

Module 3: Overview of HIV Testing Technologies

Purpose	To provide the participants with a basic knowledge of HIV testing and how HIV rapid test results are interpreted.
Pre-requisite Modules	Module 1: Overview of HIV Infection
Module Time	1 hour 5 minutes
Learning Objectives	<p>At the end of this module, participants will be able to:</p> <ul style="list-style-type: none"> • Discuss settings where HIV testing will be part of service delivery during an era of expanded services • Discuss the spectrum of testing technologies for HIV • Explain the advantages and disadvantages of HIV rapid tests • Accurately recognize individual test result as reactive, non-reactive, or invalid

Module Overview

Step	Time	Activity/ Method	Content	Resources Needed
1	2 min	Presentation	Module introduction	Slides 2-3; Prepared flipchart – content outline
2	5 min	Presentation; Discussion	Expansion of HIV Testing	Slides 4-6
3	15 min	Exercise	Spectrum of HIV Tests	Slides 7-16;
4	30 min	Presentation; Discussion	HIV Rapid Testing Technologies	Slides 17-32
5	5 min	Exercise	Interpreting Individual HIV Rapid Test Results	Slide 33; Exercise Sheets: <u>Interpreting Individual HIV Rapid Test Results</u>
6	8 min	Q&A	Key messages Summary	Slides 34-35

Material/Equipment Checklists:

- PowerPoint slides or transparencies
- Overhead projector or computer w/LCD projector
- Flipchart
- Multiple sets of samples showing reactive, non-reactive, and invalid test results from each test approved for use in-country
- Exercise sheets: Interpreting Individual HIV Rapid Tests

Teaching Guide

Slide Number	Teaching Points
1	<p><u>Module 3: Overview of HIV Testing Technologies</u></p> <p>DISPLAY this slide before you begin the module. Make sure participants are aware of the transition into a new module.</p>
2	<p><u>Learning Objectives</u></p> <p>STATE the objectives on the slide.</p>
3	<p><u>Content Overview</u></p> <p>EXPLAIN the topics that will be covered in this module.</p>
<p>Flipchart</p> 	<p>WRITE the content outline on a flipchart prior to training.</p> <p>REFER to it frequently to orient participants to where they are in the module.</p>
4	<p><u>HIV Testing Occurs in a Variety of Settings</u></p> <p>DESCRIBE the graphic on the slide by making the following points:</p> <ul style="list-style-type: none"> • HIV testing will occur in a variety of settings outside of the laboratory • The settings where testing will likely to occur during an era of expansion of services include: Testing & Counselling Centers (T & C), Antenatal Clinics (ANC), Blood Banks, Surveillance programs, TB clinics, hospitals, and Sexually Transmitted Infections (STI) Clinics <p>HIGHLIGHT the following points:</p> <ul style="list-style-type: none"> • While all settings where testing occurs can triage persons to treatment and care, tuberculosis (TB) clinics and hospitals will be the primary venues for providing anti-retroviral treatment to HIV infected persons, and for providing care to HIV affected persons. • T&C, ANC, Blood Banks, and surveillance are the primary venues for providing prevention programs.

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5	<p><u>Expansion of Testing Services</u></p> <p>EXPLAIN the following points:</p> <ul style="list-style-type: none"> ▪ Testing will need to be integrated at all levels of testing services, and that testing must be linked to referral services, e.g., ANC and VCT. ▪ To facilitate the expected high volume of testing, non-traditional test sites will need to be incorporated into the national testing strategy. These non-traditional sites must however be linked back to the lab referral network and a quality management system.
6	<p><u>Use of HIV Testing Technologies in the Continuum of Care</u></p> <p>A variety of tests are performed at different stages. HIV rapid tests play an important role in initially identifying those who are infected with the HIV virus.</p> <p>Other tests, e.g., CD4 and viral load, play an important role in determining whether therapy can be initiated, and once initiated, if the drugs are working or not..</p>
7	<p><u>Spectrum of HIV Tests</u></p> <p>POINT OUT this list reflects commonly performed test associated with HIV. Some tests are for diagnostic purposes, e.g., EIAs, rapid tests, Western Blot, and p24</p> <p>Other tests are supplemental in monitoring disease progression, such as CD4 and viral load.</p>
8	<p><u>Challenges of HIV Testing</u></p> <p>EMPHASIZE the following points:</p> <ul style="list-style-type: none"> ▪ The ability of some test to detect early infections is sub-optimal ▪ Specialized testing is required to diagnose HIV infection in infants younger than 18 months. ▪ The variability of HIV viruses impacts upon the sensitivity the specificity of HIV tests. 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. ▪ The skill required to accurately perform and interpret tests varies; from minimal to high level of skill

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9	<p><u>Enzyme Immunoassays (EIAs)</u></p> <p>STATE the points on the slide</p>
 <p>TIPS</p>	<p>Some participants may not understand certain technical terms such as microwell. Consider passing around a microtiter plate for participants to see what is meant by “microwells.”</p>
10	<p><u>Enzyme Immunoassays (EIAs) - Cont’d</u></p> <p>DESCRIBE how Enzyme Immunoassay works.</p> <ul style="list-style-type: none"> • Sample is added to micro-well plate that has been coated with HIV antigen(s). • After a series of reagent additions, incubations and washings, the plate is placed in reading device. • The reading device measures the optical density of color that develops if HIV antibody is present in the client’s sample. <p>EMPHASIZE multiple factors can affect testing. As previously described a certain level of technical skill AND functioning equipment is a must.</p>
11	<p><u>HIV Rapid Tests</u></p> <p>STATE the points on the slide.</p> <p>HIGHLIGHT an added advantage for using rapid tests is the ability to use whole blood.</p> <p>EMPHASIZE that while HIV rapid tests, in general, is considered to be low in complexity, all test must be appropriately evaluated prior to use. It is equally important that the test be validated for use in the environment where testing will occur.</p> <p>EMPHASIZE the need for appropriate training on use of test.</p>

Slide Number	Teaching Points
12	<p><u>Western Blot / Line Immunoassays</u></p> <p>STATE The Western Blot is a supplemental test for confirming HIV infection.</p> <p>EXPLAIN the cellulose strip on the right.</p> <p>DESCRIBE key issues involved:</p> <ul style="list-style-type: none"> • It lacks standardization in performance and interpretation. • Although considered a confirmation test, this assay has a high range of indeterminate results. • It is a complex test. • It is very expensive.
13	<p><u>HIV p24 Antigen</u></p> <p>STATE the points on the slide.</p>
14	<p><u>CD4 T-Lymphocyte</u></p> <p>STATE the points on the slide.</p> <p>EMPHASIZE the need for instruments to be properly maintained, such as the BD FASCount, and BD FASCaliber seen on this slide.</p>
15	<p><u>Viral Load</u></p> <p>STATE the points on the slide. The higher the viral load (number of copies of HIV in the blood), the greater the progression of the disease.</p> <p>EMPHASIZE a number of Issues exist with this test:</p> <ul style="list-style-type: none"> ▪ Kits and reagents are expensive ▪ Demanding molecular techniques ▪ Concerns over contamination ▪ Experienced technicians required ▪ Difficult/complex assays ▪ Need separate dedicated supplies, equipment (including biosafety cabinets), and air conditioned rooms ▪ Need constant source of electrical power ▪ PCR-based technologies susceptible to genetic variation and low copy number

Slide Number	Teaching Points
 TIPS	Define terms such PCR, and explain low copy number when teaching participants without lab background.
16	<p><u>Complexity of HIV Tests Varies*</u></p> <p>4 levels of complexity for HIV tests have been described in a number of WHO reports. The complexity of tests varies, from minima – level 1, to complex - level 4, in terms of equipment, and technical skill.</p>
17	<p><u>HIV Rapid Tests provides excellent tool for expansion of services</u></p> <p>TRANSITION into HIV rapid tests.</p> <p>STATE HIV rapid testing provides excellent tool for expansion of services. The remaining module will focus on HIV rapid tests.</p>
18	<p><u>HIV Rapid Tests: Advantages</u></p> <p>DISCUSS the advantages on the slide.</p> <p>EMPHASIZE If clients do not obtain their HIV results, this is a missed opportunity for therapy or preventative measures.</p> <p>ASK participants to share any stories that illustrate the adverse effects of having to wait a long time for test results.</p>
 TIPS	<p>For participants without any lab or medical background:</p> <ul style="list-style-type: none"> • EXPLAIN the term <i>reagent</i>. A reagent is a substance used in a chemical reaction to detect or produce other substances. • EXPLAIN why some lab materials need refrigeration, and others don't, emphasizing the consequences of not refrigerating materials that require refrigeration (kits e.g. Capillus, and serum quality controls).
19	<p><u>HIV Rapid Tests: Disadvantages</u></p> <p>STATE the points on the slide</p> <p>DISCUSS past and present problems in slow turn-around of results from the laboratory, and the poor come-back rate of clients to obtain their results (due to fear, cost issues, transport issues, etc.).</p>

Slide Number	Teaching Points
 <p>TIPS</p>	<p>For participants without any lab or medical background:</p> <ul style="list-style-type: none"> • EXPLAIN that although the tests are not difficult, they will require a lot of practice and supervision to become proficient. • EMPHASIZE they must not feel afraid or embarrassed to stop at any time and ask questions.
<p>20</p>	<p><u>Body Fluids Used for HIV Rapid Testing</u></p> <p>EXPLAIN that HIV tests could be performed on a wide range of body fluids. Serum, plasma, whole blood and oral fluids are used the most.</p> <p>ASK participants what each fluid is and the typical method for obtaining each.</p>
 <p>TIPS</p>	<p>For participants without any lab or medical background:</p> <ul style="list-style-type: none"> • Briefly DESCRIBE these different fluids. • INFORM them that the samples they will use for HIV rapid testing will most likely be whole blood drawn from their clients' fingertips.
 <p>TIPS</p>	<p>Slides 21, 22, 23, 25, 26, 29, and 30 are highly technical. We recommend that you do not present these slides to lay counsellors.</p> <p>If you do present these slides,</p> <ul style="list-style-type: none"> • EXPLAIN every technical term on the slides • USE analogies and examples to help participants understand.
<p>21</p>	<p><u>Three Formats of HIV Rapid Tests</u></p> <p>STATE that there are three main formats or types for rapid HIV tests, and you will explain each format in the following slides.</p>
<p>22</p>	<p><u>How Immunoconcentration Works</u></p> <p>EXPLAIN the graphic on the slide.</p> <p>DESCRIBE how Immunoconcentration works.</p> <ul style="list-style-type: none"> • Flow-through (or immunoconcentration) devices are usually cartridges, with HIV antigen attached to a membrane. • The specimen and individual reagents are each added to the cartridge in a series of steps. • Presence of HIV antibody is indicated by the development of a colored spot or line.

Slide Number	Teaching Points
23	<p><u>Tests Based on Immunoconcentration</u></p> <p>STATE the devices mentioned in the slide (DESCRIBE more fully ones used in-country).</p> <p>DESCRIBE the graphics on slide.</p>
 <p><i>Customization Notes</i></p> <p>24, 27, 28, 31</p>	<p>Modify these slides if necessary:</p> <ul style="list-style-type: none"> • Delete tests not used in your country's algorithm • Replace photos with in-country examples
24	<p><u>Reading Results: Genie II</u></p> <p>EXPLAIN how to read results from Genie II.</p> <ul style="list-style-type: none"> ▪ If non-reactive, you will only see one visible dot in the control region • If reactive, you will see either one or two visible dots. One dot for HIV 1, and the other for HIV 2 • At the control dot, Human IgG links to membrane-bound anti-human IgG <p>Note: It will be helpful to physically point to the control dot.</p>
 <p><i>TIPS</i></p>	<p>For participants without lab or medical background, you may need to explain the terms “reactive” and “non-reactive.”</p> <ul style="list-style-type: none"> • Reactive – Antibodies to HIV present in the client's blood • Non-reactive – No HIV antibodies detected
25	<p><u>How Immunochromatography Works</u></p> <p>EXPLAIN the graphic on the slide by describing how Immunochromatography works.</p> <ul style="list-style-type: none"> • Specimen is applied to a pad (filter) where it mixes with gold or selenium colloid-antigen conjugate. This mix migrates through the nitrocellulose strip to immobilized recombinant antigens and synthetic peptides at the patient window. • If HIV antibodies are present then a red line will form in the test area of the strip.
 <p><i>TIPS</i></p>	<p>SIMPLIFY the explanation when teaching participants without lab background:</p>

Slide Number	Teaching Points
26	<p><u>Tests Based on Immunochromatography</u></p> <p>EXPLAIN how lateral flow devices work.</p> <ul style="list-style-type: none"> • Capillary flow (lateral flow) devices resemble dipsticks. All of the necessary reagents are usually incorporated into the test strip embedded in the device. • Specimen (and sometimes buffer or a reagent) added to the strip flows across the reagents, and a colored line develops in the presence of antibody. • Most lateral flow devices also have an internal control that detects human IgG. This internal control indicates that specimen was added to the test strip. If no human IgG is detected, an internal control line does not develop indicating an invalid test. <p>STATE the devices mentioned in the slide (DESCRIBE more fully ones used in-country).</p>
 <i>TIPS</i>	SIMPLIFY the explanation when teaching participants without lab background:
27	<p><u>Reading Results: Determine</u></p> <p>EXPLAIN how to read test results from Determine test kit.</p> <ul style="list-style-type: none"> • The reactive reaction shows two lines <ul style="list-style-type: none"> ○ One for the control band. ○ The other for the test. A band in the test area means a reactive result. • A non-reactive reaction will show a control band only. • The control band (line) must always be present for the test results to be valid.
28	<p><u>Reading Results: OraQuick</u></p> <p>ASK participants to explain OraQuick test results on the slide.</p>

Slide Number	Teaching Points
29	<p><u>How Particle Agglutination Works</u></p> <p>DESCRIBE how particle agglutination works by describing the graphic on the slide.</p> <ul style="list-style-type: none"> • The round circles represent antigen-coated latex particles that bind to antibodies to HIV (represented by the “Y”. Note: POINT TO each figure as you describe the graphic. • Agglutination or clumping occurs when the antibodies bind to the antigen-coated particles • Agglutination assays were among the first of the rapid tests developed. • Inexperienced persons or those who do not conduct the tests frequently may have problem with differentiating the coarseness or clumping of individual particles from true agglutination. They sometimes “over-interpret” agglutination, which result in a larger number of false-positives.
 <i>TIPS</i>	SIMPLIFY the explanation when teaching participants without lab background:
30	<p><u>Tests Based on Agglutination</u></p> <p>STATE the devices mentioned in the slide (DESCRIBE more fully ones used in-country).</p> <p>DESCRIBE the 3 images on the slide, using Capillus as the main example (as it is the most commonly used agglutination test):</p> <ul style="list-style-type: none"> • Left – The blood is placed in the oval area, also called the mixing well. • Center – The specimen travels along the thin tubes in the slide. • Right – If the blood contains antibodies to the HIV virus, visible clumping or agglutination can be seen.

Slide Number	Teaching Points
31	<p><u>Reading Results: Capillus</u></p> <p>DISCUSS the three different results on the slide.</p> <ul style="list-style-type: none"> • ASK participants to describe what they see in the slide. • POINT OUT the clumping or agglutination in the reactive sample, and the smooth liquid without clumps in the non-reactive test. • MENTION that unlike other test previously discussed, there is no control line to let the tester know that the test has worked correctly. However, the test kit includes a positive and negative control, which can be used to verify on a regular basis that the test is working properly
Transition	<p>STATE that there is a third possible result – the control line is not present.</p> <p>REMINDE that when the control line fails to show, it indicates that the test has failed. The result is therefore called “invalid.”</p> <p>TRANSITION into next slide.</p>
32	<p><u>There Are Only Three Possible Outcomes for Single HIV Antibody Tests</u></p> <p>REVIEW the terms on the slide.</p> <p>EMPHASIZE these are the terms when interpreting the outcome of a <u>single</u> test.</p> <p>EMPHASIZE if a test yields an invalid result; meaning no control band or line is present, the test has failed. The test MUST be repeated using a new test device.</p>
 <i>Customization Notes</i> 33	<p>If the tests used in the exercise are not in your country’s algorithm, consider replacing them with tests that are.</p>
33 Exercise 5 minutes	<p><u>EXERCISE – Interpreting Individual HIV Rapid Test Results</u></p> <p>REFER participants to the exercise in their manual.</p> <p>ASK participants to spend 3 minutes, working alone, to identify the results.</p> <p>DISCUSS the answers as a group.</p>
 TIPS	<p>Consider having multiple sets of real samples available to pass around, with each set containing three different test results (reactive, non-reactive, and invalid) for each test approved in-country. The more participants, the more sets you will need.</p>

Slide Number	Teaching Points
34	<u>Key Messages</u> STATE the points on the slide.
35	<u>Summary</u> ASK participants to answer the questions on the slide. ANSWER any questions the participants may have.