Comparative Detection of Measles-Specific IgM in Oral Fluid and Serum from Children by an Antibody-Capture IgM EIA


In vaccinated populations, the diagnosis of measles often requires laboratory confirmation. Serum tested by EIAs has proven sensitive and specific for diagnosing measles. For comparison of detection of measles-specific IgM in oral fluid and serum samples by an antibody-capture EIA, 163 Ethiopian infants who presented for routine measles vaccination were studied. Paired serum and oral fluid samples were collected before and 2 weeks after vaccination; 269 paired samples were adequate for analyses. Of the 104 serum samples that were IgM-positive, 95 (91%) of the paired oral fluid samples were IgM-positive. Of the 165 serum samples that were IgM-negative, 156 (95%) of the paired oral fluid samples were IgM-negative. The Pearson partial correlation coefficient for optical density readings from postvaccination oral fluid compared with serum was 0.81. Oral fluid appears to be an acceptable alternative to serum for measuring measles-specific IgM antibodies by an antibody-capture EIA.

Measles remains a significant public health problem, with ~70 million cases occurring globally each year [1]. The 45th World Health Assembly has established a goal of achieving a 90% reduction in the number of measles cases by the year 1995 as a step toward global eradication of this disease [2]. The United States is currently working toward its objective of eliminating indigenous transmission of measles in 1996 [3]. Historically, in most countries, measles has been diagnosed based on clinical criteria. However, with high immunization rates, the numbers of mild or asymptomatic infections increase, and medical personnel have less experience diagnosing measles. Inaccurate diagnosis can lead to inappropriate vaccination campaigns and wasted resources or missed opportunities to prevent transmission. Therefore, laboratory confirmation has become important to measles control programs.

Currently, laboratory diagnosis of measles is usually achieved by serologic assays for measles-specific IgM or IgG antibodies. Because of the discomfort associated with phlebotomy, however, parents are often reluctant to allow their infant
or small child to be phlebotomized. Oral fluid may be a more acceptable alternative and easier to obtain from infants and small children, and researchers have demonstrated that IgA, IgG, and IgM are all present in oral fluid [4, 5]. Oral fluid has been used to determine the presence of immunoglobulins against hepatitis A and B, human immunodeficiency virus, rubella, and mumps [6–10]. Recently, Perry et al. [10] successfully used oral fluid to detect measles-specific IgM and IgG in patients with measles, using a sensitive and specific RIA. However, laboratories in developing countries may find an EIA easier to do and read than an RIA. The use of radionuclides in some settings may be almost impossible due to problems in obtaining, storing, using, and disposing of radioactive materials. Moreover, expensive equipment is required to monitor and measure radioactivity. We report here the detection of measles-specific IgM antibodies by an antibody-capture EIA for oral fluid from infants who received live attenuated measles vaccine.

Methods

Study group. Our study group consisted of 163 9-month-old infants who presented to the Tekle Haimanot Health Centre, Addis Ababa, between 24 January and 24 March 1995 for routine measles vaccination. We collected information about age, sex, race, household size, prior immunizations, history of measles infection in the household in the last year, and date of specimen collection. Blood and oral fluid were collected as described below, and infants then received live attenuated measles vaccine (Schwartz). Two weeks later, we collected postvaccination blood and oral fluid samples from the infants. For negative control samples, we used prevaccination serum and oral fluid samples from 43 healthy 12- to 15-month-old US infants presumed to be IgM-negative for measles.

Specimen collection. Blood (1 mL) was collected by heel stick, using universal precautions. Serum was separated and stored at −70°C and then shipped to the Centers for Disease Control and Prevention (CDC) for routine measles vaccination. The median age of all enrolled infants was 9.4 months (range, 8.4–12.9); 87 (53%) were boys. 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 0.8 mL of an antimicrobial preservative solution (Orasure device; donated by Epitope, Beaverton, OR). The device is designed to collect 1 mL of oral fluid. The oral fluid is a combination of saliva and gingival crevicular fluid (transudate) and has 3- to 4-fold higher levels of IgG than does saliva alone [11]. 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 0.8 mL of an antimicrobial preservative solution for transport and storage. The pads were centrifuged to recover the oral fluid plus preservative. The specimens were then frozen at −70°C and shipped to CDC on dry ice.

Specimen testing and interpretation. Sera were tested for measles-specific IgM antibodies by a previously described IgM antibody-capture EIA [12]. Briefly, goat anti-human IgM antibodies diluted in PBS were coated onto microtiter plates for 1 h at 37°C. After the plates were washed, serum diluted 1:200 in PBS with 0.5% gelatin and 0.15% Tween 20 (PBS-GT) was added to four consecutive wells, and the plates were incubated for 1 h at 37°C. After plates were washed, baculovirus-measles virus nucleoprotein or S9-uninfected cell control lysate diluted in PBS-GT with 4% normal goat serum and 0.3% sodium deoxycholate was added to duplicate cells. Plates were then incubated for 2 h at 37°C. Plates were washed, biotinylated monoclonal antibody (83VIIKK2) in PBS/GT was added to wells, and the plates were incubated for 1 h at 37°C. Plates were washed, streptavidin-peroxidase in PBS/GT was added to all wells, and the plates were incubated for 20 min. After a final wash, tetramethylbenzidine substrate solution was added, and the plates were incubated for 15 min at room temperature. The reaction was stopped by acidification, and the optical density was determined photometrically.

For each sample, we calculated the difference between the mean optical density for the antigen-positive wells and the mean optical density for the uninfected negative antigen wells (difference = PN value). Undiluted oral fluid samples were tested using the same technique. Sera were also tested for IgG antibodies by a previously described indirect EIA [13].

We used the negative control sera to determine our positive cutoff value for the serum IgM assay. We defined a positive result as any PN value that was >4 SD above the mean of the optical densities for these negative control samples. We determined the positive cutoff value for the oral fluid specimens in the same manner. The cutoffs for sera and oral fluid samples were >0.095 and >0.091, respectively.

Some oral fluid samples collected from the study infants had high background values (uninfected negative antigen wells [N] with an optical density of ≥0.13) and could not be interpreted. Since additional oral fluid was unavailable for retesting, we report the serum and oral fluid results from these infants separately and not in the table. None of the oral fluid samples from the control infants had high background values.

Data analysis. We calculated the concordance between oral fluid and serum samples for detecting the presence and absence of IgM antibodies by EIA in pre- and postvaccination specimens. Since the specimens were tested in four runs, we took into account run-to-run variability when determining our serum and oral fluid cutoff values by using variance component estimates from the analysis of variance. We also compared the PN absorbance values of serum compared with pre-and postvaccination oral fluid by using the Pearson partial correlation coefficient, adjusting for run-to-run variability.

Results

Demographic information. The median age of all enrolled infants was 9.4 months (range, 8.4–12.9); 87 (53%) were boys. Two male infants had histories of clinical measles at 7 months of age but did not have IgM or IgG antibodies in their prevaccination sera.

Laboratory samples. We collected prevaccination serum and oral fluid specimen pairs from 159 of the 163 enrolled infants and postvaccination serum and oral fluid specimen pairs from 143 of the 163 infants, for a total of 302 serum and oral fluid sample pairs. Postvaccination serum and oral fluid samples were obtained a median of 14 days after measles vaccination (range, 13–32; 142/143 of the samples were collected between 13 and 17 days). Thirty-three (18 [11%] prevaccina-
tion, 15 [10%] postvaccination) oral fluid samples had high background absorbance values in the uninfected negative antigen wells (≥0.13) and are discussed separately below. We report first on the remaining 269 serum and oral fluid sample pairs (141 pre- and 128 postvaccination).

Results from serum and oral fluid samples. Prevaccination serum samples from 3 (2%) of 141 infants were IgM-positive, and 138 (98%) were IgM-negative. Two infants with IgM-positive sera had additional serologic evidence of measles (IgG before and after vaccination, with declining IgM titers). Prevaccination oral fluid samples from 3 (2%) of 141 infants were IgM-positive, and 138 (98%) were IgM-negative. None of the infants with IgM-positive oral fluid samples had serologic evidence of measles. Postvaccination serum samples from 101 (79%) of 128 infants were IgM-positive, and 27 were IgM-negative. Postvaccination oral fluid samples from 101 (79%) of 128 infants were IgM-positive and 27 were IgM-negative (table 1).

Comparison of serum and oral fluid samples. Overall, for 104 IgM-positive pre- and postvaccination serum samples, 95 (91%) of the corresponding oral fluid samples were IgM-positive; for 165 IgM-negative serum samples, 156 (95%) of the corresponding oral fluid samples were IgM-negative (table 1). For the 3 prevaccination serum specimens that were IgM-positive, none of the corresponding oral fluid specimens were IgM-positive; for the 138 IgM-negative prevaccination serum specimens, 135 (98%) of the corresponding oral fluid specimens were also IgM-negative. For the 101 IgM-positive postvaccination serum samples, 95 (94%) of the corresponding saliva samples were IgM-positive; for the 27 IgM-negative postvaccination serum samples, 21 (78%) of the corresponding oral fluid samples were also IgM-negative.

The correlation between prevaccination serum and oral fluid PN values was low (Pearson partial correlation coefficient = 0.35), as would be expected in samples that lacked detectable measles-specific IgM antibodies. The partial correlation between postvaccination serum and oral fluid PN values was 0.81 (Pearson partial correlation coefficient).

Six prevaccination serum–oral fluid pairs gave discordant results (table 1). Two infants had samples that were IgM- and IgG-positive in serum and IgM-negative in oral fluid. As explained above, these 2 infants presumably had been infected with measles virus, and the IgM serum results represented true-positive results. A third infant was IgM-positive but IgG-negative in serum and IgM-negative in oral fluid. In addition, 3 infants had prevaccination sera that were IgM-negative and IgG-negative but had oral fluid samples that were IgM-positive (presumably false-positive results). Two of these oral fluid results, however, had PN values close to the positive cutoff value (≤0.10).

Twelve postvaccination pairs also had discordant results. Six infants were IgM- and IgG-positive in serum but IgM-negative in oral fluid; 2 of these oral fluid results had PN values close to the cutoff value (0.085 and 0.082). In addition, we found 4 infants who had seroconverted against IgG and were IgM-positive in oral fluid samples but IgM-negative in serum; 1 infant had a serum PN value close to the cutoff (0.091). Finally, 2 infants had IgM- and IgG-negative postvaccination serum, but their oral fluid results were IgM-positive; for 1 of these samples, the serum IgM result was just below the cutoff value (PN = 0.089).

Samples with high background values. Thirty-three (18 prevaccination and 15 postvaccination) oral fluid samples (from 27 infants) had high background absorbance values in the negative antigen wells (≥0.13). Twenty-four of the 33 sample pairs with high-background oral fluid specimens were IgM-negative in both serum and oral fluid (18 prevaccination, 6 postvaccination). All 9 of the remaining postvaccination serum samples were IgM-positive, and 2 of the corresponding oral fluid specimens were IgM-positive.

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

<table>
<thead>
<tr>
<th>Serum</th>
<th>Oral fluid</th>
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<tr>
<td></td>
<td>IgM+</td>
<td>IgM-</td>
<td>Total</td>
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<tr>
<td>Prevaccination</td>
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<tr>
<td>IgM+ no. (%)</td>
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<tr>
<td>IgM- no. (%)</td>
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<tr>
<td>Total</td>
<td>3</td>
<td>138</td>
<td>141</td>
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<tr>
<td>Postvaccination</td>
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<tr>
<td>IgM+ no. (%)</td>
<td>95 (94)</td>
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<tr>
<td>IgM- no. (%)</td>
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<tr>
<td>Total</td>
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<tr>
<td>Totals</td>
<td>95 (91)</td>
<td>9 (9)</td>
<td>104</td>
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<td></td>
<td>9 (5)</td>
<td>156 (95)</td>
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<tr>
<td>Total</td>
<td>104</td>
<td>165</td>
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NOTE. +, positive; −, negative.
this monoclonal antibody–based capture EIA has proven highly specific for measles IgM antibodies in serum (>99%) [13], and it should be equally specific for oral fluid. Studies are in progress to test these hypotheses. Third, the assay appeared to perform well regardless of the presence of teeth. Previously, concerns had been raised that the presence of teeth might be necessary for adequate detection of immunoglobulins; immunoglobulins are more concentrated in crevicular fluid than in salivary gland secretions [4, 5], and teeth may be needed for production of gingival crevicular fluid. However, we found that the assay worked well in 9-month-old infants with few or no teeth. Similarly, Bagg et al. [9] demonstrated that anti–rubella and anti–hepatitis A IgG could be detected in saliva from subjects with and without teeth.

Although our study supports the use of oral fluid specimens for diagnosing measles, it also suggests the need to refine this approach to diagnose recent measles infections. First, more study is needed to determine the optimal sampling time to detect IgM after vaccination. The percentage of IgM positivity for postvaccination serum and saliva in this study (79%) was lower than expected percentages of IgG seroconversion (87%) [14]. One possibility is that 14 days after vaccination may be too early to allow for maximum detection rates for IgM positivity in either serum or oral fluid specimens. Some of the discordant pairs of postvaccination serum and oral fluid sample data may have resulted from samples being collected too early in the course of the IgM response. For example, for 3 of the discordant sample pairs, the specimens with negative results (2 oral fluid samples and 1 serum specimen) had PN values near the cutoff value. Repeat specimens would need to be collected later to test this hypothesis.

Another problem we faced with the oral fluid was the set of samples with high background levels and low IgM positivity. We believe that this high background was specific to certain specimens and is not a general feature of oral fluid samples. The high background values occurred in only the study specimens and not in the control specimens, and for 19 of 27 infants with samples that had high background values, only 1 of their 2 oral fluid specimens had high background values. It is possible that specimens with high background values were not collected properly and contained too little oral fluid. Alternatively, these oral fluid samples may not have been handled properly and, consequently, may have been overgrown with bacteria that degraded the IgM antibodies. Unfortunately, residual specimens were not available to evaluate the quality of specimens by determining the concentration of IgG or IgM antibodies. Further study will be important to identify ways to optimize specimen collection and handling.

In addition to optimizing specimen collection and handling, it would be helpful to complement the IgM assay with an IgG assay that works with oral fluid. One group [15] reported good results detecting measles-specific IgG antibodies in oral fluid by using an indirect EIA, while another group [16] reported a sensitivity of only 54% by using an IgG antibody-capture RIA. We are currently evaluating our ability to detect IgG antibodies in oral fluid by using both antibody-capture and indirect EIA formats.

In summary, this study demonstrates that oral fluid is likely to be an acceptable alternative for measuring measles-specific IgM by using a monoclonal antibody–based capture EIA. This approach could simplify specimen collection for diagnosing recent measles infections and conducting measles vaccine studies, especially in children.

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References


Hepatitis B and C Virus Serologies among Japanese Americans with Hepatocellular Carcinoma

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A cohort of 5924 Japanese American men was examined between 1967 and 1970 for hepatocellular carcinoma (HCC). By 1992, 24 incident cases of HCC were histologically confirmed in the group. Frozen serum samples from the 24 men with HCC and 72 age-matched controls were tested for hepatitis B surface antigen (HBsAg), antibodies to hepatitis B core antigen, antibodies to HBsAg, and antibodies to hepatitis C virus. HBsAg was detected in 15 (62.5%) of 24 HCC cases compared with 2 (2.8%) of 72 controls (odds ratio, 43.0; 95% confidence interval, 5.7–325.5). None of the cases and only 1 control had antibody to hepatitis C virus. This study demonstrates a strong association between hepatocellular carcinoma and hepatitis B virus infection, but not with hepatitis C virus infection, among men of Japanese ancestry in Hawaii.

In recent years, the incidence of hepatocellular carcinoma (HCC) has increased substantially in Japan [1, 2]. Data from Japan [2] and some European countries [3, 4] suggest that HCC is associated more frequently with hepatitis C virus (HCV) than with hepatitis B virus (HBV); however, studies in some US populations [5] and in southern Africa [6] implicate HBV more strongly. In several investigations, persons infected with both HBV and HCV appear to have a risk of developing HCC that is greater than for persons infected with either virus alone [5, 7], although the possible additive roles of these viruses remain to be defined.

In contrast to Japanese who live in Japan, the descendants of Japanese immigrants to Hawaii have not experienced a substantial increase in the incidence of HCC in recent years [8–11]. The present study evaluated the roles of HBV and HCV among HCC patients in Hawaii and compared the results to reports of HCC patients in Japan. This study is unusual because of the availability of stored serum samples obtained many years before the diagnosis of HCC. As a result, the possible etiologic roles of HBV and HCV could be more validly assessed.

Methods

The study subjects were American men of Japanese ancestry who were born between 1900 and 1919 and lived on the Hawaiian island of Oahu. They were identified in 1965 by the Honolulu Heart Program by review of military draft records for Japanese surnames in the 1942 Selective Service registration files [12]. Of 11,148 men, 8006 (72%) agreed to enter the study. They were interviewed and had cardiovascular examinations between 1965 and 1968. The remaining men either chose not to participate (2962; 26%) or died before they could be examined (180; 2%). Of the 8006 men enrolled in the study, 7498 (94%) returned for a second cardiovascular examination between 1965 and 1970. At that time, a serum specimen was obtained and frozen. A 20% random sample of these specimens was sent by the Honolulu Heart Program to the US Public Health Service Hospital in San Francisco and were not available for subsequent studies. Serum samples from the remaining 5924 men were stored in Hawaii at −20°C. There were no diagnosed cases of liver cancer among these 5924 men at the time of their second examination.

The Japan-Hawaii Cancer Study continuously reviewed discharge records of all general hospitals on Oahu from 1967 to 1992 to identify incident cases of HCC. To reduce the possibility of