14 Laboratory diagnosis of HIV infection

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Laboratories can reliably deliver accurate human immunodeficiency virus (HIV) diagnoses in almost all specimens, except during acute HIV infection. In addition, the diagnosis of opportunistic infections, sexually transmissible infections, haematological abnormalities, such as unexplained thrombocytopenia or histological diagnosis of cancers, such as Kaposi's sarcoma or a lymphoma, should prompt the laboratory to advise the clinician to consider HIV as a diagnosis.

Introduction

It is well established now that 20-80% of the people in different parts of the world who have HIV infection do not know their HIV status.[1] It is therefore important to make use of every opportunity to offer to test people who are unaware of their status. Laboratories can play an important role in managing testing procedures by having clear and detailed test request protocols (e.g. provide relevant clinical information on forms), and by carefully evaluating all HIV test results according to the most up-to-date diagnostic protocols.[2]

HIV serology

Most people develop antibodies against HIV that are detectable by standard enzyme-linked immunoassays (ELISA) within 30 days after infection, although some seroconvert later. The vast majority of people (99%) have detectable antibodies by three months after HIV infection.[3] The 'window period' is the time it takes for a person who has acquired HIV infection to react to the virus by creating HIV antibodies.

The average window period to detect seroconversion using HIV-1 antibody tests is 22 days for subtype B. Antigen testing shortens the window period to approximately 16 days and nucleic acid amplification testing (NAAT) further reduces this period to 12 days.[3] During the window period, a person with HIV infection can transmit HIV to others although their HIV infection may not be detectable with an antibody test.

All diagnostic tests have limitations. False-positive results occur when a test is reactive, but the person does not really have HIV infection. False-negative results occur when a test is non-reactive, but the person actually has HIV infection. An HIV diagnosis should be based upon the outcome of two or more tests. However, when two test results disagree (i.e. one is reactive, the other non-reactive), the finding is said to be discordant. In this case, a third test or a test using another platform (e.g. NAAT for viral load) should be performed.

ELISA: the first generation of HIV testing

ELISA tests are usually the first HIV screening tool. A positive ELISA test result is usually observed within 3-6 weeks following infection. Very rarely, antibodies may develop up to 12 weeks after infection. Beyond the window period, ELISA tests are rarely false negative. This means if the patient has a negative test result, and is beyond the window period after the last potential exposure, the test is truly negative. An ELISA test may rarely be false positive.

False-positive ELISA results can occur in the presence of other auto-antibodies, hepatic disease, influenza vaccination and an acute viral infection, as well as from laboratory errors of procedure and specimen handling. For these reasons, positive ELISA results should always be followed by confirmatory tests.

Western blot: the confirmatory test

The Western Blot (WB) is a confirmatory test: it is only performed if an ELISA or rapid test is positive. The WB can be positive, negative or indeterminate. If no viral bands are detected, the result is negative. If at least one viral band for each of the GAG, POL, and ENV gene-product groups is present, the result is considered positive. In certain circumstances in which a few viral bands are detected but not enough to confirm infection, the result will be considered as indeterminate.

A person who has an indeterminate result should be retested, as later tests may be more conclusive. Almost all persons with HIV infection with indeterminate Western-Blot results will develop a positive result when retested one month later; persistently indeterminate results over a period of six months suggest the results are due to cross-reaction with other antibodies and do not represent true HIV infection.

Rapid testing

Rapid tests have become popular in resource-limited, remote or field settings for HIV diagnosis. These tests can be carried out with minimal training, and do not require expensive laboratory equipment for testing or biohazardous reagent disposal.[4] The tests also play a valuable role in situations where a test result is urgently required, such as testing of a source patient after a needle-stick injury and pregnant women in labour.

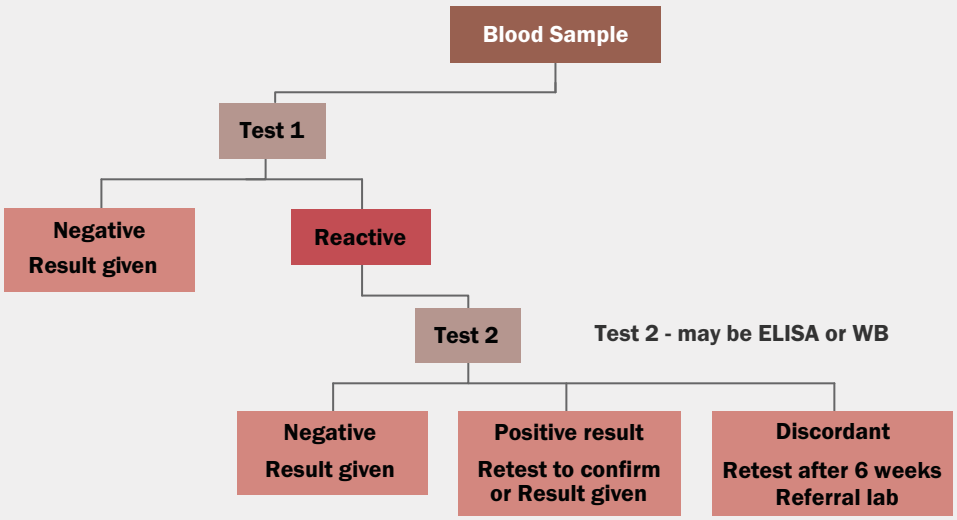
Presently non-reactive (negative) results may be reported on the result of a single test, but reactive test results must be confirmed through standard serological testing. For intrapartum women, repeat rapid testing with other test kits may be more appropriate in order to obtain a result before delivery. Currently a number of rapid screening test kits are available; four FDA-approved tests are summarised in Table 14.1.

Table 14.1: US Food and Drug Administration (FDA)–approved rapid HIV antibody tests for HIV-1 detection[5]

Rapid HIV test	Specimen type	Sensitivity†	Specificity†
OraQuick® Advance Rapid HIV-1/2	Oral fluid	99.3% (98.4–99.7)	99.8% (99.6–99.9)
Antibody	Whole blood (finger stick or venipunctures)	99.6% (98.5–99.9)	100% (99.7–100)
	Plasma	99.6% (98.9–99.8)	99.9% (99.6–99.9)
Reveal™ G-2 Rapid HIV-1 Antibody	Serum	99.8% (99.5–100)	99.1% (98.8–99.4)
	Plasma	99.8% (99.5–100)	98.6% (98.4–98.8)
Uni-Gold Recombigen® HIV	Whole blood (finger stick or venipuncture)	100% (99.5–100)	99.7% (99.0–100)
	Serum and plasma	100% (99.5–100)	99.8% (99.3–100)
Multispot HIV-1/HIV-2 Rapid	Serum	100% (99.94–100)	99.93% (99.79–100)
	Plasma	100% (99.94–100)	99.91% (99.77–100)

† 95% CI
Modified from Health Research and Education Trust.
Available at http://www.hret.org/hret/programs/hivtransmrpd.html

An algorithm using rapid tests for HIV diagnosis is presented below (Figure 14.1):



Source: WHO/CDC rapid testing guidelines 2004 [6]

ELISA: enzyme-linked immunoassay
WB: Western Blot

Figure 14.1: Algorithm using rapid tests for HIV diagnosis

However, note that the predictive value of tests depends on prevalence of infection.Table 14.2 outlines the predictive values based on a test which has a sensitivity and specificity of 99%.

Table 14.2: Predictive values for rapid HIV tests[6]

HIV prevalence	0.1%	1%	5%	10%	30%
NPV single test	100%	100%	99.9%	99.9%	99.6%
PPV single test	9%	50%	84%	92%	98%
PPV two tests	91%	99%	99.8%	99.9%	100%

NPV: Negative Predictive Value
PPV: Positive Predictive Value

For example, in a country where the population prevalence is estimated at 1%, a test with a sensitivity and specificity of 99% would only be 50%, i.e. 50% or half the reactive tests will be false positives. Two tests are required to achieve a positive predictive value (PPV) of 99%.

Nucleic Acid Amplification Test (NAAT): shortening window period and early infant diagnosis

NAAT is used to detect the presence of genetic material of the HIV virus. Various PCR assays have been designed to detect the highly conserved region of the HIV GAG gene. These assays are highly sensitive and meant to be used for early detection. For example, since 2001, donated blood in the USA has been screened with nucleic-acid-based tests, shortening the window period between infection and detectability of the virus genome to about 12-15 days. DNA and RNA tests for HIV may function as qualitative diagnostic assays that demonstrate infection, or quantitative detection systems that measure the level of circulating copies of HIV nucleic acid for prognostic or therapeutic monitoring (viral load tests).

Nucleic acid tests are also useful for resolution of cases where serological tests are inconclusive (indeterminate) and the diagnosis of HIV infection in newborns. Qualitative HIV DNA PCR tests detect proviral DNA that has been integrated in cellular DNA of the host. HIV antibody tests are not helpful in infants due to the persistence of maternal antibodies for up to the first 15 months of life. Peripheral blood mononuclear cells are recovered from whole blood from the patient, from which DNA is extracted and PCR is performed.

Most newborns with HIV infection are identified from birth within a 4-6 week post-partum period using HIV DNA PCR. Some newborns with the infection may not be detected at the time of birth reflecting the time of transmission which occurred from the HIV-positive mother. In utero infection is suspected when a newborn has a detectable DNA PCR result at 48 hours after birth, whereas transmission during labour and delivery or breast-feeding is detected 2-12 weeks later. Because of the importance of initiating therapy as early as possible, DNA PCR testing is recommended within the first 3 months to identify infants who would greatly benefit from treatment.

Venipuncture in children is not easy particularly if they are unwell; and the procedure requires an experienced phlebotomist. More recently the use of dried blood spots has been successfully applied to qualitative DNA PCR tests. This alternate specimen collection method is more acceptable in the form of capillary blood dried on specimen collection paper (Whatman#903), allowed to dry overnight, packaged and transported to a reference laboratory for testing (Figure 14.2). This specimen type has greatly improved access to early infant HIV DNA testing in remote resource-constrained settings.

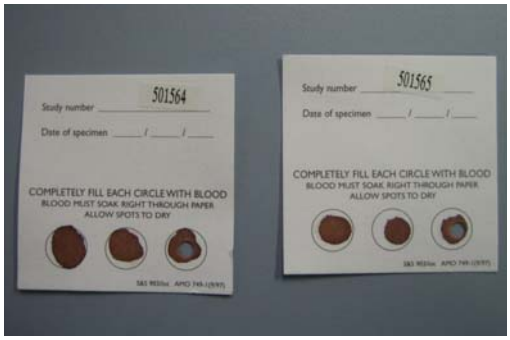


Figure 14.2: Dried blood spot specimens suitable for HIV DNA PCR testing of infants born to HIV-seropositive mothers.

HIV p24 Antigen test

p24 antigen testing may be used to help diagnose early HIV infection. Levels of p24 antigen increase significantly at about one to three weeks after initial infection. It is during this time frame before HIV antibody is produced when the p24 test is useful in helping to diagnose infection. About 2-8 weeks after exposure, antibodies to HIV are produced and remain detectable in response to the infection, making the HIV antibody test the most useful assay to diagnose an infection.

Laboratory quality assurance

Measures to ensure quality must be applied to all tasks and procedures conducted before, during and after the performance of each laboratory analytical procedure.

Inaccurate results caused by technical or transcriptional errors are preventable and an effective quality system can eliminate these errors.[4,7]

The term Quality System encompasses all quality measures related to the entire testing system (pre-analytical, analytical and post-analytical phases of testing). All steps from the correct labelling and identification, requesting of correct tests, appropriate specimen containers, appropriate transport to laboratory, correct receipt and registration procedures once the sample is received by the laboratory can influence the quality of what enters the testing process.

Once specimens enter the testing process factors such as reagents and test methods are important. Having and using appropriate quality controls, assay acceptance or rejection validity controls, worksheets and forms to minimise transcription errors, equipment calibration and performance, testing conditions such as incubation times, temperatures, humidity, power and water quality are all critical to quality output. Post analytical procedures include how results and records are managed to ensure traceability; reporting of results in a clear and unambiguous manner to ensure correct interpretation. Archiving and long-term result management may also be important.

The Quality System includes the management of both administrative and technical aspects of the laboratory. Regardless of the size or scope of the laboratory or whether in voluntary counselling and testing (VCT) clinical settings in the context of rapid tests, many of the elements of the laboratory quality system should still apply to reduce sources of error, contamination, controlled environment and traceable records and results.

Case study 14.1

A 28-year-old healthy male presents for HIV testing to his local VCT centre.

An initial rapid test yields a weakly reactive result (faint band on Determine® test)

This is followed by a second test from a different manufacturer, which yields a negative result.

- What is your interpretation?
- What is your further diagnostic approach?
- What do you tell the patient?

A number of possibilities need to be considered in the context of rapid tests and, particularly, performance of rapid tests in non-laboratory settings.

Any weakly reactive (note the terminology 'reactive' not 'positive') result should be interpreted with caution particularly in the absence of clinical or history risk assessment or without understanding the prevalence of HIV in the population being tested.

Conventional immunoassays (e.g. ELISA or chemiluminescent formats) involve carefully controlled reactions to optimally complex antibody - antigen test components. Reactions generally reach a steady state of equilibrium where the antibody in the patient's serum binds to the antigen in the tests to form a stable complex which is in turn detected.

Rapid tests or, more appropriately termed, short incubation tests do not reach a steady state of equilibrium and are usually read while the test is still in a dynamic stage of antibody-antigen complex reaction. Because of this, reactivity may be variable and more likely to be affected by environmental factors which are known to drive immunological reactions, such as, most commonly, temperature. Performance of rapid tests in uncontrolled environments may give rise to variable results such as ambient temperature being too high (common in tropical countries) and when the reactions are not adequately timed by the use of calibrated timing devices. Other variables may include reader interpretation, volume and type of sample applied to the test, reagent stability such as inappropriately stored tests, expiry date and variable manufacturer production batch.

The World Health Organization (WHO) recommends the use of a second test which has been evaluated and chosen to complement the first test usually of higher specificity than the screening test.

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Case study 14.1 (Continued)

The positive predictive value (PPV) of tests performed on individuals from populations with high HIV prevalence will be greater than that from a low prevalence population. Concordance on both screening and second test in appropriately chosen tests will greatly improve the PPV of the final test result. This scenario highlights the importance of using validated testing strategies to confirm true from false reactivity in HIV diagnostic testing. In any case, if the result of the test is unexpected or simply to confirm the initial result, a second sample of venous blood should be collected and sent for conventional HIV antibody testing in a reference laboratory.

Weakly reactive test results may be observed in early seroconversion in individuals reporting high risk exposures 3-4 weeks before testing. Seroconversion involves the development of antibodies in response to exposure to the virus. The primary care provider may be alerted to clinical evidence of acute retroviral syndrome which may include some or a number of symptoms such as fevers, non-exudative pharyngitis, adenopathy, malaise arthralgias, myalgias, headache featuring retro-orbital pain and photophobia, maculopapular erythematous rash and occasionally mild gastrointestinal symptoms.

The presence of these symptoms which may persist for up to 3 weeks may be less obvious in individuals from different racial backgrounds. In this case, the discordance in the two tests may be due to a difference in the sensitivity (limit of detection) of one test over another.

Case study 14.2

An infant is delivered by normal vaginal delivery to a woman given a short-course of nucleoside reverse transcriptase inhibitors (NRTIs) as prophylaxis during the third trimester of pregnancy. The mother has agreed to formula-feed her baby. She is anxious to know the HIV status of her child.

- What test(s) do you order?
- How will you advise the mother?

There are many benefits to determining the HIV status as early as possible in exposed infants. Antiretroviral therapy, adjunctive therapy and prophylaxis for opportunistic infections can be initiated and evaluated during the critical first 3-6 months. There may be opportunities for newborns to undertake routine immunisation schedules, provision of social and medical support and early monitoring of nutritional status are clear benefits.

When the newborn's serum is tested, the maternal IgG may be detected for up to 15 – 22 months. IgG has a half life of approximately 3 weeks, so maternal antibodies are clearly relatively slowly declining over many months in the infant without infection. In addition, maternal antibodies to HIV may be passively transferred to the newborn postnatally through breastfeeding. If a standard antibody is the only test available, then the infant must be continually monitored for seroreversion (tests converting from positive to negative) for up to 18 months. This seroreversion does not however exclude infection with HIV until the newborn becomes immunocompetent to produce its own antibody response.

Other laboratory techniques may be used to diagnose HIV infection in the newborn by the use of direct detection tests (e.g. HIV-1 p24 antigen serological tests and nucleic acid tests [DNA and RNA]). In countries with limited resources where nucleic acid tests are unavailable, p24 antigen assays may be the only way viraemia can be determined. HIV-p24 antigen tests only have a sensitivity of between 50-80% in the first 6 months but increase significantly after 6 months of age.

Testing for HIV infection in the newborn by molecular tests is recommended at 48 hours. In many resource-limited settings, breastfeeding is recommended even in HIV-positive mothers as the risk of morbidity and mortality from bottle feeding outweighs the risk of HIV transmission from breastfeeding. Therefore children must continue to be re-assessed for their HIV status until they are weaned. The use of dried blood spots may be useful in improving access to nucleic acid tests in resource-limited and remote settings as previously discussed.

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