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

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4 1 **Comparison of the performance of rapid HIV tests using samples**
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6 2 **collected for surveillance in Mozambique**
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29 Preliminary results of this study were presented at the International Conference on AIDS,
30 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.

1 INTRODUCTION

2 Mozambique has a population of around 18 million and is situated in the area of the
3 world hardest hit by the HIV/AIDS epidemic. Several of its neighbours have HIV
4 prevalence rates above 20% [UNAIDS 2008]. Up until the mid-1990s, Mozambique had
5 low HIV prevalence rates (0.4% for HIV-1 in 1988 [Barreto et al., 1993]), but more
6 recent data indicate a trend of increasing HIV-1 prevalence [Ministry of Health,
7 Mozambique, 2005]. Earlier reports also showed cases of HIV-2 [Barreto et al., 1993],
8 and among displaced pregnant women in rural Mozambique in 1994, infection rates were
9 1.2% and 2.1% for HIV-1 and HIV-2, respectively [Cossa et al., 1994]. Since then, HIV-
10 2 has not been studied, and the current national testing strategy does not include
11 differentiation between HIV-1 and HIV-2.

12
13 In settings like Mozambique, where laboratory facilities and trained staff are limited and
14 people in remote areas have to rely on very basic services, simple/rapid assays for the
15 detection of HIV antibodies are very important because they enable wide-scale HIV
16 screening [WHO/UNAIDS 1998]. By the use of algorithms comprising a combination of
17 two or more rapid tests (so-called alternative confirmatory strategies), HIV test results
18 can be achieved with a level of performance comparable to those of the more
19 conventional but expensive and complicated ELISA-Western blot combinations
20 [Andersson et al., 1997; Stetler et al., 1997; Urassa et al., 2002; WHO/UNAIDS, 1997;
21 Koblavi-Deme et al., 2001]. Thus, both negative and positive results can be reported on
22 the day and site of testing [Kassler et al, 1998], and the patient can receive immediate
23 counselling, which may encourage adoption of risk-reducing behaviours. This potentially
24 increases the number of individuals who get access to testing and who can thereby be
25 offered clinical follow-up and treatment, if necessary [Palmer et al., 1999; Galvan et al.,
26 2004.; Ekwueme et al., 2003; Rouet et al., 2004; Granade, 2005; Morin et al., 2006].
27 Some recent improvements in simple/rapid tests include the ability to test whole blood
28 specimens (in addition to serum or plasma) and storage at ambient temperatures (usually
29 up to +30° C) [WHO, 2004].

1 It is important that HIV testing strategies are evaluated in the settings where they are
2 going to be used [CDC, 2002]. In this study, the national HIV testing strategy as
3 performed by health centre staff without formal training in laboratory techniques, was
4 evaluated and compared with established and published strategies used elsewhere,
5 including an assay for differentiation between HIV-1 and HIV-2 [Andersson et al., 1997].
6 The national HIV testing strategy had not been evaluated in Mozambique before. A new
7 simple/rapid assay designed especially for conditions prevailing in tropical areas was also
8 included (DoubleCheckGold HIV-1&2). In addition to the reference assays, this study
9 included three assays not requiring a cold chain of storage that may enable simple/rapid
10 screening and confirmation in the field. This evaluation was conducted with a portion of
11 the samples collected for a seroprevalence study performed in pregnant women in order
12 to obtain baseline data on HIV-1 and HIV-2 prevalence among pregnant women in
13 Maputo.

14 15 **MATERIALS AND METHODS**

16 **Serum specimens**

17 Samples were collected in the prenatal care units of three major peripheral health centres
18 in the capital, Maputo (José Macamo General Hospital and Bagamoyo and 1° de Junho
19 Health Centres). Pre- and post-test counselling was provided by trained nurse counsellors
20 to all pregnant women as part of the national programme for prevention of mother-to-
21 child transmission of HIV (PMTCT). All pregnant women included gave informed
22 consent for participation. Peripheral blood was collected by veni-puncture (10-ml tubes)
23 at the health centre and HIV testing was performed on site by health centre staff. During
24 the years 2002-2005, a cross-sectional HIV seroprevalence study was performed for
25 which a total of 6930 samples were collected; 520 in 2002 (at José Macamo General
26 Hospital and Bagamoyo health centres only), 2715 in 2003, 2625 in 2004 and 1070 in
27 2005. Following testing at the respective health centre, 888 sera collected consecutively
28 during 2003 were coded and sent for further analyses to the Laboratory of Microbiology,
29 Department of Microbiology, Faculty of Medicine, University Eduardo Mondlane,
30 Maputo (Central Microbiology Laboratory). These samples were used for test
31 evaluations.

1

2 **Testing strategy**

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

1 HIV-positive samples as determined by the gold standard (the Capillus HIV-1/HIV-
2 2/ImmunoComb HIV-1&2 BiSpot combination). Specificity was defined as the
3 percentage of test-negative samples among the total HIV-negative samples, as determined
4 by the gold standard. Positive predictive value was determined as the proportion of true
5 positive samples out of the total number of positive samples tested by an assay. Negative
6 predictive values were derived from the proportion of true negative results out of the total
7 number of negative results given by each assay.

8 9 **Ethical clearance**

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

15 16 **Results**

17 Using the national testing strategy, i.e., screening by Determine HIV-1/2 and
18 confirmation by Uni-Gold HIV, the total HIV prevalence among all women included
19 from the three health centres was 14.0% (73/520) in 2002, 17.8% (484/2715) in 2003,
20 16.5% (433/2625) in 2004 and 20.2% (216/1070) in 2005. The difference between the
21 prevalences in 2002 and 2005 are statistically significant (Chi-square test, $p=0.03$;
22 Statistix, Analytical Software, Tallahassee, FL, USA).

23
24 The main results of the evaluation of the tests and testing strategies are summarised in
25 Table 3. There were a total of 160 HIV-1-positive and 723 HIV-negative samples
26 according to the gold standard based on Capillus HIV-1/HIV-2 and ImmunoComb II
27 HIV-1&2 BiSpot. No HIV-2-positive samples were found. For the resolution of five
28 samples with discordant results, see below.

29
30 The sensitivity of the two screening assays evaluated (Determine HIV-1/2 and
31 DoubleCheckGold HIV-1&2) was 100% for each. The 160 HIV-1-positive samples were

1 also correctly identified by Uni-Gold HIV as well as the Enzygnost Anti-HIV-1/2 Plus
2 ELISA. Thus, any combination of the simple/rapid assays and the ELISA included in the
3 evaluation correctly detected and confirmed all HIV-1-positive samples.

4
5 The specificity after initial testing was 99.6% for Determine HIV-1/2 and 99.7% for
6 DoubleCheckGold HIV-1&2. After repeated testing with the same simple/rapid test or
7 any other of the assays evaluated, the specificities were 99.9-100% (Table 3).

8
9 The positive predictive values ranged from 97.6% to 100%. The negative predictive
10 values were 100% throughout (Table 3).

11
12 Five samples showed discrepant (indeterminate) results (Table 4). By applying the
13 immunoblot assays and the p24 antigen assay, four of them showed a reactivity pattern
14 resembling recent seroconversion (for details see Table 4). Two of the samples were HIV
15 p24 antigen positive and three had western blot reactivity consistent with samples under
16 seroconversion (antibodies to p24 and gp160 only). These three samples also fulfilled the
17 criteria for HIV-1-positivity by Inno-Lia. One sample could not be given a final diagnosis
18 (sample no 441). It could be either a sample with false reactivity from a non-HIV-
19 infected individual or another seroconversion sample. Three of the four suspected
20 seroconversion samples were initially reactive with Determine HIV-1/2 but not with
21 DoubleCheckGold HIV-1&2. In contrast to this, the fourth sample was
22 DoubleCheckGold HIV-1&2-reactive but Determine HIV-1/2 negative. All four samples
23 were negative by Capillus HIV-1/HIV-2, i.e., the gold standard screening assay did not
24 detect the samples although two of them were positive by ImmunoComb II HIV-1&2
25 BiSpot (which was only used for confirmation of screening-reactive samples). If these
26 four suspected seroconversion samples (excluding sample no. 441) were designated “HIV
27 positive” and included in the sensitivity analysis, the results would be 99.4% for
28 Determine HIV-1/2, 98.2% for DoubleCheckGold HIV-1&2 and 99.4% for the Enzygnost
29 Anti-HIV-1/2 Plus ELISA. The addition of a confirmatory step with another test gave
30 sensitivities between 97.6% and 99.4% (data not shown). If the discordant samples were
31 designated “HIV negative”, the specificities would be 99.2%, 99.4% and 99.0% for

1 Determine HIV-1/2, DoubleCheckGold HIV-1&2 and Enzygnost Anti-HIV-1/2 Plus,
2 respectively (not shown).

3

4 **DISCUSSION**

5 This study was an evaluation of an HIV antibody diagnostic strategy based on
6 simple/rapid assays used for routine diagnosis in three health centres in Maputo,
7 Mozambique. The national policy for HIV diagnosis in Mozambique advocates the use of
8 Determine HIV-1/2 for screening and Uni-Gold HIV for confirmation. Final test results
9 may then be given to the persons tested at the examination site on the same day. The
10 testing is usually performed by trained health staff, which was also the case in this
11 evaluation. Both of these assays can be stored at ambient temperature (maximum +30° C).
12 A more recently introduced simple/rapid assay developed especially for field use in
13 resource-poor settings (DoubleCheckGold HIV-1&2) was also included. Similarly, this
14 test does not require refrigerated storage. According to WHO recommendations for
15 alternative confirmatory strategies, two or three assays in a carefully designed
16 combination may be applied, depending on HIV prevalence and purpose for testing
17 [WHO, 1997]. The results of this study show that a number of two or three-assay
18 combinations of these simple/rapid tests can be chosen, where none of the tests require a
19 cold chain of storage and transport. Although they are all based on
20 immunochromatographic lateral flow principles, the antigens and manufacturers are
21 different. In order to avoid false reactivity it is important that test principles and antigens
22 are different when alternative confirmatory strategies are formed. Among the assays
23 included in this study, the tendency to give false or indeterminate reactivity was low.

24

25 An ambitious plan for universal access to antiretroviral treatment (ART) for all HIV-
26 infected individuals in need in Mozambique is now being developed. Furthermore, since
27 1999, UNICEF Mozambique has been supporting the Ministry of Health for the
28 development of policy guidelines for prevention of mother-to-child transmission of HIV
29 [Peffer et al., 2002]. A national plan for the prevention of mother-to-child transmission of
30 HIV using combination ART has been launched recently [Ministry of Health,
31 Mozambique, 2004]. The program is based on a national strategy of integrating HIV

1 counselling and testing along with the provision of short-course ARV prophylaxis in
2 prenatal clinics. This requires appropriate counselling and testing services even in remote
3 areas. For this purpose, an HIV diagnostic strategy for field use based on simple/rapid
4 assays has been introduced in Mozambique as a national policy proposed by the Ministry
5 of Health. However, this testing strategy had not been previously evaluated in
6 Mozambique. This study shows that the national strategy using screening with Determine
7 HIV-1/2 and confirmation with Uni-Gold HIV has a sensitivity and specificity of 100%,
8 respectively, as compared to the gold standard chosen for the evaluation. The strategy is
9 therefore well suited for use in the program for prevention of mother-to-child
10 transmission of HIV.

11
12 The ideal test for simple/rapid diagnosis of HIV infection should be rapid, inexpensive,
13 highly sensitive and specific, easy to perform and the results should be easy to interpret.
14 The test should tolerate room temperature storage, have a long shelf life and require no
15 additional equipment or ancillary supplies [Malone et al, 1993]. The assay should
16 preferably also allow testing of whole blood, although that aspect was not evaluated here.
17 Whole blood testing is not possible with the DoubleCheckGold HIV-1&2 assay, but the
18 tests included here otherwise conform to these requirements. In the prenatal care settings
19 involved in this study, the handling of serum and plasma specimens is routine, thus whole
20 blood testing would not improve service or increase the number of women being tested.
21 However, in more remote areas of Mozambique, the ability to carry out HIV testing on
22 whole blood samples is likely to be an advantage and could lead to a higher coverage of
23 HIV diagnoses. Thus, under those circumstances, DoubleCheckGold HIV-1&2 would not
24 be an option.

25
26 This study showed that the sensitivity of the screening assays (Determine HIV-1/2,
27 DoubleCheckGold HIV-1&2) was 100% (n=160, excluding four suspected
28 seroconverters), and the initial specificity for Determine HIV-1/2 was 99.6% (720/723)
29 and for DoubleCheckGold HIV-1&2 it was 99.7% (721/723). A study performed in
30 Honduras and the Dominican Republic showed that both the sensitivity and specificity of

1 Determine HIV-1/2 was 100% [Palmer et al., 1999]. Similar results were also seen in
2 Thailand [Arai et al., 1999].

3
4 The seroprevalence data obtained in the three health centres show a high and possibly
5 increasing HIV prevalence in Maputo, Mozambique. This confirms data reported by the
6 Ministry of Health from the national sentinel surveillance system in which the 2004
7 figures show that Maputo City as well as Maputo Province have just above 20% HIV
8 prevalence among pregnant women [Ministry of Health, Mozambique, 2005]. This
9 epidemiological pattern is similar to what has been observed in neighbouring South
10 Africa, with low prevalence rates in the early 1990s that thereafter increased rapidly to
11 figures now well above 20% generally and even 30% in some provinces [UNAIDS, 2007;
12 Stuart et al. 1999; Coleman and Wilkinson, 1997].

13
14 The current national diagnostic strategy in Mozambique does not allow for differentiation
15 between HIV-1 and HIV-2, although HIV-1 and HIV-2 differentiation is needed for
16 correct treatment policies. However, this study indicates that HIV-2 is not common in this
17 part of the country, and the diagnostic strategy is therefore currently appropriate.
18 Previous early reports of HIV-2 in Mozambique mainly included samples from the
19 northern provinces, and it would be of interest to follow this up in order to assess whether
20 any alternatives to the current diagnostic strategy should be implemented. As shown here
21 for HIV-1 and previously for HIV-1 and HIV-2 [Andersson et al., 1997], the
22 ImmunoComb II HIV-1&2 BiSpot assay may then be a suitable alternative (in addition to
23 or instead of Uni-gold HIV) for differentiation between the two HIV types.

24
25 Four samples were reactive with an antibody pattern resembling what is commonly seen
26 in seroconverters. Two of them were also HIV p24 antigen-positive. This shows that all
27 discrepancies and weak reactivities will have to be carefully followed up since neither of
28 the assays evaluated here are designed for antigen detection. This could reflect the
29 situation in a high-incidence area. It should be noted that since we only used antibody
30 assays, all suspected seroconverters must have had some degree of antibody production
31 that could be picked up by at least one of the assays. Samples taken during the window

1 when only antigen is available will require specific antigen testing. Combination assays
2 for simultaneous antigen and antibody detection should be considered where feasible in
3 Mozambique, e.g., in blood donor screening. More detailed information on current
4 incidence rates could be obtained, e.g., through incidence testing of cross-sectional
5 samples via specially adapted antibody screening assays [Hu et al., 2003; Constantine and
6 Zink, 2005; Janssen et al., 1998; Soroka et al., 2005]. Another option would be to
7 establish prospective cohort studies, although this is a much more cumbersome task. The
8 fact that the screening assays evaluated in this study were reactive for some of the
9 suspected seroconversion samples may indicate that they have a higher sensitivity for
10 samples collected soon after seroconversion than the gold standard assays. However, the
11 number of discrepant samples was too small to draw a general conclusion about this. It is
12 also interesting to note that three of the four suspected seroconversion samples would
13 have been detected and confirmed by the more conventional but expensive strategy
14 comprising the Enzygnost Anti-HIV1/2 Plus ELISA followed by INNO-LIA HIV I/II
15 Score. However, by western blot, three of the four samples were indeterminate and one
16 was negative. In a clinical situation they could be followed up with further testing and/or
17 have new samples taken at a later stage, and thus would not necessarily be missed as
18 suspected HIV patients.

19
20 It can be concluded that the national diagnostic strategy for HIV based on simple rapid
21 assays was performed well by non-laboratory personnel in a routine diagnostic setting in
22 three large health centres in Mozambique. The strategy is highly sensitive and specific,
23 and the tests are robust and do not require cold storage as long as temperatures stay below
24 +30°C. The finding of high and increasing HIV-1 prevalence rates and of cases of
25 suspected recent seroconversion among pregnant women indicates a high HIV-1
26 incidence. In view of these data, combination assays for HIV antigen and antibody
27 detection should be considered in Mozambique, especially for use in blood donor
28 screening. Preventive measures against further spread of HIV should continue to have
29 high priority.

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For Peer Review

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1 **Table 1. Study outline**

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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)

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- 1 * Tests performed on site at each respective health centre by trained nurse counsellors.
- 2 All other assays were performed at the Department of Microbiology, Faculty of
- 3 Medicine, University Eduardo Mondlane, Maputo, Mozambique.

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1 Table 2. Characteristics of the assays

Test (Manufacturer)	Antigen type	Assay type	Solid surface	Specimen type	Test incubation time (min)	Storage temp. (+C°)
Screening and evaluation: Determine HIV-1/2 (Abbott Laboratories)	HIV-1 and HIV-2 RP and SP	immuno- chromatographic, lateral flow, S/R	nitrocellulose membrane	serum, plasma, whole blood (EDTA)	15	2-30
Uni-Gold HIV* (Trinity Biotech)	HIV-1gp41, gp120 RP HIV-2 gp36 RP	immuno- chromatographic, lateral flow, S/R	nitrocellulose membrane	serum, plasma, whole blood	10	2-30
DoubleCheckGold HIV-1&2 (Orgenics)	HIV-1 and HIV-2 env and gag RP	immuno- chromatographic, lateral flow, S/R	nitrocellulose membrane	serum, plasma	15	2-27
Quality control and alternative screening assay: Enzygnost Anti-HIV-1/2 Plus* (Behringwerke)	HIV-1 gp41 RP HIV-2 gp36 RP	sandwich EIA	microtitre plate	serum, plasma	90	2-8
Gold standard: Capillus HIV-1/HIV-2 (Trinity Biotech)	HIV-1 p24, gp41 RP HIV-2 gp36 RP	agglutination, S/R	latex particles	serum, plasma	7	2-8
ImmunoComb II HIV-1&2 BiSpot† (Orgenics)	HIV-1gp41, gp120 SP, HIV-2 gp36 SP	indirect EIA, S/R	plastic comb with 12 projections	serum, plasma	37	2-8
Resolution of discordant specimens: INNO-LIA HIV I/II score† (Innogenetics)	HIV-1 p17, p24, p31, gp41, p120 and HIV-2 gp36, gp105	line immunoassay	nitrocellulose strip	serum, plasma	180	2-8
HIVBlot2.2 (Genelabs Diagnostics)	HIV-1 p17, p24, p31, gp41, p39, p51, p55, p66, gp120, gp160	western blot	nitrocellulose strip	serum, plasma	137	2-8

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Innotest HIV Antigen Mab (Innogenetics)	HIV-2 gp36 SP Human polyclonal antibodies (anti-HIV-1)	double sandwich EIA	microtitre plate	serum, plasma, cell culture supernatant	120	2-8
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1 RP, recombinant protein; SP, synthetic peptide; EIA enzyme immunoassay; S/R, simple rapid; *Antibodies of any Ig class can be detected;
 2 †Discriminates between HIV-1 and HIV-2.

5 This table is a summary of information available from the manufacturers.

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1 **Table 3. Results from various combinations of assays**

Assays	Sensitivity [no. positive/no. tested (%, c.i.)]	Specificity [no. negative /no. tested (%, c.i.)]	Positive predictive values (no. true positive/no. tested positive (%, c.i.))
Determine HIV-1/2	160/160 (100%, 99.4-100%)	720/723 (99.6%, 99.1-100%)	160/163 (98.2%, 96.1- 100)
Determine HIV-1/2 → Determine HIV-1/2	160/160 (100%)	722/723 (99.9%, 99.6-100%)	160/161 (99.4%, 98.2- 100)
Determine HIV-1/2 → DoubleCheckGold HIV-1&2	160/160 (100%)	722/723 (99.9%)	160/161 (99.4%, 98.2- 100)
Determine HIV-1/2 → Determine HIV-1/2 → DoubleCheckGold HIV-1&2	160/160 (100%)	723/723 (100%, 99.7-100%)	160/160 (100%, 99.4- 100%)
Determine HIV-1/2 → Uni-Gold HIV	160/160 (100%)	723/723 (100%)	160/160 (100%)
Determine HIV-1/2→ Enzygnost Anti-HIV- 1/2 Plus	160/160 (100%)	723/723 (100%)	160/160 (100%)
DoubleCheckGold HIV-1&2	160/160 (100%, 99.4-100%)	721/723(99.7%, 99.3-100%)	160/162 (98.8%, 97.1- 100)
DoubleCheckGold HIV-1&2 → DoubleCheckGold HIV-1&2	160/160 (100%)	723/723 (100%, 99.7-100%)	160/160 (100%)
DoubleCheckGold HIV-1&2 → Uni-Gold HIV	160/160 (100%)	723/723 (100%)	160/160 (100%)
DoubleCheckGold HIV-1&2 → Determine HIV-1/2	160/160 (100%)	722/723 (99.9%, 99.6-100%)	160/161 (99.4%, 98.2- 100)
DoubleCheckGold HIV-1&2 → Enzygnost Anti-	160/160 (100%)	722/723 (99.9%)	160/161 (99.4%, 98.2- 100)

HIV-1/2 Plus

Enzygnost Anti-HIV-1/2 Plus	160/160 (100%, 99.4-100%)	719/723(99.4%, 98.8-99.9%)	160/164 (97.6%, 95.2- 99.9)
Enzygnost Anti-HIV-1/2 Plus → Enzygnost Anti-HIV-1/2 Plus	160/160 (100%)	723/723 (100%, 99.7-100%)	160/160 (100%)
Enzygnost Anti-HIV-1/2 Plus → Determine HIV-1/2	160/160 (100%)	723/723 (100%)	160/160 (100%)
Enzygnost Anti-HIV-1/2 Plus → DoubleCheckGold HIV-1&2	160/160 (100%)	723/723 (100%)	160/160 (100%)
Enzygnost Anti-HIV-1/2 Plus → Uni-Gold HIV	160/160 (100%)	723/723 (100%)	160/160 (100%)

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

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

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.