Comparison of the performance of rapid HIV tests using samples collected for surveillance in Mozambique

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<td>Melo, Josefa; Faculty of Medicine, University Eduardo Mondlane, Department of Microbiology Nilsson, Charlotte; Swedish Institute for Infectious Disease Control, Department of Immunology and Vaccinology Mondlane, Jose; Faculty of Medicine, University Eduardo Mondlane, Department of Microbiology Osman, Nafissa; Central Hospital, Department of Obstetrics and Gynecology Biberfeld, Gunnel; Swedish Institute for Infectious Disease Control, Department of Immunology and Vaccinology Folgosa, Elena; Faculty of Medicine, University Eduardo Mondlane, Department of Microbiology Andersson, Sören; Swedish Institute for Infectious Disease Control, Department of Virology</td>
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Comparison of the performance of rapid HIV tests using samples collected for surveillance in Mozambique

Josefa Melo¹, Charlotta Nilsson³, José Mondlane¹, Nafissa Osman², Gunnel Biberfeld³,⁴, Elena Folgosa¹, Sören Andersson³

¹Department of Microbiology, Faculty of Medicine, University Eduardo Mondlane, Maputo, Mozambique
²Department of Obstetrics and Gynecology, Central Hospital, Maputo, Mozambique
³Department of Virology, Immunology and Vaccinology, Swedish Institute for Infectious Disease Control, Stockholm
⁴Microbiology, Tumor and Cell Biology, Karolinska Institutet, Stockholm, Sweden

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Key words: Mozambique, HIV antibody diagnosis, simple rapid tests, alternative confirmatory strategy, evaluation, seroprevalence.

Corresponding author
Sören Andersson, MD, PhD
Department of Virology, Immunology and Vaccinology
Swedish Institute for Infectious Disease Control
SE-17182 Solna, Sweden
Telephone +46-8-4572661
E-mail: soren.andersson@smi.ki.se

Preliminary results of this study were presented at the International Conference on AIDS, Bangkok, Thailand, July 2004.
Abstract

Mozambique had low HIV prevalence until the mid-1990s, but recent data indicate increasing rates. There is little information on HIV-2. Therefore, HIV sero-prevalence was assessed among pregnant women and field-ready HIV diagnostic strategies were evaluated. A total of 6930 samples collected by three health centres from 2002-2005 were tested on site by nurses with two simple/rapid tests, Determine HIV-1/2 (Abbott; screening) and Uni-Gold HIV (Trinity; confirmation), which is the national HIV testing strategy. The prevalence of HIV was 14.0% (2002), 17.8% (2003), 16.5% (2004) and 20.2% (2005). A subset of 888 samples collected 2003 was sent to the Central Microbiology Laboratory, Maputo for evaluation of tests and testing strategies. The assays included for comparison were Capillus HIV-1/HIV-2 (Trinity), DoubleCheckGold HIV-1&2 (Orgenics) and Enzygnost Anti-HIV-1/2 Plus (Behring, reference ELISA). Confirmation of reactive samples was done by Uni-Gold HIV and ImmunoComb II HIV-1&2 BiSpot (for HIV type differentiation). The Capillus HIV-1/HIV-2 + ImmunoComb II HIV-1&2 BiSpot combination was the gold standard. The sensitivity of the rapid/simple screening assays (Determine HIV-1/2, DoubleCheckGold HIV-1&2) was 100% (N=160) and their (initial) specificities were 99.6% and 99.7%, respectively. Repeated testing and combinations of assays increased the specificity. Four suspected cases of recent seroconversion were found. Together with the increasing prevalence rates, this may indicate that Mozambique is a high-incidence area, although further studies are needed to confirm this. Testing strategies for on-site screening and confirmation based on the combination of Determine HIV-1/2, Uni-Gold HIV and DoubleCheckGold HIV-1&2 are well suited for local field use.
INTRODUCTION

Mozambique has a population of around 18 million and is situated in the area of the world hardest hit by the HIV/AIDS epidemic. Several of its neighbours have HIV prevalence rates above 20% [UNAIDS 2008]. Up until the mid-1990s, Mozambique had low HIV prevalence rates (0.4% for HIV-1 in 1988 [Barreto et al., 1993]), but more recent data indicate a trend of increasing HIV-1 prevalence [Ministry of Health, Mozambique, 2005]. Earlier reports also showed cases of HIV-2 [Barreto et al., 1993], and among displaced pregnant women in rural Mozambique in 1994, infection rates were 1.2% and 2.1% for HIV-1 and HIV-2, respectively [Cossa et al., 1994]. Since then, HIV-2 has not been studied, and the current national testing strategy does not include differentiation between HIV-1 and HIV-2.

In settings like Mozambique, where laboratory facilities and trained staff are limited and people in remote areas have to rely on very basic services, simple/rapid assays for the detection of HIV antibodies are very important because they enable wide-scale HIV screening [WHO/UNAIDS 1998]. By the use of algorithms comprising a combination of two or more rapid tests (so-called alternative confirmatory strategies), HIV test results can be achieved with a level of performance comparable to those of the more conventional but expensive and complicated ELISA-Western blot combinations [Andersson et al., 1997; Stetler et al., 1997; Urassa et al., 2002; WHO/UNAIDS, 1997; Koblavi-Deme et al., 2001]. Thus, both negative and positive results can be reported on the day and site of testing [Kassler et al, 1998], and the patient can receive immediate counselling, which may encourage adoption of risk-reducing behaviours. This potentially increases the number of individuals who get access to testing and who can thereby be offered clinical follow-up and treatment, if necessary [Palmer et al., 1999; Galvan et al., 2004.; Ekwueme et al., 2003; Rouet et al., 2004; Granade, 2005; Morin et al., 2006].

Some recent improvements in simple/rapid tests include the ability to test whole blood specimens (in addition to serum or plasma) and storage at ambient temperatures (usually up to +30° C) [WHO, 2004].
It is important that HIV testing strategies are evaluated in the settings where they are going to be used [CDC, 2002]. In this study, the national HIV testing strategy as performed by health centre staff without formal training in laboratory techniques, was evaluated and compared with established and published strategies used elsewhere, including an assay for differentiation between HIV-1 and HIV-2 [Andersson et al., 1997].

The national HIV testing strategy had not been evaluated in Mozambique before. A new simple/rapid assay designed especially for conditions prevailing in tropical areas was also included (DoubleCheckGold HIV-1&2). In addition to the reference assays, this study included three assays not requiring a cold chain of storage that may enable simple/rapid screening and confirmation in the field. This evaluation was conducted with a portion of the samples collected for a seroprevalence study performed in pregnant women in order to obtain baseline data on HIV-1 and HIV-2 prevalence among pregnant women in Maputo.

MATERIALS AND METHODS

Serum specimens

Samples were collected in the prenatal care units of three major peripheral health centres in the capital, Maputo (José Macamo General Hospital and Bagamoyo and 1º de Junho Health Centres). Pre- and post-test counselling was provided by trained nurse counsellors to all pregnant women as part of the national programme for prevention of mother-to-child transmission of HIV (PMTCT). All pregnant women included gave informed consent for participation. Peripheral blood was collected by veni-puncture (10-ml tubes) at the health centre and HIV testing was performed on site by health centre staff. During the years 2002-2005, a cross-sectional HIV seroprevalence study was performed for which a total of 6930 samples were collected; 520 in 2002 (at José Macamo General Hospital and Bagamoyo health centres only), 2715 in 2003, 2625 in 2004 and 1070 in 2005. Following testing at the respective health centre, 888 sera collected consecutively during 2003 were coded and sent for further analyses to the Laboratory of Microbiology, Department of Microbiology, Faculty of Medicine, University Eduardo Mondlane, Maputo (Central Microbiology Laboratory). These samples were used for test evaluations.
Testing strategy

The study outline is described in Table 1 and the main characteristics of the assays used are described in Table 2. All assays were performed according to the manufacturers’ instructions. The HIV-seroprevalence study was performed using the national testing strategy, which employs Determine HIV-1/2 (Abbott Laboratories, Tokyo, Japan) as the screening test and Uni-Gold HIV (Trinity Biotech, Wicklow, Ireland) for confirmation of reactive samples. These tests were performed in health centres by trained nurses. The samples were then transported to the laboratory for quality control and the evaluation presented here. The tests used in the national diagnostic strategy (Determine HIV 1/2 and Uni-Gold HIV) were evaluated together with an alternative screening assay, DoubleCheckGold HIV1&2 (Orgenics, Yavne, Israel). All three tests are simple/rapid assays, especially suited for field conditions in developing countries. The combination of Capillus HIV-1/HIV-2 (Trinity Biotech) for screening and ImmunoComb II HIV-1&2 BiSpot (Orgenics) for confirmation was used as the gold standard, based on previously published evaluations in which these were compared to western blot and polymerase chain reaction (PCR) assays [Andersson et al., 1997; Walther-Jallow et al., 1999]. The Enzygnost Anti-HIV-1/2 Plus ELISA (Behringwerke, Marburg, Germany) was included for quality control and as an alternative screening test for large-scale testing. Screening-reactive samples were tested using Uni-Gold HIV and ImmunoComb II HIV-1&2 BiSpot, and the latter was used to discriminate between HIV-1 and HIV-2. Sera that yielded discrepant results in some of the assays were retested. In cases where agreement between the different assays was not reached, the INNO-LIA HIV I/II Score (Innogenetics, Gent, Belgium) assay was employed. A few difficult samples were additionally tested by western blot (HIV blot 2.2, Genelabs Diagnostics, Singapore) and HIV-1 p24 antigen assay (Innotest HIV Antigen mAb, Innogenetics). All tests, including the gold standard, were completed before the analysis of their performance was performed. Different combinations of screening (Determine HIV-1/2 and DoubleCheckGold HIV1&2) and confirmation (Uni-Gold HIV) assays were evaluated with or without the standard enzyme immuno-assay (Enzygnost Anti-HIV-1/2 Plus). Sensitivity was calculated as the percentage of test-positive samples among the total
HIV-positive samples as determined by the gold standard (the Capillus HIV-1/HIV-2/ImmunoComb HIV-1&2 BiSpot combination). Specificity was defined as the percentage of test-negative samples among the total HIV-negative samples, as determined by the gold standard. Positive predictive value was determined as the proportion of true positive samples out of the total number of positive samples tested by an assay. Negative predictive values were derived from the proportion of true negative results out of the total number of negative results given by each assay.

**Ethical clearance**

Ethical clearance was received from the Ministry of Health, Mozambique, the Faculty of Medicine, University Eduardo Mondlane, Maputo and from the Scientific Committee of the Central Hospital, Maputo, Mozambique. Authorisation was also given by the clinical directorate of the three health centres participating in the study. All participants filled in an informed consent form and received pre- and post-test counselling.

**Results**

Using the national testing strategy, i.e., screening by Determine HIV-1/2 and confirmation by Uni-Gold HIV, the total HIV prevalence among all women included from the three health centres was 14.0% (73/520) in 2002, 17.8% (484/2715) in 2003, 16.5% (433/2625) in 2004 and 20.2% (216/1070) in 2005. The difference between the prevalences in 2002 and 2005 are statistically significant (Chi-square test, p=0.03; Statistix, Analytical Software, Tallahassee, FL, USA).

The main results of the evaluation of the tests and testing strategies are summarised in Table 3. There were a total of 160 HIV-1-positive and 723 HIV-negative samples according to the gold standard based on Capillus HIV-1/HIV-2 and ImmunoComb II HIV-1&2 BiSpot. No HIV-2-positive samples were found. For the resolution of five samples with discordant results, see below.

The sensitivity of the two screening assays evaluated (Determine HIV-1/2 and DoubleCheckGold HIV-1&2) was 100% for each. The 160 HIV-1-positive samples were
also correctly identified by Uni-Gold HIV as well as the Enzygnost Anti-HIV-1/2 Plus ELISA. Thus, any combination of the simple/rapid assays and the ELISA included in the evaluation correctly detected and confirmed all HIV-1-positive samples.

The specificity after initial testing was 99.6% for Determine HIV-1/2 and 99.7% for DoubleCheckGold HIV-1&2. After repeated testing with the same simple/rapid test or any other of the assays evaluated, the specificities were 99.9-100% (Table 3).

The positive predictive values ranged from 97.6% to 100%. The negative predictive values were 100% throughout (Table 3).

Five samples showed discrepant (indeterminate) results (Table 4). By applying the immunoblot assays and the p24 antigen assay, four of them showed a reactivity pattern resembling recent seroconversion (for details see Table 4). Two of the samples were HIV p24 antigen positive and three had western blot reactivity consistent with samples under seroconversion (antibodies to p24 and gp160 only). These three samples also fulfilled the criteria for HIV-1-positivity by Inno-Lia. One sample could not be given a final diagnosis (sample no. 441). It could be either a sample with false reactivity from a non-HIV-infected individual or another seroconversion sample. Three of the four suspected seroconversion samples were initially reactive with Determine HIV-1/2 but not with DoubleCheckGold HIV-1&2. In contrast to this, the fourth sample was DoubleCheckGold HIV-1&2-reactive but Determine HIV-1/2 negative. All four samples were negative by Capillus HIV-1/HIV-2, i.e., the gold standard screening assay did not detect the samples although two of them were positive by ImmunoComb II HIV-1&2 BiSpot (which was only used for confirmation of screening-reactive samples). If these four suspected seroconversion samples (excluding sample no. 441) were designated “HIV positive” and included in the sensitivity analysis, the results would be 99.4% for Determine HIV-1/2, 98.2% for DoubleCheckGold HIV-1&2 and 99.4% for the Enzynost Anti-HIV-1/2 Plus ELISA. The addition of a confirmatory step with another test gave sensitivities between 97.6% and 99.4% (data not shown). If the discordant samples were designated “HIV negative”, the specificities would be 99.2%, 99.4% and 99.0% for
Determine HIV-1/2, DoubleCheckGold HIV-1&2 and Enzygnost Anti-HIV-1/2 Plus, respectively (not shown).

**DISCUSSION**

This study was an evaluation of an HIV antibody diagnostic strategy based on simple/rapid assays used for routine diagnosis in three health centres in Maputo, Mozambique. The national policy for HIV diagnosis in Mozambique advocates the use of Determine HIV-1/2 for screening and Uni-Gold HIV for confirmation. Final test results may then be given to the persons tested at the examination site on the same day. The testing is usually performed by trained health staff, which was also the case in this evaluation. Both of these assays can be stored at ambient temperature (maximum +30°C).

A more recently introduced simple/rapid assay developed especially for field use in resource-poor settings (DoubleCheckGold HIV-1&2) was also included. Similarly, this test does not require refrigerated storage. According to WHO recommendations for alternative confirmatory strategies, two or three assays in a carefully designed combination may be applied, depending on HIV prevalence and purpose for testing [WHO, 1997]. The results of this study show that a number of two or three-assay combinations of these simple/rapid tests can be chosen, where none of the tests require a cold chain of storage and transport. Although they are all based on immunochromatographic lateral flow principles, the antigens and manufacturers are different. In order to avoid false reactivity it is important that test principles and antigens are different when alternative confirmatory strategies are formed. Among the assays included in this study, the tendency to give false or indeterminate reactivity was low.

An ambitious plan for universal access to antiretroviral treatment (ART) for all HIV-infected individuals in need in Mozambique is now being developed. Furthermore, since 1999, UNICEF Mozambique has been supporting the Ministry of Health for the development of policy guidelines for prevention of mother-to-child transmission of HIV [Peffer et al., 2002]. A national plan for the prevention of mother-to-child transmission of HIV using combination ART has been launched recently [Ministry of Health, Mozambique, 2004]. The program is based on a national strategy of integrating HIV
counselling and testing along with the provision of short-course ARV prophylaxis in prenatal clinics. This requires appropriate counselling and testing services even in remote areas. For this purpose, an HIV diagnostic strategy for field use based on simple/rapid assays has been introduced in Mozambique as a national policy proposed by the Ministry of Health. However, this testing strategy had not been previously evaluated in Mozambique. This study shows that the national strategy using screening with Determine HIV-1/2 and confirmation with Uni-Gold HIV has a sensitivity and specificity of 100%, respectively, as compared to the gold standard chosen for the evaluation. The strategy is therefore well suited for use in the program for prevention of mother-to-child transmission of HIV.

The ideal test for simple/rapid diagnosis of HIV infection should be rapid, inexpensive, highly sensitive and specific, easy to perform and the results should be easy to interpret. The test should tolerate room temperature storage, have a long shelf life and require no additional equipment or ancillary supplies [Malone et al, 1993]. The assay should preferably also allow testing of whole blood, although that aspect was not evaluated here. Whole blood testing is not possible with the DoubleCheckGold HIV-1&2 assay, but the tests included here otherwise conform to these requirements. In the prenatal care settings involved in this study, the handling of serum and plasma specimens is routine, thus whole blood testing would not improve service or increase the number of women being tested. However, in more remote areas of Mozambique, the ability to carry out HIV testing on whole blood samples is likely to be an advantage and could lead to a higher coverage of HIV diagnoses. Thus, under those circumstances, DoubleCheckGold HIV-1&2 would not be an option.

This study showed that the sensitivity of the screening assays (Determine HIV-1/2, DoubleCheckGold HIV-1&2) was 100% (n=160, excluding four suspected seroconverters), and the initial specificity for Determine HIV-1/2 was 99.6% (720/723) and for DoubleCheckGold HIV-1&2 it was 99.7% (721/723). A study performed in Honduras and the Dominican Republic showed that both the sensitivity and specificity of
Determine HIV-1/2 was 100% [Palmer et al., 1999]. Similar results were also seen in Thailand [Arai et al., 1999].

The seroprevalence data obtained in the three health centres show a high and possibly increasing HIV prevalence in Maputo, Mozambique. This confirms data reported by the Ministry of Health from the national sentinel surveillance system in which the 2004 figures show that Maputo City as well as Maputo Province have just above 20% HIV prevalence among pregnant women [Ministry of Health, Mozambique, 2005]. This epidemiological pattern is similar to what has been observed in neighbouring South Africa, with low prevalence rates in the early 1990s that thereafter increased rapidly to figures now well above 20% generally and even 30% in some provinces [UNAIDS, 2007; Stuart et al. 1999; Coleman and Wilkinson, 1997].

The current national diagnostic strategy in Mozambique does not allow for differentiation between HIV-1 and HIV-2, although HIV-1 and HIV-2 differentiation is needed for correct treatment policies. However, this study indicates that HIV-2 is not common in this part of the country, and the diagnostic strategy is therefore currently appropriate. Previous early reports of HIV-2 in Mozambique mainly included samples from the northern provinces, and it would be of interest to follow this up in order to assess whether any alternatives to the current diagnostic strategy should be implemented. As shown here for HIV-1 and previously for HIV-1 and HIV-2 [Andersson et al., 1997], the ImmunoComb II HIV-1&2 BiSpot assay may then be a suitable alternative (in addition to or instead of Uni-gold HIV) for differentiation between the two HIV types.

Four samples were reactive with an antibody pattern resembling what is commonly seen in seroconverters. Two of them were also HIV p24 antigen-positive. This shows that all discrepancies and weak reactivities will have to be carefully followed up since neither of the assays evaluated here are designed for antigen detection. This could reflect the situation in a high-incidence area. It should be noted that since we only used antibody assays, all suspected seroconverters must have had some degree of antibody production that could be picked up by at least one of the assays. Samples taken during the window
when only antigen is available will require specific antigen testing. Combination assays for simultaneous antigen and antibody detection should be considered where feasible in Mozambique, e.g., in blood donor screening. More detailed information on current incidence rates could be obtained, e.g., through incidence testing of cross-sectional samples via specially adapted antibody screening assays [Hu et al., 2003; Constantine and Zink, 2005; Janssen et al., 1998; Soroka et al., 2005]. Another option would be to establish prospective cohort studies, although this is a much more cumbersome task. The fact that the screening assays evaluated in this study were reactive for some of the suspected seroconversion samples may indicate that they have a higher sensitivity for samples collected soon after seroconversion than the gold standard assays. However, the number of discrepant samples was too small to draw a general conclusion about this. It is also interesting to note that three of the four suspected seroconversion samples would have been detected and confirmed by the more conventional but expensive strategy comprising the Enzygnost Anti-HIV1/2 Plus ELISA followed by INNO-LIA HIV I/II Score. However, by western blot, three of the four samples were indeterminate and one was negative. In a clinical situation they could be followed up with further testing and/or have new samples taken at a later stage, and thus would not necessarily be missed as suspected HIV patients.

It can be concluded that the national diagnostic strategy for HIV based on simple rapid assays was performed well by non-laboratory personnel in a routine diagnostic setting in three large health centres in Mozambique. The strategy is highly sensitive and specific, and the tests are robust and do not require cold storage as long as temperatures stay below +30°C. The finding of high and increasing HIV-1 prevalence rates and of cases of suspected recent seroconversion among pregnant women indicates a high HIV-1 incidence. In view of these data, combination assays for HIV antigen and antibody detection should be considered in Mozambique, especially for use in blood donor screening. Preventive measures against further spread of HIV should continue to have high priority.

Acknowledgements
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REFERENCES


WHO/UNAIDS. 2004. HIV simple/rapid assays: operational characteristics (Phase 1).

Table 1. Study outline

I. Seroprevalence study performed in 2002-2005 (6930 samples)
Screening with Determine HIV1/2 (Abbott Laboratories, Tokyo, Japan)*
Confirmation of screening-reactive samples with Unigold HIV (Trinity Biotech, Wicklow, Ireland)*

II. Evaluation of tests and testing strategies utilising 888 samples collected in 2003
a) Tests used in national test strategy:
Determine HIV1/2* for screening
Unigold HIV* for confirmation of screening-reactive samples

b) Alternative screening assay:
DoubleCheckGold HIV1&2 (Orgenics, Yavne, Israel)

c) Quality control and alternative screening assay for large scale testing:
Enzygnost Anti-HIV-1/2 Plus (Behringwerke, Marburg, Germany)

d) Gold standard:
Capillus HIV-1/HIV-2 (Trinity Biotech, Wicklow, Ireland)
ImmunoComb II HIV-1&2 BiSpot (Orgenics, Yavne, Israel; performed on reactive samples to discriminate between HIV-1 and HIV-2)

e) Resolution of discordant specimens:
INNO-LIA HIV 1/II Score (Innogenetics, Gent, Belgium)
HIV Blot 2.2 (Genelabs Diagnostics, Singapore)
Innotest HIV Antigen Mab (Innogenetics, Gent, Belgium)
* Tests performed on site at each respective health centre by trained nurse counsellors.

All other assays were performed at the Department of Microbiology, Faculty of Medicine, University Eduardo Mondlane, Maputo, Mozambique.
Table 2. Characteristics of the assays

<table>
<thead>
<tr>
<th>Test (Manufacturer)</th>
<th>Antigen type</th>
<th>Assay type</th>
<th>Solid surface</th>
<th>Specimen type</th>
<th>Test incubation time (min)</th>
<th>Storage temp. (+C°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Determine HIV-1/2 (Abbott Laboratories)</td>
<td>HIV-1 and HIV-2 RP and SP</td>
<td>immuno-chromatographic, lateral flow, S/R</td>
<td>nitrocellulose membrane</td>
<td>serum, plasma, whole blood (EDTA)</td>
<td>15</td>
<td>2-30</td>
</tr>
<tr>
<td>DoubleCheckGold HIV-1&amp;2 (Orgenics)</td>
<td>HIV-1 and HIV-2 env and gag RP</td>
<td>immuno-chromatographic, lateral flow, S/R</td>
<td>nitrocellulose membrane</td>
<td>serum, plasma</td>
<td>15</td>
<td>2-27</td>
</tr>
<tr>
<td>Enzygnost Anti-HIV-1/2 Plus† (Behringwerke)</td>
<td>HIV-1 gp41 RP, HIV-2 gp36 RP</td>
<td>sandwich EIA</td>
<td>microtitre plate</td>
<td>serum, plasma</td>
<td>90</td>
<td>2-8</td>
</tr>
<tr>
<td>Capillus HIV-1/HIV-2 (Trinity Biotech)</td>
<td>HIV-1 p24, gp41 RP, HIV-2 gp36 RP</td>
<td>agglutination, S/R</td>
<td>latex particles</td>
<td>serum, plasma</td>
<td>7</td>
<td>2-8</td>
</tr>
<tr>
<td>ImmunoComb II HIV-1&amp;2 BiSpot† (Orgenics)</td>
<td>HIV-1gp41, gp120 SP, HIV-2 gp36 SP</td>
<td>indirect EIA, S/R</td>
<td>plastic comb with 12 projections</td>
<td>serum, plasma</td>
<td>37</td>
<td>2-8</td>
</tr>
<tr>
<td>INNO-LIA HIV I/II score† (Innogenetics)</td>
<td>HIV-1 p17, p24, p31, gp41, p120 and HIV-2 gp36, gp105</td>
<td>line immunoassay</td>
<td>nitrocellulose strip</td>
<td>serum, plasma</td>
<td>180</td>
<td>2-8</td>
</tr>
<tr>
<td>HIVBlot2.2 (Genelabs Diagnostics)</td>
<td>HIV-1 p17, p24, p31, gp41, p39, p51, p55, p66, gp120, gp160</td>
<td>western blot</td>
<td>nitrocellulose strip</td>
<td>serum, plasma</td>
<td>137</td>
<td>2-8</td>
</tr>
<tr>
<td>Innotest HIV Antigen Mab (Innogenetics)</td>
<td>HIV-2 gp36 SP Human polyclonal antibodies (anti-HIV-1)</td>
<td>double sandwich EIA microtitre plate</td>
<td>serum, plasma, cell culture supernatant</td>
<td>120</td>
<td>2-8</td>
<td></td>
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</tbody>
</table>
1 | RP, recombinant protein; SP, synthetic peptide; EIA enzyme immunoassay; S/R, simple rapid; *Antibodies of any Ig class can be detected;†Discriminates between HIV-1 and HIV-2. |
2 | |
3 | |
4 | |
5 | This table is a summary of information available from the manufacturers. |
Table 3. Results from various combinations of assays

<table>
<thead>
<tr>
<th>Assays</th>
<th>Sensitivity [no. positive/no. tested (%, c.i.)]</th>
<th>Specificity [no. negative/no. tested (%, c.i.)]</th>
<th>Positive predictive values (no. true positive/no. tested positive (%, c.i.))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Determine HIV-1/2</td>
<td>160/160 (100%, 99.4-100%)</td>
<td>720/723 (99.6%, 99.1-100%)</td>
<td>160/163 (98.2%, 96.1-100%)</td>
</tr>
<tr>
<td>Determine HIV-1/2 → Determine HIV-1/2</td>
<td>160/160 (100%)</td>
<td>722/723 (99.9%, 99.6-100%)</td>
<td>160/161 (99.4%, 98.2-100%)</td>
</tr>
<tr>
<td>Determine HIV-1/2 → DoubleCheckGold HIV-1&amp;2</td>
<td>160/160 (100%)</td>
<td>722/723 (99.9%)</td>
<td>160/161 (99.4%, 98.2-100%)</td>
</tr>
<tr>
<td>Determine HIV-1/2 → Determine HIV-1/2 → DoubleCheckGold HIV-1&amp;2</td>
<td>160/160 (100%)</td>
<td>723/723 (100%, 99.7-100%)</td>
<td>160/160 (100%, 99.4-100%)</td>
</tr>
<tr>
<td>Determine HIV-1/2 → Uni-Gold HIV</td>
<td>160/160 (100%)</td>
<td>723/723 (100%)</td>
<td>160/160 (100%)</td>
</tr>
<tr>
<td>Determine HIV-1/2 → Enzygnost Anti-HIV-1/2 Plus</td>
<td>160/160 (100%)</td>
<td>723/723 (100%)</td>
<td>160/160 (100%)</td>
</tr>
<tr>
<td>DoubleCheckGold HIV-1&amp;2</td>
<td>160/160 (100%, 99.4-100%)</td>
<td>723/723 (100%, 99.7-100%)</td>
<td>160/160 (100%)</td>
</tr>
<tr>
<td>DoubleCheckGold HIV-1&amp;2 → DoubleCheckGold HIV-1&amp;2</td>
<td>160/160 (100%)</td>
<td>723/723 (100%, 99.7-100%)</td>
<td>160/160 (100%)</td>
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<tr>
<td>DoubleCheckGold HIV-1&amp;2 → Uni-Gold HIV</td>
<td>160/160 (100%)</td>
<td>723/723 (100%)</td>
<td>160/160 (100%)</td>
</tr>
<tr>
<td>DoubleCheckGold HIV-1&amp;2 → Determine HIV-1/2</td>
<td>160/160 (100%)</td>
<td>722/723 (99.9%, 99.6-100%)</td>
<td>160/161 (99.4%, 98.2-100%)</td>
</tr>
</tbody>
</table>
| DoubleCheckGold HIV-1&2 → Enzygnost Anti-
<table>
<thead>
<tr>
<th>Assay Combination</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzygnost Anti-HIV-1/2 Plus → Enzygnost Anti-HIV-1/2 Plus</td>
<td>160/160</td>
<td>723/723</td>
</tr>
<tr>
<td>Enzygnost Anti-HIV-1/2 Plus → Determine HIV-1/2</td>
<td>160/160</td>
<td>723/723</td>
</tr>
<tr>
<td>Enzygnost Anti-HIV-1/2 Plus → DoubleCheckGold HIV-1&amp;2</td>
<td>160/160</td>
<td>723/723</td>
</tr>
<tr>
<td>Enzygnost Anti-HIV-1/2 Plus → Uni-Gold HIV</td>
<td>160/160</td>
<td>723/723</td>
</tr>
</tbody>
</table>

Gold standard for the evaluation: screening by Capillus HIV-1/HIV-2, confirmation by ImmunoComb II HIV-1&2 BiSpot.

c.i., 95% confidence interval.

Negative predictive values are 100% for all assays and assay combinations included here (no false negative results).

Five samples with discordant results that could not be given a final HIV status are excluded from the analyses of sensitivity and specificity presented in this table (but are included in the text).
### Table 4. Patterns of reactivity of the samples from suspected seroconverters

<table>
<thead>
<tr>
<th>Sample</th>
<th>Determine HIV-1/2</th>
<th>DoubleCheck Gold HIV-1&amp;2</th>
<th>Uni-Gold HIV</th>
<th>ImmunoComb II HIV-1&amp;2</th>
<th>Enzygnost Anti-HIV-1/2 Plus</th>
<th>Capillus HIV-1/HIV-2</th>
<th>INNO-LIA HIV I/II Score (bands)</th>
<th>HIVBlot2.2 (bands)</th>
<th>Innotest HIV Antigen mab</th>
</tr>
</thead>
<tbody>
<tr>
<td>179</td>
<td>WEAK POS, (repeat NEGx2)</td>
<td>NEG</td>
<td>NEG</td>
<td>NEG</td>
<td>POS</td>
<td>NEG</td>
<td>POS HIV-1</td>
<td>IND (p17,p24, gp160)</td>
<td>NEG</td>
</tr>
<tr>
<td>276</td>
<td>POS</td>
<td>NEG</td>
<td>WEAK (repeat NEG)</td>
<td>POS HIV-1</td>
<td>POS</td>
<td>NEG</td>
<td>POS HIV-1</td>
<td>IND (p17,p24, gp160)</td>
<td>POS</td>
</tr>
<tr>
<td>396</td>
<td>POS</td>
<td>NEG</td>
<td>NEG</td>
<td>POS HIV-1</td>
<td>POS</td>
<td>NEG</td>
<td>POS HIV-1</td>
<td>IND (p24,gp160)</td>
<td>ND (no serum)</td>
</tr>
<tr>
<td>513</td>
<td>NEG</td>
<td>POS</td>
<td>NEG</td>
<td>NEG</td>
<td>NEG</td>
<td>NEG</td>
<td>IND (gp120)</td>
<td>NEG</td>
<td>POS</td>
</tr>
<tr>
<td>441</td>
<td>NEG</td>
<td>POS</td>
<td>NEG</td>
<td>NEG</td>
<td>NEG</td>
<td>NEG</td>
<td>IND (gp120)</td>
<td>IND (p24)</td>
<td>NEG</td>
</tr>
</tbody>
</table>

POS = positive, NEG = negative, IND = indeterminate, ND = not done

NB, sample 441 had reactivity in INNO-LIA HIV I/II Score similar to that of sample 513 but was p24 antigen-negative, western blot negative and could not be given a final diagnosis. It was excluded from the assay evaluation.