ORIGINAL ARTICLE

Serological diagnosis of HIV/AIDS infection in Brazil

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ABSTRACT

After being announced in the US and Europe, enzyme-linked immunosorbent assay (ELISA) for HIV screening was marketed in Brazil and was immediately put to use in several public and private laboratories. Newer technologies for HIV testing, such as those in the fourth generation, which detect anti-HIV antibodies and the p24 antigen, and nucleic acid-based tests have all shortened the interval between infection and disease markers detection. This brief narrative review intends to present the different HIV diagnostic test flowcharts used nationally in Brazil, from assays based only on anti-HIV antibodies to new flowcharts in which molecular tests have been included. Until 1998, Brazilian health authorities had not yet standardized an algorithm for diagnosing the HIV infection. Since then, different testing algorithms have been recommended by the Ministry of Health and these recommendations have been followed by laboratories. Considering the different scenarios in which the diagnosis of HIV has been performed, there is a need for frequent evaluation of the assays, since the quality of the results can be influenced by several biological factors of the host and the agent.

KEYWORDS: HIV infections, AIDS serodiagnosis, HIV antibodies, immunoassay, seroconversion.

RESUMO

O teste imunoenzimático do tipo ELISA foi comercializado no Brasil logo após ser anunciado nos EUA e Europa, sendo imediatamente utilizado em vários laboratórios públicos e privados. Tecnologias mais recentes para a testagem de HIV, como a de quarta geração, que detecta anticorpos anti-HIV e o antígeno p24, e os testes baseados em ácido nucleico reduziram o intervalo entre a infecção e a detecção da doença. Esta breve revisão narrativa se propõe a apresentar os diferentes fluxogramas de testes para diagnóstico do HIV utilizados nacionalmente, desde os ensaios baseados apenas em anticorpos anti-HIV até os novos fluxogramas em que foram incluídos os testes moleculares. Até 1998, as autoridades sanitárias brasileiras ainda não haviam normatizado um algoritmo para a realização do diagnóstico da infecção pelo HIV. Desde então, diferentes algoritmos de testagem foram preconizados pelo Ministério da Saúde e seguidos pelos laboratórios. Considerando os diferentes cenários em que o diagnóstico do HIV tem sido realizado, há necessidade de avaliações frequentes dos ensaios, visto que a qualidade dos resultados pode ser influenciada por diversos fatores biológicos do hospedeiro e do agente.

PALAVRAS-CHAVE: infecções por HIV; sorodiagnóstico da aids, anticorpos anti-HIV, imunoensaio, soroconversão.

INTRODUCTION

The identification in 1981 of the acquired immunodeficiency syndrome, commonly known as AIDS, became a turning point in human history. The epidemic of infection by the human immunodeficiency virus (HIV) and AIDS represents a global, dynamic and unstable phenomenon whose form of occurrence in different regions of the world depends, among other determinants, on individual and collective human behavior.[1]

Globally, there were around 38.4 million (33.9 million to 43.8 million) people living with HIV in 2021, with around 1.5 million (1.1 million to 2.0 million) registered new infections in that same year.[2]

In Brazil between 2007 and June 2021, 381,793 cases of HIV infection were reported in the Information System for Notifiable Diseases (Sinan). Although a decrease in AIDS cases has been observed in almost the entire country, especially in recent years, it should be noted that part of this reduction may be related to underreporting, due to the focus of health professionals on the COVID-19 pandemic.[3]

The importance of periodic testing as a prevention strategy in the programmatic response to the HIV/AIDS epidemic has been emphasized at the global level.[4] Technological advances have facilitated the expansion of HIV testing, with cost-effectiveness and undeniable benefits for both primary care and public health.[5-7] Having access to an early diagnosis of infection not only increases the individual's life expectancy due to the ability to begin immediate treatment, but also prevents the transmission of the virus to other people.

Tests for diagnosing the HIV infection have evolved considerably since the Food and Drug Administration (FDA) licensed the first enzyme-linked immunosorbent assay (ELISA) in the United States on March 2, 1985.[8,9] Produced by the American company Abbott Laboratories, in Chicago (Illinois), under the name "Abbott HTLV III EIA", this assay was initially licensed for the screening of anti-HTLV-III antibodies (the first name used for the virus that causes AIDS) in blood donors. Its rapid availability made it possible to protect countless individuals against HIV infection transmitted by blood transfusions and blood products.[9-11] Three months later, other companies in the United States also announced their tests and, in 1987,[12] the first Western blot (WB) assay to confirm HIV infection was approved[9]. Since then, the quality of these tests has increasingly improved, with methods that detect infection earlier and produce results faster.[9,11]

A detailed understanding of the structure of the virus, how the infection is established in the body and the causes of AIDS, is crucial not only to identify and develop new effective drugs and vaccines, but also to define strategies for the laboratory diagnosis of the infection. HIV testing is a

critical step in controlling its spread in the population. Therefore, laboratory diagnostic strategies must be continually revised in line with new findings on replication characteristics and pathogenic mechanisms of infection.[13]

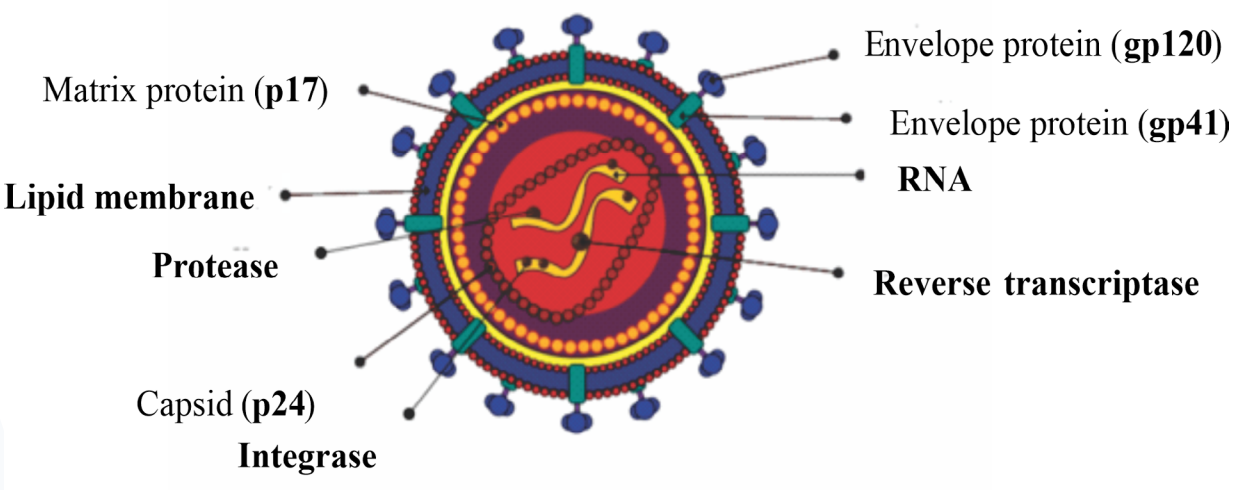
Different methodologies have been used to detect laboratory markers in HIV/AIDS infection, such as the determination of specific antibodies and circulating p24 antigen, viral load, genotyping, drug resistance and assays for recent infections.[14-19] In Brazil, these assays have been performed by specific laboratory networks.

The bibliographic survey was implemented considering the period from 1986 to 2021, using five databases — PubMed, Medline, Web of Science, SciELO and LILACS, with the following descriptors — HIV, AIDS, immunoassays, seroconversion and anti-HIV antibodies. This brief narrative review proposes to present the different test flowcharts for the diagnosis of infection used in the country over the years, from tests based only on anti-HIV antibodies up to those using newer flowcharts in which molecular tests were included. This examination aims to contextualize their usage and the results obtained. This work also alerts to the existence of several viral and host markers present during the course of the infection that can be monitored and used for identification. The kinetics and time of appearance of markers are quite consistent between different individuals and must be taken into account when choosing a diagnostic test.

HISTORY

HIV belongs to the *Retroviridae* family, *Lentivirinae* subfamily. Retroviruses are enveloped viruses that store their genetic material in the form of ribonucleic acid (RNA)[20,21] (Figure 1). They often induce cytopathic effects in infected cells and share a distinct biological feature: an early stage of primary infection, followed by a relatively asymptomatic period which can last from months to years, followed by a stage of overt disease.[22] Like all viruses, HIV can only replicate within cells, where it controls the machinery for its replication.[23] Infection begins with recognition of viral proteins by receptors on the surface of target cells.[19] Once in the infected cell, the virus must convert its RNA into deoxyribonucleic acid (DNA) through the process of reverse transcription, catalyzed by retroviral reverse transcriptase enzyme.[24] This enzyme transcribes a single-stranded viral RNA molecule into complementary viral DNA (cDNA), which can then be inserted into the host genome during the integration process. This depends on the retroviral integrase enzyme as well as host cellular cofactors. After successful addition of viral cDNA to the host genome (provirus), viral replication can be initiated.[21,23,25]

Figure 1. Basic structure of HIV.



Legend: gp: glycoprotein; p: protein; RNA: ribonucleic acid.

Source: adapted from https://bvsms.saude.gov.br/bvs/publicacoes/diagnostico_hiv_2014.pdf

Until 1986, it was believed that the human immunodeficiency virus type 1 (HIV-1) was the only agent causing AIDS, when a second type, HIV-2, was isolated.[26] Due to its wide genetic variability, HIV has been classified into these two main types, as well as into several subtypes, circulating recombinant forms (CRF – circulating recombinant forms) and unique recombinant forms (URF – unique recombinant forms). This viral diversity has an impact on diagnosis, monitoring, therapy and vaccine development.[27]

Once HIV infection has occurred, markers in the individual's bloodstream are detected in chronological order: RNA, p24 antigen and antibodies. In this context, tests for their detection are performed together to produce highly accurate and reliable results, divided into two categories: screening tests (high degree of sensitivity), designed to detect all infected individuals and confirmatory tests (high specificity) used to differentiate falsely reactive samples in screening from those that are actually from infected individuals.[28]

False-negative results in HIV antibody screening assays can also occur and be attributed to the window period, that is, the period before HIV-specific antibodies are developed. In addition, other causes of failure may occur, such as limitations of the assay itself (sensitivity and specificity); factors related to equipment/inputs (inadequate storage of reagents and lack of calibration or maintenance of equipment) and use of suboptimal algorithms for diagnosis. These failures are also likely to occur in patients who have started early antiretroviral therapy (ART), which can lead to the development of an incomplete antibody response due to virological suppression and subsequent lack of antigen.[28-31]

TREATMENT

HIV infection has been considered chronic and potentially controllable since the emergence of antiretroviral therapy and the availability of biological markers to monitor its progression.[32]

Universal and free access to ART, which began in Brazil in 1996, had a notable impact on AIDS morbidity and mortality.[33]

Currently, the recommendation is to start therapy as soon as possible after diagnosis of infection. This approach minimizes the risk of transmission, preserves the immune system, stops the spread of latent HIV reservoirs and slows the progression of the disease.[34-37]

DIAGNOSIS OF THE HIV INFECTION

Depending on the test objective, different algorithms are used. For blood, tissue and organ donor screening and epidemiological studies for example, a highly sensitive algorithm for detecting anti-HIV antibodies is recommended. When it comes to a clinical diagnosis, a positive result from the highly sensitive screening test must be followed by a confirmation test.

In 1989, the US Centers for Disease Control and Prevention (CDC) published guidelines for the serodiagnosis of HIV-1 infections. Serum samples that were repeatedly reactive in the enzyme immunoassay for anti-HIV antibodies were then subjected to a more specific supplementary test, the WB for HIV-1. In 1992, with the recommendation of screening tests for the simultaneous detection of anti-HIV-1 and anti-HIV-2 antibodies, the WB for HIV-2 was introduced as a confirmatory assay. In November 2002, the rapid HIV-1 test was approved to aid in the diagnosis of infection at the point of care. Faced with reactive results, it was necessary to detail protocols for the confirmation of rapid tests.[34,38-40]

In this context, the testing algorithm aimed to improve diagnostic accuracy. The continuous improvement of diagnostic tests has been a consequence of great advances in knowledge of the immunological and pathogenic mechanisms of infection and of the virus/host interaction obtained in research on HIV/AIDS. The discoveries of the mechanisms of virus replication, as well as the immune response of the infected individual throughout the course of the disease, have been fundamental in developing tests capable of detecting specific antibodies and HIV antigen as well as nucleic acid.[9] Thus, technological evolution provided greater sensitivity and specificity to the tests.

About three to four years after the first descriptions of AIDS (1981), its causative agent was cultivated, which led to the development and production of tests that could help health professionals

identify people living with the virus.[41] As a result, four generations of immunoassays followed, defined according to the evolution of the methodologies employed since the first commercially available assay in 1985.[28,42]

First-generation anti-HIV antibody assays were based on purified "whole virus" (viral lysate) antigens obtained from cell cultures. Detection of antibodies (Immunoglobulin G – IgG class only) bound to HIV antigens used an "indirect" approach.[11,42] While highly sensitive and useful for protecting the blood supply, these methods can lead to false-positive results, particularly when tested in low-risk individuals.[43] Thus, the need for additional tests to confirm HIV infection was soon observed,[12] such as WB and indirect immunofluorescence assay (IFA).[43]

Developed in the late 1980s, second-generation tests also detect IgG in an indirect format, but use recombinant antigens or synthetic peptides derived from HIV structural proteins to improve specificity.[42,43] With the discovery of viral type 2, simultaneous detection assays for anti-HIV-1 and anti-HIV-2 antibodies were developed.[44] Thus, the confirmatory assay for HIV-2 was added to the testing algorithm.[43]

The third-generation immunoassay that became available for the market has a "sandwich" (or immunometric) format and detects immunoglobulins of the IgG and IgM classes. It uses synthetic peptides and recombinant proteins as antigens both in a solid phase and in a conjugate form.

Used in several countries since the 1990s, fourth-generation immunoassays, also in a "sandwich" format, allow the combined detection of antigen (p24) and antibodies (IgG and IgM). They also include several assays that discriminate p24 antigen and antibody reactivity.[42,45]

At the turn of the third millennium, the world witnessed a revolution in the diagnosis of HIV infection with rapid tests (RT).[12] These tests are simple immunoassays (immunochromatographic), with results in up to 30 minutes, performed primarily in person in a non-laboratory environment (point-of-care). There are several rapid test formats, the most frequently used are lateral flow immunochromatography devices (or strips), dual-path immunochromatography (DPP) and those using immunoconcentration.[11,42] Most detect HIV-1 and HIV-2. Fourth-generation RT can differentiate between antigen and antibody reactivity.

Complementary (confirmatory) assays, performed only on samples reactive in screening tests, use different formats and principles (WB, immunoblot (IB), including rapid immunoblot (IBR) and IFA) and are less sensitive than third and fourth generation immunoassays. In cases of positivity in fourth-generation assays due to detection of p24 antigen in the absence of circulating antibodies in the blood ("serological window"), viremia may be detectable by the presence of RNA or p24 antigen.[11]

Therefore, molecular tests were also included in the algorithms as complementary, as they help to clarify the results of acute HIV infection.[40]

HIV DIAGNOSIS IN BRAZIL

The expansion of the AIDS epidemic led to the need for serological tests to diagnose HIV infection in an increasing number of people. In Brazil, the ELISA enzyme immunoassay was marketed shortly after being announced in the United States and Europe. This test was immediately used by several public and private laboratories in the investigation of suspected cases of HIV infection, as well as to determine the prevalence of the virus in different population groups.[12,46]

In 1987, the testing of anti-HIV antibodies in blood banks for the screening of donors became nationally mandatory, in order to avoid transmission of the virus to recipients of blood transfusions.[12,46,47] However, confirmatory tests for diagnostic purposes were not mandatory for patients with positive serology at screening.[12,47] Between 1987 and 1989, the creation of the Center for Serological Orientation and Support (COAS), later called the Center for Testing and Counseling (CTA), was established to offer individuals the possibility of knowing their HIV serological status based on free, confidential and anonymous tests.[46,48]

Until 1998, national health authorities had not standardized the way in which, in terms of algorithms or test flowcharts, the diagnosis of HIV infection in the country would be carried out.[12] Thus, guidelines from North America (also endorsed by the World Health Organization – WHO), were followed by most, if not all, Brazilian laboratories.[12] In this model, ELISA was the choice for initial screening, but following an important dichotomy regarding the use of confirmatory tests. In the public network, the Ministry of Health started to produce, through the Laboratory of Bio-Manguinhos Reagents of the Osvaldo Cruz Foundation (Fiocruz), the IFA for HIV-1 which had a low cost when compared to the WB.[12]

There were several cases of false-positive results in the screening for the detection of anti-HIV antibodies which were not subsequently submitted to confirmatory tests. Consequently, in June 1998 the Ministry of Health issued Ordinance SVS/MS No. 488/1998, standardizing the procedures for the laboratory diagnosis of HIV infection in individuals over 2 years of age, through a flowchart of sequential tests. The aim was to maximize the degree of reliability of the results of these tests using serum or plasma samples.[12,49] Regarding children under 2 years of age, serology cannot be used to establish a diagnosis due to the passive transfer of antibodies from seropositive mothers, which can be detected in the child's blood for up to two years after birth. In 2000, the National STD and AIDS/MS

Coordination published a flowchart regulating the use of viral load (VL) for HIV (quantitative method) in the diagnosis of infection in children in this age group.[50]

For the detection of anti-HIV antibodies, sequenced procedures in hemotherapy centers and in clinical laboratories, both public and private, became obligatory.[49] Two separate screening tests were used in the first stage (Stage I) of sequential tests. In Stage II, the IFA was used in samples with reactive or discordant results in tests performed concomitantly in Stage I. Finally, in a third stage (Step III), the WB assay was used in a sample with non-reactive or indeterminate results in the IFA or when Step II could not be performed.

After five years, with the improvement of screening tests, Ordinance GM/MS No. 488/1998 was replaced by Ordinance GM/MS No. 59, of January 28, 2003,[51] came into force, which modified the previously proposed flowchart. Initial testing (Step I) stopped performing two parallel screening tests, resulting in a significant reduction in the total cost of diagnosis.[12] In addition to the IFA, Stage II allowed the use of a type IB assay. This revision did not change the flow of Step III, except for the WB assay interpretation criteria, which needed to follow the diagnostic kit manufacturer's instructions.

In the mid-2000s, the emergence of rapid tests caused great progress in the diagnosis of HIV infection.[47] In Brazil, the use of RT began with the screening of pregnant women who had not been previously tested for the virus, conforming to the recommendations for prophylaxis of mother-to-child transmission. However, the results obtained in these tests were considered, requiring the referral of a blood sample to the laboratory to clarify the diagnosis.[47]

In the search for alternatives that would expand access to knowledge of the HIV infection status of infected individuals, especially in places in the country without a laboratory network, specific measures were adopted. The goals were to interrupt the transmission chain and provide adequate care. The use of rapid tests (RT) was regulated by Ordinance SVS/MS No. 34, of July 28, 2005.[52] Two different rapid tests (RT1 and RT2) started to be performed sequentially and, in blood samples with reactive or discordant results, a third, the RT3 was used to confirm the diagnosis.

In order to consolidate the algorithms for use in the laboratory and for rapid testing within a single document, Ordinance SVS/MS No.151 of October 14, 2009 was published,[53] authorizing the minimum flowchart for the laboratory diagnosis of HIV in individuals aged over 18 months. The diagnosis started to be performed using a flowchart divided into two stages: Stage I (screening) and Stage II (complementary). The rapid tests enabled confirmation of a negative result with only RT1, while the positive in RT1 also passed through RT2. In cases of discordant results between these two tests, it was necessary to collect a new sample by venipuncture and to have it follow the laboratory

flowchart process.[12] It is worth mentioning that within the regulation is the requirement for blood samples to be collected on filter paper using the diagnostic kits that were developed for this purpose.

On December 17, 2013, Ordinance SVS/MS No. 29 was published,[28] which regulated the diagnosis of HIV infection in Brazil and approved the use of the "*Manual t*écnico *para diagnóstico da infecção pelo HIV em adultos e crianças*" (Technical manual for the diagnosis of HIV infection in adults and children, in Portuguese)." This document described the six flowcharts, the first two (Flowchart 1 and Flowchart 2) for RT diagnosis, while the others (Flowchart 3, Flowchart 4, Flowchart 5, and Flowchart 6) dealt with laboratory diagnosis. The latest edition of the manual, which has gone through four revisions, was published in 2018. Flowchart 1 employs two rapid tests (RT1 and RT2), which contain different antigens, used sequentially in blood samples, and these can be obtained by the puncture of the digital pulp or by venipuncture (whole blood, serum or plasma). Flowchart 2 uses two rapid tests (RT1-OF and RT2) of different antigens, also used sequentially. RT1- OF is performed with a sample of oral fluid (OF), while RT2 uses a blood sample, which can be obtained by puncture of the digital pulp or by venipuncture. The diagnosis of HIV infection in a laboratory environment is performed by means of initial and complementary tests in serum or plasma samples. This method can also be used for diagnostic confirmation of samples that present discordant results in the RT of flowcharts 1 and 2. Flowcharts 3 and 6 use a fourth-generation immunoassay as an initial test (T1), differing in the complementary step for a sample with a reactive result. The complementary test (T2) in flowchart 3 a molecular assay for viral load quantification (VL), while the one in flowchart 6 is for the detection of antibodies (WB or IB). In case of discordant results between T1 and T2, the sample is submitted to another complementary test (T3). In flowchart 3, is the WB, IB or IBR is used as complementary test and in flowchart 4, the molecular test is used sequentially after a reactive initial test. Flowcharts 4 and 5 use the third-generation immunoassay as an initial test and also have a complementary step. Flowchart 4 is similar to flowchart 3 (the molecular test) as is flowchart 6 to flowchart 5 (antibody detection test).[28] It is worth mentioning that IFA was widely used as a complementary test during the first decade of the HIV epidemic, but it was replaced by WB and IB.[28] The summary of the flowcharts used in the country since 1998 is described in the Table 1.

Table 1. Flowcharts recommended by the Ministry of Health for the diagnosis of HIV infection in Brazil.

Ordinance	Publication	Stage I	Stage II	Stage III
SVS/MS n° 488	1998	Two sequential tests (immunoassay)	IFA	WB
GM/MS n° 59	2003	Immunoassay	IFA or IB	WB
SVS/MS n° 34	2005	RT1 + RT2 simultaneous tests	RT3	_____
SVS/MS n° 151	2009	Immunoassay	IFA, IB or WB	_____
		RT1	RT2	_____
SVS/MS n° 29	2013	RT1 (Flowchart 1)	RT2	_____
		RT1 (OF) (Flowchart 2)	RT2	_____
		Fourth-generation immunoassay (Flowchart 3)	MT	IB or WB
		Third-generation immunoassay (Flowchart 4)	MT	IB or WB
		Third-generation immunoassay (Flowchart 5)	IB or WB	MT
		Fourth-generation immunoassay (Flowchart 6)	IB or WB	MT

IFA:indirect immunofluorescence assay; WB: Western blot; IB: immunoblot; RT: rapid test; OF: oral fluid; MT: molecular test; SVS: Health Surveillance Secretariat; GM: Cabinet of the Minister; MS: Brazilian Ministry of Health

In this context, the testing algorithm for the serological diagnosis of HIV infection has been used for more than 20 years in Brazil. Given the large number of technological advances in the tests, the Ministry of Health has been working to develop new potential algorithms, both in laboratories and in places that enable delivery of a result during a patient's visit.

In the current era of immediate ART and pre-exposure (PrEP) or post-exposure (PEP) prophylaxis, confidently diagnosing HIV becomes increasingly complex. When used optimally, antiretroviral therapy can effectively control the replication of the virus, prevent the development of AIDS, prolong the life of its carriers, significantly reduce the risk of transmission and have an impact on incidence of the virus at a population level.[54-56]

Studies have shown that continuous treatment with antiretroviral therapy can modify the typical evolution of the HIV-specific antibody response, as well as alter the expected kinetics of the response in individuals who discontinue therapy.[37] The reduction or elimination of viral expansion by the use of ART in acute infection, below the threshold necessary for the evolution of an anti-HIV immune response, can result in a delay or a block in the formation of specific antibodies against the virus.These antibodies are the basis for the serological detection of HIV. It is critical that the results of tests based on the detection of specific antibodies or virological markers are analyzed in conjunction with the patient's clinical conditions and epidemiological data, considering that the quality of the results can be influenced.[36,57-60]

Scientific research and epidemiological surveillance are needed to determine the most appropriate assays for accurate and affordable testing algorithms.[54] Some biological parameters, however, lead to inconsistent or conflicting test results and should be investigated.[35] Even with limitations, tests for diagnosis of infection continue to play an important role in HIV prevention.[42]

FINAL CONSIDERATIONS

With the evolution of generations of serological tests, it has been possible to diagnose HIV infection at an increasingly early stage, reducing the immunological window while improving the positive predictive value and thus enabling the availability of various new options for the market. It is important to always keep in mind that the immune response, including the serological one, is very dynamic and, if a person does not meet the defined criteria for seropositivity, it is essential to undergo a serological follow-up after 15 to 30 days. This measure makes it possible to observe whether a more complete "serological conversion" would have occurred, allowing a more conclusive definition of a person´s serological status.

This narrative review shows the different scenarios in which HIV diagnosis has been performed and reinforces the need for frequent evaluations the laboratory assays. This is because the quality of results can be influenced by different biological factors of the host and agent, such as use of ART and viral diversity.

REFERENCES

1. Brito AM, Castilho EA, Szwarcwald CL. AIDS e infecção pelo HIV no Brasil: uma epidemia multifacetada. *Rev Soc Bras Med Trop*. 2001;34:207-17. https://doi.org/10.1590/S0037-86822001000200010

2. World Health Organization. *Key facts HIV* [internet]. Geneva; July 2022. [access on: Aug 11, 2022]. Available: https://cdn.who.int/media/docs/default-source/hq-hiv-hepatitis-and-stis-library/key-facts-hiv-2021-26july2022.pdf?sfvrsn=8f4e7c93_5

3. Ministério da Saúde (BR). Secretaria de Vigilância em Saúde. *Boletim Epidemiológico HIV/Aids 2021* [internet]. Brasília 2021. [access on: Feb 10, 2022]. Available: http://www.aids.gov.br/pt-br/pub/2021/boletim-epidemiologico-hivaids-2021

4. Redoschi BRL, Zucchi ME, Barros CRS, Paiva VS. Uso rotineiro do teste anti-HIV entre homens que fazem sexo com homens: do risco à prevenção. *Cad Saúde Pública*. 2017;33(4):e00014716. https://doi.org/10.1590/0102-311X00014716

5. Baggaley RF, Irvine MA, Leber W, Cambiano V, Figueroa J, McMullen H, et al. Cost-effectiveness of screening for HIV in primary care: A health economics modelling analysis. *Lancet HIV*. 2017;4(10):e465-e474. https://doi.org/10.1016/S2352-3018(17)30123-6

6. Vermund SH. Control of HIV epidemic: Improve access to testing and ART. *Lancet HIV*. 2017;4(12):e529-e576. https://doi.org/10.1016/S2352-3018(17)30166-2

7. Castejon MJ, Yamashiro R, Oliveira CAF, Brígido LFM, Generoso IP, Veras MASM, et al. Performance of rapid tests compared to conventional tests used for HIV diagnosis. *J Bras Patol Med Lab*. 2018;54(6):364-71. https://doi.org/10.5935/1676-2444.20180058

8. National Museum of American History. *Abbott HTLV III EIA (Enzyme Immunoassay for the Detection of Antibody to Human T- Lymphotropic Virus Type III in Human Serum or Plasma)* [internet]. Washington, DC; 1986. [access on: Jun 14, 2022]. Available: https://americanhistory.si.edu/collections/search/object/nmah_1322289

9. Branson BM. State of the art for diagnosis of HIV infection. *Clin Infect Dis*. 2007;45 (4):221-25. https://doi.org/10.1086/522541

10. Gallo RC, Montagnier L. The discovery of HIV as the cause of AIDS. *N Engl J Med*. 2003;349(24):2283-85. https://doi.org/10.1056/NEJMp038194

11. Buttò S, Suligoi B, Fanales-Belasio E, Raimondo M. Laboratory diagnostics for HIV infection. *Ann Ist Super Sanità*. 2010;46(1):24-33. https://doi.org/10.4415/Ann_10_01_04

12. Ferreira Junior OC, da Motta LR. Três décadas de diagnóstico de HIV: a experiência brasileira. In: Ministério da Saúde. *Histórias de luta contra a aids* [internet]. Brasília: Ministério da Saúde; 2015:258-75. [access on: Mar 5, 2022]. Available: https://www.ucs.br/ips2/wp-content/uploads/2020/09/Tres-Decadas-de-Diagnostico-de-HIV-A-Experiencia-Brasileira.pdf

13. Fanales-Belasio E, Raimondo M, Suligoi B, Buttò S. HIV virology and pathogenetic mechanisms of infection: A brief overview. *Ann Ist Super Sanità*. 2010;46(1):5-14. https://doi.org/10.4415/ANN_10_01_02

14. Centers for Disease Control. *Current trends update*: Serologic testing for antibody to human immunodeficiency virus. MMWR. 1988;36(52):833-45.

15. Kleinschmidt A, Matuschke A, Goebel FD, Erfle V, Hehlmann R. Serological markers as prognostic criteria for the course of HIV infection. *Infection*. 1991;19(2):S89-92. https://doi.org/10.1007/BF01644474

16. Constantine NT, Callahan JD, Watts DM. *HIV testing and quality control*: a guide for laboratory personnel. Durham-NC: Family Health International. 1991. 170p.

17. World Health Organization. *HIV testing methods*. UNAIDS – Technical update [internet]. Geneva; Nov 1997. [access on: Mar 5, 2022]. Available: https://www.unaids.org/sites/default/files/media_asset/testmtu_en_0.pdf

18. World Health Organization. *Joint United Nations Programme on HIV/AIDS*. Guidelines for using HIV testing technologies in surveillance: selection, evaluation, and implementation [internet]. WHO/CDS/CSR/EDC/2001.16 UNAIDS/01.22E, 2001. [access on: Mar 5, 2022]. Available: https://www.unaids.org/sites/default/files/media_asset/jc602-hivsurvguidel_en_1.pdf

19. Constantine NT, Zink H. HIV testing technologies after two decades of evolution. *Indian J Med Res*. 2005;121:519-38.

20. Yılmaz G. Diagnosis of HIV infection and laboratory monitoring of its therapy. *J Clin Virol*. 2001;21:187-96. https://doi.org/10.1016/s1386-6532(01)00165-2

21. Smith JA, Daniel R. Following the path of the virus: the exploitation of host DNA repair mechanisms by retroviruses. *Acs Chem Biol*. 2006;1(4):217-26. https://doi.org/10.1021/cb600131q

22. Levy JA. Human immunodeficiency viruses and the pathogenesis of AIDS. *JAMA*. 1989; 261(20):2997-3006. https://doi.org/ 10.1001/jama.1989.03420200087044

23. Klimas N, Koneru AO, Fletcher MA. Overview of HIV. *Psychosom Med.* 2008;70(5): 523-30. https://doi.org/10.1097/PSY.0b013e31817ae69f

24. Fuentes GM, Fay PJ, Bambara RA. Relationship between plus strand DNA synthesis removal of downstream segments of RNA by human immunodeficiency virus, murine leukemia virus and avian myeloblastoma virus reverse transcriptases. *Nucleic Acids Res*. 1996;24(9):1719-26. https://doi.org/10.1093/nar/24.9.1719

25. Craigie R. HIV integrase, a brief overview from chemistry to therapeutics. *J. Biol. Chem*. 2001;276(26):23213-16. https://doi.org/10.1074/jbc.R100027200

26. Bentsen C, McLaughlin L, Mitchell E, Ferrera C, Liska S, Myers R, et al. Performance evaluation of the Bio-Rad Laboratories GS HIV Combo Ag/Ab EIA, a 4th generation HIV assay for the simultaneous detection of HIV p24 antigen and antibodies to HIV-1 (groups M and O) and HIV-2 in human serum or plasma. *J Clin Virol*. 2011;52(Suppl 1):S57- S61. https://doi.org/10.1016/j.jcv.2011.09.023

27. Simon D, Béria JU, Tietzmann DC, de Carli R, Stein AT, Lunge VR. Prevalência de subtipos do HIV-1 em amostra de pacientes de um centro urbano no sul do Brasil. *Rev Saúde Pública*. 2010;44(6). https://doi.org/10.1590/S0034-89102010005000039

28. Ministério da Saúde (BR). Portaria SVS/MS nº 29, de 17 de dezembro de 2013. Aprova o manual técnico para o diagnóstico da infecção pelo HIV em adultos e crianças e dá outras providências. Brasília; *Diário Oficial da União*. Dec 18, 2013. Section 1; 245.

29. Kassutto S, Johnston MN, Rosenberg ES. Incomplete HIV type 1 antibody evolution and seroreversion in acutely infected individuals treated with early antiretroviral therapy. *Clin Infect Dis*. 2005;40(6):868-73. https://doi.org/10.1086/428127

30. Hare CB, Pappalardo BL, Busch MP, Karlsson AC, Phelps BH, Alexander SS, et al. Seroreversion in subjects receiving antiretroviral therapy during acute/early HIV infection. *Clin Infect Dis*. 2006;1;42(5):700-8. https://doi.org/10.1086/500215

31. Spivak AM, Sydnor ERM, Blankson JN, Gallant JE. Seronegative HIV-1 infection: a review of the literature. *AIDS*. 2010;24:1407-14. https://doi.org/10.1097/QAD.0b013e32833ac65c

32. Granjeiro A, Ferraz D, organizadores. Centros de Testagem e Aconselhamento do Brasil: desafios para a equidade e o acesso [internet]. Série Estudos pesquisa e avaliação n. 11. Brasília: Ministério da Saúde, Secretaria de Vigilância em Saúde, Programa Nacional de DST e Aids; 2008 [access on: Feb 10, 2022]. Available: https://bvsms.saude.gov.br/bvs/publicacoes/centros_testagem_aconselhamento_brasil.pdf

33. Rezende ELLF, Vasconcelos AMN, Pereira MG. Causes of death among people living with HIV/AIDS in Brazil. *Braz J Infect Dis*. 2010;14(6):558-63. https://doi.org/10.1590/S1413-86702010000600003

34. Centers for Disease Control and Prevention. *Notice to readers*: Protocols for confirmation of reactive rapid HIV tests. [internet]. MMWR Weekly. 2004;53(10):221-22. [access on: Feb 23, 2022]. Available: https://www.cdc.gov/mmwr/preview/mmwrhtml/mm5310a7.htm

35. Centers for Disease Control and Prevention. *Technical update on HIV-1/2 differentiation assays*. [internet]. 2016. [access on: Feb 23, 2022]. Available: https://stacks.cdc.gov/view/cdc/40790

36. Manak MM, Jagodzinski LL, Shutt A, Malia JA, Leos M, Ouellette J, et al. Decreased seroreactivity in individuals initiating antiretroviral therapy during acute HIV infection. *J Clin Microbiol*. 2019;57(10):e00757-19. https://doi.org/10.1128/JCM.00757-19

37. Castejon MJ, Dordetto Priscila R. Yamashiro R, Brígido LFM, Alves A A, Oliveira CAF. Antiretroviral therapy in patient living with HIV leads negative HIV serological results. *J Bras Patol Med Lab*. 2021;57:1-6. https://doi.org/10.5935/1676-2444.20210057

38. Centers for Disease Control and Prevention. *Interpretation and use of the Western blot assay for serodiagnosis of human immunodeficiency virus type 1 infections*. [internet]. MMWR Suppl. 1989;38(7):1-7. [access on: Feb 23, 2022]. Available: https://www.cdc.gov/mmwr/preview/mmwrhtml/00001431.htm

39. O'Brien TR, George JR, Epstein JS, Holmberg SD, Schochetman G. *Testing for antibodies to human immunodeficiency virus type 2 in the United States*. [internet]. MMWR. Recomm Rep. 1992; 41(RR-12):1-9. [access on: Feb 23, 2022]. Available: https://www.cdc.gov/mmwr/preview/mmwrhtml/00038078.htm

40. Branson BM, Owen SM, Wesolowski LG, Bennett B, Werner BG, Wroblewski KE, et al. *Laboratory testing for the diagnosis of HIV infection*: Updated recommendations. [internet]. June 27, 2014. [access on: Feb 10, 2022] Available: http://dx.doi.org/10.15620/cdc.23447

41. Guarner J. Human immunodeficiency virus: Diagnostic approach. *Semin Diagn Pathol*. 2017; 34(4):318-24. https://doi.org/10.1053/j.semdp.2017.04.008

42. Owen SM. Testing for acute HIV infection: implications for treatment as prevention. *Curr Opin HIV AIDS*. 2012;7(2):125-30. https://doi.org/10.1097/COH.0b013e3283506613

43. Alexander TS. Human immunodeficiency virus diagnostic testing: 30 years of evolution. *Clin Vaccine Immunol*. 2016;23(4):249-53. https://doi.org/10.1128/CVI.00053-16

44. Nkengasong J , van Kerckhoven I, Carpels G, Vercauteren G, Piot P, van der Groen G. HIV screening and confirmation: a simplified and less expensive testing algorithm. *Ann Soc Belg Med Trop*. 1992;72(2):129-39.

45. Stone M, Bainbridge J, Sanchez AM, Keatinga SM, Pappasc A, Rountree W, et al. Comparison of detection limits of fourth- and fifth-generation combination HIV antigen-antibody, p24 antigen, and viral load assays on diverse HIV isolates. *J Clin Microbiol*. 2018;56(8):e02045-17. https://doi.org/10.1128/JCM.02045-17

46. Ministério da Saúde (BR). Secretaria de Políticas de Saúde. Coordenação Nacional de DST e Aids. Diretrizes dos Centros de Testagem e Aconselhamento (CTA): manual. Brasília; 1999. [access on: Feb 24, 2022] Available: https://bvsms.saude.gov.br/bvs/publicacoes/diretrizes_cta.pdf

47. Comparini RA, da Silva ET, Pereira DCR. Estratégias de ampliação do diagnóstico da infecção pelo Vírus da Imunodeficiência Humana no Brasil, 2015. *Com. Ciências Saúde*. 2017;28(2):158-67. https://doi.org/10.51723/ccs.v28i02.210

48. Ministério da Saúde (BR). Secretaria de Vigilância em Saúde. Programa Nacional de DST e Aids. *Manual de adesão ao tratamento para pessoas vivendo com HIV e aids*. [internet]. Série A. Normas e manuais técnicos. Brasília; 2008. n.84. 130p. [access on: Feb 24, 2022]. Available: https://bvsms.saude.gov.br/bvs/publicacoes/manual_adesao_tratamento_hiv.pdf

49. Ministério da Saúde (BR). Portaria SVS/MS nº 488, de 17 de junho de 1998. Padroniza, nos serviços de saúde, o conjunto de procedimentos sequenciados, com vistas a maximizar o grau de confiabilidade dos resultados dos testes para detecção de anticorpos anti-HIV, em indivíduos com idade acima de 2 anos. *Diário Oficial da União*. Jun 18, 1998; Section 1;114.

50. Okay TS, Granato CFH. O diagnóstico molecular da infecção pelo vírus da imunodeficiência humana (HIV-1) em crianças entre dois e 24 meses. *Rev Ass Med Brasil*. 2000;46(4):298-99.

51. Ministério da Saúde (BR). Portaria GM/MS nº 59, de 28 de janeiro de 2003. Estabelece a padronização dos procedimentos sequenciados para detecção de anticorpos anti-HIV no diagnóstico laboratorial de infecção por HIV em indivíduos com idade acima de 2 anos. *Diário Oficial da União*. Jan 30, 2003. Section 1;22.

52. Ministério da Saúde (BR). Portaria SVS/MS nº 34, de 28 de julho de 2005. Regulamenta o uso de testes rápidos para diagnóstico da infecção pelo HIV em situações especiais. Brasília: *Diário Oficial da União*. Jul 29, 2005. Section 1;145.

53. Ministério da Saúde (BR). Portaria SVS/MS nº 151, de 14 de outubro de 2009. Define o fluxograma mínimo de diagnóstico da infecção pelo HIV em indivíduos com idade acima de 18 (dezoito) meses. Brasília: *Diário Oficial da União*. Oct 16, 2009. Section 1; 198.

54. Elliott T, Sanders EJ, Doherty M, Ndung'u T, Cohen M, Patel P, et al. Challenges of HIV diagnosis and management in the context of pre-exposure prophylaxis (PrEP), post- exposure prophylaxis (PEP), test and start and acute HIV infection: a scoping review. *J. Int. AIDS Soc*. 2019;22:e25419. https://doi.org/10.1002/jia2.25419

55. Deeks SG, Overbaugh J, Phillips A, Buchbinder S. HIV infection. *Nat Rev Dis Primers*. 2015;1:15035. https://doi.org/10.1038/nrdp.2015.35

56. Deeks SG, Lewin SR, Ross AL, Ananworanich J, Benkirane M, Cannon P, et al. International AIDS Society global scientific strategy: towards an HIV cure 2016. *Nat. Med*. 2016;22(8):839-50. https://doi.org/10.1038/nm.4108

57. Bongertz V, Ouverney EP, Fernandez SC, Grinsztejn B, Veloso V, Couto-Fernandez JC, et al. Anti-human immunodeficiency virus type 1 humoral immune response and highly active antiretroviral treatment. *Mem Inst Oswaldo Cruz*. 2007;102(7):817-25. https://doi.org/10.1590/S0074-02762007005000119

58. Cohen MS, Chen YQ, McCauley M, Gamble T, Hosseinipour MC, Kumarasamy N, et al. Antiretroviral therapy for the prevention of HIV-1 transmission. *N Engl J Med*. 2016;375(9):830-39. https://doi.org/10.1056/NEJMoa1600693

59. Stefic K, Novelli S, Mahjoub N, Seng R, Molina J-M, Cheneau C, et al. Nonreactive human immunodeficiency virus type 1 rapid tests after sustained viral suppression following antiretroviral therapy initiation during primary infection. *J Infect Dis*. 2018;217(11):1793-97. https://doi.org/10.1093/infdis/jiy120

60. Stoffels K, Vanroye F, Mortier V, Debaisieux L, Delforge M-L, Depypere M, et al. Chronic and early antiretroviral therapy impact human immunodeficiency virus (HIV) serological assay sensitivity, leading to more false-negative test results in HIV diagnosis. *J Infect Dis*. 2020;222(10):1660-69. https://doi.org/10.1093/infdis/jiaa271

Historic

Received	Approved	Publication
06/17/2022	09/27/2022	10/18/2022

How to cite

Jorge Castejon M, Hernandes Granato CF, de Freitas Oliveira CA. Diagnóstico sorológico da infecção por HIV/aids no Brasil. Bepa [Internet]. 18º de outubro de 2022 [citado 30º de dezembro de 2022];19:1-39. Disponível em: https://periodicos.saude.sp.gov.br/BEPA182/article/view/37710

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GOVERNO DO ESTADO DE SÃO PAULO