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Evaluation of an Enzyme Immunoassay System for Measuring Herpes Simplex Virus (HSV) Type 1-Specific and HSV Type 2-Specific IgG Antibodies

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MRL Diagnostics has developed a dual enzyme immunoassay (EIA) system that employs the recombinant Herpes Simplex Virus (HSV) type-specific glycoproteins G1 (HSV1) and G2 (HSV2) to detect HSV type-specific IgG antibodies. This system was evaluated using 155 consecutive sera previously tested in a conventional dual EIA system (Zeus) that employs multiple HSV1 and HSV2 proteins to detect type-common as well as type-specific antibodies. Sera were also analyzed by Western blot to determine the true HSV type-specific IgG reactivity pattern. Of 110 sera giving concordant reactivity patterns in the MRL and Zeus EIA systems, 108 (98%) also displayed concordant Western blot patterns; two sera gave false positive HSV2 reactivity in both EIA systems. Of 45 sera giving discordant MRL and Zeus EIA reactivity patterns, 41 (91%) displayed a Western blot reactivity pattern that matched the MRL reactivity pattern. Both the HSV1 IgG component and the HSV2 IgG component of the MRL EIA system were 100% sensitive and > 95% specific. In contrast, the Zeus HSV1 IgG EIA was 98% sensitive and 79% specific, and the Zeus HSV2 IgG EIA was 85% sensitive and 79% specific. An analysis of the distribution of index values in the MRL EIA system showed that low-positive values (1.0–3.0) were rare, but, when detected, often represented false positive results; only 11 MRL low-positive results were observed, but all 6 MRL false positive results were found within this low-positive subgroup. These findings show that the MRL dual EIA system effectively detects HSV type-specific IgG antibodies. J. Clin. Lab. Anal. 14:13–16, 2000.

Key words: herpes simplex virus; type-specific IgG; enzyme immunoassay

INTRODUCTION

Herpes Simplex Virus (HSV) types 1 and 2 exhibit a high degree of serologic crossreactivity due to extensive similarities in amino acid sequences of many proteins (1). This crossreactivity has limited the diagnostic utility of serological tests for distinguishing HSV1 from HSV2 infection. Identification of the infecting HSV types is particularly important for characterizing genital herpes infection. Although most genital herpes cases are caused by HSV2, approximately 30% of cases in some geographic regions are caused by HSV1 (2). Both HSV1 and HSV2 genital infections can be asymptomatic or go unrecognized by the patient. However, HSV2 infections tend to be more serious, as indicated by higher rates of recurrence and subclinical viral shedding, in turn leading to more frequent transmission of virus to sexual partners and neonates (3–6). Serologic identification of HSV-infected individuals and determination of the infecting HSV type are thus important components of HSV transmission control programs (7,8).

Systems now in routine use for serologic characterization of HSV infection employ a dual enzyme immunoassay (EIA) system; one EIA utilizes multiple HSV1 proteins as antigens, and a second EIA utilizes multiple HSV2 proteins. Thus, antibodies recognizing type-common antigens shared by both HSV types react in both assays (9). It is therefore difficult to determine if a positive result in both assays represents crossreactive antibodies associated with a single infection (HSV1 or HSV2), or true dual infection.

Although most HSV proteins express type-common epitopes, glycoprotein G (gG) is antigenically distinct in HSV1 versus HSV2 (10). Thus, the antibody response to gG is type-specific; antibodies recognizing gG1 are found only in HSV1 infection, whereas antibodies recognizing gG2 are found only in HSV2 infection (11,12). Dual EIA systems using recombinant gG1 and gG2 to detect only type-specific HSV antibodies have recently been described, and effectively distinguish HSV1 only infections, HSV2 only infections, and dual infections (13,14). The goal of this study was to evaluate the dual

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EIA system recently developed by MRL Diagnostics (Cypress, CA).

MATERIALS AND METHODS

Serum Specimens

Sera used in the study were 155 consecutive specimens (with volume > 0.2 ml) submitted for testing in the conventional HSV IgG dual EIA system (see following paragraph).

Conventional HSV IgG Dual EIA System

HSV1 IgG and HSV2 IgG EIA kits were purchased from Zeus Scientific (Raritan, NJ). These kits utilize multiple HSV1 proteins and HSV2 proteins, respectively, as antigens; thus, type-common as well as type-specific IgG antibodies are detected in each assay. Assays were performed according to the manufacturer's instructions. Sera giving optical density (OD) values equal to or greater than the cutoff OD (i.e., index values ≥ 1.0) were considered positive. The Zeus reactivity pattern was defined as the HSV1 IgG EIA qualitative result/the HSV2 IgG EIA qualitative result.

Type-Specific HSV IgG Dual EIA System

HSV1 IgG and HSV2 IgG EIA kits were supplied by MRL Diagnostics. These kits are commercially available, and within the United States are for investigational use only. The kits utilize purified HSV recombinant gG1 and gG2 proteins, respectively, as antigens; thus, only type-specific IgG antibodies are detected. Assays were performed according to the manufacturer's instructions. Briefly, sera diluted 1:101 in sample diluent were added to microtiter wells and incubated for 1 hr at room temperature. Following 3 washes, peroxidase-conjugated goat antihuman IgG (Fc fragment specific) was added to all wells, and incubation continued for 30 min at room temperature. Following 3 additional washes, substrate reagent (tetramethylbenzidine and hydrogen peroxide in buffer) was added to all wells; after 10 min at room temperature, stop reagent (1 N sulfuric acid) was added to all wells. OD values at 450 nm were then measured using an ELISA plate spectrophotometer. Sera giving OD values equal to or greater than the cutoff OD (i.e., index values ≥1.0) were considered positive. The MRL reactivity pattern was defined as the HSV1 IgG EIA qualitative result/the HSV2 IgG EIA qualitative result.

TABLE 1. Western blot reactivity patterns for sera giving concordant reactivity patterns in Zeus and MRL EIA systems

HSV1/HSV2 pattern in both EIA systems	No.	Western blot reactivity	
		Corcordant	Discordant
–/–	39	39	0
+/–	47	47	0
–/+	3	3	0
+/+	21	19	2[a]
Totals	110	108	2

[a]Both sera exhibited a +/– Western blot reactivity pattern.

HSV Western Blot

All sera were tested by Western blot (using in-house prepared blot strips) as described previously (15). Western blots were interpreted by three experienced readers who had no knowledge of the EIA reactivity patterns.

RESULTS

As shown in Table 1, the Zeus HSV IgG EIA reactivity patterns and the MRL HSV IgG EIA reactivity patterns were concordant for 110 of the 155 sera evaluated (71%). Western blot reactivity patterns were concordant with the EIA patterns in 108 of these 110 sera (98%); 2 sera exhibited HSV2 false positive reactivity in both EIA systems. When the 45 sera with discordant EIA reactivity patterns were analyzed by Western blot, 41 of 45 sera (91%) exhibited a Western blot reactivity pattern that matched the MRL EIA reactivity pattern (Table 2).

The performance characteristics of the Zeus and MRL HSV IgG EIA kits in comparison to Western blot are shown in Table 3. Both the MRL HSV1 IgG EIA and the MRL HSV2 IgG EIA were 100% sensitive and > 95% specific. These values were higher than the values obtained for the comparable Zeus HSV IgG EIA procedures.

To better understand the small number of false positive results obtained using the MRL EIA system, the relationship between index values and false positive results was evaluated (Table 4). The dynamic range of index values in the MRL system was much greater than in the Zeus system; index val-

TABLE 2. Western blot reactivity patterns for sera giving discordant reactivity patterns in Zeus and MRL EIA systems

HSV1/HSV2 reactivity pattern				
Zeus EIA	MRL EIA	No.	No. with WB pattern matching Zeus EIA pattern	No. with WB pattern matching MRL EIA pattern
–/–	–/+	3	1	2
–/–	+/–	3	1	2
+/–	–/+	1	0	1
+/–	+/+	5	2	3
+/+	–/+	11	0	11
+/+	+/–	22	0	22
	Totals	45	4	41

TABLE 3. EIA performance characteristics compared to Western blot

Parameter	Zeus HSV1	Zeus HSV2	MRL HSV1	MRL HSV2
True negative	46	92	57	111
False negative	2	6	0	0
True positive	95	33	97	39
False positive	12	24	1	5
Sensitivity	97.9%	84.6%	100%	100%
Specificity	79.3%	79.3%	98.3%	95.7%
Efficiency	91.0%	80.6%	99.4%	96.8%

ues > 7.00 were not uncommon in the MRL system, whereas only 2 index values > 5.00 (representing a single specimen) were observed in the Zeus system. MRL HSV1 IgG results in the low-positive range (defined as index values of 1.0–3.0) were rare (3/155 = 1.9%); MRL HSV2 IgG results in this range were also uncommon (8/155 = 5.2%). Thus, of 310 total MRL system results, only 11 results (3.5%) were in the low-positive range. Each low-positive result occurred in a separate serum; thus, 11/155 sera (7%) exhibited a low positive result in the MRL EIA system. Notably, all 6 MRL false-positive results were found among this subgroup of 11 MRL low-positive results.

DISCUSSION

Our findings show that the MRL Diagnostics dual EIA system effectively identified HSV type-specific IgG antibodies, and can be used to successfully type HSV infections. The sensitivities and specificities for the MRL system components were quite similar to the values published for the comparable EIA system manufactured by Gull Laboratories (Salt Lake City, UT) (13) and the immunoblot assay manufactured by Chiron Corporation (Emeryville, CA) (14).

As expected, the Zeus EIA system yielded many false-positive results due to crossreactive antibodies. Somewhat surprisingly, a small number of false-negative results, particularly for HSV2 IgG, were also observed in the Zeus system. Since Western blots for these specimens clearly detected HSV2 IgG antibodies, their lack of reactivity in the Zeus HSV2 IgG EIA may reflect the absence or low concentrations of the appropriate HSV2 antigens.

TABLE 4. Distributioin of index values and relationship to false positive results[a]

Index range	Zeus HSV1	Zeus HSV2	MRL HSV1	MRL HSV2
<1.00	48	98	57	111
1.00–1.50	11 (5)	22 (17)	2 (1)[b]	3 (3)[b]
1.51–2.00	13 (4)	15 (5)	0 (0)[b]	1 (0)[b]
2.01–3.00	45 (3)	8 (0)	1 (0)[b]	4 (2)[b]
3.01–5.00	37 (0)	11 (0)	8 (0)	9 (0)
5.01–7.00	1 (0)	1 (0)	11 (0)	9 (0)
7.01–10.00	0 (0)	0 (0)	48 (0)	8 (0)
10.01–14.00	0 (0)	0 (0)	28 (0)	10 (0)

[a]Data represent the number of sera with index values in the indicated range (number of false-positive sera).
[b]Sera with index values in the low-positive range for the MRL EIA system.

The small group of sera giving false-positive results in the MRL system was contained within the small group of sera giving index values in the low-positive range. This overlap suggests low-level nonspecific IgG binding as a possible explanation for false-positive results. The rarity of samples giving MRL EIA results in this low-positive range (7%), however, is indicative of the system's robust performance. A clear-cut typing result (i.e., index value < 1.0 or > 3.0) was obtained for 144 of 155 (93%) sera, and the result was 100% accurate for these 144 sera. Those rare sera with low-positive index values should be analyzed by Western blot to determine if the result is truly positive or falsely positive.

As discussed by Ashley and Wald (9), false-negative reactivity early in infection is a potential limitation of any EIA system based solely on HSV type-specific antibody detection. As determined by Western blot, 12 weeks may be required for gG-specific antibodies to develop following primary HSV infection; type-common antibodies, in contrast, appear somewhat sooner. We thus expected to identify a few sera giving a Zeus true-positive/MRL false-negative pattern for either HSV1 or HSV2. The lack of sera showing this pattern may indicate that sera submitted for testing during this early infection window are uncommon. Studies using sera from patients with clinically documented primary HSV infection are needed to elucidate the time course for the development of antibodies identified by the MRL dual EIA system.

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