Performance of Aware rapid HIV 1/2 antibody detection assays using serum and urine in a rural community-based research setting in Rakai, Uganda

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ABSTRACT

Rapid detection of HIV antibodies is of great importance in developing and developed countries to diagnose HIV quickly and at low cost. In this study, two new immunochromatographic rapid tests for detection of HIV antibodies (Aware HIV-1/2 BSP and Aware HIV-1/2 U; Calypte Biomedical Corporation USA) were evaluated in rural Africa to determine the tests’ performance and comparability to commercially-available conventional EIA and WB tests.

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This prospective study was conducted from March 2005 through May 2005, using serum and urine from respondents in the Rakai Community Cohort Survey (RCCS). Nine hundred and sixty three (963) serum samples were tested on the Aware blood rapid assay (Aware-BSP) and compared to two independent EIA for HIV plus confirmatory Calypte Western blot for any positive EIAs. The sensitivity of Aware-BSP was 98.2% and the specificity was 99.8%. Nine hundred and forty two (942) urine samples were run on 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% when compared to blood EIAs confirmed by Western blot analysis.

These results support the adoption of Aware-BSP rapid test as an alternative to EIA and Western blot assays for the diagnosis of HIV in resource-limited settings. However, the low sensitivity of the Aware urine assay with its potential for falsely negative HIV results makes the urine assay less satisfactory.

INTRODUCTION

Nearly 25 million people in sub-Saharan Africa are infected with 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 lab-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 the standard HIV tests, and the need for more expeditious point-of-care provision of HIV results, HIV rapid 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, USA has developed Aware rapid assays for 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-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 immuno-dominant 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 a suitable sample was used and the test functioned properly. 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 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 through May 2005 from consenting adults (15-49 years). The samples were collected in participants’ homes, labeled with unique computer generated identifiers, logged in, cross-checked and 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.

**Study Sample Analysis**
The blood samples were centrifuged in the laboratory and aliquots were made for testing. All the serum samples were first tested on the Aware-BSP, and the urines on the Aware-U. Laboratory staff who were blinded to Aware test results tested sera with two independent EIAs, Abbott Murex HIV-1/2 ELISA (Murex Biotech limited, UK) and Vironostika HIV Uni-Form II MicroELISA (Biomerieux, Switzerland) in accordance with each manufacturer’s instructions. A sample was considered HIV-negative if it had concordant negative results on both EIAs (n=853). However, any sample yielding one or more positive EIA results (n=110) was subjected to Western blot analysis (Calypte Biomedical, USA) for definitive characterization. WHO criteria for determining Western blot results were used to assign 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 (Microsoft corporation, version 2.6), for data cleaning and editing for range and consistency. Using STATA (version 8.2 Statacorp, Texas, USA) we calculated the performance characteristics of each Aware assay when 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% CI: 93.6-99.8%), specificity 99.8% (95% CI: 99.2%-99.9%), negative and positive predictive values were 99.8% and 98.2%, respectively. The rate of false positives was 1.8% (2/110) and false negatives 0.2% (2/853). Among the 47 samples in which the two EIA results were discordant, there was 98% agreement between Aware-BSP and Western Blot (46/47), all 47 Western Blot 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%-94.0%), specificity 99.9% (95% CI: 99.3%-100%), negative predictive value 98.6% and positive predictive value 99.0%. There were 1.1% (1/95) false positives and 1.4% (12/847) false negatives. Overall, the Aware-BSP revealed a higher sensitivity than Aware-U though 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 was satisfactory, but the Aware-U 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 poorer
performance in our study is unclear. Clade-specific differences in anti-HIV antibodies generated by the host would seem unable to explain why the serum-based assay performed better than the urine-based assay which contained the same recombinant antigens.

Several factors which 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 ART therapy may have decreased anti-HIV antibody titers causing possible false-negative test results (6). However none of the patients in whom Aware-U tests were falsely negative was on ART.

According to the World Health Organization, 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.
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 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.

ACKNOWLEDGEMENTS

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

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

REFERENCES


Table 1  Performance of Aware-BSP rapid blood test compared to serum EIA+WB

<table>
<thead>
<tr>
<th>Aware-BSP Serum test</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>108</td>
<td>2</td>
<td>110</td>
</tr>
<tr>
<td>Negative</td>
<td>2</td>
<td>851</td>
<td>853</td>
</tr>
<tr>
<td>Total</td>
<td>110</td>
<td>853</td>
<td>963</td>
</tr>
</tbody>
</table>

Table 2  Performance of Aware-U rapid urine test in comparison to serum EIA+WB

<table>
<thead>
<tr>
<th>Aware-U Urine Test</th>
<th>Double EIA plus WB if either EIA is positive</th>
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</thead>
<tbody>
<tr>
<td>Positive</td>
<td>94 (1)</td>
</tr>
<tr>
<td>Negative</td>
<td>12 (835)</td>
</tr>
<tr>
<td>Total</td>
<td>106 (836)</td>
</tr>
</tbody>
</table>

Table 3  Performance of Aware rapid HIV 1/2 antibody assays in serum and urine

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>NPV 1</th>
<th>PPV 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aware-BSP</td>
<td>98.2 (93.6-99.8)</td>
<td>99.8 (99.2-99.9)</td>
<td>99.8</td>
<td>98.2</td>
</tr>
<tr>
<td>Aware-U</td>
<td>88.7 (81.1-94.0)</td>
<td>99.9 (99.3-100)</td>
<td>98.6</td>
<td>99.0</td>
</tr>
</tbody>
</table>

1 NPV=Negative Predictive Value
2 PPV=Positive Predictive Value