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Evaluation and Reporting of Enzyme Immunoassay Determinations of Antibody to Herpes Simplex Virus in Sera and Cerebrospinal Fluid

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Several methods for evaluating and reporting enzyme immunoassay (EIA) determinations of antibody to herpes simplex virus derived from one dilution of single serum samples were studied. An EIA ratio method for serological evidence of current infection from paired serum samples was also evaluated. Optical density (OD) of the reaction at a 1:100 serum dilution and estimated titers obtained by reference of the OD of the serum dilution to a standard curve were compared to the corresponding plotted EIA titer obtained by titration to endpoint. Neither the OD per se nor the estimated titer was completely predictive of the plotted titer (correlation coefficient [*r*] of 0.824 and 0.817, respectively), and they provided only a semiquantitative measurement of antibody concentration. For an antibody status report, however, OD would be sufficient if related to the cutoff value as an EIA index (OD of sample divided by cutoff OD for positive specimens). The OD of the EIA reaction at a single dilution (1:5) of cerebrospinal fluid, on the other hand, correlated quite well with the titer obtained by titration ($r = 0.950$). For serological diagnosis of current infection, the OD ratio of convalescent-phase/acute-phase sera was determined at several dilutions. A ratio of ≥ 1.54 was calculated as a reliable index for a significant rise in antibody concentration and compatible with current infection. By determining the convalescent-phase/acute-phase serum ratio at two dilutions, 1:100 and 1:1,000, the EIA ratio method appeared to be as sensitive as or more sensitive than, complement fixation in diagnosing current infection.

Traditionally, measurements of viral antibody have been reported to physicians in terms of titers based on the assay of doubling dilutions of serum samples. With the advent of sensitive solid-phase antibody assays such as the radioimmunoassay and the enzyme immunoassay (EIA), in which very high antibody titers may be obtained, titration to endpoint can become laborious. The trend, particularly with tests from commercial houses, is to test serum samples at one dilution, determine the optical density (OD) for EIA or counts per minute for the radioimmunoassay, and report the results in relation to a standard curve, to the mean of a number of negative or low-positive standardized samples, or as an index in relation to a cutoff value. In a recent paper by de Savigny and Voller (2), the problems, advantages, and disadvantages of different methods of reporting EIA results to the physician have been reviewed. Our present report considers several of these methods using single and serial dilutions of serum and cerebrospinal fluid (CSF) in an EIA test for herpes simplex virus (HSV) antibody developed in our laboratory.

MATERIALS AND METHODS

Antigen. Crude antigen of HSV type 1 strain McIntyre was prepared by sonication (1 min, 20 kc/s) of infected BHK 21 cells suspended in Hanks balanced salt solution to 5% of the original culture fluid volume. The fluid containing the crude antigen was cleared by centrifugation at 700 × *g* for 20 min at 4°C. Control antigen was prepared in the same manner from noninfected BHK 21 cells. The antigen preparation had an infectivity titer of 10^8 PFU/ml. The virus was photochemically inactivated with 1 μg of 4,5',8-trimethylpsoralen (trioxsalen) per ml as previously described (4), with the following modifications. After the addition of the trioxsalen, 4.8-ml samples of the antigen, dispensed into T-25 plastic flasks (Corning Glass Works, Corning, N.Y.; no. 25100) were exposed at 4°C to the low-intensity UV light source with the results shown in Fig. 1. To provide a safety margin, the antigen was photoreacted for two or three times the required time for inactivation before use. Control antigen was similarly treated. By block titration with a standardized human anti-HSV serum pool, a 1:64

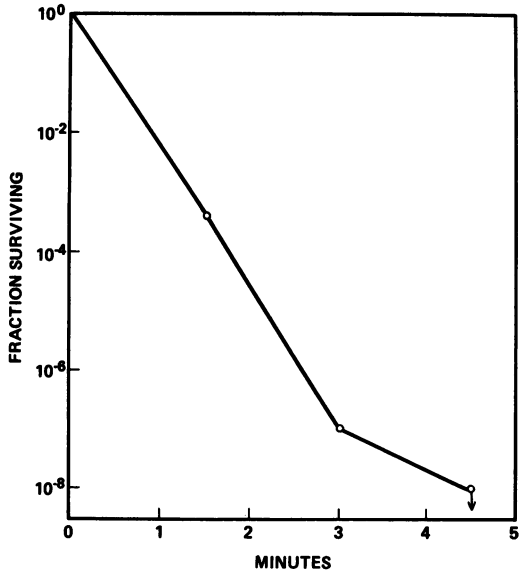

FIG. 1. Photochemical inactivation curve of HSV at 4°C by using 1 μg of trioxsalen per ml in combination with longwave UV light. After 4.5 min of irradiation (arrow), no residual infectivity was detected.

dilution of the antigen preparation contained 1 U of complement-fixing (CF) antigen per 0.025 ml before and after photochemical inactivation.

Specimens. Single serum samples and paired acute-phase (A) and convalescent-phase (C) serum samples submitted to our laboratory for viral antibody studies and single CSF samples submitted for viral isolation or for viral antibody study (or both) were tested for antibodies to HSV by CF and by EIA. Serum samples were from patients whose symptoms were suggestive of possible HSV infection, and CSF samples were taken from patients with suspected viral infection of the central nervous system.

EIA. Polystyrene beads (Precision Plastic Ball Co., Chicago, Ill.) were reacted overnight at 4°C with an optimal dilution of HSV antigen (1:100 or 1:200 dilution as determined by block tritation) or with the same dilution of control antigen in 0.005 M phosphate-buffered saline (PBS), pH 7.2. The beads were washed three times with PBS containing 0.05% Tween 20 (PBS-T), air dried, and stored at −70°C until used. For EIA, Abbott Laboratories trays (no. 7803-76), reaction tubes (no. 6171-11), and Pentawash system (no. 6118) were used. Serum or CSF samples (0.2 ml) diluted with PBS-T containing 0.5% bovine serum albumin were reacted with antigen and control coated beads. After washing three times with PBS-T, 0.2 ml of alkaline phosphatase-linked goat anti-human immunoglobulin G (IgG) (anti-H+L chain; Miles Laboratories, Inc., Elkhart, Ind.) diluted in PBS-T-bovine serum albumin to an optimal concentration (1:1,500 to 1:2,000 as determined by block titration for each lot of conjugate) was incubated with each bead. After the beads were washed, three times with PBS-T, they were transferred to reaction tubes, and 1.1 ml of substrate (1 mg of *p*-nitrophenylphosphate per ml in diethanolamine buffer, pH 9.8, containing 0.5 mM $MgCl_2 \cdot 6H_2O$ and 0.02% NaN_3) was added. The reaction was stopped by the addition of 0.2 ml of 3 M NaOH. The color reaction was read on a DU spectrophotometer at 405 nm by using a 0.7-ml microcell with a 1-cm light path. The OD of the reaction with the control coated bead was subtracted from that with the antigen coated bead. A dilution of 1:100 was used for assay of single dilutions of serum samples, and four fold dilutions starting at 1:100 were used for titration of serum antibody to the endpoint. Single dilutions of CSF were assayed at 1:5 dilution or were titrated in five fold steps to the endpoint, starting with a 1:5 dilution. The starting dilution for EIA was 1:25 for those CF-positive samples for which quantity was limited. All tests were done in duplicate.

Two incubation protocols for EIA were studied, one giving a 1-day test and the other giving a 2-day test. In the 2-day test, the coated beads were incubated with serum for 2 h at room temperature, with conjugate overnight at 4°C, and with substrate for 20 min at 37°C. In the 1-day test, the coated beads were incubated with serum and with conjugate each for 90 min at 37°C. Incubation with substrate was continued until the reactions with the low and high standard serum pools reached their mean OD as previously determined with the 2-day incubation protocol. The results by the two methods were equivalent. The 1-day test is now our standard procedure.

CF. CF antibody was determined by a standardized CF test with 2 U of antigen (5).

Statistical methods. Linear regression lines determined by the least squares method and correlation coefficients (*r*) were calculated. The logs of the reciprocal of the serum dilutions and of the titer were used in the calculations.

RESULTS

Determination of a cutoff OD to distinguish between the presence and absence of antibody and for titer endpoint. Nine serum samples lacking CF antibody to herpes simplex virus (CF titer, <1:8) were assayed repetitively at a 1:100 dilution in separate runs for a total of 115 assays. The mean of all of the OD readings was 0.0219 with a standard deviation (SD) of 0.0459. With a 0.16 OD cutoff (i.e., mean + 3 SD), 3 of 115 tests would be scored positively for antibody by EIA and thus be in disagreement with the negative CF results. With a 0.2 OD cutoff (i.e., mean + 4 SD), 1 of 115 tests (0.9%) would be scored as positive. An OD of 0.2 was therefore used as the cutoff for the presence of antibody and for endpoint titration.

Preparation of standard curve. (i) EIA titers of serum pools. Doubling dilutions of pools of serum samples with CF titers 1:8 to 1:64 were assayed in duplicate by EIA in five different runs. The mean OD at each dilution of each pool was calculated and plotted versus the reciprocal of the dilution on semilog paper, and the linear regression line was determined for the paired values. Correlation coefficients of the paired

TABLE 1. EIA results on serum pools for standard curve

Pool	Reciprocal CF titer[a]	OD			EIA titer[b]
		Mean[c]	SD	CV[d] (%)	
2	8	0.34	0.07	20.2	192
3	8–16	0.85	0.13	14.9	941
4	16–32	1.40	0.16	11.5	3,791
5	32–64	1.67	0.16	9.3	7,524

[a] CF titers of sera used in serum pools for preparation of EIA standard curve.

[b] By titration to endpoint in duplicate five times using 0.2 OD cutoff.

[c] Pools assayed in duplicate 14 times at a 1:100 dilution.

[d] CV, coefficient of variation.

values of the different pools in repeated runs varied from 0.977 to 0.999. The serum dilution at the 0.2 OD cutoff was taken as the EIA titer of each pool.

(ii) Standard curve. The standard curve was prepared by plotting the OD of the 1:100 dilution of each serum pool versus the reciprocal of the corresponding EIA titer on semilog paper. The correlation coefficients of the paired values of the standard curves in the various runs varied from 0.977 to 0.996. The means and SDs of OD values at 1:100 dilution of each serum pool were evaluated in 14 different runs, and the coefficient of variation was calculated (Table 1). The four standardized positive serum pools and one standardized negative pool were included in all subsequent runs on patients' sera as controls and for use as a standard curve in determining estimated titers from OD readings.

Stability of antigen-coated beads. Seven batches of beads prepared over a period of 26 months and stored at −70°C were checked with the standardized serum pools. No loss in activity was seen over the 2-year period tested (data not shown).

Determination of a cutoff ratio for serological evidence of current infection. In a doubling dilution test such as CF, a four fold rise in antibody titer between A and C paired serum samples is serological evidence for current viral infection. For an index of past or current infection by EIA, we investigated the use of a ratio of the OD readings of the two samples. To determine a cutoff value for a ratio which would discriminate between a stationary CF titer and a four fold or greater rise in CF titer, 21 serum pairs with stationary CF titers were assayed by EIA at a 1:100 serum dilution. The mean ratio of OD readings of C/A samples of the 21 serum pairs was 1.022 ± 0.172. A cutoff for a significant difference in OD readings between the paired sera, indicating evidence for current infection, was taken as the mean + 3 SD (i.e., C/A ratio, ≥1.54). For C/A ratio to be valid, the C specimen must be positive for presence of antibody (i.e., OD at 1:100 dilution, ≥0.2).

Study of single serum samples. Seventy-eight single serum samples were tested at 1:100 dilutions in 13 different runs to determine the interassay OD variability. The mean coefficient of variation of the OD readings of all the samples with antibody in the different runs was 14.4 ± 5.6%.

Based on the OD of the 1:100 dilution of each sample, its EIA titer was estimated from the regression line fitted to the standard curve (titer designated estimated titer), and the reciprocal was plotted on semilog paper against the reciprocal of the CF titer (Fig. 2). Eleven sera had a titer of <1:8 by CF. Nine of these were also negative by EIA. One sample with a CF titer of 1:8 was negative by EIA. The estimated titers of the other serum samples, with the exception of two samples which contained IgM antibody by indirect immunofluorescence test, generally increased with increasing CF titers. The correlation coefficient between CF titers and estimated EIA titers was 0.643 (n = 64). Samples seronegative by either method and the two samples with IgM antibody were not included in the calculations.

Comparison of EIA estimated titers and OD readings with EIA titers obtained by titration. EIA titers of 76 serum samples were determined by titration with fourfold dilutions starting with a 1:100 dilution. The titer of the sera, using a 0.2 OD cutoff, was determined in two ways. In one case the OD reading at each serum dilution was plotted against the reciprocal of the dilution of the serum on semilog paper, and the titer was read from the graph (designated as plotted titer). In the second case the linear regression line was calculated from the data points on the linear and near linear portions of the titration curve of those serum samples with sufficient antibody to have at least three data points (n = 72), and the titer at 0.2 OD was calculated from the fitted line. This titer is designated the linear regression titer. Estimated titers from 41 of the serum samples were also determined.

Plotted titers and linear regression titers were highly correlated (r = 0.993; Fig. 3). Correlations between estimated titers and plotted titers, or between the ODs at 1:100 serum dilution and plotted titers, were not as good (r = 0.817 and 0.824, respectively; Fig. 4). Correlation between CF titers and plotted titers was poor (r = 0.382; data not shown). The slopes of the antibody curves of different individuals varied (Fig. 5). The OD at the 1:100 serum dilution of patient A was 1.67, and that of patient B was 1.12, which resulted in estimated titers for patients A and B

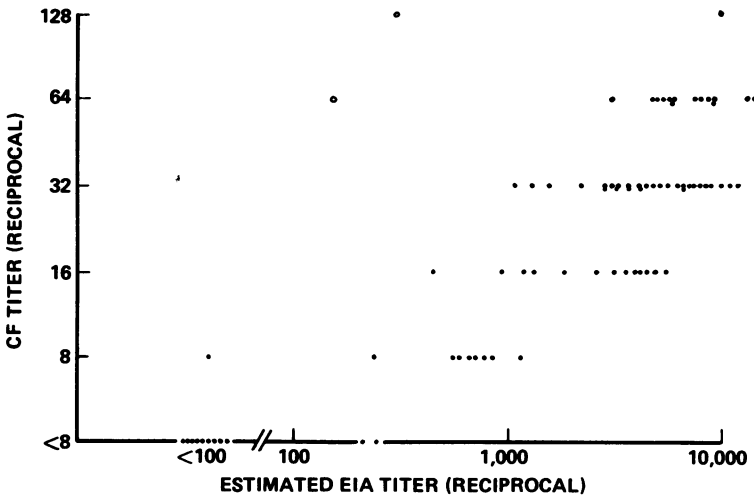

FIG. 2. Graph of CF antibody titers versus estimated EIA titers. Estimated EIA titers were determined by reference of the OD of a 1:100 dilution of each serum sample to a standard curve. The two open circles identify two samples with IgM antibody.

of 1:19,000 and 1:3,870, respectively. Yet, because of the steeper slopes of A's antibody curve compared to that of B's, their actual plotted titers and their LR titers were essentially the same, 1:4,800 versus 1:4,300.

Use of EIA ratio method for serological diagnosis of current infection. Fifty serum pairs showing a fourfold or greater rise in antibody titer by CF, and thus evidence for current or recent infection, were studied by EIA at a serum dilution of 1:100. C/A OD ratios were calculated. The OD of some A samples lacking antibody was zero or very close to zero. Therefore, to permit calculations of ratios in all cases and to avoid excessively high ratios that were meaningless, all A samples with OD <0.1 were assigned a value of 0.1. Forty-five (90%) of the 50 pairs showed a significant increase in antibody concentration by the ratio method (C/A $\geq$ 1.54). The five serum pairs not showing a significant change in OD already had CF antibody in the A sample (Table 2). These five serum pairs were tested further at fourfold dilutions. At all subsequent dilutions four of the five pairs now showed a C/A ratio of $\geq$1.54. Representative examples of antibody curves of serum pairs with the ratios at different serum dilutions are shown in Fig. 6 and 7. Because the OD of the A serum sample at 1:100 dilution may be too high to show a significant change between the ODs of the C and A samples, a more dilute sample in addition to the 1:100 dilution may be necessary. To test the efficacy of such a procedure, an additional 41 serum pairs showing a fourfold or greater rise in antibody titer by CF were checked by EIA at two dilutions, 1:100 and 1:1,000. Thirty-six of the serum pairs showed an EIA ratio of >1.54 at the 1:100 dilution (and also at the 1:1,000 dilution of those sera with sufficiently high titers), whereas five of the serum pairs had a ratio of >1.54 at the 1:1,000 dilution only. Based on these data, the EIA ratio method with two serum dilutions appeared to be as sensitive as the CF test for detecting significant changes in antibody concentrations.

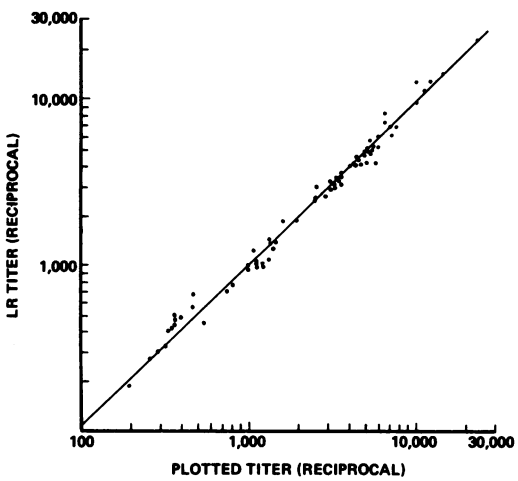

FIG. 3. Graph of linear regression titers determined from the fitted regression line for each serum sample versus the corresponding plotted titers determined by plotting the data points on semilog paper (r = 0.993, n = 72). The endpoint (titer) is the serum dilution at 0.2 OD. The slanting line is plotted from the formula of the regression line.

To determine whether the EIA ratio method could distinguish more critically significant anti-

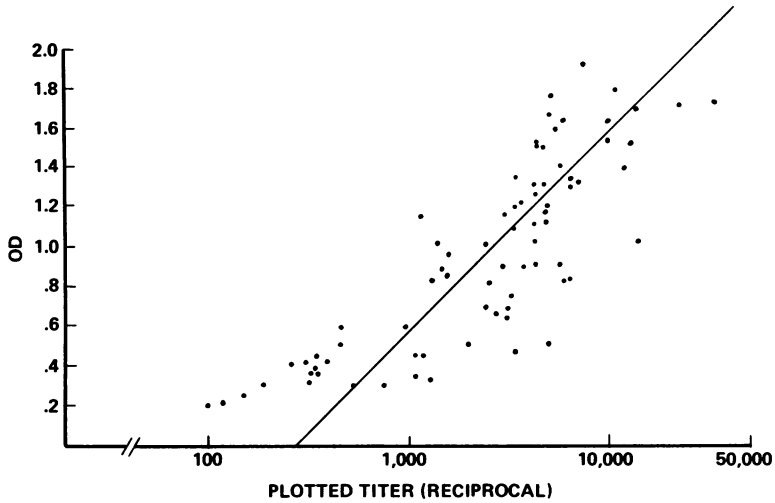

FIG. 4. Graph of the OD of a 1:100 dilution of each serum sample versus its plotted titer (determined by plotting the OD of fourfold dilutions of each serum sample on semilog paper and using as the titer endpoint the serum dilution at 0.2 OD) (r = 0.824, n = 76).

body changes among serum pairs with stationary titers by CF, 116 serum pairs with stationary CF titers or CF titers differing by only one doubling dilution were tested by the EIA ratio method. One hundred of the serum pairs (86%), as in the CF test, showed no significant change in antibody concentration at the 1:100 and 1:1,000 dilutions (EIA ratio, <1.54). Plots of the titration curves of some of these serum pairs indicated that the antibody curves of the A and C samples closely paralleled each other. Representative plots are shown in Fig. 8. Of the 16 serum pairs with EIA ratios of >1.54 (14%), 8 had IgM antibody to HSV by indirect immunofluorescence. Fourteen of these patients had a clinical picture of genital herpes, and two had a clinical picture of facial herpes.

It appears that the EIA ratio method may be more sensitive in detecting significant changes in antibody concentrations than a doubling dilution

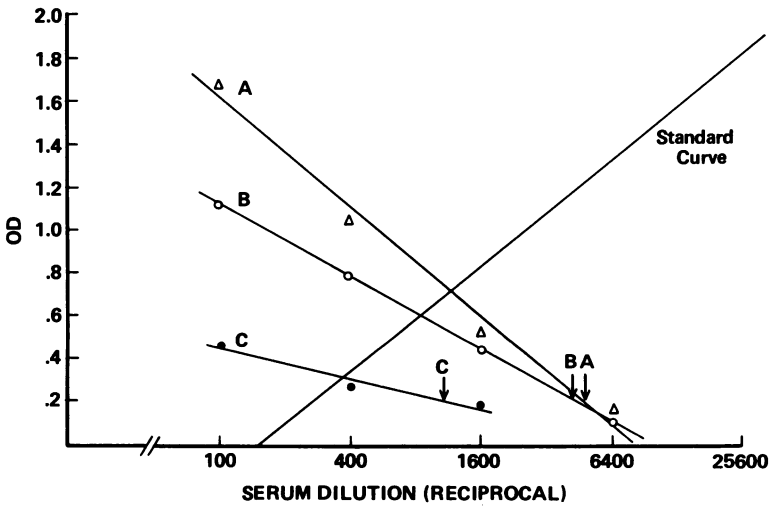

FIG. 5. Demonstration of variation in slopes of antibody curves of individual patients A, B, and C. Open and closed symbols are OD readings at the indicated serum dilutions. Slanting lines are the linear regression lines calculated from the OD data points for each patient. Arrows indicate the serum dilution at titer endpoint (serum dilution at 0.2 OD). Line A, slope (s) = −0.837, r = −0.993; line B, s = −0.566, r = −0.999; line C, s = −0.225, r = −0.976; standard curve, s = 0.772, r = 0.977.

TABLE 2. Comparison of EIA ratios method with CF technique for diagnosis of current infections[a]

A serum CF titer	No. positive by			
	CF	EIA		
		1:100 dilution[b]	Higher dilutions[c]	Total
<1:18	38	38	0	38
1:8	5	5	0	5
1:16	4	1	2	3
1:32	2	1	1	2
1:64	1	0	1	1
Total	50	45	4	49

[a] Evidence for current infection: by EIA, C/A OD ratio of ≥1.54; by CF, fourfold or greater increase in titer between A and C serum samples.

[b] Results with serum samples diluted 1:100.

[c] Five paired samples with a C/A ratio of <1.54 at 1:100 dilution were titrated at fourfold dilutions. The C/A ratios of four of the five paired samples not positive at 1:100 dilution were positive at the higher dilutions.

test such as the CF, for which the accuracy of titer is ±1 doubling dilution and results are reported in step dilutions.

CSF. A total of 153 CSF samples were checked by EIA at a 1:5 dilution and by CF at a 1:2 dilution; 55% of the samples had antibody by EIA, and 8% had antibody by CF (Table 3). Figure 9 shows a plot of reciprocals of EIA titers determined by dilution to the endpoint (plotted titers) versus the OD readings of the 1:5 dilutions of positive samples with sufficient quantity to start the titration at that dilution ($r = 0.950$, $n = 74$). The EIA titers of the majority of the positive samples fell between 1:10 and 1:100. The CSF with the highest EIA titer (1:5,100) had a CF titer of 1:128. Single or paired serum samples were available from all CSF donors. All donors having CSF antibody also had serum antibody. Of the 24 serum pairs, 5 showed a significant change in antibody titers by CF (≥4-fold) and 8 showed significant change in antibody titers by EIA (C/A ratio >1.54).

DISCUSSION

Because of the many washing steps in EIA, during which time aerosols may be produced, it was considered desirable to use an inactivated antigen. Photochemical inactivation with a psoralen derivative proved highly effective in inactivating herpes simplex virus, as was previously reported for a number of viruses (4, 6, 9), with no loss in its ability to react with antibody.

Solid-phase assays for serum antibody using viral preparations are complicated, multicomponent reactions. The viral preparation consists of multiple antigens, each with multiple determinants, whereas the antibody is a heterogeneous collection of molecules of various immunoglobulin classes and subclasses with various degrees of affinity for the various antigenic determinants. In published reports, EIA was shown to be sensitive to changes in antibody affinity, and the concentration of low affinity antibody produced early in infection was underestimated (1,

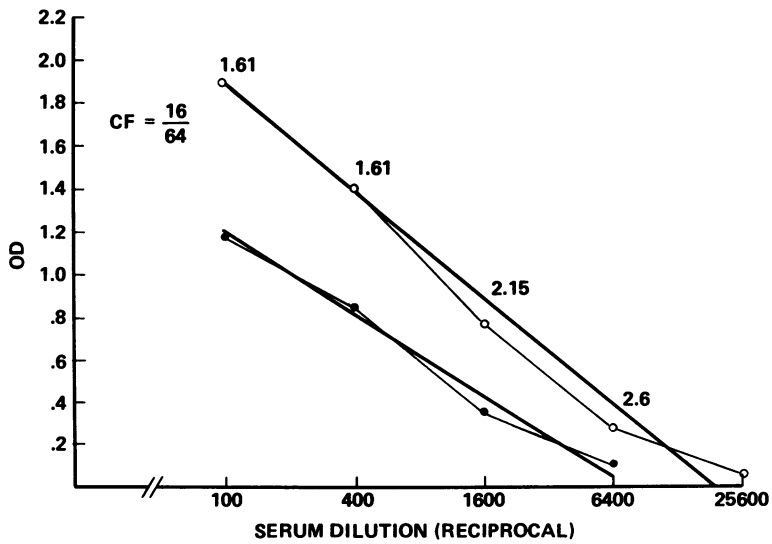

FIG. 6. A representative serum pair showing a significant change in C/A OD ratio (>1.54) at all serum dilutions. Symbols: ○, C serum; ●, A serum; —, linear regression lines of OD data points; —, plotted data points. Numbers positioned by data points are the C/A OD ratios at the indicated serum dilutions.

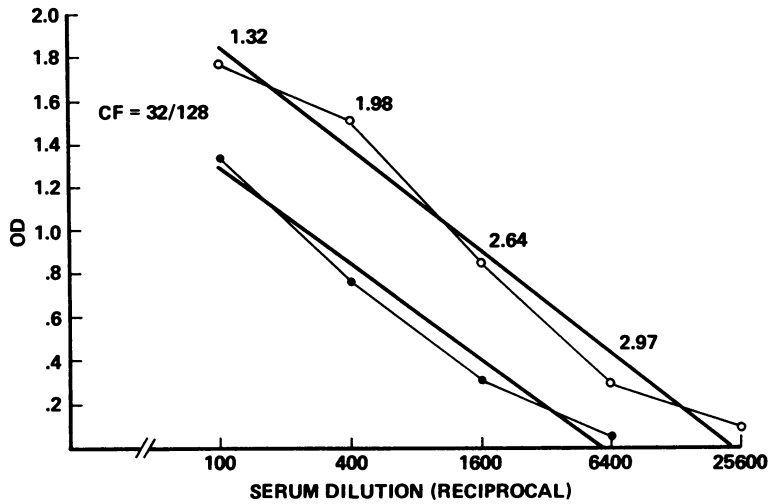

FIG. 7. Serum pair showing a significant change in C/A OD ratios at all dilutions except 1:100. Symbols, lines, and numbers as in Fig. 6.

7). The repeated washing required in the procedure may dissociate low-affinity antibody from the solid-phase antigen, and it would be lost in the wash discard.

The multicomponent nature of the serum antibody reaction with herpes simplex virus is reflected in the different antibody curves obtained with sera of various individuals. Each person's immune system will respond in its own way to antigens produced during infection, and the antibody response will be peculiar to the individual with regard to its specificity and affinity. Therein lies the problem of quantitating antibody concentration in a patient's serum with a standard curve generated by a single serum sample or by pools of serum samples. Although the antibody curves of consecutive serum samples from the same patient tend to parallel each other, particu-

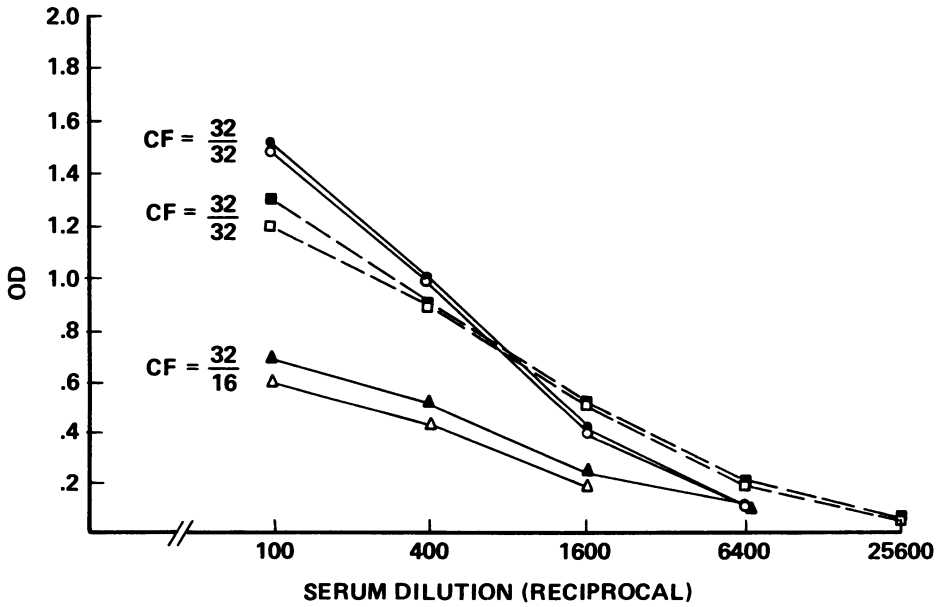

FIG. 8. Demonstration of parallelism of slopes of antibody curves of paired serum samples of patients with stationary EIA and CF titers. ODs of fourfold dilutions of each serum sample are plotted versus the serum dilutions.

TABLE 3. Comparison of CF and EIA titers of CSF[a]

CF			EIA		
Titer	*n*	Range of titers	Titer	*n*	Range of titers
<1:2	141		<1:5	69	
			>1:5	72	1:6–1:40
>1:2	12	1:2–1:128	>1:5	12	1:8–1:5,100

[a] CSF samples were titrated in fivefold dilutions. ODs at each dilution were plotted versus dilution on semilog paper. The titer was the dilution at the 0.2 OD cutoff.

larly when the antibody is not from a recent infection (Fig. 8), the slopes of antibody curves of different individuals can vary considerably from each other and from the standard curve (Fig. 5). The concentration of antigen from different individuals by solid-phase assays can be quantitated much more accurately by reference to a standard curve than can antibody from different individuals. A single, standardized antiserum is used for antigen quantitation, and the problem of differences in specificities, affinities, and classes of antibodies from different individuals does not arise (2).

The peculiarities of antigen-antibody reactions became apparent in our study. First, trying to relate an EIA titer to a CF titer for purposes of informing physicians of the meaning of an EIA titer in relation to a familiar test was not useful. Correlation between CF titers of positive sera with either EIA titers estimated from a standard curve or plotted titers by titration to endpoint was poor (r = 0.643 and 0.382, respectively). This result is not unexpected, since the two tests are not necessarily detecting precisely the same antibodies.

The correlation of estimated titer and of the OD at a 1:100 dilution with the plotted titer obtained by titration was similar (r = 0.817 and 0.824, respectively). For the purpose of reporting presence of antibody on single serum samples in a semiquantitative manner, these two methods appeared equivalent in our test system. Since the report of a titer obtained from a continuous curve implies a greater accuracy than the estimated titer warrants, we prefer OD readings. Also, the estimated titer is less reproducible than an OD reading, since it depends upon the serum samples used to prepare the standard curve which change as serum pools are exhausted. OD readings were quite reproducible in inter-assay runs (CV = 14 ± 5.6%) and more reproducible than titers of doubling dilution tests which can vary by ±1 doubling dilution in different runs (i.e., by 100%). However, they have little meaning to a clinician. To make them more meaningful in terms of relative quantity of antibody, the report of a ratio (EIA index) of the OD of the serum sample to the cutoff OD is a feasible alternative. The index gives a perspective on the amount of antibody in relation to the minimum amount detectable by the assay system. Such a report should be adequate, since nothing definitive can be concluded, relative to

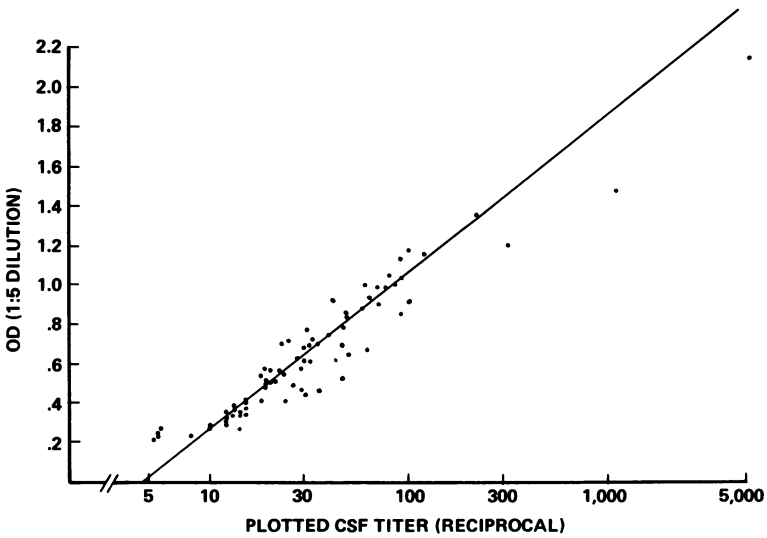

FIG. 9. Correlations between the OD of a 1:5 dilution of each CSF with its plotted titer (determined by plotting the OD of fivefold dilutions of each CSF on semilog paper and using the CSF dilution at 0.2 OD as the titer endpoint). Slanting line is plotted from the formula of the linear regression line (r = 0.950, n = 74).

current or recent infection, from results of a single serum sample, unless the assay is specific for IgM antibody.

For diagnosis of current infection, the ratio of the C/A ODs has thus far proven reliable in our laboratory, as long as two serum dilutions are evaluated. Although in our test 1:100 and 1:1,000 dilutions were satisfactory for HSV antibody, appropriate dilutions would have to be determined for other viral antibodies in this test system.

The closer correlation of CSF plotted titers and OD readings at a single CSF dilution (r = 0.950; Fig. 9) than that seen with serum samples was of interest. There may be more homogeneity in the antibody which is present in the CSF than in the antibody in the peripheral circulation. For example, in studies of CSF antibody of multiple sclerosis patients, the antibody is reported to be primarily of the IgG1 subclass (10). The proportion of patients with CSF antibody to HSV in the present study is similar to that reported by us in radioimmunoassays of CSF antibody of multiple sclerosis patients and of control patients with other neurological problems (3). Others have demonstrated, by radioimmunoassay, HSV antibody in the CSF of a high proportion of individuals without infection or demyelinating diseases (8). The EIA, like the radioimmunoassay, appears to be more sensitive in detecting low levels of antibody in CSF.

As more laboratories routinely use EIA for evaluation of viral antibody, it will become increasingly important to have a standard method for reporting results. De Savigny and Voller (2) concluded from their studies with IgG antitoxocara antibody that no method of reporting was completely satisfactory. Of all the methods they reviewed, however, they favored the estimated titer obtained by reference to a standard curve. Because of reasons just discussed, we favor an EIA index for reporting results on single specimens and C/A ratios on paired samples for serological diagnosis of current or recent HSV infection. An alternative for the latter might be EIA tests specific for IgM antibody. Whether similar methods would be satisfactory for reporting EIA results on other viral antibodies requires study.

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