

Comparative field evaluation of HIV rapid diagnostic assays using serum, urine, and oral mucosal transudate specimens ¹

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Abstract

Background: Comparative field utility of selected HIV-1 assays using homologous collections of serum, urine and oral mucosal transudate (OMT) was determined in adult populations from a tuberculosis hospital and STD clinic in Djibouti, East Africa.

Study design: Enzyme immunoassay with confirmatory Western blot was performed on all serum specimens for comparison with rapid, instrument-free assays (SUDS HIV-1, Murex; TestPack HIV-1/2, Abbott; and COMBAIDS HIV 1 + 2, SPAN Diagnostics) using various specimen sources. Delayed (48 h post-collection) testing was also performed on urine. Sensitivity and specificity for the rapid assays, in descending order, were as follows: serum SUDS HIV-1 assay (100%, 98.3%), serum COMBAIDS HIV-1/2 assay (98.4%, 99.6%), and OMT SUDS HIV-1 assay (98.4%, 94.5%).

Results: The OMT EIA optical density cutoff value was modified resulting in an improved specificity from 89.1 to 99.6%; however, sensitivity decreased from 100 to 98.5%. Urine EIA and rapid assays demonstrated unacceptable test performance for use as a screening test. © 1997 Elsevier Science B.V.

Keywords: HIV; AIDS; Oral mucosal transudate; Urine; Enzyme immunoassay; Western blot; Rapid assay

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1. Introduction

The reference standard for serologic diagnosis of HIV-1 infection is an initial screening serology, enzyme immunoassay (EIA), with a supplemental confirmatory test, typically a Western blot assay. Universal access to this diagnostic approach is limited due to test-related costs, lack of available laboratory resources and technical expertise. Rapid diagnostic, instrument-free immunoassays using serum have demonstrated sensitivities and specificities in the range of 95–99% (Constantine, 1993; Malone et al., 1993). Disadvantages in utilizing serum include the cost of venipuncture equipment, risk of HIV-1 transmission through needle stick injuries or reuse of venipuncture equipment, and occasionally individual, cultural or religious reservations against the collection of blood. Alternative specimen sources, specifically saliva and urine, have demonstrated encouraging results in both clinical evaluation and epidemiologic surveys (Tamashiro and Constantine, 1994; Constantine et al., 1994; Desai et al., 1991). Modifications of test procedures, such as increased sample volume and incubation times and using uncentrifuged urine have further optimized assay performance (Constantine et al., 1994; Holm-Hansen et al., 1993).

The current WHO Testing Strategy II for HIV-1 diagnosis employs the initial use of one ELISA or rapid instrument-free assay (highly sensitive assay), with reactive specimens further tested with a second, highly specific, ELISA or rapid assay (Sato et al., 1994). The two assays chosen should utilize different antigen preparation and/or different testing principles. An HIV-1 diagnostic testing strategy combining rapid instrument-free assays with a specimen other than blood would have obvious advantages in the developing world if proven to be reliable. In 1991, a serosurvey in the Republic of Djibouti, East Africa, revealed HIV-1 prevalence figures of 9.8, 10.4 and 36 in adult tuberculosis patients, male STD patients, and street prostitutes, respectively (Rodier et al., 1993a,b). The present study evaluated the field utility of rapid diagnostic assays for HIV infection using serum, urine and saliva compared against serum HIV-1/2 EIA with confirmatory Western blot.

2. Materials and methods

2.1. Study populations

Homologous collections of serum, urine and saliva were obtained from consenting adults from either the 'Centre Paul Faure' tuberculosis (TB) hospital or the 'Centre de Prophylaxie' STD clinic in the Republic of Djibouti from the May 15 to 27, 1993. Demographic data, medical diagnosis, current medications and tobacco and khat use were collected. Khat (*Catha edulis*) is a plant containing an amphetamine-like compound, which is chewed daily by a majority of the adult population in the Horn of Africa. A clinical evaluation of oral lesions and a urinalysis to assess for proteinuria, hematuria and/or pyuria were performed.

2.2. Specimen collection and processing

Serum was obtained through standard venipuncture sterile techniques. Saliva-based assays have been successfully undertaken using 'raw' saliva and various collection devices. The Orasure[®] saliva collection device (Epitope, Beaverton, OR, USA) uses an absorbent pad attached to a plastic stick, which was rubbed along the tooth-gum margin and then held at this site (between the lower cheek and jaw) for 2 min to enhance the relative percentage of crevicular fluid, or oral mucosal transudate (OMT), containing increased immunoglobulin concentrations (Tamashiro and Constantine, 1994). The collection pad was then transferred to a tube containing a preservative. Two simultaneous Orasure[®] collections per volunteer were pooled and then centrifuged (2500 rpm for 15 min) with the pad extract used for testing. Freshly voided urine specimens of approximately 20 ml were filtered (Cat no. X7981, Porex Technologies, Fairburn, GA, USA) and the filtrate split into two aliquots. One aliquot was refrigerated at 4°C within 2 h of receipt for same-day analysis while the other was stored at room temperature with a preservative (0.1% sodium azide). The preserved sample was analyzed approximately 48 h after collection.

Table 1
Assay characteristics of and methods for anti-HIV antibody tests

Assay	Type of test	Type of antigen ^a	Specimen volume ^b (μ l)	Diluent volume (μ l)	Time required (min)
Abbott 2nd gen. EIA	Indirect EIA	RP HIV-1/2	S 10	S 400	S 90
			O 200	O None	O 120
			U 300	U None	U 120
Murex suds HIV-1	Micro-particle assay	L/RP HIV-1	S 30	S 500	S 8
			O 500	O 100	O 15
			U 500	U 100	U 15
SPAN COMBAIDS HIV-1+2 Dipstick	Dot blot assay	SP HIV-1/2	S 100	S 100	S 20
Abbott TestPack	Dot blot	RP HIV-1/2	U 500	U None	U 5

^aAntigens: RP, recombinant protein; L, purified viral lysate; SP, synthetic peptide.

^bSpecimen codes: S, serum; O, oral mucosal transudate; U, urine.

2.3. Laboratory test procedures

All HIV-1/2 EIA (Recombinant HIV-1/HIV-2 2nd generation EIA, Abbott Diagnostics, Wiesbaden, Germany) and rapid assays were performed on-site in the Djibouti Ministry of Health Blood Bank by trained personnel of the US Naval Medical Research Unit No.3 (NAMRU-3). Aliquots of all serum, urine, and saliva samples were frozen at -70°C and transported to NAMRU-3, a WHO Collaborating Center for AIDS, in Cairo, Egypt. Serum Western blot assays (HIV-1 Blot 1.2, Diagnostic Biotechnology, Singapore) of EIA seropositive specimens were performed at NAMRU-3, as were repeat analyses of all specimens with discrepant results from the initial testing in Djibouti.

Test performance characteristics are reported after repeat testing of discrepant samples to eliminate technical errors. A non-reactive serum EIA was considered the final result (true negative) while all repeatedly reactive EIA samples were tested by HIV-1 Western blot assay. All positive samples by Western blots were considered the final result using the Centers for Disease Control (CDC)/Association of State and Territorial Public Health Laboratory Directors (ASTPHLD) criterion. To establish optimal optical density (OD) cutoff values for OMT and urine EIA tests, sam-

ple-to-cutoff OD ratios (S/CO) were calculated for each sample and both OMT and urine EIA S/CO were compared separately to the corresponding serum EIA S/CO ratio plotted on a scattergram. The optimal cutoff values for OMT and urine S/CO ratios were visually determined on the scatter diagrams. A summary of the individual assay characteristics, specimen volumes required, and test performance times is shown in Table 1. SPAN dipstick was used to test only serum and Abbott TestPack for urine only.

2.4. Statistical analysis

Statistical analyses were done using EPI-INFO version 5 (USD, Incorporated, Stone Mountain, GA) and Statistix version 4 (Analytical Software, St. Paul, MN) software packages. Means of numeric variables were compared by *t*-test or ANOVA. The χ^2 test was used to compare categorical data.

3. Results

A total of 303 volunteers was enrolled (STD clinic, $n = 189$; TB hospital, $n = 114$), of which 41 (22%) STD patients and 24 (21%) TB patients were HIV-1/2 reactive by serum EIA yielding a

Table 2

Discrepant test results recorded by initial/repeat/modified cutoff indices^a and individual HIV-1 assay test performance characteristics^b compared to EIA/Western blot results^c

HIV-1 ASSAY	False		SENS	SPEC	PPV	NPV
	Positive	Negative				
Serum						
Murex suds	4/4/NA	1/0/NA	100.0	98.3	94.1	100.0
Span combaids	2/1/NA	3/1/NA	98.4	99.6	98.4	99.6
Saliva/OMT						
EIA	27/26/1	0/0/1	100.0 (98.4) ^d	89.1 (99.6)	71.1 (98.4)	100.0 (99.6)
Murex suds	15/13/2	2/2/5	96.9 (92.2)	94.5 (99.2)	82.7 (96.7)	99.1 (97.9)
Urine (immediate)						
EIA	8/1/NA	8/6/NA	90.6	99.6	98.3	97.5
Murex suds	9/1/NA	9/5/NA	92.2	99.6	98.3	97.9
Urine testpack	3/2/NA	6/6/NA	90.6	99.2	96.7	97.5
Urine (preserved)						
EIA	17/NA/NA	11/NA/NA	82.8	92.9	75.7	95.3
Murex suds	21/NA/NA	7/NA/NA	89.1	91.2	73.1	96.9
Urine testpack	8/NA/NA	4/NA/NA	93.8	96.6	88.2	98.3

^aNA, not applicable (either no repeat testing or no modification of cutoff indices).

^bSENS, sensitivity; SPEC, specificity, PPV, positive predictive value, NPV, negative predictive value. All were calculated on the basis of repeat testing to exclude technical errors.

^cPositive samples = 64, negative samples = 238. One sample reactive by EIA but indeterminate by Western blot was not included in this table.

^dCalculated using modified cut-off values as described in the text.

total positive of 21%. All EIA reactive sera were confirmed by HIV-1 Western blot, except one which produced an indeterminate result. The latter, a TB patient, was excluded from statistical analysis and the corresponding results of the rapid diagnostic assays for this subject are presented separately. Results and test performance characteristics are summarized in Table 2.

Repeat testing of samples with discrepant results to eliminate technical errors are also shown in Table 2. There were a considerable number of modified results on repeat testing involving all three specimen sources reflecting the significant problem in test interpretation in a field setting. No repeat testing of preserved urine specimens was undertaken since no aliquot was saved. The one repeatedly EIA-seropositive, Western blot-indeterminate specimen was further evaluated with HIV-2 Western blot and p24 antigen assays, both of which were negative and was found to be negative on all assays in the study except for the OMT results.

Modifications of cutoff values for OMT EIA tests yielded only one false-positive result versus 27 with the initial cutoff value, but produced one false-negative result. Using modified cutoff values, the sensitivity and specificity of OMT EIA were 98.4 and 99.6%, respectively. The insufficient correlation between urine EIA and serum EIA ratio values, manifested by considerably scattered points on the diagram, precluded any significant improvement through an adjusted ratio-cutoff value. Modification of test interpretation parameters was also undertaken for the OMT SUDS assay. The SUDS test is a qualitative assay in which the presence of a blue color (scored from 0 to 4+) indicates a positive result. A modified cutoff with a $\geq 2+$ instead of $\geq 1+$, as a positive result found only 2, instead of 15 false-positive results, but increased the number of false-negative results from 2 to 5. The corresponding sensitivity and specificity with the new parameter were 92.2 and 99.2%, respectively. Interestingly,

the differentiation between a score of 0 and 1 + was more difficult for the technicians than differentiating between 1 + and 2 +. Modification of SUDS cutoff for urine samples, both those tested immediately and those preserved, did not improve the overall test performance.

Overall, subject evaluations revealed oral lesions, primarily gingivitis (24%), tobacco use (54%), khat use (96%), current medication use (50%), dysuria (21%), proteinuria/microhematuria (71%) and microscopic evidence of pyuria (54%). Statistical analysis for potential association of these variables with test performance was undertaken. No significant statistical association between discrepant results and these variables was found. However, the delayed urine tests (EIA and TestPack) revealed a significant association ($P < 0.05$) between discrepant results and the presence of pyuria. This suggests that bacterial overgrowth occurred, which subsequently affected the accuracy of the results.

4. Discussion

It has been demonstrated that an HIV-1/2 testing strategy using a dual combination of rapid assays on serum specimens was comparable to the 'gold standard' of a serum EIA with a confirmatory Western blot (Sato et al., 1994; Spielberg et al., 1990; Brattegaard et al., 1993). As observed in our study, the serum-based combination (initial-serum SUDS; 'confirmatory'-serum COMBAIDS) would have had a sensitivity of 98.4%, and specificity of 100%. Variable test performance characteristics in the literature have been reported when evaluating HIV-1 urine- and saliva-based testing (Holm-Hansen et al., 1993; Rodier et al., 1993a; Frerichs et al., 1994; Emmons et al., 1995), probably related to technical differences in assays, patient populations studied, and assay test performance characteristics. Comparable field evaluations of salivary HIV-1 assays have demonstrated sensitivities in the 95–100% range and specificities approaching 100% with the most consistently promising results from the specifically modified IgG antibody capture ELISA (GAC ELISA) (Constantine et al., 1994; Frerichs et al.,

1994; Crofts et al., 1991). Test modifications such as cutoff value adjustments significantly increased the specificity of both the saliva EIA and saliva SUDS assays but at the expense of decreasing sensitivity. HIV-1 UAC ELISA tests for urine have been shown to have sensitivities and specificities exceeding 99% (Connell et al., 1993); however, other field studies evaluating rapid HIV-1 assays were comparable with the reduced sensitivity of approximately 90% in our study (Holm-Hansen et al., 1993).

'Potential' common field-related confounding factors, including medication use, oral pathology and abnormal urinalysis did not affect individual test performance characteristics with the exception of pyuria. Delayed testing of urine, even with a preservative, should be avoided under the conditions used in this study. The use of alternative specimens requires consideration of preservation techniques to allow testing under field conditions since delayed analysis is unavoidable in many developing countries. Saliva-based collection systems manufactured with a preservative solution might overcome this logistical problem. Urine collection at remote sites with centralized testing centers requires adequate preservation. The selection of preservatives must be appropriate (not inhibitory) for use with the enzyme systems used in the diagnostic assays.

Alternative testing strategies might consist of using a saliva-based assay as a screening test followed by a highly specific serum-based assay or possibly a dual combination of rapid assays both testing saliva. Strategies such as these following the WHO Strategy II algorithm require further field evaluation for potential applicability. However, the widespread use of rapid assays will be feasible only if costs can be minimized. In conclusion, the sensitivity of the serum and saliva rapid diagnostic assays in this study support their use as screening assays in either surveillance or diagnostic evaluation. Urine HIV-1 testing is not recommended as a sole strategy for screening. Potential field-related confounding variables were not clearly implicated in false assay results; however, specimen processing and assay interpretation required technical adjustments (i.e. filtering specimens, SUDS and EIA OD cutoff value

modification, repeating discrepant results from the field setting in reference laboratory) emphasizing the variability that can occur in field evaluations of diagnostic assays.

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References

- Brattegaard, K., Kouadio, J., Adom, M., Doorly, R., George, J.R. and De Cock, K.M. (1993) Rapid and simple screening and supplemental testing for HIV-1 and HIV-2 infections in West Africa. *AIDS* 7, 883–885.
- Connell, J.A., Parry, J.V., Mortimer, P.P. and Duncan, J. (1993) Novel assay for the detection of immunoglobulin G antihuman immunodeficiency virus in untreated saliva and urine. *J. Med. Virol.* 41, 159–164.
- Constantine, N.T. (1993) Serologic tests for the retroviruses: Approaching a decade of evolution. *AIDS* 7, 1–13.
- Constantine, N.T., Zhang, X., Li, L., Bansal, J., Hyams, K.C. and Smialek, J.E. (1994) Application of a rapid assay for detection of antibodies to human immunodeficiency virus in urine. *Am. J. Clin. Pathol.* 101, 157–161.
- Crofts, N., Nicholson, S., Coghlan, P. and Gust, I.D. (1991) Testing of saliva for antibodies to HIV-1. *AIDS* 5, 561–563.
- Desai, S., Bates, H. and Michalski, F.J. (1991) Detection of antibody to HIV-1 in urine. *Lancet* 337, 183.
- Emmons, W.W., Paparello, S.F., Decker, C.F., Sheffield, J.M. and Lowe-Bey, F.H. (1995) A modified ELISA and Western blot accurately determine anti-human immunodeficiency virus type 1 antibodies in oral fluids obtained with a special collecting device. *J. Infect. Dis.* 171, 1406–1410.
- Frerichs, R.R., Eskes, N. and Htoon, M.T. (1994) Validity of three assays for HIV-1 antibodies in saliva. *J. Acquired Immune Defic. Syndr.* 7, 522–525.
- Holm-Hansen, C., Constantine, N.T. and Haukenes, G. (1993) Detection of antibodies to HIV in homologous sets of plasma, urine and oral mucosal transudate samples using rapid assays in Tanzania. *Clin. Diagn. Virol.* 1, 207–214.
- Malone, J.D., Smith E.S., Sheffield, J., Bigelow, D., Hyams, K.C., Beardley, S.G., Lewis, R.S. and Roberts, C.R. (1993) Comparative evaluation of six rapid serological tests for HIV-1 antibody. *AIDS* 6, 115–119.
- Rodier, G.R., Couzineau, B., Gray, G.C., Omar, C.S., Fox, E., Bouloumie, J. and Watts, D. (1993a) Trends of human immunodeficiency virus type-1 infection in female prostitutes and males with a sexually transmitted disease in Djibouti, East Africa. *Am. J. Trop. Med. Hyg.* 48, 682–686.
- Rodier, G.R., Sevre, J.P., Binson, G., Gray, G.C. and Gravier, P. (1993b) Clinical features associated with HIV-1 infection in adult patients diagnosed with tuberculosis in Djibouti, Horn of Africa. *Trans. Royal Soc. Trop. Med. Hyg.* 87, 676–677.
- Sato, P.A., Maskill, W.J., Tamashiro, H. and Heymann, D.L. (1994) Strategies for laboratory HIV testing: an examination of alternative approaches not requiring Western blot. *Bull. WHO* 72, 129–134.
- Spielberg, F., Kabeya, C.M., Quinn, T.C., Ryder, R.W., Kifuani, N.K., Harris, J., Bender, T.R., Heyward, W.L., Tam M.R. and Auditore-Hargreaves, K. (1990) Performance and cost-effectiveness of a dual rapid assay system for screening and confirmation of human immunodeficiency virus type-I seropositivity. *J. Clin. Microbiol.* 28, 303–306.
- Tamashiro, H. and Constantine, N.T. (1994) Serological diagnosis of HIV infection using oral fluid samples. *Bull. WHO* 72, 135–143.