STAINING OF BACTERIA : SIMPLE STAINING

AIM :

To study the shape, size and arrangement of different bacterial strains.

RQUIREMENTS :

Equipments	Spirit Lamp
	Inoculation needle
	Inoculation loop
	Microscope with oil immersion facility
Glasswares	Glass slide
	Cover slips
Consumables	Ethanol
	Methylene Blue
	Crystal Fuschin
	Cedar wood oil
Culture	Broth culture of bacteria

PRINCIPLE :

Simple staining method is done by employing a single dye and the cells and structures within the cell were stained according to the colour of the dye. It is used to study the shape, size and arrangement at any type at bacteria.

PROCEDURE :

A smear of bacteria was prepared, subjected to air dry and heat fixed.

↓

Stain was applied on the dried smear for stipulated time

↓

Stains	Time
Methylene Blue	50-120 sec.
Crystal Violate	2-60 sec.
Carbol Fuschin	15-.30 sec.

↓

The stain was then washed by running distilled water and blot dried.

↓

The slide was than observed under oil immersion microscope.

Experiment No. - 33 : Preparation of the Blood Smear and Identification of Different Blood Cells

Requirements :

Equipments	:	Compound microscope, Pricking needle, Micropipette, Water bath.
Glasswares	:	microscopic slides.
Consumables	:	rectified spirit, Methylated spirit, Leishman stain, Distilled water.

Principle :

Leishman stain (eosin methylene blue compound) stains the nuclei of blood cells and also granules present in them. Different blood cells having nucleus of different shapes and sizes and granules absent or present demarcates different cells.

Procedure :

The tip of ring finger is cleaned with rectified spirit. It was pricked with the disposable pricking needle and the first two drops of blood are disposed off.

↓

By cleaning with cotton dipped in methylated spirit the microscopic slides that is previously cleaned by water is touched with the fingertip at one end.

↓

Another slide inclined at 45°manner is touched with the blood drops so that the blood drop gets spread across the edge of the inclined slide and is drawn on the first slide to other end.

↓

Experiment No. - 34 : To Test the Blood Group of Human Beings on The Basis of Immuno Techniques

Requirements :

Equipments	:	Pricking needle
Glasswares	:	Microslide
Consumables	:	Antiserum-a
		Antiserum-b
		Antiserum-d

Principle :

Blood contains RBCs and RBCs contain antigens A or B, both or neither. Thus there are four groups of human beings. It contain antibodies A, B, AB or no antigen. In individuals with AB blood group no antibodies are present in the plasma. Similarly in blood group 'O' (no antigen), both the antibodies 'a' and 'b' are present in the plasma.

Procedure :

Place 1 drop each of anti A, anti B and anti D reagent separately on a slide

↓

Add 1 drop of red cell suspension to each drop of typing antiserum (20 part red cells+ 80 part normal cell)

↓

Mix cells and reagents with a clean stick. Spread each mixture evenly on a slide over an area at 10-15 mm diameter

↓

Keep for 2 minutes for agglutination.

Observation :

If clumping is observed with a and b then the blood group is AB. If no clumping is observed with either a or b antiserum then the blood group is O. If clumping of blood occurs with antiserum b, the blood group is B. Clumping of blood with antiserum a Indicates A blood group. If clumping occurs with anti D antiserum then the person is Rh positive. No clumping with anti D indicates Rh negative blood group.

The blood smear is dried rapidly by waving in air

↓

The dry film is well covered with Leishman stain, which should be evenly distributed over the entire slide.

↓

After 1 min double the quantity of distilled water is carefully added and mixed with stain.

↓

At the end of seven minutes the stain is poured off and the film is covered with distilled water for two minutes.

↓

The water is washed off with fresh distilled water and the film is gently blotted dry.

↓

The stained blood smear preparation is studied under a microscope preferably using oil immersion lens.

Observation :

The neutrophils are identified by cells having many lobed (2-10) nucleus and pale violet granules in the cytoplasm.

Eosinophils are identified by cells having nucleus possessing two lobes with cytoplasm having red colored round or coarse granules.

Basophils are identified by the presenceof kidney shaped nucleus with less numerous granules than that of the esinophils.

Lymphocytes are identified by cells having comparatively large nuclei with small amount of cytoplasm making thin rim around it in small lymphocyte and large amount of cytoplasm in large lymphocyte.

Monocytes are identified by its larger size when compared to other cells having kidney shaped or horseshoe shaped eccentric nucleus.

STAINING OF BACTERIA : SIMPLE STAINING

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	Crystal Fuschin
	Cedar wood oil
Culture	Broth culture of bacteria

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Methylene Blue	50-120 sec.
Crystal Violate	2-60 sec.
Carbol Fuschin	15-.30 sec.

↓

The stain was then washed by running distilled water and blot dried.

↓

The slide was than observed under oil immersion microscope.

STAINING OF BACTERIA: ACID FAST STAINING

AIM : To stain bacterial smear by Acid-fast staining.

REQUIREMENTS :

Equipment	Microscope with Oil immersion facility Inoculation loop Inoculation needle
Glass wares	Glass slides Cover slips
Consumables	Carbol fuschin HCI Alcohol Methylene blue Cedar wood oil
Cultures	Broth culture of bacteria

PRINCIPLE :

There are two types of staining-basic and acidic stains. Basics stains like crystal-violate sticks to the negative charges of the bacterial cell-wall. On the otherhand, the acidic stains reacts with the mycolic acid (a lipid) present in the genus mycobacterium .Carbol fuschin as the primary stain sticks to the cell walls of mycobacterium even when the cells are washed by acids. Methylene blue acts as a secondary counter stain taken by non acid fast bacilli.

PROCEDURE :

Bacterial smear prepared and subjected to air dry.

↓

Carbo I fuschin stain added.

↓

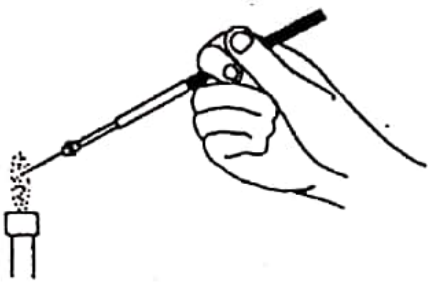
Slide allowed to dry on a hot plate.

↓

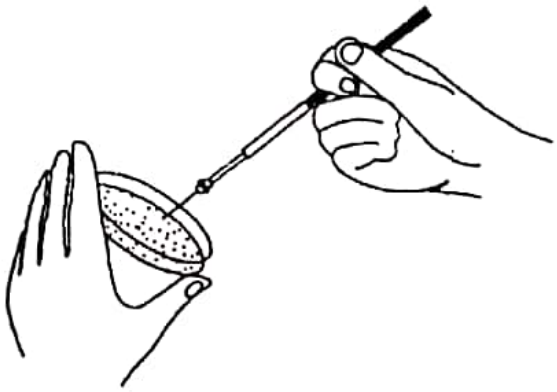
Washed with acid alcohol for removal of excess stain.

↓

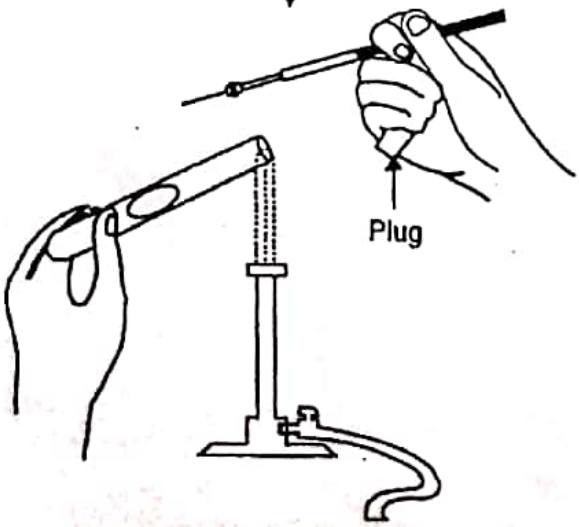
Counter stain with methylene blue.



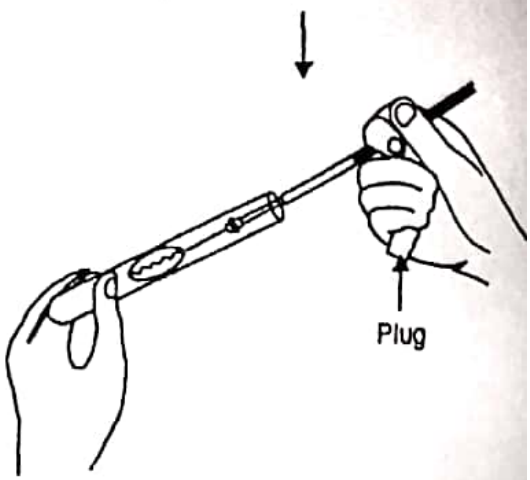
Sterilize the needle by holding the wire in a flame until it is red rot



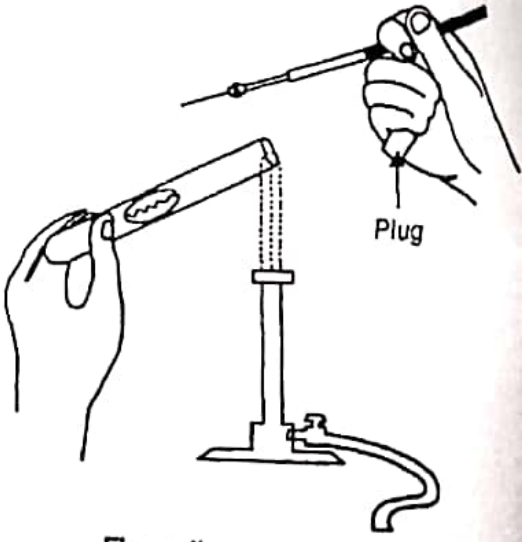
Touch the straight needle to the surface of a selected, discrete colony



While holding the straight needle and the microbial culture, uncap the agar slant and pass the neck of the tube rapidly over the Bunsen burner flame



Inoculate the slant by streaking back and forth (zig zag motion) across the surface of the agar



Flame the neck of the tube and replace the plug

Flame the inoculating needle

PROCEDURE :

Bacterial smear prepared and subjected to air dry and heat fixed.

↓

Crystal violet solution was added to the slide.

↓

Extra stain was poured of and the slide is washed with distilled water.

↓

Iodine solution called as mordant was added to the slide.

↓

. slide was washed with distilled water.

↓

Saffranine was added to the slide.

↓

The slide was washed with distilled water.

↓

A cover slip was placed.

↓

Slide was examined under a compound microscope.

OBSERVATION :

Gram-positive bacteria appears violet to black whereas gram negative bacteria are red.

PRECAUTIONS :

1. Culture of 24 hrs. (fresh) should be used otherwise the old culture of gram positive bacteria lose the staining properties and appear as gram negative.
2. During the preparation of smear, over heating should be avoided.
3. Contamination of decolourizing solution should be avoided.

Teacher

basmita panigr

Student

SUBCULTURE OF MICROORGANISMS

RQUIREMENTS :

Equipments : Inoculation needle
Inoculation loop
Autoclave

Consumables : Mixed nutrient agar stacks
Pour plates preparation of *E. coli*
Nutrient agar slants or plates

PRINCIPLE :

Sub culturing is the term used to describe the procedure of transferring of microorganisms from their parent growth source to a fresh one or from one medium to another. When transferred from a solid medium to liquid medium the term picking off is used. This technique is also used for preparing and maintaining stock culture as well as in microbiological test procedures.

PROCEDURE :

The loop was sterilized

↓

The tip of the loop was touched to the surface of the agar streak plate/pour plate.

↓

The loop was inserted to the subculture tube or slants and inoculated it by drawing over the surface in a zig-zag line and recap the tube.

↓

The culture tubes was incubated at 370.^{C} for 48-72 hours.

OBSERVATION :

After inoculation, it was observed bacterium shows a confluent and uniform growth.

STAINING OF BACTERIA : GRAM STAINING

AIM:

To stain bacterial smear to know whether the given bacterium is gram positive or gram negative.

REQUIREMENTS:

Equipment	Microscope with oil immersion facility
	Spirit lamp
	Inoculation loop
	Inoculation needle
Glass wares	Glass slides
	Cover slip
Consumables :	Crystal violet.
	Rectified spirit
	Ammonium oxalate
	Distilled water
	Potassium iodide
	Saffranine
	Cedar wood oil
Culture	Broth culture of bacteria

THEORY :

The stain was used for the first time by a Danish physician Christian gram in 1884 possessing wide application to identify an unknown bacterial culture. In this case there is generally the use of two types of dye-primary stain and counter stain, a mordant(iodine) and a decolorizing agent is frequent. Certain bacteria after being stained with primary dye (crystal violet) becomes decolourized when washed in an iodine solution followed by rectified spirit These are called gram negative which retain the colour under microscope, while those that retain the primary dye are known as gram positive bacteria.

Theory behind this is that crystal violet and iodine enter the cell and form an "insoluble dye-mordant complex", which is retained by the cell wall of gram positive bacteria. This dye-mordant complex can be extracted from the bacterium by means of an inorganic solvent ethylene alcohol. Within appropriate time, gram positive bacteria retain this colour where as gram negative bacteria completely eliminate the primary dye stain. Thus, the later applied counter stain can easily stain the gram positive bacteria.

OBSERVATION :

- The vegetative cells were observed as dark purple colour, encircled by light blue colored capsules.

PRECAUTIONS :

1. A heavy smear should be taken
2. The slide should be heated first
3. Water should be avoided while washing

Teacher

Sasmita Panigrahi
Student

STAINING OF BACTERIA : NEGATIVE STAINING

RQUIREMENTS :

Equipments	Microscope with oil immersion facility Inoculation loop
Glasswares	Glass slides Cover slips
Consumables	Nigrosin Cedar wood oil
Cultures	Broth culture of bacteria

PRINCIPLE :

A negative stain is a stain that stains the background but does not stain the bacteria. It is the simplest and fastest method for obtaining information about the cell shape, any cell breakage, cell inclusions and also about endospores.

Nigrosin is an acidic stain used for this purpose the acidic stain carries a negative charge, which repelled by the negative charge present on the surface of the bacterial cells. Hence, the bacterial cells appear unstained.

PROCEDURE :

Preparation of a bacterial smear on a slide.

↓

Add a drop of Nigrosin to it and air dry.

↓

Observation under oil-immersion microscope.

OBSERVATION :

Transparent (colourless) spherical cells were found against a blue background.

PRECAUTIONS :

1. Stain should be placed closed to one end of clean slide.
2. The thickness of the film should be uniform.
3. The slide should never be heat fixed.
4. Negatively stained preparations should not be used for cell length and width measurements.

Teacher

Sasmita Panigrahi
Student

OBSERVATION :

- The vegetative cells were observed as dark purple colour, encircled by light blue colored capsules.

PRECAUTIONS :

1. A heavy smear should be taken
2. The slide should be heated first
3. Water should be avoided while washing

Teacher

Sasmita Panigrahi
Student

STAINING OF BACTERIA : NEGATIVE STAINING

RQUIREMENTS :

Equipments	Microscope with oil immersion facility Inoculation loop
Glasswares	Glass slides Cover slips
Consumables	Nigrosin Cedar wood oil
Cultures	Broth culture of bacteria

PRINCIPLE :

A negative stain is a stain that stains the background but does not stain the bacteria. It is the simplest and fastest method for obtaining information about the cell shape, any cell breakage, cell inclusions and also about endospores.

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PROCEDURE :

Preparation of a bacterial smear on a slide.

↓

Add a drop of Nigrosin to it and air dry.

↓

Observation under oil-immersion microscope.

OBSERVATION :

Transparent (colourless) spherical cells were found against a blue background.

PRECAUTIONS :

1. Stain should be placed closed to one end of clean slide.
2. The thickness of the film should be uniform.
3. The slide should never be heat fixed.
4. Negatively stained preparations should not be used for cell length and width measurements.

Teacher

Sasmita Panigrahi
Student

STAINING OF BACTERIA: CAPSULE STAINING

AIM:

To stain bacterial smear by capsule staining.

REQUIREMENTS:

Equipment	Microscope with oil immersion facility
	Spirit lamp
	Inoculation needle
	Inoculation loop
Glass wares	Glass slide
	Cover slip
Consumables	Crystal violet
	Copper Sulphate.
	Cedar wood oil
Cultures	Broth culture of bacteria

PRINCIPLE :

- Capsules are the distinguishing external layer of some specific bacteria.
- These Capsule layers provide better identifications when stain with specific stains,
- Crystal violet acting as the primary stain ,stain the capsular material and cell wall as dark blue colour,
- Due to the non-ionic nature of capsule, primary stain is not absorbed.
- Here copper sulphate acts as a decolourization material and a counter stain.
- Copper Sulphate removes excess crystal violet and stain the capsule light blue in contrast to deep purple colour of the cell,

PROCEDURE :

A bacterial smear was prepared and air-dried.

↓

Crystal Violet was applied as primary stain for 2 mins.

↓

Excess stain was washed out by copper sulphate and observed.

OBSERVATIONS:

1. Bacteria were stained deep blue by methylene blue.
2. Bacteria were stained purple by crystal violet .
3. Bacteria were stained violet by carbol fuschin.

PRECAUTIONS :

1. Fresh culture should be used .
2. Overheating should be avoided during preparation of smear.
3. Contamination of decolorizing solution should be avoided.

Teacher

Sasmita Panigrahi
Student