Measurement of Tetanus Antitoxin in Oral Fluid

A Tool to Conduct Serosurveys

Milagritos D. Tapia, MD, Marcela F. Pasetti, PhD,* Lilian Cuberos,* Samba O. Sow, MD, MSc,† Mama N. Doumbia, MD,† Modibo Bagayogo, MD,† Karen L. Kotloff, MD,* and Myron M. Levine, MD, DTPH**

Background: Serosurveys that measure tetanus antitoxin can complement immunization coverage surveys to allow evaluation of immunization services in developing countries. Measurement of IgG tetanus antitoxin in oral fluid was investigated as a practical and noninvasive alternative to and correlate of serum antibodies.

Methods: Serum and oral fluid were collected from Malian infants, toddlers and adults (males without a history of tetanus vaccination). Specific IgG tetanus antitoxin was measured by enzyme-linked immunosorbent assay in serum (S-ELISA) and oral fluid (OF-ELISA).

Results: One hundred forty-two pairs of serum and oral fluid samples were collected from infants, 35 pairs from toddlers and 35 pairs from adults. IgG tetanus antitoxin titers measured by OF-ELISA were 100-fold lower than those measured by S-ELISA but they correlated strongly ($r = 0.90$, $P < 0.001$). All 35 toddlers who had received 2 or 3 doses of diphtheria–tetanus–pertussis (DTP) vaccine (100%) had serum tetanus antitoxin levels ≥0.15 IU/mL and 28 of 35 (80%) had oral fluid values ≥0.0015 IU/mL. Among adults lacking a history of tetanus immunization, only 6 of 35 (17.1%) had serum titers ≥0.15 IU/mL and 4 of 35 (11%) had oral fluid titers ≥0.0015 IU/mL in oral fluid.

Conclusions : IgG tetanus antitoxin in oral fluid correlates well with levels in serum. OF-ELISA values ≥0.0015 IU/mL constitute protection against tetanus and in subjects >12 months of age imply multiple prior contacts with immunization services. IgG tetanus antitoxin measured by OF-ELISA provides a logistically practical alternative for performing seroprevalence surveys.

Key Words: immunization, coverage survey, tetanus, oral fluid, Mali, serosurvey

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Surveys of the prevalence of tetanus antitoxin play an important role, particularly in developing countries, in objectively assessing the immune status of populations and the performance of immunization services. Because neonatal and maternal tetanus control in developing countries is largely based on immunization of pregnant women with tetanus toxoid, tetanus antitoxin serosurveys in women of childbearing age can identify high-risk, underserved populations.[1] Measurement of tetanus antitoxin also provides a helpful adjunct to immunization coverage surveys in 12 to 23 month olds to assess how well services of the Expanded Program on Immunization (EPI) are reaching infants. Measurement of tetanus antitoxin is especially useful because, with rare exceptions, this antibody is the result of immunization and not natural disease or subclinical exposure.[2]

Until recently, collection of serum was required to conduct population-based seroprevalence studies and to assess serologic responses to pathogens or vaccines. This is problematic in developing countries where the prevalence of human immunodeficiency virus (HIV) or hepatitis C virus infection is high because handling blood constitutes a hazard for health workers.[3] In addition, cultural beliefs about blood drawing may hinder recruitment and participation,[4] thereby biasing the sample.[5]

Since the early 1990s, investigators have analyzed oral fluid as an alternative to serum. Oral fluid, a mixture of IgA-rich saliva and IgG-rich crevicular fluid (a transudate of serum expressed at the crevice between the teeth and gums),[6] has been used in disease surveillance in the United Kingdom[7,8] and India[9] as well as outbreak investigations[10,11] and epidemiologic[12–14] studies. Results have documented high sensitivity and specificity. Several oral fluid assays are being developed as noninvasive alternatives to serum-based testing and licensed commercial kits are already available for detection of human antibodies to HIV (OraSure Technologies, Inc., Bethlehem, PA)[15] and measles (Microimmune, U.K.).[16]

Oral fluid sampling may also be well suited to assess the performance of immunization services, including immunization coverage and cold chain integrity. In underserved areas of developing countries, it is difficult to assess objectively the performance of immunization programs because surveillance systems for most infectious diseases are rudimentary and confirmatory laboratory diagnostics are not available. Estimates of immunization coverage that rely on vaccination records or parental recall may provide inaccurate results, depending on the study population.[17–20] Furthermore, because the vaccine cold chain may be faulty, infants can be vaccinated (ie, inoculated) but not successfully immunized as a result of

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From the *Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, Maryland; and the †Centre pour le Développement des Vaccins, Mali, Bamako, Mali.

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Address for correspondence: Milagritos D. Tapia, MD, Center for Vaccine Development, University of Maryland School of Medicine, 685 W. Baltimore St., Rm. 480, Baltimore, MD 21201. E-mail mtapia@medicine.umaryland.edu.

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vaccine damage caused by excessive fluctuations in temperature.[21] Whereas collecting blood on a large sample of young children for this purpose would be a daunting logistical challenge in a developing country setting, oral fluid sampling offers a more practical approach. To explore this possibility, we conducted 2 cross-sectional surveys of infants, toddlers and adults living in Kangaba, Mali, and measured IgG tetanus antitoxin in paired serum and oral fluid specimens.

MATERIALS AND METHODS

Study Site. Mali, a land-locked country in West Africa with an infant mortality rate of 122 per 1000 live births,[22] is divided into 9 administrative regions that are further apportioned into 58 cercles. Kangaba cercle, located in the Koulikoro region 100 km from the capital, Bamako, has a population 80,923 spread over 60 villages. Health care in Kangaba, including immunization, is provided at 10 health centers. These studies were completed at 2 centers that provide care for Salamalé and Kangaba villages.

Primary healthcare workers who provide immunization services are expected to document immunizations on a card (given to the parent) and in a clinic log book (in which all vaccines administered at a given facility are recorded). In most instances, the vaccination card also lists the child's date of birth or the age of first contact with health care personnel, usually for bacille Calmette-Guérin vaccination. Local health authorities currently calculate coverage for the 3-dose regimen of diphtheria–tetanus–pertussis (DTP3) by dividing the total number of doses of DTP3 vaccine administered by the number of children in the target population (children less than 1 year old). Official health statistics indicate that in 2003, 69% of 12- to 23-month-old children in Mali had received DTP3 vaccine.[22] In 2002, DTP3 coverage was reported as 83% in Kangaba cercle.

Study Review and Informed Consent. The Ethics Committee of the Malian Medical University (Faculté de Médecine, Pharmacologie et Odonto-Stomatologie- FMPOS) and the University of Maryland Baltimore Institutional Review Board approved the studies described here and community and individual consent were obtained as previously described.[23]

Study Design. The cross-sectional survey of infants comprised subjects who were primarily participating in a measles seroprevalence survey.[23] Eligible subjects were healthy infants 2, 4, 6, 8 and 9 to 10 months of age (±2 weeks) without history of previous measles vaccination, clinical measles infection or receipt of blood products in the previous month. In addition, to be eligible, 6-month-old participants had to have evidence of having received all 3 recommended doses of DPT vaccine affirmed by the infant's vaccination card or the clinic log book.

Recognizing a likely target for future serosurveys, we also enrolled healthy 12- to 23-month-old toddlers presenting vaccination cards. In an attempt to find subjects who had never received tetanus toxoid vaccination to serve as negative controls, we enrolled adult males >45 years of age without a history of vaccination against tetanus who had not received blood products, including tetanus immune globulin, in the past month and who had not served in the Malian armed forces.

Samples of blood and oral fluid were obtained from all participants at the time of inclusion. All 9- to 10-month-old participants received measles vaccination according to the Malian EPI recommendation and from these infants, a second pair of samples was obtained 3 to 5 weeks later. Dates of vaccination of the infants and toddlers, as noted on the vaccination card or the clinic log book, were recorded.

Blood samples were centrifuged and the serum was aliquoted and stored in a dry shipper (−170°C) for transport to Bamako. Oral fluid was collected using 2 sponge swabs (Oracol; Malvern Medical Developments Limited, Worcester, U.K.), each consisting of a sponge attached to a plastic stick. The first swab was inserted in one side of the mouth and rubbed along the gums for approximately 30 seconds or until saturated. The swab was then placed in its container and stored on cold packs. The same procedure was repeated for the second swab on the opposite side of the mouth. Within 4 hours, the oral fluid was expressed from the sponges in the following manner: the sponges were removed from each of the swabs and placed into a 10-mL syringe. The bottom of the syringe was placed tip-down in a cryotube and both were centrifuged in a tube shield for 20 minutes at 2500 rpm (Medilite; Thermo IEC, Needham Heights, MA). The oral fluid collected was measured and an equal volume of preservative (0.5% Tween 20, 0.01% chlorhexidine digluconate) was added. Aliquots of serum and oral fluid samples were stored and sent in a dry shipper to the Applied Immunology Laboratory at the Center for Vaccine Development in Baltimore, MD, for analysis.

Serum Tetanus Antitoxin Enzyme-Linked Immunosorbent Assay. Immulon II plates (Thermo Labsystem, Franklin, MA) were coated for 3 hours at 37°C with tetanus toxoid (Staten Serum Institute, Copenhagen, Denmark) at 5 μg/mL in phosphate-buffered saline (PBS), pH 7.4. Plates were blocked overnight at 4°C with 10% dried milk (Nestle USA, Inc., Glendale, CA) in PBS. After each incubation, plates were washed 6 times with PBS containing 0.05% Tween 20 (PBST). Samples were plated in 2-fold dilutions in 10% dried milk in PBST (PBSTM) and then incubated for 1 hour at 37°C. After washings, HRP-labeled goat anti-human IgG (ICN, Irvine, CA) diluted 1/5000 in PBSTM was added and plates were incubated for 1 hour at 37°C. Substrate solution TMB microwell peroxidase (KPL; Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD) was added for 15 minutes. The reaction was stopped by the addition of 100 μL of 1M H_2PO_4 and OD_{450} nm values were measured in an enzyme-linked immunosorbent assay (ELISA) microplate reader (Multiskan Ascent; Thermo Labsystem). Linear regression curves were calculated for each serum sample. Antibody titers were expressed in international units (IU/mL) by interpolating regression-corrected OD values of serum samples in the curve of the WHO Tetanus Antitoxin Human Immunoglobulin Reference (NIBSC #76/589, 1 IU/mL; U.K.).

Oral Fluid Tetanus Antitoxin Enzyme-Linked Immunosorbent Assay. IgG tetanus antitoxin in oral fluids was measured using an ELISA developed in our laboratory. Briefly, Immulon II plates were coated with tetanus toxoid, blocked and washed as described previously. Samples were tested in 2-fold dilution in

PBSTM starting at 1:25. Biotin–goat anti-human IgG (ICN) diluted 1/2000 in PBSTM was used as conjugate followed by HRP–avidin (Sigma, St. Louis, MO) diluted 1/400 in PBSTM; plates were incubated for 30 minutes at room temperature and then washed 10 times with PBST. TMB microwell peroxidase (KPL) was used as substrate solution. The reaction was stopped and the OD_{450} nm values were measured as described previously. Linear regression curves were calculated for each sample and titers were expressed in international units per milliliter by interpolation of OD values of serum samples in the WHO tetanus antitoxin reference curve as described previously.

Because true-negative serum samples were not available, we confirmed the specificity of the oral fluid and serum ELISAs by performing inhibition assays in which the WHO tetanus antitoxin standard (0.0002 IU/mL) as well as serum and oral fluid samples were preincubated with increasing concentrations (0.005–5 μg) of tetanus toxoid before being added to the ELISA plates.

Oral Fluid Total IgG Enzyme-Linked Immunosorbent Assay. ELISA plates were coated with goat antihuman IgG (Jackson, West Grove, PA) at 1 μg/mL in PBS. Plates were blocked and washed as described previously. Samples and standard (purified human IgG) were tested in 2-fold dilutions in PBSTM. To generate a standard curve, purified human IgG (Calbiochem, La Jolla, CA) was added to the plates at concentrations ranging from 10 to 0.156 ng/mL. HRP-labeled goat anti-human IgG (Jackson) diluted 1/20,000 in PBSTM was used as conjugate and TMB microwell peroxidase (KPL) as substrate. The reaction was stopped and the OD_{450}nm values were measured as explained previously. IgG concentrations were calculated by interpolation of the regression-corrected OD values produced by the test samples into the standard IgG curve. Samples were run in duplicate and negative and positive controls were included.

Protective Titer. Using in vivo neutralization assays, a serum antitoxin titer of 0.01 IU/mL is recognized to be protective.[2] Indirect ELISA correlates well with in vivo assays for sera having titers ≥0.15 IU/mL[24] and seroprevalence surveys that use this method conservatively use a cutoff of ≥0.15 IU/mL as evidence of a protective level of tetanus antitoxin.[2,25] In this study, we also consider a titer of ≥0.15 IU/mL as evidence of protection against tetanus.

Data Analysis. Because data were not normally distributed, they were log transformed for further interpretation. Linear regression of oral fluid IgG tetanus antitoxin titers on serum IgG tetanus antitoxin titers and oral fluid total IgG titers was performed using Epi Info (Centers for Disease Control and Prevention, Atlanta, GA). Geometric mean concentrations (GMCs) of serum IgG and oral fluid IgG tetanus antitoxin were calculated according to age groups and number of DTP doses received. Infants with unknown vaccination history and samples obtained after measles vaccination were included only in the linear regression model. Paired *t* test of serum samples obtained from 9- to 10-month-old infants at baseline and 3 to 4 weeks after measles vaccination was performed using Excel. Statistical significance was defined as $P \leq 0.05$.

RESULTS

In total, 212 pairs of serum and oral fluid samples collected from 188 participants were analyzed. There were 142 pairs (including 24 pairs of serum and oral fluid obtained from infants aged 9–10 months 24–25 days after measles vaccination) from 118 infants, 35 pairs from 12- to 23-month old toddlers and 35 pairs from adult males. Oral fluid samples were from 0.03 to 1.0 mL in volume and contained from 6.2 to 608.6 μg/mL of total IgG (Table 1). There was an excellent correlation between IgG tetanus antitoxin measured in serum and oral fluid (Fig. 1; r = 0.90, $P < 0.001$) and this correlation was stronger when the linear regression included the total IgG measured in the oral fluid sample (r = 0.95, $P < 0.001$). Linear regression analysis of IgG tetanus antitoxin levels measured in oral fluid and serum also demonstrated that oral fluid contained approximately 100-fold lower titers of tetanus antitoxin than serum; a concentration of 0.01 IU/mL in serum equaled 0.00011 IU/mL in oral fluid. The

TABLE 1. Volumes and the Concentration of Total IgG in Oral Fluid Samples by Age Group

Age	N*	Volume		Total IgG	
		Mean (mL)	Range (mL)	GMC (μg/mL)	Range (μg/mL)
2 mo	20	0.17	0.04–0.25	20.4	8.4–49.1
4 mo	20	0.28	0.06–0.66	20.9	6.2–155.4
6 mo	30	0.22	0.04–0.45	18.7	6.3–197.1
8 mo	17	0.15	0.03–0.35	33.0	11.3–164.5
9–10 mo	31	0.18	0.04–0.40	61.9	16.4–208.0
10–11 mo	24	0.14	0.04–0.29	95.8	31.9–608.6
12–23 mo	35	0.32	0.09–0.75	56.9	12.2–183.0
>45 yr	35	0.42	0.08–1.0	62.0	14.9–297.9

*N indicates the number of participants.
GMC indicates geometric mean concentration.

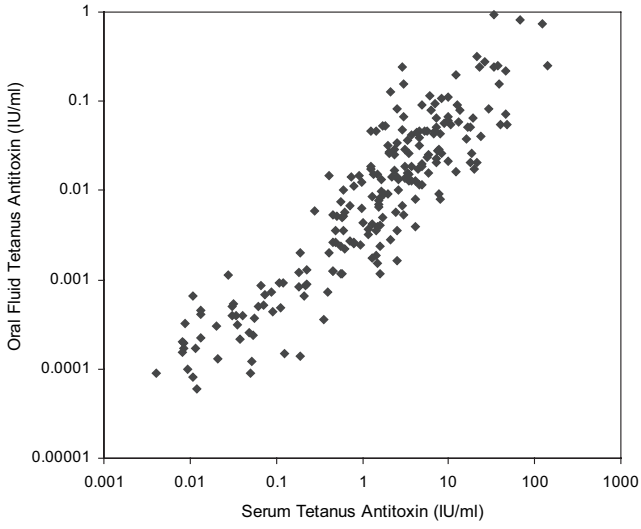

FIGURE 1. Scatterplot of IgG tetanus antitoxin concentrations measured in paired samples of serum and oral fluid collected from participants in both surveys (n = 212). Levels measured in oral fluid are approximately 100-fold lower than those measured in serum.

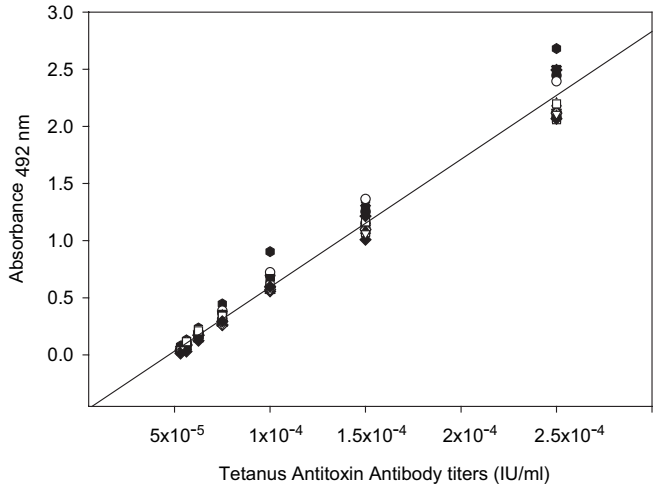

FIGURE 2. Compiled data from the Tetanus Antitoxin Standard using the oral fluid technique in 19 assays during the analysis of all oral fluid samples from this study.

analytic sensitivity of the oral fluid assay was 0.000017 IU/mL. A compilation of the dose–response curves obtained for the WHO tetanus antitoxin standard during the analysis of all oral fluid samples over 17 months is shown in Figure 2. The interassay and intraassay coefficients of variation for the standard using the oral fluid technique were 11% and 2%, respectively. The inhibition assays demonstrated a dose-dependent inhibition of tetanus antitoxin binding. The highest concentration of tetanus toxoid used for inhibition totally abrogated antibody detection and had absorbance values similar to those of blank wells, indicating that the assay specifically measures antitoxin antibodies (data not shown).

Table 2 summarizes the GMCs and ranges of IgG tetanus antitoxin measured in serum and oral fluid samples obtained from infants. Overall, similar observations can be made based on IgG tetanus antitoxin titers measured in serum and oral fluid. Among 2-month olds, the highest values were observed in samples from those who had received 2 doses of DTP vaccine (DTP2). All 4-month olds had received at least one dose of DTP vaccine and all recipients of 2 or 3 doses exhibited protective titers of IgG tetanus antitoxin. As age increased, the GMC of IgG tetanus antitoxin titers of infants who had received DTP3 decreased so that 9 to 10 month olds who had received DTP3 had lower GMC (3.2 IU/mL in serum and 0.024 IU/mL in oral fluid) than 6 month olds (7.1 IU/mL in serum and 0.218 IU/mL in oral fluid) and the GMC fell further among toddlers aged 12 to 23 months (0.98 IU/mL in serum and 0.0045 IU/mL in oral fluid; Table 3).

Toddlers who had received DTP3 vaccine had significantly higher serum IgG tetanus antitoxin titers than those who had received DTP2 (0.98 IU/mL versus 0.34 IU/mL, $P < 0.05$) (Table 3). When measured in oral fluid, IgG tetanus antitoxin titers from toddlers who received 3 versus 2 doses were not significantly different (0.0045 versus 0.003 IU/mL, $P = 0.6$). Compared with infants and toddlers, the lowest IgG tetanus antitoxin titers were observed in the adult participants, the negative controls. Twenty-nine of the 35 adult subjects (82.9%) had serum titers lower than 0.15 IU/mL (the protective level against tetanus measured by indirect IgG ELISA)[2,25] (GMC = 0.024 IU/mL), whereas 6 were above this threshold (GMC = 1.01 IU/mL). In oral fluid, the 29 serosusceptible adults had titers that ranged from 0.00009 to 0.00114 IU/mL (GMC = 0.0003); the other adults had levels that ranged from 0.00116 to 0.1066 IU/mL (GMC = 0.0065).

If one considers an immunization record that shows receipt of 3 doses of DPT as the gold standard for immunization with DTP, among the 33 toddlers who received DTP3, all had serum titers ≥0.15 IU/mL (sensitivity = 100%; positive predictive value = 94.3%) and 26 had oral fluid titers ≥0.0015 IU/mL (sensitivity = 78.8%; positive predictive value = 92.9%).

Of 173 serum specimens with titers ≥0.15 IU/mL, 159 matching oral fluid specimens had titers ≥0.0015 IU/mL (sensitivity = 159/173, 91.9%; positive predictive value =

TABLE 2. Measurements of IgG Tetanus Antitoxin in Serum and Oral Fluid Samples of 2- to 10-Month-Old Infants*

Age (months)	No. of DTP Doses*	N†	Serum			Oral Fluid		
			GMC (IU/mL)	Range (IU/mL)	Percent With ≥0.15 IU/mL	GMC (IU/mL)	Range (IU/mL)	Percent With ≥0.0015 IU/mL
2	0	8	0.727	0.012–5.8	87.5	0.0032	0.00006–0.026	87.5
	1	6	2.225	0.903–4.9	100	0.008	0.00165–0.019	100
	2	5	6.6	4.14–19.8	100	0.017	0.008–0.032	100
4	1	4	0.441	0.092–1.3	75	0.001	0.00044–0.0017	25
	2	3	12.6	12.2–13.4	100	0.064	0.016–0.2	100
	3	12	6.86	0.8–138.7	100	0.0245	0.0027–0.93	100
6	3	30	7.13	0.12–47	96.7	0.0218	0.0009–0.28	96.7
8	2	1	3.17	—	100	0.018	—	100
	3	15	3.9	0.19–46.6	100	0.018	0.00014–0.22	93.3
9–10	0	1	0.011	—	0	0.00008	—	0
	1	3	1.95	0.113–33.6	66.7	0.015	0.00048–0.24	66.7
	2	3	0.81	0.22–2.98	100	0.011	0.0009–0.155	66.7
	3	22	3.2	0.22–120.9	100	0.024	0.0009–0.75	95.5

*Includes only infants who had received a known number of diphtheria–tetanus–pertussis vaccine doses (DTP) (n = 113).
†N indicates the number of infants.
GMC indicates geometric mean concentration.

TABLE 3. Measurements of IgG Tetanus Antitoxin in Serum and Oral Fluid Samples of Toddlers and Adults

Age	No. of DTP Doses	N*	Serum			Oral Fluid		
			GMC (IU/mL)	Range (IU/mL)	Percent ≥0.15 IU/mL†	GMC (IU/mL)	Range (IU/mL)	Percent ≥0.0015 IU/mL†
12–23 mo	2	2	0.34	0.28–0.4	100	0.003	0.002–0.006	100
	3	33	0.98	0.18–6.4	100	0.0045	0.0004–0.078	79
≥45 yr	0	35	0.046	0.004–8.2	17	0.00053	0.00009–0.1066	11

*N indicates the number of persons in each group.
†The percentages of participants in each group with serum levels ≥0.15 IU/mL or oral fluid levels ≥0.0015 IU/mL are presented.
DTP indicates diphtheria–tetanus–pertussis vaccine; GMC, geometric mean concentration.

159/159, 100%). Of 39 sera with tetanus titers <0.15 IU/mL, all 39 matching oral fluid titers were <0.0015 IU/mL (specificity = 39/39, 100%; negative predictive value = 39/53, 73.6%). There was no significant difference between total IgG measured in oral fluid of those with protective titers in serum and oral fluid (n = 159) versus those who had protective titers in serum but not in oral fluid (n = 14; $P = 0.129$).

Twenty-four 9- to 10-month-old infants had 2 pairs of serum and oral fluid samples obtained at baseline and 24 to 25 days after measles vaccination (Fig. 3). These sequential specimens obtained 3 weeks apart provided an opportunity to document the repeatability of the assays. There was no significant difference between serum IgG tetanus antitoxin measurements obtained on visits 1 and 2 ($P = 0.77$); this is paralleled in the oral fluid results ($P = 0.24$).

DISCUSSION

Serosurveys that measure the prevalence of tetanus antitoxin play a useful role in public health. Among women of childbearing age, such surveys predict whether women will be protected against maternal tetanus and their infants against neonatal tetanus.[1] Similarly, these surveys can complement EPI coverage surveys, a tool that assesses the quality of immunization services by measuring the proportion of a random sample of 12- to 23-month-old toddlers who have received DTP3.[26] A weakness of EPI coverage surveys is that they are affected by the availability of vaccination records.[27] In Mali, as many as 25% of parents in urban Bamako and 35% of parents in rural areas lose their children's vaccination cards (unpublished data). Moreover, vaccines may sometimes become damaged in a faulty cold chain and lose potency, resulting in some infants being inoculated but not immunized.[24] Thus, an alternative approach to quantify immunization coverage objectively is to measure the proportion of the toddler population with protective tetanus antitoxin titers.

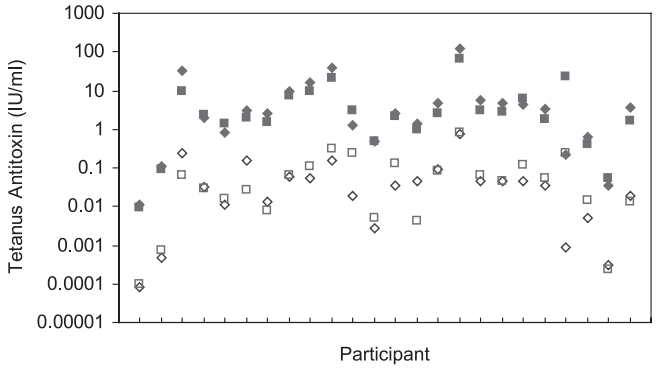

FIGURE 3. Scatterplot of tetanus antitoxin levels measured in serum (solid squares and diamonds) and oral fluid (open boxes and diamonds) samples collected from 24 9- and 10-month-old infants at baseline (visit 1: diamonds) and 3 to 4 weeks later (visit 2: squares). By paired *t* test, there is no significant difference between IgG tetanus antitoxin measurements obtained on visits 1 and 2 (serum, $P = 0.77$; oral fluid, $P = 0.24$).

The most accurate antitoxin measurement involves in vivo neutralization in animals using sera from vaccinated subjects.[2] Measurement of tetanus antitoxin in serum by double-antigen ELISA correlates well with in vivo neutralization.[28–30] However, this assay, which has only been validated with blood samples, is complicated, requires special labeled tetanus antigen and is suitable only for sophisticated laboratories. For these reasons, most serosurveys of tetanus antitoxin use the technically simple and robust indirect ELISA with tetanus toxoid as antigen and an international reference antiserum as the standard. Whereas indirect ELISA can overestimate protection at low titers,[2,31] at serum titers ≥0.15 IU/mL, results of this assay correlate closely with in vivo neutralization[24] and a value of ≥0.15 IU/mL may be considered objective evidence of protection in serosurveys.[2,25]

We investigated whether measurement of IgG tetanus antitoxin in oral fluid by indirect ELISA could be an alternative and noninvasive objective method for use in population-based surveys of tetanus immune status. Results of the studies described here indicate that the titer of IgG tetanus antitoxin measured in oral fluid is indeed an acceptable proxy for that measured in serum. With the Oracol collection device, oral fluid samples were of adequate volume and of good quality with total IgG concentrations comparable to those reported by others.[14,32,33] There was an excellent correlation (r = 0.90) between levels of IgG tetanus antitoxin measured in serum and oral fluid. Trends such as decreasing levels of antibody with increasing age among recipients of DTP3 could be clearly discerned by analyzing results obtained from either serum or oral fluid.

The oral fluid indirect ELISA assay was very sensitive with an analytic sensitivity (0.000017 IU/mL) similar to that achieved with the double antigen ELISA when analyzing blood samples.[28,30] Comparing the overall proportion of participants who had protective titers measured in serum (≥0.15

IU/mL) with those with such titers measured in oral fluid (≥0.0015 IU/mL), we found that the oral fluid assay had high sensitivity and positive predictive value. Although the negative predictive value was lower and suggests that the level of protection in a community might be somewhat underestimated by this assay, these samples represented a small proportion of these preliminary data overall (14 of 212 [6.6%]). Further testing should help to better establish the negative predictive value of this test. Overall, these data suggest that the oral fluid (OF)-ELISA can be used to survey populations with a broad age range and varying immunization status and provide valuable information regarding immunization coverage and to identify populations at risk.

A serum titer of ≥0.15 IU/mL has been successfully used in surveys of broad age groups to identify protected individuals using serum (S)-ELISA.[25,34–36] We believe that our preliminary data support the contention that a tetanus antitoxin titer of 0.0015 IU/mL in oral fluid can similarly be used to identify protected individuals in serosurveys of any age group and can offer logistic advantages. The titers of IgG tetanus antitoxin in oral fluid and serum among Malian toddlers are particularly interesting because they represent data from the target population of coverage surveys. Almost all these 12- to 23-month olds had received DTP3 and had relatively high antibody titers. Although the antibody levels in the toddlers were lower than those observed among the infant participants who got DTP3, they surpassed the protective level (0.15 IU/mL of serum) and were higher than the titers observed among the negative controls. Based on these preliminary data, we hypothesize that it may be possible to use a titer of ≥0.0015 IU/mL in oral fluid during EPI coverage surveys to objectively identify the proportion of toddlers who have received DTP3 during infancy. We plan to carry out additional serosurvey and cohort studies to address this hypothesis.

Of the adult males without a history of previous immunization against tetanus, the negative controls, only 6 lacked IgG tetanus antitoxin. It would be rare for these low titers to represent antitoxin acquired naturally in rural Mali.[2,37–41] More likely, these low titers represent either false-positives secondary to the limitations of ELISA[2,30] or participants who failed to recall tetanus toxoid vaccination sometime in the past.

Results obtained by OF-ELISA appear to be highly reproducible between assay runs and samples. The interassay and intraassay coefficients were low and although there was some variation between samples, there was no statistically significant difference between serum and oral fluid antitoxin titers measured in 2 samples collected 3 to 4 weeks apart from 9- to 10-month-old infants.

Oral fluid collection was very well accepted by the pediatric study participants. From a logistic standpoint, the only limitations were the extraction of the fluid from the device and cold storage after collection. If additional studies confirm the use and reliability of the OF-ELISA, we intend to overcome the logistic challenges of the prototype methods through the development of a robust and rapid point of use test kit.

The practical future kit must have a simple readout that can be used in developing countries to identify individual specimens with titers ≥0.0015 IU/mL. The fact that 2 kits that measure specific antibodies in oral fluid have already been licensed by regulatory authorities renders optimism that development of an oral fluid tetanus antitoxin kit is feasible.[15,16] Because there is not likely to be a market for an oral fluid tetanus antitoxin measurement kit other than public health agencies and authorities, it will be important for the kit to be inexpensive.

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