

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ



العنوان

Stianing of bacteria

Fifth modular unit



Microscopic examination of bacteria:

The morphology of bacteria can be studied by the microscopic examination

A- **Unstained preparations** used to study both **shape and motility of bacteria** suspended **in a fluid** using:

a -the hanging drop method b- wet smear

B- **Staining technique must be used** to render the structure of cells visible. These will only differentiate relatively gross individual structures

C- **Electron microscopy** complex techniques are needed to reveal those not shown by staining.

Staining will help to identify organisms and place them in their own particular group by their individual reactions to certain stains. An example is the gram stain.



Preparing of bacterial smears

A-Making of **wet** preparations:

Principle:

drop of liquid containing microorganisms on a slide covered with a coverslip

Advantages: can **observe live organisms**

Disadvantages: **dries out quickly**

Procedure:

1. Make a smear on a clean and dry microscope slide :

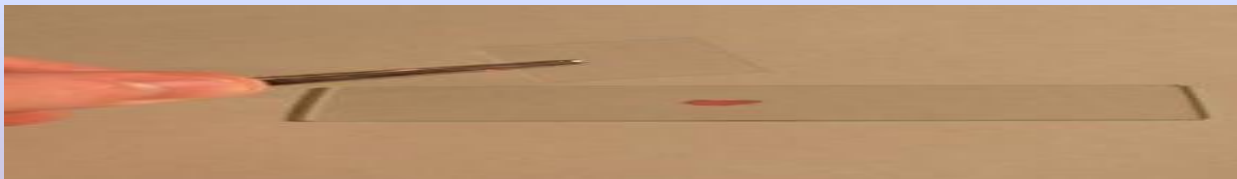
a-From **culture grow in liquid media** by putting 1-3 drops of liquid cultures on the slide using the loop or Pasteur pipette

b-From **culture grow on solid media** by emulsify the colony in a small drop of saline

c-Emulsify the specimens such as **feces** in a small drop of **saline**, **iodine**, or the required **stain**.

2-Carefully place a cover slip on to the suspension taking care that no fluid extrudes beyond the edges of the cover slip.

3- Examine microscopically as for hanging drop under power **10X or 40X**.



Hanging drop technique

drop of liquid containing microorganisms on a slide covered with a cover slip & suspended over a depression slide

Advantages of the hanging drop:

- *Easy to prepare
- *Bacteria are live so we can see bacterial motility :

Disadvantages of the hanging drop:

- *Requires special depression slide
 - *Difficult to visualize since microbes are not stained
 - *Bacteria are live and therefore slides must be disinfected
- Viewing under a Microscope

B-Making of **dry** smears

Principle

The sample to be examined (pus, sputum, urine centrifugate, cerebrospinal fluid, etc.) is treated as follows:

- it is spread in a thin layer on a glass slide
- it is dried completely
- it is fixed on the slide by heating before being stained.

General notes:

1. Use clean slides free from grease.
2. Mark the slide with a glass writing diamond - grease pencil is easily rubbed away.
3. From liquid cultures make fairly heavy smears.
4. From cultures on solid media, make thin smears.
5. Do not use water taken from rubber tubing attached to taps for making smears, as organisms may be transferred from the rubber.
6. When blotting slides, use a fresh portion of paper for each slide, to prevent transference of material

Bacterial smear

Equipment:-

- ♦ Bacteriological **Loop**.
- ♦ **Slide** (clean and dry).
- ♦ **Drop of water**

(If the culture medium is solid)

- ♦ **Bunsen burner**
- ♦ **Specimens** [growth of microorganisms in
 - **Liquid media**
 - **Solid media**
 - **Pathological samples** (liquid or solid)

C.S.F. (Cerebrospinal fluid)

Sputum

Urine (after centrifugation) Swab (pus, ear, wound, vaginal ,urethral)

A//Making of dry smears from liquid media:

1. Sterilize loop in Bunsen flame
 2. Using aseptic precautions with draw one loop full of culture
 3. Transfer this to a clean slide
 4. spread it with the loop to form a thick film of liquid.
 5. Sterilize the loop.
 - 4-Allow the film to dry without heating
 5. Sterilize the loop.
 - 4-Allow the film to dry without heating
 - 5-pass the slide 3 times through the Bunsen film flame.
- This **fixes** bacteria to the slide.
- 6-Allow the slide to cool, and then **stain the film** by the requisite method.

B//Making of dry smears from solid media:

Aseptic precautions must be observed during the manipulation of culture tubes or plates.

1. Place one **drop of distilled water** on a clean slide by sterilized loop

2. Sterilized loop.

3. With the loop transfer to the slide a small portion of the growth to be examined and Emulsify it in the drop of water until a thin homogenous film is produced.

4. Sterilize loop in Bunsen flame

5. Allow the smear to dry (air)
6. Fix by rapidly pass the slide
3 times through the Bunsen film flame.

Fixation

- ♦ **preservation of morphology but NOT internal structures**
 - Cellular enzymes are inactivated
 - Cell structures are hardened
- ♦ **kills organisms**(Organism dies) [usually results in the death of the attached microorganisms]
- ♦ **adheres specimen strongly to the glass slide**
- ♦ **promotes stain ability of specimen**

Types of fixation:

A//Heat fixing:

1-flame heating bacterial film

Pass slide through flame quickly 3-4 times

- ♦ **Heat fix too little and organisms may wash off slide**
- ♦ **Heat fix too much and organisms may be distorted**

B//Chemical fixation:

- *chemical fixatives penetrate cells

- *Preserves fine substructures and morphology

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- *Fixative chemicals penetrate cells and react with proteins and lipids; (preserves intracellular components) make them inactive, insoluble and immobile. 1. e.g.: -Acetone, Ethanol, Acetic acid, Mercuric chloride, Formaldehyde, Glutaraldehyde



Staining of smears:

Kinds of bacterial stains

1. Simple stain 2. Compound stain

b--- Special stain 3. Negative stain

Simple stain

1-only one dye, one step 2-to know the morphology of cells(shape and aggregation)

eg:-

1. Methylene blue (blue)

2. Methyl violet (blue)

3. crystal violet (blue)

4. Natural red (red)

5. Safranin (red)

6. carbol fuchsin (red)

Special stains

1-more than one dye

2-to stain special part of cells eg:-

1. Acid fast stain (Z-N stain)

2. Albert's stain

3. spore stain

4. capsule stain

5. flagella stain

a---Differential stain



Differential stain

Mo more than one dye

2-tt to differentiate between two groups of bacteria

eg:-**Gram's stain:**

1. Acid fast stain (Z-N stain) , Ziehl Nelsen stain (tuberculosis).
2. Albert's stain (*Corynebacterium*



Simple stain

- One reagent
- Usually involves basic dyes
 - Crystal violet
 - Methylene blue
 - Carbol fuchsin
- ◆ Most microbes bind basic stains (+ charged dye) because surfaces have lots of negative charges
- ◆ Typical examples are **crystal violet and methylene blue**)
- ◆ Used to stain outer surface, so used to look at morphology, size and cell arrangement

equipment :-

- Staining rack.
- loop
- Slide.
- Source of heat.
- bacterial growth
- simple stain (**Crystal violet** or diluted **Carbol fuchsin**)
- filter paper
- distil water
- 70% alcohol



procedure :-

- 1- clean and dry slide with 70% 70% alcohol
- 2-prepare bacterial smear
- 3-fixation the smear
- 4-put the slide on staining rack.
- 5- Stain the smear using any simple stain folded the smear by few drops of stain, let it for 1-2 min.
- 6-pour the stain from the slide
- 7-wash the slide by tap water upside.
- 8-Let it to dry in air or with filter paper.
- 9-exzmin the stained smear under Microscope by oil (100X)



Differential stain

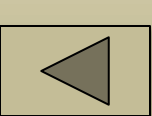
Gram's stain:

In 1884, Gram described this method which is the most important stain in routine bacteriology.

It divides bacteria into two categories depending on whether they can be decolorized with acetone, alcohol, or aniline oil after staining with one of the rosaniline dyes such as crystal violet, methyl violet, or gentian violet, and treating with iodine.

Those that resist decolorization remain blue or violet in color and are designated gram positive, those that are decolorized and take up the red counterstain such as natural red, safranin or dilute carbolfuchsin are termed gram negative.

Although many investigators have tried to uncover the mechanism of the gram reaction, no universal answer has yet been found and it is possible that more than one mechanism exists.



Reagents of gram stain

Solution 1: (primary stain)

Methyl violet 6B (CI No. 42555).....0.5 g

Distilled water100 ml

Dissolve the methyl violet in distilled water and filter. Record date and label.

Solution 2 (mordant): Logols iodine

iodine.....10 g

potassium iodide.....20 g

distilled water.....1000 ml

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Dissolve the potassium iodide in about 50 ml of water, add the iodine, dissolve by shaking and make up to the final volume.

Record date, label and store in a tightly stopper bottle.

Solution 3:(Decolorizer)

Absolute ethyl alcohol 95% or Acetone

Solution 4 (Counter stain)

Natural red OR Safranin OR dilute carbol fuchsin

prepare a smear, allow to dry and fix with gentle heat.

- 1. Stain with solution 1 crystal violet (primary stain). for 1-2 min**
- 2. Treat with Iodine (solution 2) solution (mordant) for 1-2 min.
to increase interaction between cell and dye.**
- 3. rinse with Decolorizer 95% ethanol (solution 3) and continue
application until no more color appears to flow from the
preparation.30sec.**
- 4. wash with water.**
- 5. Apply Counter stain (solution 4) safranin, for 1 min. (If dilute
fuchsin is sued, stain for 30 sec.).**
- 6. Rinse with water. Blot carefully and dry with filter paper.**
- 7. examine the stained smear under Microscope by oil (100X)**



Gram positive

Staphylococcus

Streptococcus

Pneumococci

Corynebacteria

Mycobacteria

Bacillus group

Gram negative

Coliforms

Neisseria

Vibrios

Spirochetes

Salmonella

Shigella

Hemophylus group



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