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Sensitivity of Enzyme-Linked Immunosorbent Assay, Complement Fixation, and Hemagglutination Inhibition Serological Tests for Detection of Sendai Virus Antibody in Laboratory Mice

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The enzyme-linked immunosorbent assay technique for detection of Sendai virus antibody in mice was approximately 100- and 300-fold more sensitive than the complement fixation and hemagglutination inhibition tests, respectively. The assay also permitted direct quantitative measurement of the amount of antibody on a single serum dilution rather than by the more traditional serial titration.

Enzyme-linked immunosorbent assay (ELISA) techniques for detection of antibody have been developed for a wide variety of infectious agents (3, 7) and generally have been shown to be more sensitive than other diagnostic techniques (7). Sendai is one of the most troublesome virus infections of laboratory rodents. Infection may produce a variety of undesirable sequelae, including fatal bronchopneumonia (2, 5, 9), residual lung lesions in recovered animals which may persist for months (1), and interruption of normal physiological functions including the cellular immunological response (4). Early repair lesions also may bear a striking resemblance to carcinoma in situ or metastatic carcinoma (5, 6). In the diagnosis of Sendai virus infection it is important to use the most sensitive test possible. Currently, the most useful and sensitive diagnostic tests are the complement fixation (CF) and hemagglutination inhibition (HI) tests (5).

This study compared the sensitivity of the CF and HI tests with a Sendai virus ELISA assay developed in our laboratories. Sendai antigen (strain 52) was prepared from allantoic fluid harvests of 11-day-old embryonated chicken eggs inoculated 2 days earlier with approximately 10^5 egg infectious Sendai virus doses in 0.2 ml. Allantoic fluids were clarified at 1,000 × *g* for 30 min at 5°C, and virus was pelleted from these fluids by centrifugation at 53,700 × *g* for 2 h at 5°C. The pellets were suspended by sonic disruption in 1/20 of the original volume of Dulbecco phosphate-buffered saline and frozen at −70°C. Control antigen was prepared in a similar manner from normal allantoic fluids. The enzyme conjugate used was rabbit anti-mouse immunoglobulin labeled with alkaline phosphatase (Microbiological Associates). The conjugate was stored at 4°C and appropriately diluted in phosphate-buffered saline (PBS) (pH 7.2) containing 0.05% Tween 20 immediately before use. The coating buffer (carbonate-bicarbonate), PBS-Tween, diethanolamine buffer, and *p*-nitrophenyl phosphate substrate solution were prepared as described by Voller et al. (8). The optimal dilution of virus antigen and conjugate was determined separately by box titrations as described by Voller et al. (8).

Cooke Microelisa polystyrene plates (96-well; Microbiological Associates) were coated by adding 200 μl of antigen or control antigen, optimally diluted in carbonate-bicarbonate buffer (pH 9.6), to each well and incubating in a humid chamber overnight at 4°C. The antigen solution was removed from the wells, and all wells were rinsed three successive times with PBS-Tween with 3-min incubations between each rinse. Fluids were removed, and wells were allowed to dry. Test sera were diluted in PBS-Tween, and 100 μl of each serum dilution was added to both a test antigen and control antigen well. The plates were incubated for 2 h at room temperature, rinsed with PBS-Tween three times, and dried. Diluted conjugate (100 μl) was added to each well and incubated for 2 h at room temperature, and wells were again washed with three changes of PBS-Tween. *p*-Nitrophenyl phosphate substrate (100 μl at 1 mg/ml) in diethanolamine buffer (pH 9.6) was added to each well, and after exactly 45 min the reaction was stopped by the addition of 100 μl of 1.2 N NaOH. The color reaction was read visually or by determination of optical density at 405 nm using a Gilford Stasar II spectrophotometer (Oberlin, Ohio) equipped with an aspirating microcuvette,

model no. 3018A. By visual reading, the endpoint titer was the highest serum dilution showing a discernible specific color change. Optical density of the test antigen reaction minus optical density of the control antigen reaction (the mean optical density of 269 control antigen reactions was 0.023 ± 0.019) was considered the ELISA value. An ELISA value of 0.15 or greater was considered positive.

The Sendai ELISA sensitivity was evaluated in two studies. In the first study, four mouse colonies known to be infected with Sendai virus and one colony free of infection were selected. The age of the majority of mice tested ranged from 7 weeks to 9 months, except in colony 65, where all mice tested were 5 weeks old. Approximately 20 sera from each colony were tested by HI, CF, and ELISA procedures, and the comparative sensitivity of antibody detection was evaluated (Table 1). A CF or HI titer of 1:10 was the lowest titer considered positive. Three colonies (95, 147, and 116) were actively infected as determined by the prevalence of CF antibody. The fourth colony (colony 65) had little detectable CF antibody, and colony 123 was not infected. The CF and the ELISA tests were always more sensitive than the HI test. In colonies 147 and 116 the CF and ELISA tests were equally sensitive for detection of antibody; in colony 95, however, antibody was detected in only 17 of 20 sera by the CF test, whereas all were positive by ELISA. Colony 65 presented the most striking difference in sensitivity in that antibody was not detected by HI and only 4/20 (20%) were positive by CF, whereas all were positive by ELISA. It is significant, however, that these mice were only 5 weeks old, and therefore antibody detected by ELISA was likely either maternal antibody or early antibody from a very recent infection. In all infected colonies, the serum antibody titers were consistently and significantly higher by ELISA than by either CF or HI.

A second study (Fig. 1) was designed to follow the temporal antibody response in Swiss mice (Microbiological Associates) infected intranasally with 10 50% tissue culture infective doses (Rhesus monkey kidney) of Sendai virus (strain P3193). Fifty-four mice were inoculated, and at each test interval six mice were randomly selected and sacrificed for serum antibody determination. Five mice did not become infected or seroconvert (after day 14 postinoculation), presumably because of the low dose of virus inoculated. Only mice that seroconverted are shown in Fig. 1. Antibody was not detected by any assay within the first week after inoculation; by day 14, however, antibody was detected in all mice by all three assays. The significant difference between the tests was the quantitative sensitivity of antibody detection. The HI and CF antibody response was similar to that reported previously (5), with CF titers consistently higher than HI titers. The antibody response measured by ELISA followed the same general response curve as HI and CF, except that ELISA antibody titers were approximately 300-fold higher than HI titers and 100-fold higher than CF titers. All ELISA antibody titers were in excess of 1:10,000 from weeks 6 through 16 after inoculation.

Although no attempt was made in the present study to quantitate the relationship of ELISA values and CF or HI antibody titers, it is clear that, as antibody titers increased, ELISA values likewise increased. ELISA values of CF-positive mouse sera (titers ranged from 1:10 to 1:320) tested by ELISA at a 1:80 serum dilution ranged from 0.15 to 1.40. Although there tended to be lower ELISA values with lower CF titers and higher ELISA values with higher CF titers, there was considerable overlap, and a definitive correlation between ELISA values and CF titers could not be made using these data. There was, however, a linear relationship between antibody titer measured by the ELISA test and ELISA values, which allowed the construction of a linear regression curve comparing ELISA values with ELISA titer expressed as antibody units (Fig. 2). To construct the curve, ELISA values for each twofold serum dilution from the temporal antibody response study (Fig. 1) were tabulated. To preclude any possible nonspecificity and therefore false positives, values less than

TABLE 1. *Sensitivity of HI, CF, and ELISA tests for detection of Sendai antibody*

Colony code	Virus status	No. of sera tested	Positive[a]			Titer[b]		
			HI (%)	CF (%)	ELISA (%)	HI	CF	ELISA
95	Infected	20	15 (75)	17 (85)	20 (100)	23	31	657
147	Infected	23	15 (65)	18 (78)	18 (78)	26	58	200
116	Infected	20	13 (65)	13 (65)	13 (65)	19	47	207
65	Infected	20	0 (0)	4 (20)	20 (100)	<10	25	72
123	Not infected	20	0 (0)	0 (0)	0 (0)			

[a] Tested at a 1:10 serum dilution.
[b] Reciprocal geometric mean titer of positives.

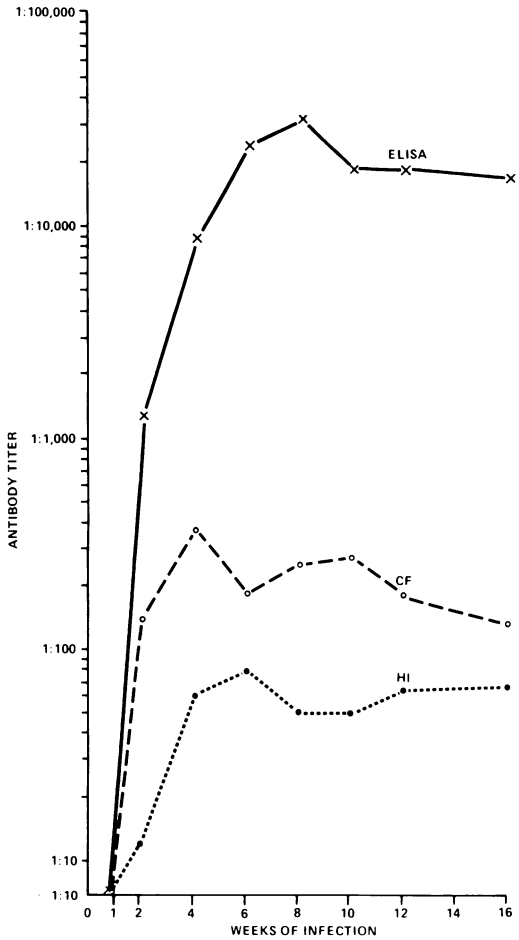

FIG. 1. *ELISA, CF, and HI antibody response in Swiss mice inoculated with 10 50% tissue culture infective doses of Sendai virus.*

0.11 arbitrarily were not considered significant and were not included in the antibody unit calculation. Thus, the ELISA values of each of the highest serum dilutions with values of 0.12 or greater were grouped, and the mean and standard error of the mean were calculated. This group was defined as containing 1 unit of antibody. In a similar manner, the ELISA values of each serial twofold-lower dilution for each serum also were tabulated. From 26 to 36 ELISA values (except nine values for 256 antibody units) comprised each antibody unit point. The mean ELISA value at the lowest detectable antibody level was 0.15 (1 antibody unit). The ELISA values and antibody units increased linearly to 0.79, which corresponded to 256 antibody units. Thus, an ELISA value determined on a single serum dilution of 1:80 could be interpreted directly and quantitatively to give the number of units of antibody or titer of that serum. This becomes significant when it is necessary to monitor for changes in antibody titer as in the diagnosis of recent infections. For example, acute- and convalescent-phase sera can be tested at a single predetermined dilution, the ELISA value calculated, and antibody units read directly from the Sendai ELISA regression curve. Diagnosis of infection can then be made by applying the conventional prerequisite for a fourfold rise in antibody titer.

The linearity of ELISA values and units of antibody also has been analyzed by using data collected in clinical trials evaluating ELISA systems for the diagnosis of rubella virus, cytomegalovirus, and toxoplasmosis infections in humans. A relationship of ELISA values to units of antibody was observed in all cases, with each infection having its own unique linear regression curve (A. J. O'Beirne, unpublished data).

Thus, the Sendai ELISA test offers certain advantages over the HI and CF tests in the diagnosis of infection in that ELISA is significantly more sensitive for detection of antibody, which is an important consideration when only low levels of antibody may be present. Also, all ELISA reagents may be standardized, and plates can be coated with antigen and stored dry at 2 to 8°C for considerable periods of time, thus enabling a diagnostic laboratory to eliminate troublesome day-to-day variables unique to HI and CF tests such as erythrocyte sensitivity, complement titer and sensitivity fluctuations,

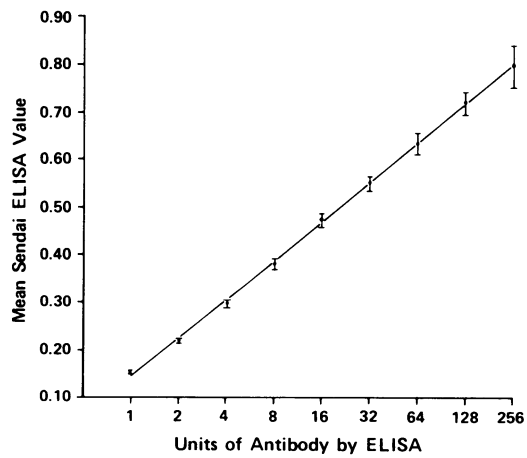

FIG. 2. *Quantitative relationship of ELISA values and antibody titer expressed as units of antibody. Results are expressed as the ELISA value per antibody unit ± standard error of the mean, showing the linear regression line. The regression equation is y = 0.0814 x + 0.06, and the correlation coefficient (r) is 1.00. See the text for discussion.*

and antigen titer variation and sensitivity. A possible disadvantage of ELISA is that only the G class of immunoglobulins is normally detected unless different conjugates are used. This is not considered to be a serious limitation in Sendai diagnosis, however. The ELISA test also has the advantage of speed, low cost, high reproducibility, ease of technical performance, and the potential for automation, not only for Sendai but perhaps for many other viruses, which would enable entire panels of viruses to be run against single serum samples in an automated fashion.

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