SRI SHANMUGHA COLLEGE OF ENGINEERING AND TECHNOLOGY

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BM8311 PATHOLOGY AND MICROBIOLOGY PRACTICALS

Department of Biomedical Engineering

Lab Manual

PREPARED BY

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ASP/CHEMISTRY

Ex. No: 1 **Date: ----------**

URINE - PHYSICAL AND CHEMICAL EXAMINATION

AIM

To determine the components of urine sample both physically and chemically

THEORY

Tests can be performed on urine samples to detect the presence of certain compounds or chemicals which may be indicative of an underlying disease. These tests are usually considered to be a part of a routine urinalysis. When performed correctly, these tests can provide valuable information to the physicians. The specimen is very easy to obtain. The first morning specimen is the preferred specimen as it is the most concentrated and has less of a chance of giving false negative results. Urine contains microscopic elements in suspension (cells, crystals etc) these elements are collected by centrifuging and a drop of the deposit is examined the slide and cover slip. As all these elements in suspension would sediment in the urine if left for a few hours, they are called urinary deposits.

URINE EXAMINATION

Preparing for urinalysis

Before your test, make sure to drink plenty of water so that you can give an adequate urine sample. You don't have to fast or change your diet for the urinalysis test.

Also, tell your doctor about any medications or supplements you're taking. Medications or supplements that can affect the results of your urinalysis include:

- vitamin C supplements
- metronidazole
- riboflavin
- anthraquinone laxatives
- methocarbamol nitrofurantoin

Some illegal drugs can affect your results as well. Tell your doctor about any substances you use before doing a urinalysis.

A. PHYSICAL CHARACTERISTICS OF URINE

The physical characteristics of urine include observations and measurements of color, turbidity, odor, specific gravity, pH and volume. Visual observation of a urine sample can give important clues as to evidence of pathology.

1. COLOR

The color of normal urine is usually light yellow to amber. Generally the greater the solute volume the deeper the color. The yellow color of urine is due to the presence of a yellow pigment, urochrome. Deviations from normal color can be caused by certain drugs and various vegetables such as carrots, beets, and rhubarb.

2. ODOR

Slightly aromatic, characteristic of freshly voided urine. Urine becomes more ammonia-like upon standing due to bacterial activity.

3. TURBIDITY

Normal urine is transparent or clear; becomes cloudy upon standing. Cloudy urine may be evidence of phosphates, urates, mucus, bacteria, epithelial cells, or leukocytes.

4. pH

Ranges from 4.5 - 8.0. Average is 6.0, slightly acidic. High protein diets increase acidity. Vegetarian diets increase alkalinity. Bacterial infections also increase alkalinity.

B. CHEMICAL CHARACTERISTICS OF URINE

BENEDICT'S TEST

Procedure:

- Take 2 ml urine sample in a measuring cylinder from the urine sample bottle.
- Take a test tube and pour the urine sample in it.
- Take 5 ml Benedict’s reagent in a measuring cylinder. Add Benedict’s reagent to the test tube that contains urine sample.
- Using a test tube holder, hold the test tube firmly and heat it for 2 minutes on the burner.
- Keep shaking the test tube while heating.
- A yellow precipitate appears which indicates the presence of sugar in urine.
- Depending upon the concentration of sugar in the urine: green, yellow, or brick red precipitates are formed.

Viva Voce

1. What is Chemical examination of urine?

2. What is chemical urinalysis?

3. What are the characteristics of normal urine?

4. What is RBC in urine?

5. What are the causes of bacteria in urine?

RESULT

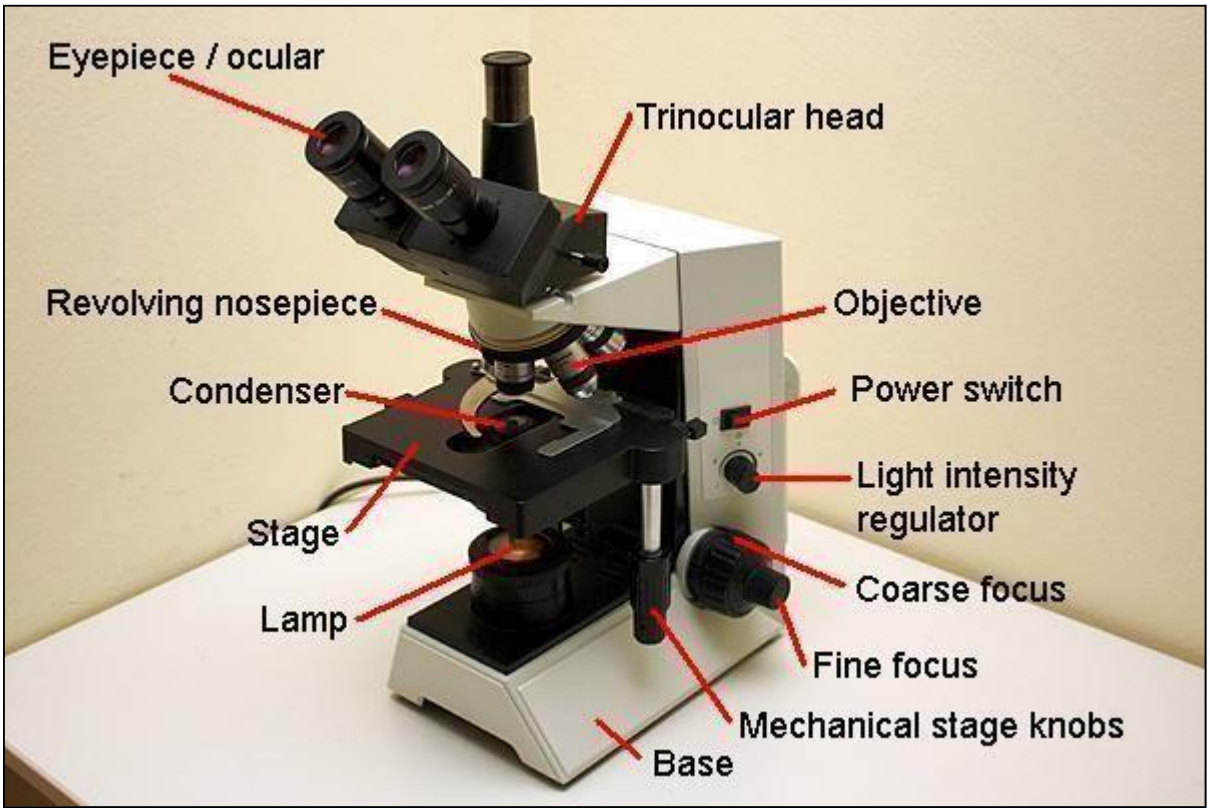


Fig: Compound Microscope

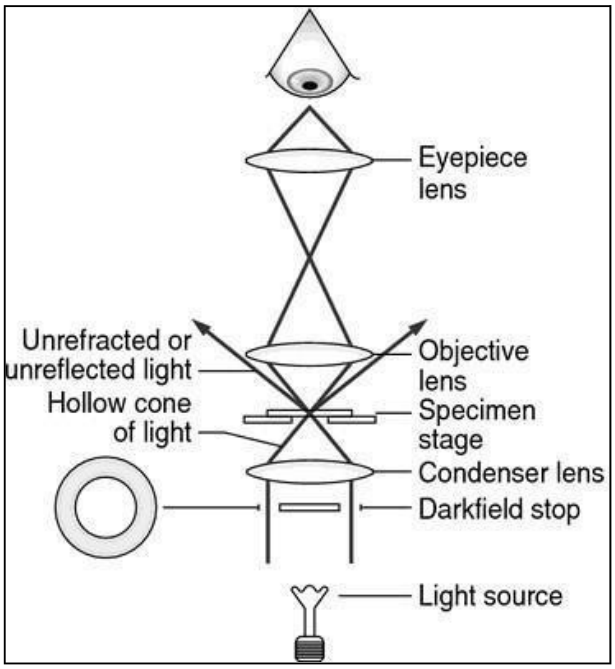


Fig: Principle of compound microscope

Ex. No: 2 **Date: ----------**

STUDY THE PARTS OF COMPOUND MICROSCOPE

AIM

To identify the parts of Compound microscope and study its working principle.

THEORY

The typical compound light microscope is capable of increasing our ability to see detail by1000 times so that objects as small as 0.1 micrometer (um) or 100 nanometers (nm) can be seen. Electron microscopes extend this range further allowing us to see objects as small as 0.5 nm in diameter or roughly 1/200,000th the size it will see with a naked eye. It is mostly used to see the cells and their structure and function.

MAGNIFICATION

The microscope has 3 magnifications:

1) Scanning
2) Low
3) High

	Magnification	**Ocular lens**	**Total Magnification**
Scanning	4x	10x	40x
Low Power	10x	10x	100x
High Power	40x	10x	400x

PARTS OF COMPOUND MIOCROSCOPE

Component	Description
Base	Metal or plastic part on which microscope rests
Arm	Somewhat C shaped pillar arising from base that supports the stage and ocular components
Stage	Flat platform attached to lower portion of arm on which slides or samples are placed
Condenser or Iris Diaphragm lever	Lever beneath opening in the stage, consists of a shutter-like group of metal leaves which regulate the amount of light coming through the slide on the stage
Condenser	Not present on all microscopes – collects illuminator light rays and focuses them – increases the resolution, enhances contrast of sample
Condenser adjustment Knob	Raises and lowers the condenser
Tube/Barrel	Cylindrical/vertical part attached at the top of arm for support of optical system
Turret	Revolving plate which bears the objective lens, attached at lower end of tube, can be turned to change the objective lens
Coarse Focus	Larger knob which moves the tube up or down rapidly to get the sample into coarse focus
Fine Focus	Smaller knob which moves the tube through short distances slowly and is used to get the sample into sharp focus
Transformer or illuminator	Controls the amount of light transferred to the sample
Eyepiece (ocular lens)	Removable, short metal tube that contains lens that fit into the top of the tube – generally 10-15x magnification
Eyepiece Focusing Ring	Adjustment used to compensate for differences between eyes
Objective Lens	Small metal tubes screwed into the turret which increase the Magnification of the sample. (often referred to as just objective)
Mirror	Used to reflect light through a sample
Mirror Axle	Used to adjust mirror to reflect light through a sample

Viva Voce

1. What are the parts of the microscope and what do they do?

2. What are the different parts of a microscope?

3. What are the uses of the compound microscope?

4. What is a monocular compound microscope?

5. What would be the magnification if you were using a 40x objective?

RESULT

Thus the parts of Compound microscope were identified and studied.

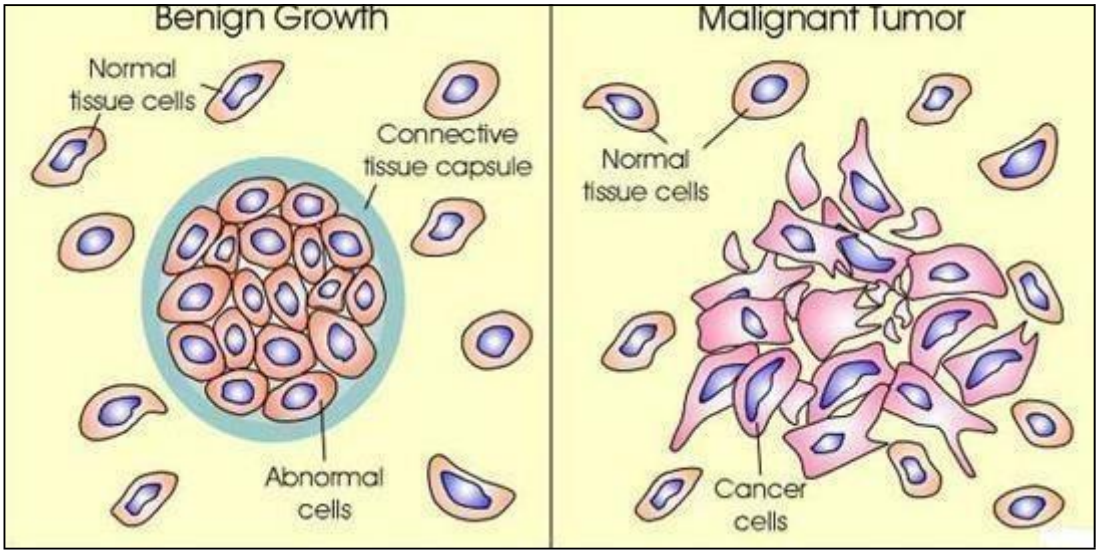


Fig: Benign Growth and Malignant tumor

Ex. No: 3 **Date: ----------**

MALIGNANT TUMOURS

AIM

To examine the histopathological slides of benign and malignant tumours.

THEORY

Tumours can grow for a variety of reasons. Benign tumours are not caused by cancer. Tumours caused by cancer are called malignant or cancerous. While the underlyingcauses for tumour growth can vary, the process by which they grow as the same normal cells in our body will naturally refresh them by dividing and this allows for dead cells to be disposed of naturally. In the case of tumours, dead cells may remain behind and form agrowth known as a tumour. Cancerv cells grow in this way as well however, unlike the cells in benign tumours, they also invade nearby tissue. Out of control growth of abnormal cells causes damage to these adjacent tissues, organs and can lead to cancerous tumours in other parts of the body. Most malignant brain tumours are caused by a cancer that started somewhere else in the body and spread to the brain through the bloodstream.

S. No.	BENIGN	MALIGNANT
1.	A slow growing self contained tumour that is not seriously harmful.	A fast growing, often fatal tumour that invades surrounding tissue and sheds that spreads throughout the body.
2.	Benign brain tumours are noncancerous. Benign brain tumours usually have clearly defined borders and usually are not deeply rooted in brain tissue. This makes them easier to surgically remove, assuming they are in an area of the brain that can be safely operated on. But even after that have been removed, they can still come back, although benign tumours are less likely to recur than malignant ones	Malignant primary brain tumours are cancers that originate in the brain, typically grow faster than benign tumours, and aggressively invade surrounding tissue. although brain cancer rarely spreads to other organs, it will spread to other parts of the brain and central nervous system

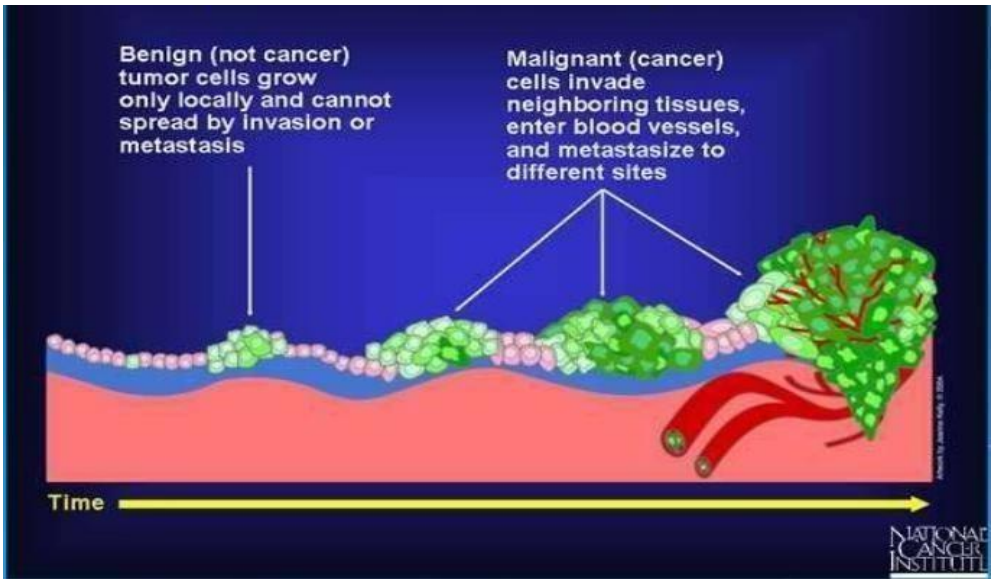
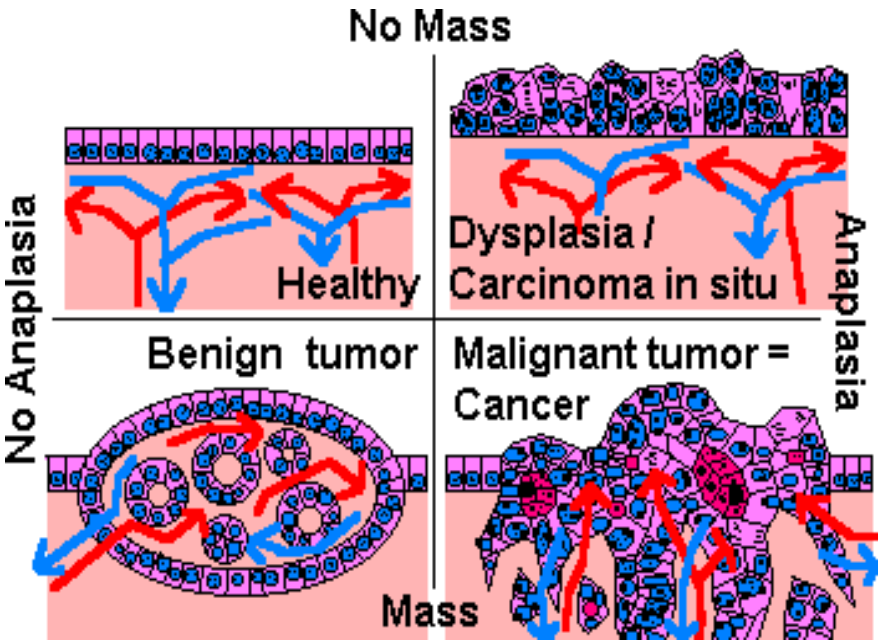


Fig: Malignant Vs Benign Tumors



PROCEDURE

1. Take a permanent slides were coated with specimen.
2. Place the slides in slide stage of microscope and observe under low power objective lens like 10x, 40x.
3. Observe the image results and record it.

Viva Voce

1. What is malignant tumor definition?

2. What does it mean to have a malignant tumor?

3. Is a malignant tumor bad?

4. Is there a cure for malignant tumor?

5. How does a malignant Tumour spread?

RESULT

Thus the histopathological slides of benign and malignant tumours were examined under microscope.

Procedure for simple staining

Ex. No: 4 **Date: ----------**

SIMPLE STAINING

AIM

To study the morphology of bacteria by simple staining method.

THEORY

In simple staining, the bacterial smear is stained with a single reagent. Basic stains with a positively charged chromogen are preferred, because bacterial nucleic acids and certain cell wall components carry a negative charge that strongly attracts and binds to the cationic chromogen. The purpose of simple staining is to elucidate the morphology and arrangement of bacterial cells. The most commonly used basic stains are methylene blue, crystal violet and carbol Fuchsin.

MATERIALS REQUIRED

Crystal Violet

PROCEDURE

1. The glass slide was washed well with soap water and a drop of saline was taken on the slide with the sterilized inoculation loop.
2. The inoculating needle is flamed, cooled for few seconds.
3. Very small amount of the organisms are picked and smeared well over the slide.
4. The smear was air-dried and heat fixed.
5. Primary stain such as crystal violet was applied
6. It was left for a minute and washed in running water.
7. The crystal violet was well fixed to the culture.
8. The bacterial cell was viewed under oil immersion objective lens.

Viva Voce

1. How does a basic stain work?

2. What are the staining procedures?

3. What is the use of methylene blue stain?

4. What is the difference between simple and differential staining?

5. What is an example of a simple stain?

RESULT

Thus the morphology of the given organism was found to be----------

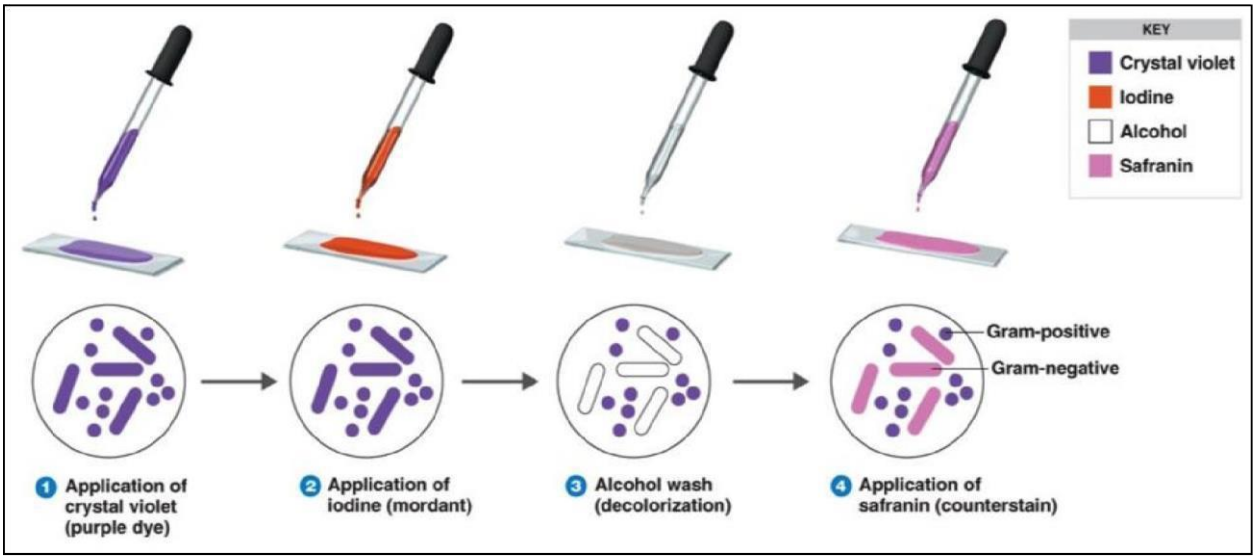


Fig: Gram staining

Ex. No: 5 **Date: ----------**

GRAM STAINING

AIM

To perform gram staining and to differentiate the bacteria into two principle groups, gram positive and gram negative.

THEORY

Three chemical reagents are used in this staining that is applied sequentially to a heat fixed smear. The first reagent is the primary stain. It gives colour to all cells in the smear .The second reagent is the mordant, which is a decolorizing agent was used. A mordant is a compound that fixes the primary stain to the cells. Based on the chemical composition of cellular components, the decolorizing agent may or may not remove the primary stain from the entire cell or only from certain cell structures.

The final reagent is the counter stain has a contrasting colour to that of the primary stain, following decolourization if the primary stain is not washed out, the counter stain cannot be absorbed and the cell or its components will retain the colour of the primary stain. If the primary stain is removed, the decolorized cellular components will accept andassume the contrasting colour of the counter stain. In this way, cell types or their structures can be distinguished from each other on the basis of the stain that is retained.

The most important differential stain used in bacteriology is gram stain, namedafter Christian gram. It divides bacterial cells into two major groups' gram positive and gram negative, which makes an essential tool for classification and differentiation of micro organisms. Gram stain uses four different reagents.

REAGENTS REQUIRED

1) Crystal Violet
2) Gram's Iodine
3) Decolorizing agent (95% Ethyl alcohol)

4) Safranin

PRIMARY STAIN

Crystal Violet

This stain was used first and it stains all the cells purple-blue

MORDANT

Gram's Iodine

This reagent serves as a mordant, a substance that forms an insoluble complex by binding to the primary stain. The resultant crystal violet-iodine complex serves to intensify the colour of the stain and all the cells will appear purple-black at this point. In gram positive cell, only these crystal violet-iodine complexes bind to the magnesium ribonucleic acid components of the cell wall. The resultant magnesium ribonucleic acid Crystal violet – iodine complex is more difficult to remove than the smaller CVI components.

DECOLORIZING AGENT (95% Ethyl alcohol)

This reagent serves a dual function as a lipid solvent and a protein dehydrating agent. Its action is determined by the lipid concentration of the microbial cell walls. In gram negative cells the high lipid concentration found in the outer layer of the cell wall is dissolved by alcohol, creating large holes in the cell wall that don't close appreciably and dehydration of the cell wall protein.

These facilitates the releases of unbound CVI complex, these cells are colorless or unstained.

COUNTERSTAIN

Safranin is the final reagent to stain red. These cells that have been previously decolorized since only gram negative cells undergo decolourisation. They may now absorb the counter stain. The gram positive cells obtain the purple blue color of primary stain.

CULTURES

About 24 hours grown cultures of *Staphylococcus sp* and *Klebsiella sp*.

PROCEDURE

1. Clean glass slide was taken and a smear was made from the given culture with the help of sterile inoculation loop.
2. The slides were air dried and heat fixed.
3. The smear was flooded with crystal violet and allowed to stand for 60 seconds.
4. The smear was then washed with tap water by holding the slide parallel to the stream of water.
5. Then the smear was flooded with gram's iodine and allowed to stand for 30 seconds and again washed with tap water.
6. The decolorizing agent ethyl alcohol was added drop by drop until crystal violet fails to wash from smear and again immediately washed with tap water.
7. It was next counterstained with safranin for 60 seconds.
8. It was then washed with tap water.
9. The slide was air dried and examined under an oil immersion objective.

Viva Voce

1. What does the Gram stain test for?

2. What does it mean to be Gram positive or negative?

3. Why is the Gram stain so important?

4. How Gram staining was discovered?

5. What color are gram positive bacteria?

RESULT

Thus the observations of the given organism shows that

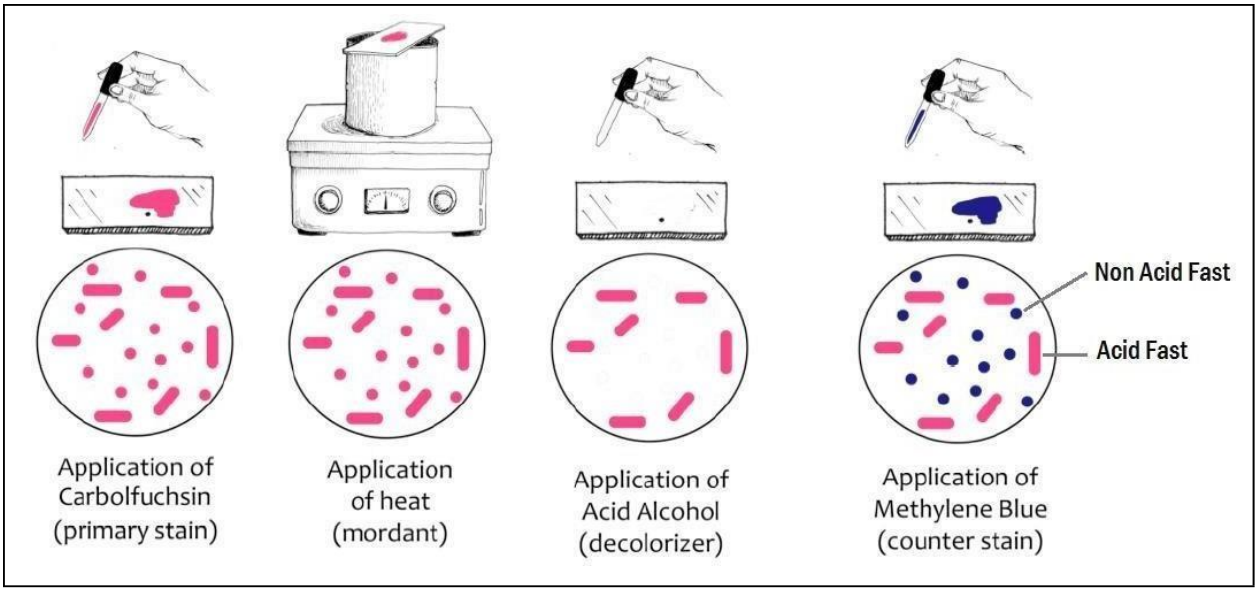


Fig: Acid Fast Bacilli Staining

Ex. No: 6 **Date: ----------**

ACID FAST BACILLI STAINING

AIM

To differentiate acid fast bacteria from non acid fast bacteria by acid fast staining.

THEORY

The ordinary aniline dye doesn't penetrate the cell wall of the tubercle bacilli. Members of the bacterial genera, Mycobacterium and Myocardial contain larger amounts of lipid substances which resist staining by ordinary methods. Therefore it is unsuitable for staining. If a powerful staining solution containing phenol with the application of heat is used it makes the dye to penetrate bacilli. Once stained the tubercle bacilli will withstand the action of a powerful decolorizing agents for a considerable time. The stain consists of basic fuchsine and phenol. The basic dye with mineral acid produces a compound which is yellowish brown in color and is readily dissolved out of all structures except acid fast bacteria. The technique also involves a counter stain to demonstrate whether or not the fuchsine has been decolorized within the cell and the second stain takes up. Any strong acid can be used as a decolourising agent upto 20% sulphuric acid by volume is usually employed. Acid alcohol may be used instead and generally given clear films.

MATERIALS REQUIRED

- Carbol Fuchsin
- Decolourising agent (20% sulphuric acid)
- Methylene Blue

PROCEDURE

1) Air dry or heat fixes the organism on a clean glass slide.
2) Flood the slide with carbol fuchsin stain.
3) Heat underside of the slide with the Bunsen burner or hot plate until steam rises without boiling.

4) Keep the preparation for 5 minutes with intermittent heating, over heating causes spattering of stain and cracking of the slide.

5) The stain must not be allowed to evaporate and dry on the slide.

6) If necessary pour on more stain to keep the slide covered with stain.

7) Wash the film with a gentle and indirect stream of tap water until colour appears in the effluent.

8) Flood the slide with the decolourising agent (20% sulphuric acid).

9) Immediately wash off with tap water.

10) Repeat the decolorizing and washing step until the film appear faintly pink.

11) Flood the smear with the counter stain (methylene blue) for 20-30 seconds.

12) Wash with tap water; blot the film into dry with absorbent paper.

13) Examine under microscope.

OBSERVATION

Acid fast bacteria appear as red and non acid fast bacteria appear as blue.

Viva Voce

1. What does an acid fast stain tell you?

2. What is the purpose of the acid fast staining technique?

3. What is acid fast bacilli test?

4. What color is an acid fast stain?

5. Why is the acid fast stain of medical importance?

RESULT

Thus the given microscopic substance appeared……..

Fig :LEUKEMIA

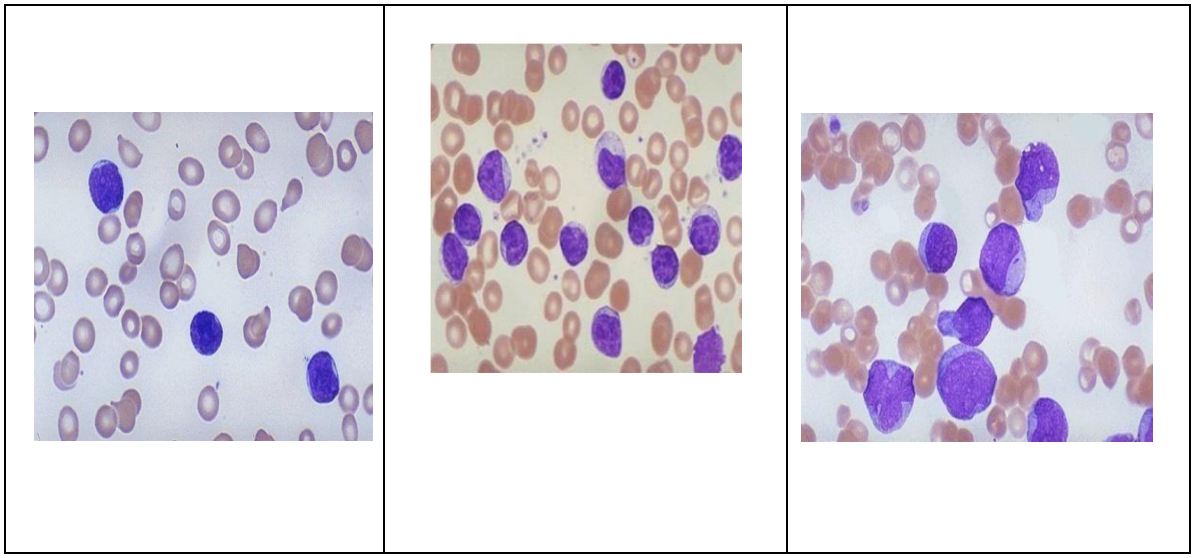
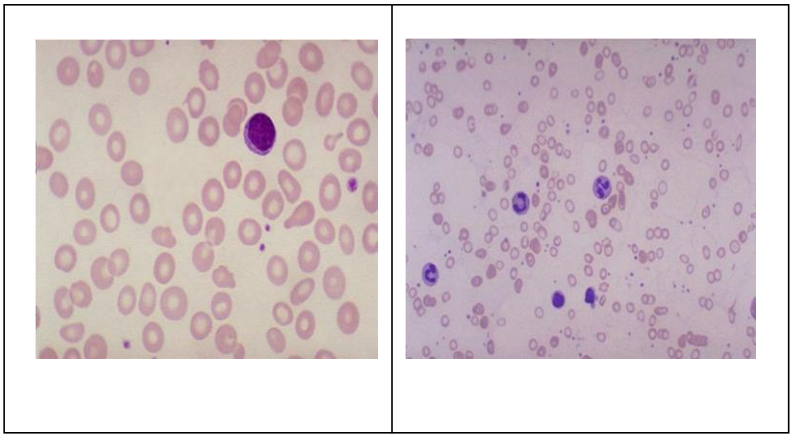


Fig: ANEMIA



Ex. No: 9 **Date: ----------**

HAEMATOLOGY SLIDES OF ANEMIA AND LEUKEMIA

AIM

To identify the microscopic permanent slides of anemia and leukemia cells

INTRODUCTION

Anemia is a medical condition in which the red blood cell count or hemoglobin is less than normal. For men, anemia is typically defined as hemoglobin level of less than 13.5 gram/100 ml and in women as hemoglobin of less than 12.0 gram/100 ml. Anemia is caused by either a decrease in production of red blood cells or hemoglobin, or an increase in loss or destruction of red blood cells. Leukemia is cancer of the blood cells. It starts in the bone marrow, the soft tissueinside most bones. Bone marrow is where the blood cells are made

APPARATUS REQUIRED

1. Microscope
2. Permanent anemia slides
3. Leukemia cells coated slides

CAUSES OF ANEMIA

- Anemia caused by blood loss.
- Anemia caused by decreased or faulty red blood cell production.
- Anemia caused by destruction of red blood cells

SYM PTOMS

Some patients with anemia have no symptoms. Others may feel tired, easily fatigued, appear pale, a feeling of heart racing, short of breath and/or worsening of heart problems.

DETECTION

Anemia can be detected by a simple blood test called a complete blood cell count (CBC). The treatment of the anemia varies greatly and very much depends on the particular cause.

CAUSES OF LEUKEMIA

- Exposed to large amounts of radiation.
- Exposed to certain chemicals at work, such as benzene.
- Had some types of chemotherapy to treat another cancer.
- Have down syndrome or some other genetic problems.
- Smoke.

SYMPTOMS

Symptoms may depend on what type of leukemia but common symptoms include:

- Fever and night sweats.
- Headaches.
- Bruising or bleeding easily.
- Bone or joint pain.
- A swollen or painful belly from an enlarged spleen.
- Swollen lymph nodes in the armpit, neck, or groin.
- Getting a lot of infections.
- Feeling very tired or weak.
- Losing weight and not feeling hungry.

PROCEDURE

1. Take a permanent slides were coated with specimen.
2. Place the slides in slide stage of microscope and observe under low power objective lens like 10x, 40x.
3. Observe the image results and record it

Viva Voce

1. What could cause anemia?

2. How do you become anemic?

3. What vitamins can cause anemia?

4. What is the cause of leukemia?

5. What are the early signs of leukemia?

RESULT

Thus the given microscopic hematology slides appeared as ---------- --.

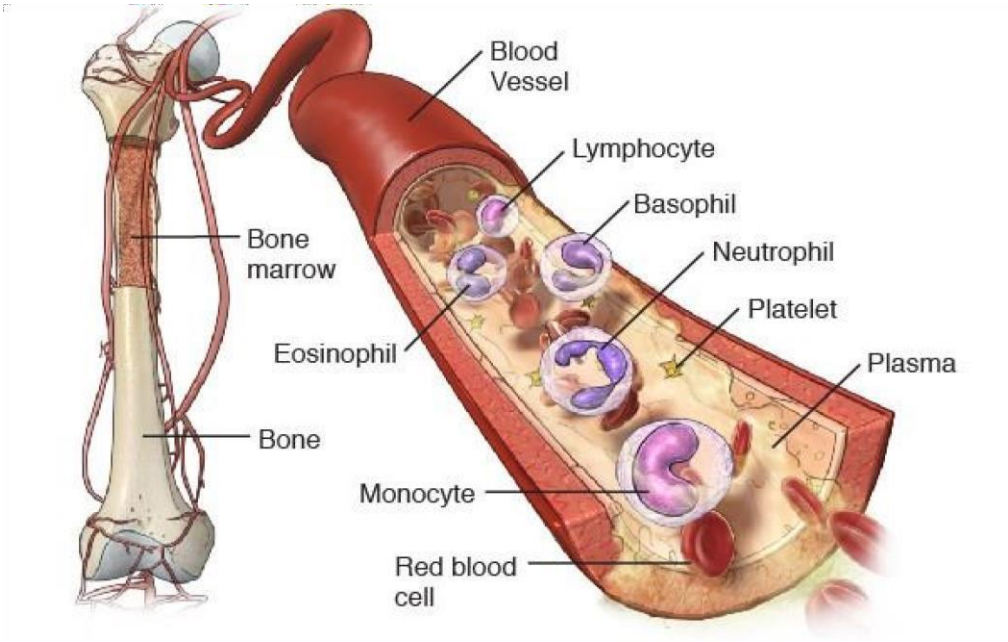
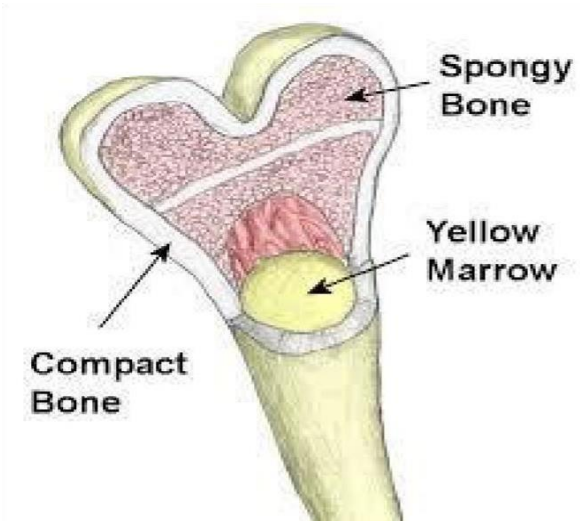
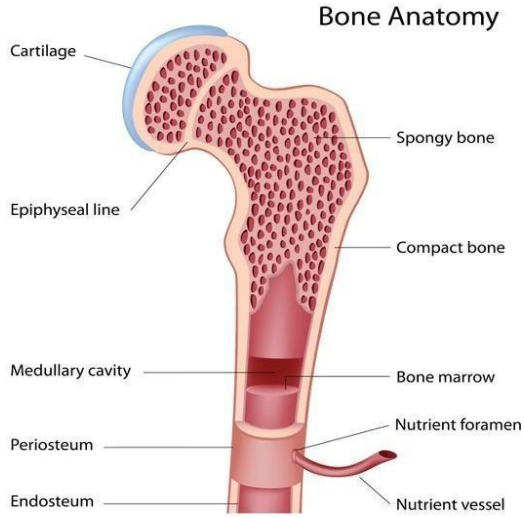


Fig: Bone marrow





Ex. No: 10 **Date: ----------**

STUDY OF BONE MARROW CHARTS

AIM

To identify the parts of Bone Marrow using Bone Marrow Charts

INTRODUCTION

The soft tissue occupying the soft cavities of bones. There are two types of bone marrow: red and yellow. Red blood cells, platelets, and most white blood cells arise in red marrow. At birth, all bone marrow is red. With age, more is converted to yellow. In cases of severe blood periods, the body can convert yellow marrow back to red marrow to increase blood cell production. Marrow is found mainly in the flat bones such as the hip, breast, skull, ribs, vertebrae, and shoulder blades, as well in long bones at the end the femur and humerous.

Examination of the bone marrow is helpful in diagnosing certain diseases, especially those related to blood and blood-forming organs, because it provides information on iron stores and blood production. Bone marrow aspiration, the direct removal of a small amount (about 1 ml) of bone marrow, is accomplished by suction through a hollow needle. The needle is usually inserted into the hip or sternum (breastbone) in adults and into the upper part of the tibia (the larger bone of the lower leg) in children. The necessity for a bone marrow aspiration isordinarily based on previous blood studies and is particularly useful in providing information onvarious stages of immature blood cells. Disorders in which bone marrow examination is of special diagnostic value include leukemia, multiple myeloma, Gaucher disease, unusual cases of anemia, and other hematological diseases.

TYPES OF BONE MARROW

There are two types of bone marrow:

- Red marrow that is responsible for producing red blood cells, white blood cells and platelets
- Yellow marrow consisting mainly of fat cells

There are a number of blood vessels and capillaries traversing through the marrow making it a very vascular organ. At birth and in early childhood most of the marrow is red. As a person ages more and more of it is converted to the yellow type. About half of adult bone marrow is red.

FUNCTIONS

1. Nutrients within to store energy and formation of blood cells. The bone marrow contains those cells that are responsible for the production of the blood cells (red blood cells, white blood cells, and platelets).
2. Red blood cells (erythrocytes) carry oxygen to the tissues.
3. Platelets or thrombocytes (derived from megakaryocytes) help prevent bleeding and aid in clotting of blood.
4. Granulocytes (neutrophils, basophils and eosinophils) and macrophages (collectively known as myeloid cells) fight infections from bacteria, fungi, and other parasites.
5. It also remove dead cells and remodel tissue and bones. B-lymphocytes produce antibodies, while T-lymphocytes can directly kill or isolate invading cells.
6. RBC live for around 170 days and rest are shorter lived and need to be replenished continuously.
7. An average human requires approximately one hundred billion new hematopoietic cells each day. This is performed by the Hematopoietic Stem Cells (HSCs).

COLOR

- Yellow/Red.

SHAPE

- Soft tissues like Jell-O.

DIRECTION

- Above and below the waist.

LOCATION

Marrow is found mainly in the flat bones such as the hip, breast, skull, ribs, vertebrae, and shoulder blades, as well in long bones at the end the femur and humerous.

DELIMITATION

Bordered by inner surface of the bones.

BONE MARROW PATHOLOGY AND DIAGNOSIS

1. Certain diseases of the bone marrow like leukemia, multiple myeloma, myelodysplastic syndrome (MDS), pancytopenia, anemia etc. require examination of the bone marrow tissue. This is called bone marrow aspiration or bone marrow biopsy.
2. A needle is used to withdraw samples of the marrow from within the bone. This is often a very painful process.
3. Bone marrow is suppressed with the use of cancer chemotherapy. This leads to severe drop in production of RBCs (leading to anemia), WBCs (leading to increased risk of life threatening infections) and platelets (leading to risk of bleeding tendencies).

Viva Voce

1. Where the bone marrow is located?

2. What is bone marrow and what does it do?

3. What are the symptoms of bone marrow?

4. Is bone marrow tissue?

5. What is bone marrow treatment?

RESULT

Thus the parts of Bones and Bone Marrow were identified using Bone Marrow Charts