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Review article

Laboratory Algorithm in HIV Infection Diagnosis

Abstract

AIDS caused by HIV is an infection disease which was defined firstly in the USA in 1981. Since then, number of AIDS patients has increased continuously. About 36.9 million people are living with HIV around the world. Approximately 15 million people living with HIV were receiving antiretroviral therapy. Early detection is important due to the high risk of transmission that precedes seroconversion and also because it provides an opportunity to improve health outcomes with an early antiretroviral therapy. HIV testing is the key part of diagnosis and prevention efforts. Many tests have been used in the diagnosis of HIV over years and with developing testing methods, accuracy of the laboratory diagnosis of HIV infection has been improved. Detecting p24 antigens, HIV 1-2 antibodies and HIV nucleic acid demonstrated that antibody testing alone might miss a considerable percentage of HIV infections detectable by these tests. This review provides updated recommendations and algorithm for HIV testing that are necessary for diagnosing HIV and offers approaches for accurate assessment of test results.

Introduction

The Human Immunodeficiency Virus (HIV) is a lentivirus, a subgroup of retrovirus, that causes HIV infection and over time Acquired Immunodeficiency Syndrome (AIDS) [1]. HIV targets the immune system and weakens people's defense systems against opportunistic infections and some types of cancer. As the virus destroys and impairs the function of immune cells, infected individuals gradually become immunodeficient [2]. Immune function is typically measured by CD4 cell count. Immunodeficiency results in increased susceptibility to a wide range of infections and diseases that people with healthy immune systems can fight off [3]. Without treatment, average survival time after infection with HIV is estimated to be 9 to 11 years, depending on the HIV subtype [4].

AIDS was described for the first time in 1981, by the United States Center for Disease Control (CDC) in a group of the gay men and in immigrants from Haiti as a result of detecting rare *Pneumocystis jiroveci* pneumonia, and severe mucosal candidiasis in Kaposi's sarcoma cases [5-9]. The virus was isolated in 1983 by F. Barre-Sinoussi and L. Montagnier at Pasteur Institute in France for the first time [10]. The International Committee on Taxonomy of Viruses has called this virus HIV (human immunodeficiency virus), as it causes severe immunodeficiency leading to AIDS [11].

Infection with HIV occurs by the transfer of blood, semen, pre-seminal fluid, vaginal fluid and rectal fluids [12]. Within these bodily fluids, HIV is present as both free virus particles and within infected immune cells. Individuals cannot become infected through ordinary day-to-day contact such as kissing, hugging, shaking hands, or sharing personal objects, food or water [13]. Men who have sex with men, people who inject drugs, sex workers, transgender people and people in prisons and other closed settings are the conditions for inclusion in a high risk population.

There are three stages of HIV infection. The symptoms of HIV vary depending on the stage of infection; acute HIV infection, chronic HIV infection (asymptomatic HIV infection or clinical latency), and progression to AIDS (the late stage of HIV infection). The first few weeks after initial infection, individuals may experience no symptoms or flu-like symptoms that can include: fever, chills, rash, night sweats, muscle aches, sore throat, fatigue, swollen lymph nodes and mouth ulcers. These symptoms can last during a few days to several weeks. The virus attacks and destroys the infection-fighting CD4 cells of the immune system. HIV can be transmitted during any stage of infection, but the risk is greatest during acute HIV infection. During this time, HIV infection may not show up on an HIV test [12,14].

The second stage of HIV infection is chronic HIV infection. During this stage of the disease, HIV continues to multiply in the body but at very low levels. People with chronic HIV infection may not have any HIV-related symptoms. AIDS is the final stage of HIV infection. Because HIV has destroyed the immune system, the body can't fight off opportunistic infections and cancer. Opportunistic infections can rarely occur during the transient CD4 lymphopenia of early HIV infection [15]. Oral and esophageal candidiasis are the most common opportunistic infections in these patients [16]. Other opportunistic infections that have been reported during acute HIV infection include CMV infection (proctitis, colitis, and hepatitis), *Pneumocystis jiroveci* pneumonia and prolonged, severe cryptosporidiosis (Figure 1) [17-19].

HIV disease continues to be a serious health issue for different regions of the world. Worldwide, there were about 2 million new cases of HIV in 2014. About 36.9 million people are living with HIV around the world, and in March 2015, approximately 15 million people living with HIV were receiving antiretroviral therapy (ART). An estimated 1.2 million people died from AIDS-related illnesses in 2014, and about 39 million people worldwide have died of AIDS-related causes since the epidemic began. Seventy percent of all people living with HIV in 2014 were living in Sub-Saharan Africa, which bears the heaviest burden of HIV/AIDS worldwide. Other regions significantly affected by HIV/AIDS include Asia and the Pacific, Latin

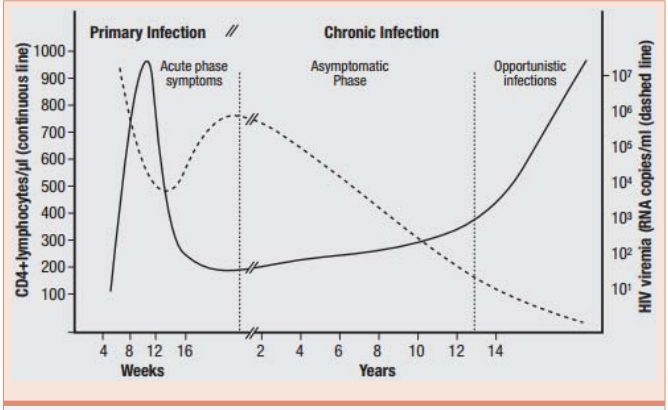

Figure 1: Clinical course of HIV infection [19] (Continuous line; CD4+lymphocytes/µl, Dashed line; HIV viremia (RNA copies/ml)).

Table 1: HIV Testing Assays and Their Window Periods [23]

HIV Test	Assay Method	Approximate Window Between Infection and Positive Test Result, day
First-generation EIA	Disrupted viral particles used to bind patient HIV antibody, detected by marker conjugated to anti-human IgG antibody	35–45
Second-generation EIA (including most rapid tests)	Synthetic or recombinant HIV antigen used to bind patient HIV antibody; detected by marker conjugated to anti-human IgG antibody	25–35
Third-generation EIA (also some rapid tests)	"Antigen sandwich": synthetic or recombinant HIV antigen used to bind patient HIV antibody; detected by marker conjugated to additional HIV antigen; detects IgM and IgG antibody	20–30
Fourth-generation EIA	Third-generation EIA method to bind patient antibody to HIV plus monoclonal antibody to bind p24 antigen; detects IgM and IgG antibodies and p24 antigen	15–20
RNA	Extraction of HIV nucleic acid, amplification by PCR or other methods; detects HIV RNA	10–15

EIA: enzyme immunoassay; Ig: immunoglobulin; PCR: polymerase chain reaction.

America and the Caribbean, Eastern Europe and Central Asia [20].

CDC recommends everyone from 13 to 64 years old to get tested for HIV at least once and that people at high risk of infection get tested more often. CDC recommends that all pregnant women should get tested for HIV as early as possible during each pregnancy. Risk factors for HIV infection include having unprotected sex (sex without a condom); having sex with many partners and injecting drugs and sharing needles, syringes or other drug equipment with others [20].

ART is recommended for all HIV-infected individuals, regardless of CD4 T lymphocyte cell count, to reduce the morbidity and mortality associated with HIV infection. ART is also recommended to prevent HIV transmission at early HIV-1 infection. Recommended initial regimens include 2 nucleoside reverse transcriptase inhibitors plus a nonnucleoside reverse transcriptase inhibitor a ritonavir-boosted protease inhibitor or an integrase strand transfer inhibitor. Alternatives in each class are recommended for patients with risk of certain concurrent conditions. CD4 cell count and HIV-1 RNA level should be monitored, as should engagement in care, ART adherence, HIV drug resistance, and quality-of-care indicators. Reasons for regimen switching include virologic, immunologic, or clinical failure and drug toxicity or intolerance. Confirmed treatment failure should be addressed promptly and multiple factors considered [21].

HIV/AIDS laboratory diagnosis

Early detection is important due to the high risk of transmission that precedes seroconversion and also because it provides an opportunity to improve health outcomes with an early antiretroviral therapy [22]. HIV tests can be classified as follows according to the purpose;

HIV screening tests: The HIV 1-2 antibody test is the most common HIV screening test. The test checks for HIV antibodies in blood, urine or fluids from the mouth. The time period from infection with HIV until the body produces enough HIV antibodies to be detected by an HIV antibody test is called the window period. Most people develop HIV antibodies within 3 months after they are infected with HIV. But the window period can vary depending on the HIV test used (Table 1) [23]. Most HIV tests, including rapid tests and home tests, are antibody tests. Their sensitivities are less than 3nd generation Enzyme Immunoassay (EIA). A combination or fourth-generation EIA test looks for both Anti-HIV 1-2 antibodies and p24 antigens. It can take 2 to 6 weeks (13 to 42 days) to develop enough antigens and antibodies for a combo-test to detect HIV [24]. In general, anyone who has a negative result on an combo-test within 3 months of a possible exposure to HIV should have the test repeated in 3 months.

Follow-up HIV tests: A reactive result on an HIV screening test must always be confirmed by a second HIV blood test. The following tests are used to confirm a reactive result on a HIV screening test. These are antibody differentiation tests, like Line immunoassay, Western blot or indirect immunofluorescence assay [25].

HIV Nucleic acid tests: HIV infection may be identified using RNA assays (qualitative or quantitative). There are a variety of methodologies used to detect HIV RNA (like reverse transcription polymerase chain reaction, transcription mediated amplification and nucleic acid sequence based amplification). Quantitative RNA assays are the standard viral load tests performed for monitoring the response to treatment of HIV infection but may be used as a supplemental diagnostic assay to confirm HIV infection [26]. FDA-approved viral load tests only detect HIV-1 [27].

HIV drug resistance tests: Drug resistance can be measured by using either genotypic or phenotypic assays. Genotypic assays detect mutations that cause drug resistance. Phenotypic assays are drug susceptibility assays in which a fixed inoculum of HIV-1 is cultured in the presence of serial dilutions of an inhibitory drug. Genotypic

testing is used more commonly than phenotypic testing because of its lower cost, wider availability, and shorter turnaround time [28,29].

For routine HIV testing, a laboratory-based fourth generation (antigen/antibody combination) assay that detects HIV p24 antigen and HIV antibodies should be used and if reactive, should be followed by a confirmatory HIV-1/HIV-2 antibody differentiation immunoassay in the first step (Figure 2) [27,30,31].

The results of serologic testing (a combination or fourth-generation EIA test) algorithms are reported as reactive or negative. The criteria for a positive test is a reactive EIA or combination assay followed by a positive confirmatory assay. A negative test is a negative screening EIA or combination assay. Indeterminate result is when the EIA or combination assay is positive but the confirmatory test is indeterminate or negative. Although rare, false negative and false positive test results can occur [32].

Specimens with a reactive or repeatedly reactive antigen/antibody combination immunoassay result should be tested with a FDA-approved antibody immunoassay that differentiates HIV-1 antibodies from HIV-2 antibodies. Reactive result on the initial antigen/antibody combination immunoassay and positive result on the HIV-1/HIV-2 antibody differentiation immunoassay should be interpreted as positive for HIV-1 antibodies, HIV-2 antibodies, or undifferentiated HIV antibodies. Specimens that are reactive on the initial antigen/antibody combination immunoassay and negatif or indeterminate on the HIV-1/HIV-2 antibody differentiation immunoassay should be tested with a FDA-approved HIV-1 nucleic acid test [27,31]. False negative results can be a result of patient related factors or due to the test itself. The most common cause of a false negative result is acute infection with HIV (window period) [23]. Another reasons for false negative results are; failure to detect certain HIV subtypes, HIV tests with low sensitivity and rare causes (immune dysfunction or agammaglobulinemia, immunocompromised due to malignancy or medications, delay in seroconversion following early initiation of antiretroviral therapy or human error during the testing process) [33]. The frequency of false positive test results is extremely rare even in low prevalence areas [34].

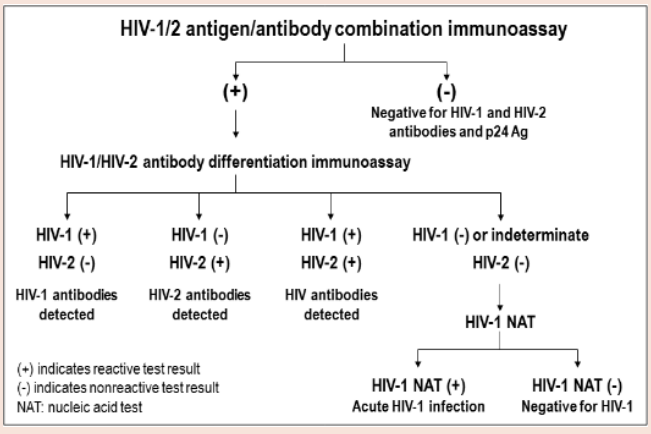

Figure 2: Recommended Laboratory HIV Testing Algorithm for Serum or Plasma Specimens [31].

Reasons for false positive results include; cross-reacting alloantibodies from pregnancy, autoantibodies (collagen-vascular diseases, autoimmune diseases, and malignancy), receipt of an experimental HIV-1 vaccine, influenza vaccination [35].

HIV serological testing is generally used to diagnose HIV infection in adults and children above 18 months of age. Because of the passage of maternal HIV antibody across the placenta to the baby, HIV serological testing in infancy cannot be used to confirm HIV infection in the infant, but does indicate maternal HIV infection and exposure of the infant. In order to diagnose HIV infection definitively in children below 18 months of age, assays that detect the virus or its components are therefore required. HIV virologic testing must be performed using assays that detect HIV-1 DNA or RNA [36]. If the mother's HIV-1 serostatus is unknown, rapid HIV-1 antibody testing of the newborn infant to identify HIV-1 exposure is essential so that antiretroviral prophylaxis can be initiated within the first 12 hours of life if test results are reactive. For HIV-1-exposed infants, it has been recommended that diagnostic testing with HIV-1 DNA or RNA assays be performed within the first 14 days of life, at 1 to 2 months of age, and at 3 to 6 months of age. If any of these test results are reactive, repeat testing is recommended to confirm the diagnosis of HIV-1 infection. A diagnosis of HIV-1 infection can be made on the basis of 2 positive HIV-1 DNA or RNA assay results. In non-breast feeding children younger than 18 months with no positive HIV-1 virologic test results, presumptive exclusion of HIV-1 infection can be based on 2 negative virologic test results, 1 obtained ≥1 month of age and 1 obtained at ≥4 months of age [27,37].

Conclusion

According to the World Health Organization (WHO), there were approximately 36.9 million people worldwide living with HIV/AIDS at the end of 2014. Of these, 2.6 million were children (<15 years old). Today, someone diagnosed with HIV and treated before the disease is far advanced can live nearly as long as someone who does not have HIV. For routine HIV testing, a laboratory-based fourth generation assay that detects HIV p24 antigen and HIV antibodies should be used and if reactive, should be followed by a confirmatory HIV-1/HIV-2 antibody differentiation immunoassay. Specimens that are reactive on the initial antigen/antibody combination immunoassay and negative or indeterminate on the HIV-1/HIV-2 antibody differentiation immunoassay should be tested with a nucleic acid test. In order to diagnose HIV infection definitively in children below 18 months of age, assays that detect the virus or its components are therefore required. HIV virologic testing must be performed using assays that detect HIV-1 DNA or RNA. Early diagnosis is very important for early treatment. Effective treatment with antiretroviral drugs can control the viral infection and reduce the risk of transmitting the virus to others.

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