CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, Nov. 1996, p. 640–644 Vol. 3, No. 6
1071-412X/96/$04.00+0


# Comparison of Saliva and Serum for Human Immunodeficiency Virus Type 1 Antibody Testing in Uganda Using a Rapid Recombinant Assay

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Received 26 February 1996/Returned for modification 8 April 1996/Accepted 18 June 1996

**The accuracy and acceptability of saliva human immunodeficiency virus type 1 (HIV-1) antibody testing were compared with serum testing in a study of paired specimens from HIV-1-seropositive and HIV-1-seronegative Ugandan adults attending a clinic for sexually transmitted diseases. Saliva collection was performed with the Omni-sal device (Saliva Diagnostic Systems, Vancouver, Wash.), and antibody testing was performed by a rapid filter paper assay (Test-Pack; Abbott Laboratories, Abbott Park, Ill.). Relative to serum testing, the sensitivity of saliva testing was 95% (195 of 205) and the specificity was 99% (295 of 297). The sensitivity of saliva testing was higher for patients with elevated levels of beta-2 microglobulin in sera and greater numbers of HIV-1-related symptoms. Pre- and poststudy interviews indicated that saliva testing did not foster inordinate fears of saliva exposure. The development of saliva tests that are inexpensive and do not require electricity is needed.**

Human immunodeficiency virus type 1 (HIV-1) antibody testing of serum samples is used widely in Uganda for counseling programs, screening of blood donations, public health surveillance, and epidemiological research. Phlebotomy is not acceptable to some people who wish to avoid the discomfort of venipuncture, who fear infection with poorly sterilized needles (3), or who believe that the amount of blood removed may be harmful. Phlebotomy also involves a risk of needlestick injury and HIV-1 transmission to clinic staff and those who dispose of contaminated sharp objects. The separation of serum from blood usually involves transporting specimens to a laboratory and prolongs the time patients anxiously wait for results. Rapid, accurate, safe, and culturally acceptable alternatives to serum HIV-1 antibody testing need to be developed and evaluated for settings in which serum testing is impractical or not widely accepted.

Saliva testing has been shown to be more acceptable than serum testing in studies of prostitutes (13) and intravenous drug users (11). Saliva testing was more acceptable because blood collection was impractical outside of clinical facilities and venipuncture was difficult with patients whose veins were collapsed by intravenous drug use.

Antibodies are present in lower concentrations in saliva than in serum (10). This explains early reports that saliva HIV-1 antibody tests may lack sufficient sensitivity for clinical use (2, 6). The lower concentration of HIV-1 antibodies in saliva has been addressed with the development of the GACELISA assay, which involves antibody capture to a solid phase to improve antibody detection in dilute specimens (5). This assay has been shown to have a sensitivity of 99% in a large study in Thailand (7). The GACELISA assay, like other microwell-based enzyme immunoassays (EIA), requires well-equipped laboratories with stable electrical power, calibrated pipettes, and EIA plate readers and washers. Such laboratories are not universally available in developing countries.

Filter paper assays offer the advantage of rapid test performance with minimal equipment. This study was designed to determine whether saliva HIV-1 antibody testing by one filter paper assay is sensitive, specific, acceptable to patients, and feasible in a Ugandan clinic population at high risk for HIV-1. The sensitivity and specificity of saliva HIV-1 antibody testing at different stages of clinical disease were assessed to define subgroups of patients for which saliva testing may be less sensitive. The effects of saliva testing on patients' beliefs about the risks of exposure to saliva from HIV-infected patients were examined in structured interviews prior to counseling and after completion of this study.

## MATERIALS AND METHODS

**Subjects.** All first-time adult visitors to the Mulago Hospital clinic for sexually transmitted diseases (STDs), located in Kampala, Uganda, were asked to participate in a precounseling anonymous interview between 14 April and 30 August 1993. Those patients desiring HIV-1 antibody testing and counseling were recruited to participate in the comparison of saliva and serum HIV-1 antibody testing. Enrolled patients provided written informed consent. Serum test results were made available to patients 3 weeks after the initial visit. Saliva test results were not provided to patients. The protocol was approved by the AIDS Research Subcommittee of the Uganda AIDS Commission and by the Committee on Human Research at University of California, San Francisco.

**Questionnaire.** Each patient underwent a precounseling anonymous interview regarding beliefs about the role of saliva in the transmission of HIV-1. The preenrollment interview was performed prior to any mention of the saliva antibody test or this study and prior to HIV-1 and STD counseling. Each patient

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enrolled in this study underwent a confidential poststudy interview immediately after blood and saliva specimens were collected. Each interview was performed in the patient's native language by a trained interviewer using a structured precoded format that had been developed in pilot testing of 20 patients from this clinic. Questions regarding HIV-1-related symptoms were developed during a survey of 7,330 STD clinic patients in Kampala (8).

**Saliva collection and analysis.** Saliva samples were collected with the Omni-sal device (Saliva Diagnostic Systems, Vancouver, Wash.). The Omni-sal device involves inserting a premanufactured cotton pad under the tongue until the pad is saturated and floppy. Approximately 1 ml of saliva is collected by this procedure. Each pad was then immediately placed in a stabilizing solution provided by the manufacturer which contains antiproteolytic and antimicrobial agents. The saliva samples were kept at room temperature for antibody testing the same day or kept at 4°C overnight. Prior to antibody testing, the cotton swab was removed from the plastic shaft with an electric vortex and a serum separator plunger filter was used to remove cotton fragments from the saliva eluate. Abbott HIV-1/HIV-2 rDNA TestPack EIA (Abbott Laboratories, Abbott Park, Ill.) was performed and interpreted according to the manufacturer's instructions for serum testing except that a larger volume of saliva was used and the TestPack filter was not used, except as noted, because the saliva eluate had been filtered during extraction from the cotton swab. Briefly, 500 μl of saliva eluate was mixed with 5 drops of diluent and poured directly onto the prewetted TestPack filter containing embedded recombinant HIV-1 and HIV-2 antigens. Then a wash buffer was poured through the filter, followed by an alkaline phosphatase-conjugated secondary antibody solution. After a 3-min incubation, a wash buffer was poured through the filter, followed by a chromogen developer. Color development was stopped after 2 min by pouring a stop solution through the filter. A positive test was indicated by +, and a negative test was indicated by − on the filter paper. All the reagents and equipment needed for the assay were supplied with the kit. All steps were carried out at room temperature. The saliva samples remaining after TestPack testing were frozen at −70°C. HIV-1 Western blotting (immunoblotting) was performed on all EIA-reactive saliva specimens with commercially available kits (Nova Path HIV-1 Immunoblot; Bio-Rad) modified for use with saliva by increasing the incubation time to overnight and by increasing the test fluid to 300 μl of the saliva eluate. These modifications were shown in prequalification testing to increase the sensitivity of the Western blot assay (unpublished data). A positive saliva Western blot was defined as one demonstrating at least two envelope bands (gp160, gp120, or gp41) according to the World Health Organization criteria (18).

**Serum analysis.** Separated serum samples were stored at −20°C for no more than 1 week prior to HIV-1 antibody EIA testing (HIV-1 Recombigen (*env* and *gag*); Cambridge Biotech Corp.). HIV-1 antibody testing was performed in singlet on all serum specimens according to the manufacturer's recommended procedures. Per the manufacturer's instructions, specimens were considered reactive when the specimen optical density (signal) was greater than the cutoff value calculated as 40% of the mean optical density of positive controls. To decrease the chance of false-positive tests because of nonspecific antibody binding, we retested in duplicate specimens that were reactive when the signal-to-cutoff (S/CO) ratio was greater than or equal to 1.0 but less than 2.0. Western blot testing (Nova Path HIV-1 Immunoblot; Bio-Rad) was performed when the S/CO ratio was repeatedly between 1.0 and 2.0, when there were discrepancies between saliva and serum antibody tests, and on 75% of reactive HIV-1 EIA with S/CO ratios of ≥2.0. A positive saliva Western blot was defined as one demonstrating at least two envelope bands (gp160, gp120, or gp41) according to the World Health Organization criteria (18). Serum samples from HIV-1 antibody-positive individuals were also tested for beta-2 microglobulin (beta-2 microglobulin EIA; Coulter Corp., Miami, Fla.) according to the test kit manufacturer's recommendations.

**Data analysis.** The laboratory staff performing the assays were blind to the results of testing for paired specimens. The identifying codes matching saliva and serum specimens were kept by two of the investigators (R.M.G. and E.M.P.). All data were entered, verified, and analyzed by using Statistical Analysis Systems software (SAS Institute Inc., Cary, N.C.). EIA plate optical densities were downloaded from the reader by using Softmax software (Molecular Devices) and exported to SAS data files for interpretation and verification. The statistical tests used are identified parenthetically in Results.

## RESULTS

**Patient characteristics.** Paired specimens were obtained from 502 patients. The median age of these patients was 26 years, and the median duration of schooling was 8 years. Of these patients, 50% were men, 52% were from the Buganda tribe, and 72% were resident in Kampala. These characteristics were representative of patients visiting the Mulago STD clinic between September 1989 and August 1993. The overall seroprevalence by serum EIA was 41% (205 of 502). Initial EIA testing indicated that 11 of 206 specimens had S/CO ratios of between 1.0 and 2.0. Of these, 10 had S/CO ratios of greater than 2.0 on repeat testing and were Western blot positive. One specimen was indeterminate by repeat EIA and Western blotting and was excluded from analysis. Western blot assays were positive for all reactive saliva EIA tests (197 of 197) and for all EIA-reactive sera (152 of 152) from patients with EIA-reactive saliva.

TABLE 1. Comparison of saliva and serum HIV-1 antibody testing results

| Saliva EIA result[a] | No. of patients with serum EIA result[b] | | Total no. of patients |
|---|---|---|---|
| | Negative | Positive | |
| Negative | 295 | 10[c] | 305 |
| Positive | 2[d] | 195 | 197 |
| Total | 297 | 205 | 502 |

[a] The initial TestPack result.
[b] The initial Cambridge Biotech EIA result.
[c] TestPack EIA were reactive for 10 of 10 sera. Western blot assays were positive for 9 of 10 sera and indeterminate for 1 of 10 sera. Repeat TestPack EIA testing without the filter was nonreactive for 8 of 10 saliva specimens and reactive for 2 of 10 saliva specimens. Among saliva specimens that were nonreactive on repeat TestPack EIA testing, Cambridge Biotech EIA were nonreactive for four and indeterminate for three and one could not be tested because of an insufficient quantity of saliva. Western blot assays were negative for one and indeterminate for four, and three could not be tested because of an insufficient quantity of saliva. Among saliva specimens that were reactive on repeat testing by TestPack EIA, one of two was also reactive by Cambridge Biotech EIA and positive by Bio-Rad HIV-1 Western blotting; the other was nonreactive by Cambridge Biotech EIA and there was an insufficient quantity of saliva for Western blot testing.
[d] Both sera were nonreactive by TestPack EIA and negative by Western blotting. Both saliva specimens were reactive by repeat testing with TestPack EIA and Cambridge Biotech EIA and were positive by Western blotting.

**Sensitivity.** Initial testing with TestPack using saliva samples from serum HIV-1 antibody-positive patients had a sensitivity of 95% (195 of 205 [Table 1]). Eight of the 10 initially false-negative saliva tests were repeatedly negative with TestPack, and 2 were positive when the same specimens were retested. Three of the eight patients with repeatedly false-negative TestPack results were recruited for repeat specimen collection and testing using a modified protocol. The saliva testing protocol was modified to include the TestPack filter that had not been used during initial testing. The modified saliva Test-Pack assay was positive for all three patients when the filter was used and was negative for two of three patients when the filter was not used. Repeat serum testing was positive for these three patients. The saliva specimens that initially tested negative could not be retested by the modified protocol because insufficient volumes of saliva remained. Sera from all 10 patients with initially false-negative saliva tests were tested with TestPack and found to be positive.

**Beta-2 microglobulin and HIV-1-related symptoms.** Mean beta-2 microglobulin levels were lower in patients with false-negative saliva tests than in patients with true-positive saliva tests (5.0 versus 3.0 μg/ml; Wilcoxon rank sum test; $P = 0.0005$). Similarly, the sensitivity of initial saliva antibody testing increased from 87% (49 of 56) for patients with normal levels of beta-2 microglobulin in sera (<3.5 μg/ml) to 98% (146 of 149) for patients with elevated levels of beta-2 microglobulin in sera (Fischer's exact test; $P = 0.005$). The sensitivity of saliva antibody testing was also higher for more-symptomatic patients (chi-square test; $P = 0.004$) (Table 2). The symptoms that have been found to be HIV-1 related in these STD patients include sweats (>1 month), cough (>1 month), weight loss, diarrhea, fevers (>1 month), and pruritic skin rash. The

TABLE 2. Sensitivity and specificity of saliva HIV-1 testing by stage of infection

| No. of HIV-1-related symptoms | HIV-1 seroprevalence (%)[a] | Specificity (%)[b] | Sensitivity (%)[c] | Mean beta-2 microglobulin level ± SD (μg/ml)[d] |
|---|---|---|---|---|
| 0 | 21.7 (36/166) | 100.0 (130/130) | 83.3 (30/36) | 4.0 ± 1.5 |
| 1–3 | 47.5 (106/223) | 99.1 (116/117) | 96.2 (102/106) | 4.8 ± 2.3 |
| 3–6 | 67.7 (42/62) | 100.0 (20/20) | 100.0 (42/42) | 5.7 ± 2.9 |

[a] No. of positive serum tests/total no. of serum tests noted parenthetically.
[b] No. of negative saliva tests/no. of negative serum tests noted parenthetically.
[c] No. of positive saliva tests/no. of positive serum tests noted parenthetically.
[d] In patients with positive serum tests.

mean beta-2 microglobulin levels were higher among HIV-1 antibody-positive patients with increasing numbers of HIV-1-related symptoms present at the time of the interview (analysis of variance; $P = 0.004$) (Table 2).

**Specificity.** Initial testing with TestPack using saliva samples from serum HIV-1 antibody-negative patients indicated a specificity of 99% (295 of 297 [Table 1]). Two saliva specimens were repeatedly positive by TestPack HIV-1/HIV-2, Cambridge Biotech HIV-1 EIA, and Bio-Rad HIV-1 Western blotting. The corresponding serum samples tested negative by all three methods. A nonreactive TestPack HIV-1/HIV-2 test of a serum sample effectively rules out infection with HIV-2. HIV-2 infection has not been found in blood bank screening of the Ugandan population (data not shown). These two patients may have had false-negative serum EIAs and Western blots. One patient had a beta-2 microglobulin level of 5.0 μg/ml, which has been found in less than 5% of HIV-1-negative Ugandans (15). Mislabeling of specimens is always a possible explanation for discrepant results, although mislabeling has not been a general problem in this setting and no discrepancies were found between patient and specimen identifiers. These two patients were not available for repeat specimen collection or PCR testing.

**Effects of saliva testing on beliefs about how HIV-1 is transmitted.** The majority of STD clinic patients understood that HIV-1 is transmitted by sexual intercourse, is possibly or very unlikely to be transmitted by saliva, and is very unlikely to be transmitted by shaking hands with an HIV-1-infected person (Table 3). In a structured interview after counseling and saliva sampling, a patient who had initially reported that exposure to saliva could possibly transmit HIV-1 was more likely to report less concern about transmission from saliva than to report more concern (Wilcoxon signed rank test; $P = 0.0001$) (Table 3).

## DISCUSSION

The specificity of saliva testing compared with that of serum testing was 99%. The sensitivity of saliva HIV-1 antibody testing was 95% when the TestPack filter was not used. The sensitivity may increase to as high as 100% when the filter is used, although only three patients were repeatedly tested with the modified protocol. Use of the TestPack filter is unlikely to compromise the specificity of the saliva assay, but this was not addressed by these data.

The sensitivity of saliva antibody testing observed in this study was less than expected on the basis of previous studies using TestPack (1, 4, 11, 16). These studies indicate that TestPack saliva antibody testing, performed in well-controlled settings, is close to 100% sensitive and specific. We doubt that inadequate saliva collection or test kit degradation in Uganda accounts for the loss of sensitivity because saliva specimen volumes were as high expected and repeat TestPack testing with new kits yielded persistently false-negative tests unless the TestPack filter was used. These data suggest that use of the TestPack filter with saliva samples is essential to provide sensitivity comparable to those of serum antibody testing and saliva GACELISA testing. The importance of the TestPack filter was surprising given that the saliva eluate was filtered during extraction from the cotton swab. We speculate that the TestPack filter may serve to slow the flow of antibodies through the antigen-embedded filter as well as remove residual debris that may interfere with antibody-antigen binding.

The increased sensitivity of saliva testing in more-symptomatic patients has not been described previously. The loss of salivary anti-HIV-1 immunoglobulin A antibodies that occurs during symptomatic HIV-1 infection (12, 17) is not expected to affect the sensitivity of TestPack because the secondary antibody used is specific for human immunoglobulin G according to the package insert (Abbott Laboratories). The hemoglobin concentration in saliva has been found to be higher in HIV-1-infected patients with low circulating counts of CD4 lymphocytes (14) probably because of frank bleeding from HIV-1-related oral pathology, such as candida stomatitis and aphthous ulcers. Transudation of serum or frank bleeding into the oral cavity would be expected to increase the concentration of immunoglobulin G in saliva (9) and probably accounts for our observation of increased sensitivity of saliva antibody testing in more-symptomatic patients. Physical examination of the oral cavity was not performed in this study, but we commonly find candidiasis and aphthous ulcers in hospitalized Ugandan patients with AIDS.

The lower sensitivity of saliva HIV-1 antibody testing in asymptomatic patients will decrease the negative predictive

TABLE 3. Effects of saliva testing on patient assessments of the risk of saliva exposure

| Mode of transmission | Patient assessments of HIV-1 risk | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Before counseling | | | | After counseling and saliva testing[a] | | | | |
| | No. of responses | % of responses | | | No. of responses | % of responses | | | $P^b$ |
| | | Very likely | Possible | Very unlikely | | Very likely* | Possible | Very unlikely** | |
| Shaking hands | 473 | 0.2 | 1.1 | 98.7 | 5 | 0.0 | 80.0 | 20.0 | NS |
| Sharing a straw | 438 | 0.2 | 13.2 | 86.5 | 58 | 1.7 | 65.5 | 32.8 | 0.0001 |
| Sharing a toothbrush | 433 | 4.4 | 61.0 | 34.6 | 264 | 1.9 | 82.6 | 15.5 | 0.0001 |
| Kissing mouth to mouth | 403 | 5.5 | 25.3 | 69.2 | 102 | 1.0 | 64.7 | 34.3 | 0.0001 |
| Sexual intercourse | 490 | 99.4 | 0.2 | 0.4 | 1 | 100.0 | 0.0 | 0.0 | NS |

[a] Among patients who responded "possible" before counseling.
[b] * versus ** by Wilcoxon signed rank test. NS, not significant.

value of saliva testing in population-based studies in which most subjects have minimal or no symptoms. The effect of lower sensitivity in residential populations is balanced by the lower prevalence of HIV-1 infection that is expected in nonclinical settings. Decreased HIV-1 prevalence tends to increase negative predictive value. We conclude that saliva testing, as performed here, is sufficiently accurate for both surveillance of low-risk populations and for the clinical evaluation of symptomatic patients.

Implementation of antibody tests in developing countries is often difficult because of the scarcity of technicians and well-equipped laboratories. In this study, saliva collection and antibody testing were performed in the STD clinic by a microbiology technician with no immunology training. Supervised performance of 10 tests was sufficient for mastery of the procedure, and the remaining tests for this study were performed without supervision or proficiency monitoring. Saliva collection was performed by the patients themselves after simple instructions. We believe that health care workers available in all regions of Uganda could master the saliva collection and testing procedures used here.

The lack of well-regulated electrical power is often another barrier to the implementation of antibody tests. Electricity was required for the refrigeration of TestPack kits and for the vortex needed to fully remove and disintegrate saliva-saturated cotton pads. A manual method to disintegrate pads, recommended by the manufacturer, involves flicking the Omni-Sal device against a hard surface. This procedure yielded the desired volume of saliva eluate mixture, but subsequent antibody testing was not sensitive (data not shown). Other manual methods for disintegrating pads should be developed so that antibody testing of saliva specimens could be performed in settings where electrical power is not available or not well regulated. Although refrigeration using electrical, kerosene, or solar power is available in many parts of Uganda, a kit that did not require refrigeration would be ideal.

The cost of assays affects how widely they can be used in developing countries. We estimate that HIV-1 antibody EIA using serum and microwell plates in Uganda costs $6.56 per determination while saliva testing would cost $7.87 per determination. These estimates include the test kits, disposable supplies, staff time, equipment depreciation, and specimen collection. Saliva testing involves less expensive specimen collection, no equipment depreciation, and less staff time, but these are relatively minor components of the total cost of HIV-1 testing in settings where laboratory facilities have been established and salaries are relatively low. Nevertheless, saliva testing using TestPack would be cost-effective in settings where facilities needed for microwell assays are not available or where performing antibody tests at the clinical facility would avoid the transport of specimens and results over long distances.

We were concerned that patients would develop an inappropriate fear of exposure to saliva after participation in this study. Exaggerated fear of exposure to saliva would compromise the capacity of families to care for ill relatives and may undermine the promotion of sexual behaviors, like condom use and monogamy, that are known to be protective against HIV-1 transmission. Our counseling included statements that the risk of HIV-1 transmission from contact with saliva is low relative to the risk from sexual intercourse and that saliva tests measure the patient's response to HIV-1 infection rather than the infection itself. During exit interviews, patients tended to indicate that exposure to saliva (e.g., sharing a straw, kissing mouth to mouth, and sharing a toothbrush) was less likely to transmit HIV-1 than they had indicated upon entry to the clinic. There were no control groups for this aspect of the study, so we cannot separate the effects of saliva testing and counseling, clinical examination and treatment, general HIV-1 and STD counseling, and repeated interviews. Nevertheless, these data suggest that HIV-1 antibody testing using saliva samples in Uganda does not foster fear of saliva when appropriate counseling is provided.

No patient who was undergoing blood sampling for HIV-1 testing and counseling refused to enroll in the comparative trial involving saliva collection. We expect that saliva testing would be preferred over serum testing by most Ugandans, especially participants in prospective studies that require repeated testing or population-based studies that require specimen collection in the field. Saliva testing for HIV-1 antibodies is specific, sensitive, and feasible in Uganda. These findings should encourage the development of less expensive, rapid saliva tests that do not require electricity.

## ACKNOWLEDGMENTS

This study was supported in part by Abbott Laboratories and by National Institute of Allergy and Infectious Diseases grant AI-32395.

We thank John Greenspan for helpful discussions and Sam Kabengera for technical assistance.

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