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Evaluation of Two Novel Immunoassays Designed to Detect HIV Antibodies in Oral Fluids

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The testing of oral fluid samples for the detection of HIV antibodies offers several advantages over the testing of blood. Our objective was to evaluate a new generation of rapid and simple assays designed specifically to detect HIV-1 and HIV-2 antibodies in oral fluids (saliva). Serum and oral fluid pairs were collected from 615 high- and low-risk individuals in the United States, Peru, and the Ivory Coast. Two different oral fluid collection devices and rapid assay systems included: (1) the Orapette/SalivaCard HIV-1/HIV-2 and (2) the Omni-Sal/ImmunoComb II HIV-1 and HIV-2. The corresponding serum pairs were analyzed by conventional ELISAs, and all reactive sera were confirmed with HIV-1 and HIV-2 Western blots. The results indicated a 100% sensitivity for both rapid oral fluid assays, including successful detection of HIV-2 antibodies. Specificities ranged from 99.8% to 100%. One sample produced a reactive result by the SalivaCard while being nonreactive by the other assays including the Western blots. Both assays performed excellently, indicating that antibodies to HIV can be detected reliably in oral fluids by simple and rapid assays. This combination of rapid testing technology and the use of easily collected oral fluid samples offers an efficient and accurate alternative to conventional testing and can be appropriately applied to a variety of testing situations for the laboratory diagnosis of HIV infection. J. Clin. Lab. Anal. 11:63–68.

Key words: rapid assays; saliva; diagnosis; retroviruses; serology

INTRODUCTION

The demonstration of specific antibodies in blood is the most commonly used means for detecting HIV infection. Rapid HIV assays, introduced in the late 1980s, added a new alternative to ELISAs for the diagnosis and monitoring of infection (1). At about the same time, oral fluid testing for HIV antibodies was successfully accomplished, although sensitivities were generally low (2–5). The low sensitivity was not due to the absence of antibodies in the fluid but rather to a relative insensitivity of the assays for detection of low quantities of immunoglobulin. This was proven later with the development of exquisitely sensitive assays, specifically designed for the testing of oral fluids for HIV IgG antibodies (6). Furthermore, the availability of specifically constructed oral fluid collection devices offered preferential collection of appropriate fluids (crevicular fluid) in a standardized manner. A detailed review of oral fluids, the use of tests to detect HIV antibodies in this medium, and the advantages and disadvantages of this testing strategy have been published (7).

Oral fluids (whole saliva) contain pure saliva from the salivary glands and crevicular fluid, which is a plasma transudate derived from the capillary bed beneath the tooth-gum margin. This transudate contains components similar to those in plasma but at a lower concentrations due to the dilutional effect of pure saliva. Hence, the testing of oral fluid for HIV is actually aimed at detection of the same type of antibodies as with tests designed for testing serum. A more detailed review of the physiology of oral fluid production and its constituents can be found elsewhere (8, 9).

The use of alternative testing algorithms, such as the use of rapid assays or alternative confirmatory strategies such as ELISAs followed by rapid assays, can offer more simplified and cost-effective approaches for the diagnosis of infection (7, 10). Rapid assays to detect HIV antibodies in serum were introduced as manufacturers strived to drive technology forward to produce tests that utilize novel principles, simple performance characteristics, and possessed the sensitivity

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necessary for detection of antibodies during established and early infection. Oral fluids as a medium for infectious disease testing have now been added as yet another alternative, appropriate in certain testing situations (7, 11–14).

Two diagnostic systems that combine the use of appropriate and easy sample collection via specifically designed oral fluid collection devices and rapid test formats for detection of HIV antibodies are evaluated in the present study. One incorporates a modified dipstick technique using plastic combs to which HIV peptides are sensitized and can differentiate between HIV-1 and HIV-2 antibodies. The second is a rapid "card" test, which differs from a typical flow-through assay in that the sample diffuses along a network (chromatographic) where interaction with HIV peptide antigens occurs. We sought to evaluate these new technologies for their accuracy when testing samples from several geographic locations and by comparing results to established testing algorithms.

MATERIALS AND METHODS

Samples

Homologous pairs of serum and oral fluid samples were collected anonymously from 615 individuals, including 585 healthy adult volunteers at the University of Maryland (Baltimore) having unknown HIV status and individuals previously classified as HIV seropositive. The former were students and employees; the latter were patients of the Dental School HIV clinic, volunteers enrolled in studies conducted at the Medical Biotechnology Center, and patients from HIV outpatients clinics. Additional sample pairs from HIV-infected volunteers from the Ivory Coast (n = 5) and Peru (n = 51) were also tested in the study. The samples from the Ivory Coast were all previously confirmed positive for HIV-2 (only), whereas the samples from Peru originated from HIV-1 positive individuals. The study was approved by the Institutional Review Board of the UMAB, and informed consent was obtained from all participants.

Blood was collected via venipuncture, and oral fluids were obtained using two different oral fluid collection devices: the Orapette (Trinity Biotech, Dublin, Ireland) and the Omni-Sal (SDS, USA). The Orapette is a rayon ball that is placed in the mouth and moved around the gum area until saturated. It contains no preservatives and collects whole saliva rich in crevicular fluid. The Omni-Sal consists of an absorbent pad on an applicator stick that is placed under the tongue until a color indicator signals appropriate sample volume. The fluid is subsequently placed in a transport medium containing preservatives. Sera were separated, oral fluids were transferred to vials, and sample pairs were stored at –20·C.

Testing

The oral fluid pairs, collected with the Orapette, were tested with the SalivaCard HIV-1/HIV-2 (Trinity Biotech), whereas the samples collected via the Omni-Sal device were tested with the ImmunoComb II HIV-1 and HIV-2 (Orgenics, Israel). Both assays are in vitro qualitative tests based on the indirect ELISA principle. In general, the SalivaCard consists of a solid matrix coated with synthetic HIV peptides (specific peptides not indicated) at a site (test site) removed from, but not connected to, the sample addition site. The sample (120 μl or three drops from the Orapette device) was added, followed by sequential additions of wash buffer, an enzyme-labeled antihuman IgG conjugate, another wash, and the addition of a substrate. Another side of the card (control site) lacked the peptide antigens to control for nonspecific binding of proteins. A blue color of equal or greater intensity in the test site as compared to the control site indicated a reactive result. The total time required to perform the assay was ~12 minutes per sample.

The solid phase of the ImmunoComb II is a plastic comb with 12 projections or teeth. Each tooth is sensitized in three spots, an upper spot with goat antibodies to human IgG, which acts as an internal control, a middle spot with HIV-2 synthetic peptides (derived from the conserved sequence of gp36), and a lower spot with HIV-1 synthetic peptides (derived from the conserved sequences of gp41 and gp120). The developing plate (reservoir) has six rows of 12 wells; each row contains a reagent solution ready for use at each different step in the assay. In the first step, oral fluid samples (100 μl) were added to the first row of wells in the developing plate. The test was performed stepwise, moving the comb from row to row, with incubations at each step. The results were observed as gray-blue spots on the surface of the teeth of the comb corresponding to HIV-1, HIV-2, and control. The assay requires ~35 minutes to complete, but 10 samples and two controls can be tested simultaneously.

Screening of the serum samples was performed with the Cambridge Biotech (Worchester, MA) Recombigen HIV-1 indirect enzyme immunoassay. Repeatedly reactive specimens were confirmed with an HIV-1 Western blot (Biotech/DuPont, Wilmington, DE) using the CDC criteria. HIV-2 positive samples were screened using a third-generation EIA (Abbott, N. Chicago, IL) and confirmed using a specific HIV-2 Western blot (Institute Pasteur, Paris, France). When using the rapid immunoassays, samples which had invalid or equivocal results when first tested were repeated as recommended by WHO (13). Oral fluid samples that yielded results in conflict with serum ELISA results were retested by the same technologist, as well as tested blindly by another person (14). Western blot assays were also performed on each sample that produced discordant results between the rapid assays and the ELISA. Testing of the oral fluid pairs was performed without knowledge of the results from the corresponding serum samples. The results obtained from the analysis of the oral fluid pairs were compared to results obtained from the ELISA/Western blots on the serum pairs. The diagnostic accuracy and usefulness of the tests were defined by the sensitivity,

specificity, predictive values, and efficiency of the assay as described elsewhere (14–16).

RESULTS

A total of 615 samples were tested by the SalivaCard and 589 by the ImmunoComb II. Several samples had to be repeated due to poor flow-through using the SalivaCard and due to equivocal reactions (weak) by the ImmunoComb II. When data were compiled, concordance using the rapid assays and oral fluids versus the ELISA using sera was 99.8% (614/615) for the SalivaCard and 100% (589/589) for the ImmunoComb II. Both rapid oral fluid assays detected all samples in which the corresponding serum pairs had been repeatedly reactive by ELISA and confirmed as positive, including the HIV-2 samples. The SalivaCard detected all 114 positive samples, including 51 from Peru and five HIV-2 positives from the Ivory Coast. The ImmunoComb II detected all 62 HIV seropositive samples, including four HIV-2 positive samples from Ivory Coast. This yielded a sensitivity of 100% for both rapid tests.

Of the samples in which the serum pairs had been classified as negative by ELISA, both the SalivaCard (n = 502) and the ImmunoComb II (n = 527) produced negative results on all but one of the oral fluid pairs. This latter sample produced a repeatedly positive result by the SalivaCard, but a negative result by the ImmunoComb II. When tested by HIV-1 and HIV-2 Western blots, this sample produced negative results. The SalivaCard, therefore, produced a specificity of 99.8% (501/502), a positive predictive value of 99.1% (114/115), and a test efficiency of 99.8% (615/616). The ImmunoComb II produced perfect test indices. All data are presented in Table 1.

DISCUSSION

A number of studies have indicated that several methods, including EIA, antibody capture assays, rapid assays, and Western blots can be used successfully to test oral fluids for HIV (7). In general, most of the assays have reported sensitivities of 95–100% and specificities between 98–100% for detecting antibodies to HIV using oral fluids. The relatively high specificity obtained by most assays using oral fluids supports the use of oral fluids as an epidemiological tool (7, 14, 17). Six of the 15 (18–32) most recent studies included 11 evaluations of EIA assays (some evaluations used the same EIA), with only four of the 11 EIAs having a sensitivity of < 98% (range 93–96%) (20, 22, 23, 27) and specificities generally between 98–100%. The less than optimal test indices from these studies were most likely due to the use of tests designed for use with serum samples, not oral fluids. In the largest comparative investigation of the use of oral fluids for detecting HIV antibodies, 1,955 subjects (300 seropositive, 1,654 seronegative, and 1 indeterminate) were tested by the GACELISA (28). The sensitivity and specificity were found to be 100% and 99.6%, respectively. A previous study of 1,880 paired samples (356 seropositive and 1,524 seronegative) demonstrated a sensitivity of 99.4% and specificity of 100% when using a commercial ELISA (36). These data illustrate the potential for accurate results when using oral fluids as a medium in surveillance studies. The consistently high specificity of HIV EIA tests with oral fluid samples may be attributable to the low background OD readings observed in negative oral fluid samples, probably due to the relatively low levels of immunoglobulin and protein in oral fluid (22, 33, 34). Several studies have shown that modifying serum-based tests such as the ELISA to perform with oral fluid samples can be accomplished. However, this has not been consistently successful with all assays (2, 35). The GACELISA was the first test introduced for use with body fluids other than blood and showed a high sensitivity, most likely attributed to its advanced technology (2, 6, 12). Improvements in oral fluid collection methods specifically designed to preferentially collect crevicular fluid from the capillary beds under the gums, optimizations of assay procedures, and advancements in technology have most likely led to the increased sensitivities observed in later studies (7).

Only a few studies have evaluated HIV antibody detection in oral fluid samples when using rapid assays. All assays were designed for use with serum or plasma samples (18, 22, 29, 30) and not oral fluids. The studies reported excellent results, although a lack of standardization might have contributed to varying results between different laboratories using the same assay. One study in Tanzania reported excellent sensitivities and specificities with 288 oral fluid samples (44 seropositive and 244 seronegative) collected by the OraSure device (Epitope, Beaverton, OR) and tested by two rapid serum assays (18). One rapid test exhibited a sensitivity of 100%, whereas the other assay had a sensitivity of 97.2%; both resulted in specificities of 100%. The second study tested whole saliva samples collected directly from 100 seropositive and 100 seronegative individuals using five HIV rapid commercial assays (22). Three of the five test kits were found to be unsatisfactory

TABLE 1. Comparison of Test Indices for Two Rapid Test Kits for Detection of HIV Antibodies in Oral Fluids From Seropositive and Seronegative Individuals[a]

	SalivaCard	ImmunoComb II
Total tested	615	589
(P/N)	(115/500)	(62/527)
ELISA total	615	589
(P*/N)	(114/501)	(62/527)
Sensitivity (%)	100	100
Specificity (%)	99.8	100
Test efficiency (%)	99.8	100
PPV (%)	99.1	100
NPV (%)	100	100

[a]P = positives, N = negatives, PPV = positive predictive value, NPV = negative predictive value.
*Confirmed by Western blots.

with oral fluid samples. Several other studies also evaluated the performance of a serum-based rapid assay modified to analyze oral fluid specimens and reported excellent results (29, 30, 31, 37).

In the present study, 615 oral fluid samples were collected anonymously and tested blindly by two novel rapid assay kits designed specifically for use with oral fluid samples. The results, when compared with conventional ELISA and Western blot testing of corresponding serum samples, were used to determine the sensitivity and specificity of the assays and to evaluate their usefulness. The results indicated a high degree of sensitivity and specificity with both rapid tests. The ImmunoComb II had perfect test indices, whereas the SalivaCard test had a sensitivity of 100% but with a slightly less than perfect specificity (99.8%). The seemingly "false-positive" result was unlikely to be a collection error, as sample collection materials were independently packaged and a corresponding false-negative was not found. In addition, the ImmunoComb II correctly classified this sample. An alternative explanation is that the serum sample produced a false-negative by the EIA, ImmunoComb II, and Western blots, and therefore represented a sample from an individual who had low levels of antibody, such that occurs during seroconversion. This can only be determined from a subsequently collected specimen.

Several factors probably contributed to the high sensitivity and specificity observed in the present study, particularly the use of extreme care to ensure matched pairs of oral fluid and serum. All collection supplies were labeled in code, and each set of supplies for one collection was contained in an individually sealable bag. One bag of collection materials was used at a time with each study subject. Also, the use of specifically designed oral fluid collection devices and the use of highly accurate immunoassays most likely contributed to the excellent results obtained in the study.

Oral fluids offer several advantages over blood as a medium for HIV testing (14). Sample collection may be safer because of the elimination of accidental needle-stick injuries and cuts from glass test tubes, and there is minimization of biohazardous waste materials. Disposable plastic devices are safer to discard than needles, lancets, and glass. Also, the infectious capacity of oral fluids can be considered negligible, making this a safe, noncontaminating medium to be used for testing (3). Oral fluids are easier and more convenient to collect, with only minimal training needed. In addition, large numbers of samples can be collected simultaneously from groups (17). As a noninvasive and painless method, oral fluid collection may be more acceptable compared to phlebotomy and offers a potential for a higher degree of collection compliance among subjects being tested for surveillance purposes, thereby reducing sampling bias (38). Individuals such as children, IV drug users, and obese people whose blood may be difficult to obtain can be easily sampled. In general, most of the volunteers in the present study, when asked, did not have complaints about either of the oral fluid collection devices, and ~75% of the volunteers preferred providing this medium rather than blood.

Conventional assays require sophisticated instrumentation such as spectrophotometers and additional equipment including incubators and washers. Therefore, rapid assays offer a viable option, particularly in developing countries where laboratory conditions might be less than optimal due to limited resources, lack of equipment, inadequate maintenance, or electrical shortages (18). In fact, oral fluid testing for HIV antibodies has been investigated in independent studies in several developing countries, including Mexico (36), Myanmar (17), Tanzania (18, 39, 40), Thailand (28, 41), Guinea-Bissau (26), the Ivory Coast (42), Peru (43), and Zaire (33). The sensitivities and specificities reported ranged from 95–100% and 98–100%, respectively. The results were similar to those demonstrated by studies performed in developed countries, including Europe (6, 23, 25, 44, 45, 46, 47), Canada (38, 48) and the United States (17).

Other advantages for considering this testing strategy include the simplicity and short turnaround times. Results are obtained in 12–15 minutes with the SalivaCard and in 36–40 minutes (10 samples) with the ImmunoComb II compared to conventional ELISAs, which usually require 3–4 hours. The ImmunoComb II provides documentation of the results as well as an internal control that indicates the presence of total IgG in the sample. Similarly, the SalivaCard has a site separate from the test site to detect the presence of nonspecific binding by substances present in the sample. Thus both systems provide a built-in quality assurance measure to invalidate any results due to nonspecific causes. This is important, especially in developing countries where formal programs for laboratory medicine are not available and quality assurance measures need improvement.

Several disadvantages of the oral fluid collection/testing systems include: (1) dislike of the taste and texture of the rayon ball and refusal to leave it in the oral cavity the entire time, (2) complaints about the length of time the Omni-Sal device required to obtain sufficient sample volume to change the indicator system (the Omni-Sal devices usually took ~2–3 minutes to become saturated, but many required 5–10 minutes), (3) problems with dry mouth due to the medications being administered and, therefore, some difficulty in providing a sufficient sample; Sjogren's or Sicca syndrome may cause dry mouth and low sample volumes (49), and (4) both assays required much larger sample volumes than serum tests, most likely for the detection of the lower levels of immunoglobulin in oral fluid.

There are many practical applications where the use of these assays would be attractive. The ease of performance allows the rapid assays to be appropriate for low-volume testing such as in small clinics, emergency rooms, dental clinics, small-scale laboratories including small blood banks where results may be needed immediately, private physician offices, point-

of-care (POC) testing, autopsy rooms, and for insurance company testing (1, 18, 50). POC testing, testing in physicians' offices, and decentralized testing reflect changes in the health care system and are appropriate situations where this testing strategy could be realized (1). These assays allow individuals with more varied credentials to perform the testing since the tests are simple and incorporate a quality control measure to assure correct performance.

In summary, technology has evolved to produce accurate, rapid, easy, and cost-effective tests with incorporated quality control measures. New advances have produced rapid tests that have the sensitivity required to detect successfully the low levels of antibody in oral fluid. Oral fluid-based tests offer alternatives for a variety of testing situations, for increased safety, and for use in certain populations (e.g., the Dai ethnic group in China and the Maasai in Tanzania and Kenya) who oppose giving blood specimens. Rapid tests offer important advantages for HIV testing, such as POC testing, for use where facilities cannot support electricity or where personnel have not been adequately trained for performing EIAs, and when results are needed immediately (i.e., emergency blood transfusions). The merging of tests that use oral fluid samples and rapid test technology now offers further possibilities for a variety of testing situations. The findings in the present study, using this combination of technologies, have shown the potential for accurate and effective HIV testing and suggest that these novel test systems can be added to the arsenal of methods to detect HIV infection.

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