

UNIT 6

Reagents used in the medical laboratory

Introduction

- **Laboratory Reagents** are high-purity chemicals for use in chemical analysis, chemical reactions or physical testing.
- **Reagents** and **stains** can be prepared or purchased from the market based on their feasibility.
- Reagents and stains are stored at room temperature in clean containers with leak-proof, airtight screw caps and stoppers.
- When selecting rapid tests, consider
 - price,
 - availability,
 - clarity of instructions,
 - size, stability,
 - storage requirements and expiry dates

Reagents for Stool tests

- For microscopy tests:
 - Reagents required are Saline, Eosin, Iodine
- Saline used for examination of motile parasites, e.g. cysts, red cells, pus cells.
- Iodine used for identifying cysts in stools.
- Eosin provides a pink background, which makes it easier to see the parasites.

Reagents for urine Gram stain

- Gram stain used to identify and classify bacteria by their Gram reaction.
- Reagents required are:
 - 0.5% Crystal violet
 - Iodine
 - Alcohol decoloriser and
 - Dilute neutral red.
- ***Gram positive bacteria*** stain dark purple with crystal violet and are not decolorized by alcohol.
- ***Gram negative bacteria*** stain red, as they are decolorized.

Reagents for TB

- To detect AFB (Acid Fast Bacillus) in sputum smears.
- Mycobacteria, unlike most other bacteria, do not stain well by the Gram technique.
- Reagents required are:
 - 70% Alcohol
 - strong Carbol fuchsin
 - 3% Acid alcohol and
 - 0.1% Methylene blue or 0.5% Malachite green.
- Methylene blue stains the background material, providing a contrast color against which red AFB can be seen.

Reagents for Malaria

- Staining thick and thin films. Thick films do not need fixing. Thin films require fixing with Methanol or Ethanol.
- Thick and thin film requires Giemsa stain and buffered saline.
- Thick film for detection of parasites and thin film for confirmation of plasmodium species.
- Thick and thin films can be made on the same slide or separate slides. Thin films must first be fixed then stained.

Human Chorionic Gonadotropin Hormone (HCG and pregnancy)

- HCG is a hormone secreted by placenta during pregnancy.
- Its production stimulates secretion of progesterone by the ovary.
- Laboratory tests for pregnancy are based on the detection of HCG hormone in urine.

Reagents

- Antiserum that contain HCG antibody
- Latex reagent coated with HCG
- Positive and negative controls.



HCG and pregnancy...

- There are two types of immunologic test commonly available and provided in a form of kit .

A. Rapid latex slide test: have two types:

- I. Indirect latex slide test.
- II. Direct latex slide test.

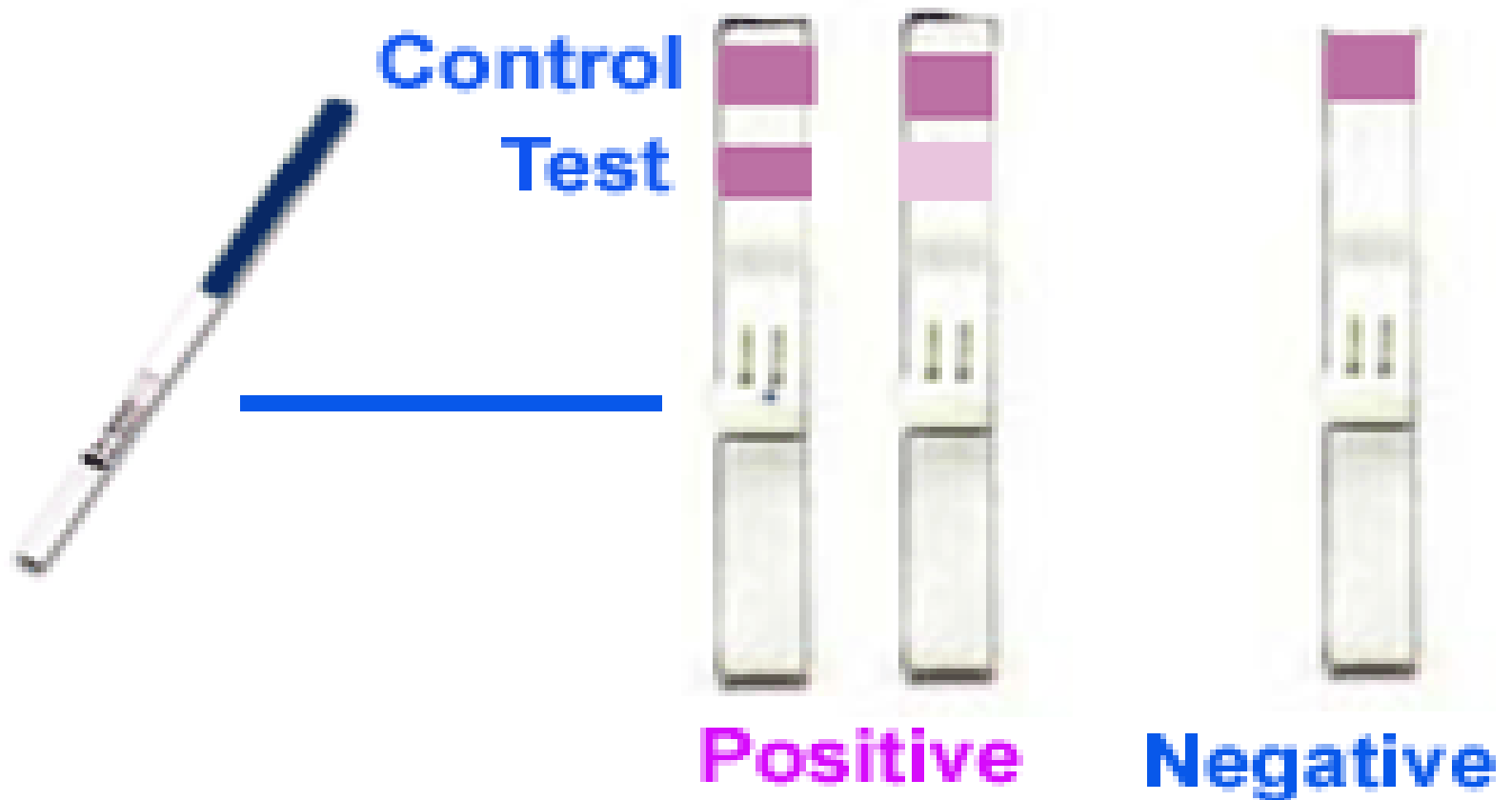
I. Indirect latex slide test

- **Principle:** Urine specimen is first treated with anti-HCG and then reacted with the latex suspension. If the urine contains HCG, the anti HCG will be neutralized and then the latter will not be available to the HCG coated latex particles for bringing about agglutination.

B. Tube test (haemagglutination inhibition technique)

HCG and pregnancy...

hCG Pregnancy Tests Results



ABO Grouping

- Determination of ABO group is necessary both for the donor as well as receiver
- The differences in human blood are due to the presence or absence of certain protein molecules called **antigens** and **antibodies**.
- The **antigens** are located on the **surface of the RBCs** and the **antibodies** are in the blood **plasma**.

ABO Grouping ...

BLOOD TYPE

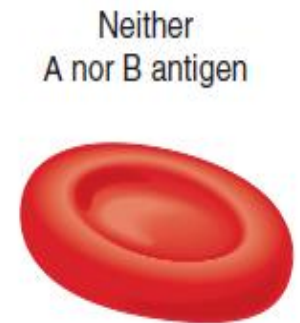
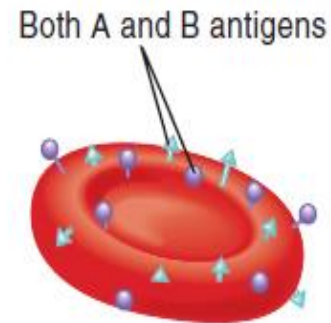
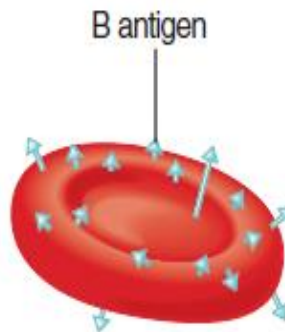
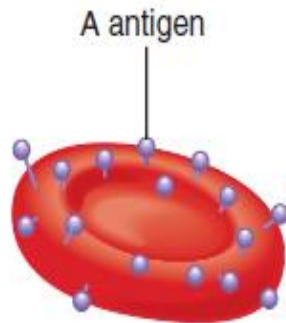
TYPE A

TYPE B

TYPE AB

TYPE O

Red blood cells



Plasma



Anti-B
antibody



Anti-A
antibody

Neither
antibody

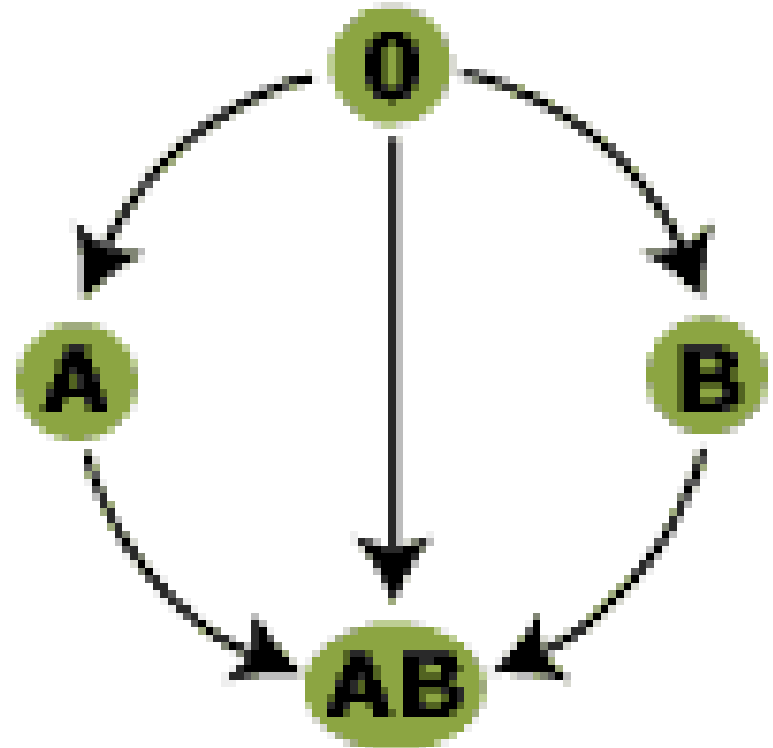


Both anti-A and
anti-B antibodies

ABO Grouping ...

Reagents

- ABO Grouping antisera: Anti-A, Anti-B and Anti-AB
- ABO Control cells (10% suspension): A cells , B cells O cells
- Anti-D antiserum (complete)
- **Blood transfusions
who can receive blood
from whom?**



Venereal Disease Research Laboratory (VDRL) Test

- Serological testing, for the diagnosis of syphilis,
- Antigen prepared from normal tissues, most commonly beef heart.
- **Principle:** heat inactivated serum is mixed with a buffered saline suspension of cardiolipin–lecithin–cholesterol antigen. This serum-antigen mixture is microscopically examined for flocculation
- **Reagents include:**
 - VDRL antigen
 - Buffer saline Diluent
 - Positive control serum
 - Negative control serum

VDRL Test...

LIMITATIONS:

- ❖ The VDRL procedure is not specific for syphilis but may demonstrate positive reactions in other:
 - reagin-producing disorders,
 - infectious disease and
 - alterations such as pregnancy or aging in normal physiology.

Widal Test

- Widal test is a serological test, which is commonly used to diagnose typhoid and paratyphoid fever.
- The patient's serum is tested for O and H antibodies.

Reagents include:

- S. typhi "O" Antigen suspension
- S. typhi "H" Antigen suspension
- Polyspecific positive control

Weil-Felix Test

- A Weil Felix test is a type of agglutination test which detects ***anti-rickettsial antibodies*** in patient's serum.
- Weil-Felix test is based on cross-reactions which occur between antibodies produced in acute rickettsial infections with antigens of OX (OX 19, OX 2, and OXK) Strains of *Proteus* species.
- Weil Felix test has similar principle and procedure with Widal test .

Limitation of Weil Felix test:

- Both Sensitivity and specificity of weil-felix test is low,

HIV Tests

There are two categories of HIV tests:

1. Antibody tests (e.g., ELISA, simple/rapid, saliva and urine, and Western blot)
2. Virologic tests (e.g., HIV antigen test, PCR, and viral culture).

Spectrum of HIV testing ...

HIV diagnosis (Antibody/Antigen detection)

- Enzyme Immunoassays (EIAs)
- Rapid tests
- Western blot (WB)

HIV Tests...

- **Challenges of HIV Testing**

- -Early detection of seroconversion
- -Early detection in infants born to HIV positive mothers
- -Effect of HIV subtypes on test performance
- -Impact of other health conditions on test performance
- -Product specific equipment
- -Technical skill

- **Antibody Tests**

- -HIV antibody tests look for antibodies against HIV.
- -Most commonly used antibody tests are EIA or ELISA, including the rapid HIV test.
- -The less commonly used is Western blot

HIV Tests...

- **Enzyme Immunoassays (EIAs)**
 - Quantitative assay to measure HIV antibodies
 - Most detect antibodies to HIV-1 and HIV-2
 - Antigens coated in microwells
 - HIV Antigen / Antibody reaction is detected by color change
 - Intensity of color reflects amount of antibody present serum
- Some assays can detect both HIV antibody and HIV antigen
- Issues:
 - Skilled lab technician
 - Large volume testing
 - Properly maintained equipment required
 - Initial cost

HIV Tests...

ELISA for HIV antibody



Microplate ELISA for HIV antibody: colored wells indicate reactivity

HIV Tests...

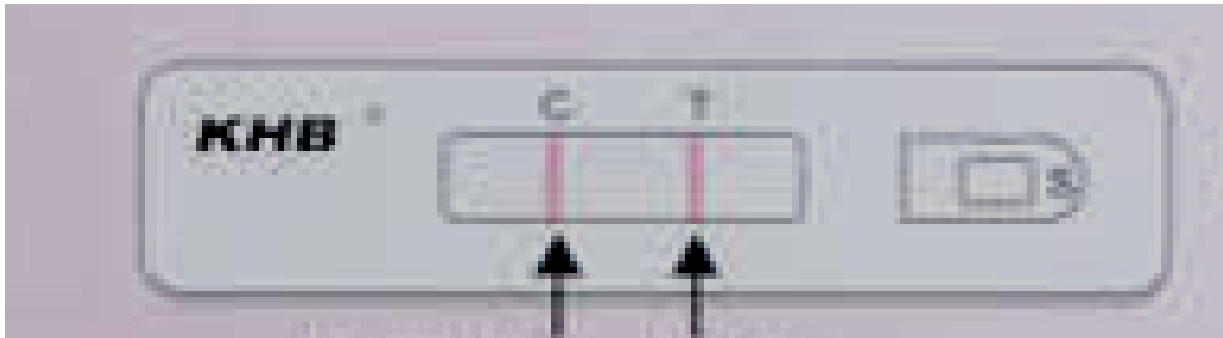
HIV Rapid Tests

- Qualitative assay to detect Ab from 5-30 minutes
- Most detect HIV 1 and HIV 2
- As reliable as EIAs
- Issues:
 - Small volumes
 - Validation of use
 - Appropriate training

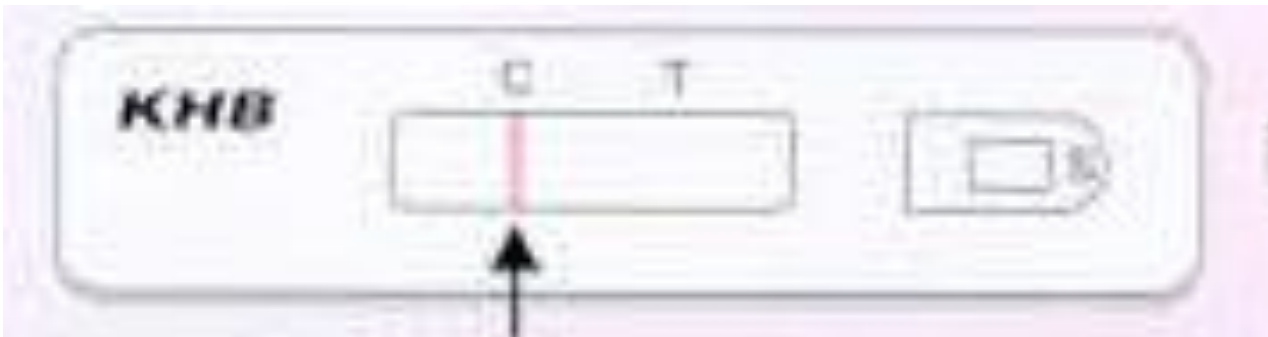
HIV Tests...

Rapid Test Kits used in Ethiopia

KHB



Reactive result



Non-Reactive result

HIV Tests...



Invalid result

HIV Tests...

STAT-PAK



Reactive



Non-reactive



Invalid result

HIV Tests...

UNI-GOLD



Reactive



Non-reactive



Invalid result