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Performance and Reliability of the Enzygnost Measles Enzyme-Linked Immuno-Sorbent Assay for Detection of Measles Virus-Specific Immunoglobulin M Antibody during a Large Measles Epidemic

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Evaluation of the Enzygnost Measles Enzyme-Linked Immuno-Sorbent Assay kit (Behring) performance to detect specific immunoglobulin M (IgM) was carried out with 3,297 single serum samples and 898 paired serum samples collected during a measles epidemic (10,184 reported cases) in Quebec, Canada. Anti-measles IgM and IgG were detected by using the Enzygnost kit with the appropriate conjugates. Complement-fixing (CF) antibody (Ab) titers were assessed by the laboratory branch complement fixation micromethod. The Centers for Disease Control's clinical measles case definition was used. A modification of the manufacturer's optical density interpretation algorithm was introduced to allow for equivocal results, in addition to positive and negative ones. These three categories differed as to their association with a significant increase in CF Ab titer and the time between the onset of symptoms and phlebotomy. The IgM positivity rate for complement fixation-confirmed measles cases was 96.6% for vaccinated subjects and 100% for nonvaccinated subjects. The daily percentage of IgM seropositivity that was detected for subjects who became IgM positive within 30 days increased gradually from 40 to 90% for sera taken 1 to 7 days after the onset of symptoms, and it plateaued at 100% for sera taken 16 to 30 days after the onset of symptoms. IgM seropositivity was strongly associated with IgG seroconversion, CF Ab titer increase, and clinical measles ($P < 0.0001$). Reproducibility was 100% for nonreactive sera and 99.1% for reactive sera. In conclusion, the Enzygnost Measles Enzyme-Linked Immuno-Sorbent Assay kit performed adequately to confirm measles virus infection during this epidemic. A second serum sample should be tested when an early-acute-phase serum sample is IgM negative.

Measles diagnosis may be confirmed by virus isolation, a significant increase in measles virus antibody titer, or detection of anti-measles virus immunoglobulin M (IgM) antibody (3). Several investigators (7, 8) advocate that anti-measles virus IgM antibody detection in acute-phase serum is sufficient to confirm a measles diagnosis (7, 8).

During the 1989 measles epidemic in Quebec, Canada, we tested 5,093 serum samples using the Enzygnost Measles Enzyme-Linked Immuno-Sorbent Assay (ELISA) kit (Behring) to confirm measles virus infection on the basis of anti-measles virus IgM (measles-IgM) seropositivity. ELISA detection of anti-measles virus IgG (measles-IgG) by using the same kit with a different conjugate and the complement fixation test were carried out on all paired sera to identify subjects who showed measles-IgG seroconversion or a significant increase in the complement-fixing (CF) antibody (Ab) titer.

Using the data bank compiled during the epidemic described here, we had the opportunity to carry out a large-scale field evaluation of the performance and reliability of the Enzygnost Measles ELISA kit in confirming clinical measles cases or contacts which induced a specific immune response, with or without symptoms, in a subject (measles virus immunogenic contact). Also, the efficacy of testing convalescent-phase serum in the case of measles-IgM-negative acute-phase serum was evaluated. Finally, the relationship between measles-IgM seropositivity, measles-IgG seropositivity, and an increase in the CF Ab titer was studied; this gave an overall picture of the serological responses observed during the epidemic.

To our knowledge, no large-scale evaluation of this kit has been carried out in North America during a measles epidemic. This evaluation provides useful information to laboratories, for the selection of the appropriate ELISA kit for the rapid confirmation of measles when outbreaks occur, and to clinicians, for the interpretation of Enzygnost Measles ELISA kit results.

MATERIALS AND METHODS

Sera studied. During the 1989 measles epidemic in Quebec (10,184 reported measles cases [2]), the Laboratoire de Santé Publique du Québec, which is the public health reference laboratory for the province of Quebec, performed measles serology on 3,297 single serum samples and 898 paired serum samples from 4,195 subjects over an 8-month period. The subjects' ages ranged from 4 months to 63 years (mean, 13 years). Females and males were equally represented. On the basis of clinical information for 1,235 subjects from two large urban communities in the province of Quebec, the subjects for whom measles serology was requested were distributed as follows: 67% clinical measles cases as defined by the Centers for Disease Control (CDC) (3), 21% clinical measles cases with rashes of unknown durations, 9% presumed measles cases with either rash or fever, and 3% asymptomatic subjects.

The first serum sample was taken, on average, 4.9 days after the onset of symptoms (median, 4 days; mode, 4 days; range, 0 to 56 days; 95th percentile, 10 days). The second

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serum sample, when available, was taken, on average, 18.5 days after the onset of symptoms (median, 18 days; mode, 20 days; range, 1 to 51 days; 95th percentile, 25 days). The time lapse between the dates that paired serum samples were obtained was, on average, 15.4 days (median, 14 days; mode, 14 days; range, 1 to 42 days; 95th percentile, 22 days). Most specimens were analyzed for the presence of measles-IgM within 24 h of receipt and were then frozen until measles-IgG detection and complement fixation tests could be carried out on the paired serum samples.

Serological tests. The Enzygnost Measles indirect ELISA kit (Behring) was used to detect measles-IgM or measles-IgG with the appropriate conjugate. Since an excess of IgG or rheumatoid factor (IgM-RF) may interfere in the IgM solid-phase enzyme immunoassay (11), measles-IgM ELISA was carried out by using sera diluted in rheumatoid factor absorbent (sheep anti-human IgG; RF Absorbent, Behring) to precipitate IgG and IgG-linked IgM-RF. One measles virus antigen-coated well and one control antigen-coated well were used for each serum sample. Working dilutions of serum samples, were 1:42 and 1:44 for the IgM and IgG ELISAs, respectively. ELISA was performed by following the manufacturer's instructions and by using a multichannel pipette (Flow), a Titertek Handiwash 100 washer (Flow), and a Multiskan MC reader (reading at 405 nm; Flow). Each run included low-positive, high-positive, and negative controls which consisted of patient serum samples that were previously tested several times in parallel with commercial controls (Behring) to assess their measles-IgG or measles-IgM status. The result for each sample was expressed as an adjusted optical density (OD) calculated by subtracting the control antigen well OD from the measles antigen well OD. This kit is designed to detect either IgG or IgM, depending on the conjugate used. The same cutoff points are used for both IgG and IgM ELISAs. According to the manufacturer's instructions, the adjusted sample OD should be interpreted as negative (OD, <0.2) or positive (OD, ≥0.3) by using fixed cutoff points. Samples for which the first OD falls between 0.2 and 0.3 (grey zone) should be retested and interpreted as negative (second OD, <0.2) or positive (second OD, ≥0.2). There is no provision for equivocal results. In our laboratory, modifications were introduced to expand the manufacturer's OD grey zone from 0.2–0.3 to 0.19–0.4 where repeated tests were to be carried out. The possibility of equivocal results was also introduced (see justification of the modifications in the Results section).

The Laboratory Branch complement fixation micromethod (15) was carried out to detect CF Ab by using measles antigen (Behring). Paired sera were analyzed within the same run. Each run included low-positive, high-positive, and negative controls which consisted of patient serum samples that were previously tested several times in parallel with commercial controls (Behring) to assess their CF Ab measles titers. A significant increase in CF Ab titer was defined as a fourfold increase between results for acute-phase and convalescent-phase serum samples (12).

Clinical definition. By following the recommendations of the CDC (3), a clinical measles case was defined as a subject who showed the following documented symptoms: fever, ≥38.3°C (if measured); generalized rash lasting at least 3 days; and cough, coryza, or conjunctivitis. Clinical information was obtained for 1,524 subjects. A confirmed case of measles was a complement fixation test-confirmed clinical case of measles for the purposes of this evaluation.

Data collection and analysis. Demographic and laboratory data were downloaded from our main laboratory data management system (EPIC; EPIC Systems Corp.) to a data base file by using dBase IV software (Ashton Tate) running on a Turbo IBM-PC XT-compatible computer. Clinical and immunization data were obtained from regional health authorities or the attending physician. Computerized demographic, clinical, and immunization information was validated with the attending physician or by comparison with information from other registers. All computerized laboratory data were validated by using laboratory files.

TABLE 1. ODs obtained on duplicate tests by using the Enzygnost Measles-IgM ELISA

OD of first test	No. (%) of samples with a second test OD of[a]:			
	<0.2	0.2–<0.3	0.3–<0.4	≥0.4
<0.2	17 (65)	9[b] (35)	0	0
0.2–<0.3	21 (23)	49 (55)	3 (3)	17 (19)
0.3–<0.4	3 (3)	24 (27)	33 (38)	28 (32)
≥0.4	0	1 (≈1)	11 (9)	103 (90)

[a] Percentages represent percentages for data in each row.
[b] Six of nine serum samples had an initial OD of >0.190.

To calculate the time lapse between the onset of symptoms and phlebotomy, the date of the first reported symptom (mostly fever) was used instead of the date that the rash appeared, which was not available for 66% of the clinically documented cases.

In a vaccinated population, such as the one studied, measles virus infection may induce an immune response with or without symptoms (4, 13). Since measles-IgM ELISA detects measles virus infections by measuring the subject's immune response, evaluation of its performance on the basis of a comparison between measles-IgM seropositivity and the presence or absence of clinical measles may be misleading. In our study, two sensitivities were estimated: one for the detection of specific immune responses as detected by complement fixation and one for the confirmation of measles cases. The sensitivities were computed by the usual formula (6). Specificity was not assessed because of the lack of appropriate data. We estimated only the measles-IgM predictive value of the absence or presence of an immune response as detected by complement fixation because of the lack of data for true asymptomatic subjects without a significant increase in CF Ab titer (nonreacting subjects). Serum samples that gave equivocal measles-IgM results were not included in the estimation of predictive values. Predictive values were calculated by the usual formula (6). Comparisons between runs to evaluate reproducibility were carried out by using contingency tables. The chi-square distribution test and Fisher's exact test were used to analyze data (14).

RESULTS

Analysis of the cutoff points and OD interpretation. Since the manufacturer's cutoff points delimit a narrow grey zone and there is no provision for equivocal results, it was necessary to verify whether this kit provided a robust enough reaction to produce results within the same category on duplicate tests. This assessment was carried out with 319 randomly selected serum samples that were tested twice for measles-IgM by the same person, with the same lot, on 2 consecutive days. Results are presented in Table 1.

These results led us to expand the grey zone from 0.2–0.3 to 0.19–0.4 and to introduce a third class of results qualified

as equivocal. Following these modifications, negative sera were defined as sera that gave an initial OD of <0.19 or an initial OD of between 0.19 and 0.3 and an OD of <0.19 when they were retested. Equivocal sera were defined as sera that gave an initial OD of between 0.19 and 0.4 and an OD of between 0.19 and 0.3 on repeat testing. Positive sera were defined as sera that gave an initial OD of ≥0.4 or an initial OD of between 0.19 and 0.4 and a subsequent OD of ≥0.3. These criteria were used to interpret both measles-IgM and measles-IgG ELISA results.

The following measles-IgM ELISA results were obtained with the first serum sample from 4,161 subjects: 1,707 (41%) positive, 198 (4.7%) equivocal, and 2,256 (54.3%) negative (34 of the 4,195 first serum samples received [0.8%] were not analyzed). The 198 measles-IgM-equivocal results would have been either positive (n = 170) or negative (n = 28) if the manufacturer's instructions had been used. Eighty-one subjects initially with measles-IgM-equivocal serum samples were clinically documented (n = 81 of 198 subjects); 55 of them (55 of 81; 67%) were clinical cases of measles. Paired serum samples were received for 23 of 55 of these subjects; 18 of 23 (78%) subjects became measles-IgM positive.

For ELISA measles-IgG (n = 1,422 serum samples), we obtained the following results: 934 (65.7%) were positive, 34 (2.4%) were equivocal, and 454 (31.9%) were negative. These 34 measles-IgG-equivocal results would have been either positive (n = 30) or negative (n = 4) if we had used the manufacturer's instructions.

The relationship between the presence of a significant increase in CF Ab titer, which indicated an immune response, and the IgM result that was obtained when testing the convalescent-phase serum from 564 subjects who were initially measles-IgM negative was studied. This permitted us to evaluate whether equivocal results were truly different from negative and positive ELISA results, with each type of result corresponding to a different rate of increase in the CF Ab titer in subjects. There was a difference between the proportion of increase in the CF Ab titer associated with measles-IgM-negative (20 of 407 [4%] with a significant increase in CF Ab titer), measles-IgM-equivocal (7 of 10 [70%] with a significant increase in CF Ab titer), or measles-IgM-positive (130 of 147 [88%] with a significant increase in CF Ab titer) convalescent-phase serum ($P < 0.0001$). There was also a difference in the proportion of subjects with significantly increased CF Ab titers associated with measles-IgM-equivocal (70% with a significant increase in CF Ab titer) or measles-IgM-positive (88% with a significant increase in CF Ab titer) convalescent-phase serum samples ($P = 0.11$).

The interval between the date that acute-phase serum samples were obtained and the date of onset of symptoms was studied for a group of 172 subjects showing measles-IgM-positive convalescent-phase serum to see the relationship between measles-IgM status and phlebotomy timing. The average interval between the onset of symptoms and acute-phase serum was 3.3, 4.4, and 5.1 days for measles-IgM-negative, -equivocal, and -positive acute-phase serum samples, respectively.

Measles-IgM ELISA sensitivity. The proportion of measles-IgM-positive subjects was 375 of 408 (91.9%) in a group of subjects showing recent measles virus immunogenic contact as indicated by a significant increase in the CF Ab titer. The remaining 33 subjects had either both measles-IgM-negative sera (20 of 408; 4.9%) or measles-IgM-equivocal first and/or second serum but no measles-IgM-positive sera (13 of 408; 3.2%).

Among the clinically documented subjects with significant increases in CF Ab titer who met the measles case definition, the rate of measles-IgM seropositivity was 96.6% (114 of 118) and 100% (30 of 30) for vaccinated and nonvaccinated subjects, respectively, giving an overall sensitivity of 97.2% (144 of 148 subjects) for the detection of specific IgM in measles cases confirmed by the complement fixation test.

Predictive values. Among measles-IgM-positive subjects (n = 461), 375 showed a significant increase in CF Ab titer, giving a positive predictive value for the detection of a recent immune response of 81.6%. Among the 86 measles-IgM-positive subjects without a significant increase in CF Ab titer who were clinically documented (n = 45), 40 (88%) were symptomatic subjects, with most of them (34 of 45; 75%) having clinical measles as defined by the CDC. Among measles-IgM-negative subjects (n = 408), 388 did not show a significant increase in CF Ab titer, giving a negative predictive value for the detection of a recent immune response of 95% (388 of 408 subjects). Among the 20 measles-IgM-negative subjects showing a significant increase in CF Ab titer, 16 (80%) were symptomatic.

Measles-IgM ELISA reproducibility. To evaluate measles-IgM detection reproducibility, only sera that gave an initial OD below 0.2 or over 0.3 were considered. Since these sera would not be retested if the manufacturer's instructions were followed, the initial result would be final and thus should be stable. Reproducibility was 65% (17 of 26) for sera with an initial OD of <0.2 and 86.2% (175 of 203) for sera with an initial OD of >0.3 (Table 1), giving an overall reproducibility of 83.8%. However, 99.1% of measles-IgM-positive sera with an initial OD of >0.4 gave an OD of >0.3 on repeat testing, thus remaining positive (Table 1).

One hundred percent reproducibility was obtained when five randomly selected measles-IgM-positive serum samples (OD, >0.4) were tested 20 times on a daily basis; the OD coefficient of variation was 14.4% on average (range, 12.1 to 15.9%). Also, 100% reproducibility was obtained when five randomly selected measles-IgM-negative serum samples (OD, <0.1) were tested 20 times on a daily basis. The ODs obtained were repeatedly below 0.1.

Measles-IgM seronegativity as a result of inappropriate phlebotomy timing. The probability of a measles-IgM-negative serum sample as a result of inappropriate phlebotomy timing was estimated by using paired serum samples from 170 clinically documented symptomatic subjects showing measles-IgM-positive acute- and/or convalescent-phase serum. The proportion of measles-IgM positive serum samples over the daily total number of serum samples received in regard to the time lapse from the onset of symptoms is shown in Fig. 1. The daily percentage of measles-IgM seropositivity increased gradually from 40 to 90% from 1 to 7 days after the onset of symptoms. Between 7 and 15 days after the onset of symptoms, most (>90%) of the sera received were measles-IgM positive. All sera taken later than 15 days after the onset of symptoms were measles-IgM positive.

Relationship between measles-IgM and measles-IgG response, increase in CF Ab titer, and clinical status. Single serum samples from subjects who met the CDC's clinical measles case definition had a significantly higher rate of measles-IgM seropositivity (457 of 616; 74%) than did symptomatic subjects who did not meet the complete CDC's case definition of measles (e.g., lacking of rash or fever) (98 of 288; 34%; $P < 0.0001$) and asymptomatic subjects (1 of 22; 4%; $P < 0.0001$) for whom a serum sample was submitted for measles-IgG detection (verification of immune status). There

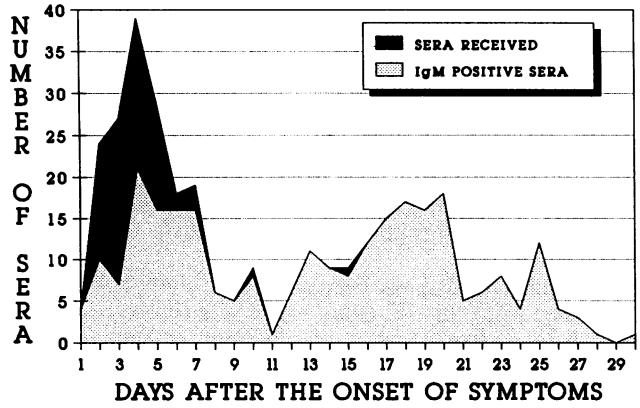

FIG. 1. Daily number of measles-IgM-positive serum samples (grey area) superimposed over the daily total number of serum samples received (black area).

was a significant association between measles-IgM seronegativity and measles-IgG seropositivity in acute-phase sera for subjects for whom both tests were performed (n = 1,422; $P < 0.0001$), for documented vaccinated subjects (n = 518; $P < 0.0001$), and for documented cases of clinical measles (n = 616; $P = 0.012$).

Within the group of subjects for whom paired serum samples were tested, measles-IgM-positive first sera (n = 266) were followed by a measles-IgM-positive second sera in 97% of the subjects. Measles-IgM was detected in the convalescent-phase sera of 147 subjects with acute-phase measles-IgM-negative sera and 47 subjects with measles-IgM-equivocal acute-phase sera. These 194 subjects included 13 subjects (7%) who had only a measles-IgM immune response, 169 subjects (87%) who had a measles-IgM immune response with a significant increase in CF Ab titer (112 of 169 subjects were also measles-IgG seroconverters), and 12 subjects (6%) who had a measles-IgM immune response with IgG seroconversion only (stable CF Ab titers). Thirty subjects with measles-IgG-positive acute- and convalescent-phase sera never became measles-IgM seropositive (20 measles-IgM-negative and 10 measles-IgM-equivocal subjects), even though they showed a significant increase in CF Ab titer. Three measles-IgM-seronegative subjects showed a measles-IgG seroconversion without a significant increase in CF Ab titer. Three subjects showed measles-IgG seroconversion and a signficant increase in CF Ab titer without measles-IgM seropositivity. Significant increases in CF Ab titers were observed in 91% of the measles-IgG seroconverters. There was a strong association between measles-IgM seroconversion and a significant increase in CF Ab titer ($P < 0.0001$). There was a strong association between measles-IgG seroconversion and measles-IgM seropositivity ($P < 0.0001$) or a significant increase in CF Ab titer ($P < 0.0001$). Measles cases confirmed only by the results from convalescent-phase serum represented 11.8% (230 of 1,937) of all the confirmed cases. Overall measles virus immunogenic contact was confirmed in 1,937 of 4,195 (46.2%) subjects; measles-IgM-confirmed immunogenic contacts represented 98.1% (1,901 of 1,937) of them.

Relationship between measles-IgM and measles-IgG response and vaccination status. The percentage of measles-IgM-positive acute-phase sera was 53.7% (446 of 829) for vaccinated subjects and 61.5% (237 of 385) for unvaccinated ones. The percentage of measles-IgM-negative sera was 40.9 and 36% for vaccinated and unvaccinated subjects, respectively. There were twice as many measles-IgM-equivocal acute-phase sera from vaccinated subjects (5.4%) than from unvaccinated subjects (2.5%).

The percentage of measles-IgG-positive acute-phase sera, when tested, was higher in vaccinated subjects (258 of 412; 62.6%) than in unvaccinated subjects (28 of 75; 37.4%). The percentage of measles-IgG-equivocal acute-phase sera was similar for the two groups (vaccinated, 3.2%; unvaccinated, 2.6%). The percentage of measles-IgG-negative acute-phase sera was 34.2 and 60% for vaccinated and unvaccinated subjects, respectively. A measles-IgG-negative, measles-IgM-positive acute-phase serum sample was detected in 64 (15.5%) of the vaccinated subjects; 61 of them had clinical cases of measles.

DISCUSSION

ELISA kits that use fixed cutoff points do not take into account daily test fluctuations as do the ones that use floating cutoff points calculated from daily results for control sera. Thus, a fixed-cutoff-point ELISA must be robust enough or have negative and positive OD clusters far enough from the cutoff point for the interpreted results to be unaffected by daily fluctuations. Our results showed that sera that gave an initial OD of between 0.19 and 0.4 produced highly variable results on repeat testing, indicating that the manufacturer's negativity cutoff point (0.2) might be too high and the positivity cutoff point (0.3) might be too low for the robustness of the technique. We proposed a different set of cutoff points that delimits an extended grey zone (OD, 0.19 to >0.4) to provide safer interpretation of the results following repeat testing. The provision for equivocal results was introduced for sera that gave an OD over but near the negativity cutoff point.

Study of the association between a significant increase in CF Ab titer and measles-IgM ELISA results indicated that measles-IgM-negative, -equivocal, and -positive ELISA results might represent different classes of measles-IgM results associated with an increasing proportion of subjects who show a specific active immune response, as indicated by a significant increase in the CF Ab titer (12). Measles-IgM-negative sera were taken earlier (3.3 days after the onset of symptoms) than measles-IgM-equivocal sera (4.4 days after the onset of symptoms) were, which, in turn, were taken earlier than measles-IgM-positive sera (5.1 days after the onset of symptoms) were, suggesting that measles-IgM-equivocal sera were taken at the beginning of the IgM response, before a high concentration of measles-IgM in blood was reached.

Modification of the OD interpretation algorithm changed the results for 4.3% (219 of 5,093) of the serum samples tested for measles-IgM and 2.4% (34 of 1,422) of the serum samples tested for measles-IgG. Our results showed that some subjects with clinical measles and measles-IgM-equivocal acute-phase serum became measles-IgM positive, suggesting that the first serum sample was taken at the beginning of the patient's immune response. Some other subjects with clinical measles and measles-IgM-equivocal acute-phase serum samples did not seroconvert, suggesting that they had a secondary immune response with a low IgM immune response. Since subjects with measles-IgM-equivocal acute-phase serum might be at the beginning of their immune response or might have a low immune response, this modification may have some beneficial consequences for clinicians in pointing out those subjects who might have been

otherwise reported as measles-IgM negative by using the manufacturer's algorithm. Even though some subjects who would have been reported as measles-IgM positive by using the manufacturer's algorithm might be reported as measles-IgM equivocal by using our algorithm, none would be reported as measles-IgM negative. Thus, this modification to the manufacturer's algorithm does not create false-negative results.

Our results showed that the measles-IgM detection sensitivity of the Enzygnost Measles ELISA was 91.9% for subjects with anti-measles virus immune responses shown by a significant increase in CF Ab titer. A measles-IgM-negative immune response in subjects with complement fixation test-confirmed immunogenic contact may indicate that these subjects had a primary immune response with transient measles-IgM (4) which were missed or that only a measles-IgM-negative secondary immune response (1) was induced in these subjects. The measles-IgM detection sensitivity of this ELISA was 96.6% for vaccinated subjects and 100% for nonvaccinated subjects, both of which had complement fixation test-confirmed measles. Similar sensitivities were reported previously when 17 serum samples from nonvaccinated subjects were tested (13) and when this ELISA kit was compared with the immunofluorescence assay by using 72 immunofluorescence assay-positive serum samples (10). Interfering IgG, which might have caused false-negative results because of competition with IgM for attachment sites in the ELISA wells, was eliminated prior to measles-IgM testing. Because of the high IgM detection sensitivity that was observed and the elimination of interfering IgG prior to measles-IgM ELISA testing, it is highly unlikely that a large number (>5%) of measles-IgM-negative sera would be false negative during such an epidemic, especially within a group of clinical measles cases for which a confirmatory serodiagnosis is requested.

This study was a field evaluation of the Enzygnost Measles ELISA kit during an epidemic. It was not a formal evaluation with a selected panel that included, among other things, true-negative sera and tricky sera, such as panels that we have used in other evaluations (9). Thus, the specificity could not be formally evaluated. Since 97% of the measles-IgM-positive acute-phase serum samples were followed by a second measles-IgM-positive serum sample, the probability of transient measles-IgM false-positive results caused by technical problems or abnormal samples was low. Measles-IgM seropositivity was strongly associated with an active immune response ($P < 0.0001$) and was more likely associated with a clinical measles case than a measles-IgM-negative result was ($P < 0.0001$). Thus, IgM seropositivity was strongly associated with recent clinical measles. Also, potential IgM false-positive results caused by the IgM-RF were eliminated prior to IgM testing. Thus, the probability of a large number of false-positive measles-IgM results leading to the false diagnosis of measles would be low during such an epidemic.

The measles-IgM positive predictive value for the detection of a recent immune response to the measles virus was 81.6%. Since most of the measles-IgM-positive subjects without a significant increase in CF Ab titer were clinical measles cases, it is reasonable to assume that they represent primary immune responses and not false-positive measles-IgM results. Thus, the positive predictive value for the detection of a specific immune response or measles cases is probably much higher than 82%. Measles-IgM negative predictive value was 95%. Among the residual 5%, most of them were symptomatic subjects, suggesting that they represent secondary immune responses.

Intrasite reproducibility for measles-IgM-positive sera (>99%) was similar to the reproducibilities of other ELISA kits tested in our laboratory (9). Results that were interpreted as equivocal were highly variable on repeat testing. These facts indicate that the technique might not be reproducible for sera that are interpreted as equivocal but is highly reproducible for sera that are clearly measles-IgM positive (OD, >0.4).

Our results and those from one other study (10) indicate that when using this kit, acute-phase sera should be taken at least 6 days after the onset of symptoms to have a 80% or greater probability of being positive in subjects with a measles-IgM response. Serum samples should be taken at least 16 days after the onset of symptoms to have a 100% probability of obtaining a measles-IgM-positive result in subjects with a measles-IgM response. This period corresponds to the optimal time for specimen collection for IgM detection (5).

Confirmed cases on the basis of results obtained with convalescent-phase serum samples represented 11.8% (230 of 1,937) of all the confirmed cases. This indicates the importance of testing a convalescent-phase serum sample when the acute-phase serum sample is measles-IgM negative. Overall, 98.1% of the measles immunogenic contacts were confirmed following detection of measles-IgM, showing the usefulness of the procedure in confirming measles cases.

Study of the relationship between measles-IgM status in acute-phase serum and vaccination status showed that there were fewer measles-IgM-positive subjects in the vaccinated group (53.7%) than in the unvaccinated group (61.5%) ($P = 0.0134$), suggesting that the primary immune response was less frequent in the vaccinated group. However, 15.5% of the vaccinated subjects had measles-IgG-negative, measles-IgM-positive acute-phase serum, and most of them had clinical measles, suggesting that cases of measles caused by primary vaccine failure (inefficient immunization) were present during this epidemic.

In conclusion, our results indicate that the Enzygnost Measles ELISA kit, with our modification to the interpretation algorithm, would perform adequately for the confirmation of cases of measles or immunogenic contact with the measles virus during an epidemic, provided that acute- and convalescent-phase sera are obtained at the appropriate times. They also indicate that measles-IgM ELISA testing of only an acute-phase serum sample may not be sufficient to confirm measles; a second serum sample taken a few days later should be tested when the one from the acute phase is measles-IgM negative, especially when the first serum sample was taken early (within 6 days after the onset of symptoms) or was taken from a symptomatic subject. In rare instances, testing of a third serum sample might be necessary to confirm immunogenic contact with the measles virus for subjects who are slow to respond.

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