Saliva-based HIV-antibody testing in Thailand

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Objective: To determine whether saliva could serve as an alternative to serum for HIV-antibody testing in an ongoing sentinel surveillance program in Thailand.

Methods: Serum and saliva specimens were collected from 1955 individuals in four of the 73 sentinel sites of the national surveillance program in Thailand. Intravenous drug users, female prostitutes, and men attending sexually transmitted disease clinics were included as participants. All specimens were collected and tested anonymously. Saliva was gathered with the Omni-Sal collection device and analyzed for the presence of HIV antibodies using the immunoglobulin G antibody-capture enzyme-linked immunosorbent assay (GACELISA) laboratory test, specially designed for low concentration body fluids. Our gold standard was serum, collected and analyzed independently from the saliva specimens, using an ELISA test for screening and Western blot for confirmation. Linkage between serum and saliva was blind to the laboratory. A set of HIV-positive and HIV-negative quality assurance samples for both serum and saliva were also analyzed blind.

Results: Findings are presented as observed in the field, and as quality assurance samples after the correction of various field and laboratory errors. The sensitivity of the GACELISA with saliva was 98.0% in the field (298 HIV-positive specimens), 100% after correction of errors (300 HIV-positive specimens), and 100% among the quality assurance samples (95 HIV-positive specimens). The specificity of the GACELISA was 99.4% in the field (1653 HIV-negative specimens), 99.6% after correction of errors (1654 HIV-negative specimens), and 100% among the quality assurance samples (96 HIV-negative specimens).

Conclusion: Our findings support other published studies that also featured the GACELISA. We conclude that saliva is comparable to serum for assessing HIV antibodies in individuals for surveillance and screening purposes.

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Introduction

As HIV continues to infect more than 14 million people throughout the world [1], simplified testing methods are needed to identify asymptomatic HIV carriers for early treatment and prevention of transmission. Serologic tests for HIV antibodies have been available since the mid-1980s, soon after the AIDS virus was first identified [2]. Most of the tests were enzyme-linked immunosorbent assays (ELISA), initially used to detect HIV-1 antibodies and later to find HIV-1 and HIV-2 antibodies. While many advocate testing blood, others have shown that saliva is an acceptable and often favorable alternative [3].

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Cost of testing is also a major consideration, especially in developing countries [4] and as Tamashiro *et al.* [5], representing the view of the World Health Organization (WHO) have noted, 'the cost of HIV testing can be reduced if body fluids other than sera (or plasma) give similar (valid) results because collection costs are lower'.

Blood of HIV-infected individuals may be viewed as a hazardous substance that occasionally leads to HIV infection among health workers [6,7]. As was recently suggested by Ippolito *et al.* [7], exposures (to infected blood) could be prevented with the use of barrier precautions, appropriate behaviors, and safer devices and techniques. Of course, the need for precautions with blood results in increased cost, both in training and supplies. Saliva is a safer medium than blood for four reasons: HIV is rarely found in the saliva of HIV-infected individuals [8,9]; when found in saliva the concentration of HIV tends to be very low [8,9]; there appear to be factors in saliva that inhibit viral infectivity [10]; and there is no possibility of needlestick injuries associated with specimen collection. Even a bite from an HIV-infected patient is unlikely to transmit infection via saliva [11]. Hence saliva can be collected without ancillary supplies such as needles, syringes, alcohol, gauze pads, bandages and rubber gloves, although special collection devices that feature an adequate volume indicator, and a stabilizing buffer to preserve HIV antibodies prior to laboratory analysis are useful. Finally, disposal of saliva collection devices is less troublesome than needles and syringes which may be stolen and reused as contaminated equipment [12,13]. Saliva is especially useful for HIV testing among groups such as intravenous drug users (IVDU) who may have collapsed blood vessels [3,14], prisoners [15], or street-based prostitutes who might otherwise refuse to be tested.

Our research on saliva for HIV-antibody testing began in Myanmar, as part of the government's ongoing surveillance program [16]. The ELISA test originally chosen for saliva had very high specificity (i.e., the proportion of individuals with no HIV antibodies who test negative) but lower sensitivity (i.e., the proportion of individuals with HIV antibodies who test positive). While we showed that saliva was more effective for HIV surveillance than serum using the existing WHO-supplied ELISA, the lower sensitivity of the saliva assay was a problem. The Myanmar serum and saliva were later tested blind with two additional ELISA, one of which was an immunoglobulin (Ig) G antibody-capture ELISA (GACELISA) specifically designed for saliva [17]. The GACELISA displayed 100% sensitivity and 99.9% specificity, and became the test of choice for a future field evaluation.

Although saliva appeared to have many advantages for HIV testing, health officials in Thailand were not convinced that saliva was comparable to serum in measuring the presence of HIV antibodies. In addition, Thai health workers lacked experience in collecting and analyzing saliva specimens. Since 1989, the Ministry of Public Health in Thailand has conducted biannual surveillance of HIV prevalence in six risk groups [18]. For the past 3 years, sentinel sites have included all 73 provinces of the country. To evaluate saliva, the Ministry of Public Health approved the collection of both serum and saliva from participants in four of the 73 sentinel sites. The intention of the investigation was to investigate the issue raised recently by Tamashiro *et al.* at WHO [5,19], of whether saliva is as valid for HIV-antibody testing as serum in an ongoing surveillance program.

Materials and methods

Study population

As part of the biannual sentinel surveillance program conducted by the Department of Public Health, serum and saliva specimens were collected between December 1992 and March 1993 from 450 subjects in Nakhon Ratchasima (Korat), 406 in Bangkok, 610 in Chonburi, and 489 in Songkla (Hat Yai), Thailand. All participants were from four risk groups: IVDU, direct prostitutes (brothel-based), indirect prostitutes (bar girls), and men attending sexually-transmitted disease (STD) clinics. The samples were collected anonymously for surveillance purposes only.

Specimen collection

Vials of blood and saliva were collected from each individual using a vacutainer system and the Omni-Sal saliva collection device [Saliva Diagnostic Systems, Inc. (SDS), Vancouver, Washington, USA], respectively. The Omni-Sal device consists of a cotton pad and a tube containing a transport medium. Antimicrobial and antiproteolytic agents in the transport medium stabilize the specimen during the period between collection and testing. The collection pad is designed to hold 1 ml fluid when fully saturated, to fit into the transport tube, and to allow contact with the transport medium. Extraction of fluid from the pad typically yields 1–1.5 ml cell-free fluid.

Each vial of serum and saliva was identified with a numbered label that provided no information linking the specimens or the identity of the individuals. Thus, laboratory personnel were completely blind to the linkage between serum and saliva specimens. The linking code was retained by one of the authors (N.S.) in Thailand and one (R.R.F.) in the United States. Serum and saliva were analyzed immediately after collection and then frozen (–20°C) and stored in Thailand. Discrepant specimens were shipped to the SDS Laboratory in Vancouver, Washington, USA where they were thawed and analyzed.

Laboratory testing

Serum (Nakhon Ratchasima)

The first assay used to test for HIV antibodies was Vironostika anti-HIV Uni-Form (Organon Teknika, Boxtel, The Netherlands). If non-reactive on the first test, the specimens were deemed negative. If reactive, they were retested in duplicate with the Abbott Testpack HIV-1/HIV-2 (Abbott Laboratories, Abbott Park, Illinois, USA). If both duplicates were non-reactive, they were reported as negative. If either one was reactive, they were confirmed with either the HIV-1 Western blot (WB; Organon Teknika) or the Biotech/DuPont HIV-1 WB (Biotech Research Laboratories, Rockville, Maryland, USA). The outcome of the WB was positive [reactive pattern following Centers for Disease Control and Prevention (CDC) criteria: two out of three bands; p24, gp41, or gp160/gp120], negative (no bands present) or indeterminant (one or more bands present, but not the reactive pattern specified by CDC).

Serum (Bangkok)

Each specimen was tested for HIV antibodies with the Wellcozyme HIV Recombinant enzyme immunoassay (EIA; Murex Diagnostics Ltd, Dartford, England, UK). If non-reactive on the first test, the specimens were deemed negative. If reactive, they were retested singly with the Vironostika anti-HTLV-III EIA (Organon Teknika). If reactive on the second test, they were confirmed with the New LAV-Blot I WB (Sanofi Diagnostics Pasteur, Marnes-La-Coquette, France) using CDC band criteria. If non-reactive with the second test, they were again tested singly with the Wellcozyme HIV Recombinant EIA (Murex Diagnostics Ltd). If non-reactive, they were reported as negative and if reactive, confirmed with the New LAV-Blot I WB (Sanofi Diagnostics Pasteur), also with CDC band criteria.

Serum (Chonburi)

Specimens were initially tested for HIV antibodies with the Vironostika anti-HIV Uni-Form (Organon Teknika). If non-reactive on the first test, the specimens were considered to be negative. If reactive, they were retested in duplicate with the Serodia HIV-1 (Fujirebio, Tokyo, Japan), a particle agglutination HIV-1-antibody test. If both were non-reactive, they were reported as negative. If either one was reactive, they were confirmed with the HIV-1 WB Kit (Organon Teknika) for HIV-1 antibodies following the CDC band criteria.

Serum (Songkla)

Each specimen was tested in duplicate for HIV antibodies with the Vironostika anti-HTLV-III EIA (Organon Teknika). If both assays were non-reactive, the specimen was negative. If either initial assay was reactive, the specimen was confirmed with the HIV-1 WB Kit (Organon Teknika) following the CDC band criteria.

Saliva (all sites)

Each specimen was tested in duplicate with the Wellcozyme HIV 1+2 GACELISA (Murex Diagnostics Ltd) for HIV-1 or HIV-2 antibodies. No change was made in the cut-off value specified by the manufacturer's instructions. If the initial assay was non-reactive, the specimen was considered negative. If the optical density reading for the sample was above 0.7 times the cut-off value (gray zone), the specimen was retested. If the sample was reactive in at least two of the three repeated tests it was considered positive, but otherwise negative.

Serum and Saliva (Vancouver)

Discrepant pairs of serum and saliva specimens were retested non-blindly in the SDS laboratory, Vancouver. All serum were analyzed by the Abbott HIVAB HIV-1 EIA (Abbott Laboratories). Those that yielded negative results, while the corresponding saliva specimens yielded positive results, were retested with the Detect HIV 1/2 ELISA (BioChem Immuno Systems, Montreal, Quebec, Canada). If unclear results were obtained with the Detect, serum was analyzed a third time with the Cambridge HIV 1/2 EIA (Cambridge BioScience Corporation, Worcester, Massachusetts, USA). Subsequently, sera of seven saliva-positive but serum-negative pairs (i.e., false-positives) were tested with the Wellcozyme HIV 1+2 GACELISA (Murex Diagnostics Ltd), following the manufacturers instructions. Saliva specimens were tested with the Wellcozyme HIV 1+2 GACELISA (Murex Diagnostics Ltd), following the manufacturer's instructions. Both serum and saliva were analyzed for band profiles with either the Cambridge Biotech HIV-1 WB Kit (Cambridge BioScience Corporation) or HIV Blot 2.2 Kit (Diagnostic Biotechnology, Singapore). WB for saliva was optimized for the lower concentration of HIV IgG antibodies by increasing the sample volume to 300 ml and decreasing the diluent buffer volume to 1.2 ml for both WB assays. Additionally, the conjugate strengths were increased for the Cambridge test by a factor of 3, and for the HIV Blot 2.2 by a factor of 1.3.

Serum (Murex Diagnostics Ltd)

Sera from six linked pairs with positive saliva and negative serum (i.e., false-positives) were sent to the retrovirology section of Murex Diagnostics Ltd, for additional analyses with the Wellcozyme HIV 1+2 (a third generation assay, different in format from the GACELISA) and the Wellcozyme HIV recombinant EIA.

Quality assurance samples

HIV-antibody-positive and HIV-antibody-negative specimens were inserted at every tenth specimen in a systematic manner after a random start in the first sampling interval. Each specimen had a random identification number and was analyzed blind by laboratory personnel. The identity of the 189 blind

serum specimens and 191 blind saliva specimens was known only to one of the authors (N.S.) in Thailand and two in the United States (E.S. and R.R.F).

Analytic procedures

Data were entered into the computer using Excel (Microsoft, Redmond, Washington, USA), Lotus 1,2,3 (Lotus, Cambridge, Massachusetts, USA) and Quattro Pro (Borland, Scott Valley, California, USA) spreadsheet programs. Data were merged, linked and analyzed using Epi Info (CDC, Atlanta, Georgia, USA). The serum assay serves as the known gold standard for the saliva findings. We measured the sensitivity (i.e., proportion of true HIV-antibody positives who test positive) and specificity (i.e., proportion of true HIV-antibody negatives who test negative) of the saliva assay with reference to the gold standard, either serum or the quality assurance samples.

Results

Linked saliva and serum specimens were collected anonymously from 1955 individuals at the four sentinel surveillance sites. While the identity of the individuals was not known, the field staff were instructed to obtain linked saliva specimens for this study only from IVDU, direct and indirect prostitutes and men attending STD clinics. The four study sites were representative of Thailand as a whole with regard to prevalence of HIV infection in the four higher-risk groups (Fig. 1). Members of the two lower-risk groups, women attending antenatal clinics and blood donors, were not included in this study.

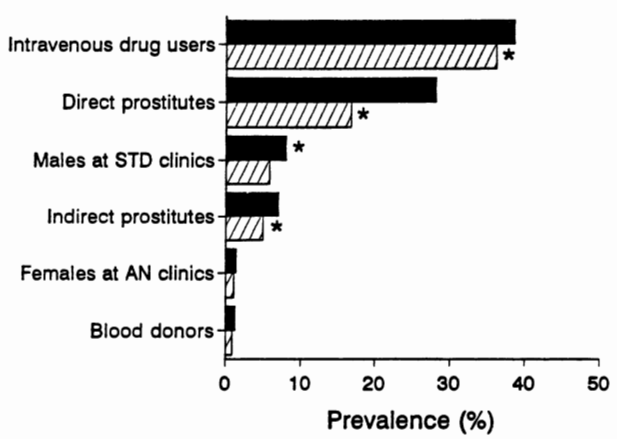

Fig.1. Sentinel surveillance for HIV infection in Thailand (■, 73 provincial sites) and study (▨, four provincial sites), December 1992–March 1993. *Groups included in the study. STD, sexually transmitted diseases; AN, antenatal.

Quality assurance samples

To assess the quality of the four regional laboratories, we prepared a set of quality assurance samples. Known HIV-antibody-positive and HIV-antibody-negative quality assurance samples were prepared in the United States and sent to Thailand at the start of the study; all came from individuals who had been been tested previously for HIV antibodies in serum or saliva. At random intervals during testing, 189 serum and 191 saliva vials were inserted among the specimens sent to the laboratories for analysis. Staff performing the analyses did not know the identity of the quality assurance samples. All serum quality assurance specimens were correctly identified in the four regional laboratories with a sensitivity and specificity of 100% (Table 1). Thus, although there were slight differences in the serum testing procedures among the four laboratories, the results were in total concordance.

Saliva was tested with the GACELISA in all four laboratories. As with the serum tests, Table 1 shows that sensitivity with the GACELISA was 100% for the 95 true HIV-positive specimens, and specificity was 100% for the 96 true HIV-negative specimens.

Table 1. Validity in the field of serum and saliva quality assurance samples.

	Quality assurance samples*	
	Positive	Negative
Serum		
Positive	91	0
Negative	0	98
Total	91	98
Sensitivity (%)	100.0	–
Specificity (%)	–	100.0
Saliva		
Positive	95	0
Negative	0	96
Total	95	96
Sensitivity (%)	100.0	–
Specificity (%)	–	100.0

*HIV-1-antibody-positive and -negative samples (blind to laboratory).

Blind field analysis

We assessed the validity of the GACELISA with specimens from the surveillance program at two stages; as observed in the field and after a non-blinded review and analysis of discrepant specimens. The interpreted result of the serum test was the standard of comparison (or gold standard) for both stages. The first stage was a comparison of all specimens as reported by the four laboratories, blind to the link between serum and saliva samples and with no adjustment made for labeling, recording or other laboratory errors. At this stage,

the laboratories reported 298 specimens as HIV-antibody-positive (15.2%), 1653 as HIV-antibody-negative and four as indeterminant, all based on the serum testing procedure described previously (Table 2). Of the 298 HIV-antibody-positive specimens, the GACELISA with saliva correctly identified 292, with a sensitivity of 98.0%. Of the 1653 HIV-antibody-negative specimens, the GACELISA with saliva correctly identified 1643, with a specificity of 99.4%. The four indeterminant serum specimens were split in the saliva analysis with two being HIV-antibody-positive and two being HIV-antibody-negative specimens (Table 2).

Table 2. Validity of saliva HIV-antibody test in the sentinel surveillance program in Thailand.

	Gold standard*		
	Positive	Negative	Indeterminant
Blind field results			
Positive	292	10	2
Negative	6	1643	2
Total	298	1653	4
Sensitivity (%)	98.0	–	
Specificity (%)	–	99.4	
Post-discrepant analysis results†			
Positive	300	7	1
Negative	0	1647	0
Total	300	1654	1
Sensitivity (%)	100.0	–	
Specificity (%)	–	99.6	

*Existing serum enzyme-linked immunosorbent assay plus Western blot confirmation; †see Table 3.

Post-discrepant analysis

Among the blind field results, there were 20 linked pairs that did not show complete agreement between serum and saliva: six false-negatives, 10 false-positives and four indeterminant for serum (Table 2). For the second stage of our analysis we reviewed the results reported by each field site to determine whether the differences were due to field or laboratory errors or to deficiencies in the GACELISA with saliva. With two exceptions, the paired serum and saliva specimens were sent to the SDS laboratory in the United States for further analysis with ELISA and WB. One serum and one specimen were lost in the field. The missing saliva sample, however, had been re-analyzed previously in the field.

The findings for the 20 discrepant pairs are shown in Table 3. Two of the 20 discrepant paired specimens were labeling errors in the field; one pair later confirmed as concordant HIV-antibody-positive and the other as concordant HIV-antibody-negative by the field staff after reviewing their records. Seven of the 10 false-positives were confirmed, two were errors in the reported saliva value, and one was a labeling error mentioned previously. The sera for the two

Table 3. Re-analysis of 20 discrepant pairs of blind field results (see top section in Table 2).

	Original (Thailand)		Re-analysis (USA)		
	Serum	Saliva	Serum	Saliva	Comment*
False-positives					
1	–	+	–	–	Label error in field
2	–	+	–	+	WB(0,2); false-positive
3	–	+	–	–	WB(0,0); saliva error
4	–	+	–	+	WB(2,1); false-positive
5	–	+	–	+	WB(2,0); false-positive
6	–	+	–	+	WB(2,0); false-positive
7	–	+	–	–	WB(0,0); saliva error
8	–	+	–	+	WB(2,1); false-positive
9	–	+	–	+	WB(0,1); false-positive
10	–	+	–	+	WB(0,1); false-positive
False-negatives					
1	+	–	+	+	Label error in field
2	+	–	+	+	WB(1,1); saliva error
3	+	–	+	+	WB(1,2); saliva error
4	+	–	+	+	WB(1,2); saliva error
5	+	–	+	+	WB(1,1); saliva error
6	+	–	+	+	WB(1,QNS); saliva error
Indeterminants					
1	±	+	?	+	WB(?,1); missing serum
	±	+	+	+	WB(1,?); false ind.†
3	±	–	+	+	WB(1,2); serum and saliva errors
4	±	–	–	–	Serum error in field

*Western blot results: 0, negative; 1, positive; 2, indeterminant (serum of pair, saliva of pair). †Confirmed in Thailand with re-analysis but missing saliva for USA re-analysis. QNS, quatity not sufficient; ?, missing value; ind., indeterminants.

saliva errors (i.e., false-positive pairs 3 and 7) on re-analysis in a non-blind manner by SDS were negative by ELISA and WB, possibly because of antibody degradation during shipment. Among the six false-negatives, five were field errors in measuring saliva and one was a labeling error mentioned earlier. The five saliva specimens all tested HIV-antibody-positive on re-analysis with GACELISA, and four of the five tested either positive or indeterminant with WB. The remaining saliva specimen had insufficient fluid for performing a WB.

Among the four indeterminants, one had a missing serum sample and could not be reviewed, the second was tested further and found to be concordant HIV-positive in the field (thereafter the saliva sample of the pair was lost), the third was concordant HIV-antibody-positive after correcting serum and saliva errors in the field, and the last was concordant HIV-antibody-negative because of a serum measuring error in the field.

Once the labeling, coding and laboratory errors had been corrected, there were 300 true HIV-antibody-positive specimens and 1654 true HIV-antibody-negative specimens. Only one specimen remained indeterminant with serum (Table 2), and might have become concordant if we had been able to locate the missing serum specimen. After these corrections,

the sensitivity of the GACELISA with saliva was 100% and specificity 99.6% (Table 2).

Table 4. Re-analysis with selected enzyme-linked immunosorbent assay (ELISA)* of paired saliva and serum for seven false-positives (see lower section in Table 2).

	SDS, Inc.				Murex Diagnostics Ltd	
Sample	Abbott	Detect	Cambridge	GACELISA†	HIV 1+2	HIV Recomb.
Saliva						
1 (10)‡				+		
2 (9)				+		
3 (4)				+		
4 (2)				+		
5 (5)				+		
6 (6)				+		
7 (8)				+		
Serum						
1 (10)‡	–	±	–	+	+	–
2 (9)	–	–		+	+	–
3 (4)	–	±	–	–	QNS	QNS
4 (2)	–	–		+	+	–
5 (5)	–	–		+	+	–
6 (6)	–	–		+	+	–
7 (8)	–	–		+	+	–

*Abbott HIVAB HIV-1 enzyme immunoassay (EIA), Detect HIV 1/2 ELISA, Cambridge HIV 1/2 EIA, Wellcozyme HIV1+2 immunoglobulin G antibody-capture ELISA (GACELISA), Wellcozyme HIV 1+2, Wellcozyme HIV Recombinant. †Followed manufacturer's instructions for saliva and dried blood spots. ‡False-positive number in Table 3. QNS, quantity not sufficient.

The seven false-positives shown in the lower section of Table 2 are presented in greater detail in Table 4 (ELISA findings) and Table 5 (WB findings). The saliva from each of the seven pairs was retested with the GACELISA and confirmed positive (Table 4). When tested with various ELISA, seven of the linked sera were HIV-antibody-negative (both HIV-1 and HIV-2), although two had gray zone optical density values with the Detect HIV 1/2 ELISA (BioChem Immuno Systems), but were negative when further tested with the Cambridge HIV 1/2 EIA (Cambridge BioScience Corporation). Six of the seven sera were also negative with the Wellcozyme HIV Recombinant assay (Murex Diagnostics Ltd) when assessed later at Murex Diagnostics Ltd, with one missing because of insufficient quantity (Table 4). When tested with the GACELISA using the dried blood spot protocol, we found six out of seven sera to be positive and one negative. The six positives were later confirmed by Murex Diagnostics Ltd with the Wellcozyme HIV 1+2 assay (Table 4). The one negative sera by GACELISA had insufficient quantity for further measurement.

The WB profile of the seven linked saliva and serum are shown in Table 5. Four of the seven sera showed single bands with the WB, but even these were weak in intensity. Conversely, three of the seven HIV-antibody-positive saliva specimens had sufficient bands on WB testing to be classified as HIV-antibody-positive by one or more criteria [20] (see Table 4). Thus, there were two groups of false positive specimens: three linked pairs that were clearly HIV-antibody-positive in saliva but mixed negative and positive in sera, and four linked pairs that were positive only by GACELISA in saliva and with the related GACELISA and Wellcozyme HIV 1+2 (Murex Diagnostics Ltd) in sera.

Table 5. Re-analysis of paired saliva and serum for seven false-positives (see lower section of Table 2).

	Western blot bands*									Western blot criteria*				
Sample	gp160	gp120	p66	p55	p51	gp41	p31	p24	p17	CRSS	WHO	CDC	ARC	FDA
Saliva														
1 (10)†	+	–	±	–	–	±	–	+	–	+	+	+	+	–
2 (9)	+	–	±	–	–	±	–	+	–	+	+	+	+	–
3 (4)	+	–	+	+	+	–	–	+	–	+	–	+	+	–
4 (2)	–	–	–	+	–	–	–	–	–	–	–	–	–	–
5 (5)	–	–	–	–	–	–	–	–	–	–	–	–	–	–
6 (6)	–	–	–	–	–	–	–	–	–	–	–	–	–	–
7 (8)	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Serum														
1 (10)†	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2 (9)	–	–	–	–	–	–	–	–	–	–	–	–	–	–
3 (4)	–	–	–	±	–	–	–	–	–	–	–	–	–	–
4 (2)	–	–	–	–	–	–	–	–	–	–	–	–	–	–
5 (5)	–	–	–	±	–	–	–	–	–	–	–	–	–	–
6 (6)	–	–	–	±	–	–	–	–	–	–	–	–	–	–
7 (8)	–	–	±	–	–	–	–	–	–	–	–	–	–	–

*Cambridge Biotech HIV-1 Western blot Kit, or Diagnostic Biotechnology (PTE) Ltd HIV blot 2.2 Kit. †False-positive number in Table 3. CRSS, Consortium for Retrovirus Serology Standardiazation; WHO, World Health Organization; CDC, Centers for Disease Control and Prevention; ARC, American Red Cross; FDA; Food and Drug Administration.

Discussion

The HIV epidemic is continuing to spread in Thailand, as it is in much of the developing world. In two recent studies of military conscripts, one reported that 12% of young men in six northern provinces tested positive for HIV antibodies [21], while the other observed an HIV-antibody-positive rate of 15.3% in the upper north subregion of the country [22]. A year earlier, Weniger *et al.* [18] had noted that 6.2% of about 4000 young men who were tested for conscription in the northern region of Thailand were HIV-antibody-positive. They anticipated that non-prostitute women and newborns would represent the majority of new infections. WHO considers surveillance programs that use sentinel groups such as IVDU or prostitutes to be important for identifying the present and future course of the HIV epidemic and for planning intervention efforts [23]. Since 1989, Thailand has had a comprehensive sentinel surveillance program that uses serum for HIV-antibody testing [18].

Although testing of serum for HIV antibodies is efficient for sentinel groups in a clinic setting where blood is already being collected, saliva appears to be more useful for street-based testing. Surveillance programs typically feature anonymous testing where findings are not made available to those tested. The benefit of the surveillance program is derived by the general population rather than the sample of tested individuals. Many people do not like to give blood, are suspicious of government medical or nursing personnel, and feel that the societal benefits of testing do not outweigh the personal inconvenience of a venipuncture. Groups such as IVDU, prostitutes or homosexuals may be more inclined to provide a saliva specimen, given the painless, non-invasive nature of the collection process. However, before saliva is accepted for HIV surveillance, government officials need to be assured that findings are comparable to serum. This was the intention of our investigation.

Validity of saliva testing

In our earlier study of the sentinel surveillance program of neighboring Myanmar, we found that saliva tested with the Cambridge Recombigen HIV-1 ELISA (Cambridge BioScience Corporation) had very high specificity (99.5–100%) but lower sensitivity (90.5–94.6%) when compared with a serum gold standard [16]. In that study there were also field errors in the measurement of both saliva and serum. For a subsequent publication, we re-analyzed all the Myanmar specimens in a blind manner using two gold standard assay methods. In that re-analysis, performed blind for linkage between saliva and serum, the specificity of the Cambridge Recombigen HIV-1 ELISA (Cambridge BioScience Corporation) was 99.9% while sensitivity was 99.3%. Also included were two additional assays for comparison, the Abbott HIVAB HIV-1 EIA (Abbott Laboratories) and the Wellcozyme HIV 1+2 GACELISA (Wellcome Diagnostics) [17]. While the Abbott assay was acceptable with saliva (sensitivity, 98.7%; specificity, 99.1%), the GACELISA performed exceptionally well with a sensitivity of 100% (based on 75 HIV-antibody-positive individuals) and a specificity of 99.9% (based on 1405 HIV-antibody-negative individuals). Given these excellent findings, we decided to further evaluate the GACELISA in the sentinel surveillance program of Thailand.

Since Archibald *et al.* [24], published their initial findings on saliva testing in 1986, 23 additional publications have appeared in the international literature that document the sensitivity and specificity of saliva as a testing medium for HIV antibodies [3,14,16,17,25–43]. Although the collection devices and the assays vary widely, more than half of the 38 reported ELISA tests presented in the 23 publications state a sensitivity of between 98 and 100%. The specificity of the 38 published assays with saliva is even higher, with 34 out of 38 exhibiting values between 99.5 and 100%.

Ten of the 24 saliva publications reported on the GACELISA with saliva, the same test used in our in-

Table 6. Published studies of HIV-antibody assays with immunoglobulin G antibody-capture enzyme-linked immunosorbent assay.

		No. subjects		%	
Authors	Collection method	HIV+	HIV–	Sensitivity	Specificity
Parry *et al.* 1987 [25]	Free drip	43	10	100	100
Crofts *et al.* 1991 [32]	Salivette	50	50	98.0	100
Klokke *et al.* 1991 [33]	Not stated	42	48	100	100
Gershy-Damet *et al.* 1992 [35]	Free drip	32	43	100	97.7
Thongcharoen *et al.* 1992 [37]	Free drip	54	55	100	100
Covell *et al.* 1993 [14]	Salivette	4	94	100	100
Hunt *et al.* 1993 [38]	Cotton swab	8	214	100	100
Connell *et al.* 1993 [42]	Salivette	50	127	100	100
Frerichs *et al.* 1994 [17]	Omni-Sal	75	1405	100	99.9
Chassany *et al.* 1994 [43]	Omni-Sal	115	451	100	100

vestigation (Table 6). With one exception, sensitivity was 100% and specificity (also with one exception) was 99.9–100%. As shown in Table 6, most of the studies are small in number with few HIV-antibody-negative and even fewer HIV-antibody-positive subjects; yet the pattern is clear with high sensitivity and specificity.

Our current study with 1955 subjects is the largest comparative investigation of saliva and serum to date, and along with our earlier study in Myanmar [17], is unique in being associated with a sentinel surveillance program. A related study of near-equal size in a clinical setting in Mexico City was reported by Soto-Ramirez *et al.* [36]. Their investigation of 1880 individuals included more HIV-antibody-positive subjects (356) but fewer HIV-antibody-negative individuals (1524) then reported here. Using the Orasure saliva collection device (Epitope, Beaverton, Oregon, USA) and a commercial ELISA (Organon Teknika), Soto-Ramirez *et al.* reported a sensitivity of 99.4% and a specificity of 100%. Their findings are similar to our results with the Omni-Sal (Saliva Diagnostic Systems) collection device and the GACELISA both here and in Myanmar [17].

Saliva-positive and serum-negative specimens

Of the 20 discrepant pairs found in the first stage of our investigation, we could explain all but eight. One of the eight was indeterminant for HIV antibodies with serum but positive with saliva. Unfortunately, the serum specimen was missing so we were not able to determine its status. Of greater interest are the seven false-positives noted in Tables 2, 4 and 5, which showed measurable HIV antibodies in the field with saliva but not with serum. It is unlikely that this discordance was caused by linkage errors since there was no parallel set of seven false-negative saliva specimens. It is also unlikely that HIV antibodies disintegrated in serum prior to analysis in Thailand since all samples were analyzed following standard laboratory procedures. Instead, we speculate that the group of seven false positives contained two subgroups: first, four specimens that reacted to the GACELISA for unknown, possibly non-specific, reasons; and second, three specimens that had measurable HIV antibodies in saliva but initially not in serum.

We hypothesize further for the second subgroup that, either HIV antibodies are easier to measure in saliva with the high sensitivity GACELISA than in serum with less sensitive conventional ELISA, or that HIV antibodies in some subjects appear earlier in saliva than serum. Our finding that sera from six of the seven pairs available for analysis were negative for HIV antibodies with both conventional ELISA and WB, but positive when analyzed with the highly sensitive Wellcozyme HIV 1+2 GACELISA (Murex Diagnostics Ltd) and the closely related Wellcozyme HIV 1+2 (Murex Diagnostics Ltd), gives credibility to the sensitive test hypothesis. Nevertheless we cannot rule out the hypothesis that HIV antibodies may appear earlier in saliva than serum since the serum findings with the GACELISA and the Wellcozyme HIV 1+2 (Murex Diagnostics Ltd) may be caused by non-specific reactions to other Ig. A large study like ours with 1955 subjects would expect to find several subjects in the early stages of immunoconversion. However, because this is a cross-sectional study and not a seroconversion panel, we could not determine the order of appearance of HIV antibodies in saliva versus serum.

Only three studies have been published that compare serum and saliva in a longitudinal manner during the period of immunoconversion [30,31,44]. Major *et al.* [31] gathered saliva and serum from subjects at regular intervals, including five subjects who seroconverted while under observation. There was no difference among the five saliva–serum pairs, with saliva and serum converting from HIV-antibody-negative to -positive in the same time interval. For the five, the average time between a negative test for HIV antibodies and a positive test varied greatly, but with a mean of 5.8 months. Thus, the data of Major *et al.* do not rule out the possibility that HIV antibodies could be measured in saliva before being evident in sera.

Behets *et al.*. [30], in a follow-up study of 315 seronegative prostitutes in Zaïre, collected both serum and saliva samples. Fourteen women seroconverted within a 20-week observation period, as defined by changes in serum. Two women had positive saliva (one of which was borderline) before serum, 12 had positive saliva and serum simultaneously, and five had negative saliva but positive serum. Of these five, three showed a p24 band on a saliva WB, or an indeterminant finding. Therefore, only two of the five saliva specimens were HIV-antibody-negative with an ELISA and completely negative with WB. The saliva was tested with the Vironostika ELISA (Organon Teknika), a serum assay that the authors optimized for saliva. Based on their larger data set, the sensitivity of the saliva ELISA was 97.9%, while specificity was 100%. The lower sensitivity suggests that 2.1% of true-positive saliva are missed by the test, as might have occurred with the three ELISA-negative saliva specimens that showed p24 bands on WB. Thus, the Vironostika ELISA (Oraganon Teknika) is not sensitive enough with saliva to detect early immunoconverters with levels that are more difficult to measure. Therefore, the study of Behets *et al.* [30], similar to the investigation by Major *et al.* [31], neither supports nor disproves the hypothesis that HIV antibodies can be measured in saliva before serum.

Connell and Parry [44] reported on nine subjects who had recently seroconverted and from whom saliva was collected within 4–43 days of serum. All

saliva specimens were reactive with the GACELISA. They concluded that at the time of seroconversion, the GACELISA is much more sensitive at detecting lower antibody concentrations in both serum and saliva, than other commercial EIA. They also hypothesized that saliva and serum are likely to seroconvert at about the same time.

Our findings, earlier in Myanmar [16,17] and now in Thailand, show that HIV antibodies can be detected in saliva as in serum. In an earlier publication [16], we showed that saliva with the Cambridge ELISA (Cambridge BioScience Corporation) provided a better estimate in a surveillance program of the prevalence of HIV than the existing serum ELISA provided by WHO. Subsequently, we showed that the GACELISA was equivalent to the serum ELISA as a screening test for HIV infection [17]. Here, in the largest study of its kind to date, we have shown that the GACELISA is a highly specific and sensitive testing assay among subjects participating in a sentinel surveillance program. The findings of 100% sensitivity and near-100% specificity with saliva are especially relevant to vaccine trials that must assess the onset of HIV infection in mobile or reluctant populations, to surveillance programs that regularly measure sentinel groups such as IVDU or street prostitutes, and to those wanting a tool for personal HIV screening [45].

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References

1. World Health Organization: **The current global situation of the HIV/AIDS pandemic.** *Wkly Epidemiol Rec* 1994, 69:7–8.
2. Kegeles SM, Catania JA, Coates TJ, Pollack LM, Lo B: **Many people who seek anonymous HIV-antibody testing would avoid it under other circumstances.** *AIDS* 1990, 4:585–588.
3. Coates R, Millson M, Myers T, *et al.*: **The benefits of HIV antibody testing of saliva in field research.** *Canadian J Public Health* 1991, 82:397–398.
4. Colebunders R, Ndumbe P: **Priorities for HIV testing in developing countries?** *Lancet* 1993, 342:601–602.
5. Tamashiro H, Maskill W, Emmanuel J, Fauquex A, Sato P, Heymann D: **Reducing the cost of HIV antibody testing.** *Lancet* 1993, 342:87–90.
6. Veeken H, Verbeek J, Houweling H, Cobelens F: **Occupational HIV infection and health care workers in the tropics.** *Trop Doct* 1991, 21:28–31.
7. Ippolito G, Puro V, De Carli G: **The risk of occupational human immunodeficiency virus infection in health care workers. Italian Multicenter Study.** *Arch Intern Med* 1993, 153:1451–1458.
8. Barr CE, Miller LK, Lopez MR, *et al.*: **Recovery of infectious HIV-1 from whole saliva.** *J Am Dent Assoc* 1992, 123:36–48.
9. Yeung SC, Kazazi F, Randle CG, *et al.*: **Patients infected with human immunodeficiency virus type 1 have low levels of virus in saliva even in the presence of periodontal disease.** *J Infect Dis* 1993, 167:803–809.
10. Robinovitch MR, Iversen JM, Resnick L: **Anti-infectivity activity of human salivary secretions toward human immunodeficiency virus.** *Crit Rev Oral Biol Med* 1993, 4:455–459.
11. Richman KM, Rickman LS: **The potential for transmission of human immunodeficiency virus through human bites.** *J Acquir Immune Defic Syndr* 1993, 6:402–406.
12. Liss GM, Crimi C, Jaczek KH, Anderson A, Slattery B, D'Cunha C: **Improper office disposal of needles and other sharps: an occupational hazard outside of health care institutions.** *Can J Public Health* 1990, 81:417–420.
13. The Hospital Infection Control Group of Thailand, Liss GM, Crimi C, *et al.*: **Disposal of needles, syringes and gloves Improper office disposal of needles and other sharps: an occupational hazard outside of health care institutions.** *J Med Assoc of Thailand* 1990, 81:417–420.
14. Covell R, Follett E, Coote I, *et al.*: **HIV testing among injecting drug users in Glasgow.** *J Infect* 1993, 26:27–31.
15. Bird AG, Gore SM, Burns SM, Duggie JG: **Study of infection with HIV and related risk factors in young offenders' institution.** *BMJ* 1993, 307:228–231.
16. Frerichs RR, Htoon MT, Eskes N, Lwin S. **Comparison of saliva and serum for HIV surveillance in developing countries.** *Lancet* 1992, 340:1496–1499.
17. Frerichs RR, Eskes N, Htoon MT: **Validity of three saliva assays for HIV-antibodies.** *J Acquir Immune Defic Syndr* 1994, 7:522–524.
18. Weniger BG, Limpakarnjanarat K, Ungchusak K, *et al.*: **The epidemiology of HIV infection and AIDS in Thailand.** *AIDS* 1991, 5 (suppl 2):S71–S85.
19. Tamashiro H, Constantine NT: **Serological diagnosis of HIV infection using oral fluid samples.** *Bull WHO* 1994, 72:135–143.
20. Constantine NT, Callahan JD, Watts DM: *Retroviral Testing — Essentials for Quality Control and Laboratory Diagnosis.* Boca Raton: CRC Press, Inc.; 1992:1–228.
21. Nelson KE, Celentano DD, Suprasert S, *et al.*: **Risk factors for HIV infection among young adult men in northern Thailand.** *JAMA* 1993, 270:955–960.
22. Nopkesorn T, Mastro TD, Sangkharomya S, *et al.*: **HIV-1 infection in young men in northern Thailand.** *AIDS* 1993, 7:1233–1239.
23. Chin J: **Public health surveillance of AIDS and HIV infections.** *Bull WHO* 1990, 68:529–536.
24. Archibald DW, Zon L, Groopman JE, McLane MF, Essex M: **Antibodies to human T-lymphotropic virus type III (HTLV-III) in saliva of acquired immunodeficiency syndrome (AIDS) patients and in persons at risk for AIDS.** *Blood* 1986, 67:831–834.
25. Parry JV, Perry KR, Mortimer PP: **Sensitive assays for viral antibodies in saliva: an alternative to tests on serum.** *Lancet* 1987, 335:72–75.
26. Johnson AM, Parry JV, Best SJ, Smith AM, de Silva M, Mortimer PP: **HIV surveillance by testing saliva.** *AIDS* 1988, 2:369–371.
27. Holmstrom P, Syrjanen S, Laine P, Valle SL, Suni J: **HIV antibodies in whole saliva detected by ELISA and western blot assays.** *J Med Virol* 1990, 30:245–248.
28. O'Shea S, Cordery M, Barrett WY, Richman DD, Bradbeer C, Banatvala JE: **HIV excretion patterns and specific antibody responses in body fluids.** *J Med Virol* 1993, 31:291–296.

29. Archibald DW, Hebert CA: Salivary detection of HIV-1 antibodies using recombinant HIV-1 peptides. *Viral Immunol* 1991, 4:17–22.
30. Behets FM, Edidi B, Quinn TC, *et al.*: Detection of salivary HIV-1-specific IgG antibodies in high-risk populations in Zaïre. *J Acquir Immune Defic Syndr* 1991, 4:183–187.
31. Major CJ, Read SE, Coates RA, *et al.*: Comparison of saliva and blood for human immunodeficiency virus prevalence testing. *J Infect Dis* 1991, 163:699–702.
32. Crofts N, Nicholson S, Coghlan P, Gust ID: Testing of saliva for antibodies to HIV-1. *AIDS* 1991, 5:561–563.
33. Klokke AH, Ocheng D, Kalluvya SE, *et al.*: Field evaluation of immunoglobulin G antibody capture tests for HIV-1 and HIV-2 antibodies in African serum, saliva and urine [letter]. *AIDS* 1991, 5:1391–1392.
34. van den Akker R, van den Hoek JAR, van den Akker WMR, *et al.*: Detection of HIV antibodies in saliva as a tool for epidemiological studies. *AIDS* 1992, 6:953–957.
35. Gershy-Damet GM, Koffi K, Abouya L, *et al.*. Salivary and urinary diagnosis of human immunodeficiency viruses 1 and 2 infection in Cote d'Ivoire, using two assays. *Trans R Soc Trop Med Hyg* 1992, 86:670–671.
36. Soto-Ramirez LE, Hernandez-Gomez L, Sifuentes-Osornio J, *et al.* Detection of specific antibodies in gingival crevicular transudate by enzyme-linked immunosorbent assay for diagnosis of human immunodeficiency virus type 1 infection. *J Clin Microbiol* 1992, 30:2780–2783.
37. Thongcharoen P, Wasi C, Louisirirotchanakul S, Parry J, Connell J, Mortimer P: Immunoglobulin G antibody capture enzyme-linked immunosorbent assay: a versatile assay for detection of anti-human immunodeficiency virus type 1 and 2 antibodies in body fluids. *J Clin Microbiol* 1992, 30:3288–3289.
38. Hunt AJ, Connell J, Christofinis G, *et al.*. The testing of saliva samples for HIV-1 antibodies: reliability in a non-clinic setting. *Genitourin Med* 1993, 69:29–30.
39. Chamnanput J, Phanuphak P: Comparison of eight commercial test kits for detecting anti-HIV antibodies in saliva specimens [letter]. *AIDS* 1993, 7:1026.
40. Holm-Hansen C, Constantine NT, Haukenes G: Detection of antibodies to HIV in homologous ses of plasma, urine and oral mucosal transudate samples using rapid assays in Tanzania. *Clin Diagnost Virol* 1993, 1:207–214.
41. Urquia M, Rodriguez-Archilla A, Gonzalez-Moles MA, Ceballos A: Detection of anti-HIV antibodies in saliva. *J Oral Pathol Med* 1993, 22:153–156.
42. Connell JA, Parry JV, Mortimer PP, Duncan J: Novel assay for the detection of immunoglobulin G anti-human immunodeficiency virus in untreated saliva and urine. *J Med Virol* 1993, 41:159–164.
43. Chassany O, Bergmann JF, Maseron MC, *et al.*: Testing of anti-HIV antibodies in saliva [letter]. *AIDS* 1994, 8:713–714.
44. Connell JA, Parry JV: Detection of anti-HIV in saliva and urine at the time of seroconversion. *Clin Diagnost Virol* 1994, 1:299–311.
45. Frerichs RR: Personal screening for HIV in developing countries. *Lancet* 1994, 343:960–962.