Comparison of Enzyme-Linked Immunosorbent Assay for Acute Measles with Hemagglutination Inhibition, Complement Fixation, and Fluorescent-Antibody Methods

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An enzyme-linked immunosorbent assay (ELISA) using commercially available reagents was compared with standard serological tests for determination of antibody titers in patients with acute measles. The measles-specific ELISA was as sensitive as complement fixation, hemagglutination inhibition, and indirect fluorescent-antibody methods. The ELISA method used was simple, sensitive, inexpensive, and reproducible and may conveniently be used in a small clinical laboratory.

The incidence of measles has decreased sharply since the introduction of live attenuated measles vaccine. Measles continues to be seen in the United States in unimmunized individuals (1, 8), in those who fail to seroconvert after immunization, and in atypical form in nonimmune persons who have received killed measles vaccine many years previously (3). Mild and modified measles may be difficult to recognize, especially by individuals who have completed medical training in the past several years. Atypical measles and measles infection in an individual with impaired immune function can be difficult to recognize even for the experienced observer (2, 7). A simple, inexpensive, reproducible, sensitive, and specific serological method for the diagnosis of measles infection is therefore an important diagnostic test and should ideally be available in reference and small clinical laboratories.

The enzyme-linked immunosorbent assay (ELISA) has well-documented advantages over complement fixation (CF), hemagglutination inhibition (HAI), indirect fluorescent-antibody method (IFA), viral neutralization assay, and radioimmunoassays. The ELISA method has been used for detection of measles antibody by Voller et al. (10) and by others (6); however, a detailed comparison of the ELISA method with other standard serological procedures has not, to our knowledge, yet been reported. We have studied an ELISA procedure which may conveniently be used in a small clinical laboratory. The technique uses commercially available reagents and is read by eye without the necessity of expensive photometric equipment. We have compared ELISA with other standard techniques presently used for the serological confirmation of measles infection.

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MATERIALS AND METHODS

Serum specimens. Seventy-six paired acute- and convalescent-phase sera were obtained from children with rash-associated, febrile illness during a statewide outbreak of measles in 1976. Sera were screened by the Communicable Disease Laboratory of the Indiana State Board of Health, and a fourfold or greater increase in CF titer was demonstrated in the convalescent-phase specimen of each pair. Sera were aliquoted and stored at -20°C. Acute- and convalescent-phase samples were subsequently tested in the same assay under identical conditions in our laboratory, and 95.7% showed a fourfold rise in titer.

Sixty-three paired serum specimens were obtained from children enrolled in an acute febrile illness study. All patients presented with fever without rash, and all had only mild upper respiratory complaints unassociated with other foci of infection. None had an illness consistent with measles.

Seven paired sera from patients showing a fourfold or greater rise in antibody titer to adenovirus, cytomegalovirus, echovirus, coxsackievirus, or blastomyces antigens were obtained from the serum bank at the Indiana University Medical Center.

Sera were obtained from 20 healthy adult volunteers, most of whom reported a history of measles many years previously.

ELISA. The ELISA was carried out in microtiter plates (Implulon, Dynatech Laboratories, Inc., Alex-
andria, Va.) according to the methods of Voller et al. (12) and Voller and Bidwell (11), with only the following modifications. The antigen was incubated in microtiter plates for 2 h at 37°C and then overnight at 4°C. Plates were washed between steps, using a washing buffer as described by Voller and Bidwell (11). Washing buffer was allowed to remain in wells for 3 min, after which it was aspirated. This was repeated twice, making a total of three washes between steps. Antisera and conjugates were each incubated at 37°C for 2 h. A 3-h incubation period at 37°C was used for the conjugate in the conjugate titration. Plates were read by eye exactly 1 h after the addition of substrate. A stopping solution was not used. Color was compared with a color standard. Equal volumes of conjugate and substrate were placed in a microtiter plate at 200 μl per well. The 1:1 mixture of conjugate and substrate was considered the undiluted sample. Then, serial twofold dilutions beginning with a 1:1 mixture were carried out to a 1:32 dilution and incubated for 1 h at room temperature. A difference in color intensity was consistently seen between the 1:8 and 1:16 samples. This color standard showed excellent reproducibility. Color greater than that of the 1:16 dilution in the color standard was considered positive in test samples.

Rubeola CF antigen, control antigen, and alkaline phosphatase conjugated to sheep anti-human immunoglobulin were obtained from Microbiological Associates, Bethesda, Md. Positive human control serum was obtained from Flow Laboratories, Rockville, Md., and p-nitrophenylphosphate was purchased from Calbiochem, La Jolla, Calif.

**CF assay.** The CF test was performed by a previously described method (9). Antigen, guinea pig complement, hemolysin, and control reagents were purchased from Flow Laboratories.

**HAI.** The HAI assay for measles antibody was performed by a standard method (5). Positive and negative controls, antigen, and reagents were purchased from Flow Laboratories.

**IFA.** The IFA assay was performed by a previously described method (4). CV-1 cells infected with the Edmonston strain of measles virus (American Type Culture Collection, Rockville, Md.) and noninfected control CV-1 cells were used for preparing slides. Fluorescein isothiocyanate-tagged sheep anti-human gamma globulin was purchased from Becton, Dickinson & Co., Research Triangle Park, N.C.

**RESULTS**

Forty-six pairs of acute- and convalescent-phase sera from patients with measles infection were assayed by CF, HAI, IFA, and ELISA techniques (Fig. 1 and 2). A fourfold or greater increase in the convalescent-phase titer was found in 95.7% by CF, 82.6% by ELISA, and 91.3% by HAI and IFA techniques. There was no statistically significant difference in seroconversion rate determined with the four methods.

![Graph](https://example.com/graph.png)

**Fig. 1.** Acute-phase (S₁) and convalescent-phase (S₂) antibody titers of 46 paired sera. (A) ELISA; (B) HAI. Bracketed area represents geometric mean titer (●) and standard error.
The absolute titers determined with ELISA were higher than those determined with the other methods. The geometric mean titers with ELISA were 26 to 45 times higher than those with IFA, CF, and HAI (Table 1). The method of visual endpoint determination yielded excellent reproducibility when performed by a single technologist in duplicate or replicate and when the results of three technologists were compared. The method was used in 40 separate assays and difficulties were not encountered. The color standard provided more reproducible results than a "standard" positive serum, which showed occasional endpoint variation of one dilution.

The titers determined with ELISA were compared with those obtained with IFA, HAI, and CF. A serum was considered positive if the titer was 1:4 or greater with IFA, 1:4 or greater with CF, 1:5 or greater with HAI, and 1:10 or greater with ELISA. Among all acute-phase serum samples, 8 of 25 with negative CF titers had titers of 1:10 to 1:50 with ELISA. Five of 18 acute-phase sera with negative IFA titers had titers of 1:10 to 1:80 by ELISA, and 3 of 6 acute-phase sera with negative HAI titers had titers of 1:10 to 1:40 with ELISA. Only 2 of 24 acute-phase sera with positive ELISA titers were negative by all other methods. Of the 22 acute-phase sera which were negative with ELISA, 16 failed to react with CF and 12 failed to react with IFA. Only 3 of the 22 failed to react with HAI, and these 3 sera were negative with all other techniques as well. Five of the 46 convalescent-phase sera had titers of less than 10 with ELISA, whereas all 5 of these had titers of 1:32 or greater with CF, HAI, and FA techniques (Table 2).

Collection dates of acute- and convalescent-phase sera and the date of onset of rash were known for 29 patients. The mean number of days from the onset of rash to collection of the acute-phase specimen was 2.0, and the mean number of days between onset of rash and collection of the convalescent-phase specimen was 17.6. Antibody was detected by HAI in 87% of acute-phase serum specimens drawn within 72
h of the reported onset of rash. Only 36% of these specimens were positive by CF, whereas ELISA and IFA results were positive in 48% within this time. Results with each technique appeared to show no further increase in titer between 15 and 19 days after the reported onset of rash (Fig. 3 and 4).

ELISA and CF were used to test sera from an additional 30 patients with clinical measles. These sera demonstrated a fourfold or greater rise in measles antibody titer when previously screened by CF. Sera from all 30 patients demonstrated fourfold or greater rises in ELISA titer. Among acute-phase sera, 15 of 30 showed CF titers of 1:4 or greater, whereas 17 of 30 demonstrated ELISA titers of 1:10 or greater. Two acute-phase sera which were unreactive by CF showed ELISA titers of greater than 1:10. All acute-phase sera which failed to show reactivity by ELISA also failed to show it by CF. Of the total 76 paired sera tested with ELISA and CF, only 3 failed to demonstrate a fourfold rise in antibody titer with CF. Two of these showed marked elevations in both acute- and convalescent-phase titers and reacted similarly with ELISA, IFA, and HAI. The third pair could not be evaluated by CF due to severe hemolysis of the acute-phase serum. This latter pair demonstrated significant rises in titer when examined subsequently with IFA and HAI. Numbers of serum pairs showing a fourfold or greater increase in measles antibody titer by all methods were as follows: CF, 73 of 76 (96%); ELISA, 68 of 76 (89%); HAI, 42 of 46 (91%); IFA, 42 of 46 (91%).

Paired sera from seven patients showing a fourfold rise in antibody titers against unrelated pathogens, including adenovirus, cytomegalovirus, echovirus, coxsackievirus, or blastomyces antigens, were tested for measles antibody by the ELISA technique. The measles antibody titer was unchanged in acute- and convalescent-phase sera, thus demonstrating no cross-reactivity between these agents. Acute- and convalescent-phase serum samples from 63 children enrolled in an acute febrile illness study were assayed by HAI and ELISA. A fourfold or greater rise in measles antibody titer was not observed in any of these paired specimens. Serum specimens from 20 healthy adults reporting a history of measles many years previously all showed detectable measles-specific antibody by ELISA.
DISCUSSION

Voller et al. (10) and others (6) have used ELISA for the determination of measles antibody titers. Kahane et al. (6) have evaluated a limited number of measles patients by using an ELISA method that employs peroxidase-linked anti-human immunoglobulin G and a measles antigen prepared in their laboratory. Good correlation was found in eight patients when measles-specific ELISA was compared with HAI and CF, although the authors felt that additional data were needed to establish a reliable statistical correlation among methods. Kahane also compared HAI and ELISA in healthy adults and found that there was excellent correlation between these methods, suggesting that the ELISA technique, unlike CF, may be a useful seroepidemiological tool for screening of measles susceptibility.

Our results show that the ELISA method, when used for detection of measles-specific antibody in paired sera from patients with acute measles, is as sensitive as the CF, IFA, and HAI methods. It is to be emphasized that the sera tested were screened initially by a CF assay, and each demonstrated a fourfold or greater increase in antibody titer. A significant difference in rates of seroconversion was not found in the 46 paired sera tested by CF in our laboratory or by the ELISA, HAI, and IFA methods. The ELISA showed excellent reproducibility between assays performed by a single technologist in duplicate or replicate and when results from three technologists were compared. The lowest initial serum dilution used was 1:10, and detection of antibody at this dilution could reliably be assessed by comparison with wells containing non-virus-infected control antigens. Detection of antibody below a dilution of 1:10 was precluded by nonspecific serum reaction with non-virus-containing control antigen. The color standard used in the assay provided a reproducible color intensity for comparison with test samples. Both a positive and a negative control serum were tested with each assay. The endpoint of the positive control serum occasionally varied by one dilution. On the other hand, the color standard showed excellent reproducibility over 40 separate assays and permitted more accurate comparisons between assays.

The absolute measles ELISA antibody titers exceeded those determined with the other techniques tested; however, ELISA was no more sensitive than IFA, HAI, and CF. Elevated ELISA titers compared with titers determined by other methods have been demonstrated with many antigens, including measles (11). Five serum pairs tested by ELISA during four different assays completely failed to show evidence of measles-specific antibody in acute- and conva-
lescent-phase samples. These five pairs showed fourfold or greater rises in titer by IFA, CF, and HAI. We could find no explanation to account for this finding. Of an additional 30 paired sera which showed CF seroconversion, all demonstrated a fourfold or greater increase in ELISA antibody titer. The frequency of CF-confirmed seroconversions that are undetectable by ELISA is therefore low. An estimate of the overall seroconversions in measles patients which are undetected by ELISA cannot be made based upon the present data, since all sera tested were pre-selected for this study by CF testing.

Comparison of techniques during acute illness reveals that the HAI assay detects antibody earlier than the CF, ELISA, and IFA techniques. This may perhaps result from a greater sensitivity of the HAI assay to immunoglobulin M antibody in early specimens. Therefore, in an HAI test of paired sera in which the acute sample was drawn late in the acute stage of illness, a fourfold or greater increase in titer may not be detected; ELISA, CF, or IFA may be more useful in this situation.

The ELISA method used in our study may conveniently be used in a small laboratory. All reagents are commercially available. Antigen-coated plates can be stored at −70°C for extended periods of time without loss of antigenicity (6). The assay could then be run in several hours, much more rapidly than most of the other standard methods. Expensive equipment, perishable supplies, infective agents, and radioactive substances need not be used, thus providing an additional advantage of ELISA over other methods, especially in a small clinical laboratory.

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LITERATURE CITED