Evaluation of the Determine™ Fourth Generation HIV Rapid Assay

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Abstract

Assays that detect p24 antigen reduce the diagnostic window period of HIV testing. Most point-of-care HIV assays have poor sensitivity to diagnose acute HIV infection as they only detect antibodies against HIV-1 and HIV-2 (HIV-1/2). This was a cross-sectional laboratory-based study that evaluated the performance of the Determine™ HIV-1/2 Ag/Ab Combo fourth generation rapid strip - currently the only rapid assay that detects both HIV-1/2 antibodies and p24 antigen. A total of 79 serum specimens (29 positive for HIV antibodies only, 14 positive for HIV antibodies and p24 antigen, 20 HIV-negative, and 16 positive for p24 antigen only) were used for the evaluation. Results were compared with those from validated fourth generation HIV ELISAs. The Determine™ Combo rapid strips had a sensitivity of 90.7% and a specificity of 100% for the detection of HIV-1/2 antibodies. Its sensitivity for the detection of p24 antigen was only 10% (3 out of 30 p24 antigen positive specimens). This implies that most acute HIV infections will be missed with this assay. The need for a point-of-care assay which can detect acute HIV infection reliably still remains, particularly for use in a high prevalence setting such as South Africa.

Keywords

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1. Introduction

The diagnosis of Human Immunodeficiency Virus (HIV) can be established by means of several different methods, including rapid HIV tests (immunochromatographic assays), enzyme-linked immunosorbent assays (ELISAs), p24 antigen assays, and polymerase chain reaction (PCR) (Guatelli et al., 2009). Only rapid tests offer point-of-care HIV testing, while all other tests need to be performed in a laboratory. Laboratory-based testing may be associated with logistical problems such as delays in the availability of results, particularly in developing countries where difficulties with transport are often encountered.

Several rapid HIV tests are available for point-of-care HIV testing, which may avoid the need for laboratory-based testing (Louie et al., 2008). The World Health Organization (WHO) has endorsed the use of rapid HIV tests in order to scale up HIV testing since 2004, particularly in resource-poor settings (WHO, 2007). Point-of-care assays form an integral part of HIV testing in high prevalence settings such as South Africa, which has the largest population of people infected with HIV in the world, with a prevalence of 16.9% among adults (aged 15 to 49) reported in 2008 (Joint United Nations Programme on HIV/AIDS and WHO, 2009). Point-of-care assays offer several advantages, for example the opportunity for health care practitioners to provide results to patients immediately, with fewer patients being lost to follow-up. Rapid diagnosis of HIV will also expedite patient referral for care (Martin et al., 2011). The disadvantages of using rapid HIV assays include limited sensitivity (especially when compared to laboratory-based HIV tests) and the problem that acute HIV infection is not detectable with the majority of rapid HIV assays available, as they only detect HIV-1/2 antibodies (Louie et al., 2008).

Acute HIV infection is defined as the period from initial exposure to the virus until the appearance of HIV-specific antibodies in the blood (termed seroconversion). Patients with acute HIV infection are usually asymptomatic, or may present with non-specific symptoms such as fever, lymphadenopathy and rash, therefore HIV diagnosis requires a high index of suspicion (Hare and Kahn, 2004). These patients typically have high HIV viral loads and detectable p24 antigen in the absence of detectable HIV-specific antibodies, and pose a high risk to spread the infection (Fiebiga et al., 2003; Hare and Kahn, 2004). Earlier diagnosis of individuals with acute HIV infection may therefore play a vital role in HIV prevention.

Fourth generation laboratory-based HIV assays have the ability to detect antibodies to HIV-1/2 and p24 antigen simultaneously, which reduces the window period to an average of 2 weeks (most rapid HIV assays only detect HIV antibodies, with an average window period of 3 to 4 weeks) (Gurtler et al., 1998; Stekler et al., 2007). Alere (Waltham, USA) has recently launched the Determine^{TM} HIV-1/2 Ag/Ab Combo, which is the first rapid fourth generation HIV assay (Alere, 2010a).

The aim of this study was to compare the performance of the Determine™ Combo assay with automated fourth generation HIV ELISAs already in use at National Health Laboratory Service Tshwane Academic Division.

2. Materials and methods

A cross-sectional laboratory-based study was conducted over a period of 3 months at the diagnostic virology laboratory of National Health Laboratory Service Tshwane Academic Division. The antibody and p24 antigen components of the Determine™ Combo assay (Alere, Waltham, USA) were evaluated separately in this study using selected serum specimens, including seroconversion specimens from the South African National Blood Service. The performance of each component was evaluated against previously validated automated assays in the laboratory.

2.1. Specimens

A total of 79 serum specimens were used for this evaluation. 63 were serum specimens that had been submitted for routine HIV serology testing to the diagnostic laboratory of National Health Laboratory Service Tshwane Academic Division, and 16 were seroconversion specimens, i.e. positive only for p24 antigen, that were obtained from the South African National Blood Service. Among the 63 routine diagnostic specimens, 29 were positive for HIV-1/2 antibodies only, 14 were positive for both HIV-1/2 antibodies and p24 antigen, and 20 were negative for both HIV-1/2 antibodies and p24 antigen. These specimens had initially been tested on automated fourth generation HIV ELISAs at National Health Laboratory Service Tshwane Academic Division, after which the specimens were stored at -20°C up to a maximum of 8 months, and were thawed for the first time during this evaluation. Three of the HIV antibody and p24 antigen positive routine diagnostic specimens were randomly selected to be re-tested for p24 antigen on the reference automated p24 antigen assay described below to assess sample integrity after storage, all three of which still tested positive for p24 antigen. The 16 seroconversion specimens were received frozen, and were stored at -20°C prior to being tested. Thirteen of the 16 seroconversion specimens belonged to HIV-1 subtype C according to nucleotide sequence analysis results provided by the South African National Blood Service.

2.2. Reference assays

The 63 routine diagnostic specimens were initially tested on one or more of the following automated fourth generation HIV assays: Cobas® HIV Combi kit on Modular E170 (Roche Diagnostics, Mannheim, Germany), HIV Ag/Ab Combo kit on AxSYM (Abbott Diagnostics, Wiesbaden, Germany) and HIV Ag/Ab Combo kit on ARCHITECT i2000 (Abbott Diagnostics). Two of the HIV 1/2 antibody reactive specimens had

confirmatory testing performed on the Determine HIV 1/2 assay (third generation rapid assay) instead of a fourth generation HIV 1/2 ELISA. The Cobas® HIV Ag kit (Roche) on Modular E170 was used for initial p24 antigen determination on the diagnostic specimens.

The 16 seroconversion specimens were screened for HIV-1/2 antibodies at the South African National Blood Service using the Prism HIV O Plus (Abbott Diagnostics, Wiesbaden, Germany), and for HIV-1 nucleic acid using the Procleix® Ultrio™ Assay on the Procleix® TIGRIS® Platform (Novartis Diagnostics, Emeryville, USA). The South African National Blood Service used the INNOTEST® HIV Antigen mAb kit (Innogenetics Diagnostics, Ghent, Belgium) on the BEP® III System (Siemens Healthcare Diagnostics, New York, USA) to test the specimens for p24 antigen. The National Institute for Communicable Diseases (a division of the National Health Laboratory Service) had performed the nucleotide sequence analysis on the seroconversion specimens for the South African National Blood Service, using an accredited in-house assay which targets the protease and reverse transcriptase genes to determine the HIV subtype. The p24 antigen result was confirmed in the diagnostic virology laboratory of the National Health Laboratory Service Tshwane Academic Division using the Cobas® HIV Ag kit on Modular E170 (Roche Diagnostics, Mannheim, Germany).

2.3. HIV testing on Determine™ Combo assay

All specimens were tested using the Determine™ Combo assay (Alere), an immunochromatographic assay designed for the qualitative detection of p24 antigen as well as HIV-1/2 antibodies. Testing was performed and results were interpreted as per manufacturer's instructions.

3. Results

The Determine™ Combo assay detected HIV-1/2 antibodies with a sensitivity of 90.7% (39 of the 43 HIV-1/2 antibody-positive specimens) and a specificity of 100% (table 1). The four HIV-1/2 antibody-positive specimens that tested negative on the Determine™ Combo assay had relatively low signal to cutoff values ranging from 1.01 to 9.60 on the automated assays.

The Determine™ Combo assay detected p24 antigen in only three of the 30 p24 antigen positive specimens, which translates into a sensitivity of 10% (table 1). Only one of the 14 specimens positive for both HIV antibody and p24 antigen (table 2), and two of the 16 seroconversion specimens (table 3) tested positive for p24 antigen using the Determine™ Combo assay. All 16 of the seroconversion specimens ordered from the South African National Blood Service tested positive for p24 antigen on the Cobas® HIV Ag kit on (Roche Diagnostics, Mannheim, Germany) (table 3). The specificity for the p24 antigen component of the Determine™ Combo assay was 100%.

4. Discussion

To the authors' knowledge this is the first study which evaluates the p24 antigen component of the Determine™ Combo assay extensively in a South African setting, while also evaluating the antibody component of the assay. In this study, the assay detected HIV-1/2 antibodies with a sensitivity of 90.7%. Rapid HIV assays are generally not as sensitive as HIV ELISAs for the detection of HIV antibodies, in particular during HIV seroconversion (Pavie et al., 2010); therefore it is not surprising that the Determine™ Combo assay did not detect HIV reliably in specimens with relatively low signal to cutoff values on automated HIV ELISAs.

The Determine™ Combo has not shown superiority for HIV antibody detection when compared to other rapid assays. An evaluation by Pavie et al. (2010) of five different rapid HIV assays on finger stick blood (FSB) and oral fluid (OF) reported 95.8% sensitivity for Determine™ Combo to detect HIV-1/2 antibodies on FSB in comparison with 94.5% for OraQuick (FSB), 86.5% for OraQuick (OF), 98.5% for Vikia (FSB), 94.9% for Determine third generation (FSB), and 99% for INSTI (FSB). The same study reported 33 invalid tests results with the Determine™ Combo assay, i.e. absence of the control line, compared to only 4 with Determine third generation (FSB), 2 with INSTI (FSB) and none with the other rapid assays. Pavie et al. (2010) also reported that all the rapid assays demonstrated inferior performance on whole blood compared to serum (Pavie et al., 2010). The local evaluation was performed on serum, not on whole blood as would typically be used for point-of-care testing. In concordance with previous literature the Determine™ Combo assay was highly specific for the detection of HIV-1/2 antibodies, (Beelaert and Fransen, 2010; Rosenberg et al., 2012).

The Determine™ Combo assay performed poorly for the detection of p24 antigen, with a sensitivity of 10%. Automated HIV p24 antigen assays, such as the Cobas® HIV Ag kit, make use of several incubation steps, as heat denaturation may aid in dissociation of non-specific antigen-antibody complexes (Schüpbach et al., 2006). The Determine™ Combo assay, being a rapid assay, does not require heat denaturation. Previously published data suggested that the presence of immune complexes in a specimen due to antibodies that bind p24 antigen may interfere with the detection of p24 antigen in an assay (Schüpbach and Boni, 1993). In order to eliminate the potential influence of HIV-1/2 antibodies on the p24 antigen component of the Determine™ Combo assay, a seroconversion panel, with samples positive for p24 antigen but negative for HIV antibodies, was also used to evaluate the assay. In the majority (87.5%) of the seroconversion specimens p24 antigen was undetectable on the Determine™ Combo assay, thereby negating the abovementioned theory.

The findings of this study correlate with two recently published evaluations of the Determine™ Combo assay in Lilongwe, Malawi (Rosenberg et al., 2012) and KwaZulu-Natal, South Africa (Chetty et al., 2012), both of which reported poor sensitivity of the assay for the detection of p24 antigen. The confirmed positive p24 antigen sample size was small in the abovementioned studies though.

During the development of the Determine™ Combo assay evaluations were performed in-house and at nine external sites in Africa, Asia, Europe and Latin America (Alere, 2010b). The performance of the Determine™ Combo's sensitivity in seroconversion samples was also assessed as part of the study in Belgium, United Kingdom, France, Thailand and Columbia. The assay's sensitivity in seroconversion samples ranged from 28.57% (Thailand) to 100% (France and Columbia). However some of the study sites tested a limited number of samples, e.g. in Columbia only one seroconversion sample was tested. It should be noted that none of these seroconversion sample evaluations were done in Sub-Saharan Africa, where HIV-1 subtype C predominates. HIV-1 subtype B is the predominant subtype found in Belgium, France and the UK, as well as South America, while the circulating recombinant form CRF01_AE prevails in South and Southeast Asia (Hemelaar et al., 2011). Taking this into consideration, the possibility exists that the Determine™ Combo assay has limited ability to detect p24 antigen in HIV-1 subtype C specimens. The predominant HIV-1 subtype in Malawi is also subtype C (Hemelaar et al., 2011), which may explain the similar findings between this evaluation and the evaluation in Malawi (Rosenberg et al., 2012).

In another evaluation of the Determine™ Combo assay, the p24 antigen sensitivity of the assay was 86.6% (58/67) when compared to the reference p24 antigen assay, namely the Innotest HIV Antigen mAb (Innogenetics, Ghent, Belgium) followed by neutralisation of HIV antigens using the Innotest HIV Antigen mAb Neutralisation Reagents kit (Innogenetics) (Beelaert and Fransen, 2010). In this study the investigators made use of primary HIV infection serum samples, supernatants from cultures of different HIV subtypes, as well as an HIV-1 p24 antigen standard to evaluate the p24 antigen component of the Determine™ Combo assay. Four supernatants of cell cultures infected with HIV-1 subtype C were used in the evaluation; the Determine™ Combo assay detected p24 antigen in all of these supernatants. According to the evaluation the Determine™ Combo assay could not detect p24 antigen in one subtype F and one group O strain. The authors mention that the results of the p24 antigen detection in the different HIV-1 subtype supernatants were dependent on the strain used in the evaluation, and cannot be generalised to all the different members of a particular subtype (Beelaert and Fransen, 2010). It should also be noted that although the Determine™ Combo assay could detect the subtype C supernatants, this may not accurately reflect test performance on patient specimens. The study by Beelaert and Fransen was further limited by the small number of HIV-1 subtype C p24 antigen samples used for the evaluation.

Limitations of this study include the relatively small number of specimens used to evaluate both the HIV-1/2 antibody and p24 antigen components of the assay. As previously mentioned, testing in this evaluation was done on serum, while whole blood would typically be used for point-of-care testing.

5. Conclusion

Despite adequate performance of the Determine™ Combo assay for HIV-1/2 antibody detection, it performed poorly for the detection of p24 antigen, and therefore cannot detect acute HIV infection reliably. Further studies with larger sample sizes are recommended to confirm whether the assay's limited ability to detect p24 antigen pertains specifically to HIV-1 subtype C specimens. A point-of-care assay which can detect acute HIV infection reliably is still needed, particularly for use in high HIV prevalence settings such as South Africa.

Conflict of interest

None of the authors have any conflict of interest to declare in the evaluation of diagnostic methods.

Ethical approval

The study was approved by the Research Ethics Committee, Faculty of Health Sciences of the University of Pretoria – Ref: 8/2012.

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Table 1

Correlation of Determine™ Combo with other assays.

	Automated 4th generation HIV ELISA[a]/p24 antigen[b] assays	Determine™ Combo assay	Correlation of Determine™ Combo with other assays
HIV antibody positive specimens	*n* = 43	*n* = 39	90.7%
p24 antigen positive specimens	*n* = 30	*n* = 3	10%
HIV antibody & p24 antigen negative specimens	*n* = 20	*n* = 20	100%

a Automated HIV ELISAs include Cobas® HIV Combi kit on Modular E170 (Roche Diagnostics, Mannheim, Germany), HIV Ag/Ab Combo kit on AxSYM (Abbott Diagnostics, Wiesbaden, Germany) and HIV Ag/Ab Combo kit on ARCHITECT i2000 (Abbott Diagnostics).

b Automated p24 antigen assays include INNOTEST® HIV Antigen mAb kit (Innogenetics Diagnostics, Ghent, Belgium) on the BEP® III System (Siemens Healthcare Diagnostics, New York, USA) & Cobas® HIV Ag kits on Modular E170 (Roche Diagnostics, Mannheim, Germany).

Table 2

Results of p24 antigen positive routine diagnostic specimens.

Specimen identifier	HIV antibody on Modular E170 (S/CO)[a]	HIV antibody on AxSYM (S/CO)[a]	P24 antigen on Modular E170 (S/CO)[a]	HIV antibody on Determine™ Combo	P24 antigen on Determine™ Combo
RS01	Pos (221.8)	[b]	Pos (6.86)	Pos	Neg
RS02	Pos (146.6)	Pos (25.7)	Pos (74.71)	Pos	Neg
RS03	Pos (718.2)	[b]	Pos (4.99)	Pos	Neg
RS04	Pos (164.1)	Pos (19.08)	Pos (15.13)	Pos	Neg
RS05	Pos (562.4)	Pos (21.38)	Pos (15.14)	Pos	Neg
RS06	Pos (308.5)	Pos (7.15)	Pos (1.47)	Pos	Neg
RS07	Pos (167.3)	Pos (22.25)	Pos (112.8)	Pos	Neg
RS08	Pos (689.8)	Pos (21.52)	Pos (57.77)	Pos	Neg
RS09	Pos (687.2)	Pos (20.96)	Pos (52.79)	Pos	Neg
RS10	Pos (619.6)	Pos (17.07)	Pos (37.87)	Pos	Neg
RS11	Pos (128.7)	Pos (10.53)	Pos (10.53)	Pos	Pos
RS12	Pos (511.3)	Pos (51.16)	Pos (32.07)	Pos	Neg
RS13	Pos (572.5)	Pos (18.73)	Pos (99.38)	Pos	Neg
RS14	Pos (1.75)	Pos (6.07)	Pos (125.7)	Neg	Neg

a S/CO = sample signal/cutoff signal.

b RS01 and RS03 initially tested positive with the Determine HIV-1/2 3rd generation rapid assay, hence further HIV testing was performed on only one automated platform.

Table 3

Results of the p24 antigen assays on the seroconversion specimens from the South African National Blood Service.

Specimen identifier	p24 antigen on INNOTEST® HIV Antigen mAb (S/CO)[a]	p24 antigen on Roche Modular (S/CO)[a]	p24 antigen on Determine™ Combo
RS64	Pos (4.58)	Pos (3.96)	Neg
RS65	Pos (3.59)	Pos (2.7)	Neg
RS66	Pos (32.03)	Pos (24.25)	Neg
RS67	Pos (28.71)	Pos (763.7)	Pos
RS68	Pos (1.71)	Pos (1.64)	Neg
RS69	Pos (12.67)	Pos (134.7)	Pos
RS70	Pos (13.11)	Pos (10.18)	Neg
RS71	Pos (6.33)	Pos (2.63)	Neg
RS72	Pos (2.32)	Pos (6.05)	Neg
RS73	Pos (31.94)	Pos (299.2)	Neg
RS74	Pos (23.7)	Pos (21.23)	Neg
RS75	Pos (6.92)	Pos (4.23)	Neg
RS76	Pos (13.91)	Pos (11.41)	Neg
RS77	Pos (7.59)	Pos (9.7)	Neg
RS78	Pos (1.33)	Pos (15.22)	Neg
RS79	Pos (21.06)	Pos (15.06)	Neg

a S/CO = sample signal/cutoff signal.