Integrative Biomedical Sciences

Short Communication

Detection of an Acute HIV-1 Infection Case in the Last Day of Diagnostic Window through a Combination of Two Fourth-Generation Screening Assays on a Brazilian Public Healthy Institution: We Close the Window

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Abstract

In 2011, an estimated 2.2-2.8 million persons became infected with Human Immunodeficiency Virus (HIV) worldwide. Although the number of persons newly infected with HIV has decreased, with significant reduction in the mortality caused by the Acquired Immunodeficiency Syndrome (AIDS), the number of persons living with HIV has increased steadily. HIV-1 testing is important for limiting the spread of infection and identifying individuals who might benefit from early initiation of antiviral therapy. Diagnostic window is the time between the Human Immunodeficiency Virus (HIV) infection and laboratory diagnosis, the period during which tests for HIV are negative in infected persons. "Detecting acute infection" has also been used synonymously with "closing the window". We report a case where the diagnostic of acute infection was made in the last day of the diagnostic window, using a combination of two fourth-generation tests.

Keywords: HIV; Diagnostic window; Fourth-generation assays

A 25-year-old female was attended in 12/01/2015 in the Clinic of Infectious Diseases of the Clinical Hospital of Faculty of Medicine of Ribeirao Preto, Brazil, presenting oral and esophageal moniliasis, leading to an investigation for Human Immunodeficiency Virus (HIV) Infection.

The patient's risks included prostitution and crack use. The screening for HIV was performed at Laboratory of Serology of the hospital in 13/01/2015, which follows

the Brazilian Ministry Health recommendations [1]. According to the manual, the nonreactive sample can be released based on a single test. If samples were reactive or borderline, they should be evaluated further with a second test, different from the first one, being necessary two reactive results to confirm the diagnosis. Furthermore, the manual recommends that all newly diagnosed individual should perform the viral load assay, a third complementary test, and the result confirms the presence of the infection, if ≥ 5000 copies/mL.

The laboratory use a fourth-generation HIV screening assay (ARCHITECT HIV Ag/Ab Combo, Abbott) and an immunoblotting assay as additional test for

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confirmation (New Lav Blot I, Bio Rad). However, this laboratory complements the screening with a second additional method (Vidas HIV DUO Ultra, Biomeriux). All assays are performed and interpreted according to the manufacturer's recommendations. The principles and procedures of these assays have been described. Briefly, the ARCHITECT HIV Ag/Ab Combo is a chemiluminescent magnetic microparticle-based immunoassay used to determine the presence of HIV-1 p24 antigen and antibodies to HIV-1 group M, group O, and HIV-2. The ARCHITECT assay uses recombinant antigens and synthetic peptides derived from the HIV transmembrane gp41 proteins of HIV-1 group M and O and gp36 of HIV-2 for antibody detection, and anti-p24 monoclonal antibody for antigen detection. Samples with signal to cut-off (S/CO) values greater than or equal to 1.0 are considered reactive and samples with S/CO values less than 1.0 are considered nonreactive. ARCHITECT HIV Ag/Ab Combo has high analytical sensitivities (sensitivity 100%, specificity 99.6%) and would be preferable for reducing the window period [2].

VIDAS HIV DUO Ultra is an enzyme-linked fluorescent assay which permits the simultaneous detection of p24 Ag and IgG antibodies against HIV-1 (including subtype O) and HIV-2. The assay comprises two reactions. The first, for the detection of anti-HIV-1 and anti-HIV-2 IgG, is performed in the lower part of the solid-phase receptacle (SPR), which is coated with synthetic peptides (gp41 and gp36). Anti-human IgG labeled with alkaline phosphatase is used as the conjugate. The second reaction, for the detection of p24 Ag, is performed in the upper part of the SPR, which is coated with monoclonal anti-p24 antibodies. During incubation, p24 Ag is released through virus lysis and binds to the monoclonal antibodies on the SPR and also to the biotinylated anti-p24 antibodies. The antibody-Ag-antibody complex binds to the alkaline phosphatase-labeled streptavidin. The final detection step is the same for both reactions. The substrate (4-methylumbelliferyl phosphate) is catalyzed by the conjugate into a fluorescent product (4-methylumbelliferone). The test value is calculated by dividing the patient reference value by the reference value of the standard. A test value greater than or equal to 0.25 is considered to be positive. Values <0.25 are considered to be negative.

The Immunoblotting assay is based on indirect ELISA

technique on a nitrocellulose strip containing all the HIV-1 constituent proteins. Inactivated HIV-1 proteins are separated according to their molecular weights by polyacrylamide gel electrophoresis in dissociating and reducing medium and subsequently electrically transferred onto a nitrocellulose membrane sheet. This assay detects antibodies against HIV-1 glycoproteins: gp160, gp 110/120 and gp 41 and antibodies against the HIV-1 proteins: p68/66, p55, p52/51, p40, p34/31, p24/25 and p18/17. The test results are considered negative, indeterminate or positive, according to the manufacturer's recommendations.

The ARCHITECT HIV Ag/Ab Combo assay detects both p24 antigen and antibodies against HIV, however does not distinguish between the two, but VIDAS HIV DUO Ultra does this distinction. For this case report the screening for HIV using ARCHITECT HIV Ag/Ab Combo assay presented a value of 10.15, considered Reactive and the screening for HIV in VIDAS HIV DUO Ultra presented a value of 3.03 for antigen, and considered Reactive too.

To detect the antibody presence, additionally, was performed the rapid test HIV TEST BIOEASY and the result was nonreactive. The additional method immunoblotting New Lav Blot I, Bio Rad, was nonreactive too, and no bands were presented. These results were consistent with HIV viremia without seroconversion, what does it mean the acute phase of infection.

This is important because in Brazil, > 95% of people are diagnosed in the chronic phase [1].

To follow up this case, another sample was sent to the laboratory in the next day (14/01/2015) and the same assays were performed. Screening in ARCHITECT HIV Ag/Ab Combo presented a value of 14.12, considered Reactive. VIDAS HIV DUO Ultra presented the value of 3.53 for antigen, considered Reactive too, but at this time the HIV TEST BIOEASY already presented a weakly Reactive result for HIV-1, while in the immunoblotting there were bands for p24 and p17, an Undetermined result according to the manufacturer's recommendations.

This mean, that in the interval of one day the antibodies against HIV started to be produced, ending the acute phase of infection.

To confirm the diagnostic another sample was draft in 15/01/2015 to perform viral load (Real Time PCR, m2000 rt, Abbott) and CD4/CD8. The result was 3.684.420 copies/mL (log 6,566), confirming the diagnostic of HIV infection. The CD4 and CD8 cells number were, respectively, 357/mm³ and 341/mm³ (Ratio CD4/CD8: 1.05).

In 2011, an estimated 2.2-2.8 million persons became infected with Human Immunodeficiency Virus (HIV) worldwide [3].

Early diagnosis of HIV infection provides opportunity for early linkage to HIV care and important benefits both on individual level, because early treatment may allow for preserved immune-system control of the virus, and on public health, because the risk of HIV transmission during the early stages of the infection characterized by high-level of viraemia can be decreased [4,5].

The traditional laboratory diagnosis of HIV infection relies upon detection of HIV-specific antibodies. The first screening assays for HIV, which used purified HIV lysates, had a relative lack of sensitivity and specificity. The second generation of assays was based on recombinant proteins and/or synthetic proteins [6]. The third generation assays, which detect both IgM and IgG, have led to a further improvement in sensitivity and specificity. The fourth generation assays (HIV Ag–Ab EIA) use combined, simultaneous detection of anti-HIV IgG and HIV core protein [7,8]. The advantage of combining antibody and antigen detection in a single immunoassay format is a reduction in the duration of negative tests during sero-conversion. This is because HIV core protein appears transiently in the blood and has been used as a marker of antigenemia prior to a detectable humoral immune response directed against HIV [9]. Most people develop detectable antibody within 3-4 weeks after infection using third generation assays [10]. However, antibody may not be present in quantities greater than the minimum concentration detectable by some assays in the early stages of infection. Detection of viral p24 protein in blood is usually earlier than

detection of antibody and remains detectable for 1-2 weeks and then disappears or falls to low levels until the onset of clinical illness [6,7]. Studies have shown that detection of HIV antigen prior to the development of detectable immune response can result in a reduction of the sero-conversion window by approximately 9 days [10-12].

An HIV infection can potentially be detected with 4th generation HIV assays within the first 2weeks after exposure [10,13,14]. However, in addition to the first diagnostic window, a so-called second diagnostic window has been reported; this second diagnostic window may occur when 4th generation HIV assays are used. This period lies between the p24 antigen detection and the anti-HIV antibody detection. The appearance of a second diagnostic window with 4th generation HIV assays was demonstrated based on a decline of HIV-1 p24 antigen prior to the detection of HIV antibody [15].

Niederhauser et al. [16], also related two cases of second diagnostic window, using fourth-generation HIV screening assays.

In other studies, no evidence was found for a second diagnostic window due to the impaired sensitivity of the antibody detection module of different 4th generation HIV assays [13,17]. These studies did not observe a significant drop in the measured index values or negative results in the transient period between the decline of antigenemia and the beginning of the sero-conversion. In this case, we also did not observe the second diagnostic window.

In addition, some HIV 4th generation assays display a decline in the index values, which parallels the decline of the p24 antigen concentration before the sero-conversion. No seronegativity for different samples was observed, but the index values dropped near the cut off [13]. This was not observed for this case, instead, the levels of p24 antigen did not dropped in two days, but increased and then the antibodies appeared.

Diagnostic window is the time between the Human Immunodeficiency Virus (HIV) infection and laboratory diagnosis, the period during which tests for HIV are

negative in infected persons. “Detecting acute infection” has also been used synonymously with “closing the window” [18]. So in this case, we close the window, because first sample was analyzed in the last day of the diagnostic window.

This case is important because this laboratory performs around 22,000 assays for HIV annually but in Brazil is unusual to detect the acute phase of HIV infection and for the first time we can demonstrated it.

This case highlights the importance to implement new and modern methodologies of fourth generation and combine them to get the best performance of the assays. In this case, if only the ARCHITECT HIV Ag/Ab Combo, had been used, with the result nonreactive in immunoblotting assay, the test would be considered false-positive and at best hypothesis a new sample would be requested further, delaying antiretroviral therapy, being harmful to the patient.

The antiretroviral therapy was initiated with tenofovir 300 mg/day, efavirenz 600 mg/day and lamivudine 150 mg/day and in 05/03/2015 another sample was sent for viral load assay, presenting 467 copies/mL and log of 2,669, demonstrating that the antiretroviral therapy were been effective.

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