LABORATORY MANUAL FOR BEGINNERS AND TECHNICIANS

A STUDY GUIDE FOR LABORATORY TECHNOLOGIST AND TECHNICIANS IN CLINIC AND HOSPITALS

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DEDICATION

This book is dedicated to God Almighty for his wisdom and knowledge. It is also dedicated to my mentor in medical science; Louis Agboro (Dr.) and staffs of Cleve-hill Clinic, Cape-land Clinic, Ever-well Clinic and Maternity and all students who desire knowledge.

PREFACE

This text titled **"Laboratory Manual for Beginners and Technicians (A study guide for technologist and technicians)"** is for the development of the skill set of technicians and technologist of **Medical Laboratory Science (MLS)** and **Science Laboratory Technology (SLT)** who may be opportune to dabble into the world of medical science in the course of their provisional industrial training program. It contains carefully researched theories of medical test; ranging from Hematology, Microbiology and In-vivo assay analysis. The text contains detailed explanation on the parameters of diagnosis with alternative methods for laboratories with less industrious equipment and facilities.

The author has gone extra mile to show practical of the diagnostic test imbedded in this text in his personal YouTube channel with step by step procedures with detailed explanation. Therefore, the link

http://m.youtube.com/channel/UCMHoxW5bcVMsOn2OzsIYoHA is a YouTube channel created to further help widen the social interaction among students and like-minds.

It is important to note that some of the methods explained in this text may not meet up with the required standard in diagnostic laboratories. However, outlining the basic biochemical principle of these analyses, the method employed by the author meets 75 to 90% of the required laboratory standard for Hematology and Microbiological analysis. However, constructive criticism of the methods employed will be greatly appreciated for the enhancement of subsequent volumes.

Students who have this material are expected to make other research on any of the contained analysis on www.microbesonline.com and other relevant text.

Odiakose Chifunanya. Hillary (Biochem. HND)
Laboratory instructor and Technologist

TABLE OF CONTENT

INTRODUCTION

SAFETY PRACTICE IN THE LABORATORY

1. Never mouth pipette. Use safe measuring and dispensing devices.
2. Do not eat drink, smoke, store food or apply cosmetics in the working area of the laboratory.
3. Use aseptic techniques when handling specimens and culture.
4. Always wash the hands after handling infectious materials, before leaving the lab and before attending to patients. Cover any open wound with a waterproof dressing.
5. Wear appropriate protective clothing (Lab coat) when working in the lab. Ensure it is decontaminated and clean.
6. Wear protective gloves, and when indicated a face mask and hear net, for all procedures involving direct contact with infectious materials. When wearing gloves, the hands should be washed with the gloves on particularly before using the phone or clerical work.
7. Centrifuge safely to avoid creating aerosols.
8. Avoid practice that could lead to needle-stick injury.
9. Do not use chipped or cracked glass wares and always deal with breakage immediately and safely.
10. Avoid spillage at the barest minimal. Work neatly and keep the bench free of unnecessary materials.
11. Decontaminate working surface at the end of each day's work and following any spillage of infectious fluid, knowing what to do when a spillage occurs.
12. Report immediately to the laboratory officer in charge, any spillage or other accident involving exposure to infectious materials and also breakage.
13. Know how to decontaminate specimen and other infectious material.
14. Do not allow unauthorized persons to enter the working areas of the laboratory.
15. Use and control the autoclave correctly…. etc

CHEMICAL REAGENTS

The following are some of the chemical reagent used in the laboratory.

1. **METHYLENE BLUE:** Is used as a counter stain for Acid Fast Bacilli (AFB).
2. **METHANOL OR METHYLATED SPIRIT:** Is used as a disinfectant
3. **ETHANOL AND ACETONE:** Used as a fixing or decolorizing agent.
4. **CRYSTAL VIOLET:** Is used as the primary stain in gram staining
5. **CARBON FUCSHIN:** Is used as the primary stain for AFB
6. **SAFARIN OR NEUTRAL RED:** Is used as the counter stain in gram staining and endospore staining (Neutral red only).
7. **FIELD STAIN A & FIELD STAIN B:** Is used in staining malaria parasite.
8. **INDIA INK:** Is used as in capsule staining (Negative stain)
9. **ACID-ALCOHOL:** Is used as a fixing or decolorizing agent.
10. **MALACHITE GREEN:** Is used as the primary stain in endospore staining.
11. **FORMALIN:** Is used as preserving specimen from surgical operation.
12. **DISTILLED WATER:** Is used as solvent to make solution.
13. **TRIS-BUFFER:** Is used in genotype test as the mobile phase.
14. **HYDROGEN PEROXIDE:** Is used to test for catalase reaction.
15. **ANTISEPTIC:** Is used as a disinfectant.
16. **LEISHMANN:** Is used in peripheral blood staining.

COLLECTION OF SAMPLES


Lithium heparin **EDTA** **Universal sterile bottle**

BLOOD:

Blood samples can be collected from a patient in two ways;

1. Use of syringe with needle.
2. Use of capillary tube with blood lancet.

USE OF SYRINGE WITH NEEDLE

Apparatus: Syringe, wet and dry swab, tourniquet and EDTA can.

Procedure:

- Look for the appropriate vain site (usually the hand)
- Tie above the spot using the tourniquet to stabilize vein.
- Clean the area around the spot with wet swab to disinfect.
- Using a new sterile syringe, insert the needle in the stabilized vein maintaining a steady entrance'
- Draw out blood, untie and withdraw the needle.
- The blood is transferred to the labeled EDTA can.

USE OF CAPILLARY TUBE WITH BLOOD LANCET

Apparatus: Lancet, Hematocrit capillary tube, wet and dry swab.

Procedures:

- Clean the surface of the finger (preferably the ring finger because it's less sensitive and painful).
- Jab deep with a lancet and wipe the first set of blood
- Put pressure on the finger to get a small ball of blood then place your capillary tube at the point of jab.
- Blood will flow into the tube by capillary action.

SEMEN

Before samples are collected, patients (Male) are advised to produce semen after 3-7 days of abstinence and the samples are rushed to the laboratory before 30 minutes.

The patient should be advised to make sure all the samples gets into the sterile can provided (especially the first part) and if condom is to be used, it must be well washed to remove the powder that coats the rubber. However, masturbation remains the best method of collection. The patient is expected to wash his genitals properly before collection of sample.

VAGINAL SWAB SAMPLE

Before samples are collected, patients (Female) are prepped on the procedure. The Lab. technician should seek consent to touch the patient and inform her of the apparatus to be inserted into her body. The laboratory scientist is expected to make the patient to feel safe and instruct the patient on the need for her utmost cooperation during the collection procedure.

The sample is to be collected using a sterile swab stick (preferably with tube), mild antiseptic with dry swab to disinfect the area around the vaginal and a Speculum.

The speculum can either be of sterile stainless steel or sterile disposable type which is inserted at the opening of the vaginal and extended to increase the opening.

The swab stick is then removed from the tube and inserted through the speculum into the vagina till it gets to the cervix.

NOTE: THIS PROCEDURE MUST FIRST BE DONE BY A PROFESSIONAL TO SHOW YOU HOW DEEP THE SWAB SHOULD BE INSERTED.

The swab stick should then be rotated to swab the surface for discharge. It is then removed and kept back inside the tube and sent back to the laboratory immediately for microscopy and culture.

NOTES

- If the patient is in her menstrual flow, it is expected that she should be dismissed to return after her period has elapsed.
- If the patient is a virgin, the test should also be dismissed as it rules out the patient from possible STDs.
- If the patient has had sex 24hrs prior to the test, it should be noted so as not to confuse sperm cells for *T. vaginalis*. The test could be postponed to the next day if necessary.
- The patient is to abstain from sexual intercourse until diagnosis and treatment is complete and declared healthy.

URETHRAL SWAB

Before the sample, the patient is expected to wash his penis with soap and water, and dried properly.

The technician collecting the sample should ask the questions as stated in the collection for HVS sample.

A sterile swab stick should be used to collect the sample while an antiseptic swab should be used to disinfect the area surrounding the tip of the penis.

The penis should be held upright and the swab stick carefully inserted just around the opening of the urethral.

NOTE: THIS PROCEDURE MUST FIRST BE DONE BY A PROFESSIONAL TO SHOW YOU HOW DEEP THE SWAB SHOULD BE INSERTED.

URINE

The patient is provided with sterile universal bottle in which he or she urinates after washing his/her hands with soap and water, and dried with clean towel. Sample can be collected from the catheter of bedridden patient with the assistance of the nurses. The sample is taken to the lab and examined at once or stored in the lab refrigerator at $4^{o}C$ if the test is not to be done immediately. The sample MUST be allowed to room temperature before commencing the test.

PUS/WOUND

No specific instruction is required to collect this sample.

PART ONE:

HAEMATOLOGY AND PARASITOLOGY

1.1 PACKED CELL VOLUME (PCV)

Packed cell volume hematological test is the measurement of the ratio of the volume occupied by red blood cells to the volume of the whole blood after centrifugation. Centrifugation refers to the separation of macromolecules of biological samples based on their density. When anti-coagulated blood is centrifuged at standard speed, erythrocytes, which are heavier than white blood cells and plasma, will settle down at the bottom. The red cells volume is what we call Hematocrit or Packed Cell Volume (PCV). The PCV is the volume of red blood cells expressed as a percentage of the whole blood. The speed of centrifugation is utmost important as higher speed or lower speed affects immensely the outcome of the separation hence giving rise to false reading. The speed should be checked and set correctly. The voltage of the power supply can greatly affect the revolutions per minute despite the user setting the right speed. Therefore, an alternative power supply (power generator) should be used in such case given that the voltage output of the power generator can be regulated.

PROCEDURE

Use venous blood or the patient can be pricked using a blood lancet at the ring finger after it has been sterilized with methanol.

Using capillary tube, the blood is collected and sealed using heat (See https://youtu.be/DCwx2P9IMWE for demonstration) and then placed in the centrifuge rotor.

The centrifuge is then switched on to spin for 5 minutes at 10,000RPM or 15 minutes at 4000RPM after which the percentage of the red blood cells is measured using a Micro-hematocrit reader.

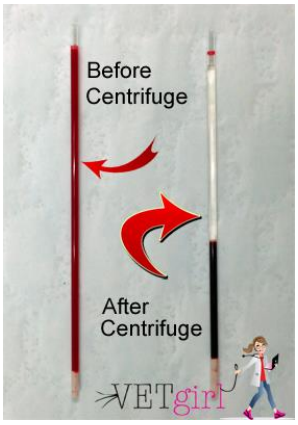

fig. 1.1a

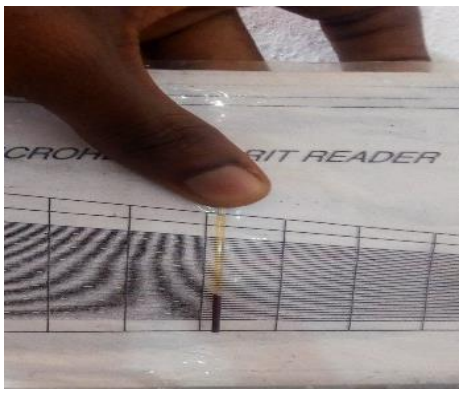

fig.1.1a

Fig.1a Capillary tube with blood sample before and after centrifugation.
Fig.1b Using a Micro-hematocrit reader to measure PCV.

Normal Range

36% - 56%

1.2 WIDAL TEST

Widal test is one of the methods that may be used to make a presumptive diagnosis of enteric fever, also known as typhoid fever, which is caused by the bacterium *Salmonella typhi*. It is an agglutination test which detects the presence of serum agglutinins (H and O) in patient serum with typhoid and paratyphoid fever. The patient's serum is tested for H and O antibodies against *S. paratyphi* (Ha, Hb, Hc, Oa, Ob, and Oc) and S. *typhi* (Hd and Od) suspensions. Although this method is easy to perform, most technicians find it difficult to be consistent in their interpretation of the reaction. This may however be subjected to the technician's discretion once certain reactions are observed.

PROCEDURE

- Collect venous blood and pour into EDTA bottle.
- Place it into the centrifuge machine and centrifuge for 5 minutes to separate the blood to get the portion known as blood serum (yellow colored supernatant) from the red cells.
- Put 1 drop each of the 4 suspensions of antigen H and O (a, b, c and d) on the tile provided in the Widal kit.
- Put 1 drop of blood serum on each drop of suspension and then using the top of a pipette or needle cap to mix it uniformly to obtain a homologous mixture.
- Spin the preparation manually (rocked) to seek out agglutination of the antigen for 2 minutes.

RESULTS

The degree of agglutination is qualitatively measured in titers of 1:20, 1:40, 1:80, 1:160 and 1:320; whereby ≥1:80 is significant for infection.

For the practical please follow this link below to access my YouTube channel to search for the practical demonstration

https://www.youtube.com/watch?v=RM2dxQpjdhQ

1.3 MALARIA TEST (MP)

Malaria is a life-threatening mosquito-borne blood disease caused by a *Plasmodium* parasite. It is transmitted by the bite of the female anopheles' mosquito. Once an infected mosquito bites a human, the parasite multiply in the host's liver before affecting and destroying the red blood cells.

In other to diagnose the severity of the disease, it is important to know that in the human body the parasite undergoes various morphological stages which may make its identification very tedious and difficult. At the end of this section you will see the various microscopic morphology of malaria cells. It is important to note that when counting the cells, parasitized cells are also included in the counting to further aid the doctor on the degree of severity.

The test involves the preparation of thick and thin film but for this text we shall only discuss the thick film because of its high percentage sensitivity to the parasite.

HOW IS THE TEST CARRIED OUT?

2ml of blood can be collected from the vain using a syringe and poured into an EDTA bottle, or 2-3 drops of blood can be collected directly from the index finger with the aid of a blood lancet.

If EDTA blood is used, mix the blood properly with the anticoagulant inside the EDTA bottle and then put 2-3 drops on a clean microscopic slide.

The blood should be smeared evenly and air dried to get a thick film.

After drying, the smear is then immersed in **Field stain A** (3-5sec), rinsed in a water jar and immersed in **Field stain B** (3-5sec) before being rinsed again and allowed to air dry.

The film is then viewed in the microscope using **X100 oil immersion objective lens.**

MICROSCOPY

What you are expected to see in this film are leucocytes (lymphocytes, neutrophils, eosinophil, monocytes), parasitized leucocytes and plasmodium parasites at different stages of their life cycle. You are expected to only count the parasitized leucocytes and the plasmodium species observed in your microscopy.

If you see cells looking like basophils, it is regarded as a parasitized cell and therefore is counted as malaria parasites. Below are examples of possible microscopic views of a thick film stain slide.

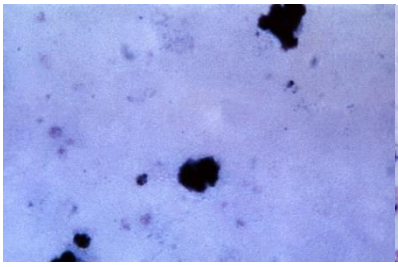

fig. 2a

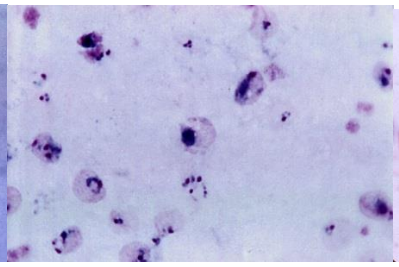

fig. 2b

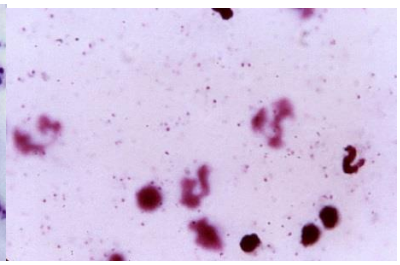

fig. 2c

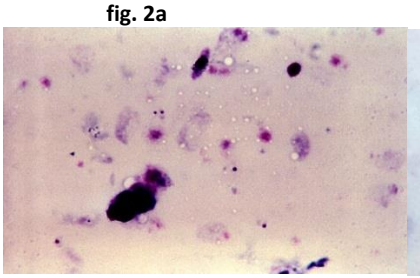

fig. 2d

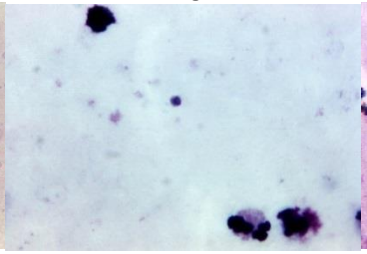

fig. 2e

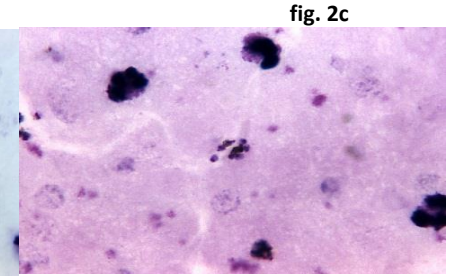

fig. 2f

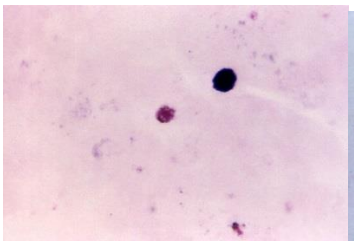

fig. 2g

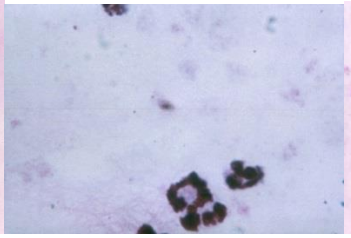

fig. 2h

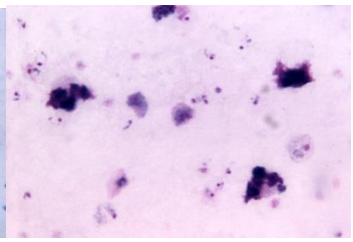

fig. 2i

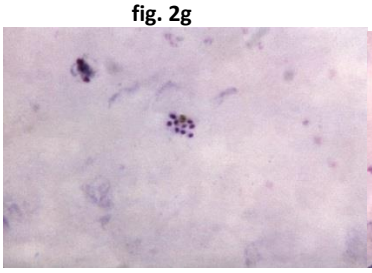

fig. 2j

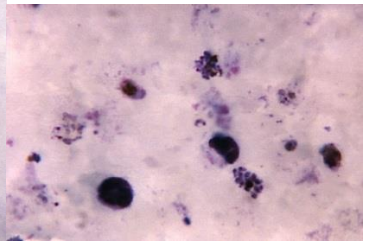

fig. 2k

fig 2a – 2k *Plasmodium spp* at different life cycle in thick blood smear. (Pictures were downloaded from https://pixnio.com/free-images/science/microscopy-images/malaria-plasmodium/)

RESULTS AND INTERPRETATION

In order to report your findings, Plasmodium or parasitized cells are recorded for every 200 cells are counted. Using a prescribed standard of 8000 cells per micro-liter of blood, the formula below is employed.

$$\frac{Parasites}{200} \times 8000$$

The unit of the result is parasite load per micro-liter of blood (p/µl).

As we well know, the plus (+) system is unreliable given that it shows no specifics on the severity of infection as well as it been a random method of reporting diagnosis. The table 1 below shows the appropriate a number of parasite per micro-liter of blood for each corresponding pluses.

PLUS	P/µl
Scanty	<40
+	40-400
++	401-4,000
+++	4,001-40,000
++++	>40,000

Table 1. Plus (+) equivalent to parasite load per µl of blood

1.4 WHITE BLOOD CELL COUNT (WBC)

This test is done to determine the number of white blood cells in the body. This test is often included with complete blood count otherwise known as full blood count. There are several types of white blood cells, and the body usually contains a percentage of each type. Sometimes, however, the WBC count can fall or rise out of health range.

WHY DO PEOPLE DO THIS TEST?

Having a higher or lower number of WBCs than normal may indicate an underlying condition. A WBC count can detect hidden infections within the body and alert doctors to undiagnosed medical conditions such as autoimmune disease, immune deficiencies and blood disorders. It also helps doctors to monitor the effectiveness of chemotherapy on patients so it is important for the technician to be very good at it.

MATERIALS NEEDED

- Venous blood (EDTA)
- Micro-pipette or 1ml insulin syringe
- Turk's solution
- Test tube
- Capillary tube
- Neubauer chamber (depth 0.1mm, 1/400mm^2)
- Cover clip

PROCEDURE

- Using micro-pipette, measure 380µl of Turk's solution and pour into a test tube. Then measure 20µl of blood and add into same test tube making a total of 400µl of solution mixture (38 units of Turk's solution and 2 units of blood if using insulin syringe).
- Mix gently and allow settling (**NB**: Avoid the formation of bubbles).
- Then prepare the Neubauer chamber by cleaning gently with dry swab and fixing a cover clip in the appropriate position.
- Using a capillary tube withdraw solution mixture from test tube and fix it on the counting area of the chamber and allow to settle for 40 to 60secs.
- View the counting chamber using **X40 objective lens with light aperture closed.**
- Count the total WBCs in the four large squares

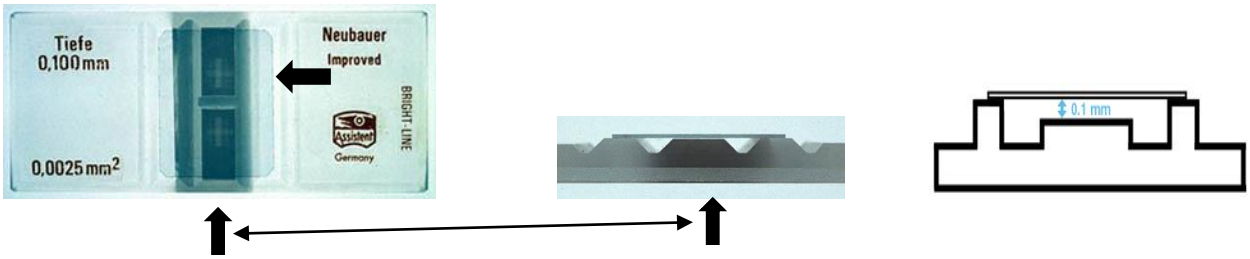

fig 3a. Neubauer chamber

The Neubauer Chamber is a special kind of microscopic slide. It comprises of two separate viewing fields indicated by the two (2) cross-like shapes seen in the first image above (L-R). The second image indicates the point at which the dilution is placed into the counting chamber of the slide after the insertion of the cover clip.

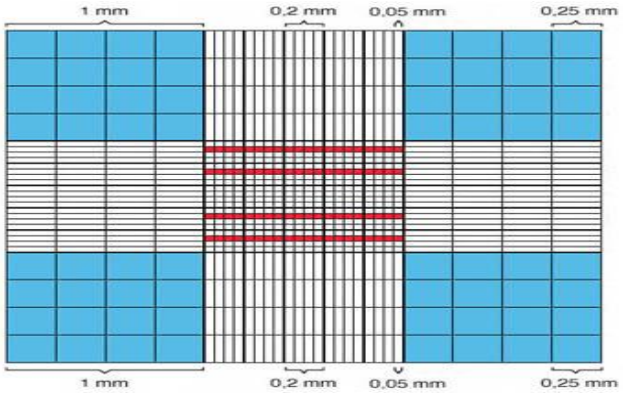

Fig 3b. Neubauer counting squares

As shown in the image above, the counting chamber comprises of nine (9) large squares of 1mm with the first, third, seventh and ninth square (4 squares) comprising of sixteen (16) smaller squares of 0.25mm each. These are the squares in which you bare expected to count the WBC of a blood sample.

Please visit my YouTube channel “Alternative microbiology” to see demonstration of this test on how to count and report White blood cell count

https://www.youtube.com/watch?v=FjG-1Ch74WI

https://www.youtube.com/watch?v=tBki311FFSA&t=115s

NORMAL RANGE

AGE RANGE	WBC's per mm^3
0-1month	9,000-30,000
1-3months	5,000-19,500
3months-2years	5,500-17,000
2-4years	5,500-15,500

Table 2. Normal ranges of white Blood cell (WBC) count

NB: Children over 2 and adults are generally from 5,000 to 10,000mm^3

1.5 PLATELET COUNT

A platelet count is a test that determines the number of platelet in a person's blood sample. They are essential for normal blood clothing. It is usually carried out to diagnose or monitor conditions that affect the number of platelet such as bleeding disorder, bone marrow disease or underlying conditions.

PROCEDURES

- 0.38ml of 1% ammonium oxalate solution (prepared by dissolving 1gm of ammonium oxalate in 100ml of DW) is added to 0.02ml of venous blood.
- The dilution mixture is then placed in the counting chamber as explained in WBC count.
- Place the Neubauer chamber now containing the dilution in a petri dish containing moistened filter paper and allow to stand for 15 minutes
- Count using X 40 objective with the light partially cut off (reduced condenser) in the squares indicated for RBC as shown below.

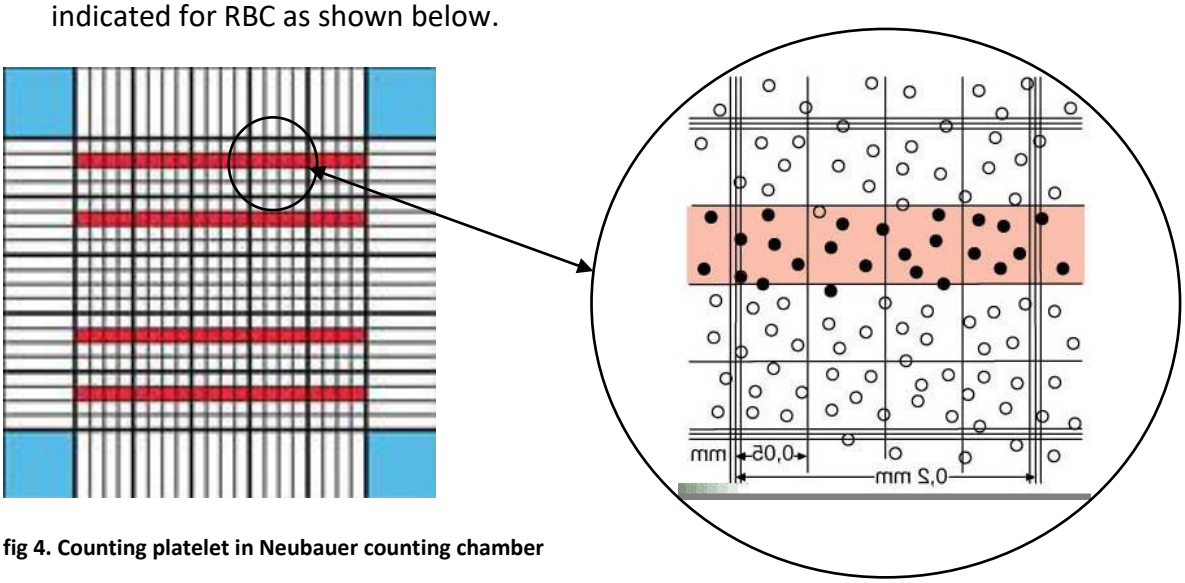

fig 4. Counting platelet in Neubauer counting chamber

- The cells found in the 80 small squares shown in the image above are counted.

NOTE: During chamber counting, all cells that lie on the left and lower border or just touch it are counted. None on the upper and right border or those that just touches it will be counted.

RESULTS

If the total cells counted are 230 for example, the total platelet count of the blood sample can be calculated by

$$Cells\ counted\ X\ 1000$$

Therefore;

$$230\ X\ 1000 = 230{,}000mm^3\ or\ 0.23\ X\ 1000000mm^3$$

Normal range is 150,000mm³ to 300,000mm³. Women are higher than men.

1.6 BLOOD GROUPING

This biochemistry diagnosis is very easy to perform by anyone and takes about 5 minutes. This test uses the principle of antigen-antibody interaction to determine the blood group/type of a given blood sample. The clumping of the blood indicates the reaction of the antibody against the antigen present in the blood sample.

Three anti-sera are required for this test. Anti-sera A, Anti-sera B and Anti-rhesus D; which helps to indicate reactions for the blood types; A, B, AB and O.

PROCEDURE:

- Using Pasteur pipette put three drops of blood separately on a blood tile.
- Put one drop of Anti-sera A on the first drop of blood and mix properly.
- Put one drop of Anti-sera B on the second drop of blood on the tile and mix properly.
- Then put one drop of Anti-Rhesus D on the third (last) drop of blood on the tile and mix properly.
- Spin/roll the mixture clockwise to observe for agglutination.

RESULTS:

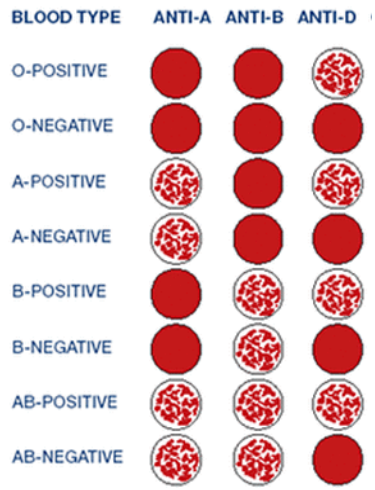

fig 5. How to read Blood group result.

1.7 HEMOGLOBIN GENOTYPE

The test implores the principle of electrophoresis on cellulose acetate paper strip which separates protein on the basic of their net charge difference and migrates towards the anode under the influence of electric field with mobility to their net charge in alkaline medium.

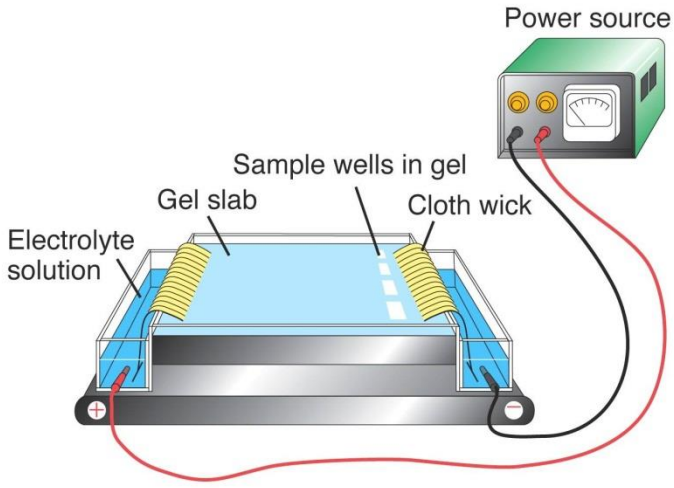

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fig 6. Electrophoresis machine for gene type

Apparatus: Cellulose acetate paper, Tris-buffer solution, blood tile, distilled water, 'AS' control gene type blood sample, a means of application of the blood sample and Electrophoresis machine.

PROCEDURES:

- Put a drop of blood test sample on the blood tile and dilute with distilled water to wash off the blood plasma.
- Do same to the 'AS' control blood sample.
- Put a reasonable quantity of Tris-buffer solution into the buffer chamber of the electrophoresis machine.
- Using a suitable applicator, immerse in diluted blood sample a make a straight make of blood at the edge of the cellulose acetate paper. Do same for the dilute 'AS' control sample at a point below the test blood sample.
- Put the acetate paper into the chamber across the well separating the cathode terminal from the anode terminal.
- Plug the machine and switch on for 5 minutes to get a result.

RESULT:

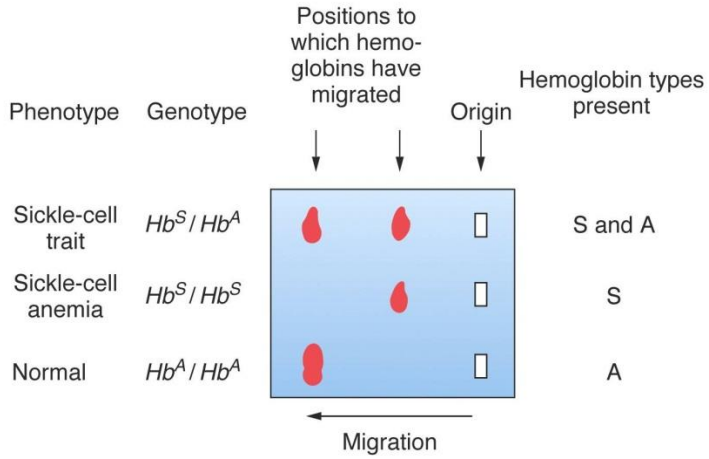


fig 7. Separation of hemoglobin after electrophoresis

1.8 CROSS-MATCHING

When a patient is suffering from any type of anemia he or she may be required to undergo blood transfusion. The procedures refer to as cross matching is done to ascertain the compatibility of the donor's blood with that of the recipient. The compatible blood types are as follows:

RECIPIENT	COMPATIBLE DONOR
A+	A+ O+
B+	B+ O+
AB+	A+ B+ AB+ O+
O+	O+
A-	A- O-
B-	B- O-
AB-	A- B- AB- O-
O-	O-

Table 3. Acceptor and Donor Blood type compatibility

PROCEDURES:

- After confirmation of the blood type, put 2ml of donor's blood and recipient's blood in EDTA bottle and centrifuge for 5 minutes.
- Screen the donor's blood for HIV (RVS), VDRL, HBSag and HCV infections.
- From the blood bag containing the donor's blood, put few drops of blood in a test tube and fill test tube with normal saline solution.
- Cork with non-absorbent swab (cotton wool) and centrifuge for 10 minutes.
- Dispense all the supernatant and add another normal saline to the test tube, cork and centrifuge for another 10 minutes.
- Repeat same procedure as previous.
- Dispense all supernatant leaving just the red cells of the donor's blood sample.
- Take drop of the red cells and place in a slide.
- Now collect one drop of the recipient blood plasma and put on the red cells on the slide.
- Mix properly and observe for agglutination.

RESULT

COMPATIBLE: No agglutination.

NON-COMPATIBLE: Presence of agglutination.

1.9 ERYTHROCYTE SEDIMENTATION RATE (ESR)

The Erythrocyte sedimentation rate (ESR) is a non-specific test to determine the rate of sedimentation of red blood cells due to the presence of suspected infection.

Apparatus: Westergren ESR tube with anticoagulant, Westergren ESR pipette and stopwatch.

fig 8a. Westergren ESR tube with anticoagulant and Westergren ESR pipette

PROCEDURES

- Pipette 2ml of blood into the Westergren ESR tube.
- Insert the Westergren ESR pipette into the tube and push until the blood flows to the 0 mark.
- The tube is allowed to stand in an upright position for 1hr
- Read the final position of the red cells after sedimentation has occurred for an hour.

fig 8b. ESR after sedimentation for 1 hour

NOTE: Avoid error of parallax.

CAUSES OF HIGH ESR

- HIV.
- Tuberculosis.
- Acute viral hepatitis.
- Pelvic Inflammation disease…. etc

NORMAL RANGE:

Females: 0-15mm/hr.

Males: 0-10mm/hr.

Elderly: 0-20mm/hr.

1.10 BLOOD CLOTTING TIME

Materials:

Clean slide, sterile blood lancet with lancet pen, Ethanol with swab, needle and Stop-watch.

PROCEDURES

- Sterilize the fingertip (ring finger) using ethanol with swab.
- Insert the lancet in the lancet pen and prick the sterilized fingertip.
- Apply squeezing pressure from bottom to top ward of finger.
- When blood appears, gently touch the finger over the slide and collect 3-4 drops of blood.
- Using a needle, try to pull the blood towards the upward direction.
- Repeat this process 3-4 times continuously.
- Follow the procedure after 30 seconds (after each 30 seconds try to pull the blood upward).
- Continue this every 30 seconds until a thread is formed by the needle with the blood.
- The time it took for the thread to be formed is the **BLOOD CLOTTING TIME**.

PART TWO:

BACTERIOLOGY AND SEROLOGY

2.1 MICRO-ORGANISMS

Micro-organisms are living organisms that are too minute to be seen with the naked eye. They can however be seen with the aid of a microscope. Micro-organisms are classified into Bacteria, Fungi, Protista, Algae and Virus.

Bacteriology is the study of bacteria, and these organisms can exist practically anywhere. The soil, air, water and even our bodies can serve as a dwelling place for these micro-organisms. For them to survive they require certain requirements in their environment such as optimum temperature, presence or absence of oxygen (aerobes and anaerobes), water, optimum pH and adequate amount of nutrient. Bacteria can either cause harm to our body (pathogenic) or aid some certain biochemical reactions in our body systems.

In this section you shall encounter some clinical bacteria and methods of isolating and identifying them either as pathogens or commensals.

LIST OF GRAM POSITIVE BACTERIA

- *Staphylococcus*
- *Streptococcus*
- *Bacillus*
- *Clostridium*
- *Corynebacterium*
- *Listeria*
- *Actinomycetes*
- *Propionebacterium*

LIST OF GRAM NEGATIVE BACTERIA

- *Escherichia coli*
- *Klebsiella*
- *Enterobacter*
- *Proteus*
- *Salmonella*
- *Shigella*
- *Serratia*
- *Pseudomonas*
- *Vibrio*
- *Campylobacter*
- *Helicobacter*
- *Haemophilus*

- *Brucella*
- *Bordetella*
- *Yersinia*
- *Neisseria*

Acid-fast stain bacteria: *Mycobacterium tuberculosis and Mycobacterium leprae*

2.2 COLONY MORPHOLOGY DESCRIPTION

Bacteria grow in solid media as colonies. A colony is defined as a visible mass of micro-organism all originating from a single mother cell. Key features of these bacterial colonies serve as important criteria for their identification. Colonial properties such as **size, shape, texture, elevation, pigmentation and effect** on growth media are sometimes useful in bacterial identification. For more information on bacteria morphology, visit https://microbeonline.com/colony-morphology-bacteria-describe-bacterial-colonies/

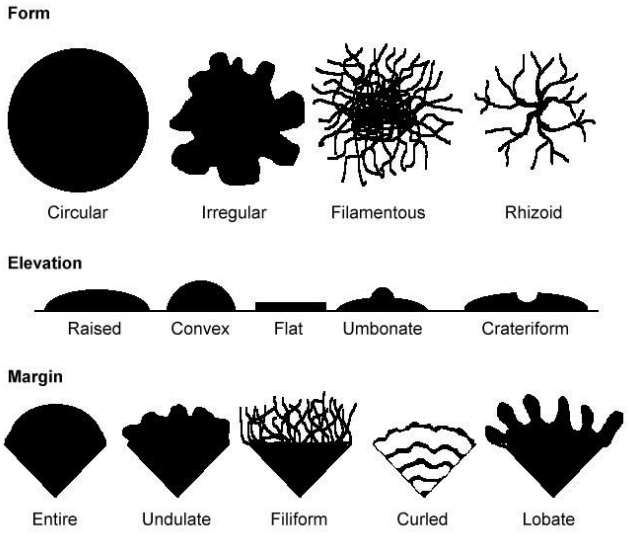


fig 9. colony morphology of bacterial growth

2.3 CHARACTERISTICS OF CULTURE MEDIA

MacConkey Agar

This selective culture media is used for isolating Gram-negative bacteria. It is a differential media for Lactose fermenters and Non-lactose fermenting Gram-negative bacteria

❖ **Colony characteristics on MAP**

- Lactose fermenting bacteria grow as pink to brick red colonies with or without the precipitation of bile. Examples include; *Citrobacter, Serratia, Escherichia coli* and *klebsiella. Enterobacter* is a weak lactose fermenter while *Citrobacter* ferments lactose after 48hrs (late fermenter)
- Non-lactose fermenting bacteria grow as colorless or clear colonies. Examples include; Proteus*, Shigella, Yersinia, Salmonella*,etc
- Gram-positive bacteria show no growth in MAP.

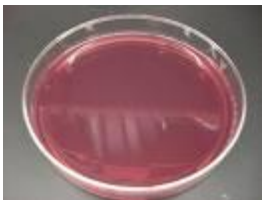


fig 10. MacConkey Agar

BLOOD AGAR

This is a differential media that is prepared when calculated volume of blood is mixed with sterilized Nutrient agar at 50^{o}C and used in isolating fastidious organism and to detect hemolysis. It encourages the growth of *Streptococci* and determines the type of hemolysis if any occurs. This media is generally used for isolation, identification and testing sensitivity of isolates.

fig 11. BLOOD Agar

Hemolysis

Hemolysis in blood agar is the complete or partial destruction of red cells in the media and the complete denaturation of hemoglobin within the cell to colorless product.

❖ **Types of Hemolysis**

- **Alpha Hemolysis** causes greenish gray or brownish discoloration around colony. Example is *Streptococcus pneumonia*
- **Beta Hemolysis** results from complete destruction of RBCs, causing the clearing of blood from the media. Example is *Streptococcus pyogenes*, *S. agalactiace, Staphylococcus aureus* etc
- **Gamma Hemolysis** causes no change in the media under the colony formed i.e they is no hemolysis. Examples include *E. coli, S. epidermidis, and P. aeruginosa.*
- **Target Hemolysis** causes the formation of double zones of hemolysis. Example is *Clostridium perfringens*.

CHOCOLATE AGAR

After the sterilization (autoclaved at 121^{o}C) of Nutrient agar (see preparation of culture media), the prepared media is kept in a Water bath and allowed to cool to 75^{o}C. A calculated amount of blood is then added which is lyzed to give chocolate color.

This culture media I best suited for the isolation of *Hemophilus influenza* at 35^{o}C-37^{O}C in a 5% CO_2 atmosphere (candle jar). It can also be suitable to isolate *Neisseria gonorrhoeae, N. meningitis* and other *Hemophilus spp.*

fig 12. Chocolate Aga

❖ **Colony characteristics on CAP**

N. meningitis appears as grayish, round, convex, smooth, moist, glistering colonies with no hemolysis of the media.

N. gonorrhoeae appears as pinkish brown and translucently smooth colony with consistent and defined margins.

H. Influenza is non-hemolytic, opaque cream to gray colonies.

S. pneumonia appears as small gray to green colony with zone of alpha hemolysis.

2.4 PREPARATION OF CULTURE MEDIA

- Different medium is weighed according to their grams per liter, dispense into water and stir to mix well (measure according to the quantity you want to prepare).
- The water is filled to the mark on the calibrated conical flask.
- The base of the autoclave is filled with water to the mark; the autoclave is covered (without knot) and switched on for 5 minutes.
- Switch off the autoclave then place inside the autoclave the prepared solution of the culture media.
- Tighten the wind knot, closed the safe valve and open the air escape valve and switch on the autoclave again.
- When they are droplet of water, close the air escape valve and wait for 15 minutes.
- When the autoclave makes a "*whiny*" sound; three to four times it should then be switched off and allowed to cool.
- The autoclave is opened when the pressure has returned to zero.
- The conical flash is removed and the sterilized media is poured into petri dish and flamed to avoid air bubbles.
- The plates are then labeled with dates and stored at $4^{o}C$

2.5 PATHOGENS AND COMMENSAL

URINE CULTURE		GENITAL CULTURE		PUS CULTURE		THROAT CULTURE	
PATHOGEN	**COMMENSAL**	**PATHOGEN**	**COMMENSAL**	**PATHOGEN**	**COMMENSAL**	**PATHOGEN**	**COMMENSAL**
N. gonorrhoeae *E. coli* *Klebsiella* *Enterococcus* *S. aureus* *S. saprophyticus* *C. jeikeium* *Acinetobacter* *Pseudomonas* *Gardenerella vaginalis* *β-hemolytic Streptococci* *Salmonella* *Providencia* *Morgnarella. Etc*	*Urethral mucosa* *Lactobacilli* *S. epidermidis* *Bacillus spp*	*N. gonorrhoeae* *S. agalactiace* *G. varginalis* *Enterococcus* *Actinomyces* *H. ducreyi* *T. palladium* *Mycoplasma* *Enterobacteriaceae* *Chlamydia* *Candida albicans* *T. vaginalis. etc*	*S. epidermidis* *Corynebacterium* *E. coli*	*P. aeruginosa* *Proteus* *E. coli* *Klebsiella* *Providencia* *Morgnarella* *S. pyogenes* *S. aureus* *Enterococcus* *C. perfringens* *M. tuberculosis.* *Etc*	*α-Streptococcus* *corynobacterium* *S. epidermidis* *Bacillus*	*S. pyogenes* *S. pneumonia* *S. aureus* *E. coli* *Klebsiella* *Citrobacter* *N. gonorrhoeae*	*α-Streptococcus* *N. meningitis* *S. epidermidis* *Candida albicans*

Table 4. Pathogens and Commensals of Urine, Genitals, Pus and Throat culture

2.6 STEPS FOR GENITAL CULTURE

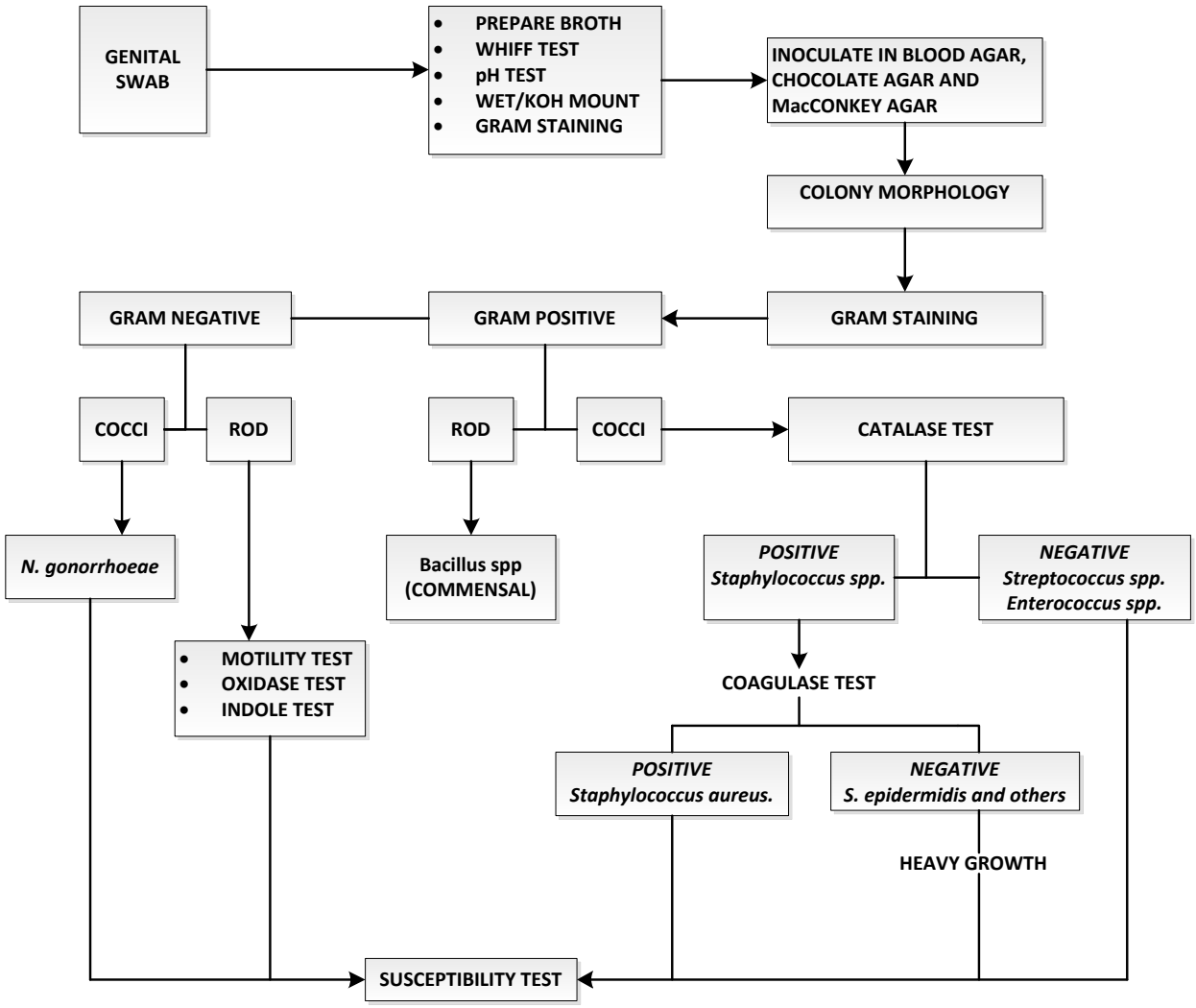


fig 13. Steps for Genital Culture

2.7 STEPS FOR THROAT CULTURE

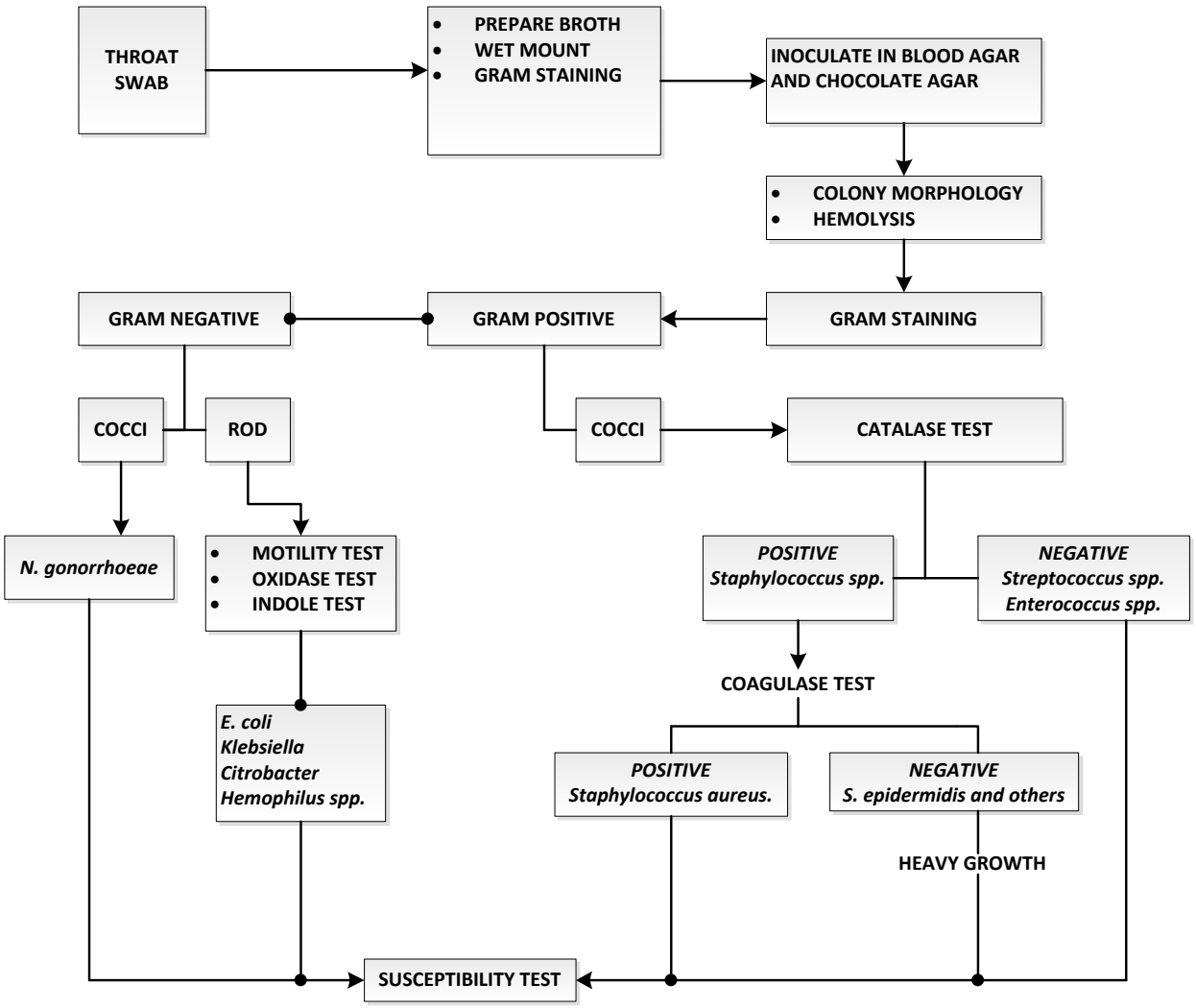


fig 14. Steps for Throat Culture

2.8 STEPS FOR PUS CULTURE

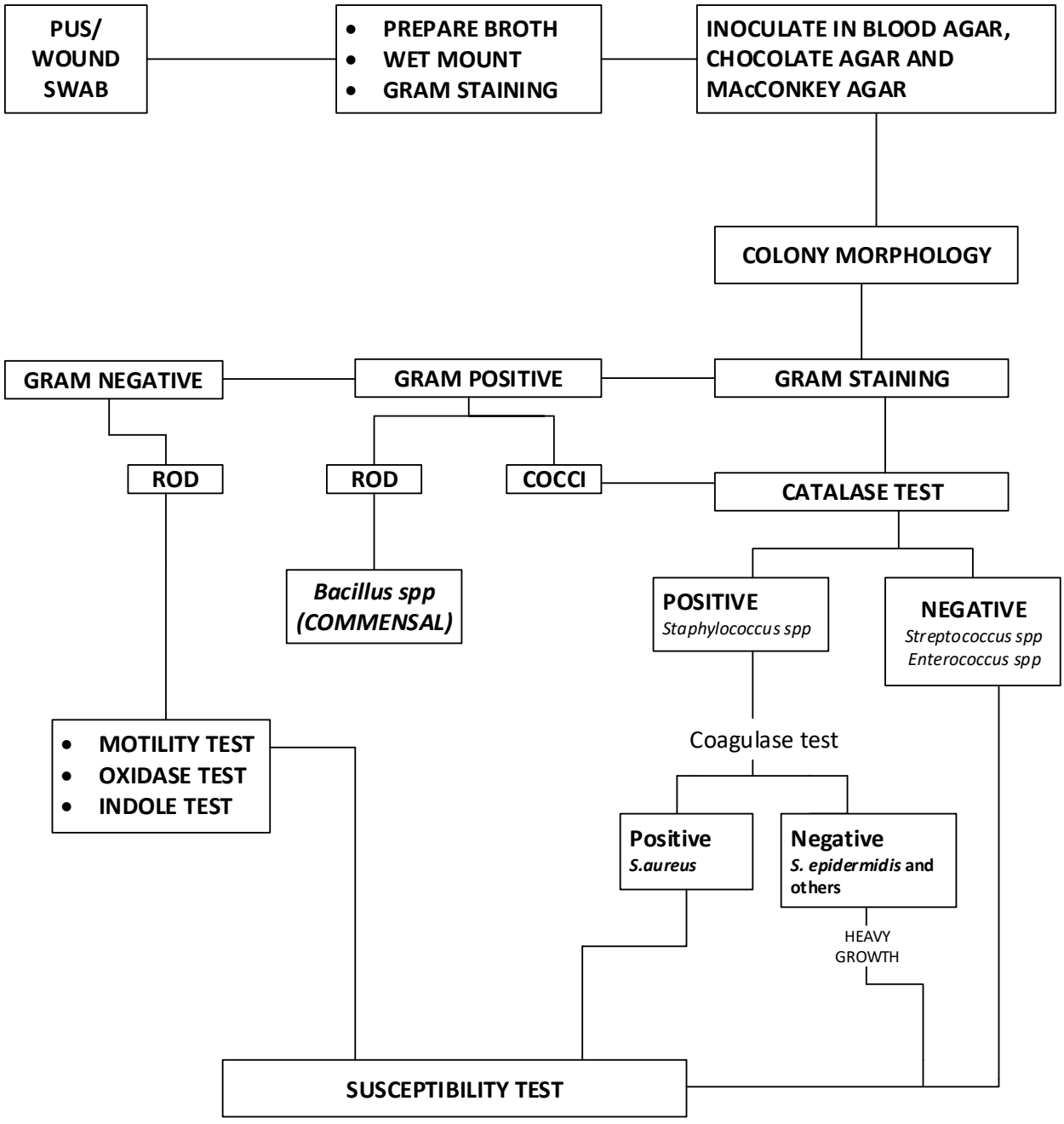


fig 15. Steps for Pus/Wound Culture

2.9 STEPS FOR URINE CULTURE

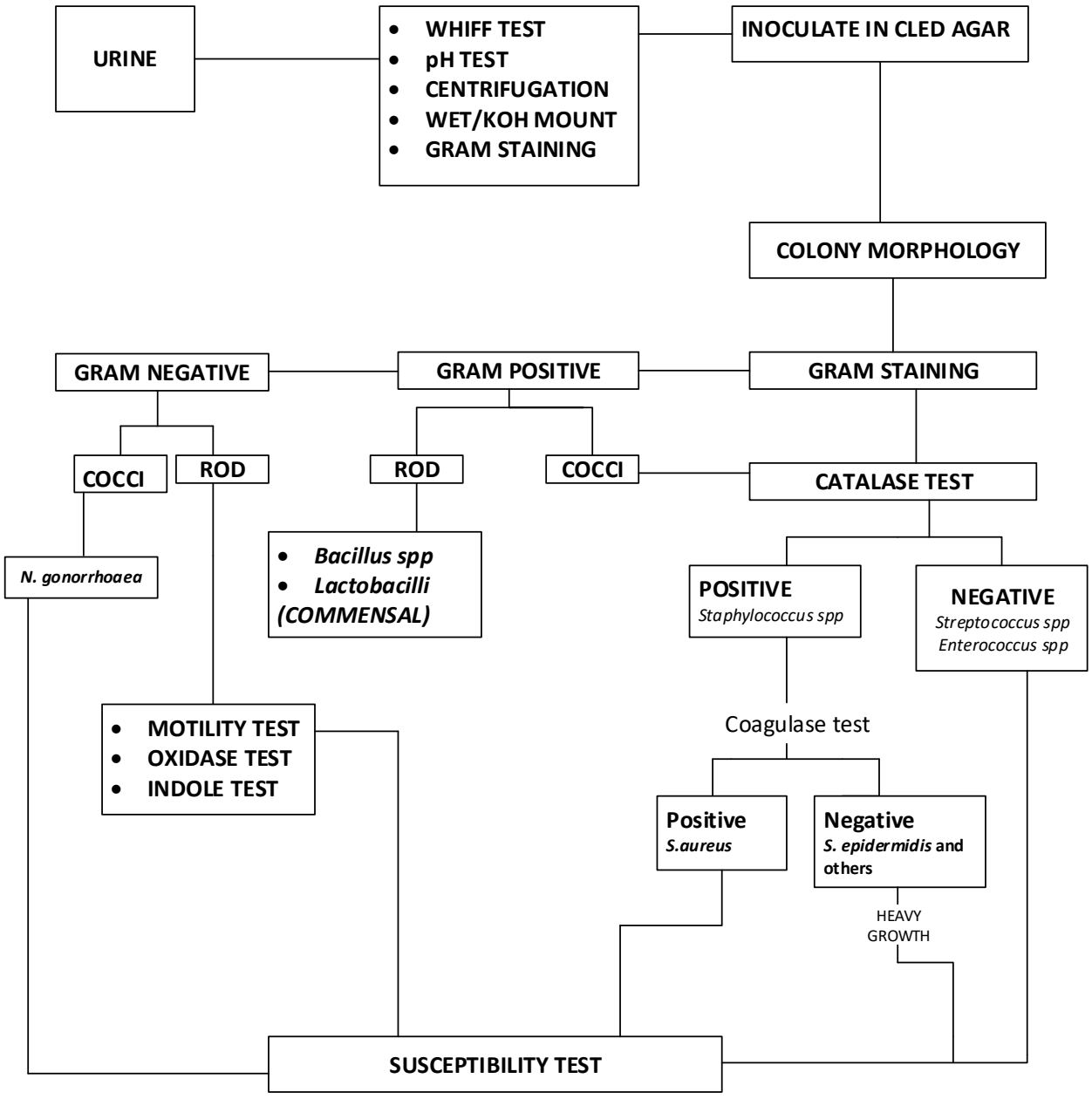


fig 16. Steps for Urine Culture

2.10 ASEPTIC TECHNIQUES

Asepsis can be defined as the absence of infectious microorganisms. However, the term is usually applied to any technique designed to prevent microorganisms from contaminating sterile material.

Observe the application of this technique by the accompanying instructions and diagrams.

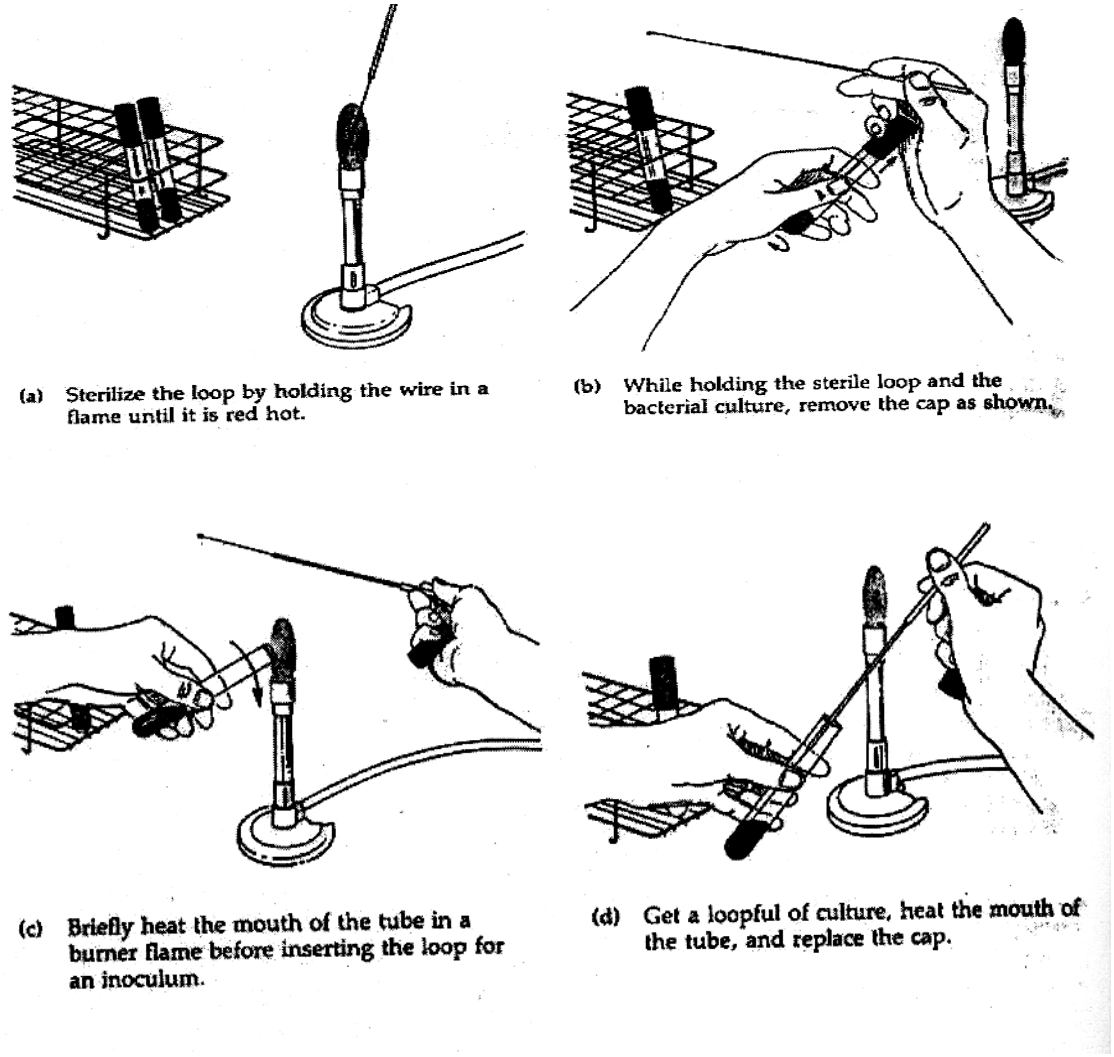


fig 17. Aseptic technique

2.11 WET MOUNT

A wet mount is prepared by mixing the vaginal sample with saline (and KOH pellets) on a glass slide, after which a cover slip is added. A diluted preparation is 8preferred to ensure the separation of the cells, which may otherwise be clumped together. Examine at a magnification of X400 for the presence of *T. vaginalis* with typical movement, budding yeasts, and clue cells.

C. albicans may form pseudomycelia, which may be observed occasionally in vaginal material. Clue cells are found in most women with bacterial vaginosis. A granular or dirty appearance of the epithelial cell cytoplasm is a less objective criterion than the loss of the cell border. The wet mount preparation is to be done immediately after the sample is collected.

Samples for Genital cultures (HVS, Urethral swab and Semen) and throat culture are first emulsified with Normal saline solution in a test tube and corked (See Broth preparation https://www.youtube.com/watch?v=sk_yJSVAN7U&t=39s). Using a loop of the broth is then placed on a microscopic slide and viewed with X10 and X40 objective lens.

Urine samples are measured into a 5ml test tube, corked and centrifuge at 4000RPM for 20 minutes. The urine after centrifugation may form sediments depending on the density of its constituents.
Pour off 4.5ml of the supernatant with 0.5ml left in the tube. Using a loop of the broth is then placed on a microscopic slide and viewed with X10 and X40 objective lens.

A Wet mount may present either of the following cells.

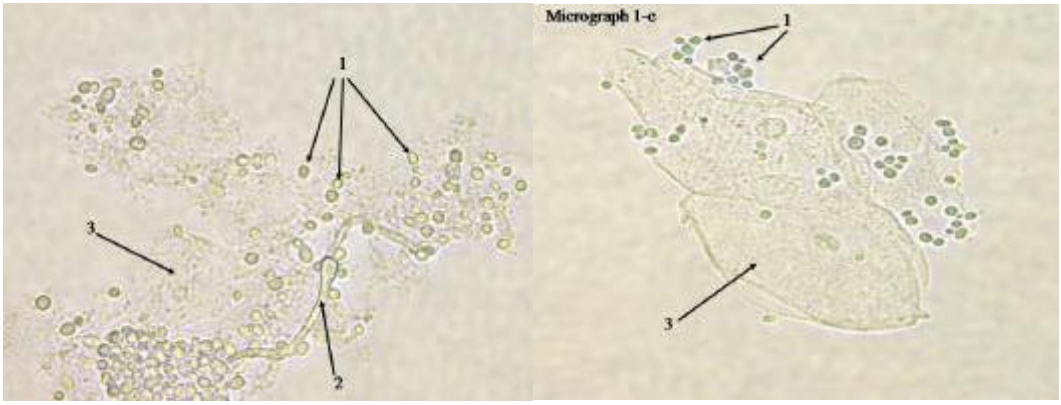


fig 18. 1) Yeast cell (2) Pseudohyphae (3) Squamous Epithelial

- **YEAST CELLS:** The cells vary in shape from circular to oval. Yeast cells are more variable in shape and are smaller than a red blood cell. It is often possible to pick out the thick cell wall of the yeast cell. In budding yeast cells, a single bud is observed.
- **PSEUDOHYPAE:** These are fragile tube- like structures that arise through elongation of the yeast form of Candida. They are called pseudohyphae because they lack true branching as seen with mold like fungi.
  The side walls are parallel to each other which are an important characteristic that helps separate pseudohyphae from artifact whose side walls vary in width. Small oval structures called bastoconidia are often seen attached along the length of the pseudohyphae. The blastoconidia are smaller in size when compared to the yeast form of Candida.

- **SQUAMOUS EPITHELIAL:** The examples shown here are typical of a normal squamous epithelial cell. A cell nucleus and the cell boundary are clearly observed. The squamous epithelial cell is a large cell with a nucleus approximately 10 microns in diameter. The cell nucleus is therefore a handy tool for assessing the relative size of red blood cells, white blood cells, and yeast cells. A white blood cell is approximately the same size as the nucleus of the squamous epithelial cell while RBC's and yeast cells will be slightly smaller than the nucleus.

Being able to observe the cell nucleus is not always the distinguishing feature between normal epithelial cells and clue cells.
As described below, the cell nucleus may be observed in a clue cell even if the cell margin is totally obscured.

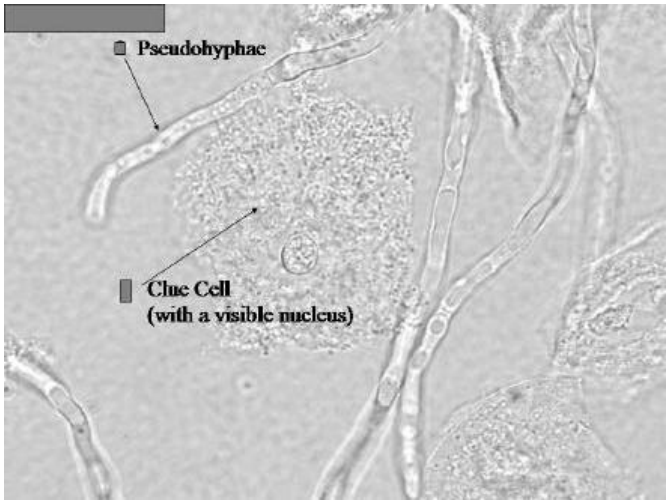


fig 19. Pseudohyphae and Clue cell (X40)

Below are further microscopic views of clue cells, yeast cells and normal epithelial cells.

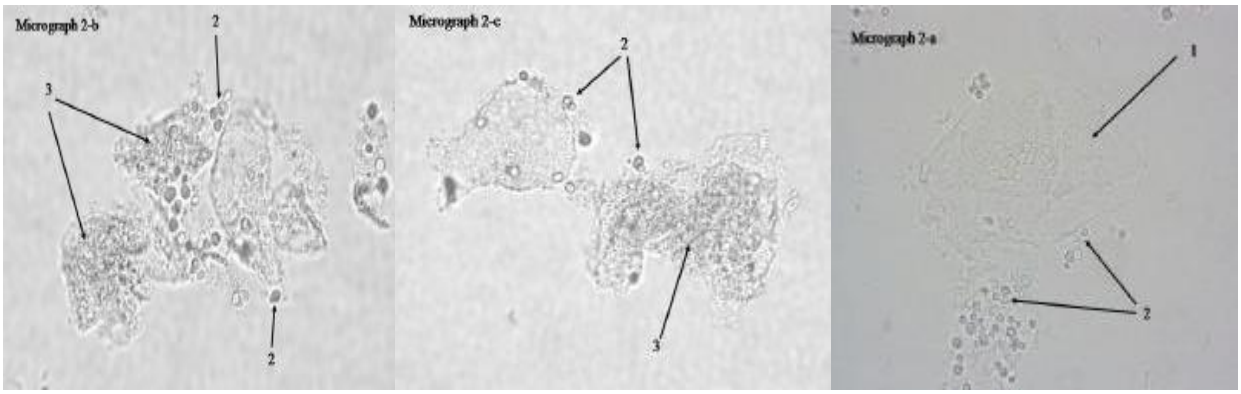


fig 20. 1) Normal Epithelial cell (2) Yeast cells easily misidentified as RBC (3) Clue cells

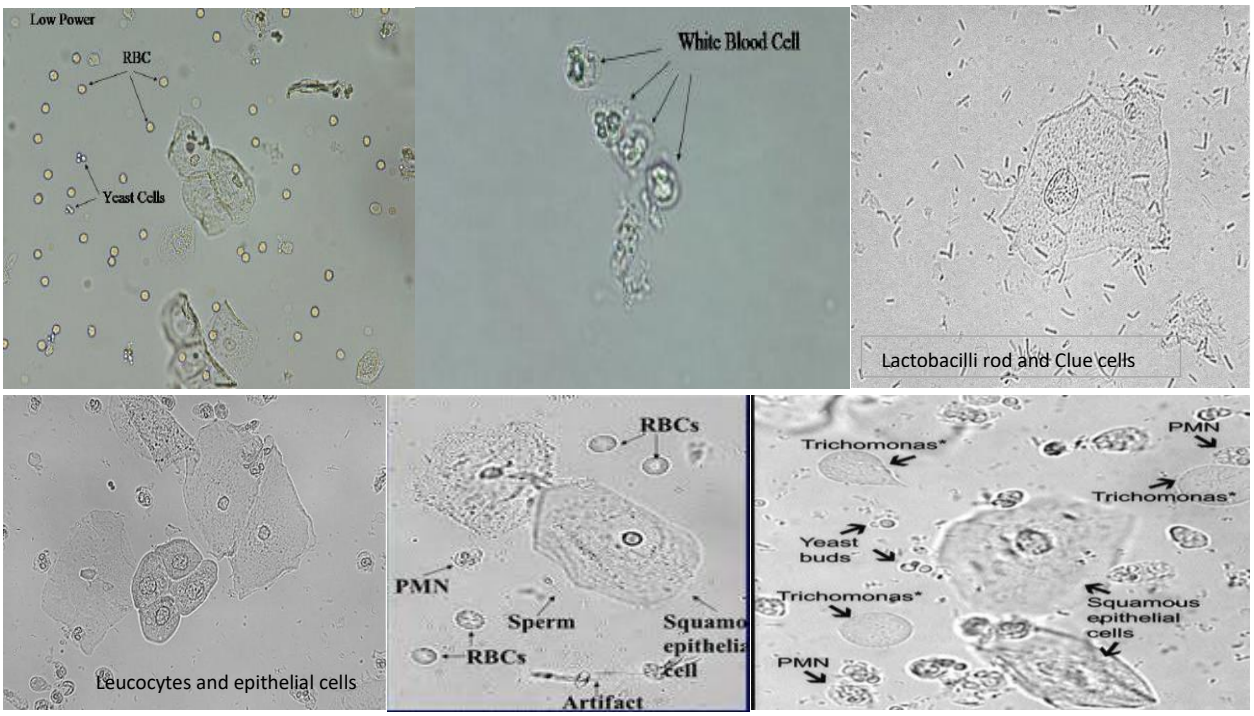


fig 21(a.) RBC, Yeast cell (X10); (b.) WBC (X40); (c.) Lactobacilli rod and clue cell (X40)

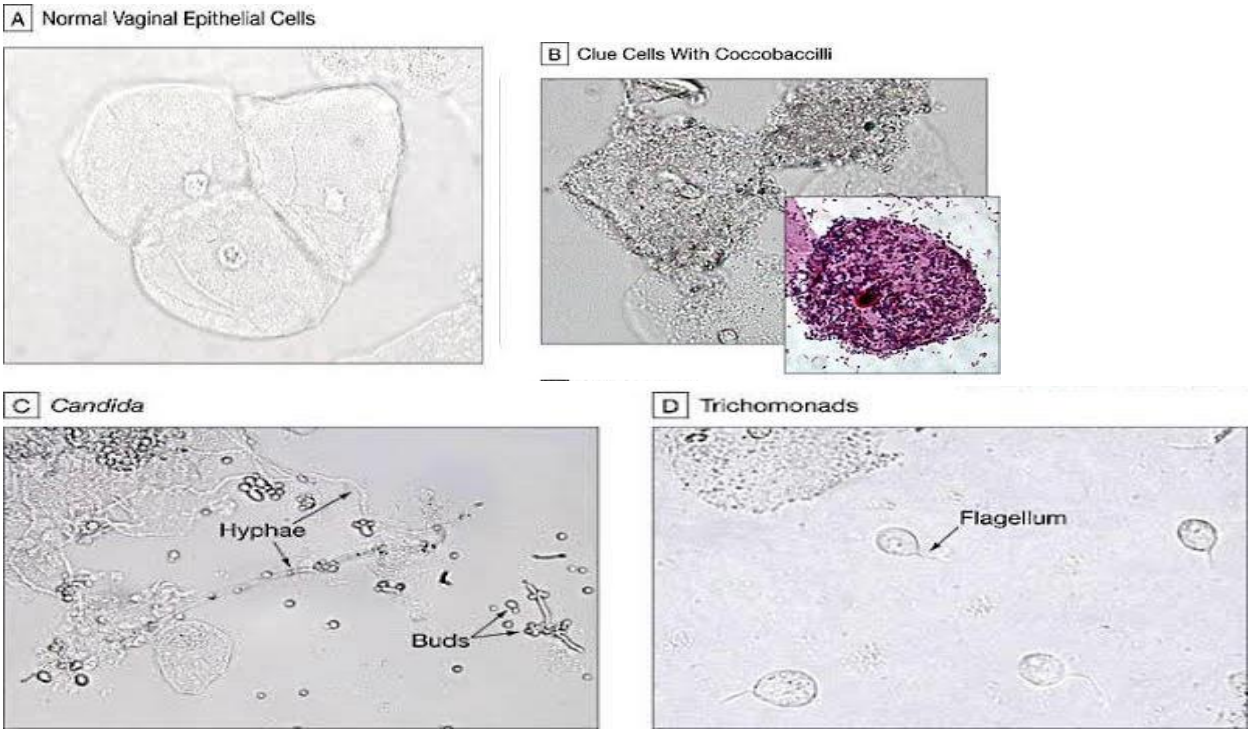


fig 22. (a) Normal vaginal epithelial cell (b) Clue cells with coccobacilli (c) Candida (d) Trichomonas vaginalis

The bacterium, ***Lactobacilli*** is a normal flora in women following the onset on menses and will persist as normal flora until menopause. Bacterial vaginosis results from the synergistic association of *Gardnerella vaginosis* (a small gram-positive or gram-variable rod) and

Mobiluncus sp. (a small curved anaerobic gram-rod). These are the bacteria which coat squamous epithelial cells. The presence of squamous epithelial cells thickly coated with bacteria is a "clue" to the detection of BV – hence the term "Clue Cell".

- **Clue cells** are squamous epithelial cells that are covered with a thick matte of bacterial cells and are associated with bacterial vaginosis. The traditional definition of a clue cell is that the bacterial overgrowth is so thick that all cell detail (such as the cell nucleus and the cellular edge) is totally obscured. As shown in the above illustration, it is sometimes possible to detect the nucleus in a clue cell by using the fine focus knob to focus throughout the cell.

- **White Blood Cells** (leucocytes) are larger than **Red Blood Cells** and are approximately the same size as the nucleus of a squamous epithelial cell. You can easily compare the relative size of the white blood cell with the size of the squamous epithelial cell nucleus by screening on low power. Under high power, the nuclear detail of the WBC becomes apparent. The WBC is characterized by a multi-lobed nucleus (usually three distinct lobes can be identified). The three lobes may not always be present (at least in the same focal field), but you should be able to see them by using the fine focus to focus up and down.

RESULTS:

Cells are counted on a field of X100 magnification and in 3-4 fields of X400 magnification and recorded as follows:

- Pus cells: 1-5(scanty), 6-15(few), >15(many).
- Yeast/Fungi: Present/Absent.
- Epithelial cells: Cells/LPF (X10 OL).
- WBC/RBC: Cells/HPF (X40 OL).
- Clue cells: Present/Absent.
- *T. vaginalis*: Present/Absent.
- *Lactobacilli*: Present/Absent.

2.12 INOCULATION TECHNIQUE

- Sterilize the work bench and the work area with ethanol or any disinfectant.
- Ensure that windows close to the work area are locked to avoid air-borne bacteria contamination.
- Sterilize the wire loop using aseptic technique (see page 39).
- Collect a loopful of broth and streak on a culture media plate as shown below.

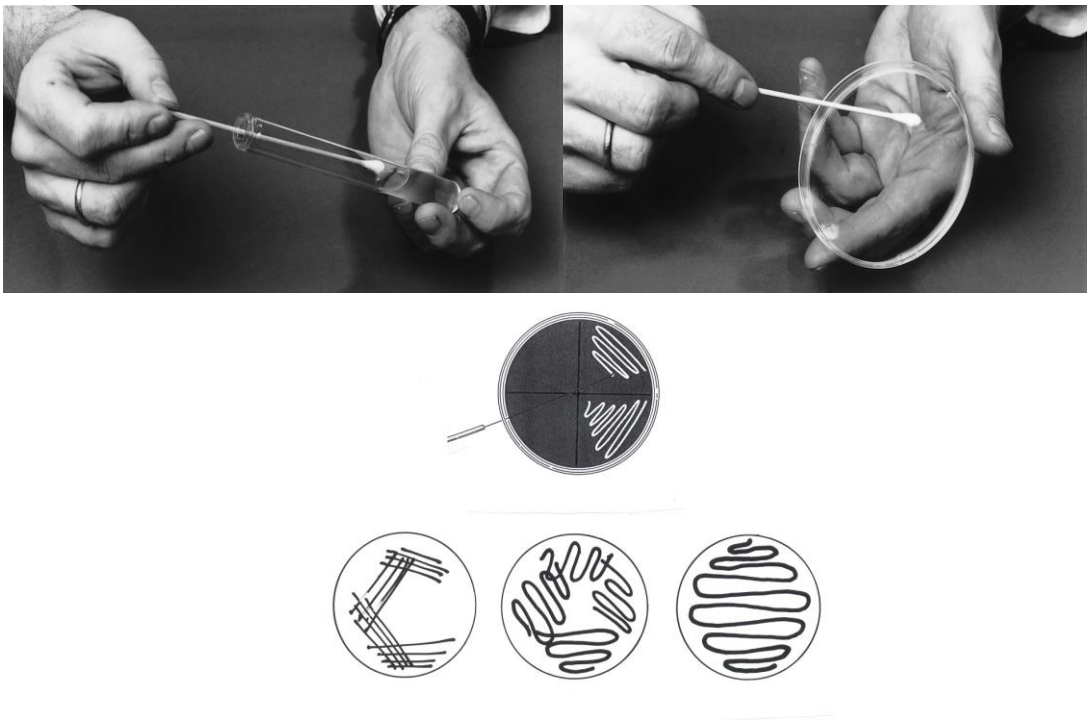


fig 23. Streaking method of inoculation

2.13 CATALASE TEST

The test is used to differentiate *Staphylococcus* from *Streptococcus* and *Enterococcus*; and *Clostridium* from *Bacillus*. Almost all Gram-negative bacilli are catalase positive therefore it is useless performing this test if the bacteria growth occurred in MAP or if the Gram staining shows Gram-negative bacteria. During aerobic respiration, microorganisms produce hydrogen peroxide. Accumulation of this substance will result in death of the organism unless they can be enzymatically degraded. Organisms capable of producing catalase rapidly degrade hydrogen peroxide as illustrated

$$2H_2O_2 \rightarrow \text{catalase} \rightarrow 2H_2O + O_2$$

$$Staphylococcus(+) \leftarrow catalase\ test \rightarrow Streptococcus(-)$$

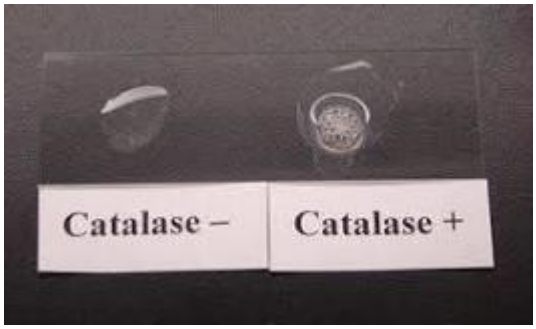


fig 24. Catalase reaction

See video via https://www.youtube.com/watch?v=lG6lriLQZuY

PROCEDURE:

- Put 1-2 drops of normal saline solution on a slide.
- Collect a loopful of isolate and emulsify with the normal saline solution on the slide.
- Add 3% hydrogen peroxide solution to the mixture and observe for rapid oxygen bubbles which signifies catalase positive. Any reaction after 15 seconds is considered false.

NOTE: Avoid using metal loop or crumbing the blood agar when collecting the isolate from BAP to avoid false positive reaction.

2.14 MOTILITY TEST

Organisms that possess the ability to propel themselves are said to be motile. This is an important characteristic that is used for identification of bacterial organisms. There are two types of movement that can be observed, one is Brownian movement caused by molecules colliding with the organism and moving around in an irregular pattern, but is not true motility. True motility appears as movement in consistent direction. There are two different methods of determining motility: **Hanging drop method** and Inoculation of semi-solid media with the micro-organism. We shall only discuss the Hanging drop preparation.

Materials:

Glass slide, broth of suspected motile micro-organism, wire-loop, cover slips and Vaseline or petroleum jelly.

PROCEDURE:

- Place a loopful of bacterial culture at the center of a cover slip.
- Use Vaseline to mark out a square as the size of a cover slip at the center of the slide.
- Place the slide on the cover slip using the marked area to touch the edges of the cover slip.

- Turn the slide over so that the culture drop adheres to the cover slip.
- View with X100 and X400 magnification, the bacteria at the edges to avoid misinterpretation of bacteria moving as a result of current with those that are actually moving.

MOTILE BACTERIA	NON-MOTILE BACTERIA
Salmonella	*Neisseria*
Enterobacter	*Nocardia*
Proteus	*Enterococcus*
E. coli	*Staphylococcus*
Citrobacter	*Shigella*
Morganella	*Streptococcus*
Providencia	*Klebsiella*
Vibro (darting motility)	*Yersina.*
Aeromonas	
Plesiomonas	
Pseudomonas.	

Table 5. Motile positive and negative bacteria

2.15 COAGULASE TEST

Coagulase test is specifically carried out to identify *staphylococcus aureus* from other *staphylococcus* species. A culture broth of unidentified *staphylococcus sp*. is place on a slide and blood serum is added to the broth and mixed together using any suitable mixing aid. The preparation is *"rocked"* and observed for agglutination which signifies a positive result.The coagulase negative species include: *S. epidermidis* and *S. saprophyticus*.

RESULT:

fig 25. Coagulase reaction

2.16 OXIDASE TEST

PROCEDURE:

- Place 2-3 drops of oxidase reagent (1% tetramethyl-paraphenylenediamine) on a piece of filter paper in a petri-dish
- Pick up a small amount of fresh growth from the MacConkey agar with a platinum loop or a clean woken stick or toothpick.
- Smear the growth across the moistened part of the filter paper.
- A positive reaction is indicated by the appearance of a dark purple color paper within 10 seconds.

RESULT:

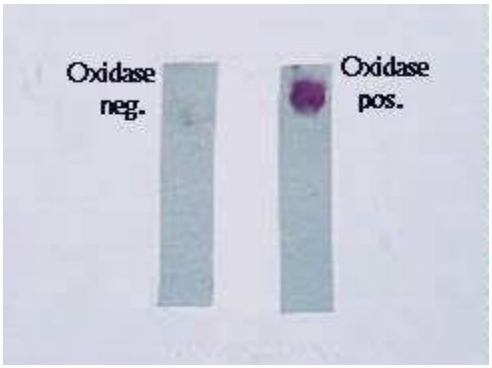


fig 26. Oxidase reaction

OXIDASE POSITVE	OXIDASE NEGATIVE
Aeromonas hydrophilia	*Acinetobacter*
Bacillus	*Klebsiella*
Burkholderia capacia	*Acetobacter*
Campylobacter jejuni	*Staphylococcus*
C. festus	*Streptococcus*
Flarobacterium	*E. coli*
Helicobacter pylori	*Enterobacter*
Kingella kingae	*Citrobacter*
Pasteurella multocida	*Proteus*
Plesiomonas aeruginosa	*Morganella*
P. shigelloides	*Shigella*
Vibro sp.	*Pasturella*
Neisseria	*Enterococcus*
Pseudomonas	All Enterobacteriaceae
Hemophillus	All Gram positive cocci.
Capnocytophaga ……etc	

Table 6. oxidase positive and negative

2.17 WHIFF TEST AND KOH MOUNT

Whiff test and KOH mount are two of the preliminary tests in investigating UTIs and STIs. It follows the same procedure as wet mount as a colony is from the bacteria culture is emulsified with normal saline and 2-3 drops of KOH solution is added. If the prepared smear gives off a bad fishy smell, it is indicative of presence of **clue cells** or ***T. vaginalis*,** hence the outcome is reported as whiff positive.

2.18 SUSCEPTIBILITY TEST OF PATHOGENIC GROWTH

Sensitivity or susceptibility test is required at the final stage of culture investigation; to identify antibiotics that are reactive and resistant to pathogenic micro-organism isolated in culture media. It is required to aid the Pharmacist and Doctor give proper prescriptions to the infected patient. Most common antibiotics used include; Augmentin(AU), Amplicin(AM), Tarivid(OFX), Pefloxacine(PEF), Ciprofloxacin(CPX), Gentamycin(CN), Streptomycin(S), Ceporex(CEP), Nalidxic Acid(NA), Septrin(SXT), Amoxil(AMX), Erythromycin(E) etc.

Materials:
Bacterial culture, wire loop, forceps and Susceptibility disc and Blood agar plate

PROCEDURE:

- Sterilize the loop using simple aseptic technique (see page 39).
- Collect a colony of the inoculum and sub-culture in BAP using the streak method.
- Sterilize the forceps using simple aseptic method.
- Using the forceps, pick up a susceptibility disc and place at the center of the BAP.
- Incubate for 24hrs.

RESULTS:
The result is based on the distance of the bacterial growth away from the antibiotics. The plus (+) system is employed to indicate high and low sensitivity. If they are no gap between the bacterial growth and the antibiotic in the disc, the isolate is reported to be resistant to the drug. If culture yield fungi growth, susceptibility test is not required.

fig 27. Susceptibility test with nutrient agar

2.19 SMEAR PREPARATION FOR STAINING TECHNIQUES

Since bacteria are so small, they are difficult to see with light microscope. Bacteria are usually colorless and therefore cannot be seen because of the lack of contrast with the surrounding medium. Simple stain is the use of basic dye to increase the contrast of cells for microscopy; the scope of simple staining technique to determine cell morphology (shape) relative size and arrangement

MAKING A SMEAR:

PROCEDURE

- Put 1 or 2 loopful of cell suspension from a culture broth on the clean glass slide. If cell is to be collected from a solid media, put one drop of water on the loop and place it on the clean slide.
- With a circular movement of the loop, spread the suspension into a thin area, approximately the size of a dome.
- Transfer a small amount from the bacteria inoculum from the media into the drop of water and spread with a circular movement.
- Allow the smear to air dry.
- While holding the slide at one end, quickly pass the smear over a flame, two to three times.

GRAM STAINING

Developed in 1884 by the Danish physician Christian gram, is one of the most important and widely used procedures in microbiology for characterizing most bacteria into two groups based on the structure and chemical difference of the cell wall.

Those organisms which retain the crystal violet (appear dark blue or violet) are designate gram positive; those which lose the crystal violet and stained by the safranin or neutral red (appear red) are designated gram negative.

Materials:

Crystal violet, Lugol's iodine, acetone, safranin or neutral red and absorbent paper.

PROCEDURE:

- ❖ Prepare a smear as discussed above.
- ❖ Cover the smear with crystal violet for 30 seconds.
- ❖ Carefully wash off the crystal violet with slow running water.
- ❖ Cover the smear with Lugol's iodine for 30 seconds.
- ❖ Wash off iodine with acetone.
- ❖ Gently wash the smear with slow running water.
- ❖ Cover the smear with safranin/neutral red for 30 seconds.
- ❖ Wash off with water and blot off using absorbent paper.

RESULTS:

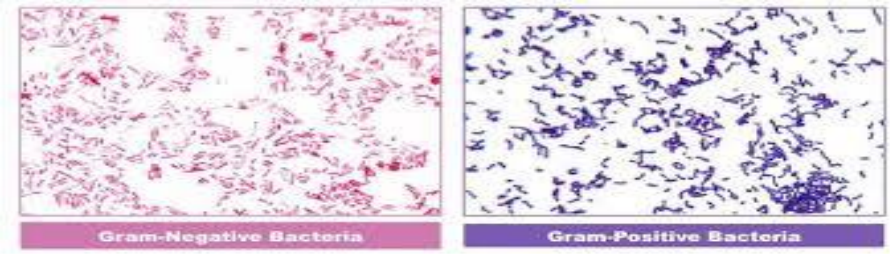


fig 28. Gram-negative and Gram-positive bacilli (Bacteria)

ACID-FAST BACILLI STAINING

While the majority of bacterial organisms are stainable by either simple or gram's staining procedures. A few genera, particularly the members of the genus *mycobacterium*, are resistant and can only be visualized by the acid-fast method. Since *M. tuberculosis* and *M. leprae* represent bacteria that are pathogenic to humans, the stain is of diagnostic value in identifying these organisms.

The characteristic difference between mycobacteria and other microorganisms is the presence of a thick waxy (lipoidal) wall that makes penetration by stains extremely difficult. Once the stain has penetrated, however, it cannot be readily removed even with the vigorous use of acid alcohol as a decolorizing agent. Because of this property, these organisms are called acid-fact, while all other microorganisms, which are easily decolorized by acid-alcohol, are non-acid- fast.

Materials:

Mycobacterium culture, Carbol fuchsin, Acid alcohol, Methylene blue and filter paper.

PROCEDURE:

- Make a smear of the bacterial culture.
- Apply Carbol fuchsin and heat and heat for 5 minutes. Do not allow stain to evaporate by continually adding the stain.
- Cool and wash off stain with tap water.
- Add acid-alcohol drop by drop until the alcohol runs clear.
- Wash off the acid alcohol with tap water.
- Counter stain with methylene blue for 2 minutes.
- Wash off methylene blue with tab water
- Blot the slide dry with filter paper.

NEGATIVE STAINING (CAPSULE STAIN)

The negative stain technique does not stain the bacteria but stains the background. The bacteria will appear clear against a stained background. The negative stain does not stain the bacteria due to the ionic repulsion of the negative charge on the bacterial surface and the acidic

stain (negative charge). No heat fixing or strong chemical are used, negative stain can be used to determine cell morphology, size and capsule.

Materials:

India ink, clean slide, distilled water and bacterial culture e.g. E. coli

PROCEDURE:

- Place a drop of India ink towards one end of the slide.
- Place a loopful of the inoculum into the drop of stain and mix with the loop.
- Place a slide against the drop of suspended organisms and allow the drop to spread along the edge of the applied slide.
- Push the slide away from the previous spread drop of suspended organisms, forming a thin smear.
- View with X100 and X400 magnification.

ENDOSPORE STAINING

Endospores are formed by members of two genera bacillus and clostridium which are of great medical importance. Endospores are metabolically inactive and are resistant to heating, various chemicals and many harsh environmental conditions. **Sporogensis** is not for reproduction, but it is resistant to unfavorable environment, Endospore can remain dormant for long time. However, Endospore may return to its vegetative or growing state.

Materials:

Culture of 2-3 days old *Bacilli* and *clostridium*, malachite green, safranin (neutral red), beaker of boiling water and piece of filter paper.

PROCEDURE:

- Make a smear of the inoculum and place a piece of filter paper over the smear.
- Cover the paper with malachite green.
- Place the smear over steam for 5 minutes and wash the smear with water.
- Cover the smear with safranin (or neutral red) for 30 seconds.
- Wash with water and blot dry.

ILLUSTRATION:

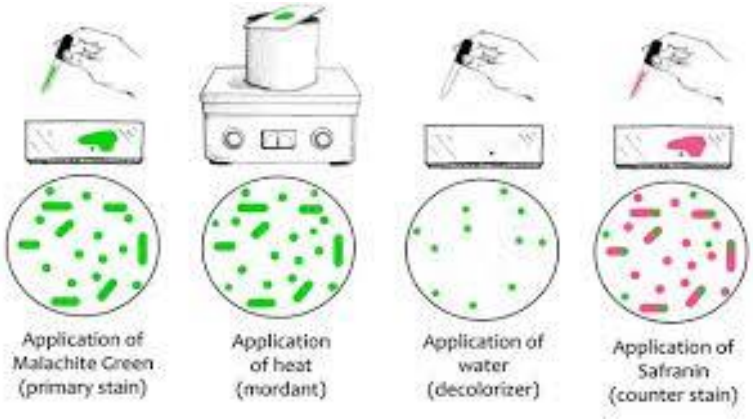


fig 29. Endospore staining

2.20 CHOLESTEROL TEST

Cholesterol test can be done in two ways.

- Use of cholesterol home test kit
- Use spectrophotometer to determine the hormone profile of the patient.

fig 30. Cholesterol home kit

PROCEDURE (HOME TEST KIT METHOD):

- Read the manual of the testing device.
- Disinfect the ring finger of the patient.
- Jab the finger with blood lancet.
- Add pressure on the finger to bring out blood and gently wipe off the blood.
- Apply pressure again to bring out blood.
- Place a drop on the indicated portion on the test strip.
- Wait for 5 seconds for test readings on the display.

USE OF SPECTROPHOTOMETER

This method uses the Beer lamberts principle of absorbance in relation to the ability of the different lipoproteins to absorb light at different spectrum with corresponds with their relative mass in the given blood sample. The blank sample is first illuminated to calibrate the machine; after which the sample is then illuminated with a cathode ray which is dispersed through monochromator or polarizer.

NORMAL RANGE of cholesterol

120-200mg/dl.

NB: The test is preferably done while the patient is fasting and before 11am. The blood sample is stored in a **Lithium Heparin bottle**.

2.21 FASTING/RANDOM BLOOD SUGAR

Fasting Blood Sugar and Random Blood Sugar are conducted to detect the sugar level of a blood sample. **FBS** is done only in the morning (before 11AM) while **RBS** can be done anytime of the day. Unlike cholesterol, the sugar test requires a different device called a **Glucometer** or **Glucose-meter** which comes with **50 coded strips, blood lancet** and a **lancet pen**. The procedures for FBS and RBS are identical to Cholesterol test with Cholesterol Home kit.

fig 31. Glucometer

Normal range

FBS: 70-100mg/dl
RBS: 70-200mg/dl

2.22 IN-VITRO ASSAY/SEROLOGY

These are biochemical analysis carried on biomolecules outside their natural environment (like the body). Biological substances such as blood and urine can be used to detect viral, infectious or abnormal conditions of the whole organism outside the body. The samples are collected from the body, processed and reacted with synthetic antigens which give specific qualitative reactions. For example, a patient can be said to be with child (pregnant) after a positive result from an in-vitro pregnancy test.

An increase in the secretion of hCG hormone in the blood is due to the formation of placenta which can only occur if the patient is pregnant. This is the basis for this in-vitro diagnosis which uses a test strip that indicates the presence of the hormone when in contact with blood serum of pregnant patients.

PROCEDURES:

- Collect blood in EDTA bottle and centrifuge for 5 minutes.
- Remove the test strip from the wrap.
- Place the sensitive end of the strip in the blood plasma and allow capillary action until the plasma reaches the antigen in the strip.
- Observe for result.

RESULT:

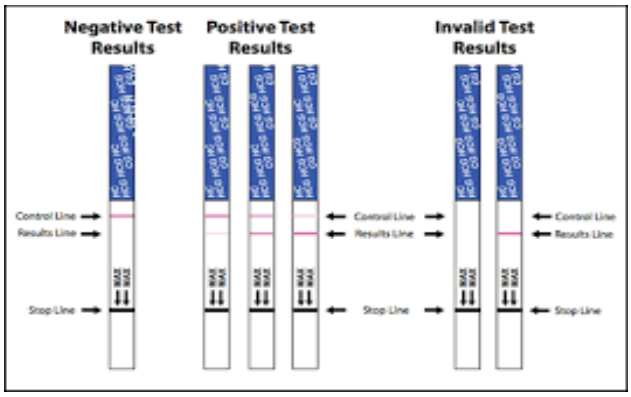


fig 32a. Rapid test strips for in vitro diagnosis

Other in-vitro analysis with similar procedure and result description include:

- **Venereal Disease Research Laboratory (VDRL)**
- **Hepatitis A, B&C (HAV, HBV or HBSag, HCV)**
- **Test for H. PYLORI**
- **Retroviral Screening (RVS)**

2.23 FECAL OCCULT BLOOD

Fecal occult blood test (FOBT) refers to blood in the feces that is not visibly apparent (unlike other type of blood in stool such as melena or hematochezia). A fecal occult blood test checks hidden (occult) blood in the stool.

PROCEDURES

- Unwrap packet and remove the sample collection device (a small capped plastic bottle containing a buffer solution)
- Remove the cap (it has a long plastic tube attached to it).
- Dip the tip of tube in 3 different spots on the stool and put back into the sample collection device (bottle).
- Shake well.
- Break the top of the cap (creating a hole) and turn the mixture at the sensitive portion of the FOB strip.
- Observe for result (same as other test strip)

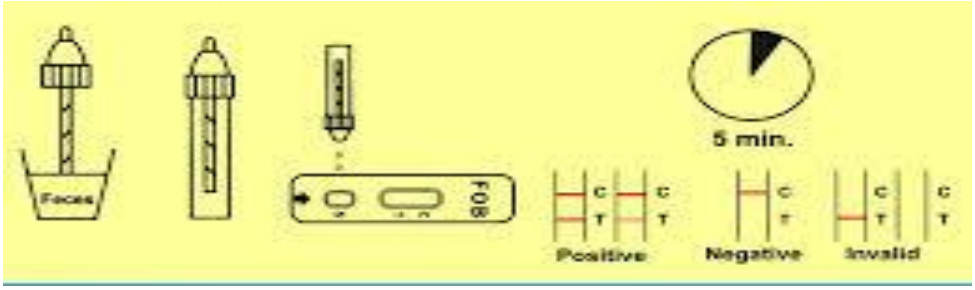


fig 32b. Illustration of in vitro assay of fecal occult blood

2.24 MANTOUX TEST

Mantoux test is a tool for screening for tuberculosis (TB) and for tuberculosis diagnosis. Tuberculin which is injected under the skin of the patient is a glycerol extract of the tubercle bacillus. Different types of tuberculin test are available. The Mantoux tuberculin skin test is the preferred type because it is most accurate. The tuberculin used is also known as Purified protein derivative (PPD). The PPD tuberculin is a precipitate of species-nonspecific molecules obtained from filtrates of sterilized, concentrated cultures.

MATERIALS:

Tuberculin and insulin syringe.

PROCEDURE:

- Using 1ml insulin syringe, withdraw 0.1ml of tuberculin

- Select a preferred site for the injection, left hand forearm dorsal surface, about 4 inches below the elbow joint without vein.
- Inject the tuberculin intradermally on the volar surface of the forearm positioning the syringe at 10-15˚ to the forearm and insert just below the epidermis (about 2mm).
- Remove the needle quickly and do not massage or use dressing. A well-defined bleb of 6-10mm in diameter should be formed if injected correctly. If the bleb is <6mm, repeat the procedure 2.5cm from the first site.
- Mark down the site, date and time on the forearm and on the patient's record.
- Instruct patient not to touch or pour water on the injection site until after diagnosis.
- Read the result after 72 hrs.

fig 33. How to administer intradermal injection

RESULTS:

Measure the diameter of the diameter of hardened swollen area. DO NOT MEASURE THE INFLAMMED AREA (REDDISH SURFACE)

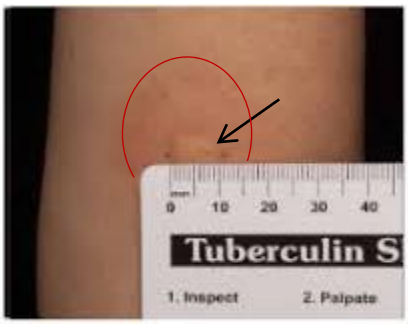


fig 34. Measuring inflammation after 48 hours

INTERPRETATION

≥ 5mm

- HIV Positive
- Recent contact with an active TB patient
- Fibrotic changes in chest x-ray
- Organ transplant

≥ 10mm

- IV drug user
- Resident or employee at high risk environment
- Mycobacteriology Lab personnel
- Children < 4 years' old
- Infant, children and adolescents exposed to high risk categories

≥ 15mm

- Person with no known risk factor for TB

PART THREE:

IDENTIFICATION OF COMMON CLINICAL BACTERIA

3.1 STREPTOCOCCUS and ENTEROCOCCUS

The streptococci are gram-positive spherical bacteria that characteristically form pairs or chains during growth. They are widely distributed in nature. *Streptococci* elaborate a variety of extracellular substances and enzymes. The streptococci are a large and heterogeneous group of bacteria, and no one system suffices to classify them. Many species of streptococci, including *Streptococcus pyogenes* (group A), *S agalactiae* (group B), and the enterococci (group D), are characterized by combinations of features, including colony growth characteristics, hemolysis patterns on blood agar (α-hemolysis, β-hemolysis, or no hemolysis).

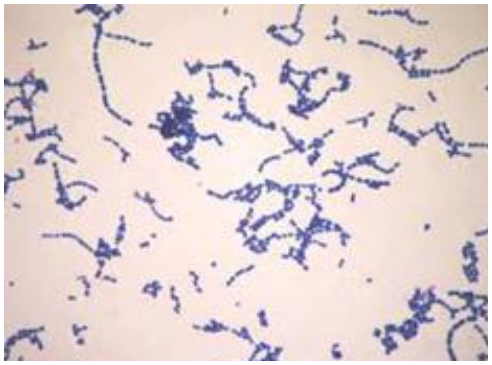


fig. 35a. Gram-positive streptococcus

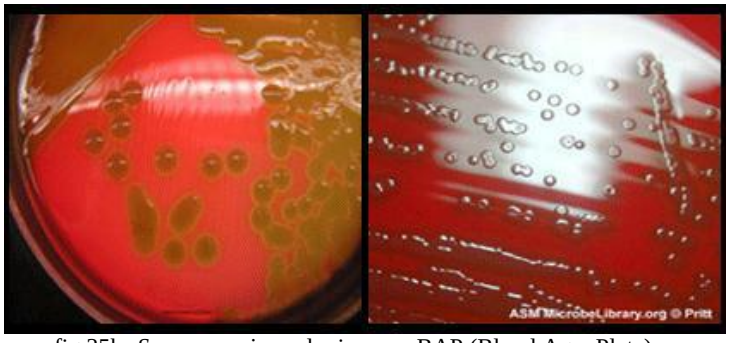
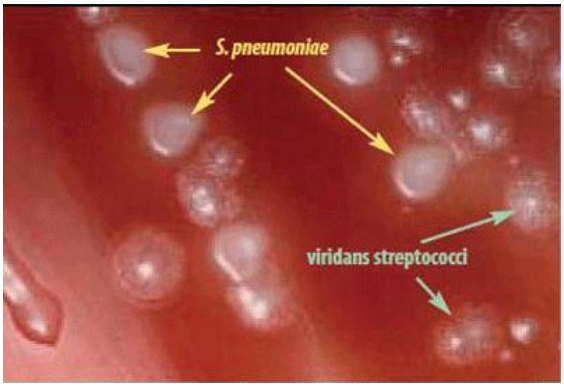


fig 35b. *S. pneumoniae* colonies on a BAP (Blood Agar Plate)



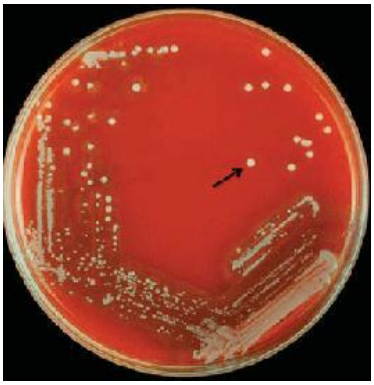


fig 35c. *S. pneumononiae* colonies have a flattened and depressed greenish center after 24-48hrs of growth on BAP.

Fig 35d. *S. viridans* retain a raised center. *S. pneumononiae* is surrounded by alpha hemolysis. *S.epidermidis* has no hemolysis.

3.2 STAPHYLOCOCCUS

The staphylococci are gram-positive spherical cells, non-sporing, non-motile, usually non-capsulate, true facultative anaerobic organisms. (1micrometer in diameter) usually arranged in grapelike irregular clusters. They grow readily on many types of media and are active metabolically, fermenting carbohydrates and producing pigments that vary from white to deep yellow. Colonies on solid media are round, smooth, raised, and glistening *S. aureus* usually forms gray to deep golden yellow colonies. *S. epidermidis* colonies usually are gray to white on primary isolation; many colonies develop pigment only upon prolonged incubation. No pigment is produced anaerobically or in broth. Various degrees of hemolysis are produced by *S. aureus* and occasionally by other species. *Staphylococcus saprophyticus* is a frequent cause of cystitis in women, probably related to its occurrence as part of normal vaginal flora.

S. saprophyticus can be distinguished from *S. epidermidis* and most other coagulase-negative staphylococci by its natural resistance to novobiocin. [**Note:** Urinary coagulase-negative staphylococcus is often presumed to be *S. saprophyticus*; novobiocin resistance can be used for confirmation.].

fig 36. (a) *S. aureus* (β-hemolysis) (b) *S. epidermidis* (no hemolysis) (c) *S. saprophiticus* (no hemolysis)

3.3 ENTEROBACTERIACAE FAMILY

Escherichia coli

Short gram-negative rods, motile, *E. coli* are facultative anaerobes and form circular, convex, smooth, flat, non-viscous colonies with distinct edges. **Lactose fermented rapidly**, some strains of *E. coli* produce hemolysis on blood agar, have metallic sheen on differential media.

fig 37. *Escherichia coli* (a) Gram-staining (b) MacConkey Agar (c) lactose fermenters and non-lactose fermenter

Klebsiella

Short gram-negative rods, motile and facultative anaerobes. Capsules are large and regular in *Klebsiella* species. *Klebsiella* colonies are large and very mucoid and tend to coalesce with prolonged incubation and **Lactose fermented rapidly**. *Klebsiella pneumonia* are very viscous and have mucoid growth.

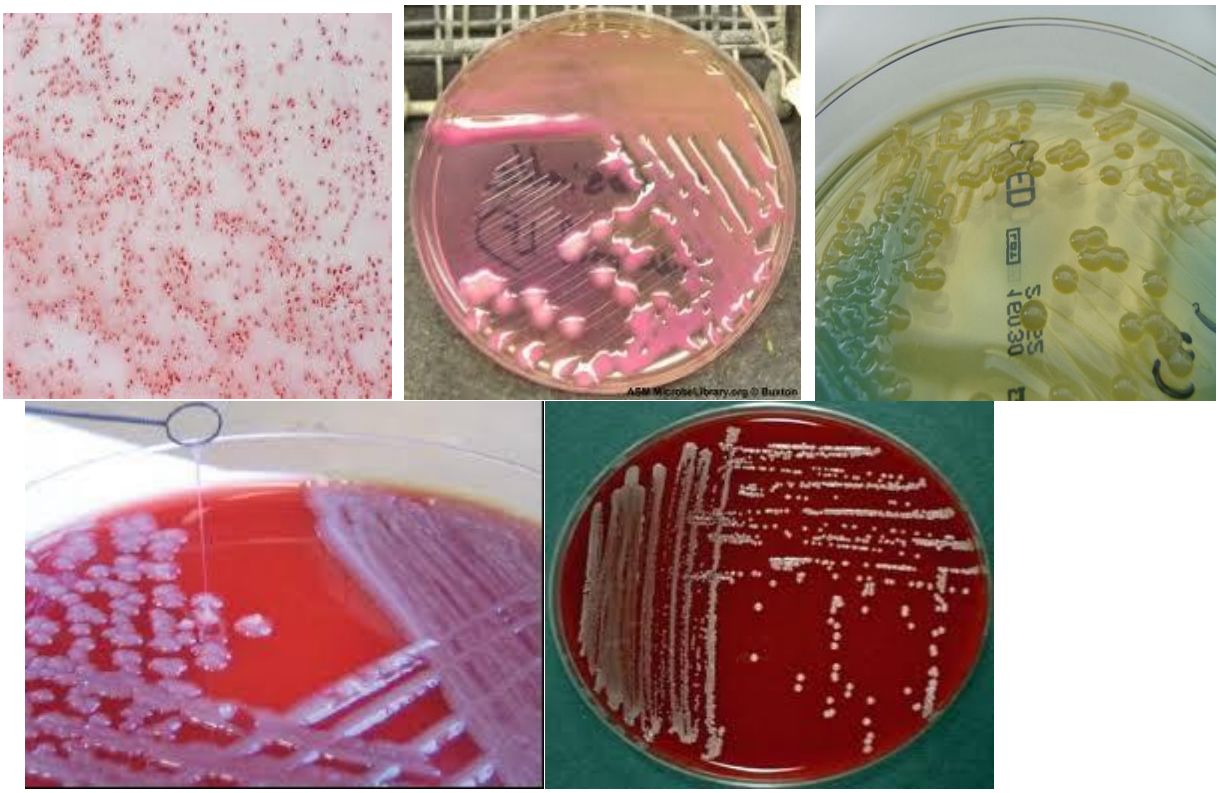


fig 38. *Klebsiellla* (a) Gram staining (b) MacConkey Agar (c) CLED Agar (d and e) Blood Agar

Enterobacter and Citrobacter

Short gram-negative rods, facultative anaerobes, most *Enterobacter* and *Citrobacter* species give positive test results for motility, produce mucoid colonies, *Enterobacter aerogenes* has small capsules. Raised colonies, no metallic sheen; often motile; more viscous growth *Enterobacter cloacae* has similar to *Enterobacter aerogenes.* They are both weak fermenters of lactose with *Citrobacter* a late fermenter which grows after 48hrs.

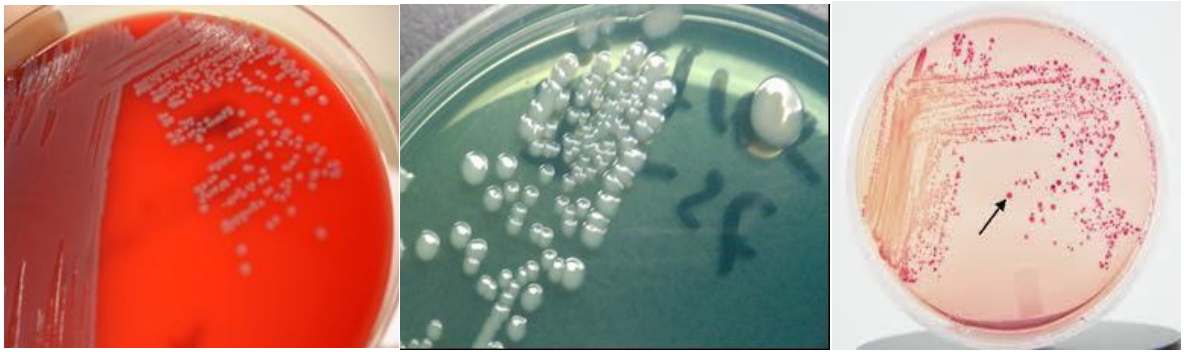


fig 39. Colony morphology in (a) Blood Agar (b) CLED Agar (c) MacConkey Agar

Serratia

Short gram-negative rods, facultative anaerobes, slowly fermented Lactose *Serratia* species produces DNase, lipase, and gelatinase. *Serratia marcescens* is a common opportunistic pathogen in hospitalized patients. *Serratia* (usually non-pigmented) causes pneumonia, bacteremia, and endocarditis. Only about 10% of isolates form the red pigment (prodigiosin) that has long characterized *S. marcescens. S. marcescens* appear as smooth, round, medium sized colonies on blood agar. Some strains produce a pinkish-orange pigment. They appear as red pigment on CLED and MacConkey agar plate.

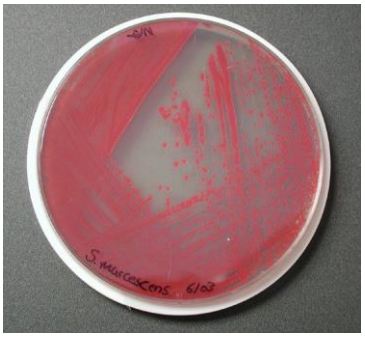


fig 40. Characteristic red colonies of serratia spp.

3.4 PROTEUS.

Short gram-negative rods, facultative anaerobes. *Proteus* species moves very actively by means of peritrichous flagella, resulting in "swarming" on solid media unless the swarming is inhibited by chemicals such as phenyl-ethyl alcohol or CLED (cystine-lactose-electrolyte deficient) medium. *Proteus* species are urease positive (smell of ammonia). The *Proteus* ferments lactose very slowly or not at all. *Proteus mirabilis* is more susceptible to anti-microbial drugs, including penicillin, than other members of, the group.

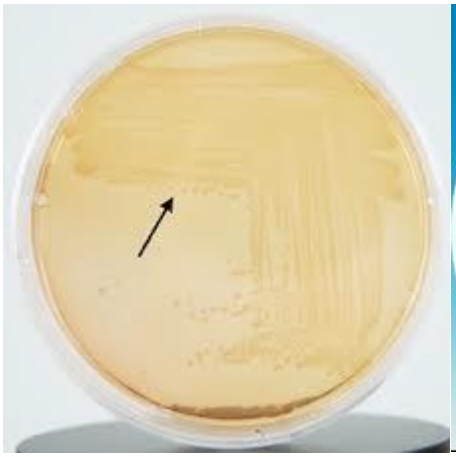


Fig.41. Proteus on MacConkey

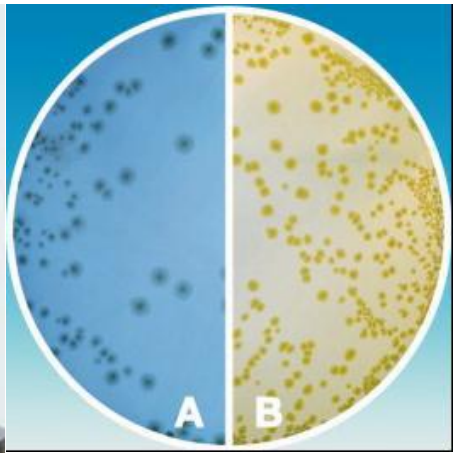


fig.42 (A) Proteus on CLED (B) E. coli on CLED

Fig.42c. Proteus on Blood Agar Plate

3.5 PSEUDOMONADS

The pseudomonads are gram-negative rods occurs as single bacteria, in pairs, and occasionally in short chains, motile, obligate aerobic, some of which produce water-soluble pigments. The pseudomonads occur widely in soil, water, plants, and animals. *P. aeruginosa* is frequently present in small numbers in the normal intestinal flora and on the skin of humans and is the major pathogen of the group. Other pseudomonads infrequently cause disease. *P. aeruginosa* grows readily on many types of culture media, sometimes producing a sweet or grapelike or corn taco–like odor. Some strains hemolyze blood. *P. aeruginosa* forms smooth round colonies with pigments which diffuse into the agar. Other *Pseudomonas* species do not produce the same pigments. *P. aeruginosa* in a culture can produce multiple colony types.

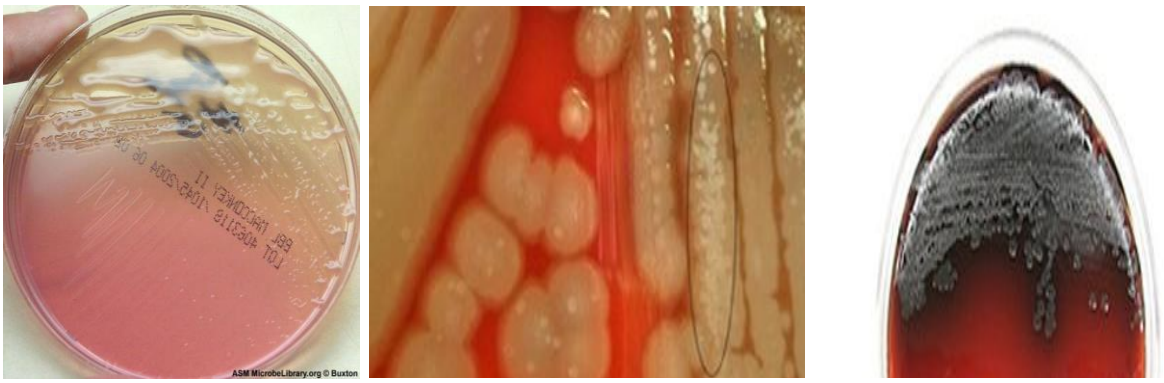


fig 43. Pseudomonas spp in MacConkey and Blood Agar

LABORATORY EQUIPMENTS AND INSTRUMENTS

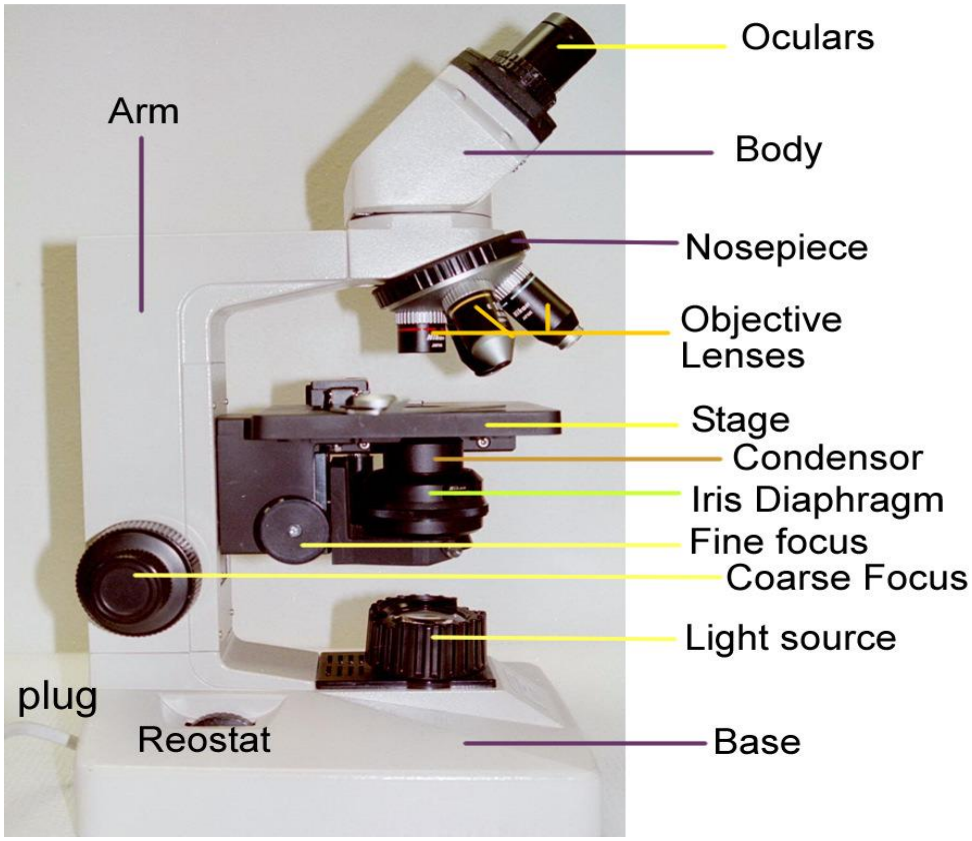


fig 44. THE STRUCTURE OF LIGHT MICROSCOPE

fig 45. Sterile cotton swab

fig 46. Bacteriological loop

fig 47. Petri-dish

fig 48. Incubator

fig 49. Hot air Oven

fig 50. Anaerobic jar

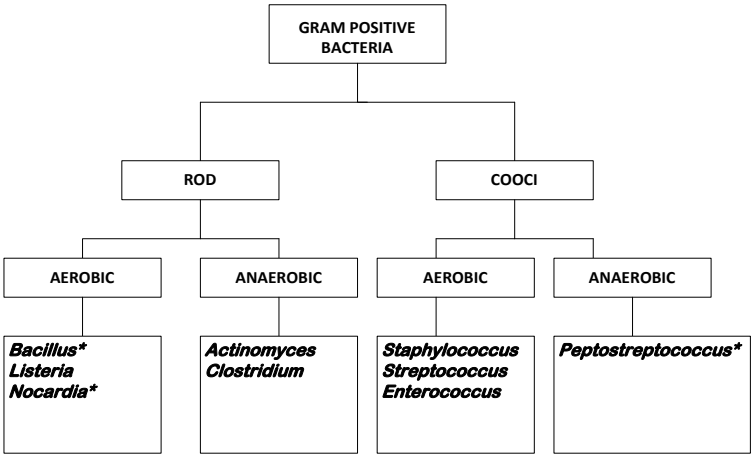
fig 51. Autoclave

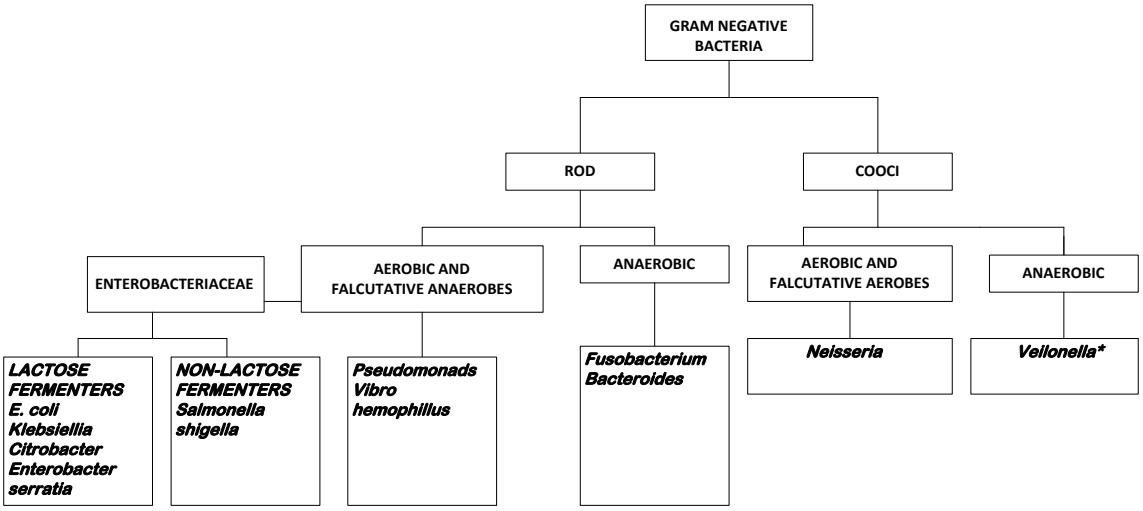
SCHEDULE MAINTENANCE OF LABORATORY EQUIPMENTS

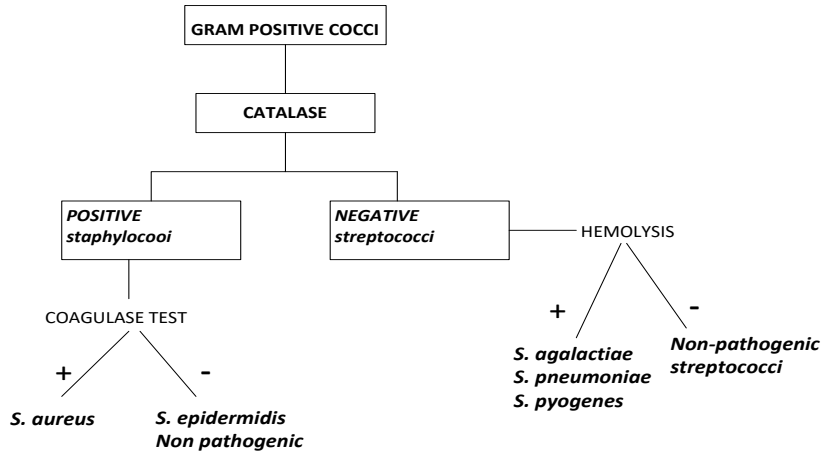
Equipment	Routine care	Monitoring	Technical maintenance and inspection
Autoclave	Clean and change water monthly	Check and adjust water level before each run. Record time and temperature or pressure for each run.	Every 6 months
Centrifuge	Wipe inner walls with antiseptic solution weekly or after breakage of glass tube or spillage		Replace brushes annually
Microscope	Wipe lenses with tissue or lens paper after each day's work. Clean and lubricate mechanical stage weekly. Protect with dust cover when not in use.	Check alignment of condenser monthly. Place a dish of blue silica with the microscope under the dust cover to prevent fungi growth in humid condition.	Annually
Refrigerator	Clean and disinfect weekly and daily during prolonged power failure.	Record temperature every morning (2-8˚C)	Every six months
Generator	Check for fuel and switch on for 5 minutes every morning.	Check and change the oil every month. Dust off sand and clean accessible areas with damped cloth.	Every 3 months.

Table 7. Maintenance of laboratory equipment

APPENDIX-1


GRAM POSITIVE BACTERIA
ROD
COOCI
AEROBIC
ANAEROBIC
AEROBIC
ANAEROBIC
Bacillus*
Listeria
Nocardia*
Actinomyces
Clostridium
Staphylococcus
Streptococcus
Enterococcus
Peptostreptococcus*


GRAM NEGATIVE BACTERIA
ROD
COOCI
ENTEROBACTERIACEAE
AEROBIC AND FALCUTATIVE ANAEROBES
ANAEROBIC
AEROBIC AND FALCUTATIVE AEROBES
ANAEROBIC
LACTOSE FERMENTERS
E. coli
Klebsiella
Citrobacter
Enterobacter
serratia
NON-LACTOSE FERMENTERS
Salmonella
shigella
Pseudomonads
Vibro
hemophillus
Fusobacterium
Bacteroides
Neisseria
Veillonella*


GRAM POSITIVE COCCI
CATALASE
POSITIVE
staphylocooi
NEGATIVE
streptococci
HEMOLYSIS
COAGULASE TEST
+
-
+
-
S. aureus
S. epidermidis
Non pathogenic
S. agalactiae
S. pneumoniae
S. pyogenes
Non-pathogenic
streptococci

APPENDIX-2

BACTERIA	MORPHOLOGY	CATALASE	COAGULASE	MOTILITY	OXIDASE	INDOLE
S. aureus	BAP: Yellow to creamy colony with β-hemolysis MAC: No growth CLED: Same as BAP	+	+	-	-	-
S. epidermidis	BAP: White colony with no hemolysis MAC: No growth CLED: Same as BAP	+	-	-	-	-
Streptococcus agalactiae *S. pyogenes*	BAP: Pin point colony with β-hemolysis MAC: No growth	-	-	-	-	-
Streptococcus pneumonia	BAP: Tiny colony with concave center and α-hemolysis MAC: No growth	-	-	-	-	-
Enterococcus	BAP: No hemolysis and diplococci on gram staining MAC: No growth	-	-	-	-	-
E. coli	BAP: Shinny opaque creamy colony (β-hemolytic is pathogenic, non-hemolytic type are commensals) MAC: Lactose fermenter with vigorous precipitation of bile CLED: Yellow colonies (commensals), pink to red colony (pathogen)	+	N/A	+	-	+
Klebsiella	BAP: Shinny white/creamy mucoid colony MAC: Mucoid Lactose fermenter with little bile ppt CLED: Yellow to blue mucoid colony	+	N/A	-	-	+
Citrobacter	BAP: White opaque colony with no hemolysis MAC: Ferments lactose after 48 hrs.	+	N/A	+	-	-
Enterobacter	BAP: White opaque colony with α-hemolysis MAC: Slow lactose fermenter, less mucoid than *Klebsiella*	+	N/A	+	-	-
Proteus	BAP: Swarming effect on blood agar. MAC: Non-lactose fermenter CLED: Translucent blue colony	+	N/A	+	-	-
Pseudomonas	BAP: Large, rough, mucoid or pigmented colony often with β-hemolysis MAC: Non lactose fermenter with green pigment CLED: Green colony with mattered surface	+	N/A	+	+	-
Neisseria spp.	No growth on MAC, gram negative cocci (bean shaped)	+	-	-	+	-
Hemophillus	No growth on MAC, gram negative cocobacilli	+	-	-	+	+
*Corynobacterium nigricans**	Black circular mucoid colony in BAP (Non-hemolytic), CLED and MAC (Nonlactose fermenter but ferments glucose and sucrose), Gram positive, cocoidial/cocobacillary to short rod-shaped non-sporulated and clustered. Causes UTI and B. vaginosis.	+	N/A	+	N/A	-
*Bacillus mycoides**	BAP: Rhizoid colony with filamentous and serrated edges. Gram staining shows gram variable hyphae	+	N/A	-	-	-

APPENDIX-3

SWAB TEST REPORT

NAME: MISS. ROSELINE OGHENERUME OVIE **AGE:** 24 YEARS **SEX:** FEMALE **LAB Nó:** 0273

TEST: HIGH VAGINAL SWAB M/C/S **NATURE OF SAMPLE:** HIGH VAGINAL SWAB

MICROSCOPY:

WET MOUNT: Epithelial (10/LPF), RBC (0-3/hpf), WBC (11/hpf), Yeast, Clue cells and Bacteria (Present) **WHIFF TEST:** POSITIVE **pH:** 8.0

T. vaginalis: PRESENT **OTHERS:**

GRAM STAINING: Gram positive cocci with many pus cells seen

ISOLATE(S): Culture yielded heavy growth of staphylococcus aureus after 24 hrs of incubation at 37°C

SUSCEPTIBILITY TEST

SENTITIVE: Augmentin (4+), Ciproflox (2+), Streptomycin (2+), Erytromycin (+), Gentamycin (3+), Septrin (2+), Ceporex (3+), Amoxil (+)

RESISTANT: Tarivid, Ampicilin

COMMENT: Pathogenic microorganism isolated

Sign: Odiakose C. H
LABORATORY SCIENTIST

*****Micro-organism(s) is open to further research and investigation.

N/A: NOT APPLICABLE.
+VE: POSITIVE/VIABLE/YES.
-VE: NEGATIVE/NON-VIABLE/NO

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