

Clinical Tract

Module on

Laboratory diagnosis and monitoring of HIV

LEARNING OUTCOMES FOR COUNSELLORS AND DATA CAPTURERS

After completion of this module the learner should:

- Identify the groups of people that need to be offered HIV testing.
- Know which tests are used to diagnose HIV infection in adults.
- Know which tests are used to test for HIV infection in children.
- Know what the CD4 count evaluates.
- Know what an HIV viral load means.
- Know how to explain to a patient what an HIV viral load of <25 means.

LEARNING OUTCOMES FOR DOCTORS, NURSES, PHARMACISTS, LABORATORY TECHNICIANS

After completion of this module the learner should:

- Describe the extent of HIV epidemiology in South Africa and Southern Africa.
- Know the different subtypes of HIV, their distribution in the world and their influence on vaccine development.
- Know the different routes and risk of HIV transmission.
- Know how this epidemiology can be monitored.

This module should be read in conjunction with the modules on pre- and post-test HIV counselling

1. INTRODUCTION

Viral infections can present in a variety of guises, namely:

- Latent viral infections
- Clinically latent viral disease
- Viral reactivation phenomena
- Chronic viral infections
- Persistent viral infections and
- A new concept of occult viral infections.

In most cases testing for the presence of a viral infection can be determined by serological assays. However, clinical viral disease such as those caused by the Herpes viruses may require other kinds of tests, including molecular analysis. Thus the concepts of diagnosing HIV infection and monitoring HIV disease need to be clearly understood. Diagnosing HIV infection usually requires serological analysis looking for analytically detectable antibody. In turn, determining HIV disease status requires tests to either detect presence of the viral nucleic acid or measuring the amount of circulating virus as well as assessing the immunological status of the individual. The expanding HIV testing repertoire also extends to include the “rapid” tests that can test body fluids other than blood.

As the usage of antiretroviral (ARV) drugs increases, drug resistance testing will become a regular feature of the HIV testing profile.

The HIV testing repertoire can be summarized as the following:

- HIV rapid tests – some even apply the ELISA principle (these tests may not require laboratory and personnel infrastructure and can be performed in the field);
- HIV antibody tests – HIV ELISA (enzyme linked immunosorbent assays) are used commonly;
- HIV Western blot method for antibody testing;
- HIV ELISA Combination assays – detection of host antibody and viral antigen are determined concurrently;
- HIV p24 antigen tests – ELISA method;
- HIV DNA qualitative assays – molecular analysis;
- HIV RNA quantitative assays – molecular analysis;
- HIV drug resistance assays – genotyping and phenotyping methods.

The CD4 cell count profile complements HIV testing by determining the immunological status of the infected individual. Impressive as the test repertoire is, practitioners must know exactly which HIV tests to order for the particular clinical circumstances. Although each of these tests perform exceptionally well under the current South African conditions, medico-legal implications abound in the event of mistakes, misunderstandings or ignorance with regard to the use of each HIV test and result interpretation.

2. GROUPS OF PEOPLE WHO SHOULD BE OFFERED HIV TESTING

In South Africa, everybody should be acutely conscious of the burden of HIV disease and at the same time aware of his or her own HIV serostatus. This also applies to our teenagers and children who need to be fully informed not only about the disease and infection, but also about the reality of living in South Africa.

In order to stress the importance of HIV testing a high-risk category is created to identify those individuals who require counseling and a good support system, namely:

- Individuals that have a risk of exposure due to their sexual activities and preferences;
- Spouses or sexual partners where risk activities are known or suspected;
- Certain professionals or employees who may be exposed to individuals' body fluids, such as ambulance personnel, rescue-team mine workers, health care professionals, to mention but a few ;
- Certain unusual clinical situations such as suspected date rape cases, contact with potential contaminated instruments or surfaces or other aspects of trauma;
- Any indications of sexual assault, abuse or rape;
- Any patient presenting with an STI syndrome;
- As part of an organized and supportive HIV workplace programme;

Other groups of people who should also be offered HIV testing, include:

- Any two people embarking on a new relationship;
- All pregnant women and any couple planning a family;
- Any patient presenting with a possible opportunistic infection such as herpes zoster shingles or tuberculosis.

3. THE INITIAL DIAGNOSIS OF HIV INFECTION

Screening for and diagnosis of HIV is currently done by testing for anti-HIV antibodies. Antibodies are the body's response to an infection. Antibodies normally begin to appear in the blood a few weeks or less after a person has become infected with HIV. The HIV antibody test detects the presence of antibodies to HIV in the blood or saliva. It is easier and cheaper to detect antibodies to HIV than it is to test for the virus itself.

Even if a person is found to be HIV antibody positive, this doesn't predict which HIV-related conditions may or may not develop. All it tells you is whether there are HIV antibodies in the blood. It is not a test for AIDS.

Table 1. Diagnostic window period intervals for the current HIV assays

Assay	Time interval to positivity
Current 3 rd generation HIV ELISAs	18-23 days
Current HIV p24 antigen assay	15-19 days
HIV DNA PCR	14-16 days
HIV RNA PCR	11-14 days

*The time intervals are approximate values. The window period intervals for the new HIV Combination assays are not indicated in this table.

Under certain circumstances, tests to detect the presence of the virus are for diagnostic purposes. These tests would include the

- HIV DNA polymerase chain reaction (PCR)
- P24 antigen assay

These tests would be reported as positive or negative, and do not determine the viral load. PCR testing can detect HIV in the blood approximately two weeks after infection or possible exposure.

HIV window period and acute seroconversion

HIV antibodies cannot be detected the day after a person becomes infected or exposed, so it is not possible to find out if one has been infected immediately after a possible risk event. Antibodies usually take between three to four weeks to appear in the blood. The time between infection and the development of antibodies is called the window period.

In the window period people infected with HIV have no detectable antibodies in their blood, but may already have very high levels of HIV in their blood, sexual fluids or breast milk. In fact, people with HIV are most infectious during this window period before their own immune system has attempted to control the virus. So one can transmit HIV to another person during this period, even though an antibody test might be negative.

However, HIV acute seroconversion syndrome is a phenomenon that cannot be ignored and more and more individuals will be presenting with these signs and symptoms. These clinical features include many aspects common to an acute viral infection such as pharyngitis, headache, malaise, myalgias, skin rash, lymphadenopathy and fever. Thus a strong clinical suspicion should prevail as these individuals may not have a reactive HIV ELISA test. In addition, many of these individuals may be screened at the clinic or consulting rooms with a rapid HIV test that may reveal an actual “false” negative analytical result. Clinics generally recommend that one should wait three weeks from the time of a possible risk before having an antibody test, to be sure that a negative result is truly negative.

It must be noted that the HIV RNA viral load assays (quantitative measurement) are required for prognostic and/or monitoring purposes of HIV disease alone and cannot serve solely in a diagnostic capacity.

The HIV antibody test

The HIV antibody test remains the most common form of screening and diagnosing HIV infection and the common methods are the ELISA and Western Blot assays. The following test principles apply, namely: Two to three different enzyme-linked immunosorbent assay (ELISA) tests are used for confirmation. One screening ELISA followed by two confirmatory ELISAs or one screening ELISA and a confirmatory Western Blot result are required to call the result reactive or positive on the first specimen;

- A second specimen must be tested in the case of an HIV-positive result in order to confirm the identity of the specimen and the reactivity of the result;
- Discordant results must be reported as such and additional HIV tests must be requested;
- HIV disease monitoring tests are not part of the diagnostic repertoire;

Limitations: False negative results may occur, although on rare occasions, in the advent of very early or acute HIV infection or if the specimen volume is inadequate or the specimen quality is inappropriate. False-positive results do occur, although the laboratory attempts to minimize this event from being reported. These false-positive results can be caused by a number of factors, namely:

- Interfering substances within the specimen;
- Severely haemolysed specimens;
- Cross reaction within the test method matrix;

- Cross-reaction events due to other underlying diseases such as autoimmune diseases, to name but a few .

The Western Blot assay, due to the nature of its design, is more prone to eliciting indeterminate results that require additional confirmation. Unfortunately human error or intervention accounts for the majority of false negative and false positive reactions. The Western Blot assay is also much more labour intensive.

HIV DNA PCR

Whereas all the previous tests test for anti-HIV antibodies, the HIV DNA polymerase chain reaction (PCR) test is a qualitative molecular assay that determines whether the HIV is present in peripheral blood mononuclear cells. It is thus reported as positive or negative.

It is the gold standard for early diagnosis of definitive HIV-infection, but does not replace the anti-HIV antibody test under normal circumstances.

Limitations: Due to the fact that this assay is highly specific and the technique amplifies DNA, false positive reactions may occur. The test is only accurate when requested at least 14 days after exposure or at least 6 weeks after the birth of a baby born to an HIV-positive mother.

Examples of where HIV DNA diagnostic testing may be useful, include:

- to determine viral infection in infants under the age of 18 months born to HIV-positive mothers (HIV ELISA might be falsely positive since the mother's HIV antibodies cross the placenta during pregnancy);
- early determination of infection in sexual assault or rape cases;
- within the occupational setting (analytical 'window period' issues);
- unusual clinical circumstances requiring detection of the viral presence;
- carrying out further investigations on HIV-1 serodiscordant sexually active partners.

P24 ANTIGEN ASSAY

P24 is an antigen present in the core of the virus. If p24, a "protein" of virus, is thus present in a blood sample, the diagnosis of HIV infection can be made.

The p24 antigen assay applies the ELISA method but due to antigen-antibody complex formation, false negative and false positive results have been reported. It is used under certain clinical circumstances for diagnostic purposes, namely to confirm HIV-infection in babies born to HIV-positive mothers or when HIV seroconversion phase is suspected.

Limitations: this assay is prone to generating more false-positive and false-negative results due to the possibility of antigen-antibody complex formations. It is also not as accurate as the HIV antibody assays, thus a negative p24 antigen result does not rule out the possibility of HIV-infection.

HIV RAPID TESTS

In this age of automated technology and sophisticated testing platforms, the term "rapid HIV testing" requires definition and the use thereof clarification. In addition, such advanced technology has subjected most types of HIV tests to rapid turnaround

principles. Conventional “rapid” HIV ELISAs are regarded to be those tests used outside the normal or existing laboratory infrastructure or those performed using a rapid ELISA device, not requiring an analyzer or routine test kit system. These conventional rapid tests are designed to ensure testing and reporting a result on site. Most people receiving HIV rapid tests can receive counselling and learn their HIV status in a single visit.

These tests are used in the field and generate on site and immediate-time results. Many of them are based on the ELISA principle and their results are accurate and reliable. They also form a key component of some VCT (voluntary, counseling and testing) programmes.

Rapid HIV testing must be conducted according to the same ethical standards as for any other HIV test. This includes pre- and post-test counselling, informed consent, privacy and confidentiality.

Although these tests are easy to perform, training should be carried out and discipline should be cultivated to ensure accurate and reproducible results. As many of these devices do not allow sufficient space for recording patient names and other identification data, care and attention should be given to correlating specimen collection, device labeling and patient identification correctly.

Blood specimens remain the standard specimen choice, but oral fluid or saliva specimens are an attractive specimen alternative, due to ease of sampling and patient compliance. These rapid tests are formatted to ensure ease of handling and processing, as well as clear and concise identification of test result validity (control line or marker) and of negative or positive patient results. They are also deemed to be accurate and reliable and they do provide an accessible, cheaper and effective form of testing for selected conditions in South Africa.

Therefore the usage of rapid HIV tests can be clarified by the following examples:

- Within the field setting, such as Health Care Centres or Clinics;
- As part of surveillance and seroprevalence studies or initiatives
- Within the clinical setting, whereby immediate and urgent clinical decisions are required;
- Under resource-constrained conditions;
- As part of the HIV management approach and treatment procedures, that may include a second type of HIV rapid test for confirmatory diagnostic purposes.

However, it should be noted that some HIV rapid tests stipulate specifically in their package insert that their tests are not recommended for diagnostic purposes alone in cases of suspected acute seroconversion syndromes or acute primary HIV infection. The burden of HIV infection in South Africa to date is sufficient to ensure an increasingly larger pool of potential HIV seroconverters.

Clinicians may need to consider clinical caution on receipt of HIV-seronegative results in individuals with a suggestive clinical history or evidence of an acute viral infection.

It is widely accepted that rapid HIV tests may be extremely sensitive by design and may generate false positive results in certain individuals. Hence the axiom that all reactive HIV rapid test results should be confirmed. Test confirmation will include checking the reactivity of the specimen and the identity of the individual.

In the same sense individuals who test rapid HIV negative without additional clinical suspicion are accepted as being HIV non-infected, at the time of testing. Such test analysis dogma may need to be revised and clinical caution may require additional viral parameters other than exclusive host antibody responses.

A new development in the form of HIV ELISA Combination testing (simultaneous analytical detection of viral p24 antigen and host antibody response to the presence of HIV) reveals encouraging data for limiting analytical mis-serodiagnosis of acute HIV seroconversion. Hence, decreasing the analytical "window period" to a shorter clinical period after HIV exposure.

4. CURRENT DEVELOPMENTS FOR SOUTH AFRICA

HIV Combination assays

As South Africa is currently experiencing all aspects of an advanced HIV epidemic, early infection and late stage disease phenomena are readily encountered. Thus HIV tests require a different approach to diagnosis and this may be covered by the combination assays detecting virus antigen concurrently with host antibody response. In essence decreasing the analytical window period to a clinically significant interval would assist many situations in South Africa. Definitive indications for such assays involve the insurance testing industry as well as for individuals with suspected acute viral infection.

Although South Africa has the an efficient and careful blood transfusion service with regard to safety and screening of donor products, such early detection combination assays would be an ideal screening tool for their wide ranging and dynamic donor pool. In practice it has been alleged that the combination assays may be prone to a higher rate of false positive results. Indications are that the combination assays deserve a definitive place in the diagnosis of all stages of HIV infection and disease in South Africa and confirmatory assays would assist with this concern as they are doing so currently.

Molecular assays

As technology advances so do the expectations for a faster turnaround time for molecular test results, in keeping with clinical reality and treatment decisions. This is now being catered for by the automated systems and the concept of real time resulting within hours. As an example, molecular assays for diagnosis of opportunistic infections in HIV-infected individuals are a technological advancement and the faster results could enable meaningful treatment decisions.

5. PRACTICAL RECOMMENDATIONS FOR HIV TESTING IN SOUTH AFRICA

- HIV tests are accurate, reproducible and are considered amongst the most reliable in the world. However, clinical acumen remains the cornerstone of diagnosis, as is the case in many other circumstances marrying laboratory results with clinical findings.
- Although HIV ELISA antibody tests remain the most common form of HIV testing in South Africa, their limitations should be fully understood by all health care professionals.

- Rapid HIV tests used in the field do have a definite role, particularly in the voluntary, counselling and testing programmes in South Africa, but exceptional cases need to be recognized and referred for further management. Result confirmation by means of further laboratory analysis may be applicable under certain circumstances.
- Although the HIV test repertoire is impressive, clinical caution should be exercised in the choice of the type of test and the interpretation of the result.
- The “analytical window” period remains a clinical concern and must be factored into the clinical assessment of HIV infection.
- HIV-infected individuals who are receiving ARVs require particular consideration for interpreting their HIV RNA viral load values and CD4 cell count readings. Discordant results may occur and a full medical history is required.
- Discordant or unexpected HIV test results should be discussed with practitioners experienced in HIV medicine.
- The CD4 cell count serves as an indicator of the immunological status of the HIV-infected individual and has no role in the initial diagnosis of HIV infection.

6. MONITORING HIV DISEASE PROGRESSION OR THERAPY RESPONSE

Laboratory tests can be used to determine disease progression as well as response to antiretroviral therapy. It becomes obvious that a combined CD4 count and RNA viral load provide a more complete laboratory analysis of the dynamic viral infection and clinical disease. However, monitoring HIV disease in certain individuals in South Africa may require some restrictions or adaptations, as dictated by cost-constraints and adherence to National Treatment Guidelines. Viral load testing can be delayed until initiation of antiretrovirals. These tests are done as baseline investigations and thereafter at yearly to six-monthly intervals. These tests should not be done during an acute illness or within a few weeks of a vaccination.

If HIV disease is regarded as a “train of death hurtling towards its demise”, then the HIV RNA viral load compares with the “speed of the train” and the CD4 count values with the “mile markers” the train still has to travel.

Hence, there is a revival in the popularity of the CD4 count as the “most reliable” clinical indicator of disease progression and/or antiretroviral (ARV) therapy response.

Some clinicians have even questioned the worth of the RNA viral load for individuals on ARV who are clinically well and immunologically robust. In reality, the HIV RNA copies/ml still reflects the “speed of the train of death” and remains a reliable and early indicator of change within the viral dynamics, notably potential lack of adherence or viral drug resistance development. Any persistent increase or change in the HIV RNA levels, particularly in patients who had previously “undetectable” levels, remain sinister and require closer monitoring or urgent clinical investigation.

The role of the CD4 T-lymphocyte count

HIV causes a viral infection but elicits an immunological disease. The most important cell involved in the immune attrition is the CD4 T-lymphocyte as it has a central coordinating role in the immune response. The CD4 count is used as a reflection of the damage incurred by the immune system as well as immune system restoration in patients on antiretroviral therapy.

Thus the level of CD4 cells in the peripheral blood is the key parameter to use in monitoring any changes within the immune response. It has the following well-defined roles:

- It stages HIV infection;
- Establishes the risk of specific HIV-associated complications;
- Determines the need for prophylaxis against opportunistic infections and
- Assesses response to antiretroviral therapy.

The CD4 count is expressed as an absolute number or a percentage of T-lymphocytes. The absolute CD4 count is subject to considerable variation and therefore a trend in a series of CD4 counts has more application than any one result. The CD4% is less subject to variation on repeated measures.

Table 2 reflects the relationship between CD4 count, CD4% and immune suppression in adults. The normal CD4 count in children is age dependent and CD4% should rather be used. In some instances where CD4 counts are unobtainable, the absolute lymphocyte count may give a rough guide to what the CD4 count may be. An absolute lymphocyte count of $1.25 \times 10^9/L$ correlates roughly with a CD4 count of less than 200 cells/ μL .

The CD4 count declines at an average of 40-80 cells/ μL per year. A more rapid drop of CD4 cells is associated with faster progression to AIDS.

The CD4 cell count has been shown to be an independent risk factor for progression to AIDS and death. However, it has two major limitations, namely it is subject to considerable variation and it only reflects existing damage to the immune system. The CD4 cell count is therefore not ideal for predicting future damage to the immune system in any given individual.

The variations that affect the CD4 cell count are:

- Inter-assay variability;
- Diurnal variation and
- Changes due to intercurrent illness.

Table 2: The CD4 count, CD4% and immune suppression in adults.

CD4 positive cell count/ μL	CD4 percentage	The immune system
>500	>29	Normal immune function
200-499	14-28	Moderate immune suppression
<200	<14	Advanced immune suppression
<50		Severe immune suppression

In children, the CD4 percentage appears to be more important and a more reliable indicator of immunological damage. It is important because of the wide range of physiological change in absolute CD4 lymphocyte values with age through childhood.

The assessment of CD8 T-lymphocytes has some value in terms of validating overall subset measurement, but is probably of limited value nowadays in the management of HIV-infected individuals. As with the CD4 percentage in adults, the CD4:CD8 ratio has been superseded by absolute CD4 cell counts.

Quantitative HIV RNA assay or viral load

This assay as the name suggests, measures the amount of circulating HIV or disease burden and is used to prognosticate the disease progression or to monitor the response to antiretroviral therapy. The result is expressed as viral copies/ml as the virus has two copies of RNA strands or as a log unit to quantify response to therapy. The viral load indicates the rate at which the immune system is being eroded.

Limitations: In the context of HIV infection diagnosis, it has been emphasized that quantitative assays should not be used as screening tests. With an uncertain pre-test likelihood of HIV presence, the remarkable sensitivity of the quantitative molecular assays means that specificity is compromised. Thus the test is vulnerable to intervention at very low signal levels and false low positive readings may be generated in HIV-non-infected individuals. This will create confusion and false impressions.

Readings from different viral load methods cannot be accurately compared with each other thus the same test method must be used for the same individual.

The viral load is a quantitative measure that can be expressed in two ways:

- the number of HIV-1 RNA copies/ml of plasma, or
- the logarithmic equivalent – \log_{10} equivalent.

Only a change of more than $0.5\log_{10}$ is regarded as clinically significant. As an example: a change in viral load from 10 000 to 100 000 copies/ml represents a $1\log_{10}$ change and is regarded as clinically significant, whereas a change in viral load from 10 000 to 30 000 copies/ml represents a $0.48\log_{10}$ change and is not clinically significant.

7. CD4 CELL COUNT AND HIV VIRAL LOAD IN CLINICAL PRACTICE

Frequency of testing

There are no hard and fast rules and clinical acumen remains the cornerstone of judgment. Viral and CD4 cell counts should be undertaken:

- At the initial clinical assessment;
- Where possible at 6 to 8 weeks after commencing antiretroviral therapy to assess initial response to therapy (this is not in the National Antiretroviral Treatment Guidelines);
- Four to six monthly thereafter if the patient has responded to therapy or is clinically stable;
- A repeat assessment of both CD4 cell count and viral load is indicated if routine measurements yield unexpected results or the individual's clinical condition changes.

The main goal of antiretroviral therapy is to achieve an undetectable viral load and sustain it as such.

Treatment failure: This is a simple concept and any viral load measurements on two separate occasions in an individual who has had a previously undetectable viral load needs further investigations and assessment. However, virological failure can be considered for the following:

- A decline in viral load of not more than one log within 8 weeks after commencing therapy;
- A sustained increase in viral load of greater than 0.6 log from its lowest point or a return to 50% of the pre-treatment value.

8. OTHER TESTS AS PART OF THE EVALUATION AND MONITORING OF THE PATIENT

Screening tests

At the initial visit a full blood count and liver functions (AST and ALT), baseline chest X-ray and in women a baseline Pap smear should be offered. In addition screening for other sexually transmitted and infectious diseases should be considered. Syphilis, which is tested serologically, is an easily treatable disease. Other infectious diseases can also be screened for, but does not form part of current National Treatment Guidelines. These would include hepatitis A, B and C, toxoplasmosis, and cytomegalovirus (CMV).

On antiretroviral therapy

Whilst on antiretroviral therapy: these tests will depend on the choice of drug and will fall under the realm of “safety bloods” to monitor possible drug toxicity.

9. FURTHER READING

- Johnson D. Southern African HIV Clinicians Society. Principles of treating HIV. *S Afr Med J* 2000;**90**(3):227-230.
- Martin D, Sim J. Southern African HIV Clinicians Society. Laboratory diagnosis of HIV infection. *S Afr Med J* 2000;**90**:105-109.
- Mc Alpine L, Gandhi J, Parry JV, Mortimer PP. Thirteen current anti-HIV-1/HIV-2 enzyme immunoassays: how accurate are they? *J Med Virol* 1994;**42**:115-118.
- Hecht FM, Busch MP, Rawal B, et al. Use of laboratory tests and clinical symptoms for identification of primary HIV infection. *AIDS* 2002;**16**(8):1119-1129.
- Mlisana KP. The role of the laboratory in HIV management. *Southern African Journal of HIV Medicine* 2000;**2**:36-37.
- Webber LM, Botes ME. Current status of oral fluid testing for HIV antibodies in South Africa. *Southern African Journal of HIV Medicine in South Africa* 2001;**4**:37-38.
- Martin DJ, Sim JGM. HIV antibody testing. *S Afr J Sci* 2000;**96**(6):272-274.
- Joint United Nations Programme on HIV/AIDS (UNAIDS) – WHO. Revised recommendations for the selection and use of HIV antibody tests. *Weekly Epidemiological Record* 1997;**72**:81-81.
- Turner BJ, Hecht FM, Ismail RB. CD4 T-lymphocyte measures in the treatment of individuals infected with human immunodeficiency virus type-1: a review for clinical practitioners. *Archives of Internal Medicine* 1994;**154**:1561-1573.
- WHO and Department for International Development, UK. Provision of antiretroviral therapy in resource-limited settings – A review up to August 2003. *Southern African Journal of HIV Medicine* March 2004:34-39.
- Regensberg LD, Hislop MS. A report back on more than four years of HIV/AIDS disease management in Southern Africa. *South African Journal of HIV Medicine* February 2003:7-10.