IDENTIFICATION METHODS OF BACTERIA

CAPSULE STAIN

SPORE STAIN

- Identification of the pathogen microorganism in many infectious diseases is one of the first steps of treatment.
- Identification procedures are continued until the genotype and specific genotype of the bacterium is determined.
- The identification with bacterial phenotypic profile is current method, gives quick results.
- The sample of bacteria is subjected to various biochemical and physiological tests and results are compared with certain tables to give type of the bacterium.

- In order to produce the microorganisms or to investigate whether microorganisms are present or not, it is referred to as **inoculation** of the specimens taken from the clinical/environmental material to the appropriate medium by using special instruments.

- To be transferred to another medium for purposes as incubating, obtaining pure culture, examining biochemical properties, etc. the microorganisms that are produced in a medium is called as **passage**.

- Examination of the various properties of microorganisms depends on obtaining it as a single colony or pure culture.

Various methods have been developed to obtain pure culture from mixed culture.

These are:

1. **Pure Culture with Single Colony Inoculation:**

   - Reduction in Petri dishes

   - Dilution Method (dilution in tube and petri dish)

   - Using a special medium

2. Pure Culture by Chemical Methods: It is aimed to obtain pure cultures by eliminating the susceptible ones by using chemical substances at specific densities.

3. Pure Culture with Physical Methods: It is aimed to obtain pure culture with taking advantage of sensitivity to various physical factors.

4. Use of Experimental Animals: Some microorganisms are obtained purely after injection of mixed cultures into sensitive laboratory animals.

INOCULATION METHODS

Materials that contain microorganisms or believed to contain microorganisms are inoculated on the medium to produce existing bacteria and their cultures.

Samples may be **liquid**, **viscous**, **solid**.

Inoculation to Solid Medium

1. Streake plate method: The material to be investigated is streaked on the one side of sterilized solid medium with loop. Then diluted in two or three areas to form a single colony on the substrate.

2. Stab and/or slant culture method: It is made with needle ended loop, usually in tubes with media solidified in upright shape.

3. Pour plate method: Serial diluted material is poured to the plates. Then mixed with cooled agar medium at 42-45 degrees.

Inoculation to Liquid Medium

Inoculating for various purposes made from solid and liquid media with the aid of a loop or pipette in tube, balloon etc .

PURE CULTURE DIAGNOSIS

In order to identify the genus, species, and type of a pure culture microorganism, some properties should be examined. These:

1. **Morphological** Examination: Examination of the cell shape, size and staining properties of bacterium.

2. **Culture** Morphology: Investigation of some properties such as temperature, oxygen, pH of the culture, colony pigment and shape which are required for bacterial reproduction.

3. Examination of **Biochemical Properties**: It is the examination of the reaction results that the bacterium made while growing in the medium added with the chemical substances.

4. Examination of **Antigen Structures**: An examination of antigenic properties with various serological reactions.

5. Determination of **Pathogenicity**: Some microorganisms are effective on various experimental animals. For this purpose, the pathogenicity of microorganisms is determined using a sensitive experimental animal.

IMPORTANCE :

- The identification of infectious pathogenic bacteria and the differentiation of these bacteria from normal flora bacteria are important in terms of disease diagnosis and treatment protocols.

- Many biochemical reactions are utilized for the identification of bacteria. Most of the biochemical tests used for this purpose are evaluated by the resultant color change of the test and the reagent added to the medium provides the color change.

BIOCHEMICAL REACTION TESTS

OXIDASE TEST

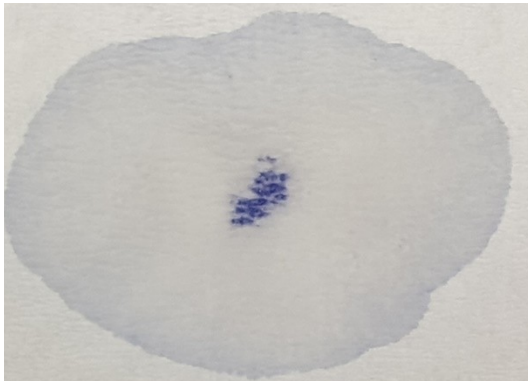
Cytochrome oxidase is the enzyme used by oxidative phosphorylating bacteria.

The cytochrome oxidase converts the phenylenediamine compounds to indophenole by oxidation.

A small amount of the suspected colony is taken with the loop and applied on the 2-3 drops oxidase indicator.

The **purple** color indicates that the test is positive.

Neisseria species oxidase +
Enterobacteriaceae species oxidase -



Oxidase +

Oxidase -

CATALASE TEST

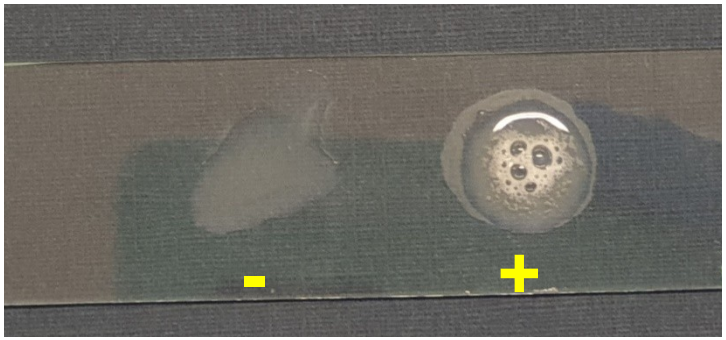
Catalase is an enzyme that breaks hydrogen peroxide (H_2O_2) into water and oxygen.

A piece of the suspected bacterial colony is suspended in a drop of saline on a clean slide and 1-2 drops of 3% H_2O_2 is added.

The formation of oxygen bubbles indicates that the test is positive.

* Erythrocyte-free nutrient

Staphylococcus species catalase + *Streptococcus* species catalase –



COAGULASE TEST

Coagulase is an enzyme that coagulates plasma by converting **fibrinogen** (soluble) to **fibrin** (insoluble).

The test can be done on the slide or in the tube.

Important for *Staphylococcus*

Slide Test

The ‘’bound coagulase’’ located on the bacterial cell surface is investigated.

A portion of the suspected bacterial colony is taken with loop and mixed with a drop of citrated rabbit plasma on the slide.

The formation of visible clusters within 10-30 seconds indicates that the test is positive.

Tube Test

The ‘’unbound (free) coagulase’’ that the bacteria secrete into the environment is investigated.

Suspected bacterial colony is suspended in 1/5 diluted citrated rabbit plasma incubated for 1-4 hours in a 37 ºC water bath.

The formation of the clot is positive for the test.

IMVIC REACTIONS

Indole

Metihyl Red

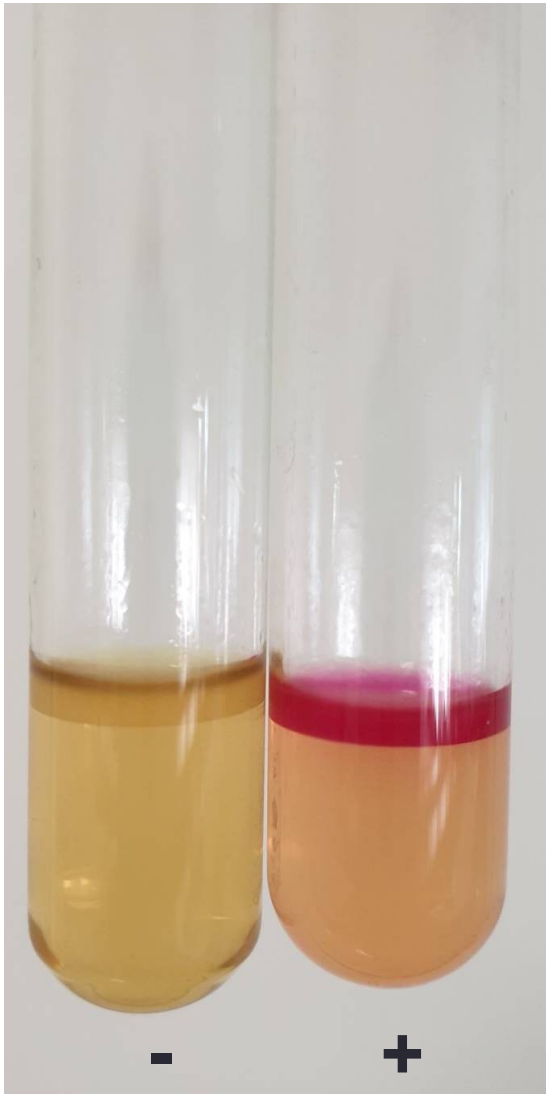
Voges Proskauer

Citrate

It is composed of several different reactions, which are used to identify species, especially in the family *Enterobacteriaceae* .

a. Indole Test:

Indole formation occurs as the result of the breakdown of tryptophan by the tryptophanase enzyme.
The bacteria to be examined is inoculated into a liquid medium containing tryptophan (liquid medium containing 1% tryptophan). After at least 24 hours of inoculation, 0.5 ml of Covacs is added by slowly pouring from the side of the tube.
The formation of a bright **red ring** at the top of the medium indicates that the test is positive.



E.coli indole **+**, *Salmonella typhi* indole **-**

b. Methyl Red Test:

It is based on the principle that bacteria ferment glucose and decrease the pH of the medium below 4.4.

The bacteria to be investigated are inoculated into buffered Clark-Lubs medium containing peptone and glucose. After at least 48 hours of inoculation, 5-6 drops of methyl red indicator is added.

The red color test is positive, the yellow color is negative.

E.coli metil red **+**
Klebsiella pneumoniae metil red **-**

c. Voges-Proskauer Test:

Some bacteria break down carbohydrates to form acetoine (acetyl methyl carbinol).
To investigate the formation of acetoine, after inoculation to Clark-Lubs medium and 48 hours of incubation Voges Proskauer indicators (α-naphtol and KOH) is added. Acetoin turns into diacetyl in the presence of KOH, and in the presence of α-naphthol, the color turns red.

The red color indicates that the test is positive.

E.coli VP -,
Klebsiella pneumoniae VP +

d. Citrate Test:

It is used to distinguish the bacteria that can only use the citrate as a carbon source.

For this purpose Simmons citrate agar medium containing citrate and bromothymol blue is used.

Citrate = oxaloacetate + acetate

Oxaloacetate = pyruvate + CO_2

pH raise

The conversion of the color of the medium to the **blue** indicates that the test is positive.

E.coli citrate **-**

Klebsiella pneumoniae citrate **+**

TSI (TRIPLE SUGAR IRON) AGAR:

It is especially used to describe members of the *Enterobacteriaceae family.*

Based on the fermentation of sugars (lactose:sucrose:glucose 10:10:1) in the medium, gas formation by fermentation and formation of hydrogen sulphide (H_2S).

- If the bottom of the culture medium is red, it means bacteria can not ferment glucose.

- Yellow at the bottom and red at the top it means bacteria can ferment glucose but not lactose and sucrose.

Phenol red is red in alkaline environment, yellow in acidic environment.

- If all of the media is yellow, it means glucose, lactose and sucrose were used by bacteria and a large amount of acid was produced.
- The upward pushing of the medium means gas formation as a result of fermentation of sugars.

- Colour change of the bottom part of the medium to blacken means H_2S is produced by bacteria

Phenol red is red in alkaline environment, yellow in acidic environment.

UREASE TEST:

$$CO(NH_2)_2 + H_2O \rightarrow 2\ NH_3 + CO_2$$

It is an experiment that examines the urea effect with the criterion of bacterium to digest urea into NH_3, and the pH of the medium to be alkaline due to ammonia.

It transforms the color of the media into pink.

https://microbiologyinfo.com/category/biochemical-test/page/2/

CARBOHYDRATE FERMENTATION:

Many bacteria digest carbohydrates to form acid.

Some form both acid and gas.

Carbohydrate medium is used which contains a carbohydrate as a substrate, an Andrade indicator as a pH indicator and a "Durham tube" to indicate gas formation.

The yellow color of the medium indicates acid formation and the presence of gas bubbles in the Durham tube indicates gas formation.

NITRATE REDUCTION:

Bacteria with nitrate reductase enzyme, reduces nitrate to nitrite. Nitrites combine with alpha naphthylamine to form a red product.

The bacteria incubated for 24-48 h at 25°C in a medium containing potassium nitrate. The addition of reagents containing alfanafilamine and sulphanilic acid to the medium, followed by the conversion of the base color to red within 30 minutes, indicates the presence of nitrites in the medium.

Members of the family *Enterobacteriaceae* generally reduce nitrates.

OTHER BIOCHEMICAL REACTIONS:

- Oxidation fermentation test
- Arginine dihydrolase, lysine and ornitine decarboxylase tests
- Jelatinase test
- Escululin hydrolysis test
- Phenylalanine deaminase test and etc.

CAPSULE

In some bacteria, it forms the outermost part of the cell envelope.

The layer around the cell wall, usually the polysaccharide structure.

In some bacteria, the capsule is mucoid.

It can be 0.2 – 10 μm thick.

Its structure is loose and contains 95-98% water. So, it does not prevent cell membrane permeability.

Capsule of *Bacillus anthracis* has polypeptide structure formed D-glutamic acid units.

- Some bacterial capsules are not in a dense layer, but in the form of a loose web of fibrils. Such capsules are called "**glycocalyx**". For example, *Streptococcus mutans*
- Virulence of pathogenic bacteria is usually directly related to their ability to form capsules.
- The capsule protects the bacterium against phagocytosis, facilitates the spread of pathogenic bacteria in the tissue.
- The capsule is not a mandatory structure for survival. It can be removed by the influence of environmental factors, as well as can be regenerated under appropriate conditions.
- They show good antigenic structure.
- Because the capsule is not easy to stain with basic dyes, it is not seen in simple stainings. It is shown with negative stainings.

SPORE

Protoplast
Spore jacket containing peptidoglycan
Shell (cortex)
Protein jacket
Exosporium (carbohydrate protein membrane)
Calcium dipicolinate

- It is a structure of some bacteria caused by unsuitable environmental conditions and it improves resistance to physical factors such as heat, dryness, inadequate nutritional conditions.
- It's not a reproductive body.
- Spore formation under inappropriate conditions – sporulation
- Formation of vegetative bacteria from spore in appropriate conditions – germination
- Bacterial spores are formed by protoplasm and a thick sheath, so they are stained with strong staining solutions and under heat treatment from bottom.

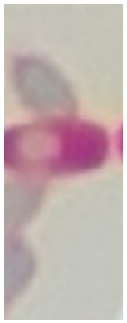
- The spores can be round or oval shaped.
- Some bacterial spores are wider than the bacterium itself and cause wideness (eg *Clostridium tetani*), some of which do not exceed the width of the cell (eg *Bacillus anthracis*).

- **<u>Spores according to settlement areas in the bacterium;</u>**

  At one end of the bacterium, Terminal

  In the middle, Central

  Between the tip and the middle, Subterminal



Topic: Capsule Staining

Method: Capsule Staining (Gins Method)

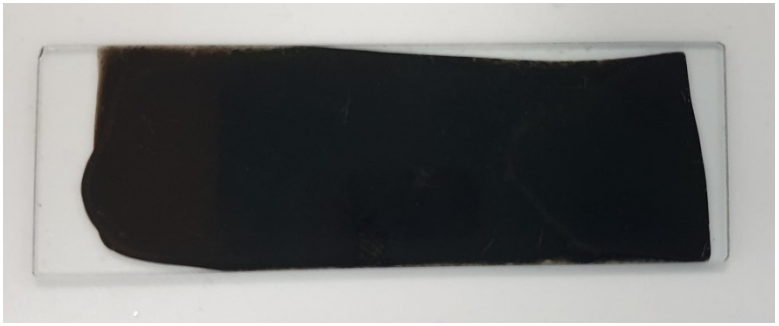
-Immobilise the slide (fixation)

-Pour methylene blue (aqueous fucsin)

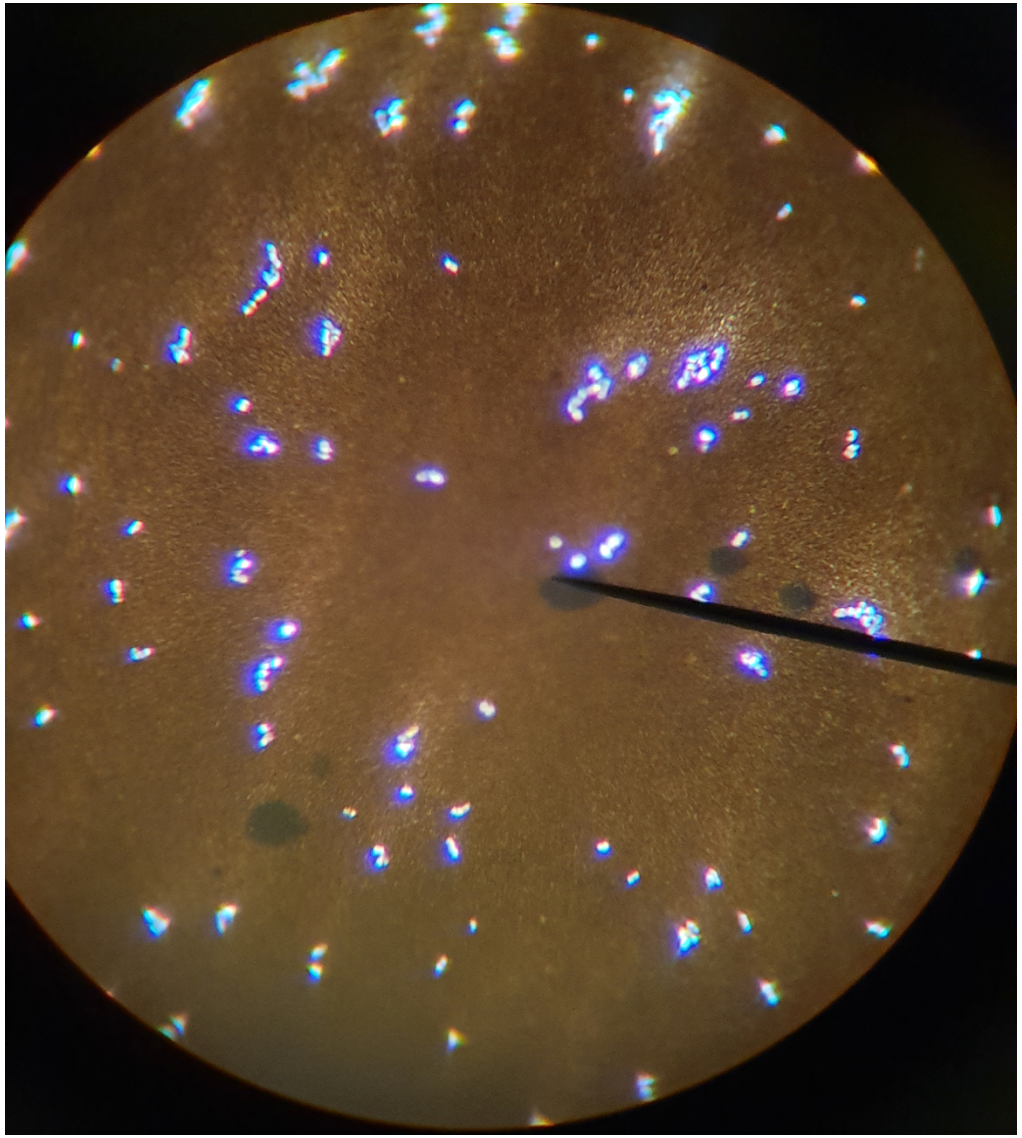
-Wait for 1 min.,

-wash gently, dry

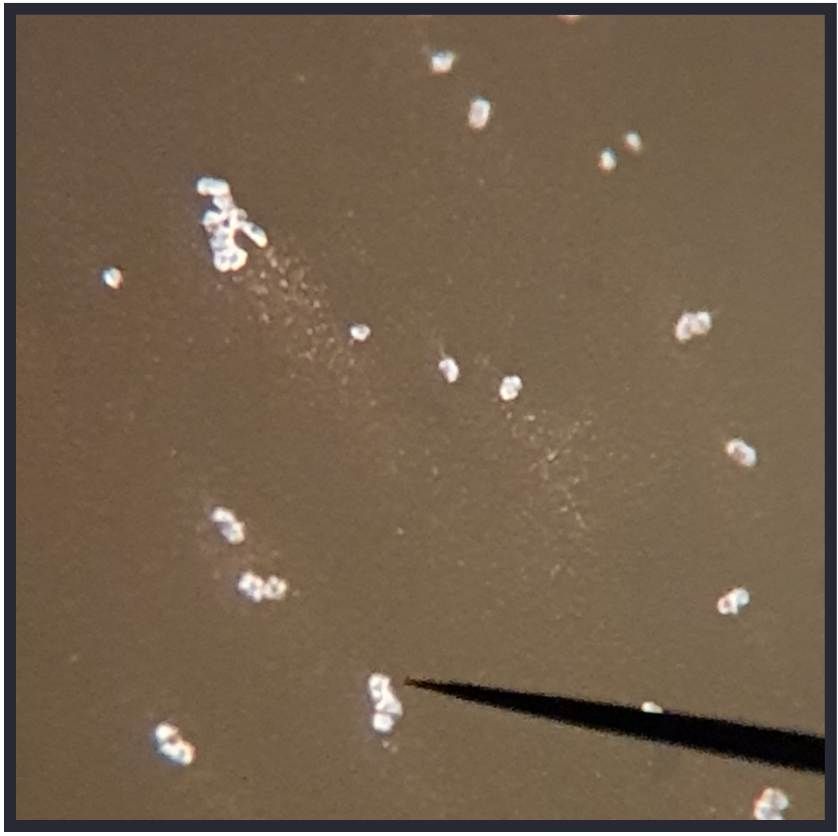
-Drop an immersion oil,
examine under 100X lens



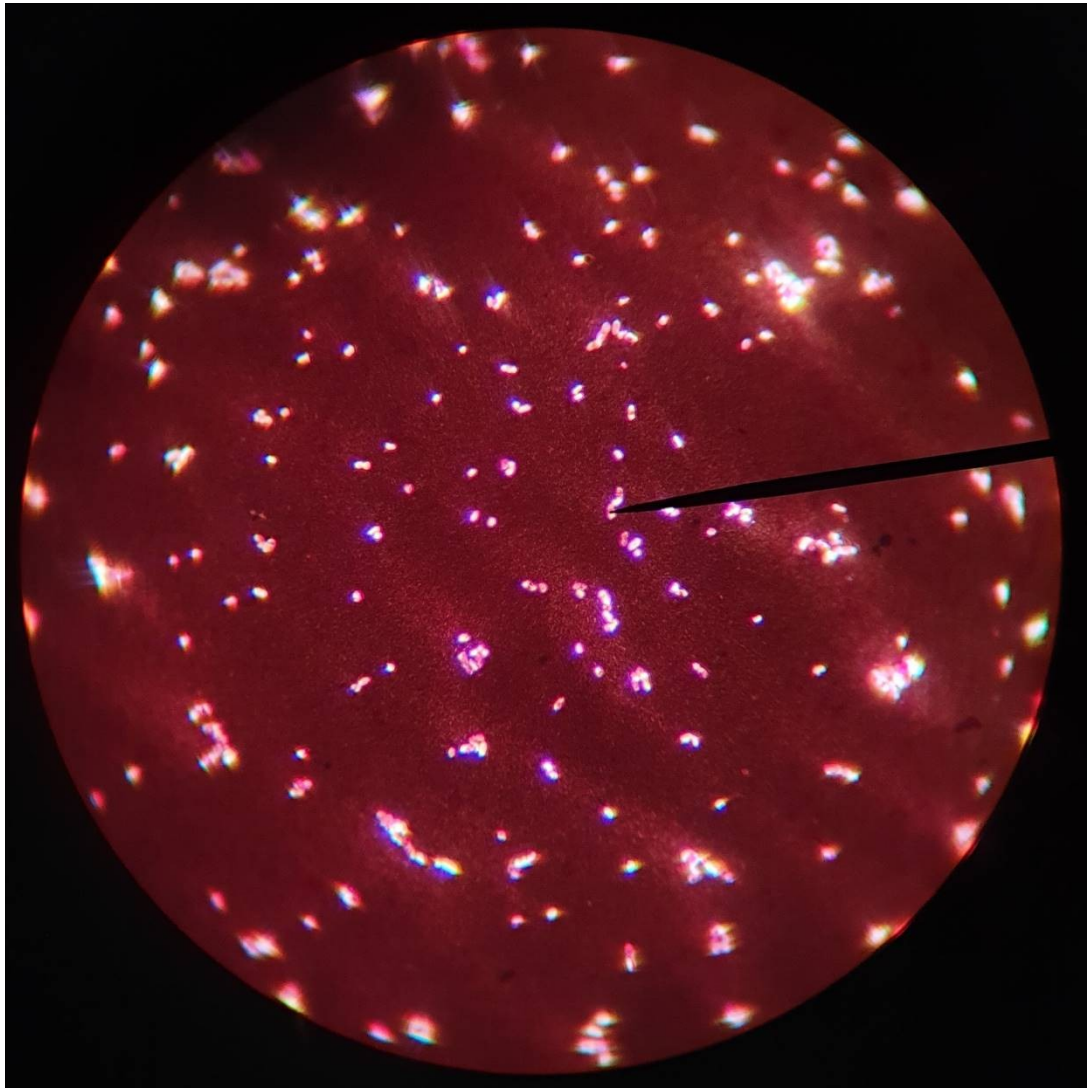
Capsule Staining Microscope Image



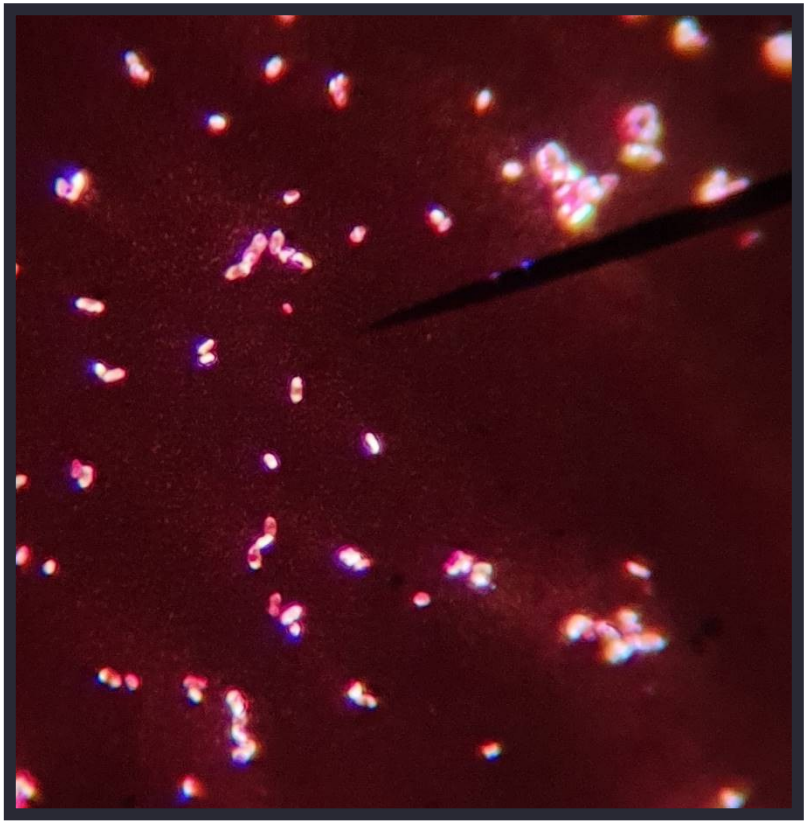
Methylene blue



Capsule Staining Microscope Image



Aqueous fucsin



Topic: Spore Staining

Method: Spore Staining (Wirtz-Conklin Method)

-Immobilise the slide (fixation)

-Pour malachite green

-6-7 min. heat slide from bottom without boiling

-Wash under water, gently

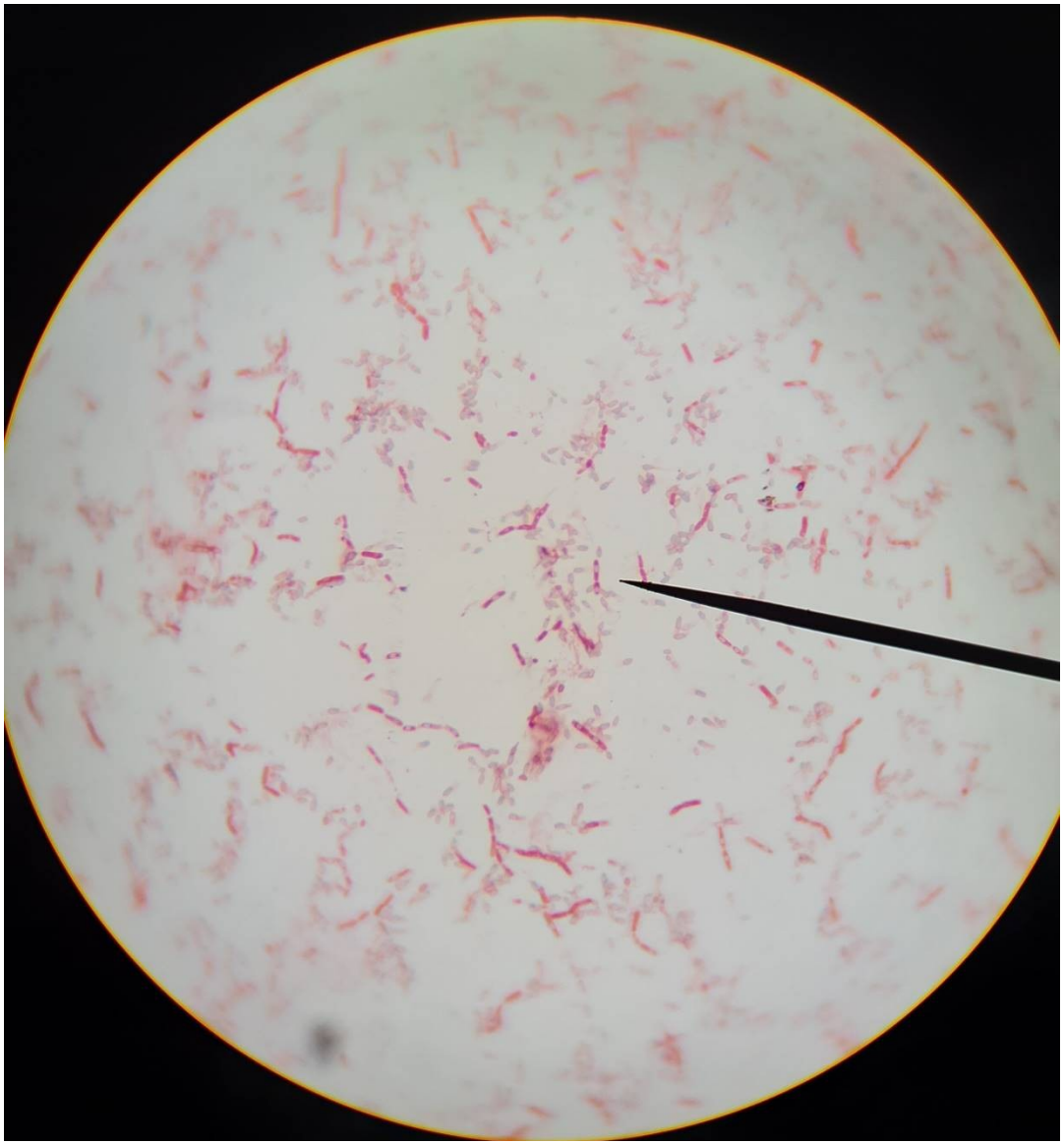
-Pour %0,5 safranine

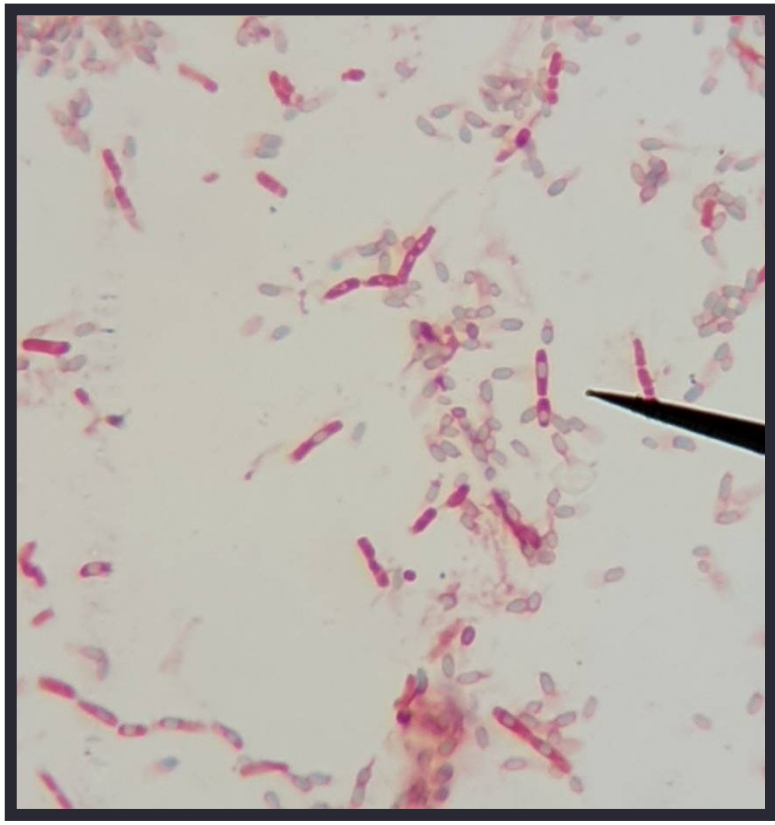
-Wait for 1 min.

-Wash under water and dry,

-drop an immersion oil,
-examine under 100X lens

Spore Staining Microscope Image





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