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Update on diagnostic tests for human immunodeficiency virus infection in pregnant women

Actualización sobre las pruebas diagnósticas de la infección por el virus de la inmunodeficiencia humana en gestantes

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ABSTRACT

Early diagnosis and proper interpretation of tests to diagnose human immunodeficiency virus (HIV) infection are of great importance during pregnancy. It is necessary to know the characteristics of such tests in order to make timely and correct decisions. The aim of this article is to disseminate the perception and interrelation of the outcomes of these tests currently used and their organization in the national diagnostic algorithm.

Key words: Pregnancy complications, VIH infections, Diagnosis

RESUMEN

El diagnóstico precoz y la adecuada interpretación de las pruebas para diagnosticar infección por el virus de la inmunodeficiencia humana (VIH) son muy importantes durante la gestación. Para ello es fundamental conocer las características de tales ensayos de manera de tomar decisiones oportunas y correctas. El presente artículo tiene como propósito divulgar el entendimiento y la correlación de los resultados de las pruebas usadas actualmente y su organización en el algoritmo diagnóstico nacional.

Palabras clave. Complicaciones del embarazo, Infecciones por VIH, Diagnóstico

INTRODUCTION

Human immunodeficiency virus (HIV) infection is a major global public health problem⁽¹⁾. This retrovirus is transmitted by sexual and blood contact and vertically from an infected pregnant woman to the product of conception during gestation, delivery or breastfeeding⁽²⁾. The risk is higher in the last trimester, specifically between 36 and 40 weeks, and increases during delivery⁽²⁾.

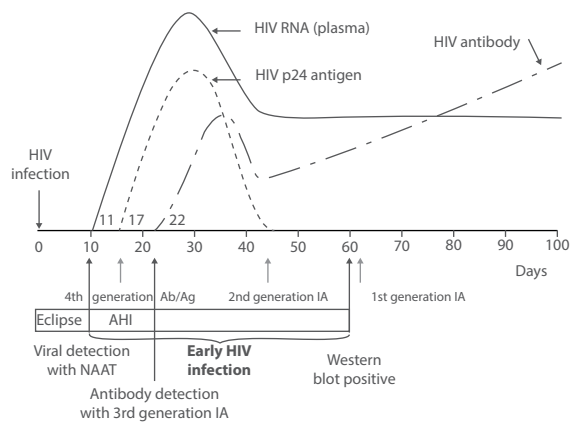
For a definitive diagnosis, the use of laboratory tests is indispensable, since no clinical manifestation is specific. There are no exclusive tests to detect this infection in pregnant women. The same tests applied in the general population are used. However, during pregnancy there are two singularities: the unavoidable need for early diagnosis to avoid vertical transmission and the higher frequency of non-specific reactions - false reagents or false positives - observed in the various tests used⁽³⁾.

For the correct interpretation of the assays, it is essential to know the natural evolution of viral replication, as well as the HIV antigens and the antibodies directed against these antigens in the different phases of the disease⁽⁴⁾. Figure 1 shows the serological evolution of HIV antigens, antibodies and viral RNA and their relationship with the various diagnostic tests during the first 100 days of infection.

After primary infection there is a window period that varies according to each assay, during which there is no possibility of detecting specific antibodies despite the existence of very high viraemia and cytotoxic activity against HIV. This suggests that cellular immunity is earlier and more important in the initial control of virus replication⁽⁴⁾.



FIGURE 1. SEROLOGICAL EVOLUTION OF HIV ANTIGENS, ANTIBODIES AND RNA AND THEIR RELATIONSHIP WITH VARIOUS DIAGNOSTIC TESTS DURING THE FIRST 100 DAYS OF INFECTION. SOURCE: HUGO AND MAINROAD, MELBOURNE, AUSTRALIA. 2023. AVAILABLE AT: [HTTPS://RAPIDHIVTESTING.COM.AU/HOW-DOES-THE-HIV-TEST-WORK/](https://RAPIDHIVTESTING.COM.AU/HOW-DOES-THE-HIV-TEST-WORK/)



Antibodies are produced on average 4-6 weeks after infection, although in some cases detectable presence may take up to 3-6 months after exposure⁽⁵⁾. Clinically, 50-90% of people present with symptoms of acute retroviral syndrome, such as fatigue, fever, skin rash, and generalized lymphadenopathy, which appear between the second and fourth week after infection⁽⁶⁾. This is phase 1 or acute infection.

Humoral and cellular immunity control virus replication after primoinfection, achieving a balance reflected in the basal viral load (VL) of enormous prognostic value. But this immune response is not sufficient to eradicate the virus and only limits its replication. This initiates a chronic infection that persists for years, which is evidence of the degree and chronicity of virus replication and the capacity of the immune system to control it for long periods. The persistence of immunosuppression and destruction of CD4 lymphocytes by HIV in the medium or long term leads to immunodeficiency, shifting the virus-host balance towards accelerated viral replication and profound immunosuppression⁽⁴⁾. This is phase 2 or chronic infection.

The final stage - phase 3 or acquired immunodeficiency syndrome (AIDS) - is clinically characterized by the appearance of opportunistic infections (OIs)⁽⁷⁾, immunologically by a decrease in the number of CD4 lymphocytes and virologically because the viral load is elevated. Both humoral and cellular responses deteriorate, as anti-p24 antibodies and against other virus pro-

teins are reduced, the quantity of neutralizing antibodies, cytotoxic action and the number of CD8 lymphocytes decline, and the activity of cellular cytotoxicity that depends on antibodies (ADCC) and natural killer (NK) cells deteriorates. All this evidence the massive destruction of the immune system by accelerated viral replication and the appearance of more aggressive mutant variants. This leads to a vicious circle due to progressive immune deterioration, as it allows a more intense replication⁽⁴⁾.

CLASSIFICATION AND INTERPRETATION OF DIAGNOSTIC TESTS

HIV types 1 and 2 are detected. The former is common throughout the world and the latter mainly in African countries. HIV-2 progresses with less morbidity and mortality than HIV-1. The AIDS phase occurs many years later and vertical transmission is lower than that of HIV-1 (10-40%)⁽⁸⁾.

No test is perfect; they all have limitations. Even if they are performed under optimal conditions, when standardized in vitro, the results can be false reactive and non-reactive and false positive and negative. However, the continuous advancement of technology optimizes existing tests and allows the emergence of new diagnostic assays with improved performance. Fifth-generation tests^(9,10) and the use of nanotechnology for HIV diagnosis are already described⁽⁹⁾. The efficiency of diagnostic tests is quantified by⁽¹¹⁾:

- Sensitivity, which relates to the number of infections that are not detected by the test.
- Specificity, which is the proportion of uninfected individuals who have a negative test.
- Positive predictive value, i.e. how many of the positive results are true positives.
- The negative predictive value, which refers to how many of the negative results are true negatives.

As mentioned above, all diagnostic tests have their own window period⁽¹¹⁾. To ensure the reliability of such tests, it is essential that each clinical laboratory has a quality assurance system, including verification of the organizational structure, daily application of internal and external



quality controls, ongoing staff training, preventive maintenance of equipment and biosafety⁽¹²⁾. Diagnostic tests are subdivided into two groups:

SCREENING TESTS

They are highly sensitive (99-100%) and adequately specific. None can detect the virus immediately after infection⁽¹⁰⁾. False reactive and non-reactive results can occur⁽¹³⁾, as will be described later. Knowledge of the serostatus of each patient's partners is also essential⁽¹²⁾, particularly in the case of a pregnant woman living in high-prevalence settings, whether these partners are regular or occasional⁽¹⁴⁾.

In these settings, the World Health Organization (WHO) recommends repeat testing in the third trimester, during labor or immediately after delivery, due to the high risk of HIV infection⁽¹⁴⁾. Similarly, HIV-negative mothers who are breastfeeding should be retested periodically during breastfeeding because of the possibility of contracting HIV and transmitting it through breast milk. Detecting infection at an early stage will allow immediate interventions and prevent transmission to the child⁽¹⁴⁾.

Screening tests are reported as reactive and non-reactive. The following tests are available⁽¹³⁾:

- Rapid tests (RT): they are characterized by obtaining immediate results in a few minutes, so in recent years they have become very important in the diagnosis of HIV⁽¹⁵⁾ in pregnant women, as they allow diagnosis from the first prenatal visit⁽¹¹⁾. The most widely used method is immunochromatography.

There are currently three versions on the national market: a) RT that only detect antibodies against HIV; b) RT that simultaneously detect antibodies against HIV and syphilis, known as dual HIV/syphilis rapid tests (HIV/Syphilis DRTs)⁽¹⁶⁾; and c) RT that detect antigens - generally p24 - and antibodies against HIV, which implies a shorter window period. None of them is applicable in pregnant women receiving antiretroviral treatment (ART)⁽¹⁶⁾.

A nonreactive result rules out HIV infection, unless a cause for a false nonreactive result is identified, which are described later. A reactive result requires following the provisions of the

Technical Health Standard No. 159-MINSA/2019/DGIESP (NTS No. 159)⁽¹⁷⁾.

RTs that only detect anti-HIV antibodies have a sensitivity similar to the enzyme-linked immunoadsorption assay (ELISA) -100%-⁽¹⁸⁾, but their specificity is lower⁽¹⁹⁾. Those that simultaneously detect antigens and antibodies have a sensitivity of 100% and a specificity of up to 96%⁽⁹⁾.

- Enzyme-linked immunoadsorption assay (ELISA): There are third-generation tests that only detect anti-HIV antibodies and fourth-generation tests that detect antigens -usually p24- and anti-HIV antibodies. In both cases they do not discriminate the type of immunoglobulin detected, but rather in a combined manner -IgM/IgG-, so it is not possible to establish whether the infection is recent or late⁽²⁰⁾. In general, their specificity is higher than that of the rapid tests, but lower than that of the confirmatory tests⁽²¹⁾.

The former are reactive as early as three weeks after infection^(9,21), and by 12 weeks after primary infection virtually all cases are reactive⁽²¹⁾. They remain so for life, except for some cases in the late phase of infection with intense immunodeficiency and great reduction of antibodies, or due to the early introduction of ART⁽¹¹⁾.

The fourth-generation tests have a high sensitivity (100%) and specificity (99%), which reduces the window period⁽⁵⁾, and can be reactive as soon as two weeks after infection⁽²¹⁾; the vast majority are reactive within 6 weeks of exposure⁽²²⁾.

A nonreactive result rules out HIV infection, unless there is a nonspecific cause of reaction, which is noted below. A reactive result will be ratified by a confirmatory test⁽¹⁷⁾. Pregnancy is among the causes of false reactive results, particularly significant in societies with low HIV incidence, i.e. pregnant women in these populations are at higher risk for false reactive results⁽⁸⁾; multiparous women have a higher rate of these nonspecific reactions⁽¹¹⁾.

- Chemiluminescent microparticle immunoassay (CLIA)

This is an immunoassay of chemiluminescent microparticles - CLIA microparticles - to qualitatively determine antigens and anti-HIV an-



antibodies in human serum and plasma. In general, it has a sensitivity of 100% and specificity greater than 95%. This assay is not considered in the HIV diagnostic algorithm of NTS No. 159⁽¹⁷⁾.

- Electrochemiluminescence immunoassay (ECLIA)

Qualitatively determines in human serum and plasma HIV-1 p24 antigen and anti-HIV-1 antibodies, including group O and anti-HIV-2 antibodies. The p24 antigen can be detected in blood as early as 2-3 weeks after infection and anti-HIV antibodies approximately 4 weeks after infection. The combined detection of p24 antigen and anti-HIV antibodies by fourth generation ECLIA tests improves sensitivity and reduces the window period compared to traditional anti-HIV assays. This assay is not considered in the HIV diagnostic algorithm of NTS No. 159⁽¹⁷⁾.

- p24 antigenemia

Some years ago, highly specific tests that only detected the p24 antigen were used, but their sensitivity was not optimal⁽²³⁾, presenting false non-reactivity; for this reason, their use decreased progressively.

Do not use screening tests in HIV-exposed newborns, but only in children older than 18 months without complete results by qualitative polymerase chain reaction (PCR-DNA-HIV) or with negative results, but when there is a risk of transmission through breastfeeding or death or unknown whereabouts of a mother whose child has not completed screening and/or follow-up⁽¹⁷⁾.

CONFIRMATORY TESTS

They are used in patients who have reactive screening assays, except in infants under 18 months of age, as detailed below. They are performed on blood or plasma and identify the presence of specific anti-HIV antibodies or directly detect the virus or any of its components. They are highly specific - greater than 99.5% - and practically exclude false positives. Their results prevail over those obtained in screening tests and are reported as negative or positive,

although some may be indeterminate. Only in the case of VL is the response quantitative. There are four commonly used methods:

- Indirect immunofluorescence (IIF)

With similar sensitivity and specificity to the Western Blot (WB) test⁽²⁴⁾, it can be positive before the WB⁽²⁵⁾, it is cheaper and has a shorter execution time due to the use of a relatively simpler technique⁽²⁶⁾. For this reason, it has relegated the WB and at the national level it currently represents 95% of confirmations⁽²⁷⁾. A positive result definitively diagnoses HIV infection; if it is negative, it is not an infection, unless there is continuous and repeated exposure. If it is indeterminate, repeat the test three to six months after the first test⁽¹⁷⁾.

- Western blot (WB)

This test basically corroborates the indeterminate results of the IIF. Methodologically, it separates the viral antigens obtained from the purified culture of HIV-1, which are distributed in nine specific bands -gp160, gp120, gp41, p66, p55, p51, p31, p24 and p17-⁽²⁴⁾, which are confronted with the specific antibodies found in the patient's serum⁽²⁸⁾; it has a sensitivity of 100%⁽²⁶⁾ and a specificity of up to 99%⁽¹¹⁾. The interpretation criteria used in Peru are those established by the CDC (Centers for Disease Control and Prevention) of the USA, which consider the test positive when p24 + (gp160, gp120 or gp41) or p41 + (gp160 or gp120) are present. A positive result corroborates HIV infection⁽¹⁷⁾, a negative result rules it out, except in the case of recent and repeated exposure to infection. When the result is indeterminate and only a few bands are shown that do not meet CDC criteria, retesting is advised after three to six months, depending on risk factors⁽¹⁷⁾.

If after six months the WB remains indeterminate (as in IIF), it would rarely be a genuine HIV infection. In this case, false positives may be due to infection by other retroviruses (HTLV-I and HTLV-II), interference by rheumatoid factor, high bilirubin values, multitransfused, presence of HLA antibodies, and autoimmune diseases such as systemic lupus erythematosus, among others^(19,24).



A limitation of WB is the different diagnostic predictive value of each of the bands. Antibodies against HIV envelope proteins are more specific, but false positives are also described⁽¹⁹⁾.

- Qualitative polymerase chain reaction (PCR DNA HIV-1)

This is an assay primarily intended for HIV-exposed children under 18 months of age, with the aim of finding out early whether they are infected with HIV. Screening tests cannot discriminate until after that age whether it is the child's own infection or passive transmission of antibodies from the mother⁽¹⁷⁾. It is not used in pregnant women, except in some cases of serodiscordant couples⁽¹⁷⁾.

- Viral load for HIV (VL)

These are high-cost assays that determine the amount of HIV circulating in the blood. They allow early diagnosis, since detection is usually made within a few days of infection, an advantageous feature compared to other tests that can take weeks to months, and is also useful for determining the effects of ART⁽¹⁾. The method of choice for measuring VL is real-time reverse transcription polymerase chain reaction (RT-qPCR)⁽²⁹⁾, whose sensitivity allows it to detect up to 1 copy/mL of viral RNA⁽¹¹⁾ and to make a diagnosis as early as 6-8 days after exposure⁽³⁰⁾.

WHO recommends periodic follow-up in the general infected population: the first test immediately after diagnosis, the second six months after starting ART, and annual testing thereafter. VL suppression is defined as a count of less than 1,000 copies/mL at least six months after starting first-line ART⁽³¹⁾. Follow-up in pregnant women has different guidelines, as will be discussed below⁽¹⁷⁾.

The CDC considers VL to be durably undetectable when it remains negative for at least six months after the first undetectable result. Under these conditions, the patient is virally suppressed and has no risk of transmission to an HIV-negative person. The degree of VL reduction after ART initiation provides prognostic information about the likelihood of disease progression⁽³¹⁾. An unsuppressed VL count - greater than 1,000 copies/mL - in patients re-

ceiving ART occurs when therapy fails to suppress it and is associated with an increased risk of morbidity, mortality, and HIV transmission; it suggests that the virus is resistant to current ART⁽³¹⁾.

As mentioned above, diagnostic tests have certain limitations expressed as false reactive and non-reactive, false positive and false negative.

FALSE NON-REACTIVE AND FALSE NEGATIVES

It is when a person infected with HIV has non-reactive or negative tests, an increasingly infrequent occurrence due to the high sensitivity of current tests. The main causes are⁽¹¹⁾:

- Window period, which is the time between HIV infection and the time when the immune system produces detectable antibodies with the tests. Fourth-generation tests reduce this period because they also detect antigens. The immune response depends on the individual, with a wide range of responses described. In this period patients are highly infectious, and each test has a specific window period.
- Certain pathological processes, such as altered B-lymphocyte function, terminal stages of AIDS and chronic diseases that cause immune collapse.
- Immunosuppressive therapy.
- Laboratory errors.

FALSE REACTIVE AND FALSE POSITIVE

Occurs when a non-HIV infected individual tests reactive or positive. It is more frequent with rapid tests and infrequent with confirmatory tests. The various causes can be classified into those that depend on the patient's condition and others on the diagnostic methodology:

- Patient's own conditions

Cross-reactions resulting from interactions with molecules present in blood, as in hypergammaglobulinemia, recent vaccinations against hepatitis B, rabies or influenza, antibodies with similar characteristics to the anti-HIV ones but against other infectious agents such as the HTLV-I and HTLV-II retroviruses, in certain he-



matological neoplasms -plasmacytoma- or in autoimmune diseases, the most common one, systemic lupus erythematosus^(11,24).

Gestation can also cause cross-reactions since the placenta normally contains molecules similar to HIV antigens^(11,24). On the other hand, some researchers argue that this type of interference is due to the presence of circulating alloantibodies, as in the case of polytransfused patients, transplant recipients and carriers of autoimmune diseases⁽³²⁾.

However, in pregnancy the mechanism postulated to explain the appearance of alloantibodies, which in this case are of the anti-leukocyte type - directed against the white blood cells of the fetus - is particularly frequent in multiparous women. This is due to the fact that in normal gestation, as a consequence of the immunological adaptation to the presence of the fetus there is a deviation towards the Th2 cytokine response that unbalances the balance with the Th1 cytokine, favoring the production of antibodies against fetal antigens inherited from the father, which in some cases can have a protective effect on pregnancy (anti-HLA antibodies) and, in others, lead to fetal or neonatal cytopenia or pregnancy loss (specific antibodies against blood cells, anti-phospholipid antibodies). It is in this context that the alloantibodies responsible for the highest rate of false-reactive and false-positive tests in pregnant women compared to the general population are synthesized⁽³³⁻³⁵⁾.

The research by Chao et al. found that in young, nulliparous pregnant women there was a higher probability of false positives in ELISA assays than in the general population. They also mention that these non-specific results are less frequent with rapid tests than with ELISA⁽³²⁾. These empirical findings still lack adequate explanation.

- Clinical laboratory-dependent conditions

These include the quality of the blood samples -failure to collect or identify them, bacterial contamination or inadequate preservation-, the quality or generation of the test used, which confers low sensitivity and/or specificity, and failure to comply with the protocol established for the execution of the test by the manufacturer of the reagent.

NATIONAL ALGORITHM FOR DIAGNOSING HIV INFECTION IN PREGNANT WOMEN

The algorithm for diagnosing HIV infection in pregnant women currently used in our country is contained in NTS No. 159, which refers to the prevention of mother-to-child transmission of HIV, syphilis and hepatitis B. It establishes the processes for screening, early diagnosis and standardization for the application of rapid tests at the same point of care⁽¹⁷⁾. This establishes the processes for screening, early diagnosis, and standardization for the application of rapid tests at the same point of care⁽¹⁷⁾.

Its scope of application includes public, private and mixed health care institutions⁽¹⁷⁾. Figure 2 shows, in a generic way, the main components of this national algorithm.

For the purposes of initiating ART, an HIV-infected pregnant woman is one in whom one of the following situations is present^(13,17):

- a. Two third-generation HIV rapid tests with reactive results, performed in different clinical laboratories.
- b. One third-generation HIV rapid test and one fourth-generation HIV rapid test with reactive results.
- c. One third or fourth generation HIV rapid test and one third or fourth generation ELISA assay with reactive results.
- d. A reactive HIV rapid test with a positive confirmatory test result (VL or IIF).

A pregnant woman who meets one of the four criteria should have a blood sample taken immediately for IIF and VL testing, without delaying the initiation of ART⁽¹⁷⁾. In pregnant women who have already started ART and in whom the results of the follow-up VL or other confirmatory test are negative, treatment will be stopped and the HIV team responsible for the facility will be informed⁽¹⁷⁾. Follow-up VL will be performed six weeks after initiating or modifying ART and then quarterly during pregnancy. In the last trimester, a VL will be obtained four weeks before the expected date of delivery (EDD)⁽¹⁷⁾.



The route of delivery will be elective cesarean section if VL results are not available at least four weeks prior to the EDD or if the VL is greater than 1,000 copies/mL⁽¹⁷⁾. Pregnant women whose VL results are less than 1,000 copies/mL four weeks prior to EDD may terminate the pregnancy vaginally, with intravenous zidovudine (AZT) at the dose and time recommended in this NTS, regardless of the ART regimen received⁽¹⁷⁾.

If the couples are serodiscordant, where the pregnant woman is negative and the sexual partner is positive, the recommendations are⁽¹⁷⁾:

1. Screen the pregnant woman/mother every trimester and before sexual exposure to HIV.
2. If the serodiscordant pregnant woman could be in the window period or presents symptoms of acute retroviral syndrome, the PCR DNA HIV-1 will be performed and the VL will be determined, because during the syndrome mother-to-child transmission is intense.

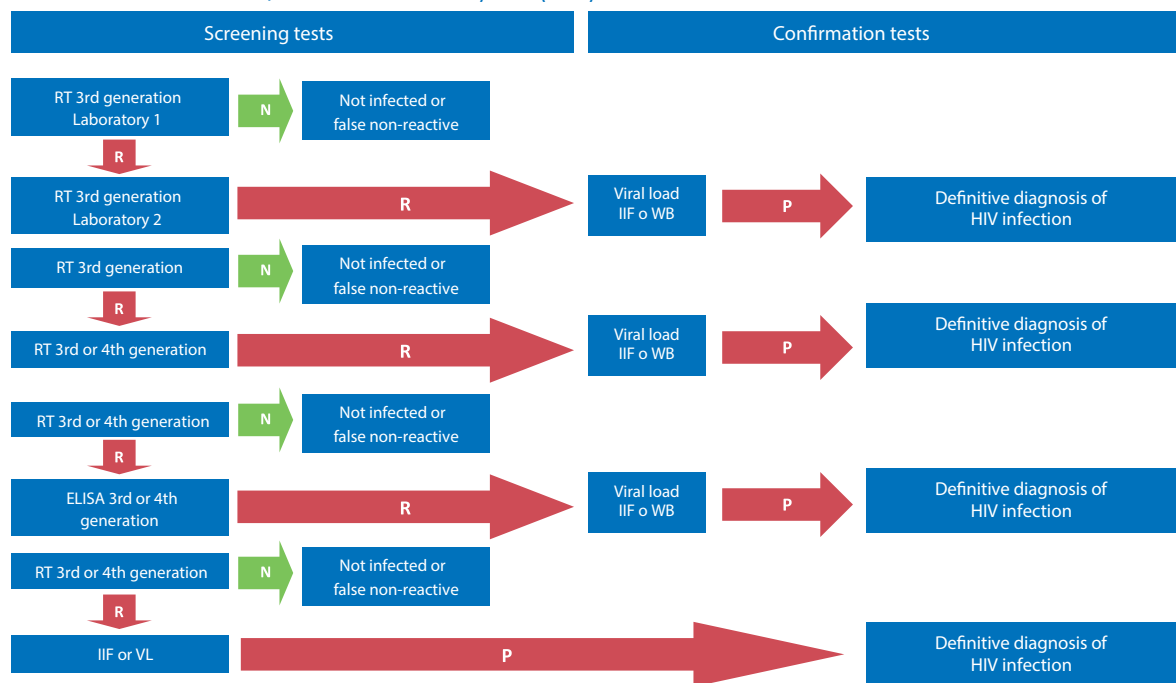
Once HIV infection has been ruled out in the serodiscordant pregnant woman, pre-exposure prophylaxis should be initiated according to the aforementioned standard⁽¹⁷⁾.

CONCLUSIONS

In our country there is a wide variety of assays to diagnose HIV infection, some of which are highly efficient for such diagnosis, but tests of controversial quality are also marketed. It is the responsibility of each clinical laboratory to adequately choose the assays for this purpose. The reliability of the tests and their results depends on that choice, coupled with the ongoing implementation of quality assurance systems. The responsibility of the treating physicians is limited to knowing their correct interpretation, on the basis of which timely and appropriate decisions will be made in the case of each pregnant woman.

Such tests should be organized under the logic of the current national algorithm which, according to the National Reference Laboratory for Sexually Transmitted Virus HIV/AIDS, a component of the National Public Health Center of the National Institute of Health, has a high reliability for detecting HIV infection in persons older than 18 months⁽¹³⁾. For those younger than that age, HIV-1 DNA PCR is used, which also takes into account the aforementioned algorithm and is also very reliable. As with any algorithm,

FIGURE 2. DIAGRAM OF THE ALGORITHM FOR THE DIAGNOSIS OF HIV INFECTION. SOURCE: NORMA TÉCNICA DE SALUD N° 159 PARA LA PREVENCIÓN DE LA TRANSMISIÓN MATERNO INFANTIL DEL VIH, SÍFILIS Y HEPATITIS B (TECHNICAL HEALTH STANDARD N° 159 FOR THE PREVENTION OF MOTHER-TO-CHILD TRANSMISSION OF HIV, SYPHILIS AND HEPATITIS B). LIMA (PERU): MINISTRY OF HEALTH. 2019.





it should be periodically reviewed by the competent technical authority in order to update it with respect to advances in technology.

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