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Comparison of HIV oral fluid and plasma antibody results during early infection in a longitudinal Nigerian cohort

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ABSTRACT

Background: Oral fluid (OF) testing is a less-invasive alternative to blood-based testing for HIV. The performance of HIV OF tests has not been extensively evaluated in serially collected paired specimens from seroconverters.
Objective: To compare paired OF and plasma test performance in a cohort of HIV-1 seroconverters from Nigeria.
Study design: Paired plasma and OF specimens from 14 seroconverters collected during 24 months of longitudinal follow up were included in the study. Plasma and OF were tested using Avioq HIV-1 Microelisa System, and first reactivity in plasma and OF specimens was compared. OF specimens reactive by Avioq were subsequently tested by OraSure HIV-1 Western blot. Genetic Systems HIV-1 Western blot was also performed on the corresponding plasma of the first 2 Avioq-OF positive time-points.
Results: Of the 14 seroconverters, 5 (35.7%) had concordant results between plasma and OF for all time points tested, whereas 9 (64.3%) showed reactivity on plasma before OF specimens early in infection. The median delay between plasma and OF reactivity was 29 days (range: 0 day–20 months) ($p < 0.0039$); the median overall delay for OF compared to RNA testing was 69.5 days. Delayed antibody response with OF was observed in both males and females regardless of viral load or HIV subtypes.
Conclusions: Results demonstrate decreased sensitivity of OF testing compared to blood-based testing with specimens obtained early after HIV infection. Programs that utilize OF testing in populations with increased risk of incident HIV infection should understand these limitations of OF testing.
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1. Background

Human immunodeficiency virus (HIV) testing is a key prevention strategy whereby infected persons can be identified and linked with life-saving medical care that can reduce forward transmission. There is evidence that the availability of less-invasive options would increase people's willingness to undertake HIV testing [1–3]. Given its minimally invasive and simple specimen collection, oral fluid (OF) testing has become a preferred choice for some high-risk populations [2–4]. However, there is evidence that some individuals who test frequently, due to known risk, do not have a high degree of confidence in OF testing and have greater trust in blood-based testing [5]. OF is a complex mixture of saliva, gingival crevicular fluid, and mucosal transudates that contain immunoglobulins such as immunoglobulin A (IgA) and immunoglobulin G (IgG). The primary reactivity to HIV antigens when using OF is due to IgG [2,3]. However, the concentration of IgG in OF has been reported to be substantially lower (average 300 times) than that in serum [6–8]. Early clinical testing with OF demonstrated problems with specimen stability and assay sensitivity due to the low antibody levels in OF [9,10]. For this reason, Orasure Inc. (OraSure Technologies, PA, USA) developed a specialized OF collection device that enhances the level of antibodies, specifically IgG, inhibits proteolytic enzymes, and ensures sufficient specimen volume [2,11].

The Orasure HIV-1 oral specimen collection device along with the enzyme immunoassays (EIAs) specifically developed for use with OF, such as the Vironostika HIV-1 (BioMerieux, France) and the Avioq HIV-1 Microelisa systems (Avioq, MD, USA), have improved the performance of OF testing [2,12]. Several studies [1,2,13–15] have compared OF and plasma test performance using the Vironostika HIV-1 Microelisa tests on OF collected with the Orasure HIV-1 oral specimen collection device, but the findings have been inconsistent. Some studies [2,13] have reported sensitivities (99.2–99.9%) and specificities (99.2–100.0%) comparable to those in blood-based tests in a mix of low and high HIV prevalence

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populations, while others [1,14,15] have shown less desirable results for OF testing. Furthermore, the performance of OF tests in serial specimens collected from before seroconversion through development of a mature immune response has not yet been evaluated.

Currently, the Avioq HIV-1 Microelisa System is the only US Food and Drug Administration (FDA) approved plate format immunoassay (IA) available for OF testing in the United States. Although this test detects only IgG, and more sensitive tests for detection of HIV infection in blood are available, the FDA approved the Avioq Microelisa System for use with OF based on a non-inferiority study in which the Avioq assay was compared to the previously approved Vironostika HIV-1 Microelisa System (package insert of Avioq HIV-1 Microelisa System). Furthermore, the Avioq assay is also approved for use with serum/plasma and thus is a useful platform for comparing the relative timing of detection of HIV antibody in OF and serum/plasma.

2. Objective

In this study, we used the Avioq HIV-1 Microelisa System to compare OF and plasma test results in a cohort of HIV-1 seroconverters from Nigeria.

3. Study design

3.1. Sample population

The participants were selected from the Recruiting Acute Cases of HIV (REACH) study conducted between May 2003 and March 2010 in Abuja and Jos, Nigeria [16]. The REACH sample population was recruited from healthcare facilities targeting populations at high risk for HIV infection, including antenatal clinic-attendees, negative partners in sero-discordant couples, commercial sex workers and taxi drivers. All participants were >18 years old, received HIV counseling and testing, and provided consent documentation prior to sample collection. The study protocol was approved by the human subjects review boards at the University of Maryland, the US Centers for Disease Control and Prevention, and the Nigerian Federal Ministry of Health's National Institute for Pharmaceutical Research and Development.

3.2. Testing algorithms to identify seroconverters

The recruitment into the study was conducted in 2 phases. During the first phase, individuals were screened by 2 rapid tests in series, Determine HIV (Abbott, IL, USA) and Unigold HIV (Trinity, NY, USA). Samples with discordant results underwent a tie-breaker third rapid HIV test (Stat-Pak HIV, Chembio, NY, USA) for the purpose of same-day, posttest counseling. The individuals who were HIV antibody-positive by the 2 initial rapid tests were considered positive for HIV antibodies. Specimens that were HIV antibody-negative or discordant based on two rapid antibody tests were pooled for screening with RNA-PCR using the Roche Amplicor v1.5 (Roche Diagnostics, Branchburg, NJ, USA) [16]. Persons with detectable HIV RNA but no antibody to HIV were offered enrollment in serial follow-up. To confirm seroconversion, HIV rapid tests and Western blot (Genetic System HIV-1 Western blot, BioRad Laboratories, WA, USA) were performed on specimens collected from individuals enrolled in this second phase of the study. Because individuals could be offered rapid test screening more than once during the study, individuals who previously tested negative but screened positive by 2 rapid tests within 6 months of their last negative test were also offered the opportunity to enroll in the longitudinal follow-up.

Table 1
Characteristics of sample population.

	Subtype	Sex	Pregnancy status	Risk group
SC12	CRF02_AG	F	Y	NO
SC24	CRF02_AG	F	Y	NO
SC29	CRF02_AG	F	N	NO
SC61	CRF02_AG	F	Y	NO
SC11	CRF02_AG	M	NA	NO
SC28	CRF02_AG	M	NA	NO
SC16	CRF02_AG/G	F	N	CSW
SC13	G	F	N	STI
SC17	G	F	Y	NO
SC20	G	F	N	CSW
SC21	G	M	NA	NO
SC26	G	M	NA	NO
SC62	G	M	NA	DC
SC27	URF	M	NA	NO

Source: Charurat et al. [16].
CSW, commercial sex workers; DC, serodiscordant couples; STI, sexually transmitted infection; NO, no identified risk; NA, not applicable.

3.3. Sample collection during follow up

During the longitudinal follow-up, plasma and OF specimens were collected at 7–10 days, 3, 5, 7, 9 weeks, and then 3, 4, 6, 8, 10, 12, 15, 18, 21, and 24 months. However, few participants missed some time points. Total 150 paired OF and plasma specimens were available for the study. Blood was collected in EDTA tubes and OF was collected using the OraSure HIV-1 oral specimen collection device. Blood and OF were processed according to the manufacturers' protocols. OF specimens were stored off of the collection pad in cryovials at −80 °C. The detailed procedures are described elsewhere [16]. All specimens had been stored at −80 °C for more than 3 years prior to testing in this study.

3.4. HIV testing

The Avioq HIV-1 Microelisa System was used to test 150 total specimens from 14 seroconverters for HIV antibody in plasma and OF. Avioq-positive OF specimens were further tested by an Orasure HIV-1 Western blot (WB) kit (Orasure technologies, PA, USA). Genetic Systems HIV-1 Western blot was performed on the corresponding plasma of the first 2 Avioq-oral fluid positive time-points.

Quantitative HIV-1 RNA viral load, HIV-1 sequencing and phylogenetic analysis were performed at the University of Maryland, Baltimore during the original characterization of the cohort. The detailed procedures have been previously published [16].

3.5. Characteristics of sample population

Fourteen individuals identified as seroconverters in the previous REACH study [16] were included in this study (Table 1). Of the 14 seroconverters, 8 were females and 6 were males. Four of 8 females were pregnant at enrollment in the study. There were 3 females from high-risk groups, 2 commercial sex workers and 1 with a sexually transmitted infection; 1 male had an HIV-positive partner. HIV risk for the remaining 10 individuals was not identified. There were 6 subtype G and 6 subtype CRF02-AG infections. One individual (SC16) had a recombinant virus consisting of CRF02 AG (pol) and Subtype G (env). The isolate from SC27 could only be partially sequenced, and some parts of the genome could not be classified and, thus, it was considered as a unique recombinant form.

3.6. Statistical analysis

The delay in antibody response was calculated as the difference in days between a positive test result on the Avioq test performed