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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

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Detection of herpes simplex virus type 2 (HSV-2)-specific antibodies by a monoclonal antibody (MAb)-blocking enzyme-linked immunoassay (EIA) was compared with detection by a strip immunoblot assay (SIA) in a sexually transmitted disease (STD) clinic population. The study population consisted of 1,683 genitourinary medicine clinic attendees (582 women and 1,101 men). Sera were tested for the presence of HSV-2 antibody by use of the blocking EIA, in which binding of the MAb AP-1 to HSV-2 glycoprotein G-2 (gG-2) is blocked by HSV-2-specific antibody. The Chiron RIBA HSV-1 and -2 strip immunoassay (SIA) utilizes HSV-1- and HSV-2-specific or cross-reactive antigens immobilized on nitrocellulose strips (HSV gB-1 and HSV gG-1 peptide bands specific for HSV-1 antibody, HSV-2 gG-2 band specific for HSV-2 antibody, and HSV gD-2 band cross-reactive for HSV-1 and HSV-2 antibodies). A total of 1,612 sera were tested by MAb-blocking EIA for HSV-2 antibody and by SIA for HSV-1 and HSV-2 antibodies. By EIA, 541 (33.6%) sera were positive for HSV-2 antibody and 1,068 sera were negative for HSV-2 antibody; 3 sera gave equivocal results. HSV-2 antibody was detected in 555 (34.4%) sera by SIA; 144 (26%) of these sera possessed only HSV-2 antibody, and 411 (74%) sera contained both HSV-1 and HSV-2 antibodies. SIA detected HSV-1 antibody in 1,155 (71.6%) sera; 744 (64%) of these sera contained HSV-1 antibody alone. Sixteen sera contained antibody against HSV but could not be typed by SIA. A total of 512 sera were positive for HSV-2 antibody by both the EIA and SIA. We concluded that the blocking EIA and SIA showed a high level of agreement in detecting HSV-2 antibody in this population. In contrast to the SIA, the blocking EIA is a useful tool for large epidemiological studies, though the SIA proved to be slightly more sensitive once sera with discrepant results were further tested.

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 (EIA) 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 immunoblot assay (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 immunoblot assay (SIA) for HSV-1- or HSV-2-specific antibodies using serum samples from a sexually transmitted disease (STD) clinic population.

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MATERIALS AND METHODS

The study population consisted of 1,683 STD clinic attendees (582 women and 1,101 men) who originally participated in a prevalence study of *Chlamydia trachomatis* infection during the period from 1986 to 1988. This cohort has been described previously (31, 32).

Serum specimens. A total of 1,612 serum specimens from the STD clinic population were available for this study, out of 1,683 specimens collected between 1986 and 1988. All specimens were stored at −20°C prior to testing.

MAb-blocking assay. Type-specific antibodies to HSV-2 were detected using an MAb-blocking EIA (with the MAb AP-1, against HSV-2 glycoprotein G-2 [gG-2]) at the Virus Reference Division, Central Public Health Laboratory, London, United Kingdom (17). This assay is a direct modification of the validated MAb-blocking radioimmunoassay (RIA) (28). Briefly, wells of Greiner microtiter plates were coated with an HSV-2-infected-cell lysate at a 1:25 dilution in phosphate-buffered saline (PBS) overnight at 4°C, followed by detergent (1.5% Triton X-100 and 0.5% Nonidet P-40 in PBS) for 30 min at room temperature. After incubation for 2 h at 37°C with PBS containing 10% fetal calf serum, wells of coated plates were incubated successfully for 1 h at 37°C with the following in PBS containing 10% fetal calf serum and 0.2% Tween 20: a 1:4 dilution of test serum, a 1:16,000 dilution of HSV-2-specific MAb, and a 1:1,000 dilution of horseradish peroxidase-conjugated anti-mouse MAb. Plates were washed three times between each stage using 0.05% Tween 20 in PBS. Colorimetric detection using tetramethyl benzidine substrate at room temperature in the dark followed, with the reaction stopped after 20 min by the addition of 2 M H_2SO_4. The optical density of each well was then measured immediately at 450 nm (and at 620 nm for reference) ($OD_{450/620}$), and the percent blocking of each serum was calculated by comparison with diluent controls and with the mean $OD_{450/620}$ of four wells containing a positive control serum (as described in reference 22, but substituting $OD_{450/620}$ measurements for measurements of counts per minute). Sera were considered positive for HSV-2 antibody if they gave a blocking value of ≥50%. This cutoff was set using the mean result plus three standard deviations for sera from a population of children (n = 213)

TABLE 1. Results of the MAb-blocking EIA for HSV-2 antibody and the SIA for HSV-1 and HSV-2 antibodies[a]

EIA result	No. of sera with SIA result					
	HSV-1	HSV-2	HSV-1 and -2	HSV NT[b]	HSV negative	Total
HSV-2 positive	8	129	383	0	21	541
Equivocal	2	1	0	0	0	3
HSV-2 negative	734	14	28	16	276	1,068
Total	744	144	411	16	297	1,612

[a] Sera were obtained from 1,612 attendees of an STD clinic in Amsterdam, The Netherlands.
[b] NT, not typeable.

considered negative for antibody to HSV-2. An in-house WB assay was used to confirm the sensitivity and specificity of the testing strategy (17).

SIA. The Chiron RIBA HSV-1 and -2 SIA (Chiron Diagnostics, Emeryville, Calif.) utilizes antigens immobilized on nitrocellulose strips as described by Alexander and colleagues (Alexander et al., Abstr. 96th Gen. Meet. Am. Soc. Microbiol. 1996). gB-1 is an HSV-1-specific peptide and gG-1 is a recombinant protein specific for HSV-1. gG-2 is a recombinant protein from HSV-2, and HSV-2 gD-2 is cross-reactive for HSV-1 and HSV-2 antibodies due to 85% conservation. The gG antigens were expressed in yeast as N-terminal-fusion proteins with superoxide dismutase; a C-terminal truncation of gD-2 was expressed in Chinese hamster ovary cells. The assay was carried out according to the instructions of the manufacturer at the Municipal Health Service of Amsterdam. Control bands for two levels of human immunoglobulin G (IgG) were also spotted on the immunostrip. A test was valid only if the human IgG control band was present. The intensity of the reaction was scored relative to that of the IgG control bands as follows: −, absent; 1+, equal to the intensity of the level I IgG control band; 2+, greater than level I and less than the level II IgG band; 3+, equal to the intensity of the level II IgG control band; 4+, greater than the level II IgG band. Antibody status was based on the banding pattern. The cross-reactive HSV-2 gD-2 band indicated only the presence of HSV antibody. The HSV-1 gG-1 and/or gB-1 band and the HSV-2 gG-2 band indicated the presence of HSV-1- and HSV-2-specific antibodies, respectively. Single-band reactivity to HSV-2 gG-2, HSV-1 gG-1, or HSV-1 gB-1 in the absence of the HSV-2 gD-2 band was interpreted as HSV antibody negative. Reactivity to only HSV-2 gD-2 was interpreted as the presence of HSV antibody, not typed.

Discrepancy analysis and gold standard. Specimens with discrepant results were further analyzed by HSV-2 WB assay using HSV-2 proteins as the antigen. This was carried out at the Institute of Virology in Rotterdam, The Netherlands, as previously described (1). Immunoblots without visible bands were treated with a colloidal gold stain (Aurodye; Jansen Life Sciences), and in some cases bands became visible after prolonged incubation, indicating the presence of HSV-2 antibody at a low concentration. Sera were considered to contain HSV-2-specific antibody if results were positive by both EIA and SIA; if the result by EIA and SIA were discrepant, the HSV-2 WB result was considered decisive. If WB retesting gave an equivocal result, then the serum was assumed to contain HSV-2-specific antibody.

Twenty-eight sera that were positive by SIA for HSV-1 and -2 antibody, but HSV-2 antibody negative by blocking EIA, could not be tested by WB. However, these samples had previously been tested by the COBAS HSV-2-specific EIA (Roche Diagnostics, Basel, Switzerland), which uses lectin-purified HSV-2 gG-2 as the antigen (15, 19).

Statistical analysis. Data were analyzed with confidence interval analysis (16) and Epi Info, version 6.3 (12).

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 by SIA for 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 gD-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 Aurodye 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-positive 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

TABLE 2. Confirmation of HSV-2 antibody status[a]

Confirmed HSV-2 antibody status	*n*	No. of samples with result			
		EIA		SIA	
		Positive	Negative	Positive	Negative
Positive	545	531	14	523	22
Negative	1,029	5	1,024	2	1,027
Total	1,574	536	1,038	525	1,049

[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) 28 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.

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

Assay characteristic	EIA			SIA		
	Ratio	%	95% CI[e]	Ratio	%	95% CI
Sensitivity[a]	531/545	97.4	95.6–98.5	523/545	96.0	93.9–97.4
Specificity[b]	1,024/1,029	99.5	98.8–99.8	1,027/1,029	99.8	99.2–100.0
Predictive value, positive test[c]	531/536	99.1	97.7–99.7	523/525	99.6	98.5–99.9
Predictive value, negative test[d]	1,024/1,038	98.7	97.7–99.2	1,027/1,049	97.9	96.8–98.6

[a] Number of true-positive test results per total number of true positives.
[b] Number of true-negative test results per total number of true negatives.
[c] Number of true-positive test results per total number of positive test results.
[d] Number of true-negative test results per total number of negative test results.
[e] CI, confidence interval.

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 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 gG-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. Vyse, 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 those 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

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. Brugha, 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.

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