Comparison of a conventional HIV 1/2 line immunoassay with a rapid confirmatory HIV 1/2 assay

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Abstract

The performance of the rapid confirmatory HIV 1/2 assay Geenius was compared with the conventional HIV 1/2 line immunoblot (INNO-LIA HIV I/II Score). One hundred HIV 1/2 confirmed positive samples from donors and patients and 136 negative screening samples from blood donors were evaluated with both assays. A 20 member performance panel consisting of different HIV 1 and 2 subtypes was also analysed. Ninety-nine of the confirmed HIV positive samples were positive with both assays. One sample was positive with the INNO-LIA HIV I/II Score but indeterminate with the Geenius HIV 1/2. From 136 negative blood donor samples (negative with a combo HIV assay and a highly sensitive ID-NAT), 125 were concordant negative. Six and five samples were incorrectly indeterminate with the INNO-LIA HIV I/II Score and the Geenius HIV 1/2, respectively. One sample was weak positive with the INNO-LIA HIV I/II Score but negative with the Geenius HIV 1/2. The 20 member performance showed equivalent results with both assays. The rapid assay showed a comparable sensitivity and specificity for confirmation for positive and negative HIV donor and patient samples as well as for a 20 member performance panel.

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1. Introduction

Several assays are currently used for the diagnosis of HIV infection. These are based primarily on the detection in combination of HIV-specific antibodies and the HIV p24 antigen. Presently there are a variety of confirmation algorithms implemented once these screening assays are reactive. These often include either a Western blot, immunoblot-based assay or immunofluorescence (IFA), whereas others use dual or triple immunoaassays, nucleic acid based testing or different combinations of these methods (Schüpbach et al., 2007; Dwyer, 2010; Torian et al., 2011; Pierce et al., 2011). In Switzerland the confirmation of HIV 1/2 screening reactive samples is performed exclusively with the INNO-LIA-HIV I/II Score assay. The sample incubation for this assay is performed usually overnight. In general most of the confirmation strategies require extensive incubation times so the confirmation of infection is delayed. In some clinical situations, such as needle-stick injuries or accidents with other potential HIV blood-contaminated instruments, a rapid HIV confirmation test would be desirable. In addition a rapid test would help when therapeutic or preventive strategies should be implemented promptly.

The federal regulations issued in 2006 by the Swiss Federal Office of Public Health (SFOPH) require all new diagnosed cases of HIV infections to be tested with the INNO-LIA HIV I/II Score assay (BAG-Bulletin, 2006). The assay generally requires an overnight incubation and can be performed automatically on a Tecan Blot analyser and results are analysed and interpreted the following morning on the scan system.

In Switzerland a specific algorithm has been devised to confirm the reactive screening HIV cases that are able to differentiate between recent and older infections (Schüpbach et al., 2007). The INNO-LIA HIV I/II Score line assay is the mandatory assay currently used in this confirmation protocol. A study was conducted to compare the performance of the rapid HIV confirmation assay Geenius HIV 1/2 to that of the conventional INNO-LIA HIV I/II Score line assay used currently in Switzerland.

2. Materials and methods

2.1. Confirmation assays

The Geenius™ HIV 1/2 Confirmatory Assay employs antibody binding protein A, which is coupled to colloidal gold dye particles
as conjugate, and HIV-1 (p31, gp160, p24, gp41) and HIV-2 antigens (gp36, gp140), which are bound to the membrane solid phase (Bio-Rad Laboratories Inc., Cressier, Switzerland). The assay can be used with needle stick injuries with whole blood, venous whole blood, serum or plasma sample material. The sample is applied to the sample and buffer well 1. After the sample and buffer have migrated onto the test strip which takes around 5 min, additional buffer is added to the buffer well 2 and incubated a further 15–20 min. This buffer facilitates the lateral flow of the released products and promotes the binding of antibodies to the antigens. In a reactive sample, the anti-HIV antibodies are captured by the antigens immobilised in the test area (bands 1–6): the colloidal gold protein A binds to the captured antibodies, producing pink/purple lines. Immunoglobulin G from sample bound to protein A which is immobilised in (C) zone of membrane solid phase and produce a pink/purple line. This control line serves to demonstrate that sample and reagents have been applied properly and have migrated through the device. Five μl of serum or plasma or 15 μl of whole blood can be used. The test procedure is very simple requiring only a few handling steps. The results can be read automatically on the Geenius reader system or manually by eye. The data collected in this study were always read automatically and verified occasionally manually. HIV 1 and 2 interpretation criteria according to the package insert are described in Table 1a and b.

In the INNO-LIA-HIV 1/II Score (Innogenetics, Gent, Belgium) recombinant proteins and synthetic peptides from HIV-1 (sgp120, gp41, p31, p24, and p17) and HIV-2 (gp36 and sgp105) and a synthetic peptide from HIV-1 group O (sgp120) are coated as discrete lines on a nylon strip with plastic backing. In addition to these HIV antigens, four control lines are coated on each strip: anti-streptavidin and three positive controls of different strengths. The assay can also be used, with human plasma or serum. The test, which usually requires an overnight incubation of up to 16 h, was performed according to the package insert. HIV-1 and HIV-2 infection interpretation criteria were according to the package insert and are described in Table 2a and b. Fully automated processing of the strips is performed by using an Auto-LIA 48 (Tecan, Männedorf, Switzerland).

### 2.2. Performance panel samples

The worldwide performance panel WWRB304 (SERACARE, Milford, MA, USA) was used to compare both HIV confirmation assays. This performance panel consisted of different 17 HIV-1 subtypes (A, B, C, CRF01_AE, CRF02_AG, D, F, G, H, J), two HIV-2 and one HIV-1/2 negative control sample.

### 2.3. Patient and donor samples

One hundred confirmed positive HIV-1 and HIV-2 plasma or serum samples were analysed with both assays. These samples had been stored previously at −30 °C, and originated from both patients and donors.

One hundred and thirty-six HIV negative plasma samples from the routine blood donor screening programme were tested with both HIV-1/2 confirmation assays. These samples had been screened with the Enzygnost HIV Integral II assay (Siemens, Marburg, Germany) and the Transcription-Mediated Amplification (TMA) Ultrio Procleix assay on the Tigris instrument (Novartis Diagnostics, Basel, Switzerland) in an individual donation format (ID-NAT) which has a sensitivity limit of 24 IU/ml.

### 3. Results

#### 3.1. Clinical sensitivity

The 100 confirmed positive patient and donor samples were used to determine the clinical sensitivity of the Geenius™ HIV 1/2 Assay. Ninety-nine concordant positive results were recorded with both assays. One sample was HIV positive according to the interpretation criteria INNO-LIA-HIV 1/II Score (gp41 2+, p24 2+, and p17+) but only indeterminate with the Geenius™ HIV 1/2 Assay (gp41 indeterminate). This sample was derived from a freshly infected individual from whom both the antibody/antigen screening and the HIV DNA assays were positive.
The performance panel WWRB304 containing 17 different HIV-1 subtypes (A, B, C, CRF01_AE, CRF02_AG, D, F, G, H, J), two different HIV-2 strains and one HIV-1/2 negative control sample gave the same result with both assays. All the 19 reactive samples tested positive (according to the interpretation criteria of Bio-Rad and Innogentics, Tables 1a/b and 2a/b) and both assays could clearly distinguish between HIV 1 and HIV 2.

3.2. Specificity

The 136 negative blood donor samples screened previously with the HIV-1/2 antigen/antibody screening EIA (Enzygnost HIV integral II) and the NAT system Procleix Ultrio on the Tigris instrument in individual donations (sensitivity limit: 24 IU/ml in the individual donation) were used to determine the specificity of the Geenius™ HIV 1/2 Assay. Concordant negative results were obtained for 125 screening negative samples. Eleven samples gave different results with the two confirmation assays. Five samples were indeterminate with the Geenius HIV 1/2 assay showing a single reactivity with band gp140 but were negative for all bands in the INNO-LIA HIV I/II Score assay. On the other hand 5 samples were indeterminate with INNO-LIA HIV I/II Score assay, with a single reactive band (gp120, one sample 1+; p24, two samples 1+ and 2+; or gp41, one sample 1+) and one sample was positive with the INNO-LIA HIV I/II Score assay (gp41 1+ and p24 1+) but all was negative for all bands with the Geenius HIV 1/2 assay.

A perfect concordance was observed when the 20 member performance panel WWRB304 was tested with both assays confirming the comparable sensitivity of both HIV confirmation assays.

4. Discussion

In Switzerland the INNO-LIA-HIV I/II Score assay is included in the mandatory confirmation algorithm for all HIV EIA or rapid assay screening reactive patient or blood donor samples (Schüpbach et al., 2007; BAG-Bulletin, 2006). At least one of the two mandatory blood drawings must be analysed with the INNO-LIA-HIV I/II Score assay in order to confirm recent or older infections. This test is specific and sensitive but generally requires a 16 h overnight incubation before the results are obtained. In emergency cases it could be very helpful to have an immediate confirmation of an HIV reactive screening result.

The Geenius HIV 1/2 test performance was believed to be less sensitive than the INNO-LIA HIV I/II Score test procedure mainly due to its short 20 min sample incubation time compared to the overnight incubation required in the INNO-LIA HIV I/II Score test. The results of our comparison did not confirm this assumption. A similar sensitivity and specificity was observed with both assays whether read automatically with the analyser or manually. From 100 HIV confirmed positive samples, 99 were concordant positive with both assays, whereas only 1 sample was indeterminate with the Geenius HIV 1/2 (gp41±). This discrepant sample was also only weakly positive (gp41 2+, gp24 2+, p17±) with the INNO-LIA-HIV I/II Score assay but indeterminate in the Geenius HIV 1/2 assay. The sample was the only samples which stemmed from a patient with a fresh HIV infection and was positive with the antibody/antigen screening assay and the HIV DNA. A Chinese study has recently compared 3 HIV confirmatory assays including the INNO-LIA-HIV I/II Score assay (Wantai-RIBA, INNO-LIA-HIV I/II Score and the MP-WB HIV Blot 2.2 WB). The Wantai-RIBA and the INNO-LIA-HIV I/II Score showed similar results, whereas the MP-WB HIV Blot 2.2 WB was less sensitive (Wang et al., 2011).

A similar specificity was observed between the two confirmatory assays when 136 screening negative were analysed. Whereas 125 samples were negative for both confirmatory assays, 10 different samples were negative for one assay but indeterminate for the other assay (see Section 3.1). One sample tested positive with the INNO-LIA-HIV I/II Score assay but was negative with the Geenius HIV I/2 assay. This result is likely to be false positive as the sample was previously negative in the Enzygnost HIV Integral II screening assay as well as the HIV ID-NAT assay. A similar case concerned a false reactive INNO-LIA-HIV I/II Score assay with a repeatedly borderline reactive anti-HIV screening assay. In that case a second screening assay and a Western blot were negative and five months later a seroreversion with the initial reactive HIV screening assay was observed (Seme et al., 2006).

It is important that HIV confirmation assays are able to differentiate between HIV-1 and HIV-2 infections due to differences in the therapeutic treatments for these two viruses. Since both assays gave a perfect concordance with the performance panel WWRB304 the Geenius HIV I/2 assay is thus able to clearly differentiate between HIV 1 and HIV 2 infections.

There still remain two slight advantages for the INNOL-LIA-HIV I/II Score compared to the rapid Geenius HIV 1/2 assay. Unlike the INNOL-LIA-HIV I/II Score assay, which detects specific HIV antigen bands, the version of the Geenius HIV 1/2 assay evaluated here was unable to distinguish between recent from older HIV infections (Schüpbach et al., 2007, 2012). Analysis of recent HIV infection using a later version of the Geenius HIV assay has in the meantime been presented at the 2014 Conference on Retroviruses and Opportunistic Infections (Keating et al., 2014). The data presented shows the assay can provide valuable information on the likely duration of infection.

Furthermore, when several series of samples are analysed the INNOL-LIA-HIV I/II Score assay is able to be performed on an automatic analyser and thus requires less technical hands-on time. However in contrast, for single or few samples the Geenius HIV I/2 assay in combination with the Geenius Reader is practical alternative as the results can be read and documented without the use of sophisticated instruments.

In summary an accurate, reliable and rapid identification of a HIV infected individual is essential for the immediate introduction of effective post-infection prophylaxis when HIV transmission is most active. This study has shown that the rapid Geenius HIV 1/2 confirmation assay has the same sensitivity and similar specificity as compared to the conventional INNOL-LIA-HIV I/II Score assay with the additional advantage of requiring only 45 min to conduct the test. As one sample from a freshly infected individual was positive with the INNOL-LIA-HIV I/II Score but only indeterminate with the Geenius HIV I/2 assay further investigations to specifically determine the exact sensitivity of the Geenius HIV 1/2 assay should be performed. If the sensitivity of the Geenius HIV I/2 assay proves to be equivalent to other established HIV confirmation assays it would be useful as an alternative HIV confirmation assay in emergency case situations.

Conflict of interest

There is no conflict of interest.

References


