DROSOPHILA GENETICS

Drosophila melanogaster is commonly called the fruit fly or vinegar fly. It is an Arthropod belonging to the class Insecta and order Diptera. They are commonly found in residential areas, vegetable markets and in cooler areas where fresh or fermenting fruits are available. The flies are attracted to ripe fruits such as banana, guava and papaya.

Drosophila melanogaster is used for research in the field of genetics and molecular biology. The fruit fly is the best specimen for genetic studies as they have a short life cycle of about 10 -12 days at 22^0C and with high reproductive potential. It produces a large number of progenies, which is a prerequisite for statistical analysis of the results. They are called as 'Cinderella of genetics' as they are used for almost all genetic experiments, such as mutations and sex linkage.

Drosophila melanogaster is a holometabolic insect. Life cycle includes egg, larva, pupa and adult.

CULTURE AND MAINTAINANCE OF *DROSOPHILA*

Drosophila's like any other animals require an optimum temperature for its survival, growth and breeding. The optimum temperature for the maintenance of *Drosophila* is 20 – 25 ^{0} C. The temperature around and above 31^0 C makes the flies sterile and reduce the oviposition and may also result in death. At lower temperature, the life cycle is prolonged and the viability may be impaired.

Different media are used to culture and maintain Drosophila in the laboratory. The most commonly used are;

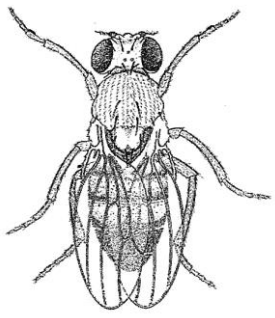
1) Wheat cream agar media

2) Banana culture media.

WHEAT CREAM AGAR MEDIA

The commonly used food media for the maintenance of *Drosophila* is the wheat cream agar medium. The ingredients required for preparing the media are;

- 1000ml distilled water
- 100g of wheat flour (rava)
- 100g of jaggery
- 10g of Agar agar
- 7.5ml of propionic acid
- Yeast granules
- culture bottles
- cotton (sterilized)



Preparation of Media:

Wheat flour and jaggery are boiled in one liter of distilled water till a paste is formed. To this agar-agar and yeast granules are added after cooling. Propionic acid is added to avoid fungal infection. Immediately it is poured into sterilized bottles and plugged with sterilized cotton. As the condition of the medium deteriorates with time, the flies have to be transferred from old to new cultured medium once in three weeks.

BANANA CULTURE MEDIUM

The ingredients required are;

- 15 to 20 ripe bananas
- 4 ml of propionic acid
- 10 g Yeast powder
- culture bottles
- cotton (sterilized)

Preparation of Media:

Peel and mash about 15-20 over ripe bananas. Add 4 ml of propionic acid and 10 gm of yeast powder mix well. Yeast enhances the growth of the larvae. Propionic acid prevents fungal infection. Add the medium to about 15 wide mouthed bottles. Take care not to drop the media onto the sides of the bottle or to the neck. The medium is ready to culture the flies. If these bottles are kept open for 1 to 2 days, flies will enter into the bottle, and then plug the mouth of the bottle with sterilized cotton. The adult flies lay eggs, which develops into larvae. Larvae after 10 days are best suited for dissection of salivary gland chromosomes. This medium is stable for 20 days and can sustain one generation.

Whenever the flies have to be analyzed either for routine observation or for experiments, they have to be anesthetized to make them inactive. The procedure is to transfer the flies from the media bottle to another empty wide mounted bottle referred to as etherizer. The mouth of this bottle is to be covered with a stopper sprayed with ether. It takes about a minute to anesthetize the flies. After this, the flies are transferred on to a glass plate for observation under the microscope. If the etherized flies revive before the completion of observation, they have to be re- etherized using a re- etherizer. The re- etherizer is nothing but ether-soaked filter paper fitted in a Petri plate which has to be placed over the flies on a glass plate. The over etherized flies will have their wings and legs extended at right angles to the body and such flies are considered to be dead.

The flies should be handled with a soft painting brush. In the process of handling the flies, care should be taken not to damage them. The flies to be discarded after observation should be transferred to a bowl or jar with coconut oil. This is called morgue.

MORPHOLOGY OF *DROSOPHILA*

The body of an adult *Drosophila* is divided into head, thorax and abdomen.

HEAD – The head is composed of six fused segments and has a pair of antennae with plumose aristae and a proboscis of licking type with mandibles. They also have a pair of compound eyes and 3 simple eyes called ocelli in between the compound eyes.

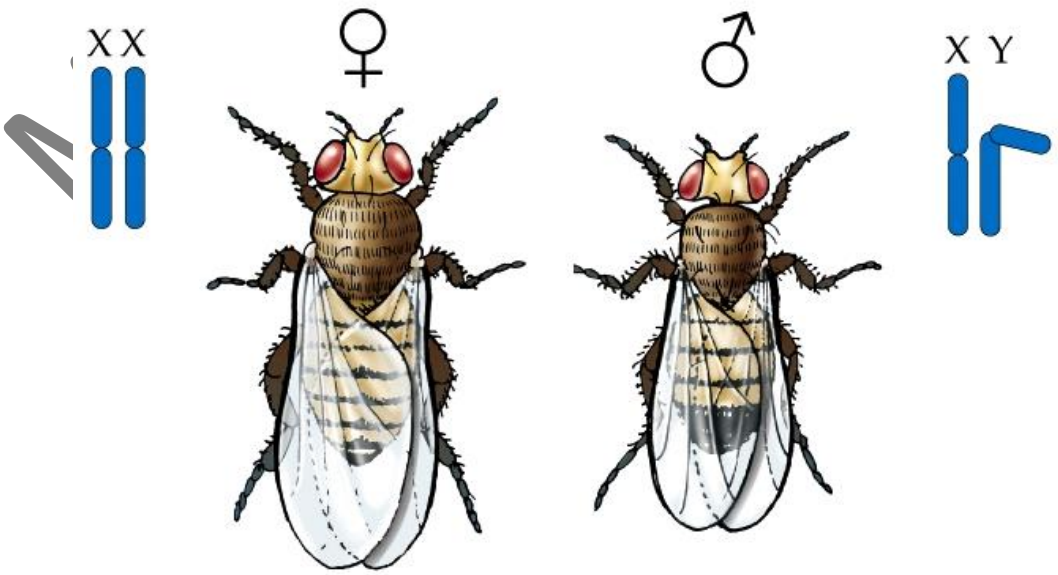
THORAX- The thorax is composed of 3 fused segments namely prothorax, mesothorax and metathorax. The prothorax has the first pair of legs, mesothorax has a pair of wings and 2nd pair of legs. The metathorax has a pair of reduced wings called halters or balancers and 3rd pair of legs.

ABDOMEN- Generally females are larger than males. The abdomen consists of 7 to 8 segments in females and 5 to 6 segments in males.

DIFFERENCE BETWEEN MALE AND FEMALE *DROSOPHILA*

Sexual dimorphism in *Drosophila* is well marked. Generally, the males are smaller than females. Differentiation of sex in adults is as follows;

MALE	FEMALE
1. Smaller in size	1. Larger in size
2. Abdomen is rounded	2. Abdomen is pointed
3. Three dark bands are present on dorsal side of the abdomen	3. Five darks bands are present on the dorsal side of the abdomen
4. Genital plate is pigmented	4. Vaginal plate is light colored
5. The first tarsal segment of first pair of legs bears sex comb	5. sex comb is absent



MUTANTS of *DROSOPHILA MELANOGASTER*

Mutation is a heritable change in genetic material and is a source of genetic variation. A mutant phenotype is a heritable deviant from the standard phenotype and is caused due to mutation. A mutation is said to be dominant if it expresses or manifest in the heterozygous condition i.e. one of the homologous chromosomes carries the mutant allele, while the other possesses standard allele. A Mutation is said to be recessive if it requires a homozygous state for its expression, wherein the same mutant allele is present on both the homologous chromosomes.

A recessive mutation on the X- chromosome of male is expressed since the Y- chromosome does not carry the corresponding allele and this is referred as hemizygous condition.

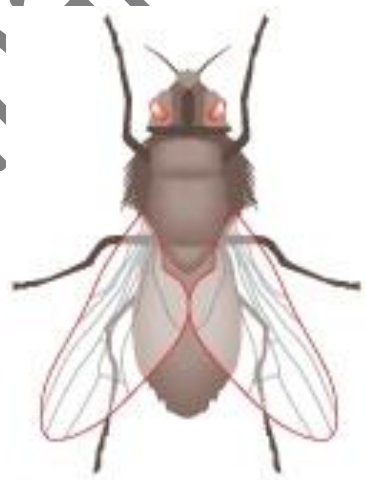
Generally, the mutants are designated by symbols. The wild type is denoted by '+'and the mutant phenotype designated by '_ 'symbols.

BAR EYE: It is a Mutation related to eye, wherein the size of the eye is reduced compared to standard eye

Bar eye: B

Location: 1- 57.0

Phenotype - Eye is reduced to narrow vertical bar in males and in homozygous females. Heterozygous females (B /+) have kidney or bean shaped eyes. The mutation is due to a duplication of small part of the X-chromosome identified as 16 A region.



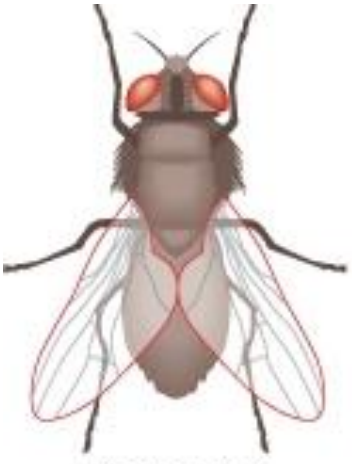
Bar-Eyed

SEPIA EYE: A Mutation related to eye color, instead of standard red eye they have sepia colored (reddish brown) eyes.

Sepia eye: Se

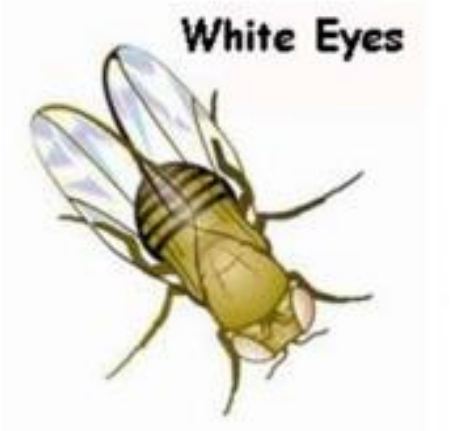
Location: 3-26.0

Phenotype - Eye color is brown at the time of eclosion (hatching). Darken into sepia and become black with age. Pigments of Ocelli are wild type. Chromatographically, sepia eye is characterized by absence of red pigments and accumulation of yellow pigments.



Sepia–Eyed

WHITE EYE: Sex linked mutation related to eye colour, instead of standard red coloured eyes, colorless eyes are noticed.

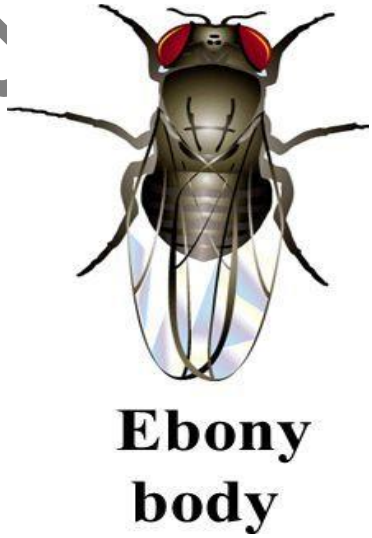


White eye: W

Location: 1-1.5

Phenotype- The white eye locus is involved in the production and distribution of ommochrome (brown) pigments found in the compound eye and ocelli of the adult flies. White eyed *Drosophila* mutants have colourless compound eyes, ocelli, Malpighian tubules and testis, due to inability to produce ommochrome pigments.

EBONY BODY: A Mutation related to body colour. Colour of the body is darker than wild type.



Ebony body - e

Location – 3-70.7

Phenotype: Body colour in this mutation varies from shining black to slightly darker than wild type, depending on the allele. Puparium is much lighter than wild type. It can be classified throughout larval period by darkened colour of spiracle sheaths. Heterozygotes for dark alleles have slightly dark colour body than normal type. These are sensitive to polarized light.

YELLOW BODY: A Mutation related to body colour. Mutants have yellow coloured body.

Yellow body: y

Location: 1-1.0

Phenotype: Body colour is yellow with body hairs and bristles brown but with yellow tips. The wing hairs and veins are yellow. The larval mouthparts are yellow to brown.

VESTIGIAL WING: A Mutation related to wing. These mutants have extremely reduced wings.

Vestigial wing: Vg

Location: 2-67.0

Phenotype –Wings reduced to vestigial, usually held at right angles to the body. Wing veins still visible, halters are reduced and bristles are erect.

The vestigial locus seems to be mainly involved in the development of wing margin. These mutants are recessive visible or recessive lethal or dominant with invisible phenotype over wild type.

Vestigial wings

PREPARATION OF SALIVARY GLAND CHROMOSOMES OF *DROSOPHILA MELANOGASTER*

AIM:

To study the structural details of polytene chromosomes in salivary glands of *Drosophila melanogaster.*

INTRODUCTION:

The polytene chromosomes are giant chromosome, first discovered by Balbiani (1881) in the salivary gland cells of *Drosophila*. The term polytene chromosome was suggested by Coller due to the presence of many threads like structure of the chromosome.

The polytene chromosome of *Drosophila* is about 200µ in length, the enormous size of the chromosome is due to the endomitotic duplication of chromonema which is resulted by non-disjunction. The polytene chromosomes are also found in the foregut, midgut and Malpighian tubules of larva.

REQUIREMENTS:

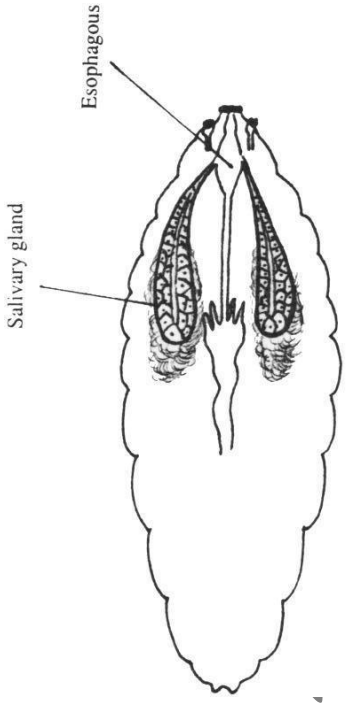
Dissection and compound microscopes, III - instar larva, physiological saline (0.7% NaCl), LactoAcetoOrceinstain (LAO), 15% acetic acid (CH_3COOH), 1N HCl, coverslips, glass slides, micro needle and nail polish or wax for sealing.

PROCEDURE:

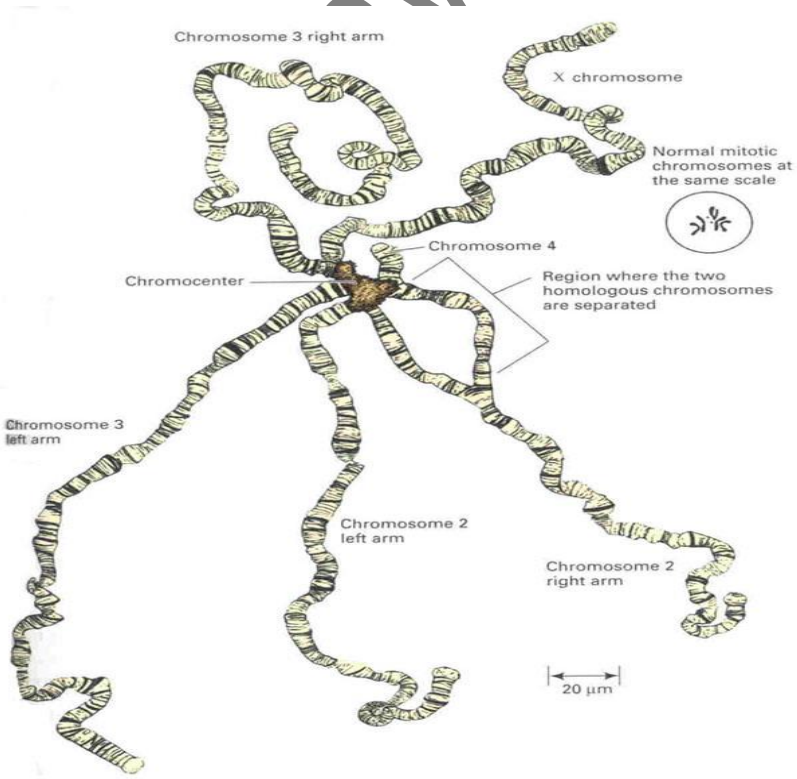
Dissect out salivary gland of III - instar larva in physiological saline. Place it in 1N HCl for 2-3 minutes. Transfer it into 2% LAO stain and keep for about 30 minutes. Squash it with 15% CH_3COOH; seal the edges of cover slip using nail polish/wax. Observe under the compound microscope.

RESULT AND DISCUSSION:

The polytene chromosomes are the giant chromosomes present in the salivary gland cells of Drosophila larva. These are usually 200 times larger than the normal somatic chromosomes. During the developmental stages, duplication occurs without separation. As a result, the number of chromosomal threads increases and the number of chromonemata may go up to 2000.Hence, they are named polytene chromosomes. In *Drosophila* (2n=8), the centromere of all the eight chromosomes are fused to form the chromocenter. The chromosomes hence appear radiating from the chromocenter. Each arm is 20µ wide. The arms also show alternate dark (heterochromatin) and light (euchromatin) bands. Sometimes the chromonemata show lateral loop formation called as **Balbiani rings** or **chromosome puffs.**



Salivary gland in Drosophila larva



Polytene Chromosome

MOUNTING OF SEX COMB OF *DROSOPHILA*

AIM:

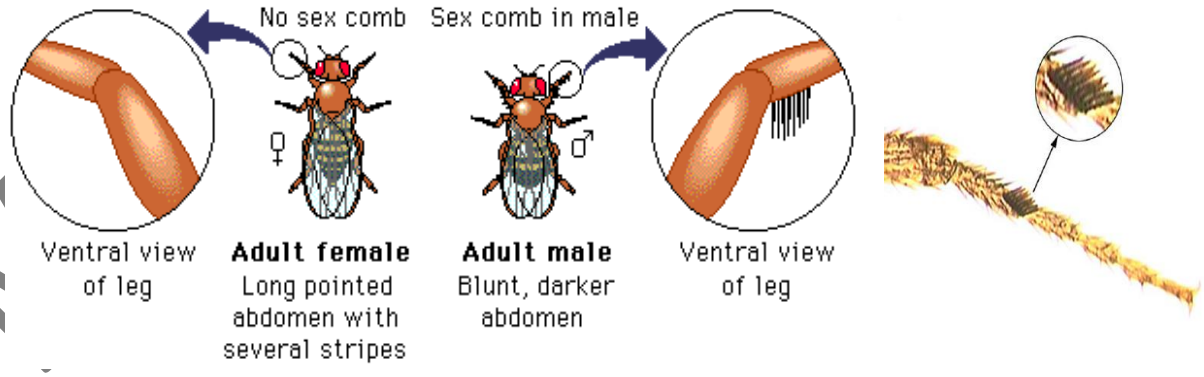
Sex comb is the tiny black coloured comb like structure found on the 1st tarsal segment of first pair of legs. The sex comb is present only in males.

PROCEDURE:

For temporary mounting of sex comb

- The male and female *Drosophila* can be identified by the size and shape of the abdomen. The male flies are selected for mounting of the sex comb.
- The male flies are taken into a clean test tube and cotton wool immersed in ether is used to plug the test tube.
- An etherised male fly is taken on to a clean slide and placed under a dissection microscope using pair of forceps. The first pair of legs are removed and placed on another slide and the fly is discarded.
- The first pair of legs is taken with a drop of KOH. This is observed under dissection microscope. A small comb like structure is seen attached to the 1st tarsal segment. This can be detached using a sharp blade and the leg is discarded.
- The sex comb is mounted using glycerine.
- For the permanent mounting the sex comb is upgraded using different grades of alcohol 70%, 80%, 90% and absolute alcohol. The material is cleaned using xylol and then mounted using DPX or Canada balsam.

OBSERVATION: Presence of sex comb in the male flies only



Mounting of Genitalia in *Drosophila Melanogaster*

Aim:

To mount the genital plate of *Drosophila melanogaster*.

Description:

The genital plate is located in the abdominal region of the male and female flies, but in the males the genital plate is more prominent and used as a copulatory pad. It is also called the epandrium. The genital plate is horseshoe-shaped, and it is bent in the posterior region. It is divided into 2 parts, the heel and the toe.

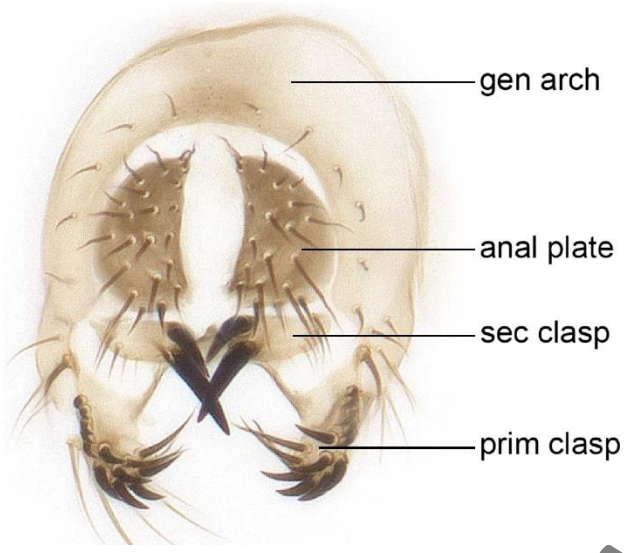
Inside the arch, a pair of anal plates and a pair of primary claspersis are present. On the primary claspers, 6–19 dark spines, or bristles are present, which are very thick. Their number is species-specific. The main function of the genital plate is to hold the female to transfer the sperm into her genital organ during copulation.

Materials:

- Male flies
- 40% KOH
- Cresosote solution
- Cavity slides
- Glycerine
- Cover slips

Procedure:

- Remove the last abdominal segment of the male Drosophila melanogaster.
- Transfer the segment into 40% KOH taken in a cavity slide and allow it to sit for 15 minutes.
- Blot out the KOH, use 2 or 3 drops of Cresosote solution, and allow it to sit for about 20 minutes.
- Remove the pellicle organ and clean the genital plate.
- Transfer the genital plate onto a clean plain slide and mount the genital plate with glycerine.



Genital plate of *Drosophila melanogaster*

HUMAN GENETICS

Preparation of buccal smear for localization of sex chromatin

AIM:

To study the localization of sex chromatin or Barr bodies in human buccal epithelial cells.

PRINCIPLE:

Murray C. Barr (1943) discovered a small body in the interphase nucleus of epithelial cells in female cats. It stained intensely with the stains having affinity for DNA. These bodies are named as **sex chromatin** or **Barr bodies**. A Barr body is the inactive X chromosome in the female somatic cell of mammals. It is rendered inactive by lyonization, in those species in which the sex is determined by the presence of Y or W chromosome rather than the diploidy of the X (Lyon hypothesis). This is called dosage compensation in which expression of genes between different biological sexes are equalized.

The sex chromatin can be obtained from the interphase nucleus of oral epithelium. The number of Barr bodies present in the nucleus of normal female is one. If the nucleus contains the bar bodies, it is referred to as sex chromatin positive (usually a normal female is considered as sex chromatin positive). The Barr body in the buccal smear of female appears as a round disc usually attached to the nuclear envelop.

MATERIALS REQUIRED:

Buccal epithelial cells, spatula, distilled water, glass slide and microscope.

REAGENTS REQUIRED:

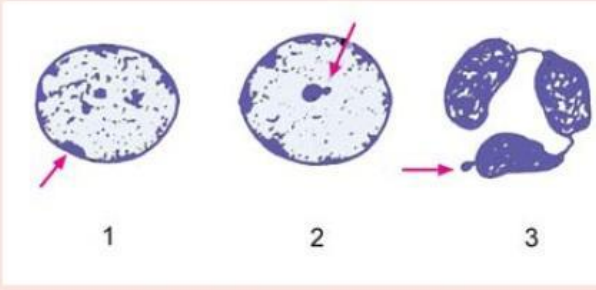
Giemsa stain, 95% alcohol.

PROCEDURE:

- Rinse the mouth thoroughly with tap water, draw the edge of metal spatula or spoon firmly over the buccal mucosa. Discard the material for the first time.
- Again, scrape the mucosa gently to obtain healthy epithelial cells from a deeper layer. Spread it over a small area on a clean dry slide and air dry it.
- Fix the dried slide in 95% alcohol for 5 mins.
- Stain the material with Giemsa stain for 20 mins. Wash the slide with the distilled water.
- Immediately observe the slide under microscope.

OBSERVATION:

- The buccal smear of female shows one dark stained Barr body attached to the nuclear membrane with in the cell. Hence, she is sex chromatin positive.
- Sex chromatin is not found in buccal epithelial cells of male. Hence, he is sex chromatin negative.



Sex chromatin. 1 – Typical position deep to nuclear membrane. 2 – As a nucleolar satellite in a neuron. 3 – As a drumstick in a neutrophil leucocyte.

DISCUSSION:

M C Barr discovered the Barr bodies in the interphase nucleus of epithelial cells in female cats. Later it was observed in the human females. The number of Barr bodies present in the nucleus is always 1 less than the total no of X chromosomes present. The sex chromatin or Barr bodies are discoidal in shape and are usually found attached to the nuclear membrane. The presence of Barr bodies in vegetative cells is a characteristic feature of normal female, one of the X chromosomes becomes inactive and highly condensed. Thus, it appears as a discoidal structure.

BLOOD TYPING

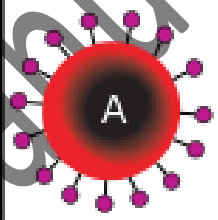
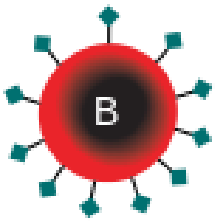
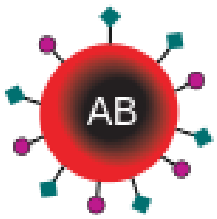
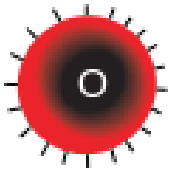
AIM: Determination of ABO blood group and Rh factor.

INTRODUCTION: Karl Landsteiner in 1900 discovered blood groups in man. In human beings with respect to blood cells there are two antigens, A and B and two serum antibodies that agglutinate them. They are grouped into four groups A, B, AB, and O based on the presence or absence of antigen on the plasma membrane of Red blood cells (RBC). Those with antigen A belong to blood group A and they have anti-B, antibody in blood serum. Those with antigen B belong to blood group B and have anti -A. Those with both A and B antigen belong to blood group AB where both antibodies are absent. Those with neither antigen belong to blood group O, have both types of antibody.

PRINCIPLE: It is based on the principle of agglutination. The antigen-antibody reaction that occurs between a particular antigen {agglutinogen} and specific antibody {agglutinin} leads to the clumping or agglutination of cells. When the cells involved are erythrocytes the reaction is termed as HAEMATAGLUTINATION.

Normally human erythrocytes will clump or agglutinate when mixed with anti-A or anti-B[antisera]. If the cell membranes of erythrocytes possess antigen-A, agglutination is seen with anti-A and for antigen -B, agglutination is seen with anti -B.

Rh factor is identified using anti-D serum. On adding anti-D, if agglutination is seen it indicates a +ve result for Rh and is designated Rh+^{ve} and otherwise Rh-^{ve}.

	Group A	Group B	Group AB	Group O
Red blood cell type				
Antibodies in Plasma	 Anti-B	 Anti-A	None	 Anti-A and Anti-B
Antigens in Red Blood Cell	 A antigen	 B antigen	 A and B antigens	None

MATERIALS REQUIRED: Blood samples, antisera A, B and D, 70% alcohol, micro slides, sterilised lancet, cotton, applicator sticks and surgical spirit.

PROCEDURE:

1. Take a clean dry 3 cavity microslide and label the cavities as A, B, and D.
2. Sterilize the fingertip with cotton dipped in 70% alcohol or surgical spirit and allow it to dry [surface sterilization].
3. Prick the disinfected area of the finger with a sterile lancet.
4. Squeeze the fingertip and allow a drop of blood to fall into each cavity area of the slide labelled as A, B and D.
5. Add one drop of each antisera A, B and D into the respective cavities without touching the dropper tip to the blood sample.
6. Using separate applicator sticks mix the antisera and blood droplet in a circular motion
7. Swirl the slide for about two minutes.
8. Observe for the presence of agglutination reaction and record the blood group.

If the antigen-antibody reaction is not clear, the slide can be observed under the microscope for confirmation.

INTERPRETATION:

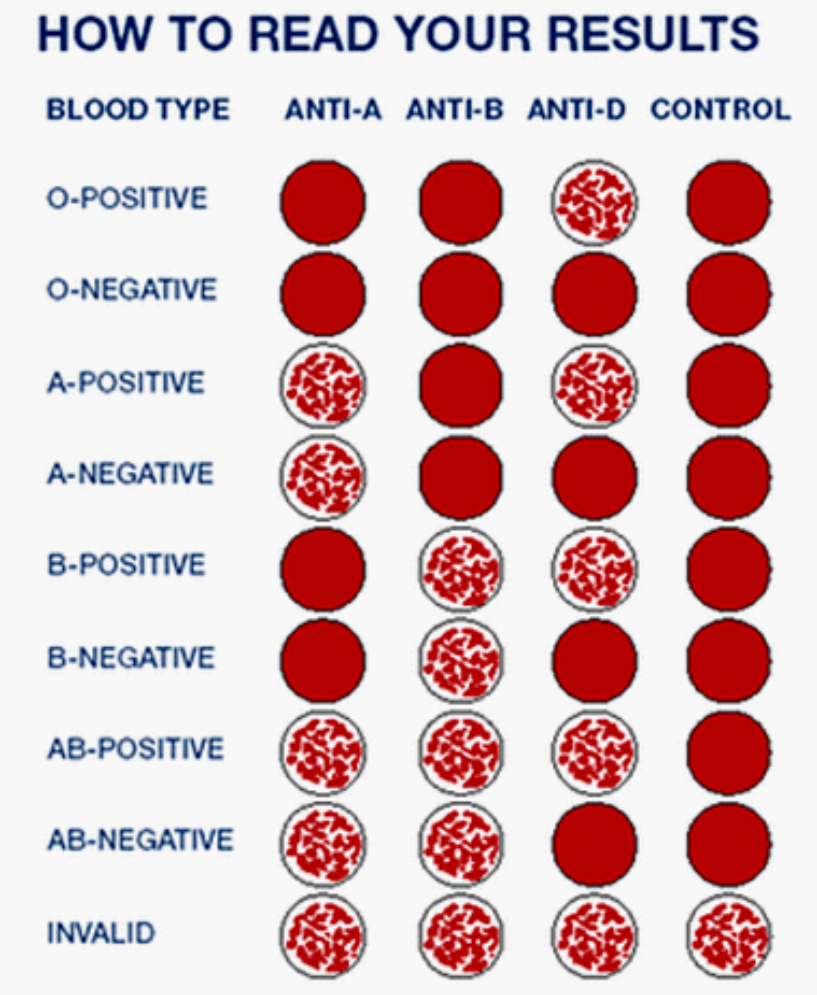
- Agglutination of erythrocytes indicates a positive result for haemagglutination.
- No agglutination of RBCs indicates a negative result.
- Agglutination in first cavity, with anti – A confirms blood group A.
- Agglutination in second cavity, with anti – B confirms blood group B.
- Agglutination in both the cavities (with anti A and anti B) confirms blood group AB.
- Lack of agglutination in both the cavities confirms blood group O.
- Agglutination in third cavity, with anti D indicates Rh^{+ve} result and lack of agglutination confirms Rh^{-ve}.

Observation:

Agglutination observed in cavity /s -----.

Result:

The given blood sample was assigned to the group ------.



PREPARATION OF BLOOD SMEAR AND STUDY OF BLOOD CELLS

Differential staining of WBC cells

AIM:

Preparation of blood smear for the identification of cell types.

PRINCIPLE:

Differential staining is the staining of the given material for which different component of the specimen being stained shows varying affinities thereby resulting in their differentiation.

Leishman's stain is a differential stain which contain methylene blue (basic in nature and hence combines with acidic material) and eosin (acidic in nature and hence combines with basic material). Basophil granules take up methylene blue and look deep purple in colour. Eosinophilic granules take up eosin and look red to orange in colour. Neutrophilic granules are amphophilic because they take up both and appear reddish brown.

MATERIALS REQUIRED:

Human blood samples, 70% alcohol, surgical spirit, Leishman's/ Wrights stain, lancet, blade, micro slide, micro scope, Petri plates, cotton, distilled water.

PROCEDURE:

1. Disinfect the finger with cotton dipped in 70% alcohol or surgical spirit.
2. Prick the finger with a sterile lancet to get few drops of blood.
3. Place a drop of blood on one end of a clean slide.
4. Make a thin smear of the blood by holding another slide at an angle of 45^0.
5. Allow the smear to dry (air drying).
6. Place the slide in a Petri dish and stain it with Leishman's stain for five to seven minutes.
7. Destain the slide by keeping it in distilled water for 15 to 20 minutes.
8. Wash the slide with the distilled water.
9. Air dry the smear and observe under the microscope.

OBSERVATION AND RESULT:

Different types of WBC along with erythrocytes were observed in the blood smear.

DISCUSSION:

LEUCOCYTES:

- Make a small part of blood's volume, normally about 1%
- Do not contain haemoglobin and hence colourless.
- They are not limited to blood alone and occur elsewhere in the body as well, most notably in spleen, liver and lymph nodes.
- The life span is about 1-4 days.
- Protect the body by destroying the pathogenic microorganisms.

Based on the size, nature of the cytoplasm, shape of the nuclei, staining characteristics (tinctorial properties), leucocytes are classified into two major groups as granulocytes and agranulocytes.

GRANULOCYTES (POLYMORPH NUCLEAR LEUCOCYTES)

- They are active, amoeboid and develop from the bone marrow.
- The cytoplasm is granular.
- Irregular lobed nuclei connected by fine threads are visible.

Involved in fighting against the disease-causing germs, they can leave the blood vessels, move to the site of infection to destroy the invaders.

Based on the tinctorial properties of their granules, granulocytes are classified into 3 types as follows:

1. NEUTROPHILS:

- They are more in number (50-70%).
- Actively phagocytic and characterized by the presence of cytoplasmic granules that stain pinkish with neutral stains.
- Lobed nucleus consisting of 2-5 parts connected by thin chromatin strains.

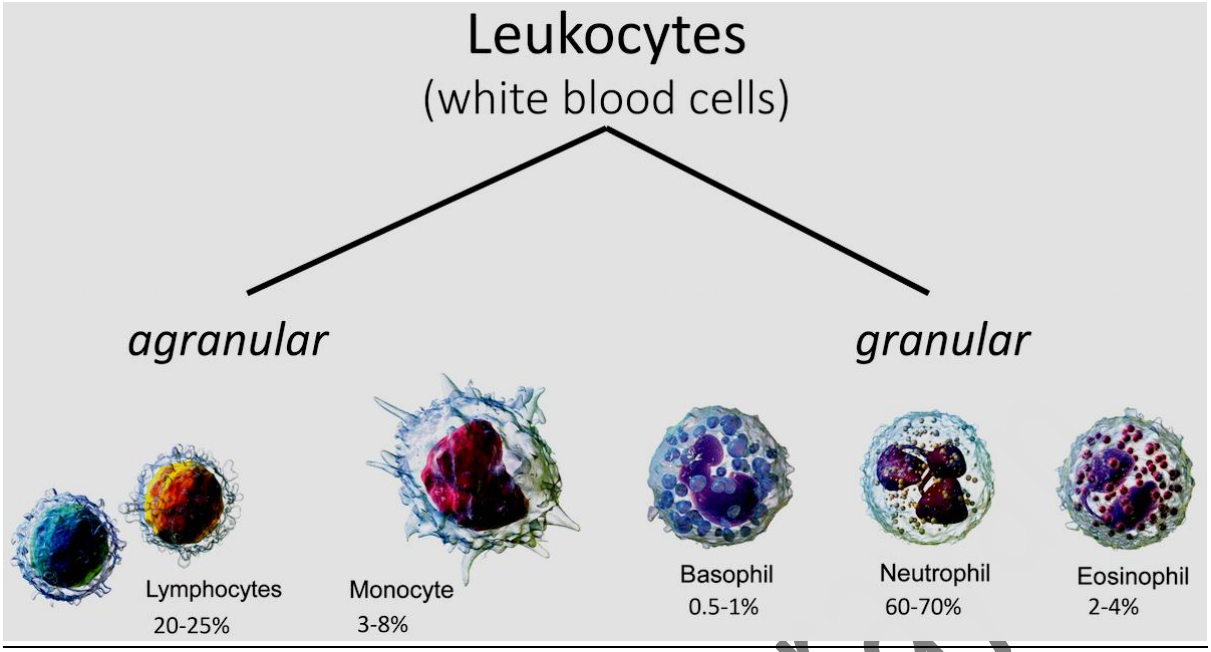
2. EOSINOPHILS:

- Constitute only 1-4% of leucocytes.
- Contain coarse, uniform sized cytoplasmic granules that stain deep red with acidic stain like eosin.
- Nucleus is distinctly bilobed.

They leave the capillaries, enter the tissue and produce antihistamines that destroy antigen-antibody complex.

3. BASOPHILS:

- Constitute only 0.5-1% of the leucocytes.
- Almost similar to eosinophils in shape and size of their nuclei.
- They have fewer, but more irregularly shaped cytoplasmic granules that stain deep blue with basic stain like methylene blue.
- They contain heparin, histamine and serotonin.
- They leave the capillaries, enter the tissues and are believed to be involved in allergic reactions.



AGRANULOCYTES :(MONONUCLEAR LEUCOCYTES)

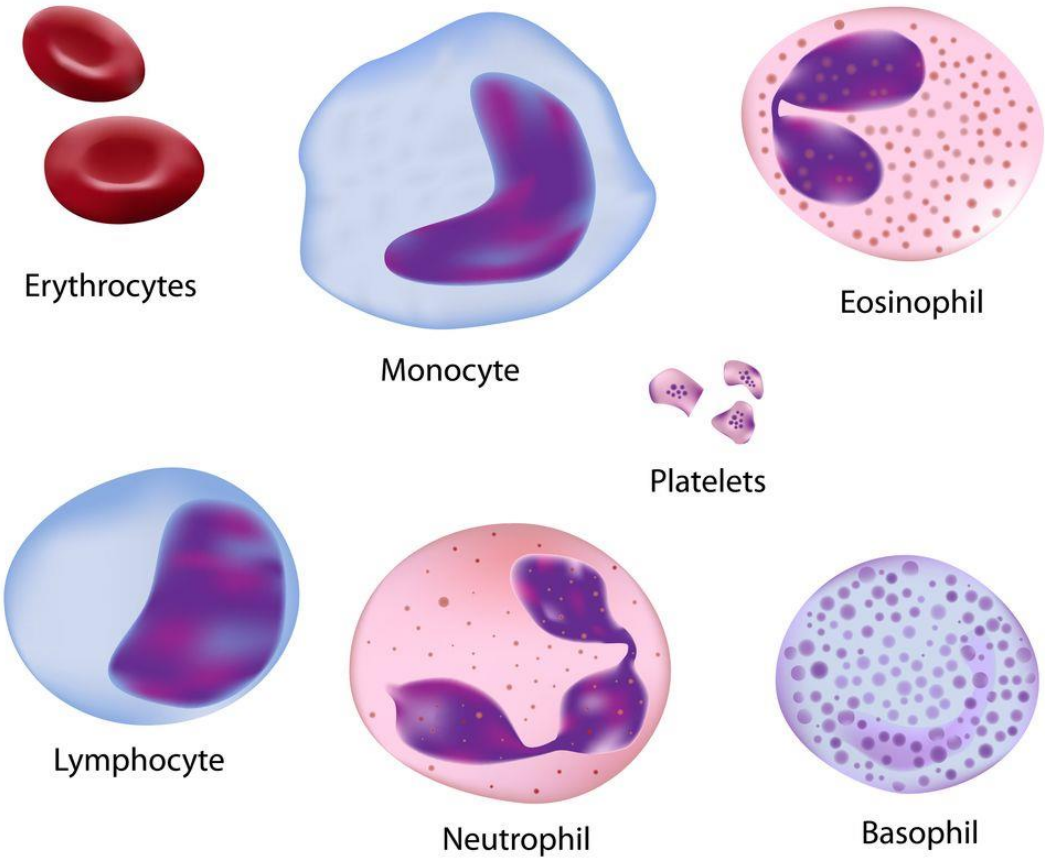
- These leucocytes develop from the lymphoid and myeloid tissue.
- They are almost round with a roughly round or indented nucleus.
- The cytoplasm is non granular or less granular.
- There are two types of agranulocytes which differ in their origin and functions.

1. LYMPHOCYTES.

- Smallest WBC which constitutes about 30 % (20-40%) is leucocytes.
- Produced in lymph nodes.
- They are comparatively less mobile.
- Contain relatively a large round nucleus, surrounded by a thin layer of cytoplasm.
- Involved in production of antibodies concerned with immunity.
- There are two types of lymphocytes viz. T-lymphocytes and B-lymphocytes.
- In cellular immunity, the body produces large number specifically sensitized lymphocytes which attached to specific invaders and destroy them.
- In humoral immunity circulating antibodies like immunoglobulin are produced which are capable of attacking the invaders and toxins produced by micro-organisms.

2. MONOCYTES:

- Largest cells of the blood, but comprise only about 2-6% of leucocytes
- They have a large horse shoe shaped nucleus.
- They are actively mobile and phagocytic.
- They escape from the blood vessel into the tissue and engulf bacteria, then they destroy the invading micro-organisms.


Erythrocytes
Monocyte
Eosinophil
Platelets
Lymphocyte
Neutrophil
Basophil

BIOTECHNOLOGY

GRAM STAINING OF BACTERIA

AIM:

To identify bacteria using gram staining.

PRINCIPLE:

Gram staining method, the most important procedure in Microbiology, was developed by Danish physician Hans Christian Gram in 1884. Gram staining is still the cornerstone of bacterial identification and taxonomic division. This differential staining procedure separates most bacteria into two groups on the basis of cell wall composition:

1. Gram positive bacteria *(thick layer of peptidoglycan-90% of cell wall)*- **stains purple**
2. Gram negative bacteria *(thin layer of peptidoglycan-10% of cell wall and high lipid content)* –**stains red/pink**

Nearly all clinically important bacteria can be detected/visualized using Gram staining method, the only exceptions being those organisms;

1. That exists almost exclusively within host cells i.e. Intracellular bacteria (e.g., Chlamydia)
2. Those that lack a cell wall (e.g., Mycoplasma)
3. Those of insufficient dimensions to be resolved by light microscopy (e.g., Spirochetes)

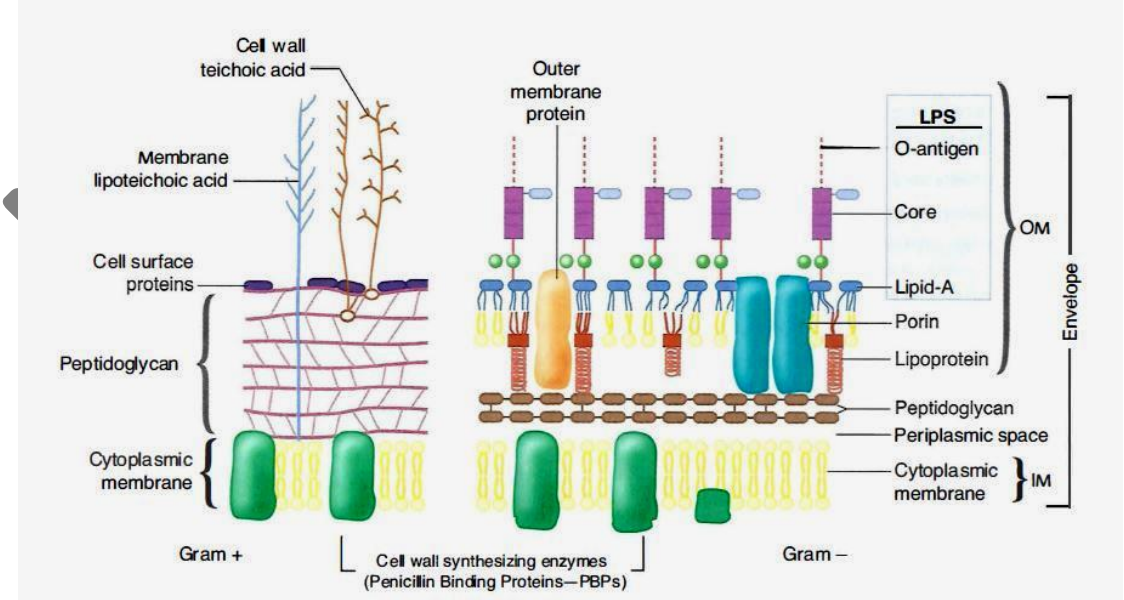


IMAGE: Cell wall of Gram Positive and Gram-Negative Bacteria

Gram staining techniques involves following steps:

1. **Fixation of clinical materials** to the surface of the microscope slide either by heating or by using methanol. (Methanol fixation preserves the morphology of host cells, as well as bacteria, and is especially useful for examining bloody specimen material). We, in our lab heat fix the cells.
2. **Application of the primary stain (crystal violet).** Crystal violet stains all cells blue/purple
3. **Application of mordant:** The iodine solution (mordant) is added to form a crystal violet iodine (CV-I) complex; all cells continue to appear blue.
4. **Decolorization step**: The decolorization step distinguishes gram-positive from gram-negative cells. The organic solvent such as acetone or ethanol, extracts the blue dye complex from the lipid-rich, thin walled gram-negative bacteria to a greater degree than from the lipid poor, thick walled, gram-positive bacteria. The gram-negative bacteria appear colourless and gram-positive bacteria remain blue.
5. **Application of counter stain (safranin):** The red dye safranin stains the decolorized gram-negative cells red/pink; the gram-positive bacteria remain blue.

The differences in cell wall composition of Gram positive and Gram-negative bacteria accounts for the Gram staining differences. Gram positive cell wall contain thick layer of peptidoglycan with numerous teichoic acid cross linking which resists the decolorization.

In aqueous solutions crystal violet dissociates into CV+ and Cl – ions that penetrate through the wall and membrane of both Gram-positive and Gram-negative cells. The CV+ interacts with negatively charged components of bacterial cells, staining the cells purple.

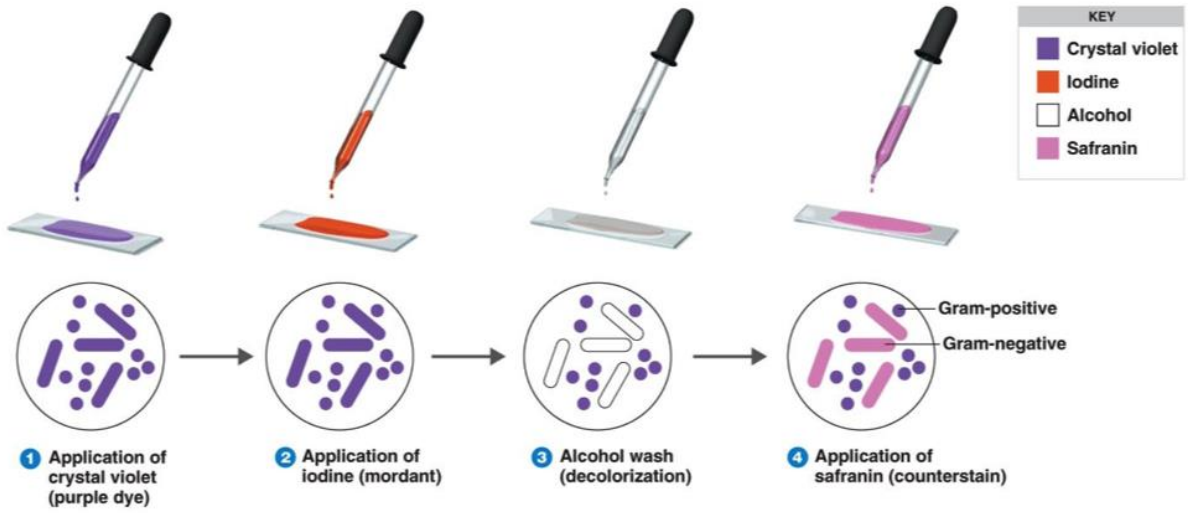
When added, iodine (I- or I3-) interacts with CV+ to form large crystal violet iodine (CV-I) complexes within the cytoplasm and outer layers of the cell. The decolorizing agent, (ethanol or an ethanol and acetone solution), interacts with the lipids of the membranes of both gram-positive and gram negative bacteria.

The outer membrane of the Gram-negative cell (lipopolysaccharide layer) is lost from the cell, leaving the peptidoglycan layer exposed. Gram-negative cells have thin layers of peptidoglycan, one to three layers deep with a slightly different structure than the peptidoglycan of gram-positive cells. With ethanol treatment, gram-negative cell walls become leaky and allow the large CV-I complexes to be washed from the cell.

The highly cross-linked and **multi-layered peptidoglycan** of the gram-positive cell is dehydrated by the addition of ethanol. The multi-layered nature of the peptidoglycan along with the **dehydration** from the ethanol treatment

traps the large CV-I complexes within the cell. After decolorization, the gram-positive cell remains purple in colour, whereas the gram-negative cell loses the purple colour and is only revealed when the counterstain, the positively charged dye **safranin**, is added

METHOD: Fix material on slide with methanol or heat. If slide is heat fixed, allow it to cool to the touch before applying stain.



Gram Staining Procedure/Protocol:

1. Flood air-dried, heat-fixed smear of cells for 1 minute with **crystal violet** staining reagent. Please note that the quality of the smear (too heavy or too light cell concentration) will affect the Gram Stain results.
2. Wash slide in a gentle and indirect stream of tap water for 2 seconds.
3. Flood slide with the mordant: **Gram's iodine.** Wait 1 minute.
4. Wash slide in a gentle and indirect stream of tap water for 2 seconds.
5. Flood slide with **decolorizing agent (Acetone-alcohol decolourizer)**. Wait 10-15 seconds or add drop by drop to slide until decolorizing agent running from the slide runs clear.
6. Flood slide with counterstain, **safranin**. Wait 30 seconds to 1 minute.
7. Wash slide in a gentile and indirect stream of tap water until no colour appears in the effluent and then blot dry with absorbent paper.
8. Observe the results of the staining procedure under oil immersion (100x) using a Bright field microscope.

Results:

- Gram-negative bacteria will stain pink/red and
- Gram-positive bacteria will stain blue/purple.

QUALITATIVE DETECTION OF ACETIC ACID IN YEAST CULTURE

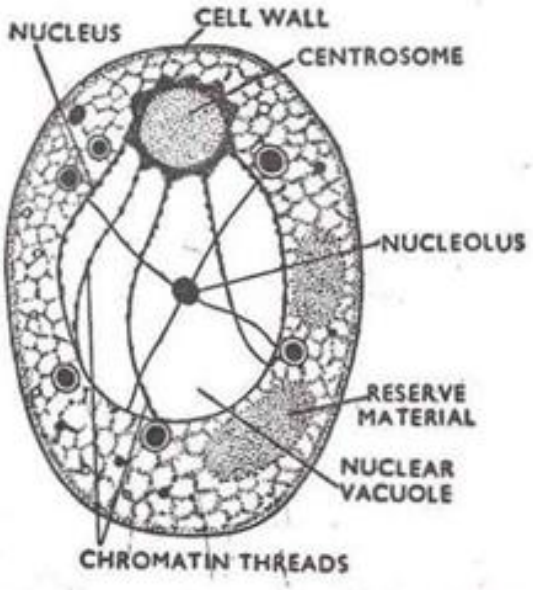
AIM:

To determine the presence or absence of acetic acid in yeast cells

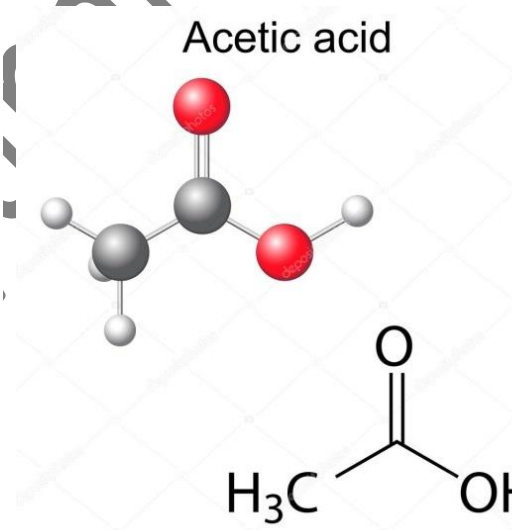
PRINCIPLE:

Yeast (Saccharomyces) is a unicellular achlorophyllous eukaryote. At high concentration of sugar, they undergo hyperosmotic stress response and liberate acetic acid by the oxidation of acetaldehyde present in the sugars. This acetic acid is liberated when heated with ferric chloride and forms a brick red colour of ferric acetate.

$$FeCl_3 + 3\ CH_3COOH \dashrightarrow (CH_3COO)_3\ Fe + 3\ HCl$$



Diagrammatic representation of parts of a yeast



REQUIREMENTS: Test tubes, test tube stand, Spatula, Spirit lamp.

REAGENTS: Peptone, Dextrose, Dry Yeast Granules, 2% Ferric Chloride and Distilled water

PROCEDURE

PART –A

- Yeast is cultured in a sabourand's dextrose medium, which is prepared by adding peptone(10g), and dextrose (40g) in 1l of distilled water
- The above culture media is distributed in 100 ml flasks and autoclaved at 121 ^{O}C at 15Psi for 20 minutes
- A suspension of yeast granules in sterile distilled water is prepared.

- This suspension was poured into the medium under aseptic conditions.
- The conical flasks were incubated at room temperature for 8-10 days on a shaker
- When there is complete turbidity in the medium, it shall be used for experiment

PART- B

- Take 1 ml of 2% FeCl3 solution in two test tubes
- One test tube is used as control without yeast. To the other test tube 2 ml of yeast culture is added
- Heat both the test tubes
- The test tube with yeast on heating will give reddish brown colour indicating the presence of acetic acid in the medium

RESULT: The experiment shows yeast culture liberates acetic acid

ISOLATION OF DNA FROM LIVER TISSUE
(Ethanol Precipitation method)

AIM:

To isolate DNA from a given animal tissue (liver or any other tissue)

PRINCIPLE:

DNA is polar due to its highly charged phosphate backbone. Its polarity makes it water-soluble (water is polar) according to the principle "like dissolves like".

Because of the high polarity of water, illustrated by its high dielectric constant of 80.1 (at 20 °C), electrostatic forces between charged particles are considerably lower in aqueous solution than they are in a vacuum or in air.

This relation is reflected in Coulomb's law, which can be used to calculate the force acting on two charges $\mathbf{q_1}$ and $\mathbf{q_2}$ separated by a distance **r** by using the dielectric constant $\boldsymbol{\varepsilon_r}$ (also called relative static permittivity) of the medium in the denominator of the equation ($\boldsymbol{\varepsilon_0}$ is an electric constant):

$$F = \frac{1}{4\pi\varepsilon_o} \frac{q_1 q_2}{r^2}$$

At an atomic level, the reduction in the force acting on a charge results from water molecules forming a hydration shell around it. This fact makes water a very good solvent for charged compounds like salts. Electric force which normally holds salt crystals together by way of ionic bonds is weakened in the presence of water allowing ions to separate from the crystal and spread through solution.

The same mechanism operates in the case of negatively charged phosphate groups on a DNA backbone: even though positive ions are present in solution, the relatively weak net electrostatic force prevents them from forming stable ionic bonds with phosphates and precipitating out of solution.

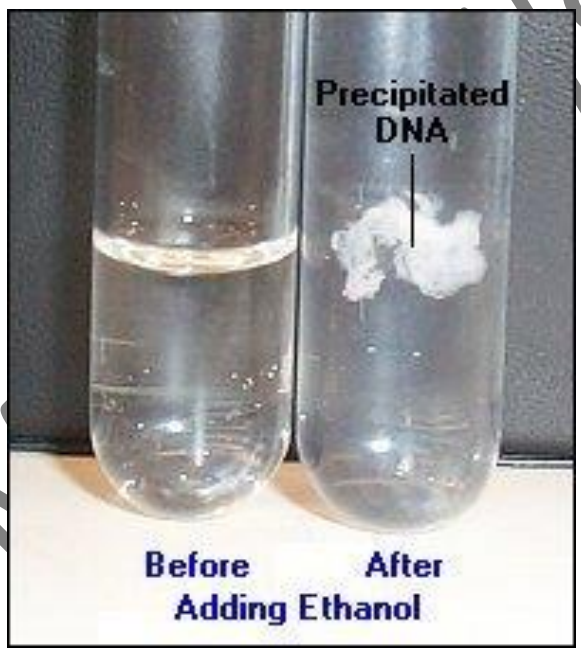
Ethanol is much less polar than water, with a dielectric constant of 24.3 (at 25 °C). This means that adding ethanol to solution disrupts the screening of charges by water. If enough ethanol is added, the electrical attraction between phosphate groups and any positive ions present in solution becomes strong enough to form stable ionic bonds and DNA precipitation. This usually happens when ethanol composes over 64% of the solution. As the mechanism suggests, the solution has to contain positive ions for precipitation to occur; usually Na+, NH4+ or Li+ plays this role

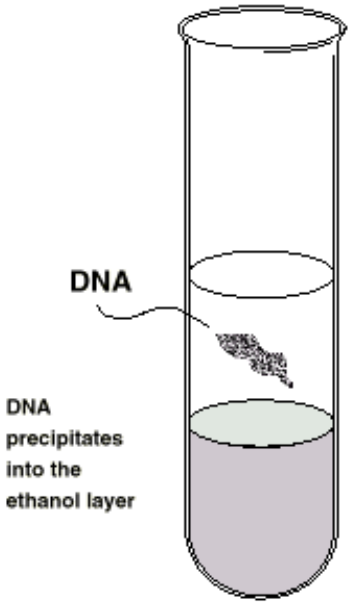
REQUIREMENTS:

Ethanol (chilled- refrigerated), Saline Sodium Citrate (SSC), 12% NaCl solution, ^{P}H water, Centrifuge tubes, 30ml test tubes and tissue sample.

METHOD:

- Grind about 200mg (2g) of the tissue sample in about 5ml of Saline Sodium Citrate (SSC) in a homogenizer or with a mortar and pestle.
- Transfer the homogenate into a centrifuge tube and make up the volume to 10ml with SSC.
- Centrifuge at 3000 rpm for 8 minutes and discard the supernatant.
- Re-homogenize the sediment with 5ml of SSC.
- Adjust the volume to 10ml, centrifuge at 3000 rpm for 8 minutes and discard the supernatant.
- Then suspend the sediment in 10ml of 12% NaCl solution and centrifuge at 10000 rpm (at least 7000 rpm) for about 15 minutes.
- Transfer the supernatant into 30 ml test tube and add 2-3 volumes of chilled absolute alcohol (ethanol).
- Gently mix it by inverting the tube, the white fibrous DNA precipitates.





RESULT: By the addition of ice-cold ethanol, DNA stands were precipitated as a white fibrous mass.

CATALASE TEST

AIM:

To identify the presence of catalase in a bacterial colony

PRINCIPLE:

Catalase is an enzyme, which is produced by microorganisms that live in oxygenated environments to neutralize toxic forms of oxygen metabolites; H2O2. The catalase enzyme neutralizes the bactericidal effects of hydrogen peroxide and protects them. Anaerobes generally lack the catalase enzyme.

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

Catalase mediates the breakdown of hydrogen peroxide H2O2 into oxygen and water. To find out if a particular bacterial isolate is able to produce catalase enzyme, small inoculum of bacterial isolate is mixed into hydrogen peroxide solution (3%) and is observed for the rapid elaboration of oxygen bubbles occurs. The lack of catalase is evident by a lack of or weak bubble production.

Catalase-positive bacteria include strict aerobes as well as facultative anaerobes. They all have the ability to respire using oxygen as a terminal electron acceptor.

Catalase-negative bacteria may be anaerobes, or they may be facultative anaerobes that only ferment and do not respire using oxygen as a terminal electron acceptor (i.e. Streptococci)

Percentage of H2O2 used on catalase test:

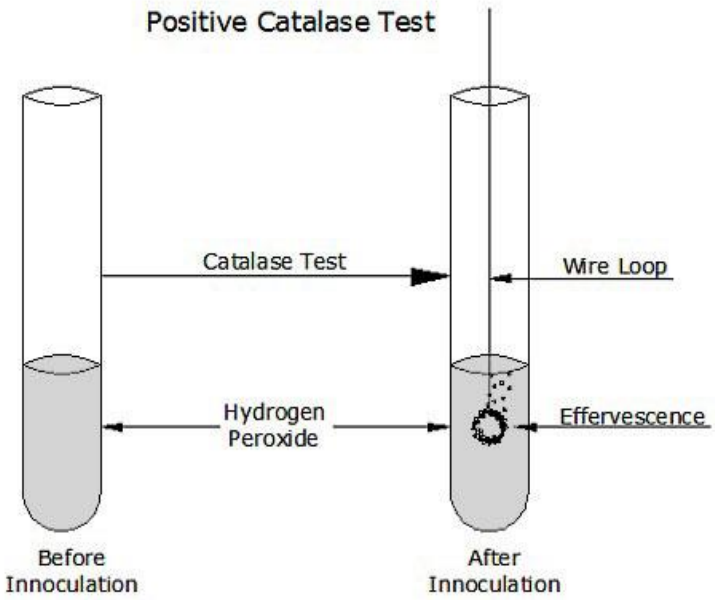
1. For routine testing of aerobes, 3% hydrogen peroxide is used.
2. 15% H2O2 solution: for the identification of anaerobic bacteria Catalase test is used to differentiate aerotolerant strains of *Clostridium* (catalase negative) from *Bacillus* species (catalase positive).

Procedure of Catalase test (Slide Test)

1. Transfer a small amount of bacterial colony to a surface of clean, dry glass slide using a loop or sterile wooden stick
2. Place a drop of 3% H_2O_2 on to the slide and mix.
3. A positive result is the rapid evolution of oxygen (within 5-10 sec.) as evidenced by bubbling.
4. A negative result is no bubbles or only a few scattered bubbles.
5. Dispose of your slide in the biohazard glass disposal container.

Tube Catalase Test-Procedure and Results

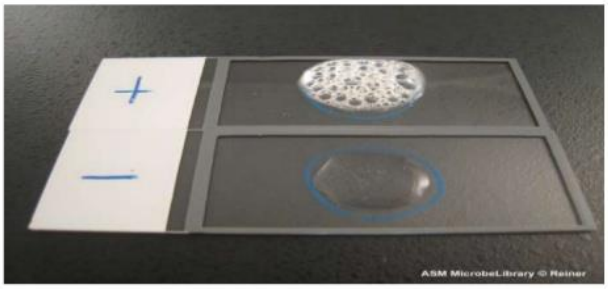
1. Add 4 to 5 drops of 3% H_2O_2 (Hydrogen peroxide) to in a test tube
2. Using a wooden applicator stick, collect a small amount of organism from a well-isolated 18- to 24-hour colony and place into the test tube *(Note: Be careful not to pick up any agar (esp if using Blood Agar).- Explanation in precaution below)*
3. Place the tube against a dark background and observe for immediate bubble formation (O2 + water = bubbles) at the end of the wooden applicator stick.



Interpretation

c. +ve result ⟶ rapid evolution of O_2 as evidenced by bubbling.

d. –ve result ⟶ no bubbles or only a few scattered bubbles.



Results:

- Catalase Positive reactions: Evident by immediate **effervescence (bubble formation)**
- Catalase Negative reaction: **No bubble** formation (no catalase enzyme to hydrolyse the hydrogen peroxide)

Uses Catalase Test Results

1. The catalase test is primarily used to distinguish among Gram-positive cocci: members of the genus ***Staphylococcus*** **are catalase-positive**, and members of the genera *Streptococcus* and *Enterococcus* are catalase-negative.
2. Catalase test is used to differentiate aerotolerant strains of *Clostridium*, which are catalase negative, from ***Bacillus*** **species, which are positive.**
3. Semiquantitative catalase test is used for the identification of *Mycobacterium tuberculosis.*
4. Catalase test can be used as an aid to the identification of **Enterobacteriaceae**. Members of Enterobacteriaceae family are **catalase positive.**

Precautions while performing catalase test

1. Do not use a metal loop or needle with H_2O_2; it will give a false positive and degrade the metal.
2. If using colonies from a blood agar plate, be very careful not to scrape up any of the blood agar as blood cells are catalase positive and any contaminating agar (carryover of red blood cells) could give a false positive.
3. Because some bacteria possess enzymes other than catalase that can decompose hydrogen peroxide, a few tiny bubbles forming after 20 to 30 seconds is not considered as positive test.

TRANSLOCATION IN *Rheo Discolor*

Aim: To observe the translocation occurring in the genome of *Rheo discolor.*

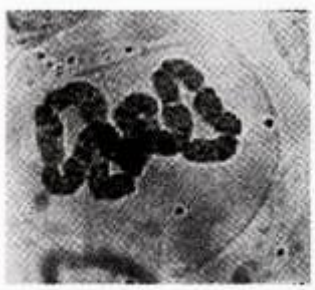
Principle: Translocation is a chromosomal aberration associated with the relocation or transfer of chromosomal segment from one chromosome to another. It is believed that there is a permanent structural heterozygosity in rheo and there is no chromosomal pairing. It causes an unusual mode of meiosis with chromosome ring occurring due to reciprocal translocation between the distal segments
of all the chromosomes (Darlington 1929, and Sax 1931).

Requirements: Rheo flower buds, slides, coverslip, 1N HCl, Aceto-orcein, 45%Acetic Acid, Spirit lamp, Dissection needles, Droppers and compound microscope.

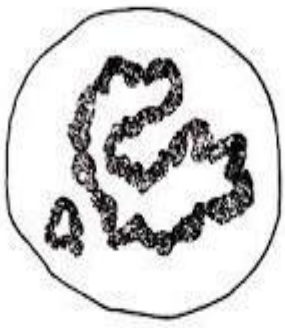
Procedure:

1. The young flower buds of rheo discolour is taken.
2. On a clean glass slide, anthers of the buds are removed with the help of a needle.
3. The material is then heat fixed with 1N HCL for 30 seconds.
4. Remove 1N HCL (using blotting paper) and stain it with aceto-orcein and keep it for 20-30 minutes.
5. Using 45% acetic acid, remove excess stain.
6. Cover it with a coverslip and squash the material.
7. Observe the preparation under the microscope.

Observation: Due to the reciprocal translocation between 12 chromosomes, all the chromosomes are attached end to end to form a ring of chromosome at metaphase during meiosis. This type of chromosomal aberration occurs naturally in plants. The presence of ringed chromosome in meiosis is a characteristic of translocation heterozygotes.



A ring of 6 pairs of chromosomes (12) in Rhoeo with heterozygote translocations (Courtesy: Prof. Sumitra Sen)



Diagrammatic representation