

Comparison of Immunofluorescence and Enzyme Immunoassay for Detection of Measles-Specific Immunoglobulin M Antibody

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During a measles outbreak, 283 serum specimens from 221 suspected cases of measles were tested by immunofluorescence and enzyme immunoassay for the presence of measles-specific immunoglobulin M (IgM) antibodies by using commercially available reagents. There was 97% agreement between the two assays; thus, the choice of the method for diagnostic testing is a matter of convenience and experience. In all 62 cases of measles from which a single blood sample was available, measles IgM-specific antibodies were detectable by both methods. Fifty percent of the 62 cases were positive within 3 days after onset of the rash. This increased to 91% 10 days after onset of the rash.

The demonstration of measles-specific immunoglobulin M (IgM) antibodies in single serum specimens collected as early as possible after onset of the rash is now an accepted confirmatory test for suspected cases of measles (5, 9). Most studies of measles IgM serology have involved small numbers of cases, and there are very few data available on the performance of commercially available reagents for measles-specific IgM antibody detection. More than 10,000 cases of measles were reported in the provinces of Quebec and Ontario in the first 6 months of 1989 (1, 7). This outbreak provided the opportunity to compare the ability of an immunofluorescence (IFA) test and an enzyme immunoassay (EIA) to detect the presence of measles-specific IgM antibodies at the earliest possible time after onset of the rash.

Source of sera. During the outbreak, 283 blood specimens (53% single, 40% paired, 7% multiple) from 221 patients were received in our laboratory for investigation of a morbilliform rash. The median age of the patients was 12 years (range, 3 months to 69 years). The requests for testing originated from private physicians, emergency departments, and outpatient clinics. Dates of onset of the suspected rash were usually provided, but precise records of measles immunization were not available.

IFA tests for measles-specific IgM antibody. The sera were stored at 4°C for no more than 3 days and were inactivated at 56°C for 30 min prior to testing. IgG and rheumatoid factor (RF) were removed with staphylococcal protein A (Zysorbin; Zymed, San Francisco, Calif.) and sheep anti-human IgG (RF Absorbent; Behringwerke AG Diagnostika, Marburg, Germany) as described previously (11). The final dilution of the original serum specimen after this two-step procedure was 1:20.

The tests were performed by using the Virgo kit for the detection of antibody to measles virus, lots 6157, 6275, 6347, 6382, 6603, and 6653 (Electro-Nucleonics, Inc., Columbia, Md.). The supplied anti-human IgG was replaced with a fluorescein isothiocyanate-conjugated caprine antibody to human IgM (Microbiological Research Corp., Bountiful, Utah). Positive and negative control sera were pretreated as described above and were run with each test. The test

procedure was performed as described in the kit protocol, except that the primary incubation with the test sera was increased to 90 min. Results were read and scored independently by two technologists who had no knowledge of the EIA results, since the IFA tests were performed first.

EIAs for measles-specific IgM antibody. The sera were stored at -80°C before testing and were not inactivated. The Enzygnost Measles EIA antibody detection test (plate lot 406 451A, conjugate lot 4011049B, positive control lot 406319A, RF Absorbent lot 407039A; Behringwerke AG Diagnostika) was used exactly as specified in the test protocol for the detection of measles-specific IgM. The final dilution of the test sera after treatment (using only RF Absorbent) was 1:42. The plates were read at 405 nm by using a microplate reader (MR 600; Dynatech Laboratories, Alexandria, Va.).

On initial testing, results for 23 serum specimens were discordant or equivocal in one test or the other. Five serum specimens were unavailable for retesting; of the other 18 serum specimens, results for 9 of them were concordant after retesting. Three serum specimens remained positive by IFA and negative by EIA; five serum specimens gave the opposite pattern. One serum specimen was EIA equivocal and IFA nonspecific on initial testing; on retesting, it became EIA positive but remained IFA nonspecific. This serum specimen and the 5 serum specimens which were not available for retesting were omitted from the comparison (Table 1), which is therefore based on 277 serum specimens. In total, 69 serum specimens were positive and 200 serum specimens were negative by both tests. Concordant results were thus obtained in 97% of the serum specimens tested, as follows: $(69 + 200)/(69 + 3 + 5 + 200) \times 100$.

Among the 65 serum specimens that were positive by both methods on first testing, 62 were single serum specimens collected from cases for which the precise date of onset of the rash was known. The cumulative percentages of positive measles-specific IgM tests obtained in these 62 cases as a function of time after onset of the rash are represented in Fig. 1.

In approximately 50% of the measles cases from which a single serum was available, measles-specific IgM antibodies were detected by both methods within 3 days after onset of the rash. Ten days after onset of the rash, 91% of the cases were confirmed in the laboratory.

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TABLE 1. Comparison between IFA and EIA for the detection of measles-specific IgM antibody in 277 serum specimens

IFA IgM result	No. of EIA IgM results		Total
	+	-	
+	69	3	72
-	5	200	205
Total	74	203	277

In 1989, there was a major resurgence of measles in Canada and the United States (3, 13). As vaccination programs take effect, young physicians and nurses are less familiar with the disease than older physicians and nurses were. As a result, the possibility of misdiagnosis increases and implementation of outbreak control measures may be delayed. Thus, the rapid laboratory confirmation of suspected cases of measles has become an important component of the surveillance of immunization programs and of the control of measles outbreaks in Canada and the United States (5, 9).

Several studies of measles virus-specific IgM antibodies as a means of rapid laboratory confirmation of disease have been carried out (10). These studies indicate that measles-specific IgM antibodies are detectable at the time of appearance of the rash in most cases (4, 8, 12). Most of these studies have involved radioimmunoassay or EIA procedures developed in-house, often in antibody-capture formats. By contrast, most of the EIA kits for IgM detection presently marketed in North America are not IgM capture assays, and there are scant data on the performance of commercial kits for the detection of measles-specific IgM.

Of 65 serum specimens which were positive on initial testing for measles IgM antibody, 62 of them were unique in that no second specimen was received; this reflects a common pediatric practice not to draw a second blood specimen when the first has tested positive. It was thus not possible, in the majority of cases, to relate the early detection of measles-specific IgM antibody to subsequent increases in the level of IgG antibody. In the three cases in which it was possible to do so, significant rise in measles-specific IgG levels occurred in two cases, and seroconversion occurred in one case.

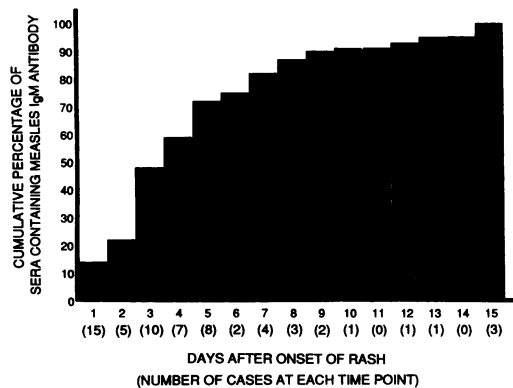


FIG. 1. Detection of measles-specific IgM antibodies by IFA and EIA as a function of time after the onset of the rash in 62 cases of measles.

By using a commercially available EIA IgM detection kit, cumulative detection rates of 72 and 91% were achieved within 5 and 10 days of the rash, respectively. Since these results were obtained with single serum specimens, it is likely that earlier sampling of some of the patients might have resulted in still earlier laboratory confirmation. These detection rates are comparable to those obtained with non-commercial IgM capture antibody assays, in which corresponding rates of 84 and 86% have been reported (8). These results are also in agreement with earlier studies carried out by hemagglutination-inhibition following IgM separation by sucrose gradient ultracentrifugation, which demonstrated that measles-specific IgM antibody peaks 10 days after onset of the rash (2).

The IFA test used in this study was an adaptation of a commercially available fluorescent-antibody slide test. Overall, there was a 97% agreement with the EIA, and the discrepant results were equally distributed between the two tests. None of these discrepant results were equivocal or weak positives; i.e., the test results by either method were clear-cut. No explanation could be found for these discrepancies. More importantly, the IFA test was as good as the EIA in its ability to detect the presence of IgM shortly after onset of the rash in single serum specimens from 62 cases of measles.

This degree of agreement is much higher than that observed by other workers. Using the same measles virus antigen slides (Virgo), Kleiman et al. (6) evaluated the IFA test for the detection of measles-specific IgM antibodies by comparison with complement fixation and hemagglutination-inhibition in paired serum specimens from 35 patients with measles. Their IFA test was positive for 31 and 64% of serum specimens collected within 5 days and between days 11 and 45 following onset of the rash, respectively. These investigators concluded that the IFA test had a limited value as a rapid diagnostic test for measles. The discrepancy between their conclusion and ours probably reflects differences in procedures such as the serum storage temperature (-20 versus 4°C), the method of removal of IgG and RF (Sephadex A-50 columns versus a two-step procedure), as well as the length of the primary incubation period (60 versus 90 min).

Since a measles outbreak exists in a community whenever a single case of measles is confirmed (5), laboratory confirmation should be available through small diagnostic laboratories. Our results demonstrate that, for such laboratories, the choice of the method, EIA or IFA, for measles-specific IgM antibody is a matter of convenience and experience.

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