Optimal Timing of Specimen Collection after Rash Onset for Diagnosis of Measles IgM Antibody

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Summary

Antibody detection is the most versatile and commonly used method for measles diagnosis. Detection of specific IgM antibodies in a single serum specimen collected within the appropriate time after rash onset can provide a good presumptive diagnosis of current or recent measles infection and is the test of choice for rapid diagnosis of measles cases. So, optimal timing for collection of a single serum specimen to diagnose measles by IgM capture Enzyme Immunoassay (EIA) was evaluated. 399-paired sera were tested for measles IgM antibody. 149-paired sera were measles IgM negative. Two hundred fifty paired sera had at least one IgM positive. 223-paired sera were positive in both first and second samples. 19-paired sample were negative in first and positive in second sample and 8-paired sera were positive in the first and negative in the second samples. 85% and 100% of first specimens within 7 and 7-21 and 94% of all second samples were IgM positive at 28th days after rash onset, respectively. Analysis of data indicates that a single serum specimen collected between 7 to 27 days after rash onset can be used to diagnose most cases of measles with an IgM capture EIA.

***Key words*:** Measles, IgM Capture EIA, Optimal time

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Introduction

Measles is a highly contagious disease and was described by Iranian physician Razi in the 10th century and credited with distinguishing smallpox from measles (Mirchamsy 1979). Despite the development of a successful live attenuated vaccine, measles remains a major cause of mortality in children, particularly in developing countries. Non-specific nature of the prodromal signs and the existence of mild cases make clinical signs unreliable as the sole diagnostic criteria of measles disease. Moreover, many measles cases in previously vaccinated or immunosuppressed individuals do not meet the clinical case definition. In addition, as disease prevalence falls, many medical practitioners will be inexperienced in recognizing measles from other clinically similar diseases. Therefore, confirmation of measles virus infection must be made using laboratory-based methods (Cutts *et al* 1995). A standard method for diagnosing measles is to detect measles-specific immunoglobulin M (IgM) in the serum of infected person (Tipples *et al* 2003, Bouche *et al* 1998, Hummel 1992, Helfand *et al* 1999). There are two formats for IgM tests (Arista *et al* 1995). The first and most widely available is the indirect format. IgM tests based on the indirect format require a specific step to remove IgG antibodies (Ratnam *et al* 2000). Problems with removal of IgG antibodies can lead to false positive tests or, less commonly, false negative results. The second format, IgM capture, does not require the removal of IgG antibodies. This is the preferred reference test for measles. Detection of specific IgM antibodies in a single specimen collected in appropriate time after rash onset can provide a good presumptive diagnosis of current or recent measles infection and confirmation the disease in the early stages of an outbreak will be very helpful in preventing of the expansion of outbreak (Helfand, *et al* 1997).

Materials and Methods

Serum specimens. The sera were selected from a subset of sera specimens from the suspected measles cases (maculo-papular rash and fever of equal or more than

38.5°C with cough, coryza or conjunctivitis) that were collected as part of the measles surveillance program in Iran 2003. The sera were tested in the reference measles laboratory, at the School of Public Health, Tehran University of Medical Sciences. All the sera accompanied by questionnaire forms. The chosen sera should meet the following criteria: "*Availability of acute and convalescent sera samples, at least one of the specimens was IgM positive and date of rash onset was available.*" Two hundred and fifty paired sera were selected following to the proposed criteria.

IgM capture EIA. IgM capture EIA was used for detection of measles specific IgM. The borderline results were classified as negative. The serum samples were tested for measles-specific IgM antibodies by using a monoclonal-based antibody-capture enzyme immunoassay (EIA). Microtiter plates were coated with goat anti-human IgM antibodies diluted (1:800) in phosphate-buffered saline (PBS), incubated for 1 h at 37°C, and washed. Then, serum diluted 1:200 in PBS with 0.5% gelatin and 0.15% Tween 20 (PBS-GT) was added to four consecutive wells. The plates were then incubated for 1 h at 37°C and washed. Baculovirus-measles virus nucleoprotein or sf 9-uninfected cell control lysate diluted (1:2500) in PBS-GT with 4% normal goat serum and 0.3% sodium deoxycholate was added to duplicate wells. The plates were then incubated for 2 h at 37°C and washed. Biotinylated monoclonal antibody (83VIIKK2) in PBS-GT was added to the plates, and the plates were incubated for 1 h at 37°C and washed. The plates were then incubated at 37°C with streptavidin-peroxidase in PBS-GT for 20 min and washed again. Tetramethylbenzidine substrate was incubated for 15 min at 37°C, and the reaction was stopped by acidification. Finally, optical densities for antigen-positive and antigen-negative wells were determined photometrically (Erdman *et al* 1991 and 1993).

IgM-EIA results were expressed as the average of differences in measured optical density values between duplicate wells of positive antigen (P) and negative tissue culture control antigen (N). Specifically, a sample was considered IgM positive if $P - N$ was ≥ 0.10 and P/N was ≥ 3.0. A sample was considered IgM borderline if

either (i) P − N was ≥ 0.09 but <0.10 and P/N was ≥3.0 or (ii) P − N was ≥ 0.10 and P/N was ≥2.0 but <3.0. (Positive/ Negative serum control and all the materials were provided by measles Laboratory in Center for Disease Control, Atlanta, Georgia).

Results

Two hundred and fifty paired sera were collected. 223-paired sera were positive in both first and second samples. 19-paired sample were negative in first and positive in second sample and 8 paired sera were positive in the first and negative in the second samples. In order to achieve the optimal time for the specimens collection at the beginning after rash onset, the first specimen among patients whose their second specimens were measles IgM positive was classified according to days of sampling (Table 1).

Table 1. *Distribution of presence or absence of measles IgM antibody in the first serum specimens after the days of rash onset among patients whom their second specimens were IgM antibody positive.*

Days after rash onset	IgM positive		IgM Negative		Total
	No	%	No	%	
1	9	69.3	4	30.7	13
2	31	88.5	4	11.5	35
3	31	93.9	2	6.1	33
4	41	93.3	2	4.7	43
5	22	95.6	1	4.4	23
6	25	89.3	3	10.7	28
7	17	89.4	2	10.6	19
8-21	47	97.9	1	2.1	48
Total	223	92.1	19	7.9	242

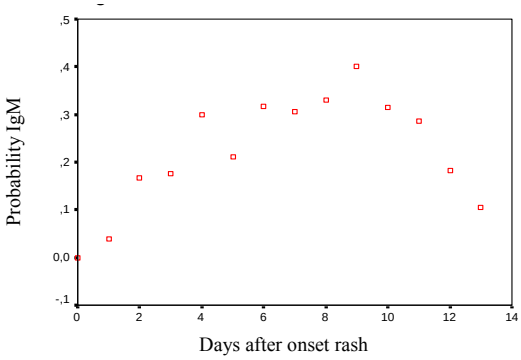
In order to achieve the optimal time period and identifying the end point of this time period for the specimens collection the second specimens among patients whose their first specimens were measles IgM positive was classified according to days of sampling (Table 2). The hazard function curve, in Figure 1, showing trend of measles IgM antibody negativity rate for first specimens over time for persons whose second specimens were IgM positive.

Table 2. *Distribution of presence or absence of measles IgM antibody in the second serum specimens after the days of rash onset among patients whom their first specimens were IgM antibody positive.*

Days after rash onset	IgM positive		IgM Negative		Total
	No	%	No	%	
10-11	12	100	0	0	12
12-13	20	95.2	1	4.8	21
14-15	22	91.7	2	8.3	24
16-17	26	100	0	0	26
18-19	24	96	1	4	25
20-21	25	100	0	0	25
22-23	20	95.2	1	4.8	21
24-25	19	95	1	5	20
26-27	20	95.2	1	4.8	21
28-29	15	93.7	1	6.3	16
30-31	14	100	0	0	14
32-33	6	100	0	0	6
Total	223	96.5	8	3.5	231

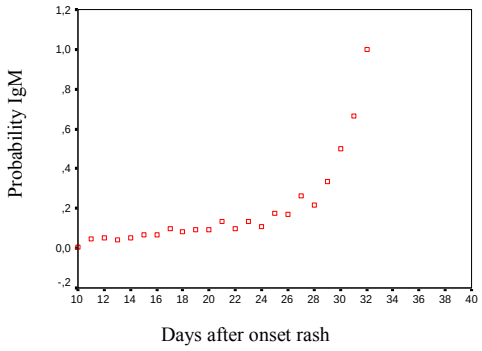
It's shows that the probability of measles IgM antibody negativity increases (i.e. probability of measles IgM antibody positivity decreases) during the first week after onset of rash and started to decrease after the first weak.

Figure 1: *Hazard function curve showing trend of measles IgM antibody negativity rate for first specimens over time for persons whose second specimens were IgM positive.*



The hazard function curve, in Figure 2, showing trend of measles IgM antibody (Ab) negativity rate for second specimens over time for persons whose first specimens were measles IgM Ab positive. It showed that the probability of measles IgM Ab negativity from the days 10 to 27 after onset of rash was less than 0.2 and sharply increased.

Figure 2: *Hazard function curve showing trend of Measles IgM Ab negativity rate for second specimens over time for persons whose first specimens were IgM positive.*



Discussion

Currently, most countries with a measles elimination goal that have conducted the initial catch-up campaign are implementing measles case-based surveillance with laboratory confirmation (Weekly Epidemiological Record, 2002). The aim of surveillance system is to detect all cases and to facilitate a rapid response to prevent a potential outbreak. Confirmation of all cases of suspected measles by a measles virus specific IgM assay is recommended, especially where the incidence of measles is low (Riddell *et al* 2002). Detection of specific IgM antibodies by capture EIA proved to be a rapid method with sensitivity and specificity (97% and 99% respectively) for confirming cases of clinical measles (Erdman *et al* 1991, Hummel *et al* 1992).

In the present study, the optimal timing for collection of a single serum specimen to diagnose measles by capture EIA was evaluated. Two hundred fifty out of 399 patients suspected measles cases, were positive at least in one of their paired sera. For achieving the earliest and best time for serum specimen collection, measles IgM antibody in first specimens according to days sampling after the rash onset among patients whom their second specimens were positive was analyzed. For evaluating, the period of persisting and declining the antibody measles IgM antibody positive in second specimens among patients whom first specimens were positive

according to days sampling after the onset of rash was analyzed. These finding shows that after the 7th day of rash onset most of measles cases will be IgM positive (Figure 1) and antibody will persist for at least 27 days (Figure 2). So the optimal time for collection of serum samples is between 7 to 27 days after onset of rash (Armitage *et al* 2002).

The results of studies performed in a variety of countries shows almost the similar results. In a study, using indirect commercial kits to detect measles IgM antibody, in 93 paired sera, showed that 56.3% and 98.4% of serum specimen were IgM positive during the first 5 days and after day five of rash onset, respectively (Mayo *et al* 1991). In another study which was carried out on 170 paired serum for timing of serum specimen collection in determining the measles IgM by commercial kits in the first, 7th day and between 7 to15 days after rash onset 40%, 90% and > 90% and after 15 days most of the cases were measles IgM positive, respectively (Ozanne *et al* 1992). Similarly, Helfand *et al*, using IgM capture EIA showed that 77% developed IgM within 72 hours and 100% at four to 11 days after rash onset and more than 90% of persons remained IgM positive for 28 days after measles natural infection (Helfand *et al* 1997). The time of appearance of protein-specific antibodies is critical for the sensitivity of the assay after the rash onset. Nucleoprotein specific antibodies may appear somewhat earlier during measles infection and are thought to be the most abundant antibodies early after the onset of rash (Griffin 1991).

In conclusion, the optimal time for collection of sera is between 7 days and 27 days after rash onset. However, if the specimens are to be taken within 7 days, the positive result is reliable but the negative result might be false-negative, therefore, the serum should be obtained for repeat testing 7-27 days after rash onset or even more. These finding may also help to guide the interpretation of IgM results from persons with suspected measles cases.

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