4. Drenth JPH, van Uum SHM, van Deuren M, Pesman GJ, van der Ven Jongekrijg J, van der Meer JWM. Endurance run increases circulating IL-6 and IL-1ra but downregulates ex-vivo TNF-α and IL-1β production. J Appl Physiol **1995**;79:1497–503.
5. Helle M, Boeije L, de Groot E, de Vos A, Aarden L. Sensitive ELISA for interleukin 6. Detection of IL-6 in biological fluids: synovial fluids and sera. J Immunol Methods **1991**;138:47–56.
6. Lopez Cortés LF, Cruz Ruiz M, Gomez Matos J, Jiménez Hernandez D, Palomino J, Jiménez E. Measurement of levels of tumor necrosis factor-α and interleukin-1β in the CSF of patients with meningitis of different etiologies: utility in the differential diagnosis. Clin Infect Dis **1993**;166:534–9.
7. Tilman Steinmetz H, Herbertz A, Bertram M, Diehl V. Increase in interleukin-6 serum level preceding fever in granulocytopenia and correlation with death from sepsis. J Infect Dis **1995**;171:225–8.
8. van Deuren M, van der Ven Jongekrijg J, Demacker PNM, et al. Differential expression of proinflammatory cytokines and their inhibitors during the course of meningococcal infections. J Infect Dis **1994**;169:157–61.
9. Keuter M, Dharmana E, Gasem MH, et al. Patterns of proinflammatory cytokines and inhibitors during thyroid fever. J Infect Dis **1994**;169:1306–11.
10. Haagen Nielsen O, Brynskov J, Bendtzen K. Circulating and mucosal concentrations of tumour necrosis factor and inhibitor(s) in chronic inflammatory bowel disease. Dan Med Bull **1993**;40:247–9.
11. Al Janadi M, Al Balla S, Al Dalaan A, Raziuddin S. Cytokine profile in systemic lupus erythematosus, rheumatoid arthritis, and other rheumatic diseases. J Clin Immunol **1993**;13:58–67.
12. Gause A, Keymis S, Scholz R, et al. Increased levels of circulating cytokines in patients with untreated Hodgkin's disease. Lymphokine Res **1991**;11:109–13.
13. Friedland JS, Suputtamongkol Y, Remick DG, et al. Prolonged elevation of interleukin-8 and interleukin-6 concentrations in plasma and of leukocyte interleukin-8 mRNA levels during septicemic and localized *Pseudomonas pseudomallei* infection. Infect Immun **1992**;60:2402–8.

Diagnosis of Measles with an IgM Capture EIA: The Optimal Timing of Specimen Collection after Rash Onset

Rita F. Helfand, Janet L. Heath, Larry J. Anderson, Edmond F. Maes, Dalya Guris, and William J. Bellini

Department of Pediatrics, Emory University; Division of Viral and Rickettsial Diseases, National Center for Infectious Diseases, and Immunization Services Division and Epidemiology and Surveillance Division, National Immunization Program, Centers for Disease Control and Prevention, Atlanta, Georgia

The optimal timing for collection of a single serum specimen to diagnose measles by using a monoclonal antibody–capture EIA was evaluated. Results of testing paired serum samples from 166 measles cases with at least 1 IgM-positive specimen were analyzed. Among persons whose second samples were IgM-positive, the seropositivity rate for first samples was 77% when collected within 72 h and 100% when collected 4–11 days after rash onset. Among unvaccinated persons whose first samples were IgM-positive, the rate for IgM positivity of second specimens declined from 100% at 4 days to 94% at 4 weeks after rash onset, then declined further to 63% at 5 weeks. Some previously vaccinated persons became IgM-negative during the third week after rash onset. In general, a single serum specimen collected between 72 h and 4 weeks after rash onset can be used to diagnose most cases of measles with an IgM capture EIA.

Measles continues to be a major health problem worldwide, with ~45 million cases globally each year [1]. The Pan American Health Organization is working toward the elimination of measles from Central and South America [2]. These elimination efforts have created renewed interest in sensitive and specific diagnostic assays that can be used by countries throughout the world to diagnose measles infections.

Currently, many different serologic techniques are used to diagnose measles; these are based on detecting either IgM or IgG antibodies [3, 4]. Serologic assays that detect IgG antibodies, including hemagglutination inhibition, RIA, plaque reduction neutralization, and microneutralization, have the disadvantage of requiring acute- and convalescent-phase specimens to measure a rise in IgG antibodies. Assays that detect IgM antibodies, such as RIAs and EIAs, often can be used to diagnose measles by testing only a single serum specimen. RIAs are reliable but have the disadvantage of requiring adequate facilities to store, use, and dispose of radioactive material. Most commercially available EIA kits use an indirect format. Although this format is relatively simple to use, its increased risk of false-positive results [5] and lower sensitivity can lead to misclassification of individual cases and outbreaks of measles-

Received 12 April 1996; revised 29 July 1996.

Reprints or correspondence: Dr. Rita Helfand, Respiratory and Enteric Viruses Branch, Centers for Disease Control and Prevention, 1600 Clifton Rd., Mailstop G-17, Atlanta, GA 30333.

The Journal of Infectious Diseases 1997;175:195–9

0022–1899/97/7501–0032$01.00

like illness. For instance, one such assay had an estimated sensitivity and specificity of 86% and 81%, respectively [6].

An antibody-capture EIA configuration for the detection of measles-specific IgM has proven more sensitive and specific (97% and 99%, respectively) and reduces the chance of a false-positive result due to rheumatoid factor [7, 8]. In addition to the assay design, the timing of specimen collection can affect the ability to detect measles infection reliably. In this report, we describe the effect that the timing of specimen collection after onset of rash illness has on the ability to detect measles IgM antibody response by using an antibody-capture EIA.

Materials and Methods

Serum specimens. We selected a subset of specimens from patients with measles infection. This subset was identified from the >6000 clinical specimens tested in the Measles Laboratory, Centers for Disease Control and Prevention, over the last 5 years from measles outbreaks, vaccine trials, studies of rash illness, and isolated cases of presumed measles infection. The patients chosen met the following conditions: availability of 2 serum specimens, at least 1 of the serum specimens was IgM-positive, date of onset of rash was available, and the patient had no history of measles vaccination in the 8 weeks before onset of rash illness.

Capture EIA. The IgM assay was done as described by Hummel et al. [8]. EIA results were expressed as the average difference in measured optical density values between duplicate wells of positive antigen (P) and negative tissue culture control antigen (N) for each serum specimen. The cutoff value was calculated at >3 times the mean P − N value for the antibody-negative control sera. In addition, P/N ratios of ≥3 were required to account for high background signal in occasional specimens. If only one of these conditions was met, the specimen was considered borderline.

Data analysis. We calculated the rates of IgM positivity for the first specimens by time after rash onset. To do this, we limited our analyses to subjects for whom the diagnosis of measles infection could be made by using the second specimen alone. We also plotted the rates of decline of IgM positivity for the second specimens by time after rash onset for all persons and by vaccination status. To do this, we created Kaplan-Meier curves and limited our analyses to subjects whose first specimens were IgM-positive. Borderline results were classified as negative results when we identified events used in calculating the Kaplan-Meier curves. We then compared the Kaplan-Meier curves of vaccinated and unvaccinated persons by using the modified Wilcoxon test [9]. Finally, we compared categorical variables using the Mantel-Haenszel χ^2 test and continuous variables using the stratified Wilcoxon test [10].

Results

Patients and vaccination status. We included paired specimens collected between 1988 and 1995 from 166 persons. The median age was 11 years (range, 0–56). The first blood specimen was collected a median of 3 days (48–72 h) after onset of rash (range, 1–11 days; $n = 166$), and the second blood specimen was collected a median of 23 days after onset of rash (range, 6–47; $n = 160$).

Information about vaccination status was available for 157 persons (95%); of these, 77 (49%) had been vaccinated previously (5 had been vaccinated twice). These previously vaccinated persons had been vaccinated a median of 13 years before onset of illness (range, 15 weeks to 18 years; $n = 76$). The date of vaccination was not available for 1 person, but this person had a confirmed case of measles from an outbreak and presumably had not been vaccinated immediately prior to illness. The previously vaccinated population was older (median age, 15 years; range, 1–26) than the unvaccinated population (median age, 1 year; range, 0–56; $P = .0001$, Wilcoxon rank-sum test).

Overall seropositivity rates. Both first and second serum specimens were IgM-positive for 130 (78%) of 166 persons. Of the first specimens, 143 (86%) were IgM-positive, 5 (3%) were borderline, and the remaining 18 (11%) were IgM-negative. Among the second samples, 153 (92%) were IgM-positive, 2 (1%) were borderline, and 11 (7%) were IgM-negative.

Seropositivity of first specimens over time. Table 1 shows the IgM results of first specimens by day after rash onset for persons whose second samples were IgM-positive. The seropositivity rate was 77% (77/100; 95% confidence interval [CI], 69%–85%) for specimens collected within 3 days (<72 h) after rash onset. Vaccination status did not affect this rate of IgM positivity ($P = .229$, Mantel-Haenszel χ^2, controlling for day after rash onset; $n = 93$). The seropositivity rate was 100% (53/53) for first specimens collected on days 4–11 after rash onset.

Seropositivity of second specimens over time. Figure 1A shows the rate of decline of IgM positivity for second specimens by time after rash onset for 137 of the 143 persons whose first serum specimens were IgM-positive (the date of collection of second specimens was unavailable for 6 persons). The rate of IgM positivity dropped from 100% at 4 days after rash onset to 90% by 4 weeks after rash onset, then declined to 65% during the fifth week after rash onset.

We next determined rates of IgM positivity for second specimens both by week after rash onset and by vaccination status for persons whose first samples were IgM-positive (table 1, figure 1B). We had vaccination information for 130 of the 137 persons reported in the previous paragraph. For the 68 unvaccinated persons, the rate of IgM positivity declined from 100% at 4 days after rash onset to 94% by 4 weeks after rash onset, then declined further to 63% during the fifth week after rash onset. For the 62 previously vaccinated persons, the IgM positivity rate fell to <90% during week 3 (figure 1B). The difference in the rates of decline of IgM positivity for vaccinated compared with unvaccinated persons approached but did not achieve statistical significance ($P = .09$; modified Wilcoxon test). Borderline IgM results were classified as negative IgM results when we identified events used to calculate the Kaplan-Meier curves.

Table 1. IgM results for first specimens by day after rash onset from 153 persons whose second specimens were IgM-positive and for second specimens by week after rash onset and by measles vaccination status from 130 of 137 persons whose first specimens were IgM-positive.

Serum sample	IgM^+	$IgM^{+/-}$	IgM^-	Total	% IgM^+
First sample, all persons					
Day 1	20	3	5	28	71
Day 2	37	1	8	46	80
Day 3	20	1	5	26	77
Day 4	24	0	0	24	100
Day 5	20	0	0	20	100
Day 6	2	0	0	2	100
Day 7	5	0	0	5	100
Day 8	1	0	0	1	100
Day 11	1	0	0	1	100
Total	130	5	18	153	85
Second sample, vaccinated					
Weeks 1–2	19*	2†	0	21	90
Week 3	19	0	1	20	95
Week 4	14	0	2	16	88
Week 5	5	0	0	5	100
>5 weeks	0	0	0	0	NA
Total	57	2	3	62	92
Second sample, unvaccinated					
Weeks 1–2	4‡	0	0	4	100
Week 3	5	0	0	5	100
Week 4	29	0	2	31	94
Week 5	16	0	6	22	73
>5 weeks	6	0	0	6	100
Total	60	0	8	68	88

NOTE. Measles vaccination status was unknown for 7 persons. Day 1, ≤24 h after onset of rash; NA, not applicable.

* All samples were collected during week 2.

† Collected on days 12 and 13 after onset of rash.

‡ 1 sample collected during week 1.

Finally, we tried to assess if time since vaccination or type of vaccine received was associated with an early decline of IgM positivity (within 3 weeks after rash onset). To do this, we looked at the subset of previously vaccinated persons whose first specimens were IgM-positive and whose second specimens were collected within 3 weeks after rash onset. In this subset, 3 of 41 persons had IgM results that were borderline (2 in week 2) or negative (1 in week 3). These 3 persons had all received measles vaccine in or after 1979 (an improved stabilizer for measles vaccines was introduced in 1979) and had been vaccinated 1 year, 12 years, and 4 months previously. Persons who had been vaccinated before 1979 (n = 29) were more likely to be IgM-positive in the 3 weeks after rash onset than persons vaccinated in or after 1979 (n = 12; P = .008 by Mantel-Haenszel χ^2, controlling for week of specimen collection). We also found that persons whose second specimens were borderline or negative within 3 weeks after rash onset were more likely to be vaccinated more recently than persons who were IgM-positive (P = .056, stratified Wilcoxon test, controlling for week of specimen collection).

These two variables are not independent; as one would suspect, persons who were vaccinated in or after 1979 were significantly more likely to have been vaccinated more recently than persons who were vaccinated before 1979 (P = .001, stratified Wilcoxon test, controlling for week of specimen collection). We were unable to determine which of these factors was the predominant one.

Discussion

On the basis of this study, a single serum specimen can be used to diagnose most measles cases with an antibody-capture IgM EIA if the specimen is collected between 72 h and 4 weeks after rash onset. In this study, we limited our analyses to specimen pairs with at least 1 IgM-positive sample. This definition excludes some cases of measles that did not have detectable IgM antibodies in either the first or second samples but might have been diagnosed on the basis of a 4-fold rise in IgG titers. Because we do not routinely diagnose measles cases on the basis of a 4-fold rise in IgG titers, we do not have a cohort of patients diagnosed in this manner to compare with results obtained when using the IgM assay. However, data from Erdman and colleagues [7, 11] suggest that the IgM capture EIA is sufficiently sensitive, even in previously vaccinated persons (97% [7] and 96% [11]), and that missed cases would be rare.

In the present study, all first specimens collected between 72 h and 11 days after rash onset were IgM-positive. Within the first 72 h after onset of rash illness, however, only 77% of specimens were IgM-positive. These results are consistent with those of Lievens and Brunell [12], who found that 3 of the 4 IgM-negative acute-phase specimens from 25 measles cases (all had second sera that were IgM-positive) were collected within the first 72 h after onset of rash illness. Our data suggest that serum collected during the first 3 days after rash onset will still allow detection of most cases of measles infection. This observation is encouraging because in some instances, the only opportunity that medical and public health personnel have to obtain clinical specimens from persons with suspected measles may be when the persons present for medical care, which usually occurs within the first 72 h after onset of rash illness. However, if EIA is negative within the first 72 h after rash onset, investigators should consider collecting a second sample later in the course of illness if it is important for infection management or outbreak control.

The results of our review also predicted a seropositivity rate of ≥90% from all measles cases with specimens that were collected within 4 weeks of rash onset (≥94% from unvaccinated persons), with the seropositivity rate declining for persons whose specimens were collected >4 weeks after rash onset (figure 1). These findings are consistent with those of

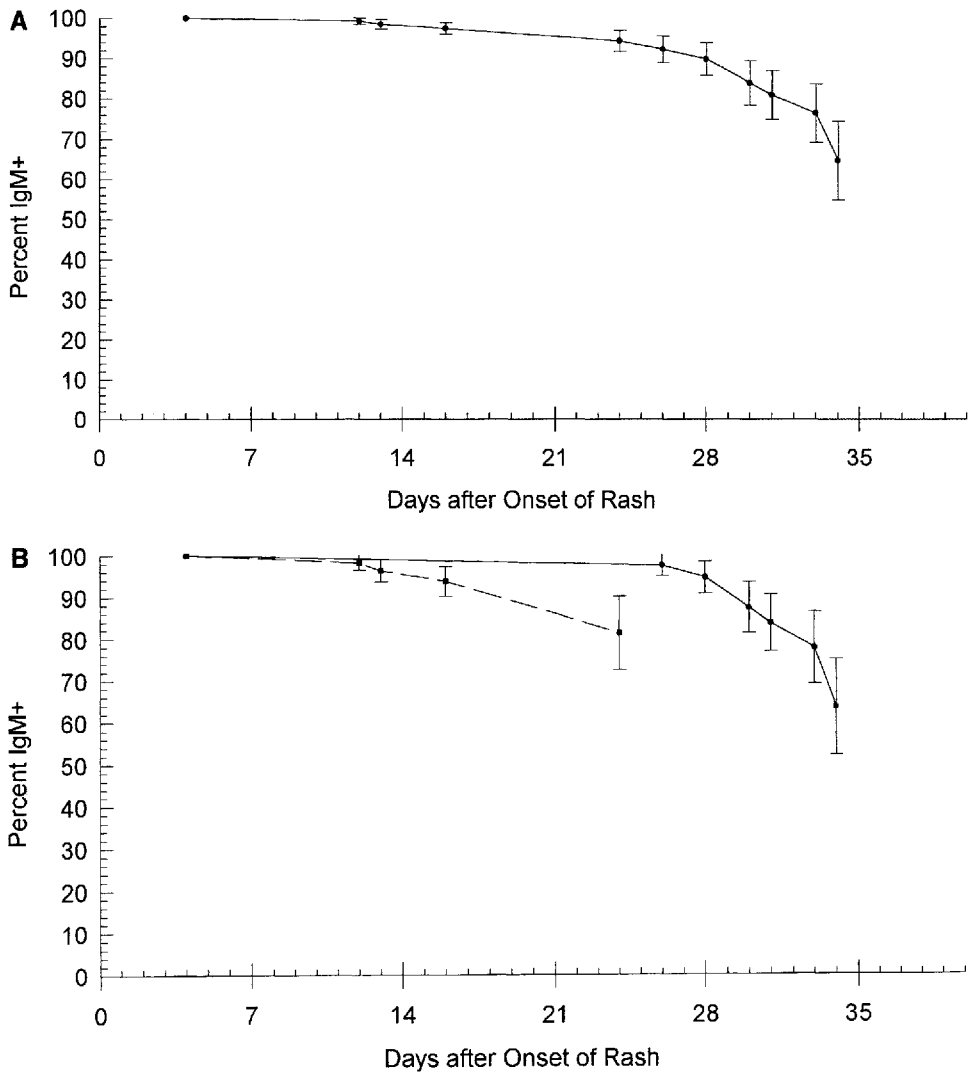

Figure 1. Kaplan-Meier curves showing decline of IgM positivity rates for 2nd specimens over time (**A**) for all 137 persons whose first specimens were IgM-positive and by vaccination status (**B**), including 68 unvaccinated (solid line) and 62 vaccinated (dashed line) persons whose first specimens were IgM-positive Borderline results were classified as IgM-negative when identifying events used to calculate curves.

Vuorimaa et al. [13], who demonstrated, using an RIA, that IgM antibodies became undetectable in 29 of 30 persons with measles by 1–3 months after rash onset. Similarly, Arista et al. [14] demonstrated, using an IFA test, an indirect EIA, and a capture EIA, that the first IgM-negative specimens were collected >30 days after the onset of rash [14].

We found that the rate of IgM positivity for 62 previously vaccinated persons fell below 90% during the third week after rash onset (figure 1B). However, the significance of this finding is unclear. On one hand, we may have overestimated the rate of decay of IgM positivity for these previously vaccinated persons. We classified the 2 borderline specimens collected during week 2 as negative when identifying the events used for calculating the Kaplan-Meier curves. If these borderline specimens were considered IgM-positive, there would be no apparent difference in rates of IgM positivity over time between the vaccinated and unvaccinated groups (table 1); however, the number of IgM-negative results would be too small ($n = 3$) to generate a Kaplan-Meier curve.

On the other hand, we may have underestimated the rate of decay of IgM positivity for some previously vaccinated persons. It is possible that the type of measles vaccine received, time since vaccination, or number of doses of vaccine could affect the duration of IgM positivity. For example, many vaccines produced starting in 1979 are more heat-stable due to an improved stabilizer, which may result in a more immunogenic product [15]. Thus, persons vaccinated before 1979 may be more likely to have IgM responses similar to those of unvaccinated persons. It is also feasible that time since vaccination may affect the duration of the IgM response to measles infection, possibly due to waning immunity, with recently vaccinated persons having a shorter duration of IgM positivity.

Our data do not allow us to differentiate between the effects that time since vaccination and type of vaccine received might have on the duration of IgM positivity. Similarly, we could not evaluate the potential effect that receiving a second dose of vaccine may have on the duration of the IgM response (only 5 persons in our study had received 2 doses of measles vaccine).

Further study will be needed to understand better the rate of decline of IgM positivity among previously vaccinated persons.

In conclusion, our data suggest that, in general, a single serum specimen can be used to diagnose most measles cases if collected between 72 h and 4 weeks after rash onset by using an IgM capture EIA. In previously vaccinated persons, there may be a small increased risk of not detecting an IgM response to measles when specimens are collected >2 weeks after rash onset.

Acknowledgments

We thank Howard Gary, Jr., for statistical support; James P. Alexander, Jr., John O'Connor, and Steve Redd for editorial suggestions; Alberto Cohen-Abbo for his role in conducting one of the studies that provided specimens; Ciro de Quadros, Jean Marc Olivé, and colleagues at the Pan American Health Organization for facilitating one of the studies that provided data for this project; and the following persons and institutions for providing us with specimens and epidemiologic information: Chuck Jennings, Jill McCready, Anthony Polloi, state health departments throughout the United States, participating ministries of health abroad, and the following members of the Venezuelan Measles Rash Illness Working Group: Nelly Barboza, Ruth Detraglia, Airton Fischmann, Osvaldo Godoy, Laura Hernandez, Tulia Hernandez, Maria Luzardo, Gustavo Martinez, Evelyn Nino, Belquis Pinto, and Jesus Querales.

References

1. World Health Organization. Expanded programme on immunization information system. Geneva: WHO, **1995**; report no. 95.2:WHO/EPI/CEIS.
2. de Quadros CA, Olivé JM, Harsh BS, et al. Measles elimination in the Americas. Evolving strategies. JAMA **1996**;275:224–9.
3. Bellini WJ, Rota PA. Measles (rubeola) virus. In: Lennette DA, ed. Diagnostic procedures for viral, rickettsial, and chlamydial infections. 7th ed. Washington, DC: American Public Health Association, **1996**:447–54.
4. Cutts FT, Brown DWG. The contribution of field tests to measles surveillance and control: a review of available methods. Rev Med Virol **1995**; 5:35–40.
5. Jenkerson SA, Beller M, Middaugh JP, Erdman DD. False positive rubeola IgM tests [letter]. N Engl J Med **1995**;332:1103–4.
6. Mayo DR, Brennan T, Cormier DP, Hadler J, Lamb P. Evaluation of a commercial measles virus immunoglobulin M enzyme immunoassay. J Clin Microbiol **1991**;29:2865–7.
7. Erdman D, Anderson L, Adams D, Stewart J, Markowitz L, Bellini W. Evaluation of monoclonal antibody–based capture enzyme immunoassays for detection of specific antibodies to measles virus. J Clin Microbiol **1991**;29:1466–71.
8. Hummel K, Erdman D, Heath J, Bellini W. Baculovirus expression of the nucleoprotein gene of measles virus and utility of the recombinant protein in diagnostic enzyme immunoassays. J Clin Microbiol **1992**;30: 2874–80.
9. Gehan EA. A generalized Wilcoxon test for comparing arbitrarily singly-censored samples. Biometrika **1965**;52:203–33.
10. Lehmann LE. Nonparametrics: statistical methods based on ranks. San Francisco: Holden-Day, **1975**.
11. Erdman DD, Heath JL, Watson JC, Markowitz LE, Bellini WJ. Immunoglobulin M antibody following primary and secondary vaccination and natural virus infection. J Med Virol **1993**;41:44–8.
12. Lievens AW, Brunell PA. Specific immunoglobulin M enzyme-linked immunosorbent assay for confirming the diagnosis of measles. J Clin Microbiol **1986**;24:391–4.
13. Vuorimaa TO, Arstila PP, Ziola BR, Salmi AA, Hanninen PT, Halonen PE. Solid-phase radioimmunoassay determination of virus-specific IgM antibody levels in a follow-up of patients with naturally acquired measles infections. J Med Virol **1978**;2:271–8.
14. Arista S, Ferraro D, Cascio A, Vizzi E, di Stefano R. Detection of IgM antibodies specific for measles virus by capture and indirect enzyme immunoassays. Res Virol **1995**;146:225–32.
15. Markowitz LE, Katz SL. Measles vaccine. In: Plotkin SA, Mortimer EA, eds. Vaccines. 2nd ed. Philadelphia: WB Saunders, **1994**:229–76.