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)\textsuperscript{v}\textsuperscript{v}

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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 instrument-ation (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.

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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   |

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

| 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.

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 99.2%, respectively (Table 3). The rate of false-positive results was 1.1% (1/95) and the rate of false-negative results was 0.2% (2/853). Among the 47 samples in which the two EIAs 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.

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%).

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.

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