



The Brazilian Journal of INFECTIOUS DISEASES

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Original article

Evaluation of rapid tests for human immunodeficiency virus as a tool to detect recent seroconversion

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ARTICLE INFO

Article history:

Received 5 April 2012

Accepted 15 May 2012

Available online 7 September 2012

Keywords:

HIV-1 incidence

Detuned assay

Surveillance

Rapid test

ABSTRACT

The identification of recent HIV infection is important for epidemiological studies and to monitor the epidemic. The objective of this study was to evaluate two rapid tests that are easily available to the Brazilian scientific community for using as markers of recent HIV infection. The Rapid Test – HIV-1/2 Bio-Manguinhos (Bio-Manguinhos/Fiocruz, Brazil) and the Rapid Check HIV 1&2 (NDI-UFES, Center for Infectious Diseases, Universidade Federal do Espírito Santo) were tested, using 489 samples with HIV positive serology, from blood donors, previously classified as recent or long-term infection by serological testing algorithm for recent HIV seroconversion (STARHS) or LS-HIV Vitros assay methods. The samples were diluted prior to testing (1:50 and 1:100 for the Rapid Test – HIV-1/2 Bio-Manguinhos, and 1:500 and 1:600 for the Rapid Check HIV 1&2). Negative samples were considered recent infection, whereas those showing any color intensity were associated with long-term infection. The best dilutions were 1:100 for HIV-1/2 Bio-Manguinhos test (Kappa = 0.840; overall agreement = 0.93), and 1:500 for the Rapid Check HIV 1&2 (Kappa = 0.867; overall agreement = 0.94). The results suggest that both rapid tests can be used to detect recent seroconversion.

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Introduction

Despite the great progress achieved in preventing new human immunodeficiency virus (HIV) infections and the reduction of

the annual number of acquired immunodeficiency syndrome (AIDS)-related deaths, the number of people living with HIV continues to grow worldwide. In 2010, 34 million people were estimated to be infected globally, a number 17% higher than in 2001, and the prevalence was nearly three times higher than

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<http://dx.doi.org/10.1016/j.bjid.2012.08.013>

that found in 1990. In the same period, there were 2.7 million new cases, which means over 7,000 new HIV infections per day, and 1.8 million annual deaths from AIDS.¹ There is evidence of success in HIV prevention in diverse settings, such as the reduction on the annual rate of new HIV infections around the globe, as well as a lower prevalence of HIV among young people in several countries.^{2,3} Efforts to prevent HIV transmission should focus on recent infections, due to their greater impact on the spread of the epidemic.

The identification of populations at risk of HIV infection is a priority for public health and epidemiological surveillance. In recent years, epidemiologists have used different laboratory tests to estimate the incidence of HIV in certain populations, or to check the number of new infections in a given period of time.⁴⁻⁷ These strategies are important tools to identify populations with high rates of ongoing transmission, to understand the patterns of transmission, to monitor and direct resources and prevention programs (especially in low-income countries, where the concentration of cases is higher), and to assess the future of the HIV epidemic.⁸⁻¹¹

Likewise, for studies that identify subtypes of HIV-1 or patterns of drug resistance in infected individuals, the identification of recent infected individuals can provide important information about the dynamics of the epidemic.¹²⁻¹⁴

Detection of early infection can also be of clinical significance to the infected individual. The identification of people at an early stage of infection may accelerate the onset of treatment, improving long-term prognosis. Early treatment, along with appropriate counseling and partner notification, can help reduce secondary transmission by the newly infected partners.¹⁵⁻¹⁷

The determination of incidence from conventional epidemiological methods is complex and expensive. The development of tools that could indicate whether a person was infected recently has always been a priority for HIV research. In 1998, Janssen et al.¹⁸ described the serological testing algorithm for recent seroconversion (STARHS) to estimate the incidence of HIV-1 in different populations. The technique was based on the principle that antibody titers against HIV increase gradually over the initial months after seroconversion. The method was based on enzyme immunoassays (ELISA – enzyme-linked immunosorbent assay) of the first generation (Abbott, and later manufactured by Organon Teknika). Subsequently, studies have shown that this technology could show unspecific recent infection results among with other HIV subtypes, since the test is based on HIV-1 subtypes B antigen. Another problem is that the companies discontinued the first generation tests. In 2002, Parekh et al.¹⁹ developed a new IgG capture enzyme immunoassay (BED-EIA), which is based on the detection of gradual increase in the proportion of HIV-specific IgG 1 and total IgG after seroconversion. This test showed a similar sensitivity to detect specific antibodies to the various HIV-1 subtypes. These methods can differentiate individuals with recent seroconversion from long-term infection within about six months after seroconversion, and can estimate HIV incidence using a single cross-sectional study.²⁰ However, false positive results also occurred among long-term infection. Factors such as heterogeneity of the immune response to HIV-1 genotype and use of antiretroviral therapy may cause misclassification. Thus, these assays are used at a

population level, and should not be used as a diagnostic tool, since they may be misleading for the individual case definition of recent infection.

Another limiting factor for their use was the need for special equipment to perform the immunoassays, and the many hours required to obtain the results.^{21,22} In places with limited resources, where the majority of diagnostic tests are performed using HIV rapid tests, these disadvantages may be of concern, and for those reasons, it is necessary to develop and validate simple tests that can be used continuously, regardless of any commercial source.²¹

The development of simple, rapid tests with high sensitivity and specificity was a major breakthrough in the diagnosis of HIV infection. These tests provide results in a very short time (minutes), do not require special laboratory equipment, and are, in addition, very economical, having been widely used as diagnostic methods, particularly in regions with limited resources and high prevalence of HIV infection.²³⁻²⁵ The availability of a rapid test that also has the ability to accurately determine the incidence would be a useful epidemiological tool to assess the HIV epidemic in these regions.^{25,26}

Recently, some researchers^{8,27,28} evaluated the use of rapid tests for the diagnosis of recent infection. The aim of the present study was to evaluate rapid tests manufactured in Brazil for this purpose.

Materials and methods

Two HIV rapid tests (RT) were analyzed: Rapid Test - HIV-1/2 Bio-Manguinhos (Bio-Manguinhos/Fiocruz, Brazil) and Rapid Check HIV 1&2 (NDI-UFES, Center for Infectious Diseases, Universidade Federal do Espírito Santo). Both tests are provided solely by the Ministry of Health, and are not commercialized. These assays consist of specific HIV-1/2 antigens adsorbed on special membranes. The results can be visualized in the form of bands on the membranes. For the purposes of the present research, the original assays were modified exclusively by dilution of samples, and the reagents and materials were not changed.

A total of 489 HIV-positive serum samples from the Epidemiology Recipient and Donor Evaluation Study (REDS)²⁹ were used for this evaluation. Of these, 147, 155, and 187 were from blood donations of the years 2007, 2008, and 2009, respectively. Samples from 2007 had previously been evaluated by the STARHS method (Vironostika HIV-1 microElisa System; bioMérieux – Raleigh, NC, USA), while in the 2008 and 2009 samples, the LS-HIV Vitros Assay method (Ortho Diagnostics, Raritan, NJ) was utilized.³⁰ The results obtained during the REDS were used to classify the samples as a recent or long-term infection.

First, four samples classified as recent infection and four samples classified as long-term infection were selected to onset of standardization. Serial dilutions were made from 1:10 to 1:10,000 in negative human serum. From this preliminary test, the best two dilutions were selected to evaluate a large number of samples. Since these are tests whose results are obtained by colorimetric bands, the reading was visual, on a properly lit and flat surface. Non-reactive samples after dilution, that is, with no band at the location indicated by the

Table 1 – Comparison of different rapid test dilutions and the reference ELISA tests (STARHS and LS-HIV).

	Reference ELISA		
	Long-term	Recent	Total
HIV 1/2 Bio-Manguinhos			
1:50			
Long-term	330 (96.2%)	13 (3.8%)	343
Recent	35 (24%)	111 (76%)	146
1:100			
Long-term	324 (94.5%)	19 (5.5%)	343
Recent	14 (9.6%)	132 (90.4%)	146
Rapid Check HIV 1 & 2			
1:500			
Long-term	327 (95.9%)	14 (4.1%)	341
Recent	13 (9%)	132 (91%)	145
1:600			
Long-term	316 (92.7%)	25 (7.3%)	341
Recent	7 (4.8%)	138 (95.2%)	145
HIV-1/2 Bio-Manguinhos 1:50 – Kappa coefficient = 0.755; Overall agreement = 0.90.			
HIV-1/2 Bio-Manguinhos 1:100 – Kappa coefficient = 0.840; Overall agreement = 0.93.			
Rapid Check HIV 1&2 1:500 – Kappa coefficient = 0.867; Overall agreement = 0.94.			
Rapid Check HIV 1&2 1:600 – Kappa coefficient = 0.848; Overall agreement = 0.93.			

colorimetric test, were associated with cases of recent infection; those that had any staining intensity at the location indicated were associated with long-term infections.

For the Rapid Test - HIV-1/2 Bio-Manguinhos, the 1:50 and 1:100 dilutions were evaluated, as both had similar results to the samples tested. Samples were diluted in plasma and 5 µL of the final dilution was used for testing.

For the Rapid Check HIV 1&2 assay, the 1:500 and 1:600 dilutions were used. Samples were diluted in negative plasma and 10 µL were used for testing.

Apart from the dilutions, the tests were performed according to the manufacturer's instructions.

Statistical analysis was performed using the kappa coefficient, which assesses the agreement beyond chance of the results with the reference methods (STARHS and LS-HIV Vitros assay). The sensitivity and specificity of each assay were determined with a confidence interval of 95%.

Results

Table 1 shows the test results obtained by the HIV-1/2 Bio-Manguinhos using the best two dilutions (1:50 and 1:100), and

the tests results by the Rapid Check HIV 1&2 at dilutions 1:500 and 1:600. Of the 146 samples defined as recent infection by reference ELISA, 111 (76%) and 132 (90.4%) were considered to be new infection by HIV-1/2 Bio-Manguinhos at dilutions of 1:50 and 1:100, respectively. Of the 343 long-term infection samples, 330 (96.2%) and 324 (94.5%) were considered to be long-term by the HIV-1/2 Bio-Manguinhos in 1:50 and 1:100 dilutions, respectively. Therefore, the 1:100 dilution was chosen for this test. For the test Rapid Check HIV 1&2, of 145 samples defined as recent infection by reference ELISAs, 132 (91%) were considered recent infection at a dilution of 1:500, and 138 (95.2%) at a dilution of 1:600. Of the 341 samples defined as long-term, Rapid Check detected 327 (95.9%) when at a dilution of 1:500, and 316 (92.7%) at a dilution of 1:600.

Table 2 summarizes the results obtained by comparing each test with each reference ELISA. The overall concordance was similar, ranging from 0.90 to 0.95.

Discussion

RTs are a practical and economical method for the diagnosis of recent HIV infection. Especially in places with limited

Table 2 – Comparison of sensitivity, specificity, kappa coefficient, overall agreement, positive predictive value (PPV), and negative predictive value (NPV) of tests at their best dilutions.

Parameters (95%CI)	Bio-Manguinhos x STARHS n = 147	Bio-Manguinhos x LS-HIV n = 342	Rapid Check x STARHS* n = 145	Rapid Check x LS-HIV n = 341
Sensitivity	0.9889 (0.9396-0.9997)	0.8972 (0.8531-0.9318)	0.9775 (0.9212-0.9973)	0.9524 (0.9183-0.9752)
Specificity	0.8772 (0.7632-0.9492)	0.9213 (0.8446-0.9678)	0.8571 (0.7378-0.9362)	0.9438 (0.8737-0.9815)
Kappa coefficient	0.8831 (0.8047-0.9616)	0.7656 (0.6906-0.8407)	0.8516 (0.7632-0.9399)	0.8740 (0.8157-0.9322)
Overall agreement	0.9456 (0.8956-0.9762)	0.9035 (0.8672-0.9326)	0.9310 (0.8768-0.9664)	0.9501 (0.9214-0.9707)
PPV	0.9671 (0.8555-0.9702)	0.9701 (0.9393-0.9879)	0.9158 (0.8408-0.9629)	0.9796 (0.9530-0.9933)
NPV	0.9804 (0.8955-0.9995)	0.7593 (0.6675-0.8363)	0.9600 (0.8629-0.9951)	0.8750 (0.7918-0.9337)
Rapid test HIV-1/2 Bio-Manguinhos in 1:100 dilution; Rapid Check HIV 1 & 2 in 1:500 dilution.				

resources, to estimate HIV incidence, using an RT could be an important tool for guiding prevention programs, identifying populations with high transmission rates, as well as understanding the patterns of transmission.

Incidence calculation by prospective cohort studies is expensive, and generally the samples are non-representative of the general population. Mathematical models created for these estimations are based on epidemiological data on HIV prevalence, diagnosis of AIDS, or death rates. However, they are difficult to standardize or require complex statistical methods.⁶ Because of this complexity and the limitations of methods to estimate HIV incidence, different laboratory techniques have been developed, seeking to distinguish recent infections from long-term infections.^{7,11,18,19,21,27,28,31-44} These tests, however, depend on the calibration panels, which are not always available in a given country. In the current study, techniques that are easily accessed by the Brazilian scientific community and public services were evaluated in order to facilitate the development of epidemiological studies needed to detect incident cases of HIV infection.

The results suggest that, by modifying the sample dilution, HIV-1/2 RTs had a similar performance as the reference ELISAs (STARHS and LS-HIV Vitros assay) to discriminate between recent and long-term infection.

The overall agreement between the RTs and the reference tests was 93% on average. As these tests are visually interpreted, it was not possible to calculate the coefficient of variation.

In 2005, Soroka et al.²⁸ modified three protocols for the detection of recent seroconversion by RT. Determine HIV-1/2 (Abbott Laboratories), OraQuick Advance HIV-1/2 (OraSure Technologies), and SeroStrip HIV-1/2 (Chem-Bio) were used, and obtained a general agreement of 95% for the tested samples. More recently, Kshatriya et al.²⁷ also compared Determine HIV-1/2 and OraQuick Advance HIV-1/2 tests, and showed an agreement of 97% and 93%, respectively.

The samples used in the present study were previously tested by the reference methods STARHS and LS-HIV Vitros assay.²⁹ Similar results were observed with both assays. However, in 2011, Kassanjee⁴⁵ suggested caution in the use of LS-HIV Vitros Assay, since it has high false-recent rates.

Unfortunately, the results of both tests were not available for the same sample, thus it was not possible to determine the agreement between the two tests in the tested samples. In this sense, the limitation of this study is the same as reported by the majority of other investigators seeking to standardize tests for this purpose, that is, the lack of availability of specific samples in an adequate volume that would allow for the evaluation of a test series, comparing performance among them.

Another limitation of this study is that the interval between infection and seroconversion could not be determined. The STARHS and LS-HIV Vitros assay tests were standardized for a window period of 170 days using samples with the window period previously known. The fact that this study's methodology has shown good correlation with these methods does not necessarily imply that the window period is the same.

Conclusion

It is still premature to use this technology to define incidence. However, it could be used to screen recently infected individuals, who would need to be confirmed by a follow up-sample, or to be used in studies comparing the relative rate of newly infected individuals in different populations.

Conflict in interest

All authors declare to have no conflict of interest.

Acknowledgements

Samira Barbosa Girardi received a Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) grant. The rapid test was freely offered by the Brazilian Ministry of Health's AIDS Program.

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