiology of herpes simplex types 1 and 2 infection of the genital tract in Edinburgh, 1978–1991. Genitourin. Med. 69:381–383.
11. Slomka, M. J., R. L. Ashley, F. M. Cowan, A. Cross, and D. W. Brown. 1995. Monoclonal antibody blocking tests for the detection of HSV-1 and HSV-2 specific humoral responses: comparison with Western blot assay. J. Virol. Methods 55:27–35.
12. Van Doornum, G. J., M. J. Slomka, M. Buimer, J. Groen, J. A. Van Der Heek, I. Cairo, A. Vyse, and D. W. Brown. 2000. Comparison of a monoclonal antibody-blocking enzyme-linked immunosorbant assay and a strip immunoblot assay for identifying type-specific herpes simplex virus type 2 serological responses. Clin. Diagn. Lab. Immunol. 7:641–644.
13. Wald, A., J. Zeh, S. Selke, R. L. Ashley, and L. Corey. 1995. Virologic characteristics of subclinical and symptomatic genital herpes infections. N. Engl. J. Med. 333:770–775.
14. Wald, A., J. Zeh, S. Selke, T. Warren, A. J. Ryncarz, R. Ashley, J. N. Krieger, and L. Corey. 2000. Reactivation of genital herpes simplex virus type 2 infection in asymptomatic seropositive persons. N. Engl. J. Med. 342:844–850.