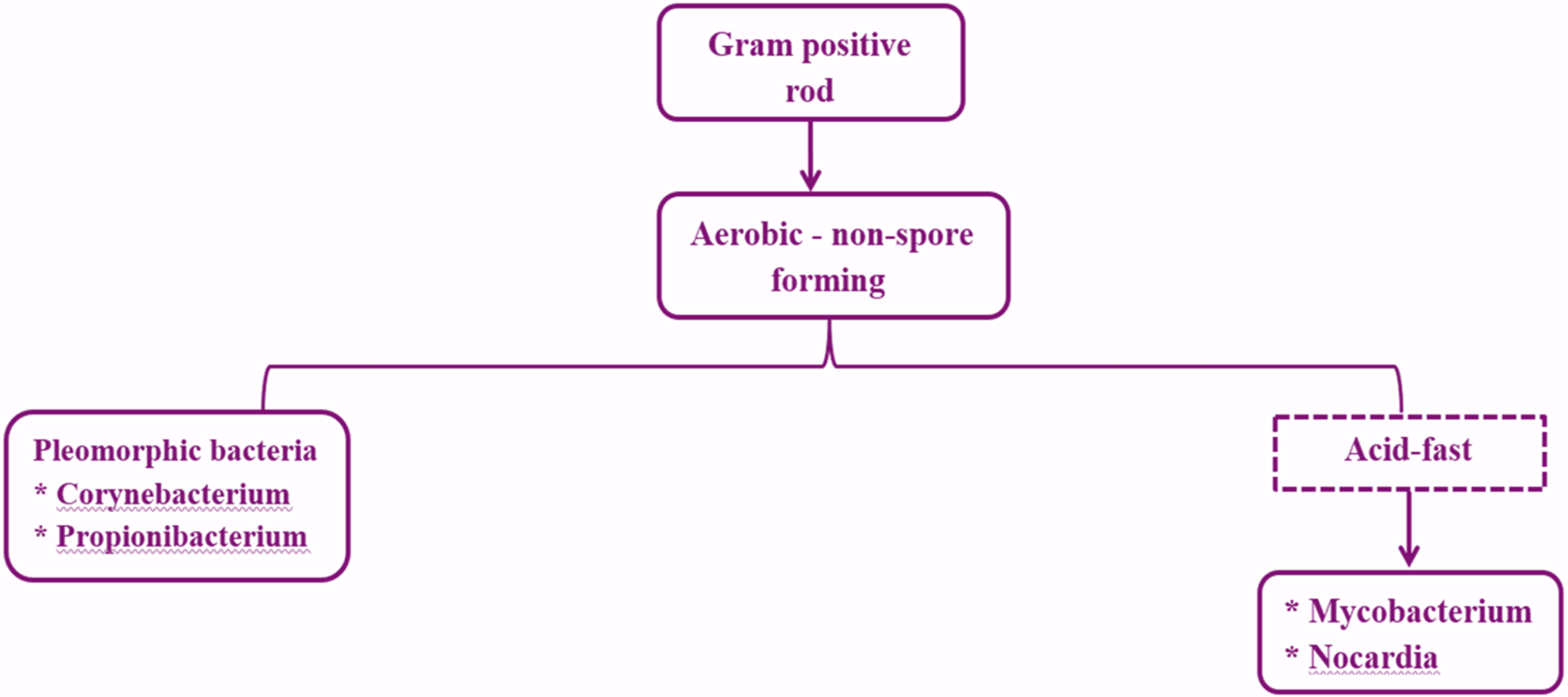
Bactria Classification

Mycobacterium spp

Lab 6

Domain: Bacteria
Phylum: Actinobacteria
Class: Actinobacteria
Order: Actinomycetales
Suborder: Corynebacterineae
Family: Mycobacteriaceae
Genus: Mycobaterium . . . . . . include over 190 species.

Species: ***Mycobacterium tuberculosis* ..... *cause* *tuberculosis***
Mycobacterium bovis ..... cause* *tuberculosis* *in cattle
Mycobacterium leprae* ..... *cause (Hansen disease) **-** ***leprosy***


Gram positive rod
Aerobic - non-spore forming
Pleomorphic bacteria
* Corynebacterium
* Propionibacterium
Acid-fast
* Mycobacterium
* Nocardia

Detection of infection methods

1. Skin Test

For the skin test, your doctor or nurse will inject a very small amount of the protein from TB under your skin. It's important not to scratch the area, even if it itches. Scratching could make the test results harder to read.

Your doctor will ask you to come back in two or three days to see if the injection spot is swollen. Redness at the location of the injection is normal and is not a sign of a TB infection. A hard, swollen lump of five or more millimeters may mean you have TB. Five millimeters is about the size of a pea.

2. Bacterial Culture

There are two types of solid culture media for the primary isolation of mycobacteria, those that have coagulated egg as a base and those that have agar. Middlebrook's medium which is an agar based medium and Lowenstein-Jensen medium which is an egg based medium.

Mycobacterium tuberculosis **colonies are small and buff colored when grown on either medium. Both types of media contain inhibitors to keep contaminants from out-growing MT. It takes 4-6 weeks to get visual colonies on either type of media.**

Lowenstein Jensen Medium (LJ Medium)

Lowenstein Jensen Medium (LJ Medium) is a selective medium that is commonly used for the cultivation and isolation of Mycobacterium (notably *Mycobacterium tuberculosis*) from specimens containing mixed flora.

Principle

[1]**Asparagine and Potato Flour** are sources of nitrogen and vitamins in Lowenstein-Jensen Medium. [2]**Monopotassium Phosphate and Magnesium Sulfate** enhance organism growth and act as buffers. [3]**Glycerol and the Egg** Suspension provide fatty acids and protein required for the metabolism of mycobacteria. When heated, the egg albumin coagulates, thus providing a solid surface for inoculation. Glycerol serves as a carbon source and is favorable to the growth of the human type tubercle bacillus while being unfavorable to the bovine type. [4]**Malachite green** acts as an inhibitory agent toward microorganisms other than mycobacteria.

Medium Preparation

1. Suspend 37.2 grams of Lowenstein Medium Base in 600 ml distilled or deionized water containing 12 ml of Glycerol and boil with constant agitation.

2. Autoclave at 121°C for 15 minutes. Cool to 45- 60 °C.

3. Prepare 1000 mL of a uniform suspension of fresh eggs under aseptic conditions. Avoid whipping air into suspension during the collection and mixing.

4. Aseptically mix the 1000 mL of egg suspension with 600 mL of the sterile Lowenstein-Jensen Medium cooled to 50 – 60°C, avoiding air bubbles.

5. Dispense into sterile screw cap tubes or bottles. Arrange in a slanted position.

6. Place in water bath or autoclave at 85°C for 45 minutes to coagulate the medium.

Method

1. Inoculate the Lowenstein Jensen Media with specimen after decontamination and neutralization.

2. Incubate medium in a CO_2 atmosphere at 35-37°C. Protect from light. Tubed media should be incubated for one week with loosened caps to allow the circulation of CO_2 for the initiation of growth. Caps should be tightened after one week in order to prevent dehydration of media.

3. Examine the media within five to seven days, and weekly thereafter for up to eight weeks.

Colonies of *Mycobacterium tuberculosis* on Lowenstein-Jensen (LJ) Medium


1216

Lecithinase (Phospholipase-C) production.

To determine the ability of microorganisms to produce the enzyme lecithinase.

Principle:

Bacterial lecithinases are of special interest because of the possible role of these enzymes in pathogenicity. Lecithinases or phospholipases are enzymes released by bacteria that have the ability to destroy animal tissues.

Phospholipid complexes are usually emulsifying agents occurring in **tissues**, **serum** and **egg yolk**. Lecithin is a normal component of the egg yolk.

In egg yolk agar, bacterial lecithinases break down this lecithin to an insoluble diglycerides resulting in an opaque halo, surrounding the colony when grown on the medium.

Lecithinase activity is used to characterize several gram positive and gram negative bacteria. *Clostridium perfringens* is lecitinase positive while most of the other strains of Clostridium are lecithinase negative.

The medium (Egg-yolk agar).

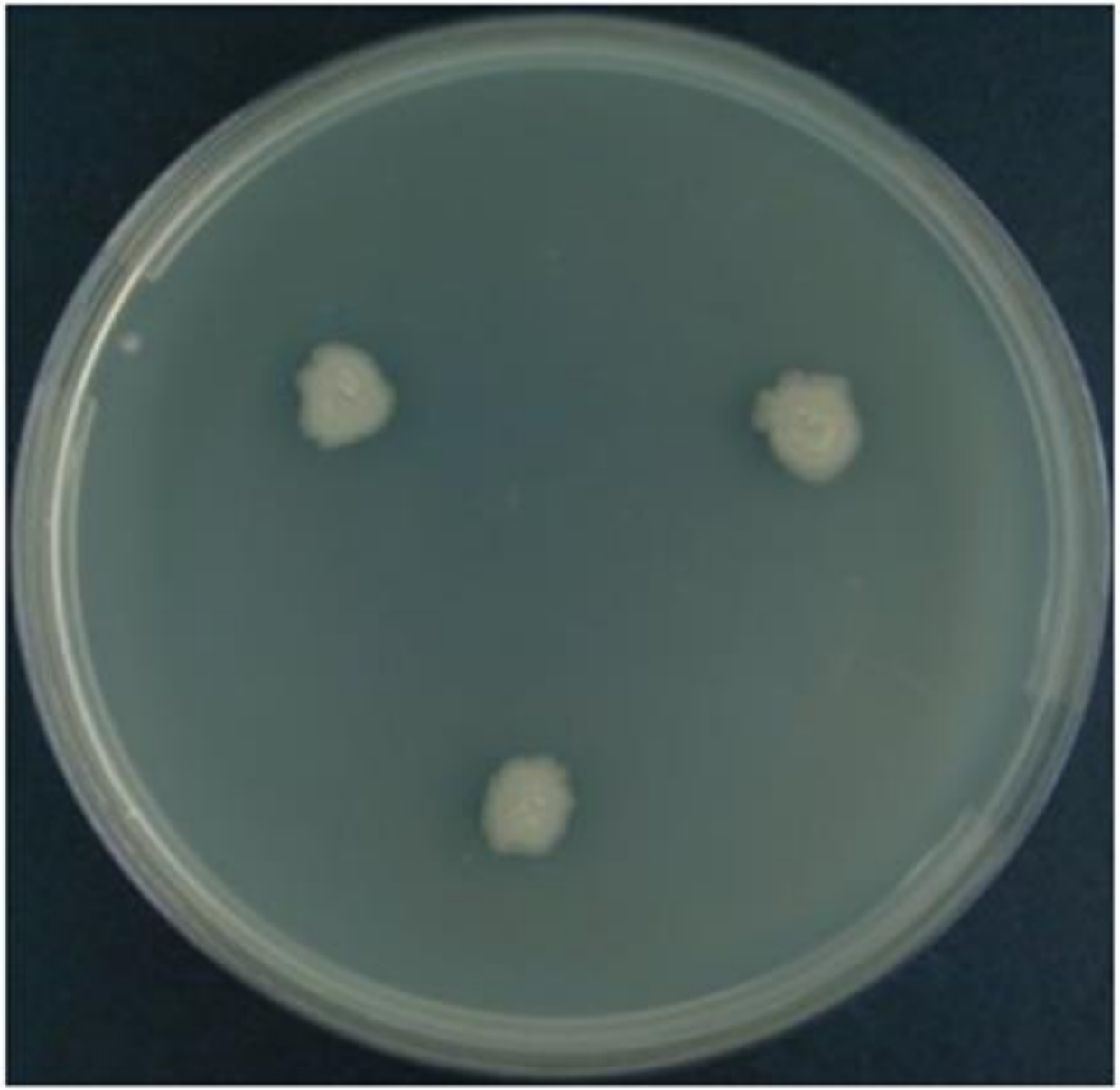
Nutrient agar, sterile……..85 ml

Egg-yolk agar suspension……...15 ml

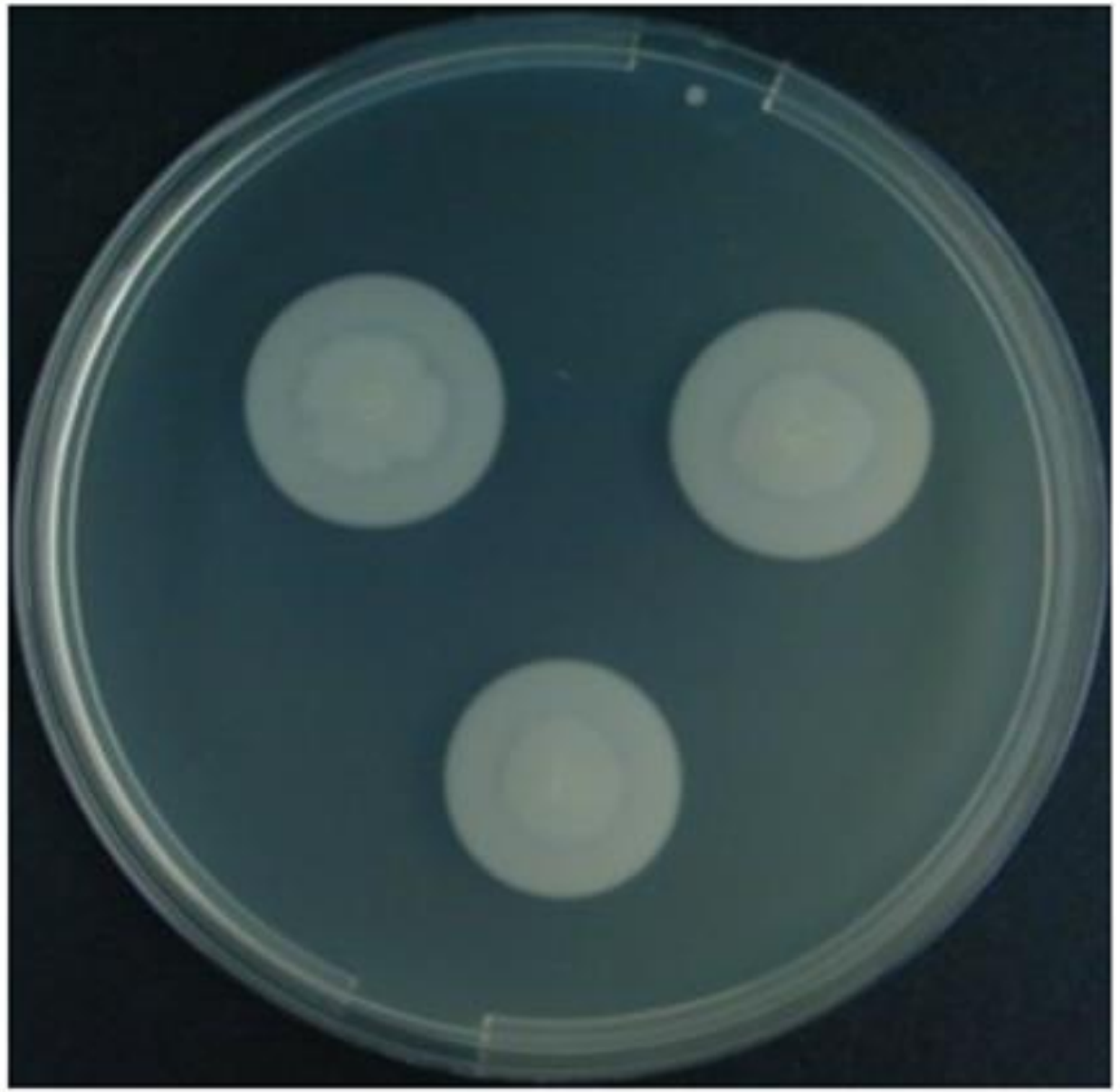
The Nutrient agar was melted and cooled at 55°C, the egg-yolk was added, then poured in plates.

The Method:

Several colonies (2-5) were picked off from bacterial culture by sterile stick then inoculated onto Egg-yolk agar plate and incubated at 37°C for 24 hours. Lecithinase-producing colonies were surrounded by zones of opalescence.



Negative



Positive

NAGLER TEST