# AMREF DIRECTORATE OF LEARNING SYSTEMS

## DISTANCE EDUCATION COURSES

## INTEGRATED HIV/AIDS PREVENTION, TREATMENT AND CARE

**Unit 2**
**Clinical Laboratory in Diagnosis & Treatment of HIV/AIDS**

**Allan and Nesta Ferguson Trust**

# Unit 2: The Clinical Laboratory in Diagnosis and Treatment of HIV/AIDS

A distance learning course of the Directorate of Learning Systems (AMREF)

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## ABBREVIATIONS

| | |
|---|---|
| **AIDS** | Acquired Immune Deficiency Virus |
| **ART** | Antiretroviral therapy |
| **ARV** | Antiretroviral |
| **AZT** | Zidovudine |
| **CBC** | Complete Blood Count |
| **C&S** | Culture & sensitivity |
| **DAART** | Directly Administered ART Therapy |
| **DTC** | Diagnostic Testing and Counselling |
| **ELISA** | Enzyme-linked imunosorbent Assay |
| **HAART** | Highly active antiretroviral therapy |
| **HIV** | Human Immunodeficiency Virus |
| **IEC** | Information Education and Communication |
| **MTCT** | Mother to Child Transmission |
| **OIs** | Opportunistic Infections |
| **PEP** | Post-exposure Prophylaxis |
| **PCP** | Pneumocystis carinii pneumonia |
| **PLWHA** | People Living With HIV/AIDS |
| **RNA** | Ribonucleic acid |
| **VCT** | Voluntary Counselling and Testing |
| **WB** | Western Blot |
| **WHO** | World Health Organisation |

# UNIT 2: THE CLINICAL LABORATORY IN DIAGNOSIS AND TREATMENT OF HIV/AIDS

## IINTRODUCTION

Welcome to Unit 2 of this course on Integrated HIV and AIDS prevention, treatment and care..

In the first unit you learned some basic information on HIV and AIDS including historical background, biology and epidemiology of HIV and AIDS. Health care providers like you all too often miss the diagnosis of HIV. You need to know the many presentations of HIV disease and use a systematic framework to ensure a proper diagnosis. In this unit therefore, you will learn how to make an initial assessment, what questions to ask when taking a history and what to look for in a physical exam. You will learn how to take a sexual history and when and how to advise patients to consider HIV testing.

You will also learn about the various serologic and other laboratory tests available for diagnosing HIV infection and AIDS, as well as how they work and how they are used in the management of HIV and AIDS.

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## UNIT OBJECTIVES

By the end of this unit you will be able to:

- Describe why establishing trust between the caregiver and the patient is essential;
- Identify the questions to ask in taking a patient's history and what to look for on a physical exam;
- Discuss why it is important to take a proper sexual history;
- Take sexual history using open-ended questions and listening skills;
- Identify clinical and lifestyle clues using an algorithmic approach to HIV testing,
- Describe when and how to advise patients to consider HIV testing;
- Identify the screening tests used to diagnose HIV;
- Identify tests used in the monitoring of patients with HIV infection;
- Describe the recommended strategies for diagnosis of HIV in resource limited
- Settings;
- Discuss the use of the clinical laboratory in HIV treatment and care in Resource limited settings.

# SECTION 1: PATIENT ASSESSMENT

## Introduction

Welcome to Section 1 of our Unit on Clinical Laboratory in the diagnosis and treatment of HIV/AIDS. In this section we will discuss how to make an initial assessment, what questions to ask when taking a history and what to look for in a physical exam. We will also discuss how to take a sexual history and when and how to advise patients to consider HIV testing.

Let's us start by looking at the objectives of this unit.

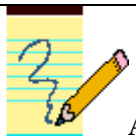
### Section Objectives

By the end of this section you will be able to:

1. Describe why establishing trust between the caregiver and the patient is essential;
2. Identify the questions to ask in taking a patient's history and what to look for on a physical exam;
3. Discuss why it is important to take a proper sexual history;
4. Take a sexual history using open-ended questions and listening skills;
5. Identify clinical and lifestyle clues using an algorithmic approach to HIV testing, and describe when and how to advise patients to consider HIV testing.

## Clinical Assessment of Patients Seeking HIV Care

All patients seeking HIV care in health care settings should have a complete medical history taken and a thorough physical examination. Before you read on, do the following activity. It should take you five minutes to complete.

**ACTIVITY**

Why should we carry out a comprehensive clinical assessment for patients seeking HIV care in a health facility?

_____

_____

_____

_____

_____

Check if your answers included the following:

The purpose of carrying out a comprehensive clinical assessment is to:

- Confirm the presence of HIV infection if not previously or reliably done;
- To stage the HIV disease;
- Detect the presence of any existing illnesses particularly the common and serious opportunistic infections. Screening for Tuberculosis (TB) should be carried out in all patients;
- Review concomitant medications, including traditional therapies, alcohol, cigarette use and non-prescribed drug use;
- HIV infection is a sexually transmitted infection; thus all HIV positive patients should be assessed for symptoms of STIs and syndromic management provided where indicated.

## History Taking

This starts with an initial assessment of the client or patient whereby you seek to establish rapport and put the patient at ease before asking him or her for the information you need to make a diagnosis. It is made up of the following steps.

***Establishing trust is essential***

You should remember that most patients are anxious and frightened by the mention of HIV; it is a life threatening disease with stigma attached to it. In order to gain a patient's trust you should empathize, share knowledge without being patronizing, provide reassurance and remain non-judgemental. Trust between you and patient is essential in order to obtain accurate information and care for the patient.

***The patient interview***

The interview is a way to establish trust between the patient and the health worker. Interviews have three main functions:

- to gather information,
- to handle emotions, and
- to manage behavior.

You need to develop the specific skills for each of these functions. Doing so takes time. This unit does not deal with this particular set of skills, however, you will learn more about these and related skills in the next Unit on Counselling. All professionals caring for people with HIV/AIDS should get special training on these skills.

Here is a brief overview of the three functions of the interview and the skills associated with them, with examples.

***Information gathering***

*SKILLS*

• Questioning

Using open-ended questions that cannot be answered with a simple "yes" or "no"

*"Tell me about how things have been going since your last visit."*

• Facilitation

"*Go on…I am listening." (including non-verbal nodding)*

• Direction

*"I understand that many things are bothering you…could we focus on the diarrhoea for just a minute?"*

• Summarising

*"So, from what I understand, you have had a lot of nausea and some cramping, you have taken all of the pills each day this week and you want some help with these symptoms…do I have it all right?"*

***Emotion handling***

Emotion handling is especially important in caring for PLWHA and their families. Here you require to practice empathy, reassurance, address time limitedness, education, and support in order to handle emotions.

*SKILLS*

• Empathy

*"I can see that you are very discouraged."*

• Reassurance (Understandability)

*"It is understandable that you are sad…look at what has happened in the last month: you lost your best friend, you are feeling weak and your son is not doing well in school…anyone faced with all that would be sad."*

- Time limitedness

*"It might help to keep remembering that these symptoms last for no more than one month in most people, and you've been through this for three* weeks now."

- Education about illness

- Support /partnership

*"I want you to know that I will be here to help you through this."*

***Behaviour management***

Behaviour management is used to achieve both medication adherence and lifestyle change (such as risk reduction). You can accomplish this best through education and motivational skills.

*SKILLS*

- Authority/modelling

*"I have seen this drug work in many patients."*

- Conditioning

*"You really did well this week….you remembered most of your pills."*

- Trait and choice attribution

Trait attribution

*"You do a good job of keeping track of things at work and caring for your children, so you probably can keep track of these medicines."*

Choice attribution

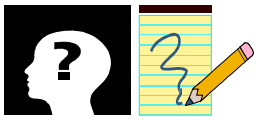
*"It is up to you to decide what method you want to use to remind yourself of when to take which pills."*

- Rehearsal and affirmation of intent

Through rehearsal, you help the patient think through a typical day; review what they will be doing about their medication and say what they intend to do.

## History Taking Specific to HIV/AIDS

In addition to the usual aspects of history taking, you should address history specific to HIV and AIDS. What do you think should be included in history taking specific to HIV and AIDS? Respond to this question by doing the following activity before you read on.



ACTIVITY

What should history taking specific to HIV and AIDS include?

_____
_____
_____
_____

_____

Now confirm your answers as you read the following discussion.

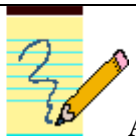
History taking specific to HIV and AIDS should include the following:

- Previous tests for HIV. If previous HIV tests were done, find out why the patient was tested and what were results?
- Presence of HIV-associated signs and symptoms;
- History of sexually transmitted diseases and other infectious diseases;
- Other medical diagnoses, for example, malignant or premalignant conditions;
- Mental health history (look for signs of depression);
- Family history: age and health of children, HIV in other family members;
- Medications taken regularly;
- Social history;
- Sources of support (family, friends, community, health care providers)

If appropriate, ask if the client remembers ever having been g treated for HIV. If yes, then ideally you would then find out about the pre-therapy, CD4+ cell count, HIV viral load and treatment, including duration/adherence.

## Taking Sexual history

Before you proceed, do the following activity. It should take you 5 minutes.

**ACTIVITY**

Why is it important to take sexual history?

_____

_____

_____

_____

Taking sexual history is important for the following reasons.

- Taking an effective and comprehensive sexual practice and lifestyle history is an integral part of medical management;
- Taking a sexual history helps to determine the possibility of past exposure. Emphasize eliciting information about behaviour that might have placed the person at risk;
- You will decide to recommend testing on the basis of clinical and lifestyle information obtained from a patient's history and from physical exam.

**How would you go about taking sexual history?**

**How to take sexual history**

You should approach it in the following way:

1. Try to begin with the least sensitive issues.
2. Put patient at ease by asking other relevant details, such as any history of symptoms or signs of concern to the patient and details of past illnesses, including STDs, etc.

3. Explain that taking a sexual history is important in order to assess the person's overall health and determine what tests to do.

4. If possible, ask questions in the context of a general medical history.

5. The interview should move from open-ended to close-ended questions.

6. In the examples below, most are closed-ended questions on the assumption that the interview has progressed from the initial, more open-ended stage. For example move from: *"Please tell me about how you see your risk for HIV?"* to *"When was the last time you engaged in sexual intercourse without a condom?*"

7. Listen carefully to the responses and ask clarifying questions.

8. Make sure that the patient understands the terms you are using. If possible, use the patient's vocabulary, and be culturally sensitive.

9. Modify your questions to suit the situation and the responses.

10. Be sure all questions about sexual practices are free of any assumptions regarding sexual orientation or monogamy.

11. Be sure to establish whether the patient has had unprotected sex at any time, and especially during the last three months, or has at any time had problems using condoms (for example, breakage).

12. Elicit a history of sexual contacts, taking the most recent first and working back from there.

**Questions for sexual history**

The following questions are mostly close-ended and should be asked only after there has been time for more open-ended discussion and the development of rapport.

a) In order to initiate a more detailed discussion of sexual history in relation to potential exposure you should ask questions such as:

- *Tell me what part sexual activity plays in your life right now? (If necessary, ask "Are you sexually active?")*
- *Can you describe for me what you think about your risk for HIV infection? Why do you think you may/may not be at risk?*
- *Have you ever had a sexually transmitted infection? (It helps to give examples.) Do you know if any of your sexual partners have developed a sexually transmitted disease or AIDS?*

b. To elicit more details about the number and sex of partners and the use of condoms:

- *Have you ever had, or do you currently have, sex with men, with women or both?*
- *How many sexual partners have you had? (If possible, determine the number of partners in the patient's lifetime, during the past year and in the past three months.)*
- *Do you use condoms?*
- *If so, how often?*
- *When did you begin using condoms?*
- *If not, what was your reason for not using condoms?*

c. Questions to identify sexual practices:

- *What form of sex do you usually have with your partner?*
- *Do you have vaginal intercourse?*
- *Do you have anal sex? (This may require additional explanation or description.)*
- *Do you have oral sex? (This may require additional explanation or description.)*

d. Questions to elicit (nonsexual) lifestyle clues that could predispose to risk of HIV infection:

- *Injecting drug use*
- *Do you smoke cigarettes, drink alcohol, or use other drugs?*
- *If the patient currently injects drugs: Do you share needles or other drug equipment?*
- *If the patient is a former injecting drug user:*
    - *When did you stop injecting drugs?*
    - *Did you share needles or other drug equipment? If so, until when?*
- *Blood products*
  - *Have you ever had a blood transfusion?*
  - *Have you ever had surgery or a major accident?*
  - *Did you receive any blood as a result of this surgery/accident?*

## The Physical Examination in Clinical Diagnosis of HIV Disease

The diagnosis and staging of HIV disease in a person living in a resource-limited country like Kenya is not as easy and quick as one might think. You need to do a good clinical examination and thorough interview; this can easily take 20 minutes per patient. The common findings to look for during a physical examination include:

- Oral thrush;
- Macular rash on palate as a sign of Kaposi's sarcoma;
- Herpes zoster scar;
- Florid nature of skin manifestations, a hallmark of HIV;
- Condition of the pectoralis, temporalis, biceps, gluteus and shin cover muscles as a clue to wasting. Ask yourself if hair is standing up straight. HIV is a wasting disease like cancer and TB;
- Lymphadenopathy usually not >2.5 cm.

In Kenya and indeed many other resource-limited countries, health care workers sometimes use the WHO AIDS case definitions and staging system which was adapted for countries with limited clinical and laboratory diagnostic facilities. Where laboratory monitoring is available, one should use a further refinement of the WHO staging system. I hope you recall that we discussed this staging system in Unit 1. We shall keep referring to it through out the course.

### What Signs Should You Look For?

When conducting a physical examination, you should look out for the following signs of HIV:

- ***General appearance***: look for evidence of wasting, marked fat loss in extremities, face and buttocks;
- ***Skin:*** rash, popular, macular, vesicular or ulcerative lesions;
- ***Eyes:*** examine conjunctiva for changes (retinal opacification, cotton wool spots);
- ***Oropharynx:*** (often yields earliest evidence of HIV) examine for thrush, etc.
- ***Lymph nodes***: non-tender or minimally tender lymphadenopathy, regional adenopathy, extremely tender lymph nodes;
- ***Lungs:*** rales;
- ***Gastrointestinal***: hepatosplenomegaly;
- **Neurology:** dementia, headache, seizures, focal neuropathies;
- ***Pelvic exam***: discharge, ulcers, abscesses.

## WHO case definitions for HIV/AIDS Surveillance in countries with limited clinical and laboratory diagnostic facilities

The WHO has developed the following guidelines to assist people who have no access to laboratory facilities be able to diagnose HIV/AIDS.

1. **Where HIV testing facilities are *not* available**, diagnose patients clinically based on major and minor signs and symptoms. The presence of at least two major signs and at least one minor sign fulfill the case definition for HIV/AIDS.

***What are the major signs?***

- Major signs include:

  - Weight loss more than 10 percent of body weight;
  - Chronic diarrhoea ( more than 1 month);
  - Prolonged fever (more than 1 month).

***What are the minor signs?***

- Minor signs include:

  - Persistent cough for more than one month (in case of TB, do not use this criterion);
  - Generalized pruritic dermatitis;
  - History of herpes zoster;
  - Oropharyngeal candidiasis;
  - Chronic progressive or disseminated herpes simplex infection;
  - Generalized lymphadenopathy.

The presence of either generalized Kaposi's sarcoma or cryptococcal meningitis suffices for the case definition of AIDS. This method has the problem of low sensitivity and specificity.

**2. Where HIV testing is available:** a positive HIV test together with the presence of one or more of the conditions below fulfils the case definition for HIV/AIDS:

- Weight loss more than 10 percent of body weight, or cachexia—with diarrhoea or fever, or both—for at least one month and not known to be the result of a condition unrelated to HIV infection;
- Cryptococcal meningitis
- Tuberculosis (pulmonary or extrapulmonary);
- Kaposi's sarcoma;
- HIV encephalopathy: neurological impairment that prevents independent daily activities and not known to be the result of a condition unrelated to HIV infection;
- Esophageal candidiasis;
- Life-threatening or recurrent episodes of pneumonia;
- Invasive cervical cancer.

## Summary

That brings us to the end of this section. In this section you have learnt how to make an initial assessment of a client who comes for HIV testing, what questions to ask when taking a history and what to look for in a physical exam. You have also learnt how to take a sexual history and when and how to advise patients to consider HIV testing.

In the next section you will learn about laboratory diagnosis of HIV.

# SECTION 2: LABORATORY DIAGNOSIS OF HIV INFECTION

## Introduction

Welcome to the second section of this Unit. As we have seen in the previous section making a diagnosis of HIV infection and AIDS is not easy, and at times could be misleading. You will therefore need to carry out a laboratory test in order to make conclusive diagnosis. In this section we are going to discuss how to go about laboratory diagnosis of HIV and the testing options and strategies available to us.

First let us look at the objectives of this section.

### Objectives

By the end of this section, you should be able to:

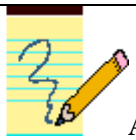
- Discuss the spectrum of testing technologies for HIV;
- Explain the advantages and disadvantages of HIV ELISA and rapid tests;
- Accurately recognize individual test result as reactive, non-reactive, or invalid;
- Apply the knowledge to manage patients with HIV infection.

## Introduction to Laboratory Tests

HIV infection is usually not noticed in the first few years after infection. While later certain signs and symptoms may suggest HIV infection or AIDS, these signs and symptoms are not specific for HIV infections or AIDS. Therefore the only definite evidence of HIV infection or AIDS is through a laboratory test.

There are two broad classes of tests may be distinguished for HIV diagnosis. Those which tests for the presence of HIV antibody formed in reaction to the virus and those that detect the actual virus (HIV) in the blood.

Start by finding out if you know the following terms commonly used in relation to laboratory tests for HIV.

**ACTIVITY**

What are the meanings of the following terms?

Sensitivity _____

*Specificity* _____

*Predictive values*_____

I am sure you were able to define the terms as follows:

- ***Sensitivity*** means proportion of samples that test positive by the test. In other words, sensitivity is the likelihood that a test result will be positive when antibodies to HIV are present.

- ***Specificity*** means the number of samples that are truly negative that test negative by the test. In other words, specificity is the likelihood that a test result will be negative when HIV antibodies are not present.

- ***Predictive values*** are calculated by combining the sensitivity and specificity with the prevalence of the disease in a given area. Predictive values are useful in determining how reliable a particular individual's test result is. It measure whether or not an individual actually has the disease, given the result of the screening test. (Accuracy of HIV serology is excellent.)

**Use of Laboratory Tests in HIV and AIDS**

Laboratory tests are used for different reasons in HIV and AIDS. These include:

- For diagnosis of HIV;
- For staging of HIV disease;
- For making decisions on initiation of ARV or prophylaxis;
- To assess response to ARV;
- To assess prognosis;
- To assess toxicity profiles of ARVs .

The tests used for diagnosis depend on the following factors:

a) Objective of the test: Is the test for diagnosing HIV infection, surveillance or to screen blood and blood products

b) Sensitivity and specificity of the test being used;

c) Prevalence of HIV infection in the population being tested.

It is important to note that no single test stands above all others. Also no combination of tests is the most appropriate for all testing situations.

## Testing Procedure for HIV Infection

In order to test a person for HIV infection, proper handling of test devices should be carried out. The following are important points to be observed:

- Infection control and universal precautions;
- Proper labeling;
- Proper sample collection procedures;
- Using the required sample volume per test;
- Use of proper buffer solution per test;
- Correct timing per test;
- Interpretation of results;
- Proper record-keeping;
- Proper disposal procedures.

The client should be as comfortable as possible during the test. Explanation and reassurance should be provided and factors that affect the test performance should be explained. Some of the factors affecting test performance are:

- Storage and handling of test kits;
- Changes in the environment;
- Calibration of equipment; external and internal controls;
- Degeneration of reagents;
- Sample collection technique;
- Quality of sample;
- Use of equipment.

These factors should be understood by the HCW and should be considered as part of quality assurance in testing for HIV infection.

There are two testing methods used in HIV and AIDS. These are:

- Serological Methods
- Viral detection methods

Let us discuss each in turn starting with serological tests.

## Serological Tests

Serological Tests are used to look for antibodies or antigens in a patient specimen. If our body is infected with any virus, it produces antibodies that fight the virus. After some weeks, these antibodies can be detected in the blood with an HIV antibody test. Antibody tests reliably detect the antibodies to the virus three months after HIV infection occurred. The period which lies between infection with the virus and an antibody test being able to identify the infection is called the "window period".

There are three types of antibody tests used in our settings:

These are:

- Elisa Test
- Western Immunoblot
- Rapid Tests

The two most commonly used HIV antibody tests for diagnosis of HIV infection are the HIV rapid tests and the standard ELISA tests. Previously standard ELISA and Western blot tests were the two most commonly used HIV tests. These tests are used to check for the presence of antibodies (not the virus) against the virus, most commonly found in the patient blood, but also in urine or saliva.

Two tests are used to make a diagnosis of HIV infection; one to for screening and the other for confirmation of the positive results. The tests used for screening purpose are designed to detect HIV antibodies in all individuals with HIV infection. They have high degree of sensitivity and therefore produce few false negative results. The tests used as confirmatory (supplemental) tests are designed to identify individuals who are not HIV infected but who have reactive or positive screening test results. They have a high specificity and therefore produce few false positive results. Rapid or ELISA tests or a combination of both can be used as screening and/or as confirmatory HIV tests.

Let us briefly discuss each type of test in turn.

**ELISA (enzyme-linked immunosorbent assay )/ EIA (enzyme immunoassay)**

The long HIV tests also known as ELISA are suitable for testing large samples. The methodology is relatively simple but requires expensive equipment and personnel who have a certain level of skill to accurately perform and interpret tests. Generally results take long to be known and therefore most patients are given a return date for results. It is also a very *sensitive* test, but not entirely *specific*, that is, it can detect antibodies to antigens other than HIV, thus making it possible to give a false positive. Should you get a positive (or indeterminate) ELISA result , this means that the sample needs to be tested further by western blot or a different ELISA test.

**Western Immunoblot Test**

Previously Western Blot test was used as the Gold Standard for confirmation of all positive screening tests, but due to its complexity, high cost and high numbers of false positive other alternative strategies (Algorithms) were developed by World Health

Organization for use especially in resource limited countries. It detects antibodies to a larger number of specific anti-HIV antibodies and is considered to be very specific for HIV. Samples yielding a negative result are reported as negative. However, if only a few types of anti-HIV antibodies are present in a sample, this may yield an indeterminate western blot. In this case a further sample should be collected at a later point in time and tested to confirm the diagnosis.

**Rapid Tests**

The rapid tests were developed in the late 1980s and can give results in less than 15 minutes. Examples of rapid tests which are found in Kenya are Uni-gold and Determine. They are recommended for use in VCT sites as they do not require a laboratory and are very simple.

A positive result is indicated by the appearance of a coloured dot or line.
If the test is negative, it shows that the person was not infected three months ago. If they have not put themselves at risk since then, they are HIV negative. If they have been at risk more recently, they need to come back for a follow-up test at the end of three months, during this time, they should practice safer sex. If the test is positive it shows that the person has been infected by HIV and that they are infectious to others.

When this test is positive it shows whether someone is well or ill or whether the person will become ill. It does not show when someone became infected with HIV, only whether they have been infected with HIV.

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Rapid tests are useful in situations where immediate results are important to management decisions, such as:

- Cases of occupational exposure, where the use of post-exposure prophylaxis (PEP) may be possible;
- STD clinics and emergency rooms, where seroprevalence rates are high, but follow-up may be impractical or compliance with follow-up poor ;
- Identification of HIV positive pregnant women in antenatal clinics or labour wards in order to provide follow-up MTCT prevention services.

***Advantages of HIV Rapid Tests***

- Same-day diagnosis
- Robust and easy to use
- Test time under 30 minutes
- Most require no refrigeration
- Minimal or no equipment required
- Minimum technical skill

***Disadvantages of HIV Rapid Tests***

HIV rapid tests also have a few disadvantages:

- Test performance varies by product;
- Refrigeration is required by some products, e.g., Capillus;
- Reader variability in interpretation of results;

- Limited end point stability of the results, i.e., reading should be done in a short time window.

## WHO Screening Tests

HIV infection is diagnosed by a positive HIV test. However, many low-income countries cannot afford expensive Western tests. WHO has therefore recommended testing strategies based on a combination of screening tests that do not require expensive Western Blot (WB) confirmation assays. HIV testing has become much more widely available than initially predicted, and the diagnosis of HIV purely on clinical features has become less frequent.

These strategies works on the principle of two screening assays being used in tandem. When these tests are performed in tandem, the results produced are highly accurate, reliable, and appropriate to protect the blood supply or assist in the diagnosis of HIV infection. There are two types of algorithms: parallel testing algorithms and serial algorithms.

- In a parallel testing algorithm, the specimen is simultaneously tested by two tests.

- In the serial algorithm all specimens are tested by a first test that is highly sensitive.

### Challenges of HIV Testing

There are several challenges associated with HIV testing:

- The ability of some test to detect early infections is sub-optimal;
- Diagnosing HIV infection in infants younger than 18 months can be difficult;
- Some tests may not be able to detect antibodies produced against specific HIV subtypes. For example, early generation of HIV test kits could not detect antibodies produced against strains of group O;
- Cross reactivity with other health conditions or infections decreases performance
- of the assay, e.g., cytomegalovirus and Epstein-Barr virus;
- Some technologies require specific equipment that must be properly maintained.
- Personnel need a certain level of skill to accurately perform and interpret tests varies (from minimal to high level)

***False positive results***

The current tests are of very highly sensitivity but a positive result will sometimes be obtained even when there are no HIV antibodies in the blood. This is known as false positive results.

False positive result is rare but may occur due to the following conditions:

- Due to presence of autoantibody arising from autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosis

- After influenza immunization which may cause a temporary false-positive antibody test

- Because of technical or clerical errors or improper interpretation. Additional testing can identify this.

- Thawing/unthawing of stored serum may interfere with the antibodies and therefore give false positive results

- All positive results must be confirmed by another test and all confirmed positive result from the second test confirms that the individual is infected with HIV.

***False-Negative results***

False-Negative result may occur when the blood tested gives a negative result for HIV antibodies when in fact the person is infected and the result should have been positive. If this occurs, it is advisable to discuss the likelihood of a false-negative test result with patients if their history suggests that they have engaged in behaviour that has put them at risk of HIV infection. Repeated testing over time for high-risk patients may be necessary before they can be reassured that they are not infected with HIV infected.

False negative results may occur in newly infected patients during the window period when very low levels of antibodies are being produced. It may occur also at the end stage of HIV due to poor quality of antibodies or due to prolonged immune reconstitution with HAART. Other causes include atypical host response, agammaglobulinemia, or results of HIV infection with HIV-1 group N or O or infection with HIV-2 (ELISA results may produce 20-30% False Negative results in HIV-2 infection) as well as results of technical error

***Indeterminate results***
Indeterminate results may occur in people with clinical signs meeting WHO stages III or IV; during sero-conversion phase; due to presence of autoantibody; due to infection with O strain or HIV-2 or due to technical errors. In asymptomatic individuals, a second blood sample should be obtained after a minimum of two weeks. The second sample should be tested using appropriate strategy and if still indeterminate a confirmatory assay should be used. If confirmatory test result is also indeterminate, follow-up of the person over a longer period may be required (3 to 12 months). If the results remain indeterminate after one year, the person is considered to be HIV-negative.

## Interpreting HIV test

There are only three possible outcomes for single HIV antibody tests. These are:

- Reactive or “Positive”
- Non-reactive or “Negative”.
- Invalid.

If a test yields an invalid result, the test has failed. The test MUST be repeated using a new test device.

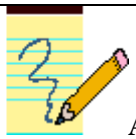
## Viral Detection Tests

Earlier in this section I mentioned that there are two main testing method used in HIV and AIDS. These are Serological Methods and Viral detection methods.

Viral detection methods are tests that can look for the virus itself or parts of the virus (antigen testing and RNA viral load testing), damage to the immune system, or other aspects of the body's response to the effects of the virus. These include HIV DNA PCR, which looks for the DNA (genetic material) of the virus.

The other tests known as P24 identifies actual HIV viral particles in blood but generally is only positive from about one week to 3 - 4 weeks after infection with HIV. The P24 protein cannot be detected until about a week after infection with HIV because it generally takes that long for the virus to multiply to sufficient numbers so that they can be detected. After one week, again the P24 proteins cannot be detected because the body produces antibodies to HIV which bind the P24 protein and eliminate it from the blood. During this time, the P24 test will register negative even in people who are infected with HIV. At that point, the regular HIV antibody test will then be positive. Later in the course of HIV, P24 protein levels rise again and become detectable.

Therefore this test cannot be used for HIV diagnosis in all people but can be used to detect early HIV infection and to screen donated blood for HIV.

**ACTIVITY**

Give an example of an immunological monitoring test

_____

_____

Give an example of a virological monitoring test

_____

_____

Now compare your answers with the information given below.

## Immunological Monitoring

Immunological monitoring is done by carrying out:

- CD+4 absolute counts;
- CD4:CD8 Ratio;
- CD4 Percentage;
- CD4+ T-Cell Counts.

### *Use of CD4T Cells in HIV Management*

As you saw in unit 1, CD4 cells have a central role in immune function. Reduction in number and quality of CD4 cells causes a weakened immune system. CD4 cells measure current strength of the immune system. Normal values ranges from 500-1400 cells/ml of blood.The CD4 Count provides:

- Benchmark for initiating prophylactic medications and ART;
- Guidance on the management of symptomatic patients;
- Information on the efficacy of ARVs;
- Monitoring ARV Therapy.

The median increase in CD4 count is 100 to 200 cells per year. The magnitude of increase in CD4 cells depends on the baseline CD4 count and adherence. The "CD4 response," as evidenced by rising CD4 counts, is much slower than the "viral load response" and may take several months or years to be complete. A reasonable frequency of CD4 count measurements in patients on antiretroviral therapy is every 6 months.

### *Factors Affecting CD4 Count*

- Diurnal variation; CD4 count is usually low in the morning and high in the afternoon. You should therefore stick to appropriate to each patient.

- Active opportunistic infection (OIs). Therefore, treat OI's before checking CD4 Count.

## Virological Monitoring

Virological monitoring is done by determining the viral load and viral resistance. Viral load and viral resistance testing are not widely available in resource limited countries but are slowly becoming increasingly available.

### *Viral Load*

Currently both international and Kenya guidelines emphasize the use of CD4 count and patients clinical status in deciding when to start a patient on ART.
Where available viral load can be used in the following situations:

- Diagnosing children less than 18 months of age
- Determine response to therapy
- Determine treatment failure
- Deciding when to initiate therapy

***Resistance Testing***

Resistance testing is expensive and generally not available in many countries. However where available it can be use in the following situations:

- Can be used to identify drugs that HIV is resistant to;
- Help in the management of patients in whom treatment has failed;
- Useful in research settings and for surveillance.

## Use of the Clinical Laboratory in HIV Treatment and Care in Resource Limited Settings.

After a diagnosis of HIV in a patient, such a patient needs to be monitored using both clinical assessment and laboratory markers. The HIV/AIDS monitoring is essential in guiding when to recommend initiation of therapy. Clinical monitoring will include staging of the HIV/AIDS disease using either the presence or absence of HIV-related signs and symptoms using the WHO staging system. Various laboratory methods can be used to monitor the HIV disease progression and to guide whether the patient will need antiretroviral therapy or not. Laboratory monitoring for patients who are not on drugs is done to provide information about the stage of illness; to enable the clinician to make decisions on treatment and to give information on prognosis of the patient. Patients who are on drugs are monitored to assess their response to treatment with antiretroviral drugs and to detect any possible toxicity associated with the antiretroviral drugs. There are many different tests which can be used for monitoring of HIV positive patients. These tests check the haematopoietic (blood) system, liver, kidney and for presence of any opportunistic. Common monitoring tests for people with HIV/AIDS include:

- Complete Blood Count
- CD4 and CD8 Cell Tests
- Blood Chemistry Tests
- Blood Sugar and Lipid Tests
- Tests for Pathogens Other Than HIV
- HIV Viral Load Tests.

**The Complete Blood Count**

Once of the tests that is carried out to monitor the management of HIV and AIDS is complete blood count.

**Why is a complete blood count test done in the management of HIV and AIDS?**

Whole blood is made up of various types of cells suspended in a liquid known as plasma. The complete blood count (CBC) is an inventory of the different cellular components of the blood, that is, the red blood cells, white blood cells, and platelets. This test is important because people with HIV may have low blood cell counts (cytopenias) due to chronic HIV infection or as a side effect of medications, particularly those drugs that damage the bone marrow, where all blood cells are produced. Blood cell counts are typically reported as the number of cells in a cubic millimeter of blood (cells/$mm^3$) or as a percentage of all blood cells.

The CBC looks at numbers of various different types of blood cells which includes red blood cells, platelets and white blood cells.

**Red blood cells (erythrocytes)**
Red blood cells (erythrocytes) carry oxygen from the lungs to the body's cells, bound to a molecule called haemoglobin. Anaemia is a condition characterized by a reduction in the number of red blood cells, often leaving a person fatigued, weak and short of breath. Anaemia is common in HIV positive people. HIV itself and various Opportunistic Infections such as *Mycobacterium avium* complex (MAC) can affect red blood cells and their oxygen-carrying capacity. In addition, drugs such as AZT (zidovudine, Retrovir) may lead to low red blood cell counts due to bone marrow suppression. White blood cells (leukocytes) carry out the body's immune responses.

**White Blood Cell Count (WBC)**
White blood cell count is the total number of white blood cells in a quantity of blood. A healthy adult normally has 4,000-11,000 white blood cells/mm3. An increase in WBC often indicates that a person is actively fighting an infection or has recently received a vaccine. Decreased WBC (leucopoenia) can leave a person vulnerable to various pathogens and cancers.

- ***White Blood Cell Count Differential:***
    - This is a report of the proportions of different types of white blood cells as a percentage of the total number of white cells; these percentages may be multiplied by the WBC to obtain absolute counts. People with HIV should be especially concerned with neutrophil and lymphocyte levels; in particular CD4 and CD8 cell counts (see discussion below).
- ***Neutrophils:***
    - Neutrophil is a type of cell that fights bacterial infections. Neutrophils normally make up about 50-70% of all white blood cells. Various anti-HIV drugs (especially AZT), and cancer chemotherapies that suppress the bone marrow may lead to low neutrophil levels (neutropenia). The risk of bacterial infection increases when the absolute neutrophil count falls below about 500-750 cells/mm3.

- ***Lymphocytes:***
    - There are two main types of lymphocytes; B cells which produce antibodies that fight foreign invaders in the body and T cells which target infected or cancerous cells and help coordinate the overall immune response. A normal lymphocyte count is about 20-40% of all white blood cells. The typical differential does not include specific subsets of T cells, but because CD4 and CD8 cell counts are important to people with HIV, they are measured separately
- ***Platelets***
    - Platelets (thrombocytes) are necessary for blood clotting. A normal platelet count is about 130,000-440,000 cells/mm3. Low platelet counts (thrombocytopenia) can lead to easy bruising and excessive bleeding and may be caused by certain drugs, autoimmune reactions, accelerated destruction by the spleen, or HIV disease itself.

**Liver Function Tests**
(Alanine transaminase (ALT); Aspartate transaminase (AST); Bilirubin; Alkaline phosphatase (AP); and Gamma glutamyl transpeptidase (GGT)), also known as the hepatic panel, are laboratory tests that help measure how well the liver is working. The liver carries out many vital bodily functions; when it is not working properly, levels of various enzymes, proteins, and other substances in the blood may rise or fall. Elevated liver enzyme levels may be a sign of liver damage caused by factors such as viral hepatitis, heavy alcohol consumption, or drug toxicity. Because several anti-HIV medications are known to cause liver damage people taking antiretroviral therapy should have their liver function monitored regularly. Anyone with significantly elevated liver enzymes should be tested for hepatitis A, B, and C.

**Kidney function tests** (Blood urea nitrogen (BUN) and Creatinine)
These are important for people with HIV because certain anti-HIV drugs and medications used to treat OIs may cause kidney damage, especially in those with a history of kidney problems. To assess kidney function, urine is usually analyzed in addition to blood tests. The presence of protein, glucose, or red or white blood cells in the urine may be a sign of kidney damage or some other abnormal condition.

***Elevated blood lipid*** **(fat**) (Triglycerides and Cholesterol); and glucose (sugar) levels have been correlated with the use of protease inhibitors (PIs) and for patients on PI may need regular monitoring of their blood fat and sugar levels.

**Other blood chemistry test** which may be important include Albumin; Amylase; Creatine phosphokinase (CPK); and Lactate (or lactic) dehydrogenase (LDH).

**CD4 and CD8 Cell Tests***:*
HIV primarily targets CD4 cells. As HIV disease progresses, CD4 cell counts decline, typically by about 30-100 cells/mm3 per year (depending on viral load), leaving a person increasingly vulnerable to infections and cancers. The CD4 cell count is therefore a valuable tool for gauging HIV disease progression. It provides information about when anti-HIV therapy is indicated and how well it is working. Effective treatment can halt HIV replication and restore CD4 cell levels.

**Viral load tests:**
**T**hese measure the amount of HIV RNA (genetic material) in the blood. The presence of RNA indicates that the virus is actively replicating (multiplying). Along with the CD4 cell count, viral load is one of the most valuable measures for predicting HIV disease progression and gauging when anti-HIV treatment is indicated and how well it is working. Viral load is expressed either as copies of RNA per milliliter of blood (copies/mL) or in terms of logs. A log change is an exponential or 10-fold change. For example, a change from 100 to 1,000 is a 1 log (10-fold) increase, while a change from 1,000,000 to 10,000 is a 2 log (100-fold) decrease. If the level of HIV is too low to be measured, viral load is said to be undetectable, or below the limit of quantification.

However, undetectable viral load does not mean that HIV has been eradicated; people with undetectable viral load maintain a very low level of virus. Even when HIV is not detectable in the blood, it may be detectable in the semen, female genital secretions, cerebrospinal fluid, tissues, and lymph nodes.

**Tests for Pathogens**
These are performed only if a person is experiencing symptoms of a specific infection. These includes tests for toxoplasmosis; syphilis; viral hepatitis; a sputum test for Pneumocystis carinii pneumonia (PCP); a stool (fecal matter) test for parasitic infections such as cryptosporidiosis, or a cerebrospinal fluid test (spinal tap) for brain infections such as cryptococcal meningitis.

In resource limited settings many of the laboratories may not be able to generate all the above haematological parameters but at minimum haemoglobin and White Blood Cell Count Differential can be performed to aid in the management of HIV/AIDS patients.
It is therefore necessary that people with HIV receive at least a haemoglobin and White Blood Cell Count Differential every six months, and more often if they are experiencing symptoms or taking drugs associated with low blood cell counts. Routine monitoring of haemoglobin in patients receiving AZT is recommended. Measurement can be done before the initiation of AZT and at 4, 8 and 12 weeks of AZT treatment.

## SUMMARY

You have now come to the end of this unit. We hope you enjoyed it . Now go back to the objectives at the beginning of this unit. If there is any objective that is not clear to you, go back to the relevant section and read it again. After that, take a well deserved rest before you complete the attached assignment for this unit.

Good Luck!