

Introduction to the principles
and procedures of various tests
performed in blood Chemistry

INTRODUCTION

The laboratory for the diagnosis of Diseases using HUMAN specimens such as Stool, Urine, Blood, Sputum and Swabs like Highly vaginal swab (HVS), requires a personnel who is very Wise and Smart because it's from the Laboratory results where the Clinician decides to which drug(s) and under what Dose will be effective to cure the disease a patient suffered from.


Witness is a key important things to consider when diagnosing diseases, Reporting or Working in the lab.



IMPORTANT NOTE

Working as a team in Hospital place such that a clinicians, nurses, pharmacists, attendants, secretary, cashiers, surgeon, e.t.c and the Lab Personnel works in a cooperative manner will increase the Quality of the Results obtained from the lab.

The **Quality of the sample** collected by Somebody else that sometimes the lab personnel not participated will depends on the relationship between the lab and other areas like the Wards.



GENERAL OVERVIEW

Remember;

70% of **MEDICAL DECISIONS**
depends on **LABORATORY RESULTS**.

- Serological tests are Quick Tests which should be carried out Carefully.
- Non Serological tests may be Short, Moderate or Long processes depends on the type of test performed.

GENERAL OVERVIEW

- ❑ Each test must be performed under a **STERILE** Environment/place.
- ❑ To achieve this;
 - i. Make sure you separate the Samples according to Type and the Source of the sample.
 - ii. Use a specific Bench or Station for a Specific Samples.
 - iii. Perform each test carefully, make sure you don't Mix up samples.
 - iv. Samples from the same Patient must have the same Patient ID, and Every Subsample should contain the ID from the Main Sample. (Eg. 5a, 5b, 5c from main sample ID 5).
 - v. Follow the Procedures of each Test Carefully...!

LABORATORY LAYOUT

The laboratory layout must fulfill at least Minimum requirements for construction to Prevent Contaminations and Protection of the Environment and the Personnel as the samples may contain **Infectious Materials**.

NB;

ALWAYS WEAR PPE (Personal Protective Gears)

Be Extra Carefully.....!!!!!!

LABORATORY LAYOUT



LABORATORY LAYOUT

General laboratory Units.

- **RECEPTION.**

This includes;

- i. Sample receiving and registration
- ii. Sample collection (Eg. Blood sample) and
- iii. Provision of sample containers (eg. Stool, Urine and Sputum containers) to the patients

SPECIMEN SUBMISSION FORM....!!!?

LABORATORY LAYOUT

▪ LABORATORY TESTS UNIT

This is also subdivided in to different SECTIONS depends on the type/mode of Test performed and the Nature of the Specimens handling.

This includes;

- i. Serology/BT section
- ii. Clinical Chemistry (Biochemistry) section.
- iii. Haematology Section
- iv. Parasitology section and
- v. Microbiology section

LABORATORY LAYOUT

SAMPLE PROCESSING AREA



LABORATORY LAYOUT

▪ STORAGE UNIT

Samples and Reagents are normally stored depending on their Storage Conditions such as Temperature, Humidity, Pressure,...

This includes the storage of;

- I. Reagents (Buffers, Solutions e.t.c)
- II. Some Instruments/ Equipments and
- III. Laboratory Documents

MATERIAL SAFETY DATA SHEAT(MSDS).!



SEROLOGY/BT SECTION
LABORATORY TESTS
PERFORMED

RBG

This is a Direct **Blood Glucose test**.
Instrument used can be **GlucOPlus**.



HB

This is the direct method of
Haemoglobin Determination.

Instrument used can be **HemoCue 201+**

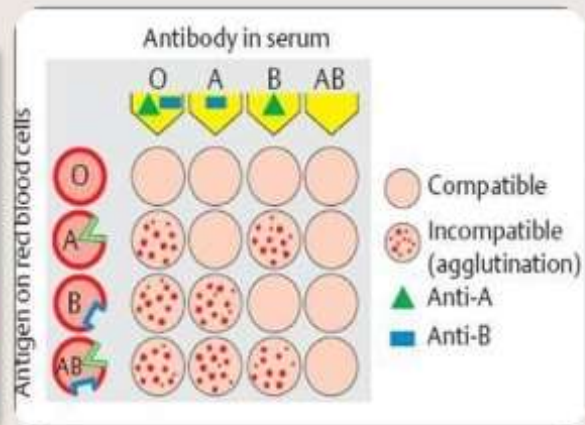


BG & X-MATCH

Blood Grouping and Typing using **ABO system (Antisera) in a Tile.**



Major and/or Minor **Cross Matching in a Tile or Test Tube.**



RF-TEST

This stands for **Rheumatoid Factor** test.

USE; **RF-Reagent** (Milky) and a **BLACK tile.**



POSITIVE REACTION:



NEGATIVE REACTION:

ELITex Bicolor RF:
Milky agglutinates on a blue background.



ELITex Bicolor RF:
Homogeneous purple suspension.

WAALER-ROSE Bicolor:
Brown agglutinates on a blue background



WAALER-ROSE Bicolor:
Homogeneous green-brown suspension

INTERPRETATION:
Presence of Rheumatoid Factor

INTERPRETATION:
Absence of Rheumatoid Factor

WT-TEST

This stands for **WIDAL TEST** which is the test for *Salmonella spp.* In **Serum. (Typhoid)**, Using Direct Aggl. Test. Use; **Salmonella Reagents (Antigen "O", "H",)** Using a Sterile Tile.



BRUCELLA-TEST

This is a Blood test for **Brucellosis**.

Use; **Brucella Reagent** and a sterile tile



mRDT/BS-Test

This is a **Malaria Parasite** detection by Rapid diagnostic tests.

Use. **mRDT** and a **Buffer** (Whole blood)



mRDT/BS-Test

This is a **Malaria Parasite** detection by Rapid diagnostic tests.

Use. **mRDT** and a **Buffer** (Whole blood)

OR;

B/S_____Prepare a **blood smear** for Parasite (*P. falciparum, e.t.c*)

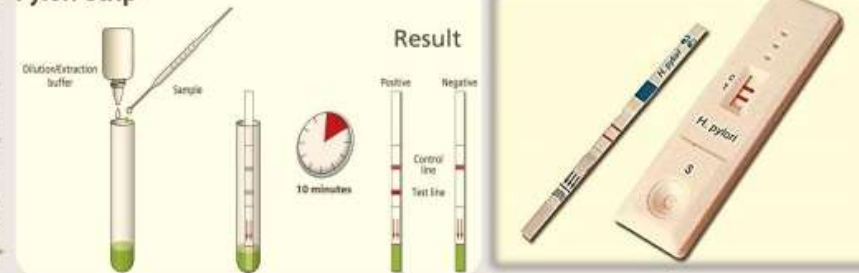
Identification and Count..!? By **Using Compound Microscope.**

HP-TEST

This is a rapid test for the detection of *Helicobacter pylori* antigens from the Serum/Plasma ie. In case of **Peptic Ulcers**.

USE. H.Pylori Ab Rapid test Strip

Pylori-Strip



VDRL-TEST

This stands for Venereal Disease
Research Laboratory test (A rapid Blood test
for *Treponema pallidum*)-**Syphilis**

Use; **SD BIOLINE Syphilis 3.0**



PITC-TEST

Rapid test for **HIV** (**Human Immunodeficiency Virus**).

Use; **SD BIOLINE HIV 1/2 3.0**



HBsAg-TEST

Tests for **Hepatitis B surface antigen** in Human Serum/Plasma cases of Systemic Disease primarily-Liver.

Use. **HBsAg Rapid Test Strip.**



HCV-TEST

This is a Test for **Hepatitis C virus** that looks for the genetic material (RNA) or Antibodies against the virus. **(Liver Problems leads to Inflammation)**

Use. **HCV Rapid Test Strip.**



UPT-TEST

This is a rapid test for testing
**PREGNANCE using Urine (Urine
Pregnancy Test)/ HCG test**





CLINICAL CHEMISTRY SECTION
LABORATORY TESTS
PERFORMED

CLINICAL CHEMISTRY

**AUTOMATED
(ROBOTIZED)
MACHINES**

**Follow the
Protocols
SOPs**

**SEMI-
AUTOMATED
MACHINES**

**Prepare Solutions
and Reagents
According to
SOPs**

STANDARD OPERATING PROCEDURE (SOPs)...!?

CLINICAL CHEMISTRY

Common Instruments;

- i. **Screen Master 3000 Analyzer.**
- ii. **Cobas Integra 400 plus and Rochel platform**
- iii. Water Bath
- iv. Lab Rotator ____ For Mixing.
- v. Centrifuge
- vi. Spectrophotometer
- vii. Roller Mixer.....e.t.c

CLINICAL CHEMISTRY

SCREEN MASTER 3000 ANALYZER.



COBAS INTEGRA 400 PLUS, ROCHEL PLATFORM.



LFT AND/OR RFT

Tests Performed under this unit includes;

- i. **LFT___LIVER FUNCTION TESTS.**
- ii. **RFT___RENAL FUNCTION TESTS.**

EXAMPLES.

- Creatinine Kinase, Urea/BUN
- Lipid Profile ie...Cholesterol Level, Triglycerides e.t.c
- Albumin, Glucose, Lactate.....!
- **ALAT___ALANINE TRANSAMINASE.**
- **ASAT___ASPARTATE TRANSAMINASE.**
- **ALP___Alkaline Phosphatase**
- **ACP___Acidic Phosphatase.**
- E.t.c

E.S.R (sed rate test)

This stands for **ERYTHROCYTE
SEDIMENTATION RATE.**

Use; **ESR Tubes, Wax and ESR Stand**



URINALYSIS

Urine Chemistry and Urine sediments are Commonly analyzed by using Disposable Strips and Microscopy simultaneously.

Equipment's that may be Used are;

- ❖ Disposable Strips (Multi-stick strips)
- ❖ Urine Chemistry Analyzer Machine (CYBROW)
- ❖ Centrifuge, Urine Test Tubes e.t.c

URINALYSIS

DISPOSABLE STRIPS.



CYBROW MACHINE.

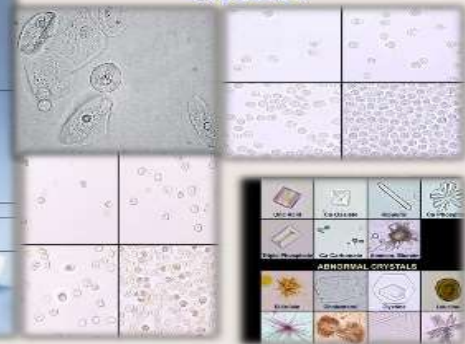


URINALYSIS

MICROSCOPE



SOME FEATURES SEEN





HAEMATOLOGY SECTION
LABORATORY TESTS
PERFORMED

CBC/FBP

Measurement of all **Haematological Parameters**. Results can be Printed..!

CBC__COMPLETE BLOOD COUNT.

FBP___FULL BLOOD PICTURE.

This may involve other accessories like;

- i. **Computer System**
- ii. **Printer**
- iii. **Roller Mixer**
- iv. **E.t.c**

CBC/FBP

Instruments that may be used are;

- ❑ **ABX Micros 60**
- ❑ **Cell Dyn 3500/3700 analyzer's**
- ❑ **Accessories ie. Computer system**

These instruments can Display all Parameters and Send the information's to the Computing system for **Printing** and **Graphical representations** of Cell Counts/Differentials..

CBC/FBP

ABX MICROS 60



CELL DYN ANALYZER





PARASITOLOGY SECTION
LABORATORY TESTS
PERFORMED

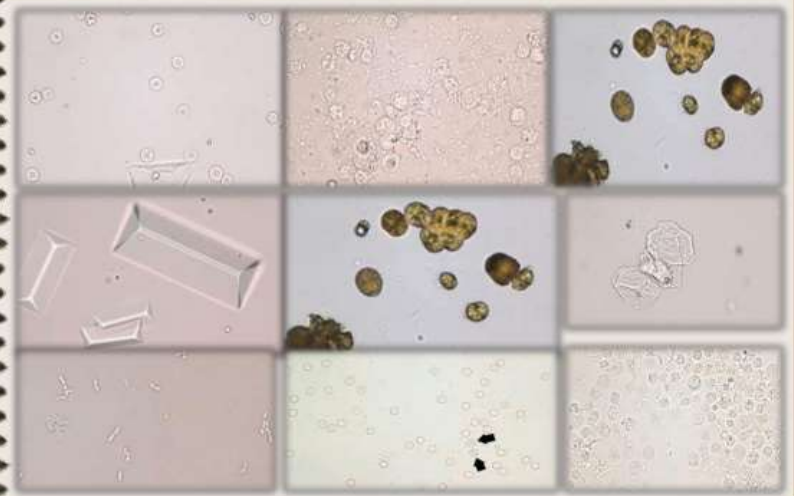
URINE SED.

Urinalysis and Urine Sediments in Parasitology are performed to observe the presence of either Eggs, Larva or Adults from Worms like *Schistosoma haematobium* and presence of Abnormal Cells due to the parasitic, Fungal or Bacterial infections...!

Use;

Microscope, Microscopic slides, cover slip, and a Light Microscope

URINE SED.



STOOL ANALYSIS

Analysis of stool for Parasitic infection
example Microscopic examination of Eggs
from worms, Proglottids, Pus cells, MQs, Fat
Droplets and some Protozoa.

Requirements

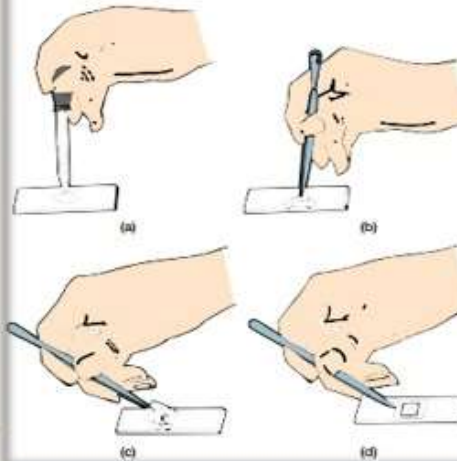
- Light Microscope
- Spreaders (Sticks)
- NS _____ NORMAL SALINE
- Microscopic Slides and Cover slips

Wet Preparation.....!!!!?

STOOL ANALYSIS

WET PREPARATION

water. (c) Place the edge of a coverslip on the slide so that it touches the edge of the water. (d) Slowly lower the coverslip to prevent forming and trapping air bubbles.



Wts this?

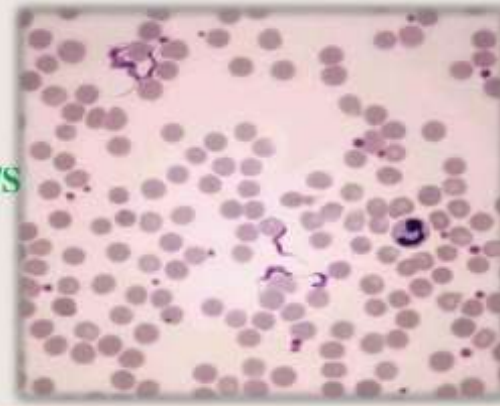


BLOOD ANALYSIS

Some parasites such as Filarial Worms/ Extracellular parasites and Most Protozoa (Intracellular Parasites) can be detected from the Peripheral Blood of an infected individual.

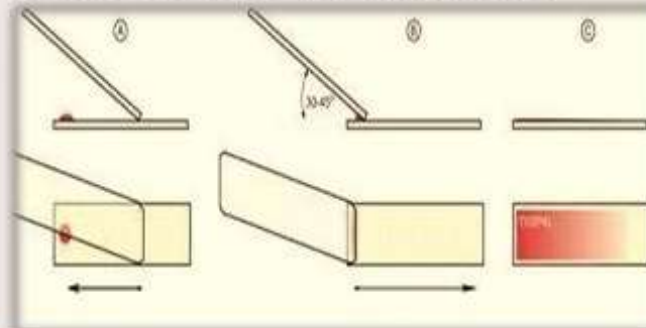
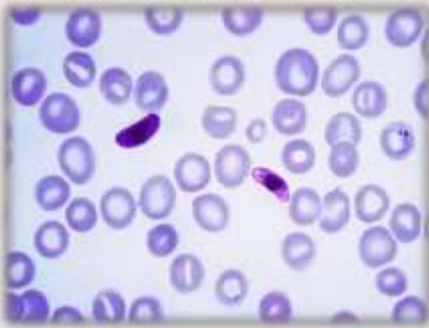
Tests for;

- MALARIA PARASITES (B/S)
- Trypanosomes
- Leishmania and
- Other Blood Parasites



B/S

BLOOD SMEAR preparation, that can either be **WET, THICK** or **THIN**, followed by Staining ie. **GIEMSA'S Stain**. Mostly used in the detection and counting of Malaria Parasites per Volume of Blood used...e.t.c





MICROBIOLOGY SECTION
LABORATORY TESTS
PERFORMED

CLINICAL MICROBIOLOGY

In Microbiology, what is Most Commonly done is **ISOLATION**, **IDENTIFICATION** and **REPORTING** of Pathogenic Microorganisms of Clinical Importance Eg. **AFB** and **Enterobacteriaceae**

Microorganisms Can be cultured in a Plate/Broth for Identification using **Automated machines** or Trained Eyes then **Staining** (Mostly **Gram's stain** and **ZN-Stain**).

CLINICAL MICROBIOLOGY

**BD BacT/ALERT
3D system**



**MICROBIAL
GROWTH IN A
PLATE**



CLINICAL MICROBIOLOGY

Samples collected for Diagnostic
Microbiology includes;

- i. Blood and CSF
- ii. **Sputum**
- iii. Rectal Swab
- iv. **Vaginal swab**_____HVS
- v. Stool
- vi. Urine
- vii. Other Swabs.....E.t.c



MICROBIAL CULTURE

By using specific **MEDIA** for a particular **SUSPECTED** Microorganism from a Clinical Specimens, Perform a Microbial Culture.

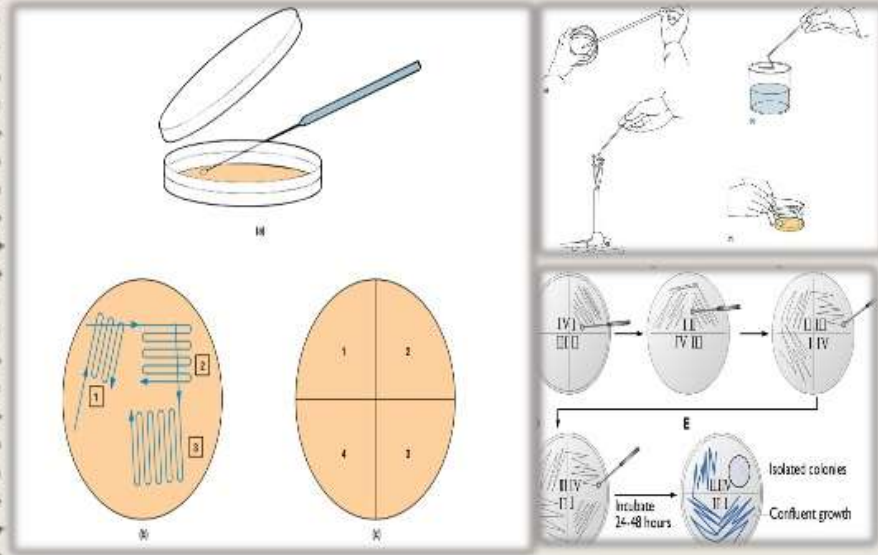
EXAMPLES;

- ❑ **STOOL Culture,**
- ❑ **URINE Culture,**
- ❑ **BLOOD Culture and**
- ❑ **SPUTUM + SWABS Culture.**



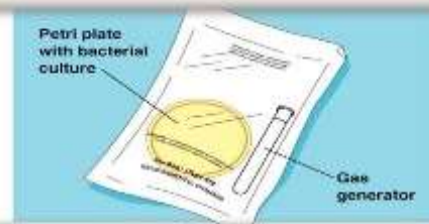
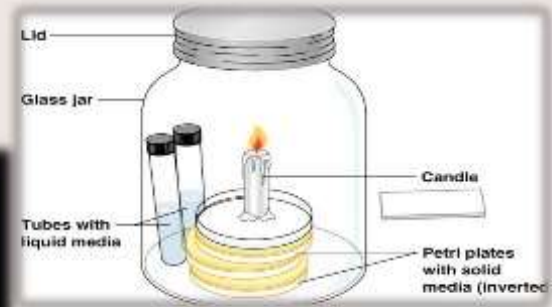
Maintain The Microbial Growth Condition...!?

MICROBIAL CULTURE



MICROBIAL CULTURE

ANAEROBIC ENVIRONMENTS



SUB CULTURE



(a) With a wax pencil, label the medium to be inoculated



(b) Shake the primary culture tube to suspend the bacteria



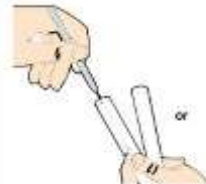
(c) Place both tubes in the palm of one hand to form a V



(d) Flame the inoculating loop or needle along full length



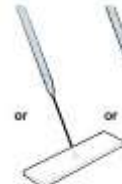
(e) Remove the caps from the tubes and flame the necks of the tubes. Do not place the caps on the lab bench



(f) Cool the loop or needle and pick up bacteria



Streak the surface of a slant



Place the bacteria on slide



Streak the bacteria on petri plate



(g) Reflame the neck of the tubes



(h) Recap the tubes



(i) Reflame the loop or needle

ISOLATION OF PURE CULTURE

1. Growth of *Pseudomonas aeruginosa* on Cetrimide agar
2. Growth of *Staphylococcus aureus* on nutrient agar
3. Growth of *Staphylococcus aureus* on Mannitol salt agar
4. Growth of *Escherichia coli* (green sheen) on Eosin methylene blue agar
5. Growth of *Klebsiella pneumoniae* on Eosin methylene blue agar
6. Growth of *Streptococcus* spp on blood agar
7. Growth of *Salmonella* spp on Salmonella- Shigella agar
8. Growth of *Escherichia coli* on MacConkey agar
9. Growth of *Shigella* spp on Salmonella-

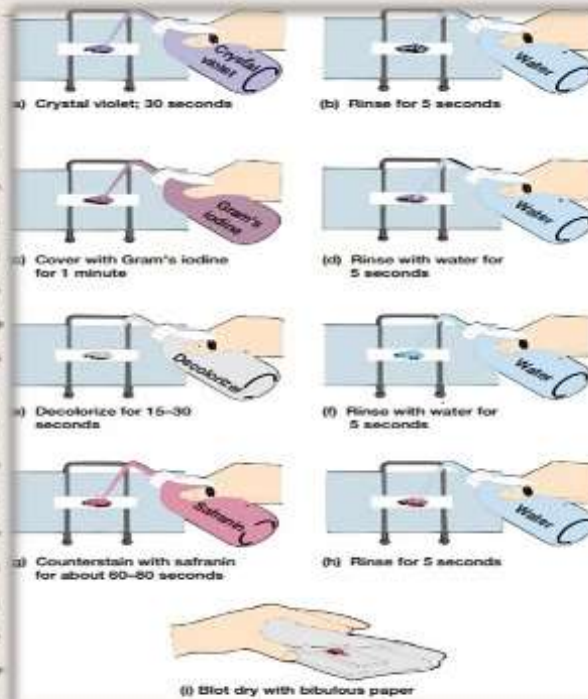


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9. Growth of *Shigella* spp on Salmonella- Shigella



GRAM'S STAIN

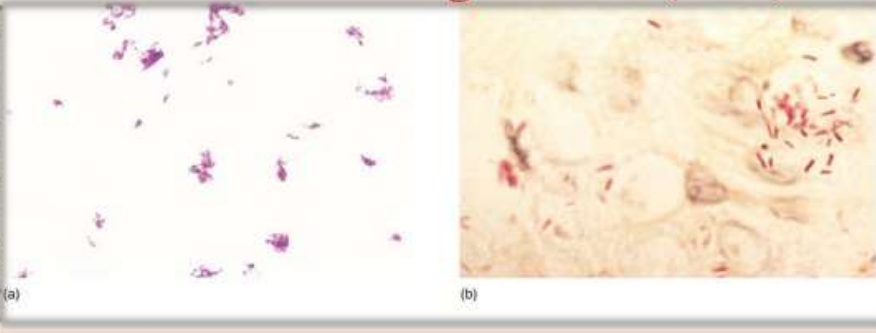


NB;
STANDARD
OPERATING
PROCEDURES
ARE
THERE...!!!

PRINCIPLES.?

AFB / ZN STAIN

In a given **SPUTUM sample**, the Mostly suspected organisms are *Pneumococcus* spp. And *Mycobacterium tuberculosis* Which is the one of **Acid Fasting Bacteria (AFB)**.



Ziehl-Neelsen (ZN STAIN)



(a) Apply carbolfuchsin to saturate paper and heat for 5 minutes in an exhaust hood



(b) Cool and rinse with water for 30 seconds



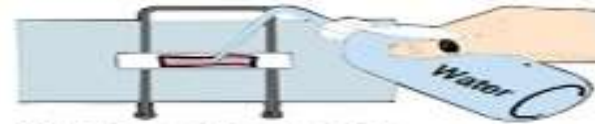
(c) Decolorize with acid-alcohol until pink (10-30 seconds)



(d) Rinse with water for 5 seconds



(e) Counterstain with methylene blue for about 2 minutes

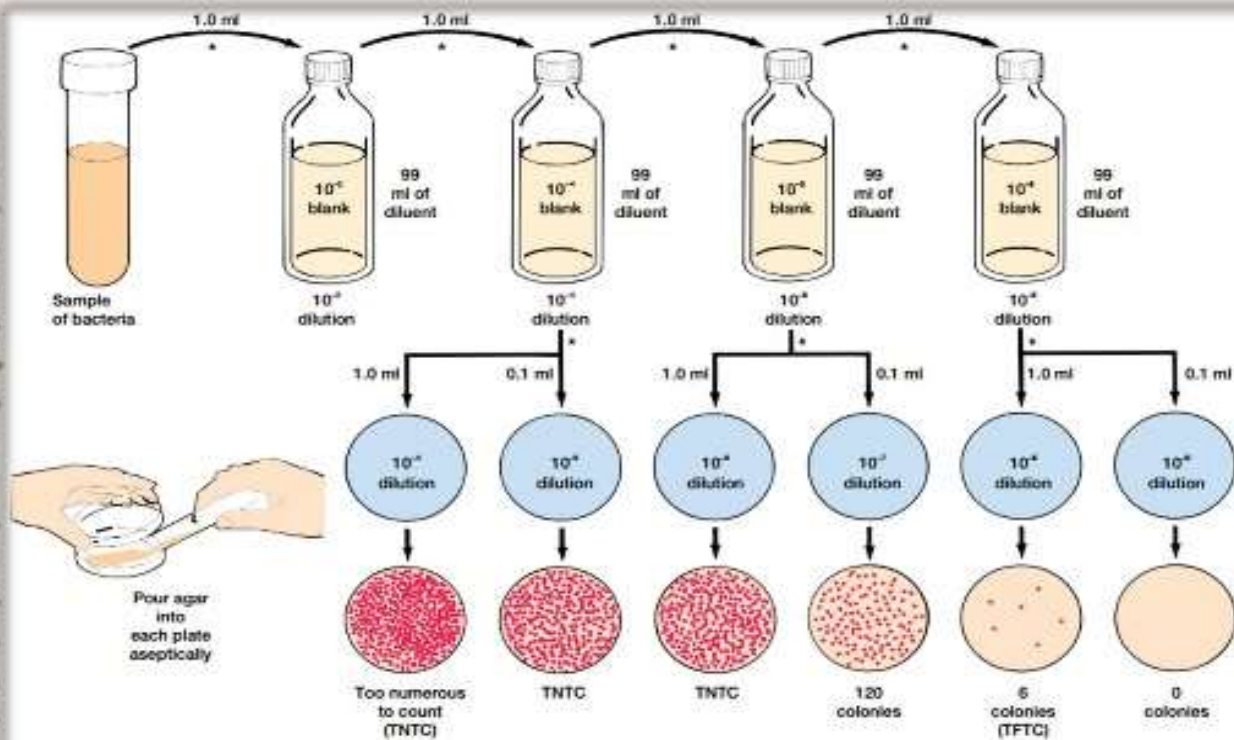


(f) Rinse with water for 30 seconds



(g) Blot dry with bibulous paper

QUANTITATIVE METHOD



*Discard pipette after each transfer.

BIOCHEMICAL TESTS

If happens that, you need to perform a biochemical test for further identification though it's very rare, you may use your Microbiology knowledge based on;

- Available methods
- Laboratory capacity and
- Purpose

The most common tests are;

**Triple sugar iron test (TSI), IMViC Test,
Coagulase and urea**

TRIPLE SUGAR IRON REACTION (TSI)

Interpretation. (a) The tube on the left has a yellow butt (acid), red slant (alkaline), H₂S production as indicated by blackening of the agar, and no gas production. (b) The tube on the right shows no H₂S formation, a yellow slant (acid), gas production, and an acid butt. Note that the gas on the bottom has lifted the agar.



(a) (b)

	Tube a	Tube b
Slant	K	A
Butt	A	A
Gas	-	+
H ₂ S	+	-

Their Interpretation. (a) The tube on the left has a red butt (alkaline), red slant (alkaline), and no acid or H₂S production. (b) The tube on the right has a yellow slant (acid), yellow butt (acid), and no gas or H₂S production.

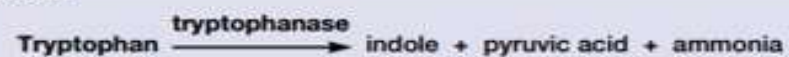


(a) (b)

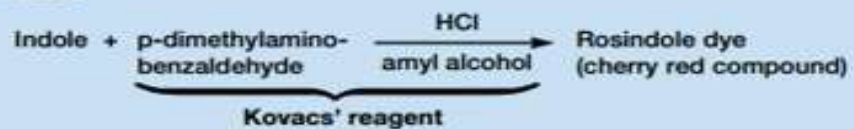
	Tube a	Tube b
Slant	K	A
Butt	K	A
Gas	-	-
H ₂ S	-	-

INDOLE TEST (IMViC)

Biochemistry within bacteria



Biochemistry within tubes



Kovacs' reagent

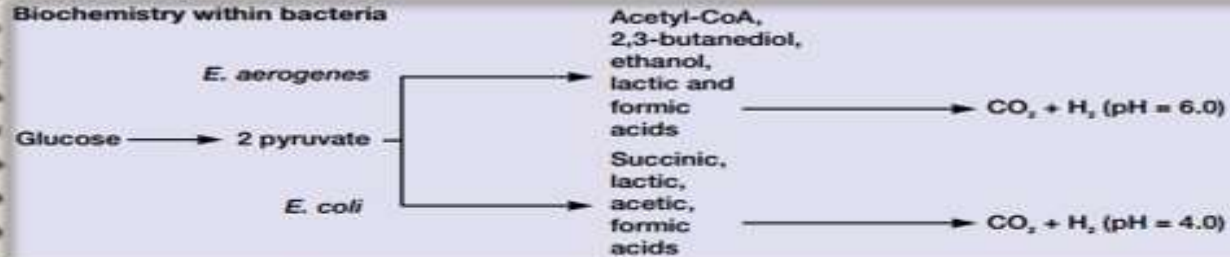


Indole -

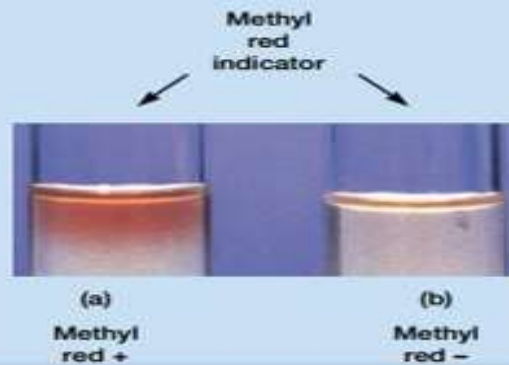
Indole +

METHYL RED TEST (MR)

Biochemistry within bacteria



Biochemistry within tubes



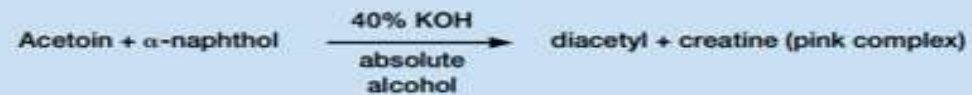
Biochemical Test of Staphylococcus aureus

Voges-Proskauer (VP) Test

Biochemistry within bacteria



Biochemistry within tubes



Barritt's reagent



(a)

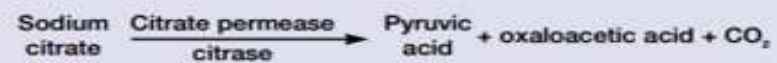
VP+

(b)

VP-

CITRATE TEST (IMViC)

Biochemistry within bacteria



Biochemistry within tubes



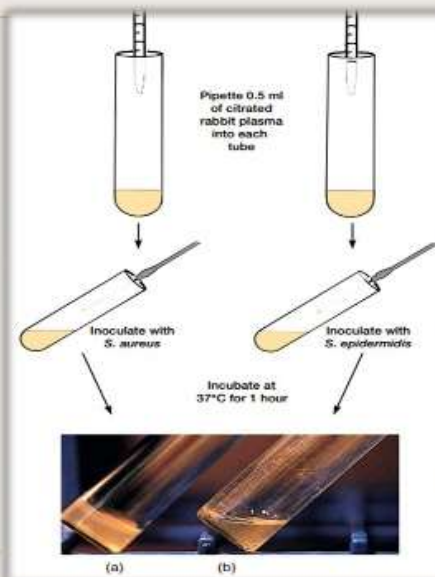
(a)

(b)

Citrate +

Citrate -

COAGULASE TEST



INTERPRETATION

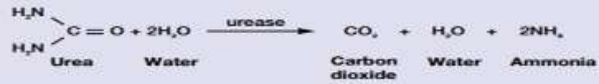
a) TUBE = +VE

b) TUBE = -VE

Observe; **Precipitation in Tube (a)**

UREA HYDROLYSIS TEST

Biochemistry within bacteria



Biochemistry within tubes

Ammonia + phenol red → deep pink



(a)

(b)

(c)

(d)

API 20E MANUAL



(a)

All tests: positive



(b)

All tests: negative

ANTIMICROBIAL SUSCEPTIBILITY TEST

DILUTION TESTS

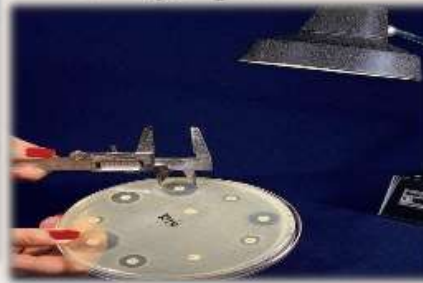
- ❖ Broth microdilution
- ❖ Agar dilution test
- ❖ McFarland turbidity

Step	Resulting Organism Concentration
Standardize suspension to McFarland 0.5	1.5×10^8 CFU/mL
Add 0.75 mL from step 1 to 25 mL water diluent (1:33 dilution)	$4-5 \times 10^6$ CFU/mL
Use inoculator prong set to inoculate wells of MIC tray (each prong delivers 0.01 mL, which results in an additional 1:100 dilution)	$4-5 \times 10^4$ CFU/100- μ L well
	$4-5 \times 10^8$ CFU/mL

DISK DIFFUSION TESTING

(**Kirby-bauer Test**)

MEDIA; Mueller-Hinton agar plate



ANTIMICROBIAL SUSCEPTIBILITY TEST



FIGURE 5-2 The Bacteric susceptibility on a sheep blood agar plate. *Staphylococcus aureus* (A) is on top and *Staphylococcus aureus* (B) is on the bottom.

VIRAL LOAD

This is basically refers to the measure of the number of viral particles esp. **HIV viruses** in a bloodstream.

Therefore;

Viral load is the term used to describe the amount of HIV in the Blood.

For the HIV Diagnosis, two things are important;

- CD4+ COUNT AND
- VIRAL LOAD

CD 4+ COUNT

The **CD4+** count and **Viral load** are very important especially in providing the informations about;

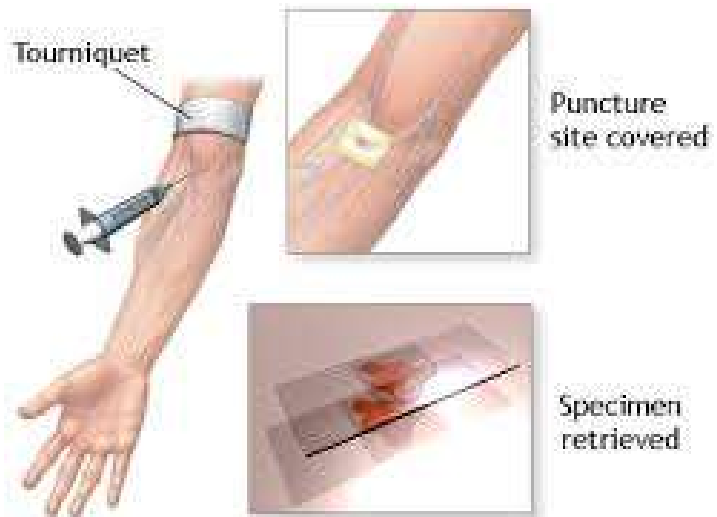
- I. The health of the immune system of a Patient.
- II. The progression of HIV in their body
- III. How their Body responds to HIV Therapy
- IV. How the Virus itself responds to HIV Therapy

Collect Blood Samples from those individuals and prepare a SERUM/PLASMA...!?

Infectious Materials.....USE PPE!!!!

A **false positive** is an error in binary classification in which a test result incorrectly indicates the presence of a condition such as a disease when the disease is not present, while a **false negative** is the opposite error where the test result incorrectly fails to indicate the presence of a condition when it is present.

	Test says you don't have it	Test says you do have it
You really don't have it	TRUE NEGATIVE	FALSE POSITIVE
You really do have it	FALSE NEGATIVE	TRUE POSITIVE



ADAM

Total WBC Count Test Procedure

url: www.kitstronglit.com | Email: info@kitstronglit.com

WBC Count Calculation:

$$\text{Total WBC (count) / mm}^3 = \frac{\text{Total Count} \times 10}{\text{Total Count} \div 4,000}$$



**SEE ALSO
OTHER EQUIPMENS**

BSL

FUME HOOD

BIOSAFETY
CABINET



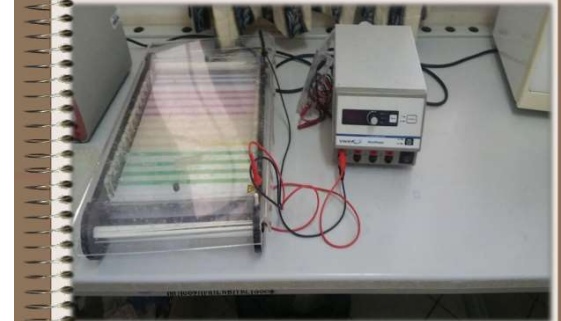
REFRIGERATORS



INCUBATOR



GEL ELECTROPHORESIS



CENTRIFUGE



TRASH



Segregation of Waste in color coded Bags

YELLOW BAGS	RED BAGS	BLUE BAGS	BLACK CARBOY
Infectious waste, bandage, syringes, cotton or any other things in contact with body fluids, human body parts, placenta	Plastic waste such as catheters, syringes, needles, scalpels, scalpels, scalpels, scalpels, scalpels	All types of glass bottles and broken glass articles, used and discarded medicines	Needles without syringes, blades, sharps and all metal articles



IMPORTANT SYMBOLS



TRASH

5 Ways of Treating Medical Waste

Incineration



Typically for pathological waste, and pharmaceutical waste. Never for plastics.

Thermal (Autoclaving)



Typically for sharps waste, and infectious waste. Never for pathological waste.

Non-Incineration Systems

Irradiative (Microwave)



Typically for sharps waste, and infectious waste. Never for pathological waste.

Chemical



Typically for chemical waste, and liquid waste (e.g. generated from laboratory cleaning).

Biological (Enzymes)



Undeveloped and rarely used technology for medical waste disposal.

BioMedicalWasteSolutions.com/Medical-Waste-Disposal/

TRASH



DON'T PUT THESE IN THE SHARPS CONTAINER



Medication*



Medication Wrappers



Aerosols or Inhalers



Garbage



Batteries of Any Type



Cauterizers



Liquids



Fluorescein



Hazardous and Chemical Waste



Radioactive Waste



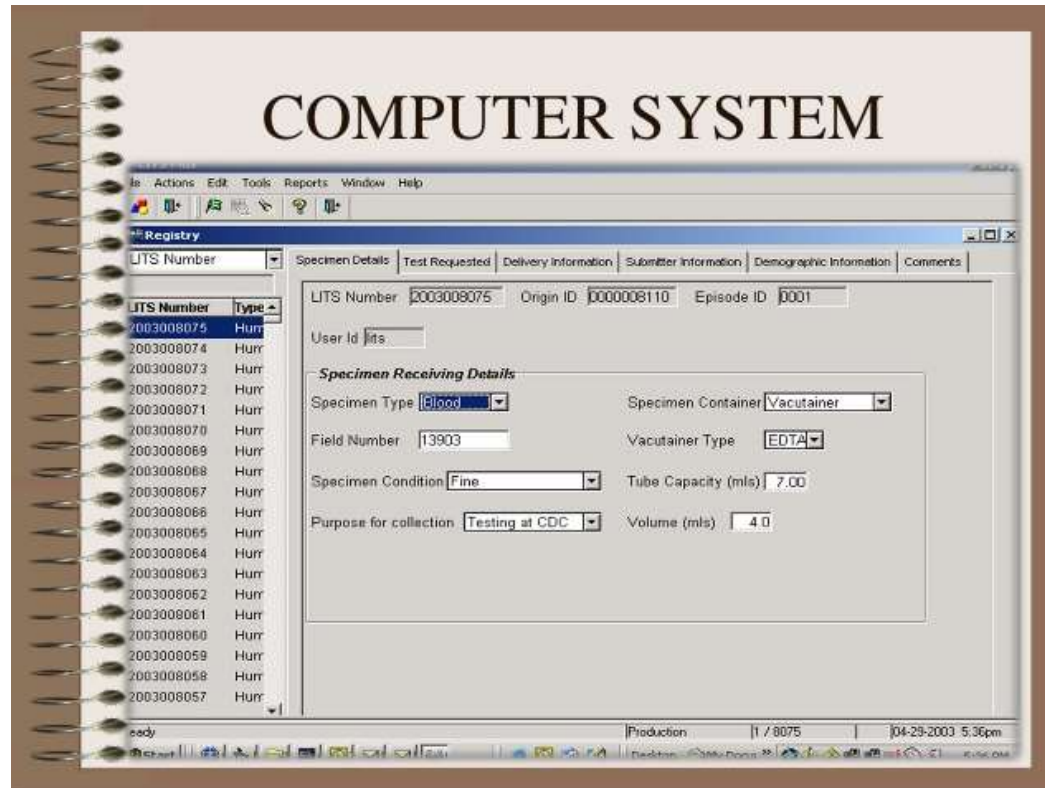
Fixatives and Preservatives



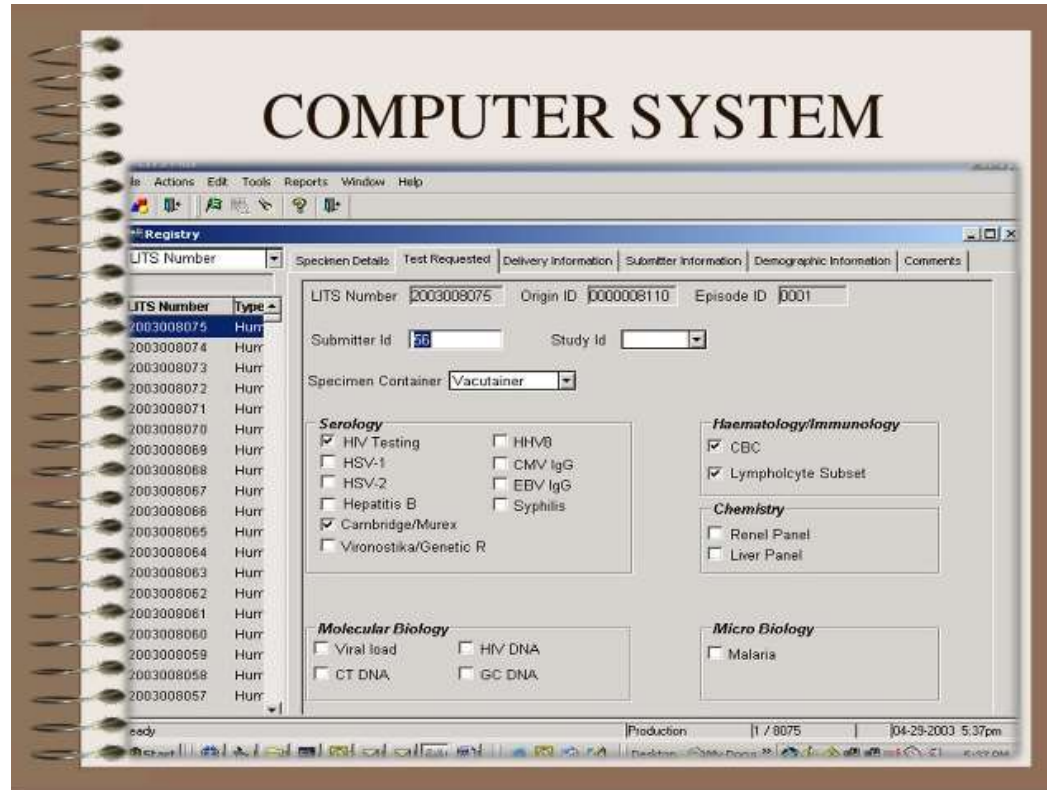
Red Bag Waste
(non-sharps regulated medical waste)

*Please follow all federal, state, and local regulations.

COMPUTER SYSTEM



COMPUTER SYSTEM



REPORTING OF RESULTS

- ❖ Final report goes to **Physician**.
- ❖ The **VALIDITY** of this report is dependent upon:
 - i. Appropriateness of specimen
 - ii. Proper collection and adequacy of specimen
 - iii. Appropriate transport to lab
 - iv. Use of media of known quality
 - v. Culture and isolation by knowledgeable personnel using equipment known to be operating correctly
 - vi. Confirmation by tests of known quality
 - vii. Results interpreted and reported by professional staff
 - viii. No transcription or computer errors