Comparison of a Monoclonal Antibody-Blocking Enzyme-Linked Immunoassay and a Strip Immunoblot Assay for Identifying Type-Specific Herpes Simplex Virus Type 2 Serological Responses

G. J. J. VAN DOORNUM,† M. J. SLOMKA,‡ M. BUIMER,† J. GROEN,† J. A. R. VAN DEN HOEK,† I. CAIRO,† A. VYSE,† AND D. W. G. BROWN‡

Municipal Health Service, Amsterdam, The Netherlands, and Institute of Virology, University Hospital, Rotterdam, The Netherlands, and Central Public Health Laboratory, London, United Kingdom

Received 29 November 1999/Returned for modification 8 March 2000/Accepted 5 May 2000

The diagnosis of primary or recurrent genital herpes simplex virus (HSV) infections, which are mainly caused by HSV type 2 (HSV-2), is based on clinical symptoms, culture of clinical specimens, viral detection by nucleic acid amplification, and HSV antigen detection assays (4, 30, 34). HSV-1 and HSV-2 are closely related (13), and for the study of humoral responses to HSV infection, complement fixation assays, enzyme-linked immunoassays (ELISA) with crude antigens, immunofluorescence assays, and neutralization assays all lack specificity due to the cross-reactivity of antibodies against HSV-1 and HSV-2 (3, 4, 5). Assays using type-specific HSV antigens which can be used to differentiate between HSV-1- or HSV-2-specific antibodies have been described (2, 7, 8, 18, 21, 24, 28; D. Alex- ander et al., Abstr. 96th Gen. Meet. Am. Soc. Microbiol. 1996, abstr. C-101, p. 18, 1996), with the immunoassay (Western blotting [WB]) considered the “gold standard” because it has been most extensively validated (1, 4). An alternative to WB which does not require affinity-purified antigen is detection of type-specific antibody by blocking monoclonal antibody (MAB) (28). Serological assays and especially type-specific assays can be used in seroepidemiological surveys and other studies of the transmission of genital herpes (10, 26, 29).

The objective of the present study was to compare an MAB-blocking EIA for HSV-2 antibody detection with a strip im-

Comparison of a Monoclonal Antibody-Blocking Enzyme-Linked Immunoassay and a Strip Immunoblot Assay for Identifying Type-Specific Herpes Simplex Virus Type 2 Serological Responses

The diagnosis of primary or recurrent genital herpes simplex virus (HSV) infections, which are mainly caused by HSV type 2 (HSV-2), is based on clinical symptoms, culture of clinical specimens, viral detection by nucleic acid amplification, and HSV antigen detection assays (4, 30, 34). HSV-1 and HSV-2 are closely related (13), and for the study of humoral responses to HSV infection, complement fixation assays, enzyme-linked immunoassays (ELISA) with crude antigens, immunofluorescence assays, and neutralization assays all lack specificity due to the cross-reactivity of antibodies against HSV-1 and HSV-2 (3, 4, 5). Assays using type-specific HSV antigens which can be used to differentiate between HSV-1- or HSV-2-specific antibodies have been described (2, 7, 8, 18, 21, 24, 28; D. Alexander et al., Abstr. 96th Gen. Meet. Am. Soc. Microbiol. 1996, abstr. C-101, p. 18, 1996), with the immunoassay (Western blotting [WB]) considered the “gold standard” because it has been most extensively validated (1, 4). An alternative to WB which does not require affinity-purified antigen is detection of type-specific antibody by blocking monoclonal antibody (MAB) (28). Serological assays and especially type-specific assays can be used in seroepidemiological surveys and other studies of the transmission of genital herpes (10, 26, 29).

The objective of the present study was to compare an MAB-blocking EIA for HSV-2 antibody detection with a strip im-

Comparison of a Monoclonal Antibody-Blocking Enzyme-Linked Immunoassay and a Strip Immunoblot Assay for Identifying Type-Specific Herpes Simplex Virus Type 2 Serological Responses

The diagnosis of primary or recurrent genital herpes simplex virus (HSV) infections, which are mainly caused by HSV type 2 (HSV-2), is based on clinical symptoms, culture of clinical specimens, viral detection by nucleic acid amplification, and HSV antigen detection assays (4, 30, 34). HSV-1 and HSV-2 are closely related (13), and for the study of humoral responses to HSV infection, complement fixation assays, enzyme-linked immunoassays (ELISA) with crude antigens, immunofluorescence assays, and neutralization assays all lack specificity due to the cross-reactivity of antibodies against HSV-1 and HSV-2 (3, 4, 5). Assays using type-specific HSV antigens which can be used to differentiate between HSV-1- or HSV-2-specific antibodies have been described (2, 7, 8, 18, 21, 24, 28; D. Alexander et al., Abstr. 96th Gen. Meet. Am. Soc. Microbiol. 1996, abstr. C-101, p. 18, 1996), with the immunoassay (Western blotting [WB]) considered the “gold standard” because it has been most extensively validated (1, 4). An alternative to WB which does not require affinity-purified antigen is detection of type-specific antibody by blocking monoclonal antibody (MAB) (28). Serological assays and especially type-specific assays can be used in seroepidemiological surveys and other studies of the transmission of genital herpes (10, 26, 29).

The objective of the present study was to compare an MAB-blocking EIA for HSV-2 antibody detection with a strip im-
TABLE 1. Results of the MAb-blocking EIA for HSV-2 antibody and the SIA for HSV-1 and HSV-2 antibodies\textsuperscript{a}

<table>
<thead>
<tr>
<th>EIA result</th>
<th>HSV-1</th>
<th>HSV-2</th>
<th>HSV-1 and -2 NT\textsuperscript{b}</th>
<th>HSV negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV-2 positive</td>
<td>8</td>
<td>129</td>
<td>383</td>
<td>0</td>
</tr>
<tr>
<td>Equivocal</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HSV-2 negative</td>
<td>734</td>
<td>14</td>
<td>28</td>
<td>16</td>
</tr>
<tr>
<td>Total</td>
<td>744</td>
<td>144</td>
<td>411</td>
<td>16</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Sera were obtained from 1,612 attendees of an STD clinic in Amsterdam, The Netherlands.

\textsuperscript{b} NT, not typeable.

TABLE 2. Confirmation of HSV-2 antibody status\textsuperscript{a}

<table>
<thead>
<tr>
<th>HSV-2 antibody status</th>
<th>n</th>
<th>EIA</th>
<th>SIA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Positive</td>
<td>545</td>
<td>531</td>
<td>14</td>
</tr>
<tr>
<td>Negative</td>
<td>1,029</td>
<td>5</td>
<td>1,024</td>
</tr>
<tr>
<td>Total</td>
<td>1,574</td>
<td>536</td>
<td>1,038</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Sera were considered HSV-2 antibody positive or negative when the MAb-blocking EIA for HSV-2 antibody and the SIA for HSV-1 and HSV-2 antibodies gave the same results. Sera with discrepant results were tested by WB to confirm HSV-2 antibody presence. Sera excluded from this table are (i) 3 with equivocal EIA results, (ii) 26 sera which were HSV-2 antibody negative by EIA but reactive by SIA and the COBAS HSV-2 antibody assay, and (iii) a further 7 sera with discrepant results which were of insufficient volume for WB.

**RESULTS**

HSV-2-specific antibody results were obtained by MAb-blocking EIA and SIA for 1,612 sera (Table 1). In the EIA, 541 (33.6%) sera were positive and 1,068 sera were negative for HSV-2 antibody, with 3 sera giving equivocal results. SIA detected HSV-2-specific antibody in 555 (34.4%) serum specimens; 144 (26%) of these 555 sera were positive for HSV-2 antibody only, and 411 (74%) sera contained HSV-1 and HSV-2 antibody. Another 16 sera were positive for HSV antibody but could not be typed. HSV-1 antibody was detected by SIA in 1,155 (71.6%) sera, with 744 (64%) of these sera containing HSV-1 antibody alone and 411 (36%) sera containing antibody to both HSV types. Comparison of the EIA and SIA results revealed that 512 of the 541 EIA HSV-2-positive sera were also positive for HSV-2 antibody by SIA, resulting in a concordance of 94.6% for the SIA relative to the EIA. Of 555 SIA HSV-2-positive sera, 512 were EIA positive, giving a concordance of 92.3% for the EIA relative to the SIA (Table 1).

**Pattern of SIA bands in sera with discrepant results.** Eight serum specimens with discrepant results were HSV-2 antibody positive by EIA and HSV-1 antibody positive by SIA. Twenty-one sera were HSV-2 antibody positive by EIA and negative by SIA for HSV-1 and HSV-2 antibodies. Eighteen of these 21 sera were reactive for SIA by the gG-2 band. However, these 21 samples were interpreted as SIA negative due to the absence of reactivity to the HSV-2 gD-2 band.

Forty-two sera were HSV-2 antibody negative by EIA and positive by SIA for HSV-1 and/or HSV-2 antibody (Table 1). Twenty-eight of these 42 sera contained both HSV-1 and HSV-2 antibodies according to the SIA results. The distribution of reactivity to HSV gg-2 (the antigen against which the MAb used in the blocking EIA was raised) was as follows: 16 sera had a score of 1+, 4 sera were 2+, and only 8 sera were 3+. The remaining 14 of these 42 EIA HSV-2-negative sera contained only HSV-2 antibody, as determined by SIA. The distribution of reactivity to HSV gg-2 was as follows: 2 sera had a score of 1+, 5 sera were 2+, 6 sera were 3+, and 1 serum specimen was 4+. Another 16 serum specimens were negative by EIA, but the SIA revealed HSV antibody that could not be typed due to reactivity only to HSV gg-2; 9 sera were 1+, 6 sera were 2+, and 1 serum specimen was 3+.

**Discrepancy analysis by HSV-2 WB.** Confirmation by WB of discrepant SIA and EIA HSV-2 antibody results is reported in Table 2. Five of the eight EIA HSV-2-positive and SIA HSV-1-positive sera were available in sufficient quantity to be tested by HSV-2 WB. Four of these five were HSV-2 antibody negative, and one serum specimen tested positive by HSV-2 WB. Nineteen of the 21 EIA HSV-2 antibody-positive and SIA HSV-1- and HSV-2-negative sera could be tested by HSV-2 WB. Of these, one serum specimen was HSV-2 WB negative, three reacted equivocally, and two were positive. For the remaining 13 serum specimens, bands were seen on the immunoblots only after Aurodyte colloidal gold stain was added, indicating the presence of HSV-2 antibody at a low concentration.

Thirteen of the 14 EIA HSV-2-negative and SIA HSV-2-negative sera were tested by HSV-2 WB. Of these, nine were positive, two specimens were equivocal, and two showed no bands, i.e., they were WB negative. Fifteen of the 16 EIA HSV-2-negative samples that were not typeable by SIA were tested by HSV-2 WB. Twelve of these...
samples were WB negative, two gave equivocal results, and one was positive for HSV-2 antibody by WB.

Three sera tested equivocal by EIA. Two of these sera were EIA HSV-2 equivocal and SIA HSV-1 positive and were reactive for HSV-2 antibody by the COBAS HSV-2 EIA. The third EIA HSV-2 equivocal serum was SIA HSV-2 positive but tested negative by COBAS HSV-2 EIA.

Twenty-eight serum specimens were negative for HSV-2 antibody by EIA and reactive by SIA for HSV-1 and HSV-2. All 28 sera were reactive when tested by the COBAS HSV-2-specific EIA and were considered to contain HSV-2-specific antibodies.

**Sensitivity and specificity of EIA and SIA relative to the gold standard (HSV-2 WB).** Sensitivity and specificity characteristics of both assays (presented in Table 3) are based on the findings reported in Table 2. Eighty of the 87 sera with discrepant EIA and SIA results could be tested by WB (n = 52) or by COBAS HSV-2 (n = 28). The calculated assay characteristics (Table 3) are based on the assumption that the WB assay used to resolve discrepant EIA and SIA results was the gold standard. Seven sera with equivocal WB results were assumed to contain HSV-2-specific antibody.

In addition, these data were recalculated to incorporate results for the 28 sera that were negative by EIA but positive by SIA for HSV-1 and HSV-2 antibodies and that were HSV-2 antibody positive when tested by COBAS EIA. Here it was assumed that the COBAS results provided confirmation of the presence of HSV-2 antibody. Using this adjustment, the sensitivity of the blocking EIA decreased from 97.4 to 92.6%.

**DISCUSSION**

This study demonstrates a high degree of concordance between the HSV-2-antibody-specific EIA and the EIA HSV-1 and -2 SIA. Analysis of the EIA HSV-2 antibody-positive and SIA HSV antibody-negative serum specimens by HSV-2 WB indicated the presence of HSV-2 antibody in 15 of 19 specimens, albeit at a low concentration. Similarly, 9 of 13 of the EIA HSV-2 antibody-negative and SIA HSV-2 antibody-positive specimens could be confirmed by HSV-2 WB. Of the EIA HSV-2-negative and SIA nontypeable serum specimens, most (12 of 15) could not be confirmed by HSV-2 WB, whereas all 28 EIA HSV-2-negative and SIA HSV-1- and HSV-2-positive sera were confirmed as HSV-2 antibody positive by the COBAS assay.

Discrepancies between the assays may be due to differences in the presented antigens or in their ability to detect a type-specific humoral response following primary infection. In the blocking assay, an MAb (AP1) raised against gG-2 was used. Humoral responses to all HSV-2 infections may not include reactivity to this epitope. Differences between the sensitivities of both assays were magnified by the group of 28 EIA HSV-2-negative and SIA HSV-1- and HSV-2-positive sera which had been tested by COBAS EIA using gG-2 as the antigen. In this group, 20 sera gave a weak reaction against the HSV-2-specific gD-2 antigen spotted on the SIA. A detailed analysis of differences between various gB-based serologic assays was reported recently (27). No single explanation was found, although inconsistent test results were partly associated with weakly positive specimens.

The correlation between the presence of HSV-2-specific antibody detected by EIA and primary episodes of HSV-2 genital herpes has previously been studied in this population by Van de Laar et al. (31). In that study, medical history and clinical presentation were used to determine whether a symptomatic patient had primary genital herpes, and swabs from genital lesions were obtained for viral culture. A low rate of detection of HSV-2-specific antibody in current primary episodes of genital herpes was obtained. Only 19 of 34 (56%) sera from primary HSV-2 episodes contained HSV-2 antibody (31). The findings of that study were confirmed by the present study, as the SIA detected exactly the same sera containing HSV-2 antibody in the subgroup of individuals with primary HSV-2 infection. According to the literature, HSV type-specific antibody may not be detected reliably within 8 weeks of onset (2, 11, 20, 28). In the present study, the interval between onset of primary infection and the development of specific antibodies could not be determined, as only one serum specimen was available from cases of primary HSV infection. As discussed earlier, for the population in the present study, sensitivity differences between the EIA and SIA were not accounted for by differences in the ability to detect type-specific antibody during a primary episode.

In the development of the original MAb-blocking RIA, WB results using HSV-1 and HSV-2 antigens were used separately to validate the assay in a panel of sera collected from 64 individuals with culture-typed genital herpes (28). Twenty-one sera were obtained from patients with HSV-2 first episodes, and 25 sera were from patients with HSV-2 recurrences. In that study, WB analysis demonstrated greater sensitivity than the MAb-blocking RIA for detecting HSV-2 antibody following HSV-2 first episodes, with sensitivities of 19 of 21 (91%) and 16 of 21 (76%) true positives, respectively.

Other comparative studies of recently developed commercial HSV type-specific antibody assays have been described (14, 18, 19; A. J. Vye, M. J. Slomka, D. W. G. Brown, D. Lewis, and J. A. Corney, EUROGIN Int. Conf. Herpes Viruses Genital Pathol., 1996). The commercial availability of these assays offers possible diagnostic tools for the clinical management of patients with genital herpes. However, the role of serological testing in the care of HSV-infected individuals remains to be established. A retrospective study suggested that

---

**TABLE 3. Sensitivity, specificity, and predictive values of the MAb-blocking EIA and the SIA for HSV-2 antibodies**

<table>
<thead>
<tr>
<th>Assay characteristic</th>
<th>EIA</th>
<th>SIA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ratio</td>
<td>%</td>
</tr>
<tr>
<td>Sensitivity&lt;sup&gt;a&lt;/sup&gt;</td>
<td>531/545</td>
<td>97.4</td>
</tr>
<tr>
<td>Specificity&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1,024/1,029</td>
<td>99.5</td>
</tr>
<tr>
<td>Predictive value, positive test&lt;sup&gt;c&lt;/sup&gt;</td>
<td>531/536</td>
<td>99.1</td>
</tr>
<tr>
<td>Predictive value, negative test&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1,024/1,038</td>
<td>98.7</td>
</tr>
</tbody>
</table>

<sup>a</sup> Number of true-positive test results per total number of true positives.
<sup>b</sup> Number of true-negative test results per total number of true negatives.
<sup>c</sup> Number of true-positive test results per total number of positive test results.
<sup>d</sup> Number of true-negative test results per total number of negative test results.

---

**CI, confidence interval.**
serology would be more useful in diagnosing and managing recurrent disease than for primary HSV infection (25). Recurrent genital herpes is often asymptomatic or atypical (23, 35). The assays evaluated in the present study may be useful for the diagnosis of recurrent genital herpes. The case has been presented for screening to identify asymptomatic HSV-2 infections among STD clinic attendees. Here it is argued that this screening can provide a means of controlling the spread of genital herpes (9). Several issues require clarification before such an approach can be introduced, although according to some authors the patients are prepared to accept the findings of type-specific serology (6, 22; R. Brugh, D. Brown, A. Meheus, and A. Renton, Editorial, Sex. Transm. Infect. 75:142–144, 1999). Similarly, the benefit of using these tests to diagnose genital herpes just before term must be determined and will be dependent on the incidence of neonatal herpes. For example, in The Netherlands there is a very low incidence of neonatal herpes, with approximately half of the cases due to transmission of HSV-1 by health care workers (33).

We conclude that both the SIA and the blocking EIA are suitable assays for the detection of antibody against HSV-2, showing a high level of agreement in detecting HSV-2 antibody in the population studied. With regard to the applicability of either assay, the blocking EIA is a useful tool for large epidemiological studies, whereas the SIA proved to be a slightly more sensitive method.

REFERENCES


