CLINICAL AND VACCINE IMMUNOLOGY, June 2007, p. 738–740 Vol. 14, No. 6
1556-6811/07/$08.00+0 doi:10.1128/CVI.00442-06

Performance of Two Commercial Immunochromatographic Assays for Rapid Detection of Antibodies Specific to Human Immunodeficiency Virus Types 1 and 2 in Serum and Urine Samples in a Rural Community-Based Research Setting (Rakai, Uganda)▽

S. C. Kagulire,[1]* P. D. Stamper,[3,4] P. Opendi,[1] J. L. Nakavuma,[2] L. A. Mills,[1,3] F. Makumbi,[1] R. H. Gray,[4] D. Serwadda,[1] and S. J. Reynolds[3,5]

Rakai Health Sciences Program, Kalisizo, Uganda[1]; Makerere University, Kampala, Uganda[2]; Johns Hopkins University School of Medicine, Baltimore, Maryland[3]; Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland[4]; and NIAID, National Institutes of Health, Bethesda, Maryland[5]

Received 21 November 2006/Returned for modification 31 January 2007/Accepted 27 March 2007

Rapid detection of human immunodeficiency virus (HIV) antibodies is of great importance in developing and developed countries to diagnose HIV infections quickly and at low cost. In this study, two new immunochromatographic rapid tests for the detection of HIV antibodies (Aware HIV-1/2 BSP and Aware HIV-1/2 U; Calypte Biomedical Corporation) were evaluated in rural Africa to determine the tests' performance and comparability to commercially available conventional enzyme immunoassay (EIA) and Western blot (WB) tests. This prospective study was conducted from March 2005 through May 2005 using serum and urine from respondents in the Rakai Community Cohort Survey. Nine hundred sixty-three serum samples were tested with the Aware blood rapid assay (Aware-BSP) and compared to two independent EIAs for HIV plus confirmatory Calypte WB for any positive EIAs. The sensitivity of Aware-BSP was 98.2%, and the specificity was 99.8%. Nine hundred forty-two urine samples were run using the Aware urine assay (Aware-U) and linked to blood sample results for analysis. The sensitivity of Aware-U was 88.7% and specificity was 99.9% compared to blood EIAs confirmed by WB analysis. These results support the adoption of the Aware-BSP rapid test as an alternative to EIA and WB assays for the diagnosis of HIV in resource-limited settings. However, the low sensitivity of the Aware-U assay with its potential for falsely negative HIV results makes the urine assay less satisfactory.

Nearly 25 million people in sub-Saharan Africa are infected with human immunodeficiency virus (HIV), and most of these people are unaware that they are infected (7). Knowledge of serostatus via antibody testing is the current entry point for most HIV prevention and treatment programs, and there have been recommendations to scale up HIV testing in developing countries to improve access to and utilization of antiretroviral care (2). However, the currently available conventional laboratory-based enzyme immunoassays (EIAs) require instrumentation (incubators, mechanical washing, and optical reading devices) and expertise, are expensive, and do not provide same-day results. Given the limitations of standard HIV tests, and the need for more expeditious point-of-care provision of HIV results, rapid HIV tests have been developed to be quicker, less expensive, and easier to perform. Rapid tests have been found to be cost-effective and to have increased the proportions of individuals receiving their HIV results (3, 4). However, there has been limited evaluation of some of the newly emerging HIV rapid tests. We therefore undertook an evaluation of two HIV rapid tests, Aware-BSP for blood and Aware-U for urine, in the Rakai District of southwestern Uganda. A preliminary evaluation of these tests in Thailand revealed good diagnostic properties (6). However, it was imperative to assess the performance of the new assays in a resource-limited rural sub-Saharan African setting, where different HIV clades are prevalent.

MATERIALS AND METHODS

Aware rapid assays. Calypte Biomedical Corporation has developed Aware rapid assays for the detection of HIV antibodies in blood (Aware-BSP) and urine (Aware-U). These are in vitro immunochromatographic rapid tests for the qualitative detection of antibodies to HIV type 1 (HIV-1) and HIV-2 in human serum, plasma, whole blood, and/or urine specimens. Both the blood and urine assays work on similar principles; however, the blood assay uses diluted samples for testing, while the urine assay does not require sample dilution. The test strip contains synthetic peptides representing the immunodominant regions of the HIV-1 gp41 and HIV-2 gp36 transmembrane proteins. A protein A antibody immobilized on the nitrocellulose membrane is used as a procedural control for the test and control zones. The endpoint of the assay is the visual detection of bound protein/colloidal gold conjugate on the nitrocellulose membrane. The control line will appear in all valid tests, indicating that a suitable sample was used and that the test functioned properly. The appearance of two lines on the test strip (i.e., test zone and control zone) is indicative of a positive reactive sample. The appearance of only one line on the test strip (in the control zone) indicates that the sample did not contain detectable HIV antibodies.

Study sample collection. This evaluation was conducted using specimens from a survey visit in an ongoing community cohort surveillance study in the Rakai District of southwestern Uganda. The Rakai Health Sciences Program (previously called the Rakai Project) has conducted cohort surveillance in 44 rural communities since 1994 (8). For this study, freshly collected urine and blood specimens were obtained between March and May 2005 from consenting adults (15 to 49 years of age). The samples were collected in participants' homes, labeled with unique computer-generated identifiers, logged in, cross-checked, reviewed by another staff member as a quality control measure, and then transported in a cold box to the testing center at the program's laboratory.

* Corresponding author. Mailing address: Rakai Health Sciences Program, Uganda Virus Research Institute, P.O. Box 49, Entebbe, Uganda. Phone: 256-41-323-255. Fax: 256-41-323-252. E-mail: ckagulire@yahoo.com.

▽ Published ahead of print on 11 April 2007.

TABLE 1. Performance of Aware-BSP rapid blood test compared to serum EIA plus WB

Aware-BSP serum test result	No. of samples tested by double EIA plus WB if either EIA was positive		
	Positive	Negative	Total
Positive	108	2	110
Negative	2	851	853
Total	110	853	963

Study sample analysis. The blood samples were centrifuged in the laboratory, and aliquots were made for testing. All serum samples were first tested with the Aware-BSP assay, and the urine samples were tested using the Aware-U assay. Laboratory staff who were blinded to Aware test results tested sera with two independent EIAs, Abbott Murex HIV-1/2 ELISA (Murex Biotech limited, United Kingdom) and Vironostika HIV Uni-Form II MicroELISA (bioMerieux, Switzerland), in accordance with each manufacturer's instructions. A sample was considered to be HIV negative if it had concordant negative results by both EIAs ($n = 853$). However, any sample yielding one or more positive EIA results ($n = 110$) was subjected to Western blot (WB) analysis (Calypte Biomedical) for definitive characterization. WHO criteria for determining WB results were used to assign the final HIV status of such samples (9). The results of the Aware-BSP and Aware-U tests were compared to their corresponding blood EIA/WB results in order to determine the performance of the Aware assays in HIV detection. Infection with HIV-2 is rare in our setting, and specific testing for HIV-2 was not performed as part of this study.

Statistical analysis. The data were reviewed by laboratory quality control staff, and data entry was performed by two independent persons using Foxpro (version 2.6; Microsoft Corporation) for data cleaning and editing for range and consistency. Using STATA (version 8.2; Statacorp, TX), we calculated the performance characteristics of each Aware assay compared to the serum EIA/WB results as the "gold standard."

RESULTS

Tables 1 and 2 show the performance characteristics of the Aware rapid tests. A total of 963 blood samples were tested. The overall HIV prevalence was 11.4% (110/963) in our study. Female respondents represented 602/963 or 62.5% of subjects.

The sensitivity of Aware-BSP was 98.2% (95% confidence interval [CI], 93.6 to 99.8%), specificity was 99.8% (95% CI, 99.2% to 99.9%), and negative and positive predictive values were 99.8% and 98.2%, respectively (Table 3). The rate of false-positive results was 1.8% (2/110), and the rate of false-negative results was 0.2% (2/853). Among the 47 samples in which the two EIA results were discordant, there was 98% agreement between Aware-BSP and WB (46/47); all 47 WB results were negative.

A total of 942 urine specimens were tested using Aware-U and were compared to their corresponding serum EIA/WB results. The sensitivity was 88.7% (95% CI, 81.1% to 94.0%), specificity was 99.9% (95% CI, 99.3% to 100%), the negative predictive value was 98.6%, and the positive predictive value was 99.0%. There were 1.1% (1/95) false-positive results and 1.4% (12/847) false-negative results. Overall, the Aware-BSP assay revealed a higher sensitivity than the Aware-U assay, although specificity was nearly identical in the two tests.

TABLE 2. Performance of Aware-U rapid urine test compared to serum EIA plus WB

Aware-U urine test result	No. of samples tested by double EIA plus WB if either EIA was positive		
	Positive	Negative	Total
Positive	94	1	95
Negative	12	835	847
Total	106	836	942

TABLE 3. Performance of Aware rapid HIV-1/2 antibody assays in serum and urine[a]

Test	Sensitivity (95% CI)	Specificity (95% CI)	NPV	PPV
Aware-BSP	98.2 (93.6–99.8)	99.8 (99.2–99.9)	99.8	98.2
Aware-U	88.7 (81.1–94.0)	99.9 (99.3–100)	98.6	99.0

[a] Values are percentages. NPV, negative predictive value; PPV, positive predictive value.

DISCUSSION

The Aware-BSP rapid test had high sensitivity (98.2%) and specificity (99.8%), whereas the Aware-U had lower sensitivity (88.7%) but high specificity (99.9%). Thus, the performance of Aware-BSP assay was satisfactory, but the Aware-U assay did not provide satisfactory results in this setting. In a prior study in Thailand (6), the Aware urine rapid test demonstrated high sensitivity (99.0%) and specificity (100%). The reason for the poorer performance in our study is unclear. Clade-specific differences in anti-HIV antibodies generated by the host would seem to be unable to explain why the serum-based assay performed better than the urine-based assay, which contained the same recombinant antigens.

Several factors that might explain the poorer performance of the urine assay may have to do with the biology of urine antibody testing. Variation in urine pH may affect antigen-antibody reaction time (1), and we were unable to control urine pH. The low viscosity of urine may have allowed rapid rates of sample migration, thus decreasing antigen-antibody exposure times (1). It has been suggested that patients on all-antiretroviral therapy may have decreased anti-HIV antibody titers, causing possible false-negative test results (6). However, none of the patients for whom Aware-U tests were falsely negative were on all-antiretroviral therapy.

According to the WHO, an ideal test for the rapid diagnosis of HIV infection should be rapid, inexpensive, highly sensitive and specific, and easy to perform and interpret (10). In addition to these characteristics, ideal rapid tests should be able to be stored at room temperature, should have long shelf lives, and should require no additional equipment or auxiliary supplies in order to be performed (4). The blood-based rapid assay in this study fulfills these criteria and, as such, serves as a powerful tool for HIV diagnosis.

The urine test may provide an alternative in situations in which drawing blood is impractical or unsafe or in which patients refuse blood testing. However, more than 10% of HIV-positive cases would be missed due to the poor sensitivity of the Aware-U assay. Notification of false-negative results could have serious personal and public health consequences.

In summary, the Aware blood-based rapid test is sensitive and specific in a laboratory setting. However, further assessment in a field setting such as prenatal clinics, delivery rooms,

and emergency rooms is needed before the utility of the Aware-BSP test can be fully evaluated. The Aware urine-based rapid test demonstrated suboptimal sensitivity in our study, and thus, we have reservations about recommending its use.

ACKNOWLEDGMENTS

This work was supported by the U.S. National Institute of Allergy and Infectious Diseases International Centers for Excellence in Research Program (ICER).

We thank Calypte Biomedical Corporation and Intercross, Kenya, for freely offering the testing kits. We also thank all the Rakai staff and, most importantly, the cohort participants themselves.

REFERENCES

1. **Constantine, N.** 14 February 2006, accession date. HIV Insite Knowledge Base Chapter. http://hivinsite.ucsf.edu/InSite?page=kb-02-02-01.
2. **De Cock, K. M., R. Bunnell, and J. Mermin.** 2006. Unfinished business—expanding HIV testing in developing countries. N. Engl. J. Med. **354:**440–442.
3. **Kassler, W. J., B. Dillon, C. Haley, W. K. Jones, and A. Goldman.** 1997. On-site rapid HIV testing with same day results and counseling. AIDS Res. Hum. Retrovir. **11:**1045–1051.
4. **Kendrick, S., K. Krok, D. Withum, R. Rydman, B. Branson, and R. Weinstein.** 2005. Outcomes of offering rapid point-of-care HIV testing in a sexually transmitted disease clinic. J. Acquir. Immune Defic. Syndr. **38:**142–146.
5. Reference deleted.
6. **PRNewswire.** 15 July 2004, posting date. Calypte Biomedical presents results of its Rapid HIV-1/2 field trial tests at the XV International AIDS Conference in Bangkok. Overall accuracy: blood rapid 100%, oral fluid rapid 99.8%, urine rapid 99.6%. http://www.aegis.com/news/PR/2004/PR040743.html.
7. **UNAIDS.** 30 May 2006, posting date. Global facts and figures. http://www.who.int/hiv/mediacentre/200605-FS_globalfactsfigures_en.pdf.
8. **Wawer, M. J., N. K. Sewankambo, D. Serwadda, T. C. Quinn, L. A. Paxton, N. Kiwanuka, F. Wabwire-Mangen, C. Li, T. Lutalo, F. Nalugoda, C. A. Gaydos, L. H. Moulton, M. O. Meehan, S. Ahmed, R. H. Gray, and the Rakai Project Study Group.** 1999. Control of sexually transmitted diseases for AIDS prevention in Uganda: a randomised community trial. Lancet **353:** 525–535.
9. **WHO.** 14 February 2006, accession date. Criteria for analysis of HIV Western blot assays. http://www.who.int/entity/diagnostics_laboratory/publications/en/hiv_panel_18_march_2003_%20EQAS_report.pdf.
10. **WHO.** 14 February 2006, accession date. HIV test kit evaluations. http://www.who.int/diagnostics_laboratory/evaluations/hiv/en.