

The effect of timing of sample collection on the detection of measles-specific IgM in serum and oral fluid samples after primary measles vaccination

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SUMMARY

This study compares the timing of the rise and decline of measles-specific IgM in serum samples and in oral fluid samples. Two hundred and eighty 9-month-old infants presenting for routine measles vaccination in Addis Ababa, Ethiopia, were enrolled. Paired serum and oral fluid samples were collected before and 1, 2, 3 or 4 weeks after measles vaccination. Samples were tested by using a modified antibody-capture enzyme immunoassay. For the 321 IgM-negative pre- and post-vaccination serum samples, 317 (99%) of their corresponding oral fluid samples were IgM-negative. Among the 130 IgM-positive serum samples, 75% of their paired oral fluid samples were IgM-positive, with the percentage rising to 87% after oral fluid samples collected ≥ 3.5 weeks after vaccination were excluded. Among the post-vaccination serum samples, the percent IgM-positive peaked in week 3 and declined to 79% in week 4. For post-vaccination oral fluid samples, the percent IgM-positive peaked in weeks 2 and 3, and then declined to 43% in week 4. This modified antibody-capture enzyme immunoassay appears to detect vaccine-induced measles-specific IgM in the first 3 weeks after vaccination.

INTRODUCTION

Many regions of the world, such as the Americas and Eastern Mediterranean Region, have announced regional measles elimination goals [1] (44th Session, Regional Committee for the Eastern Mediterranean Region, World Health Organization, October 1997). As vaccination coverage improves as a result of these efforts, the number of wild-type measles infections decreases and medical personnel have less experience with diagnosing measles clinically. Therefore, health care providers are relying increasingly on laboratory

methods, such as the detection of measles-specific IgM, to diagnose measles. Furthermore, surveillance for measles will require laboratory confirmation of measles cases. Many physicians and laboratory scientists have expressed an interest in a non-invasive diagnostic test for measles to reduce the difficulties in obtaining blood from young children and to reduce the risks associated with phlebotomy in areas with high rates of infection with the human immunodeficiency virus.

Preliminary studies have demonstrated that oral fluid, a combination of saliva and gingival crevicular fluid, may be an alternative to blood for detecting measles-specific IgM [2–6]. Oral fluid samples are already being used to diagnose measles infection by

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the PHLS Communicable Disease Surveillance Centre and the Virus Reference Division at the Central Public Health Laboratory in England [7].

We reported previously the detection of measles-specific IgM in serum and oral fluid by using an antibody-capture enzyme immunoassay (EIA) [6]. The concordance between serum and oral fluid for the presence or absence of measles-specific IgM before and 2 weeks after measles vaccination was high: 91% of persons with IgM-positive serum samples had IgM-positive oral fluid samples, and 95% of persons with IgM-negative serum samples had IgM-negative oral fluid samples. However, approximately 10% of oral fluid samples had to be discarded because of high background in the negative (antigen only) wells. The optical densities of many of the discordant samples were near to the positive cut-off values. This finding suggests that for some persons the IgM levels may not have reached their peak in oral fluid or serum at 2 weeks after vaccination. Therefore, concordance between serum and oral fluid may be increased by using samples collected earlier or later than 2 weeks. This study evaluated the timing of the rise and decline of measles-specific IgM in both oral fluid and serum samples in order to determine the timing of maximal concordance between the two types of samples. It also tested a modified IgM EIA that eliminates the problem of high background.

METHODS

Study group

We enrolled 280 9-month-old infants presenting for routine measles vaccination to Tekle Haimanot Health Centre, Addis Ababa, between August 1996 and January 1997. After obtaining informed consent, we collected serum and oral fluid samples before and either 1, 2, 3 or 4 weeks after routine measles vaccination (Schwartz vaccine). Infants were enrolled into sequential weeks according to when they presented for measles vaccination (e.g., subjects 1–4 were enrolled into weeks 1–4, respectively; subjects 5–8 were enrolled into weeks 1–4, etc). We also collected information about age, sex, prior immunizations, history of measles infections in the household in the last year, and date of specimen collection. We attempted to enroll 70 infants per week. We chose this sample size to provide a 95% probability of obtaining an estimate of the proportion IgM positive that was between 0.85 and 0.95 if the true proportion was 0.90.

Specimen collection

We collected 1 cc of blood by heelstick, using universal precautions. Samples were centrifuged, and sera were frozen at -70°C and shipped to the Centers for Disease Control and Prevention (CDC) on dry ice. Oral fluid samples were collected using a commercially available device that consists of a hypertonic saline-saturated cotton-fibre pad on a plastic stick (Orasure device, Beaverton OR). The specimens were collected by rubbing the device between the cheek and gum several times and then leaving it in place for 2 min. The pads were then placed in an antimicrobial preservative solution for transport and storage. The pad and preservative were centrifuged, and the recovered fluid specimens were frozen at -70°C and shipped to CDC on dry ice.

Specimen testing and interpretation

Sera were tested for measles-specific IgM by a previously described monoclonal antibody capture-based EIA and for measles-specific IgG by an indirect EIA [8]. EIA results for both assays 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).

The purpose of this study was to compare the time course of IgM in serum and oral fluid samples rather than to determine rates of IgM positivity. To optimize this comparison, we calculated study-specific cut-off values by using the pre-vaccination serum and oral fluid samples rather than using the standard serum cut-off [9]. Therefore, the rates of seropositivity based on these values will not be comparable to those determined with the standard cut-offs. After excluding three obvious outliers for the IgM assay and eight outliers for the IgG assay (out of 280 sample), the positive IgM and IgG serum cut-offs (0.075 and 0.087, respectively) were calculated as the mean P-N plus four standard deviations. The outliers were excluded because they were presumably seropositive with P-N values ≥ 1.7 times the value for the other samples.

Oral fluid samples were tested for measles-specific IgM by modifying the serum monoclonal antibody-capture-based EIA. Briefly, goat anti-human IgM antibodies diluted in phosphate-buffered saline (PBS) were coated onto microtitre plates overnight at 4°C . After the plates were washed, undiluted oral fluid samples were added to four consecutive wells and the plates were incubated for 1 h at 37°C . Plates were

Table 1. Comparison of IgM positivity against measles in pre- and post-vaccination serum and oral fluid samples in children

Serum	Oral fluid*, n (%)		
	IgM ⁻	IgM ⁺	Total
Pre-vaccination			
IgM ⁻ no. (%)	250 (99)	2 (1)	252
IgM ⁺ no. (%)	1 (25)	3 (75)	4
Total	251	5	256
All post-vaccination			
IgM ⁻ no. (%)	67 (97)	2 (3)	69
IgM ⁺ no. (%)	31 (25)	95 (75)	126
Total	98	97	195
First 3 weeks post-vaccination			
IgM ⁻ no. (%)	54 (96)	2 (4)	56
IgM ⁺ no. (%)	11 (13)	74 (87)	85
Total	65	76	141

* Excludes oral samples from run 1 that were not available for retesting (see methods for details).

washed and baculovirus-expressed measles virus nucleoprotein or Sf9 cell control lysate diluted in PBS with 0.1% Tween20 (PBS/T) and 3% skim milk was added to duplicate wells. Plates were then incubated for 2 h at 37 °C. Next, biotinylated nucleoprotein-specific monoclonal antibody (83VIIKK2) diluted in PBS/T/milk with 5% normal goat serum and 1.5% heat-inactivated normal monkey serum was added to washed plates and incubated for 1 h at 37 °C. After another wash, streptavidin-peroxidase conjugate in PBS/T was added to wells and incubated for 20 min at 37 °C. After a final wash, tetramethylbenzidine substrate solution was added to wells and incubated for 15 min at room temperature. Finally, the chromogenic reaction was stopped by acidification, and the optical density was determined photometrically. Using the pre-vaccination oral fluid samples, we calculated a positive P-N cut-off (the mean P-N plus four standard deviations) as 0.061. We excluded three obvious outliers that were presumably IgM positive with P-N values > twice the other 277 samples.

The pre- and post-vaccination oral fluid sample pairs were randomly assigned to testing runs and to plates within a run. A total of three runs was required to test all samples. We included on each plate two individual negative controls from healthy adults, a standard pooled negative control sample made from pooled oral fluid from healthy adults, and two mock positive control samples made by adding IgM-positive sera to the pooled negative control oral fluid sample.

The first run was retested because the pooled positive and negative control samples from this run gave much higher P-N values than those from the other two runs. No oral fluid was available for retesting 22 of the 95 (23%) pre-vaccination and 28 of the 79 (35%) post-vaccination samples from this run. The 50 oral fluid samples that were not retested will be reported separately, using a calculated positive P-N cut-off of 0.283, based on pre-vaccination oral fluid tested in the original first run.

RESULTS

Demographic information

The median age of 279 of the 280 enrolled infants was 9.5 months (range 7.3–13.0); 141 infants (50%) were male. Seven infants had histories of suspected natural measles infection at 4–9 months of age, but all were IgM and IgG negative before measles vaccination.

Laboratory samples

We collected pre-vaccination serum and oral fluid sample pairs from 278 of 280 infants and post-vaccination serum and oral fluid sample pairs from 223 of 280 (80%) infants, for a total of 501 specimen pairs. As mentioned earlier, one of the three runs of oral fluid samples required retesting, and insufficient oral fluid samples were available for retesting for 22 pre-vaccination and 28 post-vaccination samples. The collection times of the remaining 195 post-vaccination specimen pairs were distributed fairly evenly between weeks 1 and 4 (43–49 samples/week; see table 2).

We included the 256 pre-vaccination and 195 post-vaccination paired samples when comparing the ability of the EIA to detect measles-specific IgM in serum and oral fluid. Table 1 shows the comparative detection of IgM in serum and oral fluid both before and after vaccination. Overall, of the 321 IgM-negative pre- or post-vaccination serum samples, 317 (99%) of their corresponding oral fluid samples were IgM-negative. Similarly, for the 130 pre- or post-vaccination samples that were IgM-positive, 98 (75%) of their paired oral fluid samples were IgM-positive. The concordance of the oral fluid samples with the IgM-positive serum samples increased to 87% (77/89) after 54 post-vaccination samples collected 3.5 weeks or longer after vaccination were excluded. All of the 21 discordant samples collected after week 3 were

Table 2. Percentage of post-vaccination serum and oral fluid samples that were IgM-positive against measles, by week after vaccination

Week after vaccination	Serum		Oral fluid*		P-Value† (No. tested)
	% IgM+ (No. tested)	95% CI	% IgM+ (No. tested)	95% CI	
1	3.8 (53)	±5.3	4.4 (46)	±5.7	Undefined§ (46)
2	83.7 (49)	±10.3	78.1 (41)	±12.7	0.180 (40)
3	94.4 (54)	±6.3	80.4 (46)	±11.6	0.008 (46)
4	79.2 (48)	±11.5	43.5 (46)	±14.3	0.001 (46)
> 4	50.0 (8)		12.5 (8)		0.083 (8)
Total	64.2 (212)	±6.5	49.2 (187)	±7.2	0.001 (186)

Includes only persons whose pre-vaccination serum samples were IgM- and IgG-negative.

* Excludes oral fluid samples from run 1 that were not available for retesting (see methods for details).

† P-value by McNemar's test. The test is based upon paired samples only (the percentages IgM+ for this subset are essentially unchanged).

§ The McNemar's test was undefined because there were no discordant pairs.

IgM-positive in serum and IgM-negative in oral fluid, consistent with the finding that measles-specific IgM has a shorter duration in oral fluid than in serum samples.

We included only samples from infants who were both IgM and IgG negative before vaccination when evaluating the timing of IgM and IgG positivity by week after vaccination. Overall, sera from 265 (95%) of 278 infants were both IgM and IgG negative before vaccination. From these 265 infants, 212 post-vaccination serum sample results and 187 post-vaccination oral fluid sample results were available for analysis of the timing of the rise of IgM.

The pattern of IgM positivity over time after vaccination is different for serum than for oral fluid samples (Table 2). In serum, IgM positivity rose from 4% in week 1 to 84% in week 2, peaked at 94% in week 3 and declined to 79% by week 4. The median P-N optical density also peaked in week 3, as demonstrated previously (9). In contrast, the oral fluid IgM-positivity similarly rose to 78% in week 2, remained stable at 80% through week 3, and then declined to 43% in week 4. The median P-N optical density for oral fluid was slightly higher in week 2 than week 3 (data not shown), although this difference is not statistically significant ($P = 0.18$, stratified Wilcoxon rank sum test, controlling for run).

Insufficient volume prevented our retesting 22 pre-vaccination and 28 post-vaccination oral fluid samples from the original first run. Their results were

as follows. Of the 34 pre- and post-vaccination serum samples that were IgM-negative, 31 (91%) of their paired oral fluid samples were IgM-negative. Of the 16 post-vaccination serum samples that were IgM-positive, 14 (88%) of their paired oral fluid samples were IgM-positive.

DISCUSSION

In this study, we found that 99% of sample pairs that were IgM-negative in serum were also IgM-negative in oral fluid. Only 75% of sample pairs that were IgM-positive in serum were also IgM-positive in oral fluid. However, this low concordance of IgM-positive samples was driven in part by the rapid decline in IgM-positivity in oral fluid samples collected 3.5 weeks or longer after vaccination. When paired samples collected 3.5 weeks or longer after measles vaccination were excluded, 87% of sample pairs that were IgM-positive in serum were also IgM-positive in oral fluid.

The temporal patterns of IgM positivity in serum and oral fluid were different. In general, IgM positivity in oral fluid had a lower and broader peak than that in serum. The serum IgM rose through weeks 2 and 3, then fell in week 4 to levels similar to week 2. In contrast, IgM in oral fluid reached a level similar to that in serum in week 2, remained at this level through week 3, and then fell precipitously in week 4. One possible reason for detecting an earlier and broader

maximum rate of IgM positivity in oral fluid is that this assay may also detect some salivary IgA, which peaks earlier than IgM. Unlike serum IgA, salivary IgA is present in dimers connected by the same J chain that is present in pentameric IgM (10). The anti-human IgM antibodies used in the first step of the antibody-capture EIA are polyclonal (ICN, catalogue no. 55073), so some of the antibodies could be directed against this J chain.

As mentioned, testing of oral fluid identified fewer IgM-positive persons than did testing of serum. It is possible that some sensitivity for detecting measles-specific IgM in oral fluid was lost for some samples because of the additional freezing and thawing of the specimens, a process that can degrade IgM; the specimens used in this study were originally tested with the technique used in 1995 (6), and were then refrozen before being retested with the modified technique. Another possibility for the reduced rate of IgM-positivity of oral fluid samples is that some of the samples had inadequate volumes of gingival crevicular fluid. Other investigators measure total IgG by using radial immunodiffusion in order to verify the adequacy of sample collection [3]. However, the assay does not work with components in the Orasure device. We attempted without success to identify a method to verify the adequacy of sample collection (such as measurement of total IgM or total IgG by a capture EIA, measurement of total protein, and setting a minimum volume of oral fluid collection).

The modified IgM assay appears to detect vaccine-induced measles-specific IgM in oral fluid with 87% relative sensitivity compared with serum in the first 3.5 weeks after vaccination. This assay still needs to be tested on oral fluid samples that are IgM positive due to natural measles infection. When conducting these studies, it will be important to take into consideration the possibility that the timing of the rise and fall of IgM may be different for serum and oral fluid.

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