Detection of Antibodies to Human Immunodeficiency Virus Type 1 in Oral Fluids: A Large-Scale Evaluation of Immunoassay Performance

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Paired serum and oral-fluid (OF) specimens ($n = 4,448$) were collected from blood donors and patients attending local sexually transmitted disease clinics in Trinidad and Tobago and the Bahamas and were tested for the presence of human immunodeficiency virus type 1 (HIV-1) antibodies. Sera were tested by Abbott AB HIV-1/HIV-2 (rDNA) enzyme immunoassay (EIA), and positive specimens were confirmed by Cambridge HIV-1 and HIV-2 Western blotting (WB). OF specimens were collected with the OraSure collection device and were tested by Murex GACELISA and by two EIAs from Organon Teknika (the Oral Fluid Vironostika HIV-1 Microelisa System [OTC-L] and the Vironostika HIV-1 Microelisa System [OTC-M]). EIA-reactive OF specimens were confirmed by miniaturized WB (OFWB). GACELISA detected all 474 HIV-1 seropositive specimens (sensitivity, 100%). OTC-L detected 470 positive specimens (sensitivity, 99.2%), while OTC-M detected 468 positive specimens (sensitivity, 98.8%). Specificities ranged from 99.2 to 100% for the three assays. Concordance of OFWB with serum WB was 99.4%, and banding patterns determined by the two methods were similar. The immunoglobulin G (IgG) concentration of OF specimens ranged from 0.21 to 100 µg/ml, with a mean of 17.1 µg/ml. Significant differences in OF IgG concentrations were observed between HIV antibody-positive and HIV antibody-negative persons (31.94 versus 15.28 µg/ml, respectively [$P < 0.0001$]). These data further confirm the suitability of OF specimens for detection of HIV-1 antibodies. Currently available HIV-1 antibody assays provide sensitivities and specificities with OF specimens comparable to those achieved with serum specimens.

The use of oral fluid (OF) as a specimen for the detection of antibodies to infectious agents has become increasingly popular since the initial description of the technique in the 1980s (1, 2, 33). OF is a mixture of saliva, mucosal and bacterial products, and gingival crevicular fluid (34, 36). The use of OF for human immunodeficiency virus (HIV) antibody testing has generated particular interest in the AIDS research community since OF is easier to collect than serum or plasma samples and patients are more willing to provide OF than blood (7). Specialized collection devices for OF which ensure sufficient specimen volumes, stabilize immunoglobulins, inhibit proteolytic enzymes, and retard microbial growth have now been developed. One of these devices (OraSure; Epitope, Inc., Beaverton, Oreg.) and an associated enzyme immunoassay (EIA) and Western blot (WB) method have recently been licensed by the U.S. Food and Drug Administration (FDA) for detection of HIV antibodies in OF.

Immunoglobulins in OF are of the immunoglobulin A (IgA) and IgG classes, but investigations have shown that the primary reactivity to HIV antigens is due to IgG derived from gingival crevicular fluid (10, 18, 32) or possibly from local synthesis (26). Although the concentration of IgG in OF is substantially lower than that in serum (by 800 to 1,000 times) (32, 34), modification of existing EIAs has resulted in sensitivities and specificities comparable to those observed in matched serum tests (3, 6, 11–15, 20, 22–25). Confirmation of HIV antibodies

by serum WB assays modified for OF specimens, however, is significantly affected by the reduced IgG concentrations. These methods generally have not yielded banding patterns similar to those of matched serum specimens tested by conventional WB assays (2, 3, 12, 13, 23, 38). Recently, an FDA-approved WB method which improves specific HIV antibody detection in OF was introduced (17). Our previous report described a miniaturized WB technique which allowed detection of HIV antibody banding patterns consistent with those derived from matched serum specimens (20).

These recent advances affecting the use of OF specimens have led to the initiation of large-scale studies to compare HIV antibody assay results for OF specimens with those from matched serum specimens in field evaluations (15, 17, 35). In this study, we evaluated current OF testing strategies in a large survey including sites of low and high HIV prevalence to compare the sensitivities and specificities of HIV antibody assays with OF specimens to those of routine serum HIV antibody tests.

MATERIALS AND METHODS

Study population. The patient population was selected from areas of high and low HIV prevalence. Blood donors were recruited from the blood collection center in Port-of-Spain, Trinidad and Tobago, which has a seroprevalance of approximately 0.3%. The high-prevalence sites included the Queen's Park Counseling Center, an HIV clinic in Port-of-Spain, Trinidad and Tobago, and the Comprehensive Health Clinic, a sexually transmitted disease (STD) center in Nassau, Bahamas. All participants came to the sites for either routine HIV screening or blood donation. Subjects were informed of their HIV status through channels previously established at the collection sites. Each participant received an explanation of OF collection and provided consent documentation prior to

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TABLE 1. Demographic data of study participants
at the three survey sites

Characteristic	No. of subjects from[a]:			
	Site 1	Site 2	Site 3	All sites
Sex				
Male	1,552	634	821	3,007
Female	449	367	600	1,416
Not reported	1	1	23	25
Age (yr)				
<10	0	1	4	5
10–19	81	112	155	348
20–29	702	394	570	1,666
30–39	747	290	485	1,522
40–49	376	132	128	636
50–59	84	47	47	178
>60	11	24	30	65
Not reported	1	2	25	28
Reason for providing specimen				
Blood donor	2,000	0	2	2,002
STD	1	404	624	1,029
High-risk contact	0	243	165	408
HIV contact	0	32	40	72
Intravenous drug use	0	15	19	34
Symptoms compatible with AIDS	0	137	23	160
Other	0	169	546	715
Not reported	1	2	25	28
Total subjects	2,002	1,002	1,444	4,448

[a] Site 1, blood collection center, Port-of-Spain, Trinidad and Tobago; site 2, Queen's Park Counseling Center, Port-of-Spain, Trinidad and Tobago; site 3, Comprehensive Health Clinic, Nassau, Bahamas.

sample collection. Epidemiologic and demographic data were collected for each survey participant.

Sample collection. Serum specimens were obtained by venipuncture and used for reference antibody testing. OF samples were collected simultaneously in duplicate with the FDA-licensed OraSure collector (Epitope, Inc.). The device, consisting of a fiber pad on a plastic wand, is placed between the cheek and lower gum, agitated gently to induce flow from the gingival crevicular spaces, and held in place for an additional 2 minutes. The pad was placed into a transport tube containing a preservative solution and stored at 4°C until shipment (approximately 10 to 14 days) to the Centers for Disease Control and Prevention (CDC), Atlanta, Ga. Upon arrival, the collectors were centrifuged at 2,000 rpm in a TJ-6 centrifuge (Beckman Instruments, Fullerton, Calif.) for 10 min and the two OF from each individual were pooled. The samples were aliquoted, with a portion retained for immediate testing and the remainder placed into long-term storage at −20°C per the manufacturer's instructions.

EIA. All serum specimens were tested at CDC by the Abbott AB HIV-1/HIV-2 (rDNA) EIA kit to detect antibodies to HIV-1 and HIV-2. Specimens found initially to be reactive were retested in duplicate, and specimens reactive in at least two of the three tests were confirmed by WB. OF specimens were tested by an IgG immunocapture assay, GACELISA (Murex Diagnostics, Dartford, England), and by two indirect EIAs, the Oral Fluid Vironostika HIV-1 Microelisa System (OTC-L) and the Vironostika HIV-1 Microelisa System (OTC-M) (Organon Teknika Corporation [OTC], Research Triangle Park, N.C.). OTC-L and GACELISA were designed for OF and were used according to the manufacturers' protocols. OTC-M is currently licensed for serum testing but has not been recommended or thoroughly evaluated for use with OF specimens. OF samples (100 μl) were diluted with equal volumes of the specimen diluent provided in the kit and were tested according to the OTC-M assay's protocol for serum or plasma.

WB. Sera which were reactive with the Abbott EIA were tested with an HIV-1 WB assay (Cambridge Biotech Corporation, Cambridge, Mass.) according to the manufacturer's protocols. OF specimens were assayed by miniaturized WB (OFWB) as previously described, with a few modifications (20). Following specimen incubation, the conjugate (goat anti-human IgG H+L alkaline phosphatase; Kirkegaard and Perry Laboratories, Gaithersburg, Md.) was diluted 1:250 in milk diluent, added to the lanes of the miniblot apparatus, and incubated for 1 h. The blots were developed with NBT-BCIP (nitroblue tetrazolium–5-bromo-4-chloro-3-indolylphosphate) (Kirkegaard and Perry) single-component sub-

strate for 10 min, washed with deionized water, and air dried prior to scoring. Two experienced personnel scored each blot and recorded all visible bands. Criteria for positive results were based on the definition devised by the CDC and the Association of State and Territorial Public Health Laboratory Directors (5).

Quantitative IgG immunoassay. Quantitation of human IgG in OF was performed according to the in-house immunoassay method previously described (20). Specimens were diluted 1:1,000 in 0.15 M phosphate-buffered buffered saline, pH 7.2, so that the optical density measurements for the samples would fall on a standard curve. Final absorbance readings were taken with an SLT microplate reader equipped with SOFT2000 software (Tecan U.S., Hillsborough, N.C.). The software prepared a standard curve with the best fit for a second-order polynomial and calculated individual specimen IgG concentrations from the averages of duplicate tests referenced from the standard curve.

RESULTS

Demographic data. Specimens were collected from 4,448 individuals at three sites (Table 1). The blood collection center ($n = 2,002$) was a low-HIV-prevalence site (0.25%). The two STD clinics (Queen's Park Counseling Center [$n = 1,002$] and Comprehensive Health Clinic [$n = 1,444$]) were high-HIV-prevalence sites (18 to 20%). Overall, the male-to-female ratio was approximately 2:1, but the ratio varied according to the site: the blood collection center had an approximate ratio of 3:1, whereas the two STD clinics had ratios closer to 3:2. The age distribution of the sample population was similar at the three sites, with the majority of the population (70%) between 19 and 39 years old for all three sites. The principal reasons for testing at the STD clinics were the presence of existing STD or recent contact with an HIV-infected or high-risk individual. Approximately one-third of the respondents at the Comprehensive Health Clinic did not indicate a primary reason for being tested.

EIA. Fourteen specimens did not have complete sample sets (which prevented completion of the testing algorithm) and were removed from the analysis. Additionally, two sets of specimens (matched serum and OF) were determined to have been labeled in error in the field and were also removed from the data set. Initial testing of the serum specimens by EIA (Abbott) and WB identified 474 HIV-1 antibody-reactive, 3,948 HIV antibody-negative, and 8 indeterminate specimens. Since the true infection status of the eight indeterminate specimens could not be ascertained, they were not included in the rest of the analysis. One sample had an unusual HIV-1 WB banding pattern and was determined to be an HIV-2 infection by additional HIV-2 serum EIA and WB testing. This specimen was included in the 4,422 specimens used in the analysis of EIA and WB performance.

The abilities of the three EIAs to detect HIV antibody in the OF specimens compared with the matched serum specimens is shown in Table 2. GACELISA detected HIV-1 antibodies in all of the 474 seropositive specimens, for a sensitivity of 100%. OTC-L detected HIV-1 antibody in 470 specimens, for a sensitivity of 99.2%, while OTC-M detected HIV-1 antibody in 468 specimens, for a sensitivity of 98.8%. The HIV-2 specimen was detected as antibody positive by all three OF EIAs. The

TABLE 2. Sensitivities and specificities of serum
and OF EIAs versus serum WB[a]

Serum WB result	No. of specimens					
	GACELISA		OTC-L		OTC-M	
	P	N	P	N	P	N
P	474	0	470	4	468	6
N	8	3,940	33	3,915	0	3,948

[a] P, HIV-1 antibody positive; N, HIV-1 antibody negative.

FDA-licensed OTC OF EIA (OTC-L) had the largest number of antibody false-positive specimens, 33, followed by GACELISA, with 8. These OF specimens were repeatedly reactive by OTC-L or GACELISA but were found to be HIV antibody negative by OFWB and were HIV antibody negative by the matched serum assay results. OTC-M did not have any repeatedly reactive HIV-1 antibody-negative OF samples. The specificities of OTC-L, GACELISA, and OTC-M were 99.2, 99.8, and 100%, respectively. The positive predictive values were 98.3% for GACELISA, 100% for OTC-M, and 93.5% for OTC-L. The negative predicative values were ≥99.9% for all assays, including 100% for GACELISA. Analysis of the data according to the HIV prevalence in the population did not reveal any differences in performance by any of the OF EIAs (data not shown).

To compare the performance of the OF EIAs with that of the serum EIA, we examined the relationship of the initial EIA signal/cutoff (S/CO) ratios of the serum specimens tested by the Abbott EIA with the initial S/CO ratios of the matched OF specimens tested by the three OF EIAs (Fig. 1). This data includes all specimens which were initially HIV antibody positive by a given EIA (including false-positive specimens). In general, the S/CO ratios for the Abbott EIA for the HIV antibody-positive serum specimens were >14. The matched OF specimens tested by the three OF EIAs had S/CO ratios indicative of the performance of the assay. GACELISA had the highest S/CO for most of the reactive specimens (>6 [range, 6 to 11] [Fig. 1A]), whereas the S/CO ratios for OTC-M clustered between 4 and 8 (Fig. 1B) and those for OTC-L clustered between 4 and 6 (Fig. 1C). All three EIAs displayed excellent discrimination between HIV antibody-positive and HIV antibody-negative specimens.

After assimilation of all data, only six discordant results were observed among OF specimens from seropositive individuals. All of these OF specimens were HIV antibody positive by GACELISA and OFWB but were repeatedly nonreactive by at least one of the two OTC assays. EIA absorbance values of these specimens with GACELISA were low, and fewer virus-specific bands were observed on the OFWB than on the matched serum WB. However, both the serum WB and OFWB assays detected HIV-1 antibody bands sufficient to score these specimens as HIV antibody positive (except for one OFWB specimen, which was classified as indeterminate).

WB results. OFWB has been shown to yield banding patterns comparable to those observed for matched serum specimens which were also tested by WB (20). In this study, we compared OFWB banding patterns to those generated with matched serum specimens by using the FDA-licensed Cambridge WB. For this analysis we selected the 474 specimens which were HIV antibody positive by serum; 473 of these specimens were HIV-1 antibody positive by OFWB. The one remaining specimen was indeterminate by OFWB (p24 only) but HIV-1 antibody positive by serum WB (p24 and gp160). The two methods produced similar antibody-reactive banding patterns, particularly with the glycoprotein and polymerase gene products (Table 3). Differences in antigen-specific antibody detection were mostly associated with the *gag*-related antigens, particularly the p17 antigen and, to a lesser extent p24.

IgG analysis. The mean IgG concentration for all specimens was 17.13 µg/ml, with a median value of 13.85 µg/ml. Measurements ranged from 0.21 to >100 µg/ml. No significant differences in IgG concentration were noted between males (16.69 µg/ml) and females (17.93 µg/ml) or among any of the age groups. A significant difference was noted between HIV
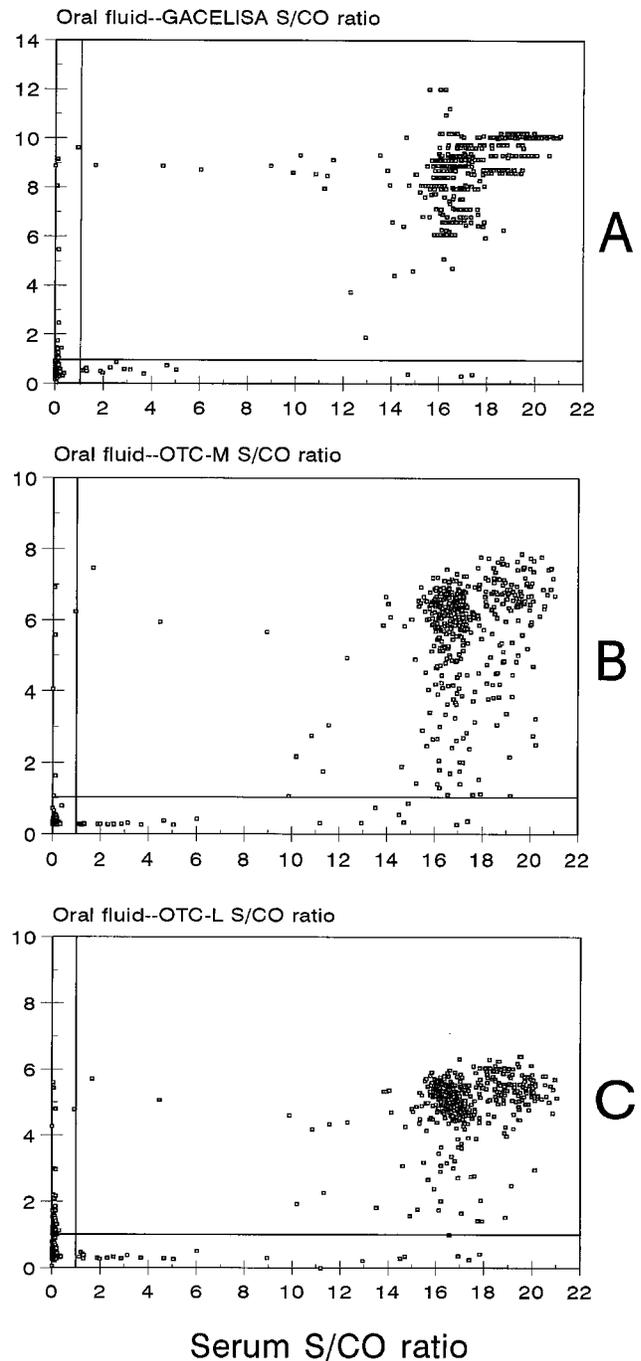

FIG. 1. Correlation of S/CO ratios for OF specimens tested by GACELISA (A), OTC-M (B), and OTC-L (C) with S/CO ratios for specimens tested by serum EIA (Abbott). Data include all specimens initially HIV antibody reactive by any of the OF or serum EIAs and 250 HIV antibody-negative specimens.

antibody-positive individuals (31.94 µg/ml) and the seronegative population (15.28 µg/ml) ($P = 0.0001$).

DISCUSSION

The low concentration of IgG in the oral cavity (approximately 0.10% of the concentration of IgG in serum) significantly affected early studies of EIA performance with OF specimens (1, 3, 12, 37). Mortimer and Parry suggested a minimal

TABLE 3. Comparison of detection of antibodies to HIV-1 proteins by WB in 474 matched serum and OF specimens

WB antigen	No. (%) of HIV antibody-positive specimens	
	Serum	OF
gp160	474 (100)	474 (100)
gp120	466 (98.3)	470 (99.2)
p66	469 (98.7)	462 (97.5)
p55	380 (80.1)	365 (77.0)
p51	465 (98.1)	451 (95.1)
gp41	471 (99.4)	459 (96.8)
p31	457 (96.4)	452 (95.4)
p24	469 (98.7)	431 (90.9)
p17	435 (91.8)	267 (56.3)

IgG concentration of 0.5 μg/ml to ensure sensitive EIA performance (32). The OraSure device has been designed to be simple to use and to provide a stable, homogeneous sample which is enriched for gingival crevicular fluid, which is known to have higher concentrations of IgG (10, 32). Direct comparisons of IgG concentrations in saliva and OF collected by various devices have been based on small sample sets but have shown higher concentrations of IgG in OF from collection devices (32). Although above 0.5 μg/ml, the mean concentration of IgG in OF collected by OraSure in this study (n = 4,448) was lower than that found in previous studies. The IgG concentrations in OF collected by OraSure in a study by Cordeiro et al. (10) were corrected for dilution, whereas the data presented here represent the concentrations of IgG in the fluid as collected. The OF IgG concentrations determined for the OraSure specimens were comparable to whole saliva concentrations (32). In this large data set, a significant increase in IgG concentration was noted in the HIV antibody-positive samples versus the HIV antibody-negative specimens. While the reason for this increase in IgG concentration in OF is not known, a similar increase has been previously noted with a small sample set and was hypothesized to be the result of local immune stimulation (26).

The OraSure OF collector has been licensed by the FDA in conjunction with an EIA and WB for sample testing. To date, two large-scale published studies have used the OraSure collector and OTC-L with excellent results (sensitivities, 99.5 and 99.9%) (17, 35). Emmons et al. found 100% sensitivity and specificity with OraSure OF and a different modified EIA (13). The sensitivity of OTC-L was slightly lower (99.2%) in this study than in the previous studies. The four undetected HIV antibody-positive OF specimens in this study did not have antibodies directed to all of the HIV antigens, as determined by serum WB and OFWB. The natural lower concentration of IgG in OF may contribute to the inability of EIAs to detect such specimens, especially when the concentration of HIV-specific antibodies is low (9).

Capture-antibody assays have been proposed as a method of providing greater sensitivity in the detection of low concentrations of HIV-specific antibody (33). This study represents the first OraSure OF specimen set to be evaluated by GACELISA, a capture-antibody assay designed for OF, urine, and dried blood spot specimens. In other studies, GACELISA has consistently provided sensitivities and specificities comparable to those found with matched serum tests using whole saliva as well as Omni-Sal (Saliva Diagnostic Systems, Vancouver, Wash.) and other OF specimens (8, 11, 12, 15, 16, 25, 27, 30, 32, 37). GACELISA has also been shown to discriminate between HIV antibody-positive and HIV antibody-negative spec-

imens despite the low IgG concentrations in OF specimens (20). GACELISA detected all seropositive specimens in our study with S/CO ratios for most specimens of >6. The six OF specimens with low concentrations of HIV-specific antibody were detected by GACELISA, which is consistent with the ability of this assay to detect seroconversion in samples with low concentrations of IgG (9).

Substantial modifications have been made to WB procedures to compensate for the low IgG concentrations in OF (3, 12, 13, 15, 23, 28, 29, 35). Initial attempts at OFWB were further complicated since dilution of the sample was required to adapt OF to WB assays designed to test serum specimens. As a result, early OFWBs did not correlate well with matching serum WBs (4, 13, 23, 31, 36). Seropositive specimens were often classified as indeterminate by OFWB when the important criterion WB bands could not be detected. By increasing the incubation period to 3 h and reducing the dilution of the OF specimen, the recently licensed OFWB method greatly improved the comparability of OFWB with matched serum results (17).

A miniaturized WB procedure for OF specimens has been shown to provide essential equivalency of WB banding patterns between matched serum and Omni-Sal OF specimens (20). In this study, the miniaturized method also provided similar banding patterns for matched OraSure OF and plasma specimens (Table 3). The lower rate of detection of the p17 band has been previously observed with serum samples and is thought to be due to less p17 antigen present on the immunoblot (19). The miniaturized method also offers the additional advantages of reducing testing time, lowering OF volume requirements, and reducing the dilution of the sample.

Although EIA and WB now provide excellent diagnostic capabilities for HIV antibodies, easy collection and rapid testing of the specimen fluid would be of great advantage in certain settings where immediate results are required. OFs have been tested with current rapid HIV antibody assays in limited investigations. The results have shown excellent correlation with matched serum samples (6, 21, 22, 29). We are currently conducting a study with a subset of the specimens discussed in this report to optimize rapid testing procedures for OF specimens and to determine the effectiveness of these assays for detecting HIV antibodies in OF specimens.

This study represents the largest data set used to date to test OF specimens and provides additional support for the use of OF for detection of HIV antibodies. The OraSure device has been shown to provide an adequate specimen with sufficient IgG to be detected in modified EIAs and miniaturized WB methods optimized for OF specimens. In addition, the use of OF collectors has been shown to improve patient participation in epidemiologic investigations (3, 7, 28). Recent FDA licensure of the OraSure device and an associated EIA-WB method now provides an alternative to routine serological HIV testing.

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