Enzyme-Linked Immunosorbent Assay for Detection of Measles Antibody

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An enzyme-linked immunosorbent assay (ELISA) was developed for the detection of measles immunoglobulin G antibody (MEASELISA). This assay was found to be comparable to the measles hemagglutination inhibition (HAI) test. Approximately 500 sera from three centers were tested by MEASELISA and the HAI test. MEASELISA demonstrated values of greater than 99% for sensitivity, specificity, and accuracy. Values were very precise, with a mean coefficient of variation of 5.4%. MEASELISA values were shown by linear regression analysis to increase as HAI titers increased. A coefficient of determination of 1.00 was obtained from test center three. MEASELISA values were found to be linearly related ($r^2 > 0.97$) to MEASELISA titers, thus enabling quantitation of measles antibody from a single value. Also, data are presented that show MEASELISA to be equivalent to complement fixation for evaluating paired sera for the presence of a significant increase in antibody levels to measles virus.

Measles virus infections in the United States have decreased dramatically since the use of live attenuated measles vaccine (1). However, many individuals remain susceptible to measles virus, owing to vaccine failure and non-immunization. Because of the numerous complications that can occur with measles virus infection (8), it is important to have a diagnostic and screening test to identify these nonimmune individuals.

The detection and quantitation of measles antibody is usually performed by one of three methods: virus neutralization, complement fixation (CF), or hemagglutination inhibition (HAI) (3, 11). The HAI test has currently gained acceptance as the method of choice in the diagnosis of measles virus infection and in the evaluation of the immune status of an individual. However, there is a requirement for pretreatment of the serum to remove nonspecific inhibitors and agglutinins which, if not removed, adversely affect the sensitivity and reliability of the HAI test (3). This prerequisite adds to the cost, complexity, and time needed to conduct the HAI test.

The microplate enzyme-linked immunosorbent assay (ELISA) described by Voller and Bidwell has been shown to be a simple, economical, sensitive, and specific serological assay for a variety of infectious agents (7, 9, 10, 13). ELISA has been shown to be a reliable assay for the detection and quantitation of measles virus antibody (2, 4, 6, 12). We now report the development and evaluation of a commercially available ELISA assay for measles virus (MEASELISA).

MATERIALS AND METHODS

Clinical sera. A test population consisting of 503 sera was tested by both the standard HAI method and the MEASELISA test system. These sera were kindly provided by the following people: C. Crump, Virginia Health Department; Y. Patel, Maryland Health Department; and D. Madden, National Institutes of Health (NIH). In addition to these samples, 17 serum pairs showing titer rises to measles virus by diagnostic CF were provided by NIH.

HAI test. The HAI test procedure used in this study followed the procedure outlined by the Centers for Disease Control. In this procedure, heat inactivation followed by absorption with 50% rhesus monkey erythrocytes is used in the pretreatment of the sera. These tests were performed by the three suppliers of the sera before the sera were tested by the ELISA system.

ELISA. The ELISA used in this study was developed in the Research and Development Department of Whittaker M. A. Bioproducts. The antigen (Edmonston strain) was cultured in roller bottles of MK-2 cells. The control antigen consisted of uninoculated MK-2 cells processed in the same manner as the antigen. At 5 days postinoculation, the antigen was harvested, and alkaline was extracted with 0.1 M glycine buffer as previously described (5). The antigen and control antigen were coated, using a carbonate buffer (pH 9.6), onto a Removawell microtiter plate (Dynatech Laboratories, Inc., Alexandria, Va.). The optimal coating dilution was determined by block titration of the antigen. The test was run by using the

TABLE 1. Intra- and interassay precision of MEASELISA values obtained with nine sera[a]

Serum	Intraassay[b]						Interassay[c]		
	Assay 1			Assay 2			Mean	SD	CV (%)
	Mean	SD	CV (%)	Mean	SD	CV (%)			
A	0.23	0.01	4.35	0.22	0.01	4.55	0.23	0.01	4.35
B	0.37	0.01	2.70	0.36	0.02	5.56	0.36	0.02	5.56
C	0.75	0.02	2.67	0.77	0.04	5.20	0.76	0.03	3.95
D	0.52	0.03	5.77	0.51	0.01	1.92	0.51	0.02	3.92
E	0.63	0.02	3.23	0.63	0.02	3.23	0.63	0.02	3.23
F	0.22	0.02	9.10	0.24	0.01	4.16	0.23	0.02	8.80
G	0.59	0.02	3.39	0.59	0.02	3.39	0.59	0.02	3.39
H	0.24	0.03	12.50	0.24	0.01	4.17	0.24	0.02	8.33
I	0.80	0.03	3.75	0.83	0.02	2.50	0.82	0.03	3.66

[a] The following MEASELISA mean coefficients of variation were obtained with sera having a high- (serum samples C, E, G, and I), mid- (serum samples B and D), or low- (serum samples A, F, and H) range positive value. Intraassay: high, 3.42; mid, 3.99; low, 6.47. Interassay: high, 3.56; mid, 4.74; low, 7.16.

[b] Each serum was tested four times on each of 2 days. CV, Coefficient of variation.

[c] Variation was obtained by combining MEASELISA values observed on assay 1 and assay 2.

following procedure, with all incubations being at room temperature (20 to 25°C).

(i) The antigen- and control antigen-coated microtiter plate was filled with phosphate-buffered saline solution (pH 7.4) containing 0.05% Tween 20. Immediately after filling, the plate was emptied, refilled, and allowed to soak for 3 min. This procedure was carried out two more times to give a total of 3 washes. After the last wash, the plate was dried on a paper towel.

(ii) Each well was then filled with 250 μl of serum diluent containing control antigen, bovine serum albumin, and phosphate-buffered saline–0.05% Tween 20. A total of 10 μl of each test serum was then added to an antigen and control well. The plate was placed on a shaker for 2 min.

(iii) After 45 min, the contents were emptied, and the washing procedure described in step i was repeated.

(iv) Each well was then filled with 250 μl of alkaline phosphatase-conjugated rabbit anti-human IgG; optimal dilution was predetermined by block titration of the conjugate.

(v) After 45 min, the plate was emptied and washed as in step i.

(vi) The plate was then filled with 250 μl of the substrate, para-nitrophenyl phosphate.

(vii) After 45 min, the enzymatic reaction was stopped by adding 50 μl of 1 N sodium hydroxide solution to each well. The plate was then placed on a shaker for 2 min. After mixing, the absorbance value of each well was determined by using a spectrophotometer set at 405 nm. The final absorbance value (MEASELISA value) for each serum was determined by subtracting the control well values from the antigen well values.

(viii) A calibration procedure was established with one high-titered positive, one low-titered positive, and one negative serum, each with an established value. These values were then used to compare with the test values obtained for the three sera. This comparison was done by using standard linear regression analysis to find the coefficient of determination (r^2). A final r^2 value greater than or equal to 0.95 was required for an acceptable test. The controls and patient samples were then read from this regression line. This procedure provides the user with a method to correct for any fluctuation in temperatures during the test or for fluctuations due to other errors in kinetics that would affect the performance of an enzyme-substrate system.

RESULTS

Determination of critical values for MEASELISA. The calibrated mean MEASELISA value (E-405 antigen value minus E-405 control antigen value) of all test sera which had <1:8 HAI titers (63 of 503) was 0.04 with a standard deviation of 0.04. The upper limit of the 99.73% confidence limit was 0.16, whereas the 99% confidence limit was 0.14. Therefore, 0.16 was chosen as the lower limit of the positive population and 0.13 as the upper limit of the negative population. The intermediate values of 0.14 and 0.15 were interpreted as equivocal. The positive population was divided into quartiles according to MEASELISA values. Sera which produced values in the lowest quartile were considered to be low-range positive, whereas samples which

TABLE 2. Comparison of mean MEASELISA values and HAI titers obtained at NIH[a]

HAI titer	MEASELISA	
	Mean	SE
<8	0.07	0.061
8	0.25	0.029
16	0.34	0.050
32	0.43	0.044
64	0.53	0.057
128	0.62	0.071
>128	0.73	0.141

[a] Total number of samples, 218; coefficient of determination, 1.00.

TABLE 3. MEASELISA values for serial twofold
dilutions of positive sera

Serum	Dilution					r^{2a}	Slope
	0	1:2	1:4	1:8	1:16		
M1	0.44	0.30	0.20	0.16	0.09	99	−0.12
M2	0.52	0.38	0.29	0.19	0.15	99	−0.11
M3	0.64	0.54	0.43	0.30	0.23	99	−0.11
M4	0.50	0.39	0.26	0.16	0.10	100	−0.12
M5	0.67	0.54	0.40	0.32	0.24	98	−0.11
M6	0.58	0.50	0.39	0.29	0.20	100	−0.10
M7	0.47	0.35	0.21	0.12	0.06	100	−0.13
M8	0.46	0.33	0.24	0.18	0.10	97	−0.09
M9	0.52	0.36	0.22	0.09	0.04	100	−0.15
M10	0.79	0.58	0.42	0.32	0.20	98	−0.14

[a] r^2, Coefficient of determination. Linear regression
compared MEASELISA values to $\log_2$ of dilution.

gave values in the highest quartile were consid-
ered to be high-range positive. Samples which
gave MEASELISA values in the second and
third quartiles were considered to be midrange
positive. Thus, the following interpretation of
values was established: negative for values
≤ 0.13; equivocal for values of 0.14 to 0.15; low-
range positive for values of 0.16 to 0.29; mid-
range positive for values of 0.30 to 0.52; and
high-range positive values ≥ 0.53.

Immune status comparison. MEASELISA and
the HAI test were performed on a total of 503
sera from three test centers. Of these sera, 436
were positive by both tests (including 11 sera
that were HAI positive at a 1:4 dilution; see
below), and 63 were negative by both tests; 2
sera that were positive by the HAI test were
negative by MEASELISA. Two sera were not
included because of equivocal MEASELISA
values of 0.15. There was 99.5% sensitivity,
100% specificity, and 99.6% accuracy. HAI ti-
ters were considered positive if $\geq 1:8$. Eleven
sera were found to be discrepant in that they
were positive by MEASELISA and negative at
1:8 by HAI. These results were considered to be
resolved when repeat testing by HAI showed the
sera to have antibody to measles at a 1:4 serum
dilution.

Precision study. To establish the variation
which occurs with MEASELISA, we tested a
serum panel of nine coded positive sera in two
separate assays performed on different days.
Each serum was tested four times on a single
plate for each assay. The MEASELISA values
obtained from each day's assay were used to
establish the intraassay precision. The interas-
say precision was determined by using the
MEASELISA values obtained from both as-
says. The means, standard deviations, and coef-
ficients of variation obtained for each of the nine
samples are listed in Table 1. The mean coeffi-
cients of variation for sera having MEASELISA

values in the high, mid-, or low range are sum-
marized in Table 1. The precision data demon-
strate the high degree of reproducibility and
comparability of MEASELISA both within and
between tests and days.

MEASELISA values compared with HAI titers.
MEASELISA values were found to increase
proportionately with increasing HAI titers by
regression analysis. Data obtained from NIH are
presented in Table 2. Although the data show
that HAI titers cannot be accurately predicted
from a MEASELISA value, they indicate that
MEASELISA values accurately reflect relative
antibody levels to measles virus.

**MEASELISA values compared with MEA-
SELISA titers.** MEASELISA values were deter-
mined in duplicate for serial twofold dilutions of
10 sera positive for IgG antibodies to measles
virus. Dilutions were prepared in normal human
serum negative for IgG antibodies to measles
virus. Each test serum was diluted until a nega-
tive MEASELISA value was obtained, i.e.,
<0.14. The MEASELISA values were found to
be linear with $\log_2$ of dilution when analyzed by
linear regression. The data are summarized in
Table 3. All sera showed a coefficient of deter-
mination $(r^2) \geq 0.97$.

**Ability of MEASELISA to detect a rise in
antibody titer.** For kits produced by Whittaker
M. A. Bioproducts, a critical ratio is employed
to detect a rise in antibody titer, as previously
described (9). The critical ratio is found by
dividing the value of the convalescent serum
sample by the value of the acute sample. The
critical ratio is interpreted to be highly indicative
of an active infection if the value is ≥ 1.47; such
values correspond in significance to a fourfold
increase in the level of antibody. The MEA-
SELISA value for the convalescent serum must
be ≥ 0.16. In addition, owing to the decrease
seen in the critical ratio at initially high MEA-
SELISA values, it is necessary that the MEA-
SELISA value of the acute serum be ≤ 0.40 for
the critical ratio to be appropriately applied.

Seventeen actual acute and convalescent se-
rum pairs which demonstrated at least a fourfold
increase in CF titer were run on MEASELISA.
The results are shown in Table 4. MEASELISA
detected a positive rise in antibody titer for each
clinically significant CF antibody titer rise. In
addition, critical ratios were determined for
fourfold dilutions of positive sera. The data are
presented in Table 5. The critical ratio indicated
a significant rise in antibody titer in 100% of the
serum pairs constructed by making fourfold dilu-
tions of positive sera.

DISCUSSION

The data from the immune status study clearly
indicate that MEASELISA is equivalent to the

standard HAI test. This conclusion is based on results showing that the overall sensitivity, specificity, and accuracy of the MEASELISA test were >99% relative to the standard HAI test. When one considers that the HAI test is not a perfect reference assay, it is theoretically possible that the MEASELISA test may be

TABLE 4. Critical ratios for actual diagnostic serum pairs determined by CF

Serum pair	CF titer	MEASELISA value	Critical ratio
1	1:4	0.44	1.66
2	1:64	0.73	
3	1:4	0.26	2.96
4	1:64	0.77	
5	1:4	0.11	1.91
6	1:32	0.21	
7	1:4	0.36	3.22
8	1:16	1.16	
9	<1:4	0.14	4.07
10	1:8	0.57	
11	1:4	0.23	4.43
12	1:64	1.02	
13	<1:4	0.15	4.47
14	1:16	0.67	
15	1:4	0.25	1.64
16	1:16	0.41	
17	1:4	0.24	3.33
18	1:32	0.80	
19	1:4	0.28	4.21
20	1:32	1.18	
21	<1:4	0.05	8.40
22	1:32	0.42	
23	1:8	0.22	3.32
24	1:64	0.73	
25	1:4	0.29	4.03
26	1:64	1.17	
27	<1:4	0.11	7.56
28	1:64	0.83	
29	1:4	0.22	2.68
30	1:64	0.59	
31	1:4	0.10	10.00
32	≥1:128	1.00	
33	1:4	0.10	4.10
34	1:32	0.41	

TABLE 5. Critical ratios for paired sera representing physical fourfold increases in measles antibody[a]

Serum	Dilution	MEASELISA value	Critical ratio (×4)
M1	1:16	0.09	2.22
	1:8	0.16	1.88
	1:4	0.20	2.20
	1:2	0.30	
	Undiluted	0.44	
M2	1:16	0.15	1.93
	1:8	0.19	2.00
	1:4	0.29	1.79
	1:2	0.38	
	Undiluted	0.52	
M3	1:16	0.23	1.87
	1:8	0.30	1.80
	1:4	0.43	1.49
	1:2	0.54	
	Undiluted	0.64	
M4	1:16	0.10	2.60
	1:8	0.16	2.44
	1:4	0.26	1.92
	1:2	0.39	
	Undiluted	0.50	
M5	1:16	0.24	1.67
	1:8	0.32	1.69
	1:4	0.40	1.68
	1:2	0.54	
	Undiluted	0.67	
M6	1:16	0.20	1.95
	1:8	0.29	1.72
	1:4	0.39	1.49
	1:2	0.50	
	Undiluted	0.58	
M7	1:16	0.06	3.50
	1:8	0.12	2.92
	1:4	0.21	2.24
	1:2	0.35	
	Undiluted	0.47	
M8	1:16	0.10	2.40
	1:8	0.18	1.83
	1:4	0.24	1.92
	1:2	0.33	
	Undiluted	0.46	
M9	1:16	0.04	5.50
	1:8	0.09	4.00
	1:4	0.22	2.36
	1:2	0.36	
	Undiluted	0.52	
M10	1:16	0.20	2.10
	1:8	0.32	1.81
	1:4	0.42	1.88
	1:2	0.58	
	Undiluted	0.79	

[a] Each test serum was serially diluted to 16-fold. The highest dilution then served as the simulated acute specimen.

more sensitive, specific, and accurate than the HAI test. The difference between the two assay methods may reflect false HAI results due to incomplete removal of nonspecific inhibitors or agglutinins, rather than incorrect MEASELISA results.

The data show that MEASELISA values and MEASELISA titers have a high degree of linear correlation. Combined with data showing that, as HAI values increased, MEASELISA values increased in a linear fashion, a single MEASELISA value can be used to accurately quantitate measles virus antibody in terms of low, medium, or high levels. MEASELISA values for a particular HAI titer were quite varied; however, the mean value for a particular titer was meaningful. This variation is due in part to the discrete twofold dilution scheme of the HAI test compared with the continuous nature of the MEASELISA test. Variation between HAI titer and MEASELISA value can also be accounted for by the observation that only HAI antibodies are measured in the HAI assay. Theoretically, ELISA will measure other functional antibodies, such as complement-fixing antibodies, as well as the HAI antibodies.

Based on the precision characteristics of ELISA kits manufactured by Whittaker M. A. Bioproducts, a critical ratio (convalescent/ acute) ≥ 1.47 can be interpreted as highly suggestive of a significant rise in antibody level with a type 1 probability error of <0.001. With this method, MEASELISA accurately detected diagnostic rises in antibody in 17 actual diagnostic serum pairs and 30 serum pairs constructed by preparing fourfold dilutions. Therefore, MEASELISA was at least as sensitive as standard serological methods for quantitating antibody to measles virus.

The results reported here agree with other work (2, 4, 6, 12) demonstrating that ELISA shows good agreement with the HAI and CF tests, with the ELISA generally being more sensitive. In summary, MEASELISA is a useful alternative to the labor-intensive HAI assay for detecting and quantitating measles virus antibody.

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