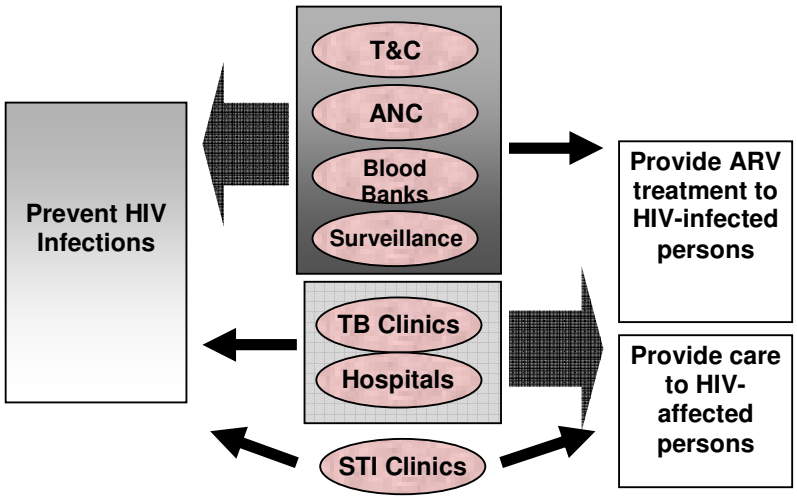
Source: U.S. CDC and WHO. *HIV Rapid Testing Training Package, Participant Manual*.
http://wwwn.cdc.gov/dls/ila/hivtraining/participants.aspx

Module 3

Overview of HIV Testing Technologies

Purpose	To provide you with a basic knowledge of HIV testing and how HIV rapid test results are interpreted.
Pre-requisite Modules	Module 1: Overview of HIV Infection
Learning Objectives	At the end of this module, you will be able to: • 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
Content Outline	Expansion of HIV rapid testing Spectrum of HIV diagnostic tests Challenges with HIV testing Spectrum of HIV testing technologies Advantages and disadvantages of HIV rapid testing Three formats of rapid tests Reading individual test results
Handouts	Exercise #1: Interpreting Individual HIV Rapid Tests Exercise #2: Interpreting Individual HIV Rapid Tests
Notes on Customization	Delete examples of HIV rapid tests not used in your country's algorithm and replace with in-country examples.

HIV Testing Occurs in a Variety of Settings



HIV testing occurs 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

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.

Expansion of Testing Services

Testing will need to be integrated at all levels of testing services, and 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 laboratory referral network and a quality management system.

Use of HIV Testing Technologies in the Continuum of Care

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.

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.

Spectrum of HIV Tests

The list below reflects commonly performed test associated with HIV. Some tests are for diagnostic purposes, e.g., EIAs, rapid tests, Western Blot, and p24. Other tests are supplemental in monitoring disease progression, such as CD4 and viral load.

- HIV diagnosis (Antibody/Antigen testing)
  - Enzyme Immunoassays (EIAs)
  - Rapid tests
  - Western blot (WB)
- Early diagnosis in infants
  - p24
  - DNA/RNA PCR
- Initiation and monitoring of ART
  - CD4
  - Viral Load

Challenges of HIV Testing

There are several challenges associated with HIV testing:

- 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. However, people have limited access to this testing.
- 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)

Enzyme Immunoassays (EIAs)

EIA is a quantitative assay that measure HIV antibodies. Most EIAs can detect antibodies to HIV-1 and HIV-2. Here is how Enzyme Immunoassay works.

- 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.

Multiple factors can affect testing such as skilled lab technician, large volume testing, and properly maintained equipment. A certain level of technical skill AND functioning equipment is a must.

HIV Rapid Tests

HIV rapid tests are qualitative assays that detect HIV antibodies. Most of them can detect HIV 1 and HIV 2. These tests are as reliable as EIAs.

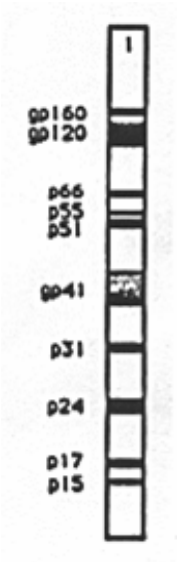
One advantage of HIV rapid tests is its ability to use whole blood. While HIV rapid tests in general are considered to be low in complexity, all tests must be appropriately evaluated prior to use and personnel be properly trained. It is equally important that the test be validated for use in the environment where testing will occur.

Western Blot / Line Immunoassays

The Western Blot is a supplemental test for confirming HIV infection. It detects antibodies to specific HIV antigens on cellulose strip.

key issues involved include:

- 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.



HIV p24 Antigen

HIV p24 antigen is the core protein of the virus. EIA detects p24 antigen before antibody can be detected. p24 is usually detected 2 to 3 weeks after HIV infection, and detected about 6 days before antibody tests become reactive.

A modified p24 antigen assay is used for diagnosis of pediatric HIV-1 infections and blood bank safety (high incidence countries).

The issues for this type of testing include high level of complexity (i.e., level 4) and equipment used for this testing must be properly maintained.

CD4 T-Lymphocyte

CD4 T-lymphocyte counts are used for determining clinical prognosis, assessing criteria for antiretroviral therapy, and monitoring therapy. CD4 counts may be performed using either manual or automated methods.

Performing CD4 T-lymphocyte counts requires high level of technical skill for test performance and interpretation. It is also very important to have properly maintained instruments such as the BD FACSCount, and BD FACSCalibur.

Viral Load

This is a quantitative molecular assay that measures amount of HIV in blood products. The higher the viral load (number of copies of HIV in the blood), the greater the progression of the disease.

This assay is used to predict disease progression, assist with deciding when to initiate anti-retroviral therapy, and monitor response to anti-retrovirals.

A number of Issues exist with this test:

- 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

Complexity of HIV Tests Varies

Four 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.

- Level 1: No additional equipment and little or no laboratory experience needed
- Level 2: Reagent preparation or a multi-step process is required; centrifugation or optimal equipment
- Level 3: Specific skills such as diluting are required
- Level 4: Equipment and trained laboratory technician are required

HIV rapid testing provides excellent tool for expansion of services. The remaining module will focus on HIV rapid tests.

HIV Rapid Tests: Advantages

HIV rapid tests have the following advantages:

- Increases access to prevention (VCT) and interventions (PMTCT)
- Supports increased number of testing sites
- Same-day diagnosis and counseling
- Robust and easy to use
- Test time under 30 minutes
- Most require no refrigeration
- None or one reagent (a substance used in a chemical reaction to detect or produce other substances)
- Minimal or no equipment required
- Minimum technical skill

Information Box

Certain kits and reagents require refrigeration as specified by the manufacturers. If they are not stored according to manufacturers' instructions, the quality of the tests will be compromised.

HIV Rapid Tests: Disadvantages

HIV rapid tests also have a few disadvantages:

- Small numbers for each test run
- Quality Assurance/Quality Control at multiple sites
- Test performance varies by product
- Refrigeration 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

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.).

If clients do not obtain their HIV results, this is a missed opportunity for therapy or preventative measures.

Body Fluids Used for HIV Rapid Testing

HIV tests could be performed on a wide range of body fluids. Serum, plasma, whole blood and oral fluids are used the most. The samples used for HIV rapid testing will most likely be whole blood drawn from clients' fingertips.

Information Box

Serum, plasma, whole blood, oral fluids: what is the difference?

Serum is the liquid part of the blood without the red blood cells.

Plasma is liquid part of the blood containing an anticoagulant without the red blood cells.

Three Formats of HIV Rapid Tests

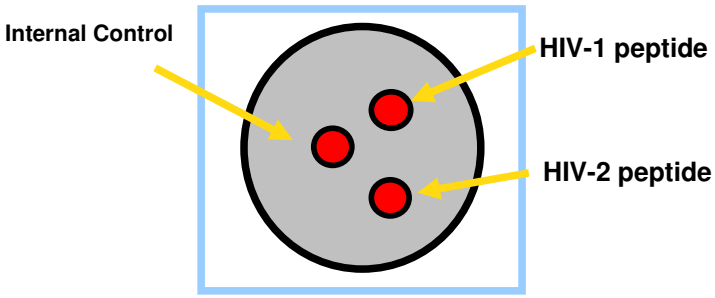
There are three main formats or types for rapid HIV tests:

- Immunoconcentration (flow-through device)
- Immunochromatography (lateral flow)
- Particle agglutination

Read on to find out more about each format.

How Immunoconcentration Works

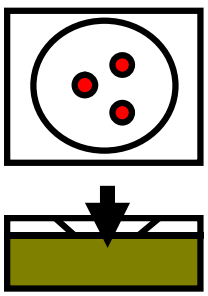
HIV antibody links to bound HIV peptide antigens forming the color spot



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.

Tests Based on Immunoconcentration

Some examples of flow-through devices are Multi-Spot and Genie II.



Reading Results: Genie II

If the result is non-reactive, you will only see one visible dot in the control region

If the result is 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

Non-reactive

Reactive

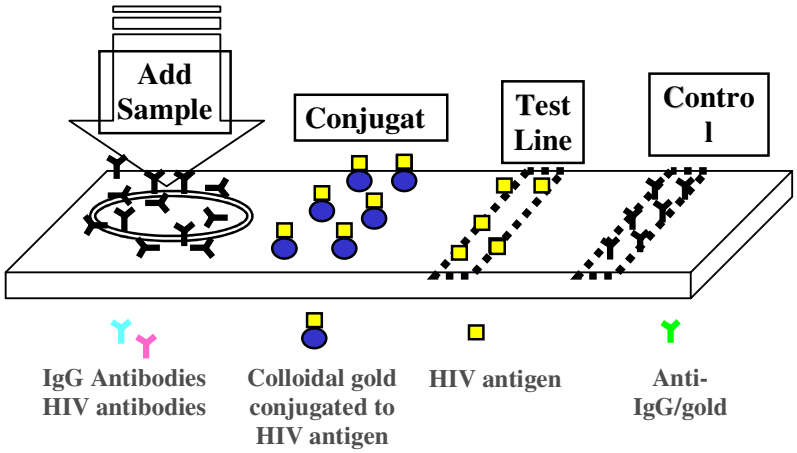
Information Box

Definition of reactive and non-reactive

Reactive means antibodies to HIV are present in the client's blood.

Non-reactive means there are no HIV antibodies detected.

How Immunochromatography Works

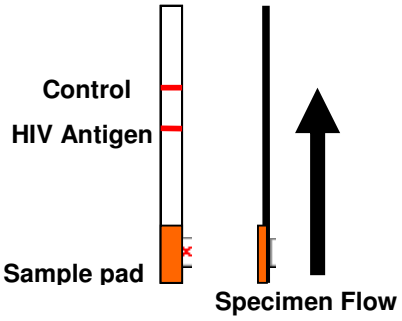


Specimen is applied to a pad (filter) where it mixes with gold or selenium colloid-antigen conjugate. This mix migrates through the nitrocellulose strip to immobilized recombinant antigens and synthetic peptides at the patient window. If HIV antibodies are present then a red line will form in the test area of the strip.

Tests Based on Immunochromatography

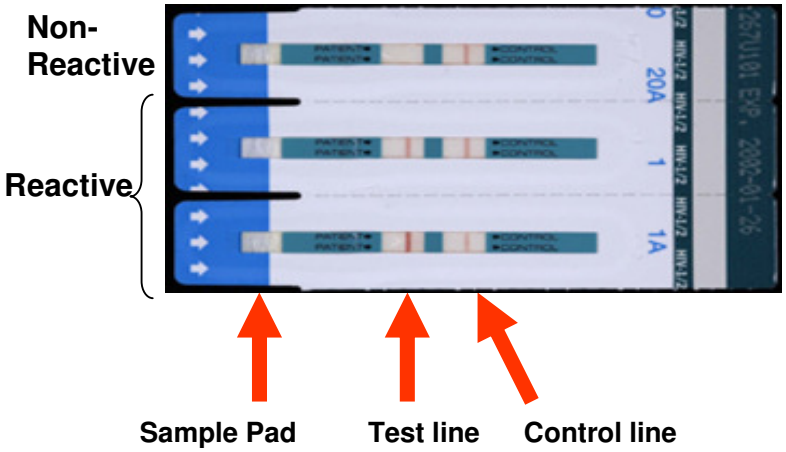
Some examples of lateral flow devices include:

- Determine
- Hema-Strip
- OraQuick
- Unigold



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.

Reading Results: Determine



The reactive reaction shows two lines: one for the control band, and 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.

Reading Results: OraQuick

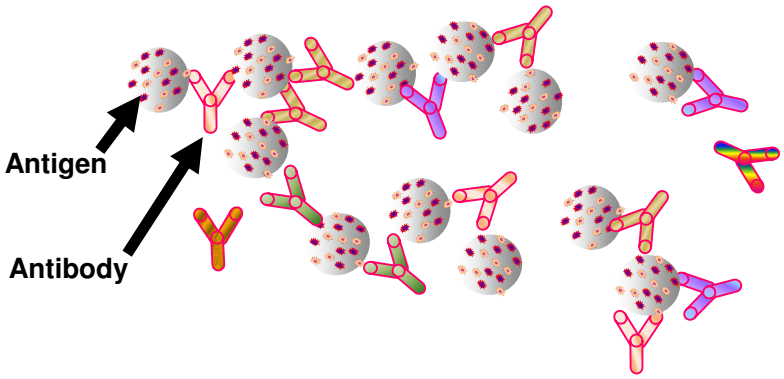
Non-Reactive

Reactive

The test device on the far left indicates a non-reactive result, while the device in the center and the one on the far right show reactive results.

How Particle Agglutination Works

Anti-HIV antibodies bind to the antigen-coated latex particles.



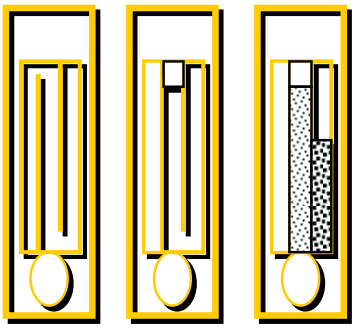
Agglutination assays were among the first of the rapid tests developed. The round circles represent antigen-coated latex particles that bind to antibodies to HIV (represented by the "Y"). Agglutination or clumping occurs when the antibodies bind to the antigen-coated particles.

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.

Tests Based on Agglutination

Examples of agglutination devices include:

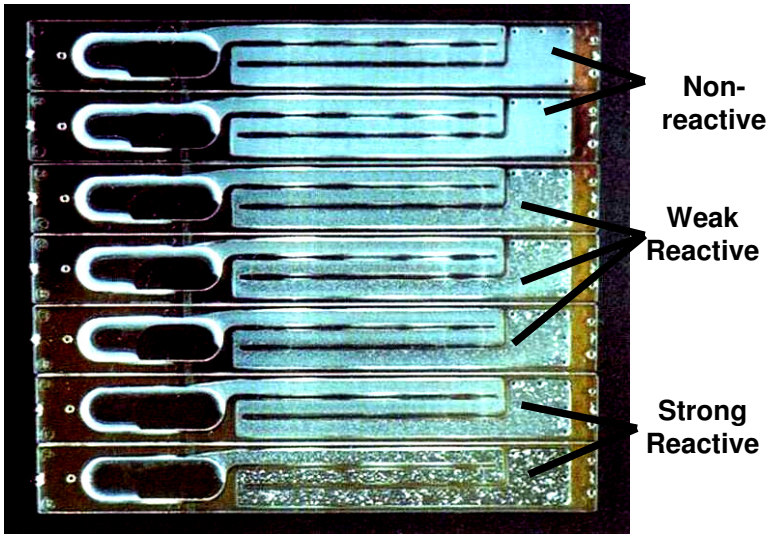
- Capillus
- Serodia



Look at the three images above:

- On the left – The blood is placed in the oval area, also called the mixing well.
- In the center – The specimen travels along the thin tubes in the slide.
- On the right – If the blood contains antibodies to the HIV virus, visible clumping or agglutination can be seen.

Reading Results: Capillus



Look at these slides. Did you notice the clumping or agglutination in the reactive sample, and the smooth liquid without clumps in the non-reactive test?

Unlike other test previously discussed, there is no control line in Capillus 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.

Besides “reactive” and “non-reactive,” there is a third possible result – the control line is not present. When the control line fails to show, it indicates that the test has failed. The result is therefore called “invalid.”

There Are Only Three Possible Outcomes for Single HIV Antibody Tests

In summary, the three possible outcomes for a single HIV antibody test are:

- Reactive or “Positive” – when both test band and control band are present.
- Non-reactive or “Negative” – when only the control band is present.
- Invalid – when no control band is present.

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

Exercises

Interpreting Individual HIV Rapid Test Results

At the end of this module, you will find two exercise sheets. Study the examples and write your interpretation of the test results in the space provided.

Key message

- HIV rapid tests can be as reliable as EIA
- All tests require attention to training, supervision, and monitoring at points of service.
- As testing is expanding and decentralized, training, supervision, and monitoring must follow accordingly and become all the more important.

Module Review

Find out how much you have learned by answering these questions.

Where is HIV rapid testing likely to occur during an era of expansion of services?_____

_____

_____

What is the intended use for:

- EIAs _____

- Western Blot _____

- p24 _____

- CD4 _____

- Viral Load _____

What are rapid tests?

_____

_____

_____

Why use rapid tests?

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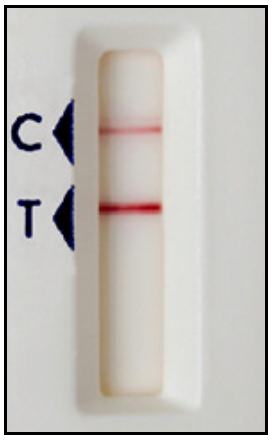
Exercise #1: Interpreting Individual HIV Rapid Tests

Instructions: **Interpret the test results in the following examples. Write your interpretation of the test result on the line provided below each example.**



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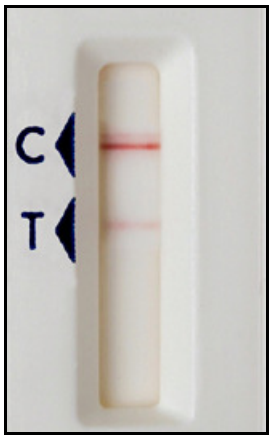

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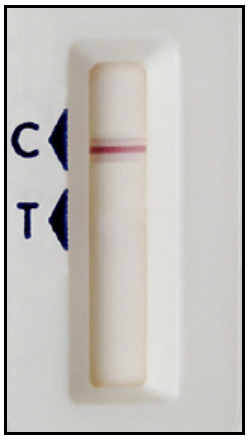

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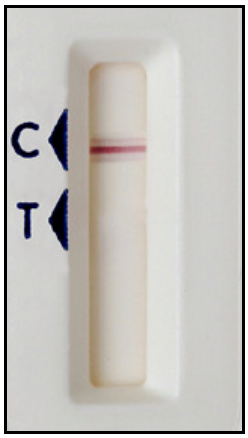

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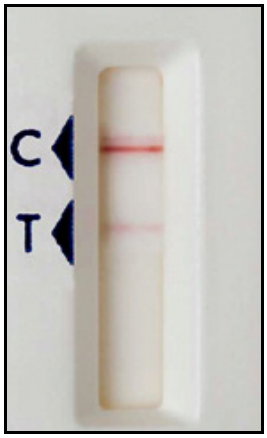

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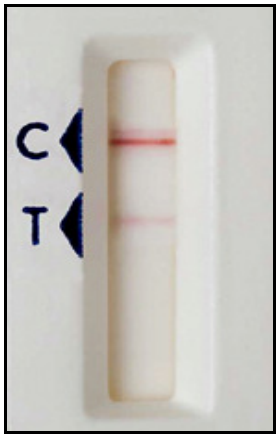

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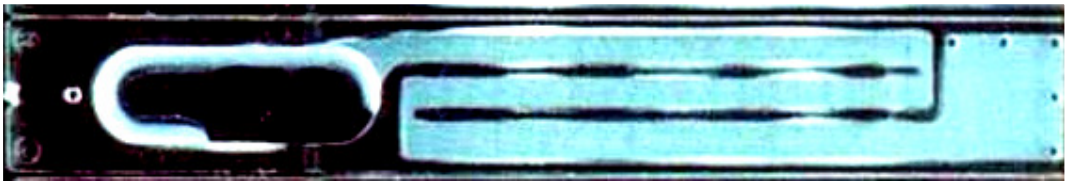
Exercise #2: Interpreting Individual HIV Rapid Tests

Instructions: **Interpret the test results in the following examples. Write your interpretation of the test result on the line provided below each example.**



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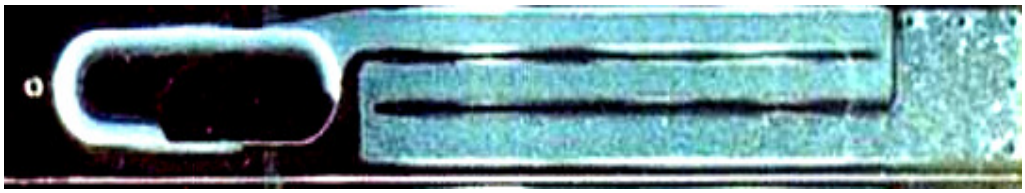

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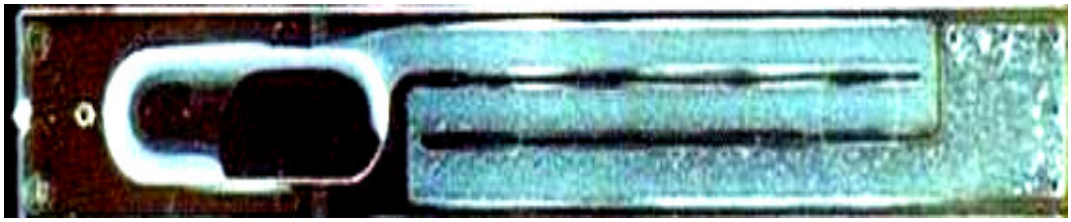

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EC.710 D-Lab: Medical Technologies for the Developing World
Spring 2010