



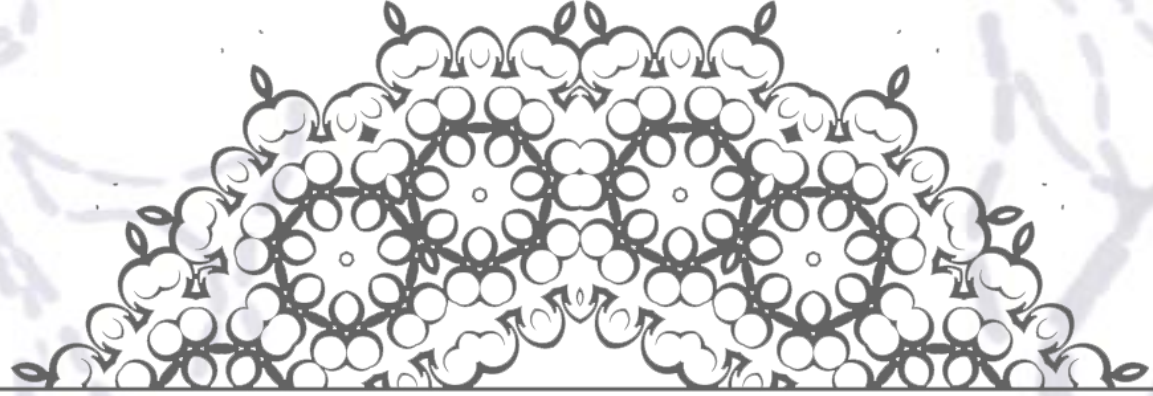
460 MBIO

Medical Bacteriology

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2017

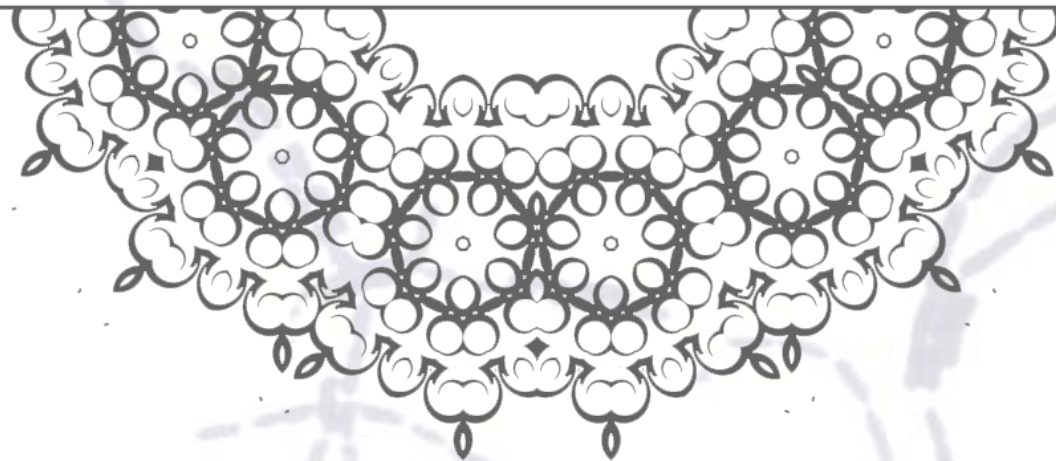




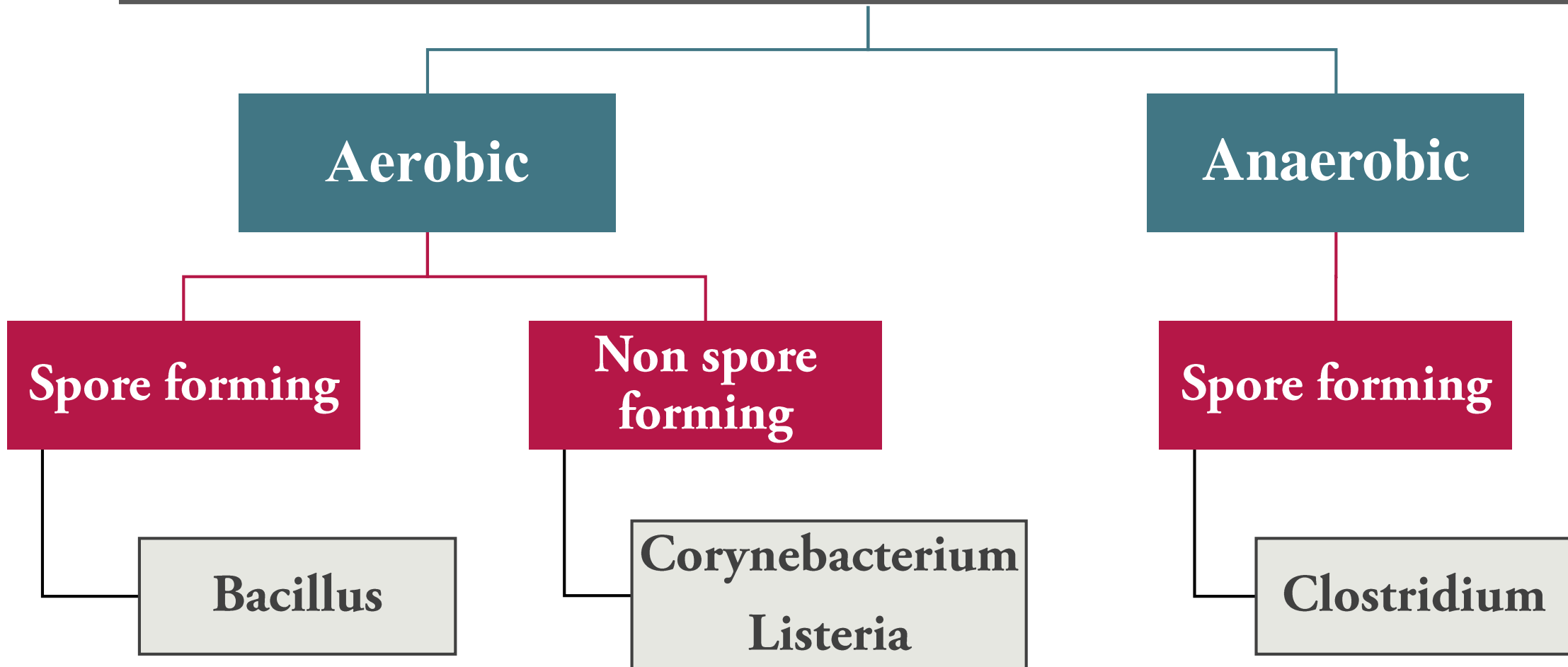
Lab 3

Gram Positive Group

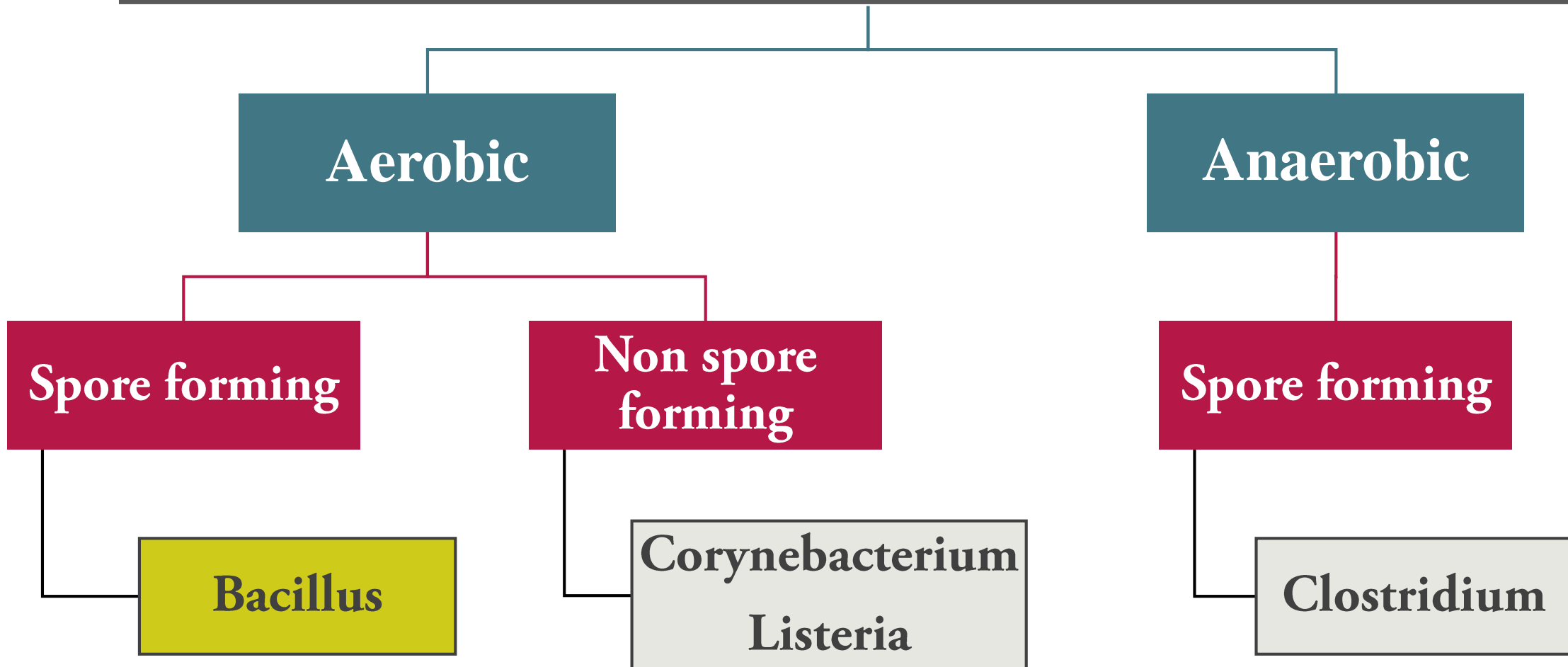
(Bacilli)



Gram positive bacilli divided into two groups according to Oxygen requirements :



Gram positive bacilli divided into two groups according to Oxygen requirements :



❖ General Characters of *Bacillus* sp. :

Very large gram positive bacilli, arranged in long chains.

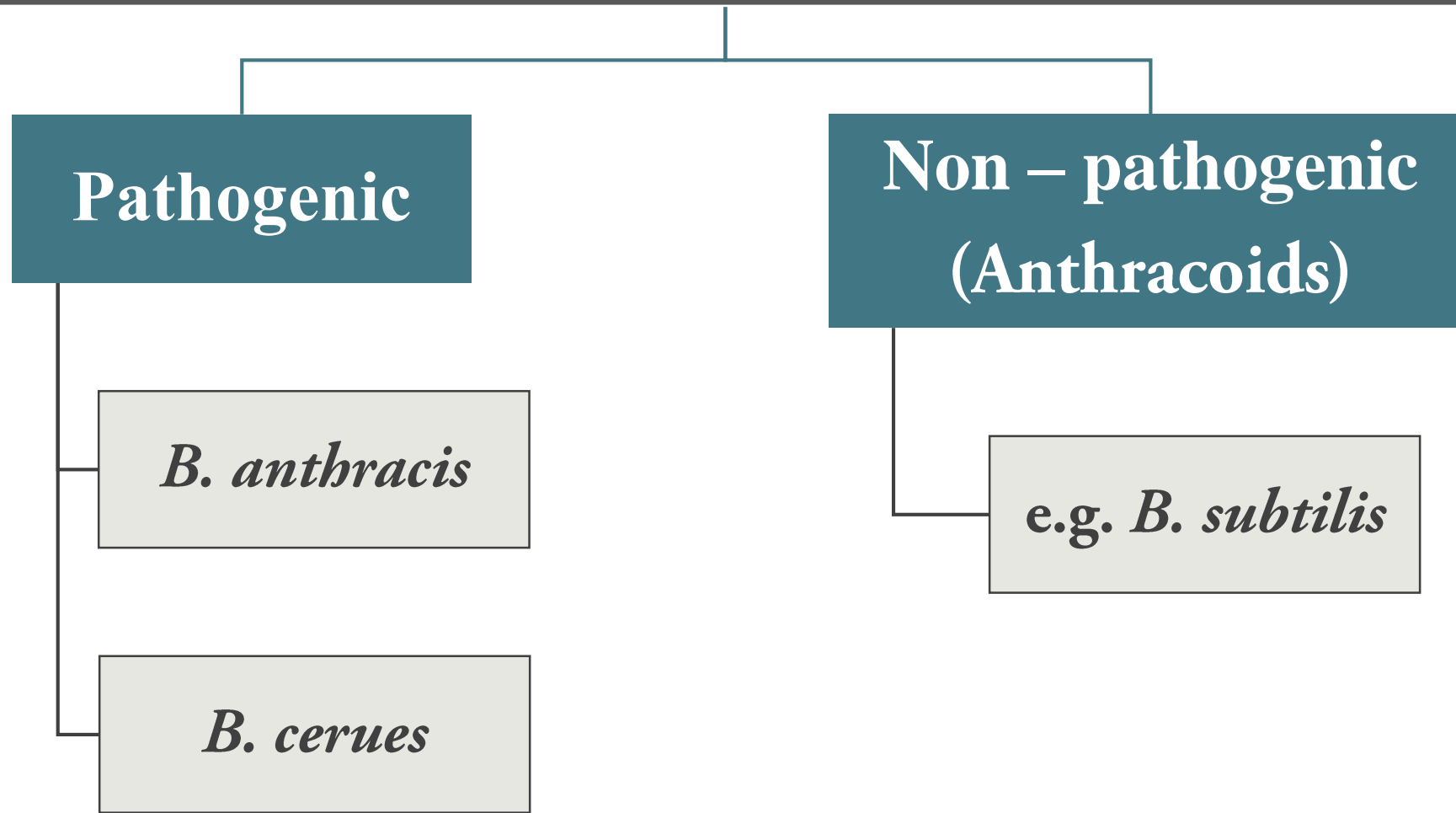
Motile except *B. anthracis*.

Spore forming outside the host, capsulated inside it.

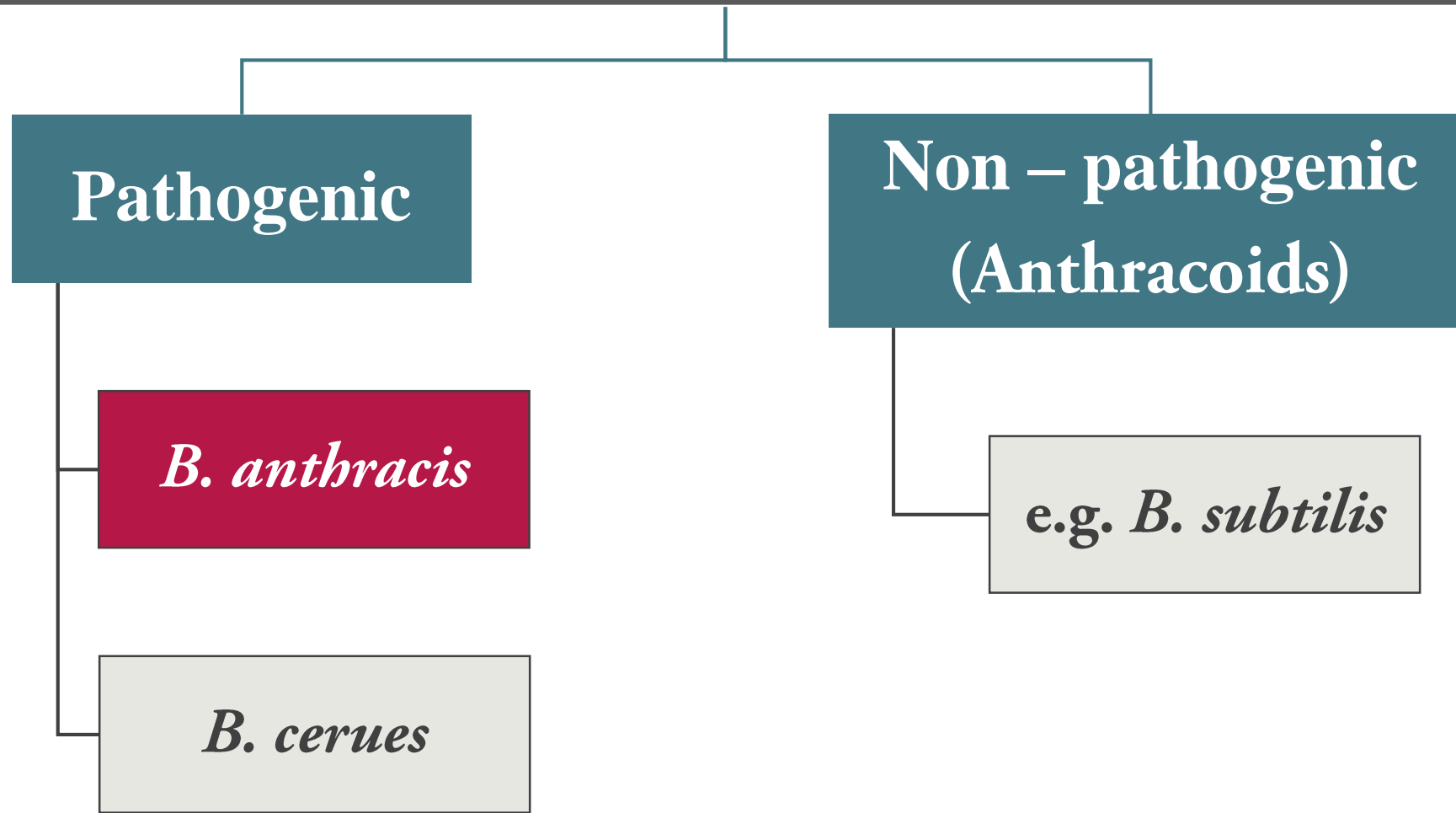
Non fastidious and facultative anaerobic.

Catalase positive, it's found naturally in soil (as habitats).

Classified Aerobic Spore Forming Bacillus spp., According to Pathogenicity to:



Classified Aerobic Spore Forming *Bacillus* spp., According to Pathogenicity to:

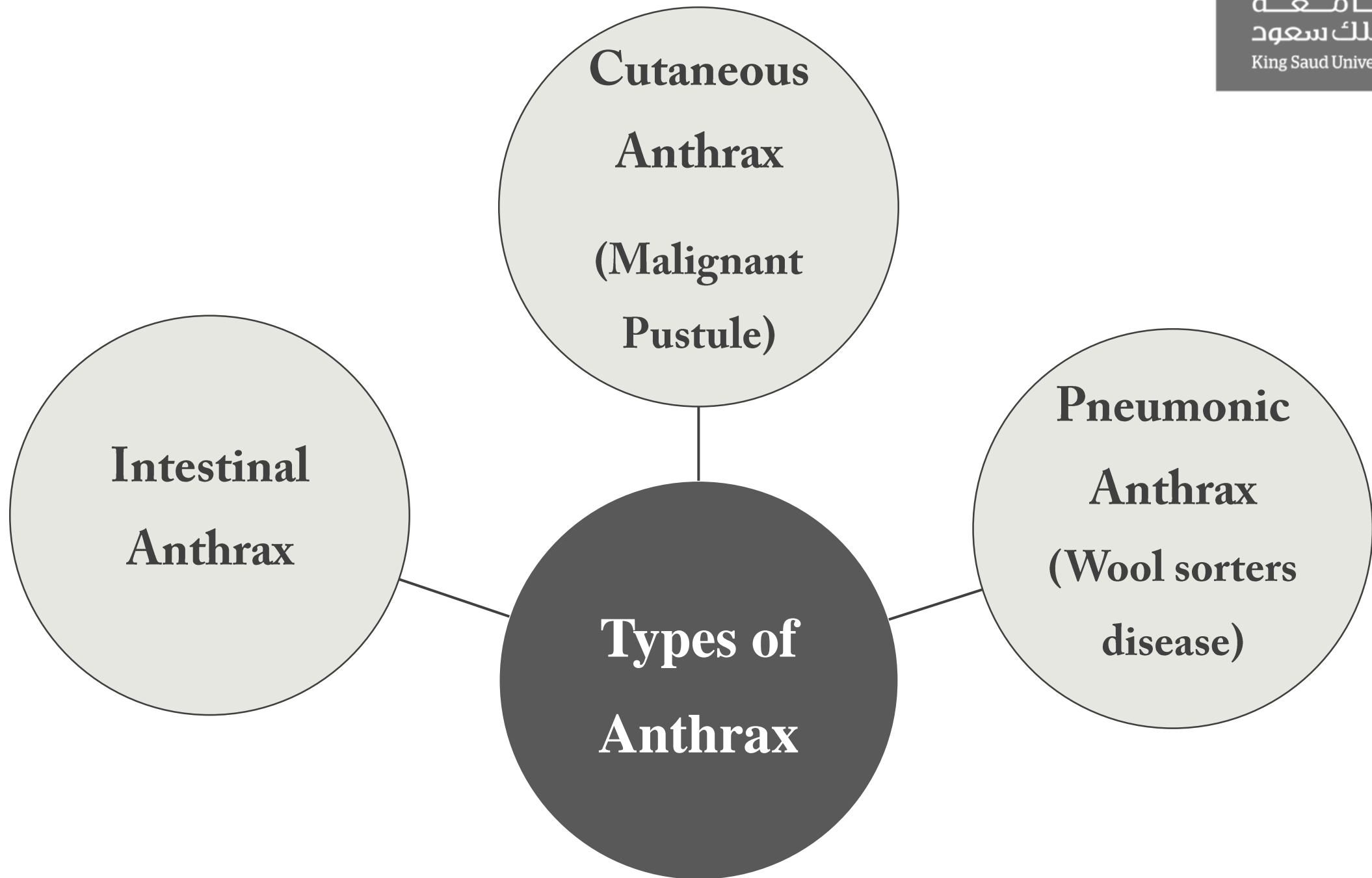


❖ Disease caused by *Bacillus anthracis* :

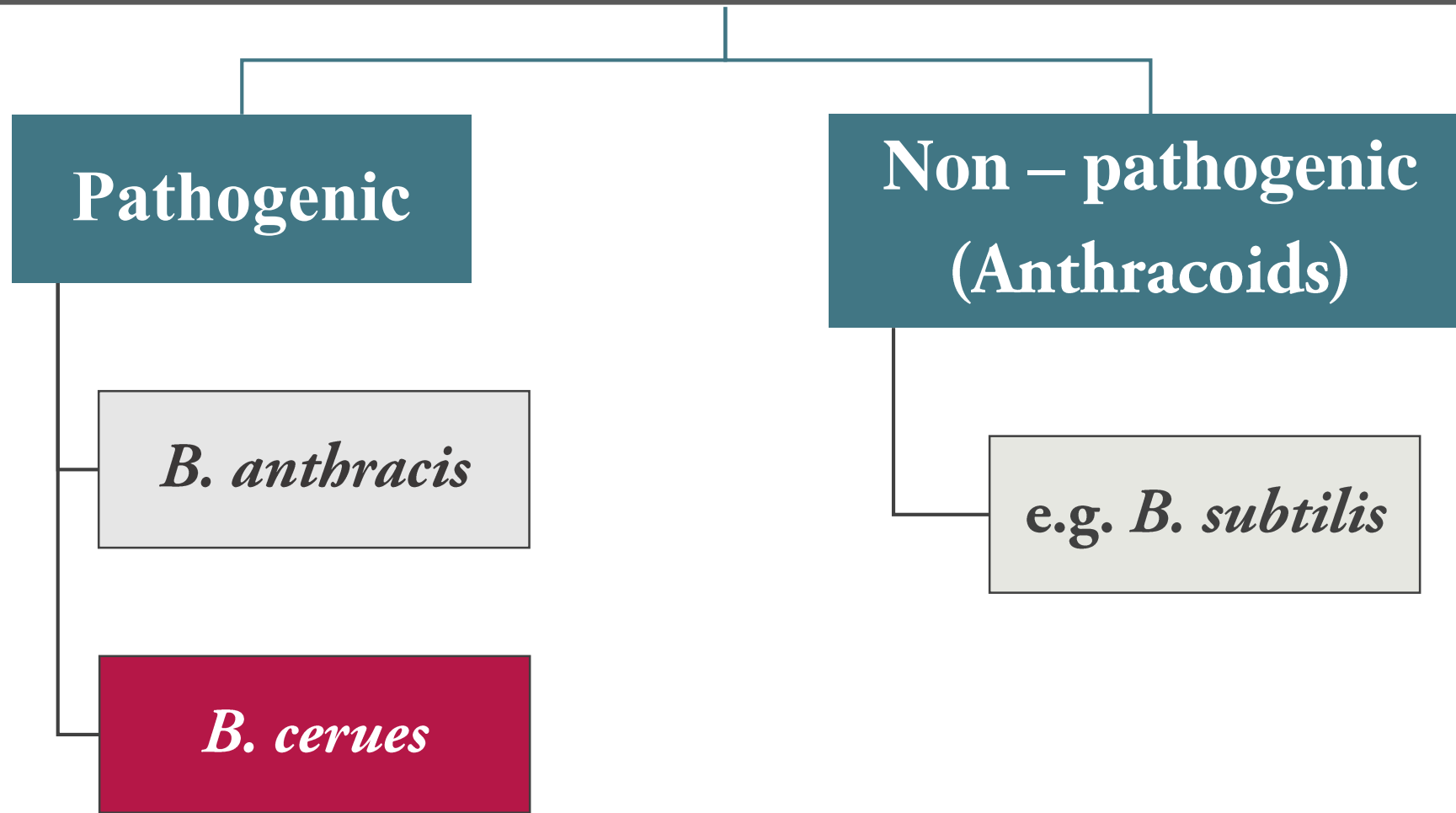
Anthrax is an acute infectious disease in man & animal.

It is zoonotic and occupational disease.

Direct person-to-person spread of anthrax is extremely unlikely to occur.



Classified Aerobic Spore Forming *Bacillus* spp., According to Pathogenicity to:



❖ General Characters of *Bacillus cereus* :



Normal habitat of soil.



May isolated from food such as grains and spices.



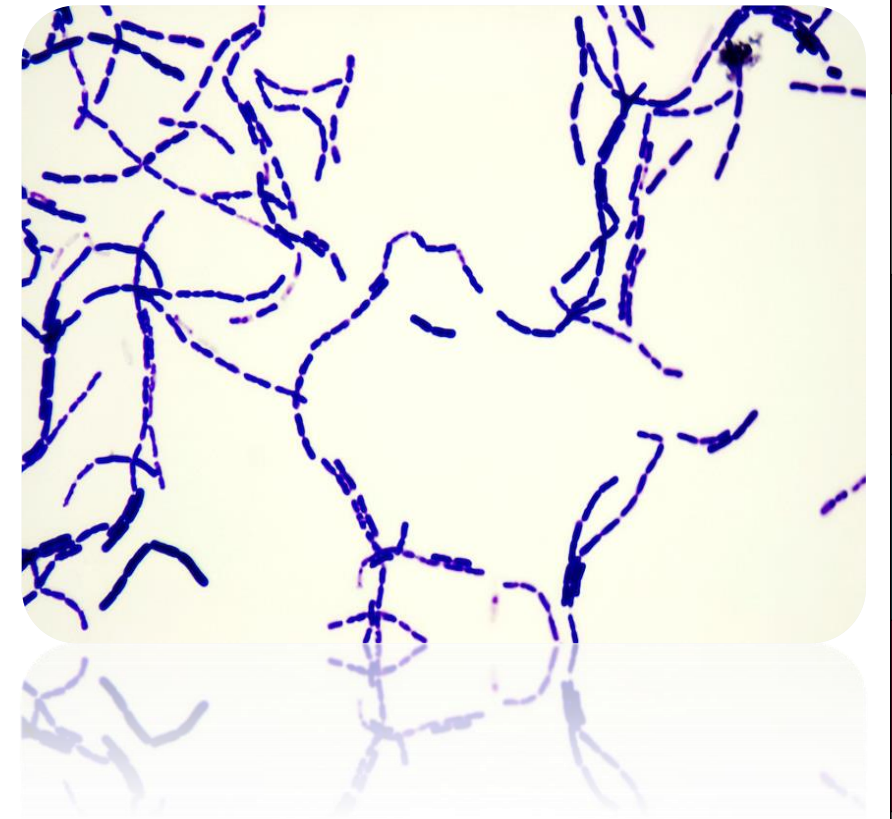
It's causes **two** types of food poisoning.

Comparison between *B. cereus* Poisoning

	Emetic Form (short incubation)	Diarrheal form (long incubation)
Cause	Heat stable enterotoxin	Heat labile enterotoxin
Symptoms	Nausea, vomiting, abdominal cramps.	Abdominal cramps and diarrhea
Incubation period	1 – 6 Hours	8 – 16 Hours
Resembles	<i>Staphylococcus aureus</i>	<i>Clostridium perfringens</i>

❖ Identification of *Bacillus* Spp. :

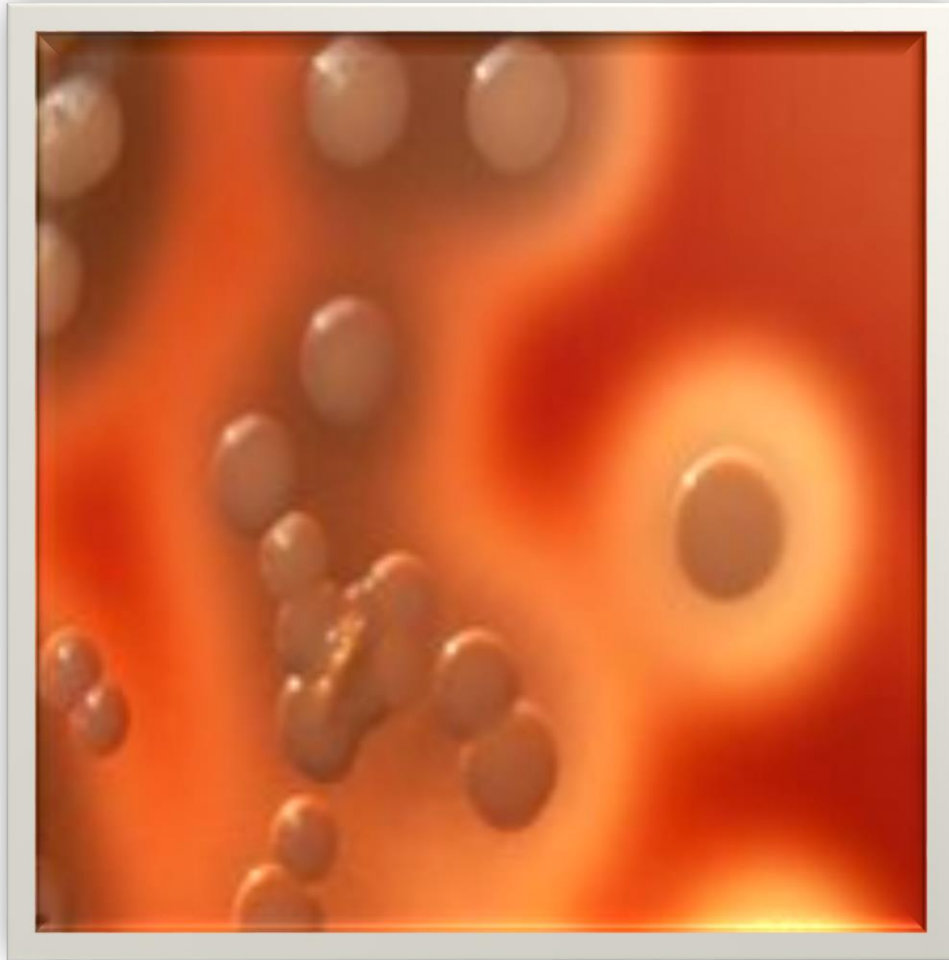
- Specimen.
- Morphology :
 - Macroscopical (Cultural characteristics)
 - Microscopical (Gram stain, spore stain)
- Biochemical test (Starch Hydrolysis)

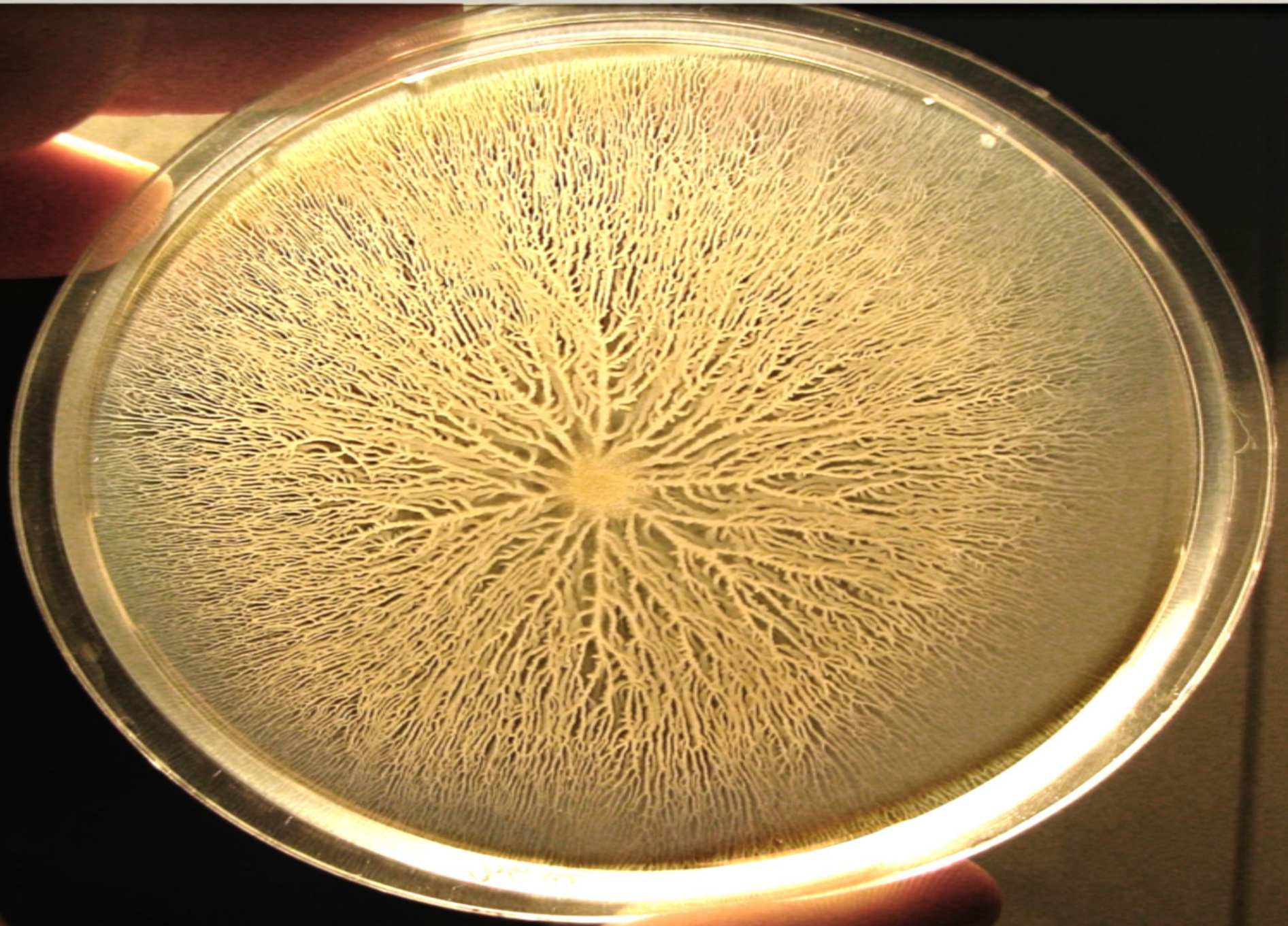


- **1st : Specimen :**
 - Pustular exudates in malignant pustule.
 - Sputum in pneumonic anthrax.
 - Stool in intestinal anthrax and food poisoning by *B. cereus*.
 - Stool specimen is emulsified and heated to 80 °C to kill non spore forming microorganism.

2nd : Morphology, Macroscopical

O ₂	Nutrient Agar	Blood Agar
Observation	<ul style="list-style-type: none"> ▪ The colonies appears mucoid or smooth colonies. ▪ Rough colonies are relatively a virulent ▪ Sub culture on gelatin medium results in inverted fire tree appearance. 	<ul style="list-style-type: none"> ▪ Bacillus species grow well on blood agar showing a double zone of hemolysis. ▪ <i>B. anthracis</i>, which grows well on blood agar without any hemolytic effect.





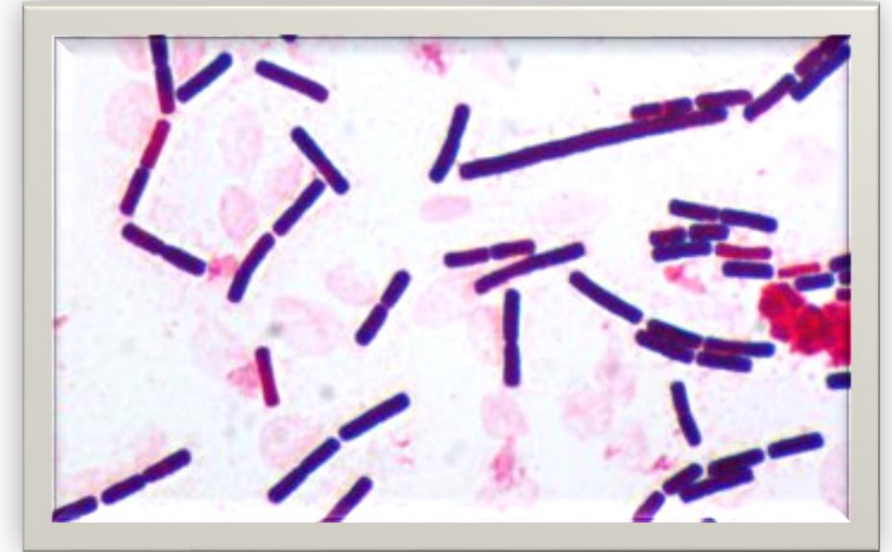
■ 2nd: Morphology, Microscopical :

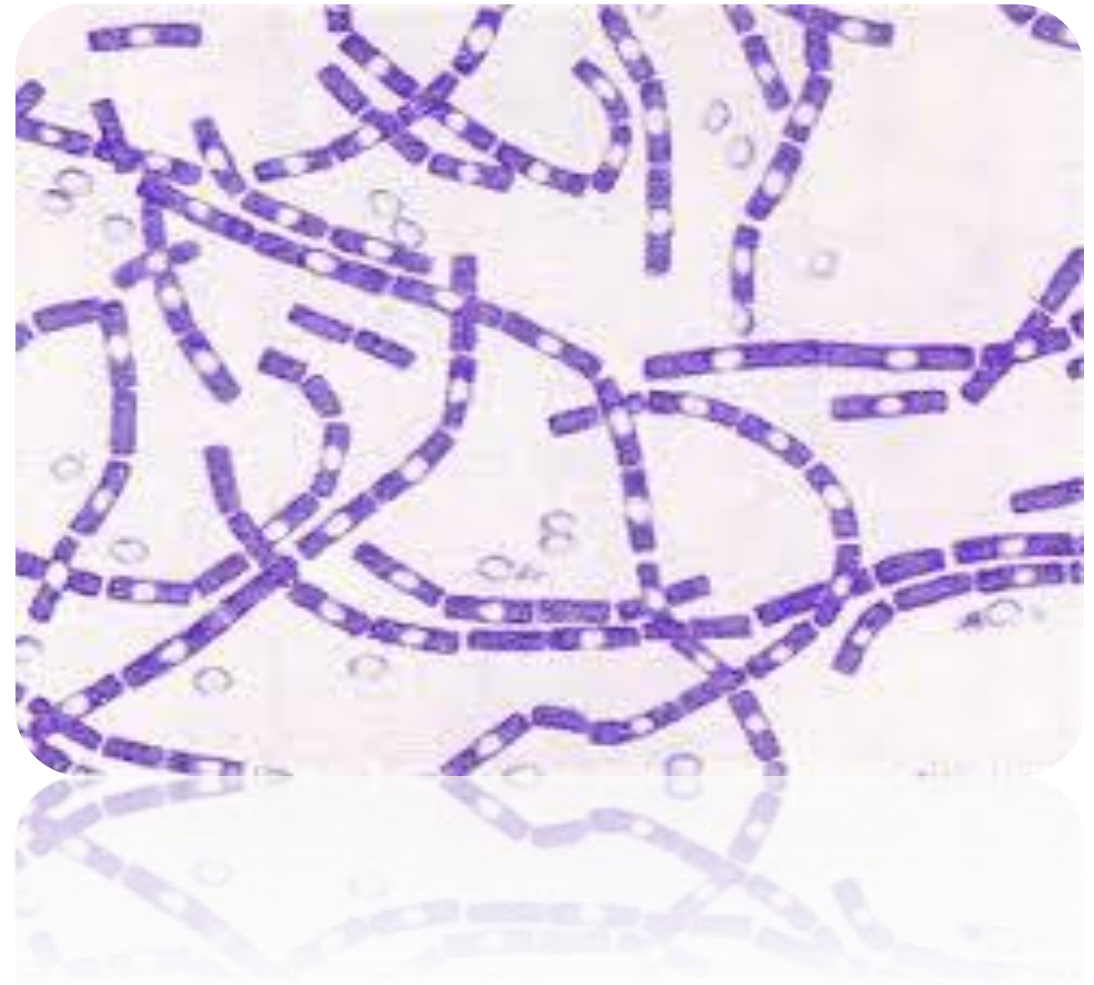
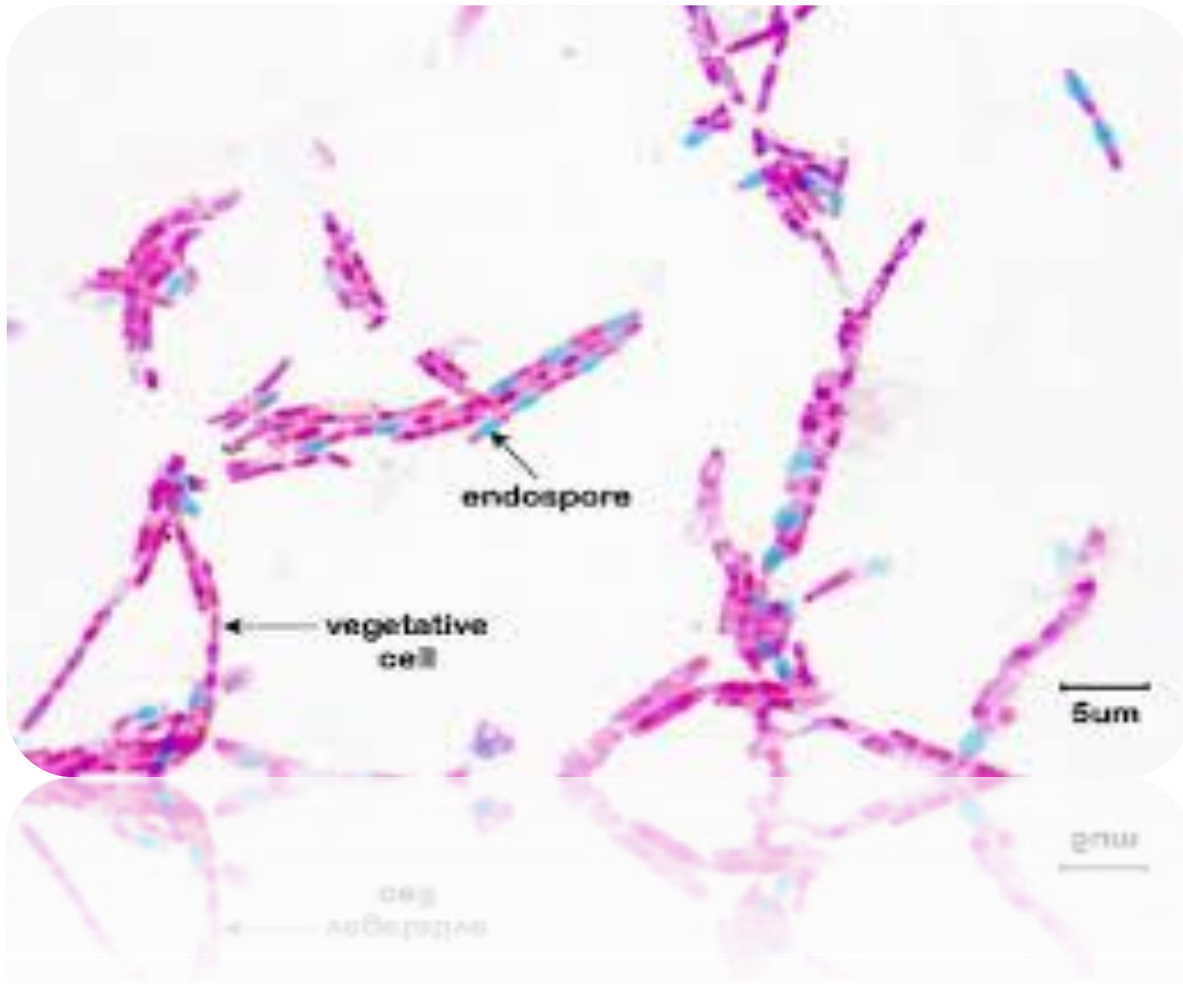
- Gram stain

- Gram positive bacilli, arranged in chains.

- Spore stain

- Bacillus spores are oval & central.
- By spore staining technique (Malachite green & safranin) , the spore appears green while the vegetative cells appear red.





■ 3rd : Biochemical test (Starch Hydrolysis):

- Principle :

- This test check the ability of microorganism to produce hydrolytic enzymes such as amylase to hydrolyzed starch.
- Exoenzymes are excreted out by microorganism to breakdown large or complex substances (starch) into smaller ones (sugars) so that they can enter the cell for food.

Method and Results

Procedure

- Inoculate nutrient agar plate containing 1% Starch with the microorganism.
- Incubate the plate at 37 °C for overnight.
- After incubation, flood the plate with Iodine solution.

Result

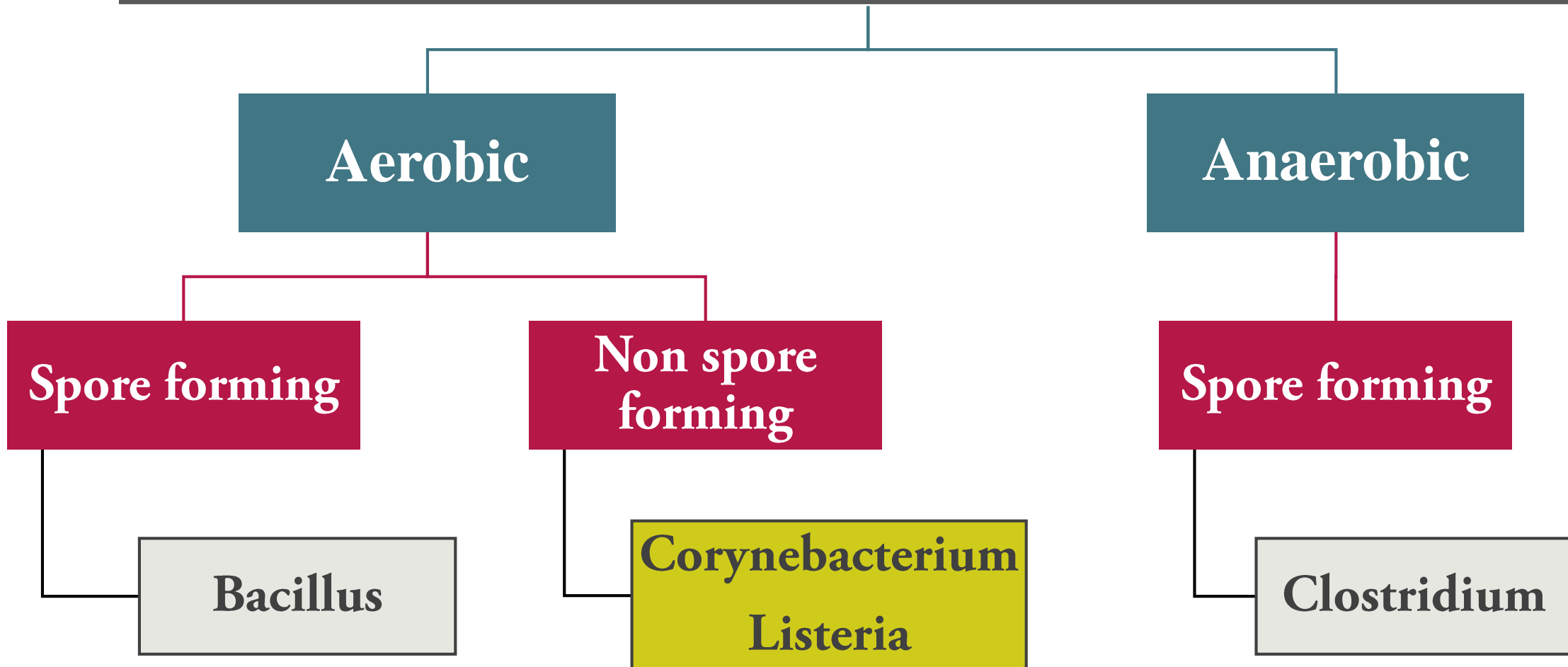
- Activity of amylase is indicated by a clear zone around the growth while the rest of the plate gives blue color after addition of iodine solution

Starch + Iodine → Blue color

Glucose + Iodine → No reaction



Gram positive bacilli divided into two groups according to Oxygen requirements :



❖ General Characters of *Corynebacterium* sp. :

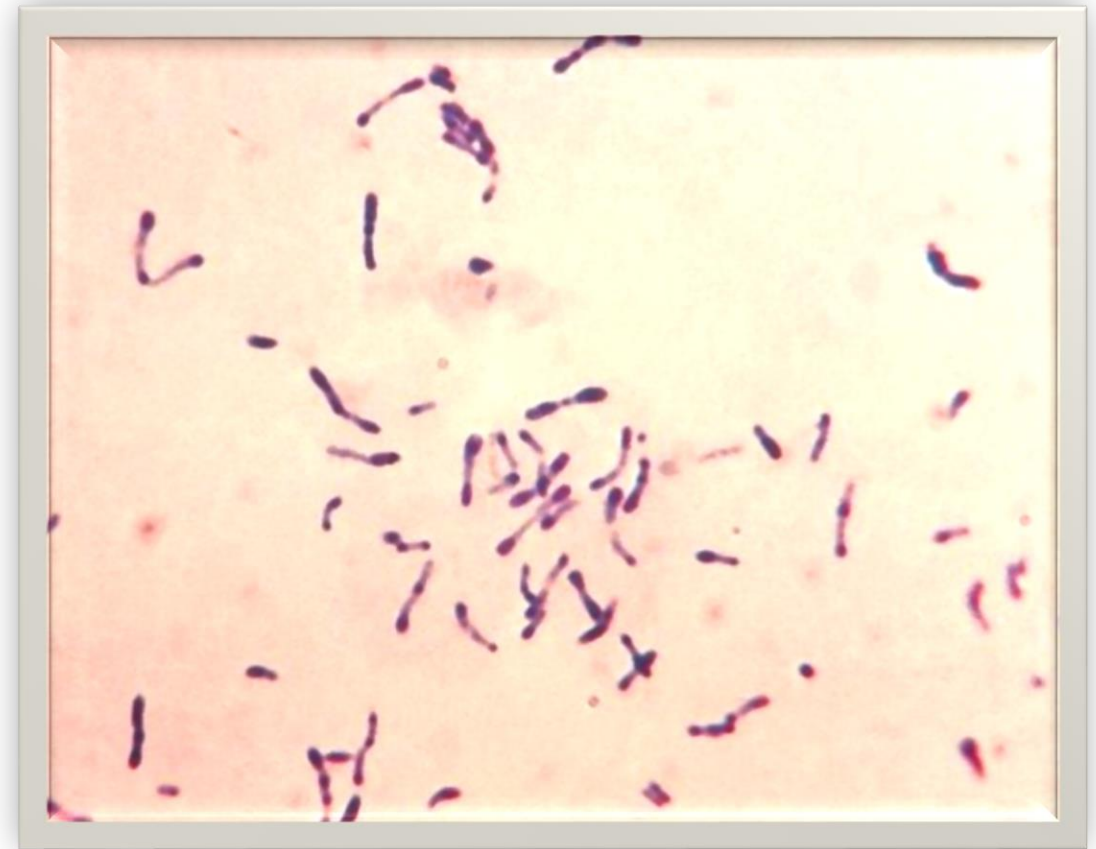
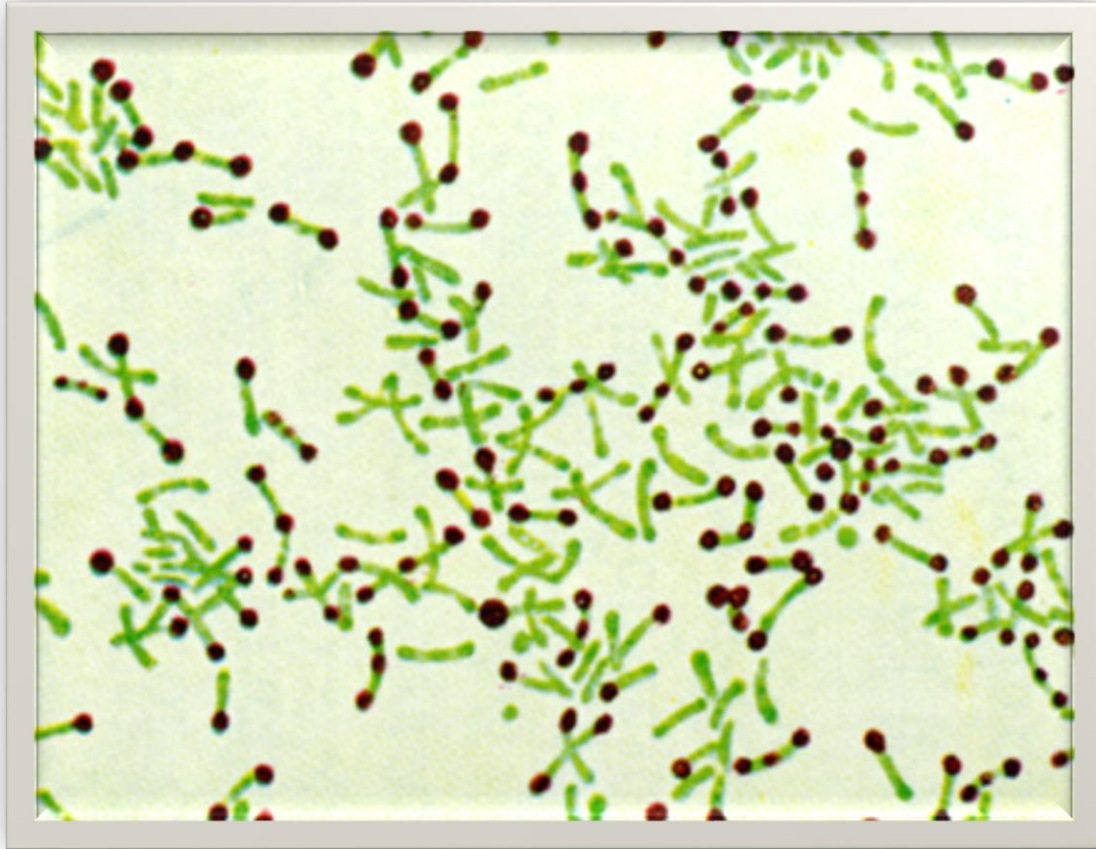
Gram positive bacilli, club shaped and beaded.

3 Non (non motile – non spore forming – non capsulated)

Facultative anaerobic.

Catalase positive but Oxidase negative.

C. diphtheriae is fastidious while diphtheroids are non-fastidious.



Species of *Corynebacterium*

Pathogenic

C. diphtheriae

is the only pathogenic members of this genus, it caused "Diphtheria"

Non pathogenic

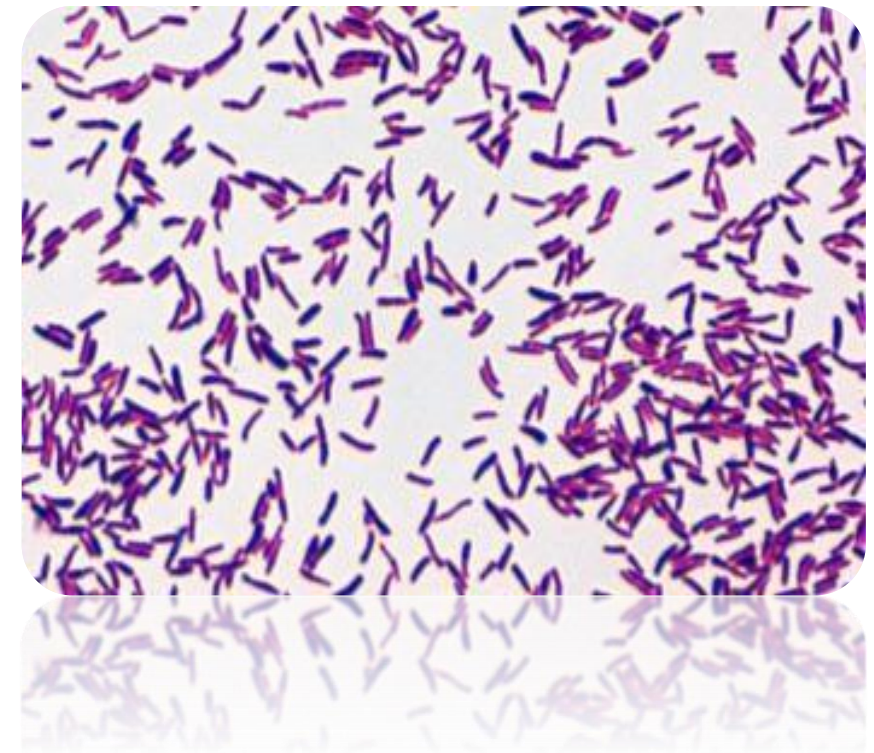
Commensal as "**Diphtheriods**"

C. hofmannii, *C. acne*

It's normal flora for RT, vagina and skin.

❖ Identification of *C. diphtheriae* :

- Specimen.
- Morphology :
 - Macroscopical (Cultural characteristics)
 - Microscopical (Gram stain)
- Detection of Exotoxin (*In vivo* and *In vitro*)



Diagnosis of Diphtheria

Clinical Diagnosis

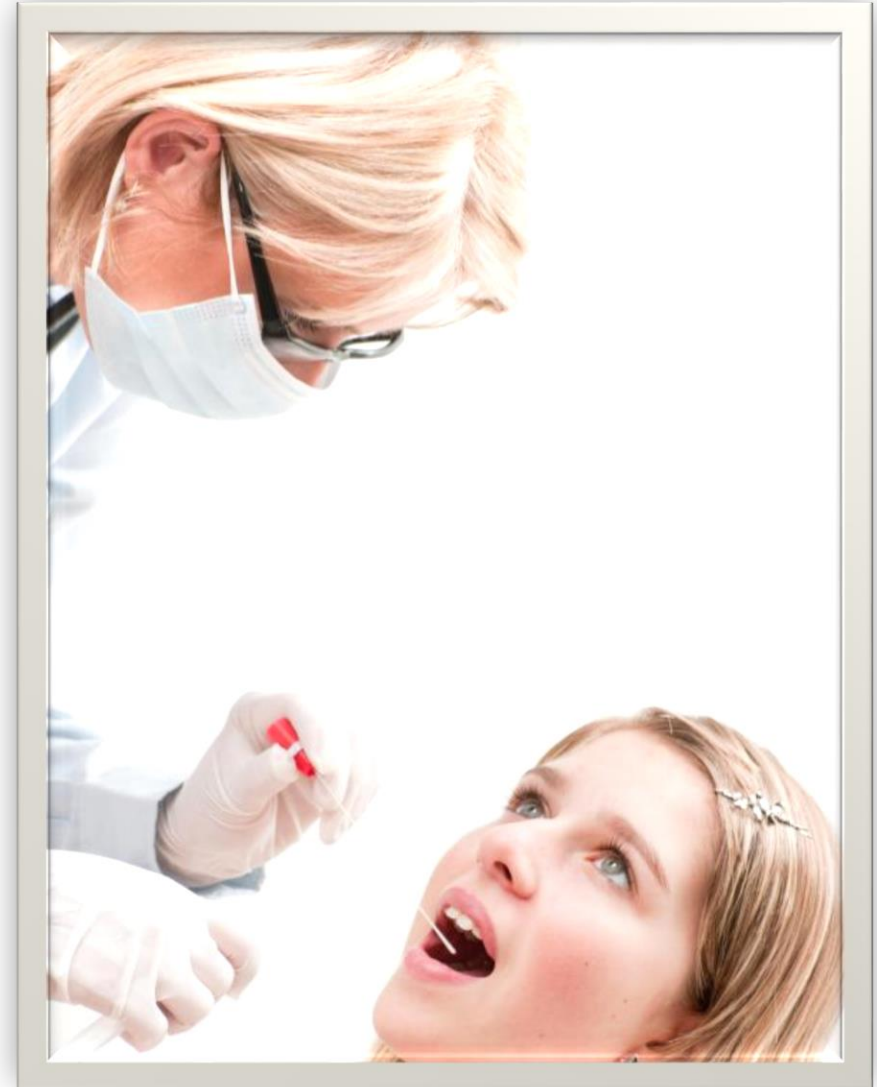
Specific treatment must be never delayed for laboratory results

Laboratory Diagnosis

To confirm the clinical manifestation

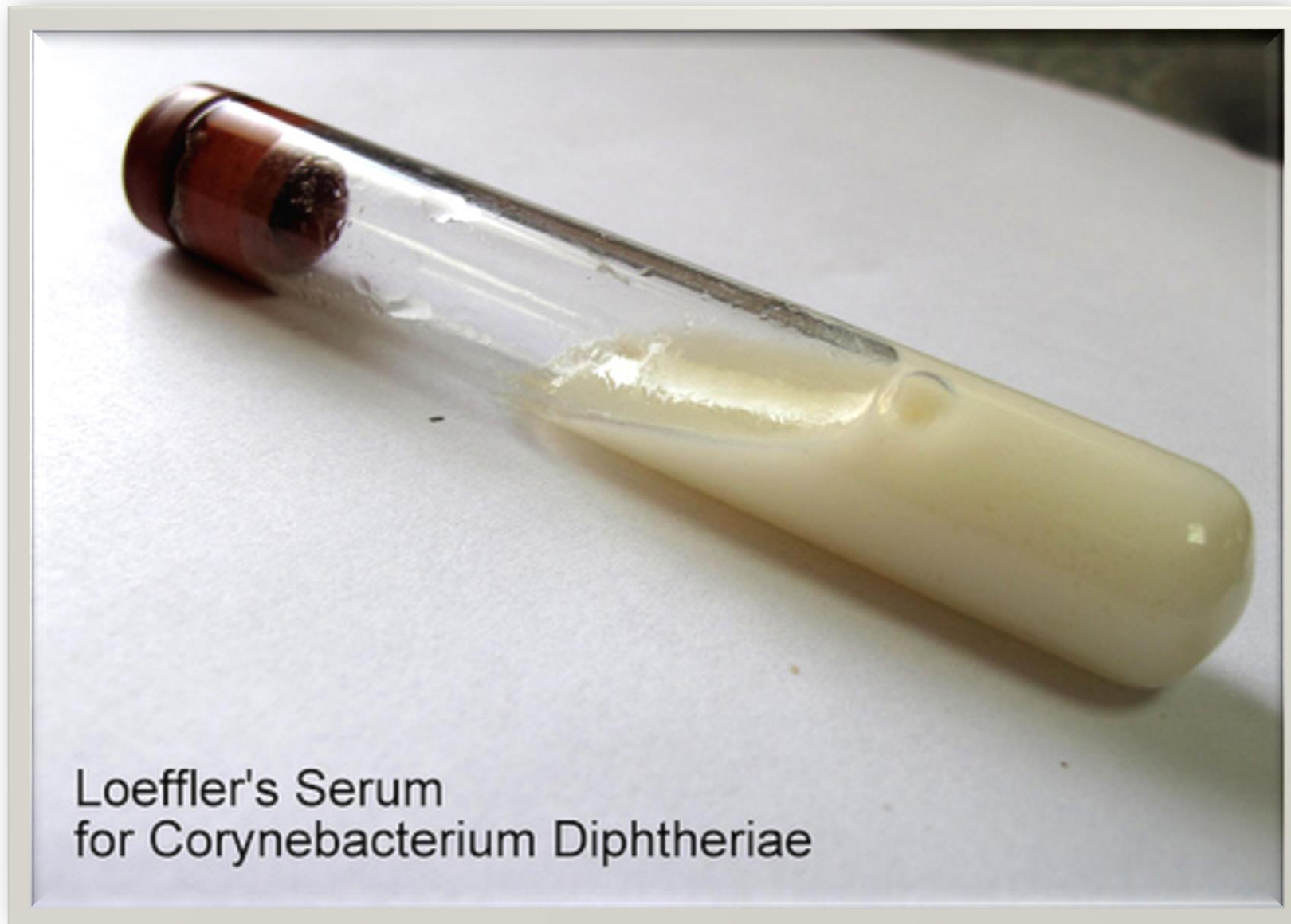
■ 1st : Specimen :

- A swap from nose, throat or other suspected lesions..



2nd : Morphology, Macroscopical

1	Loeffler's serum medium
Description	<ul style="list-style-type: none"> ▪ Corynebacteria grow much more readily than other respiratory pathogens. ▪ Used to enhance the characteristic microscopical appearance of corynebacterial. ▪ Very rapid test, colonies growth in 6 -8 Hrs.
Observation	<p>The colonies of <i>C. diphtheriae</i> are small, granular, grey, smooth and creamy with irregular edges.</p>



2nd : Morphology, Macroscopical

2

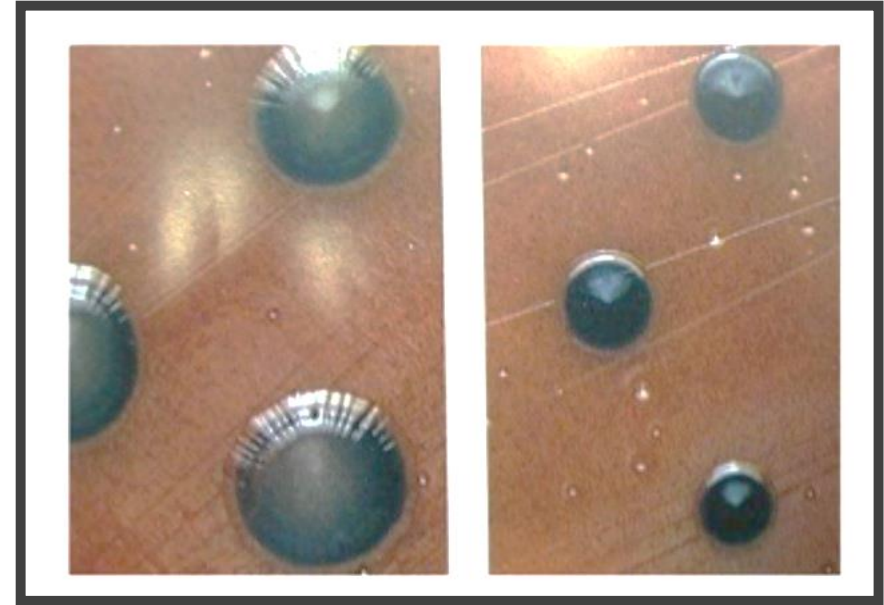
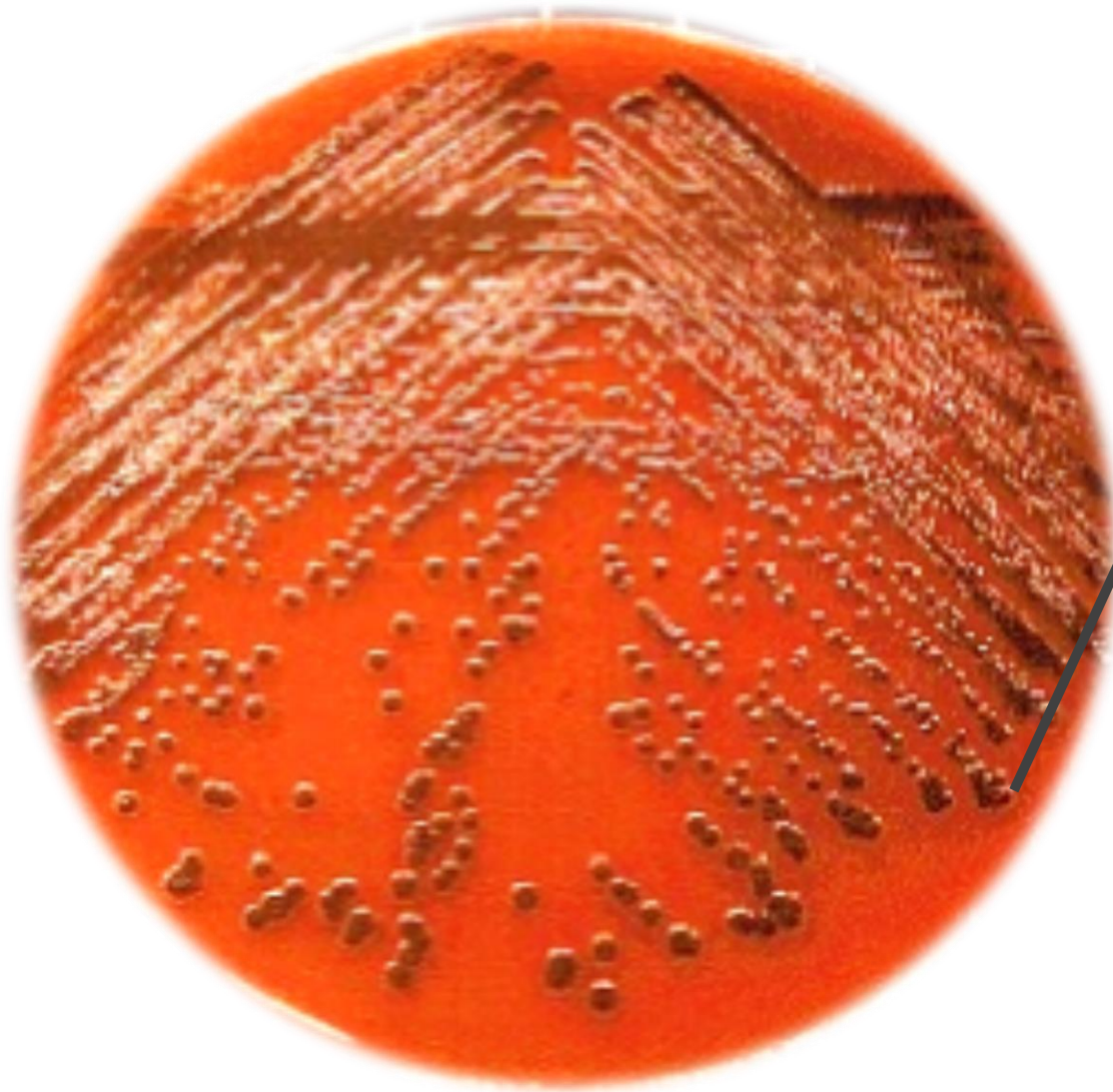
Tellurite blood agar (Mcloed's blood agar) Potassium tellurite

Description

- It is selective medium for isolation of *C. diphtheriae*
- Growth slowly, the colonies appears after 48 Hrs.

Observation

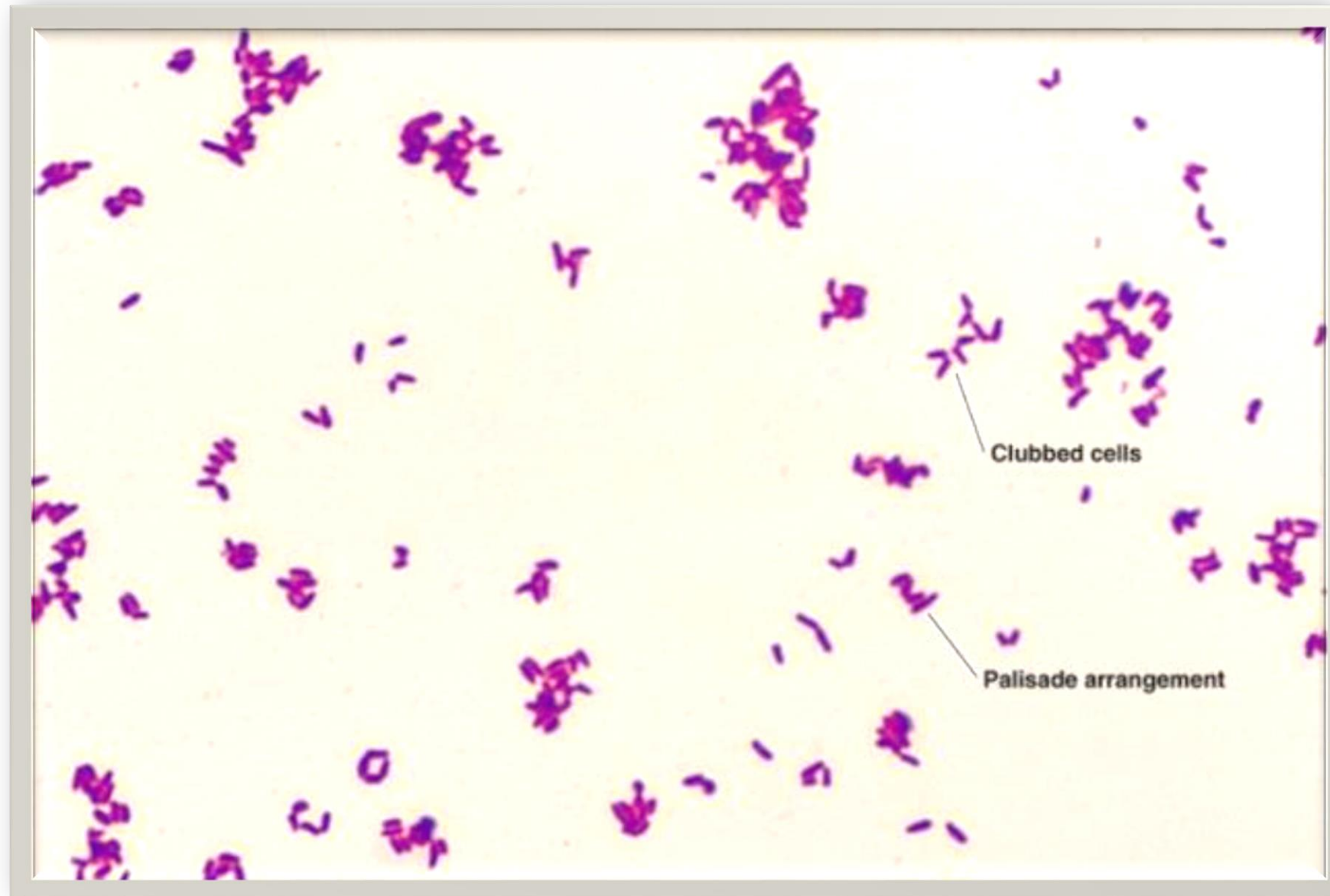
- On blood agar containing potassium tellurite, the colonies are grey to black with halo, because the tellurite is reduced intracellular.



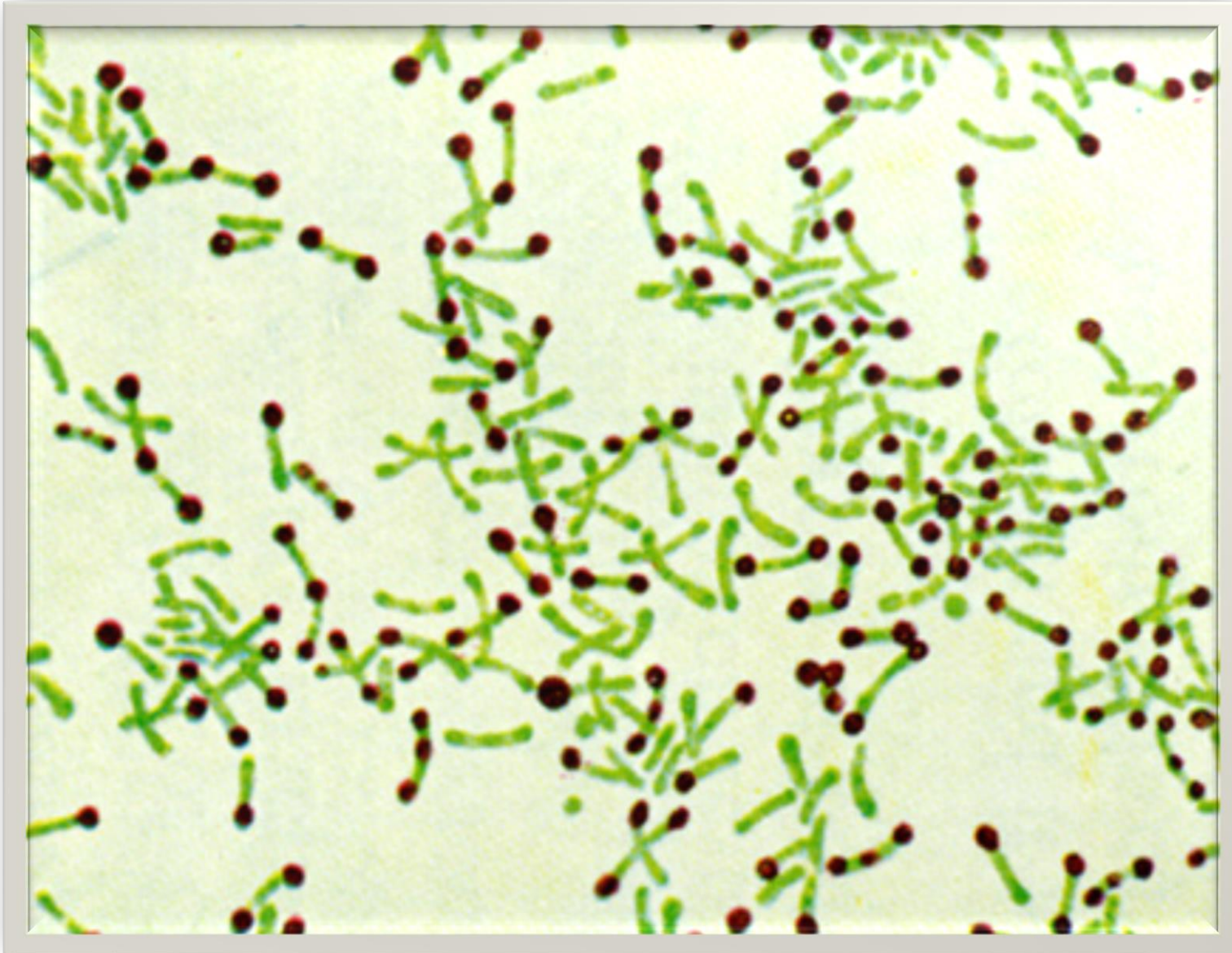
■ 2nd: Morphology, Microscopical :

- Gram stain

- Gram +ve bacilli, non spore forming and non motile
- Club shaped (Coryne= club) arranged at acute angles or parallel to each other (Chinese letters appearance).
- Beaded (metachromatic granules).



- **Polychrome methylene blue stain**
 - *C. diphtheriae* appears beaded due to the presence of intercellular “Metachromatic or volutin” granules.
 - Volutin granules staining by use Albert’s stain for 3 min after smear fixation, then wash it with Albert’s Iodine for 2 min.
 - The granules appear **red** while the rest of organism appears **blue**.



Isolation of organisms

- Diagnosis from Suspect Carrier

Collect specimen

- Swap from throat or nose

Inoculation on media, for 24 Hrs in 37°C

- On:
 - Loeffler's media
 - Blood tellurite agar

Test for toxigenicity

Detection of exotoxin

Two guinea pigs are used

In vivo

In vitro

1. As test

2. As control

Injected with diphtheria antitoxin

Both injected with Isolated bacteria

If both live

Diphtheroid or non-toxigenic

If control live and test die

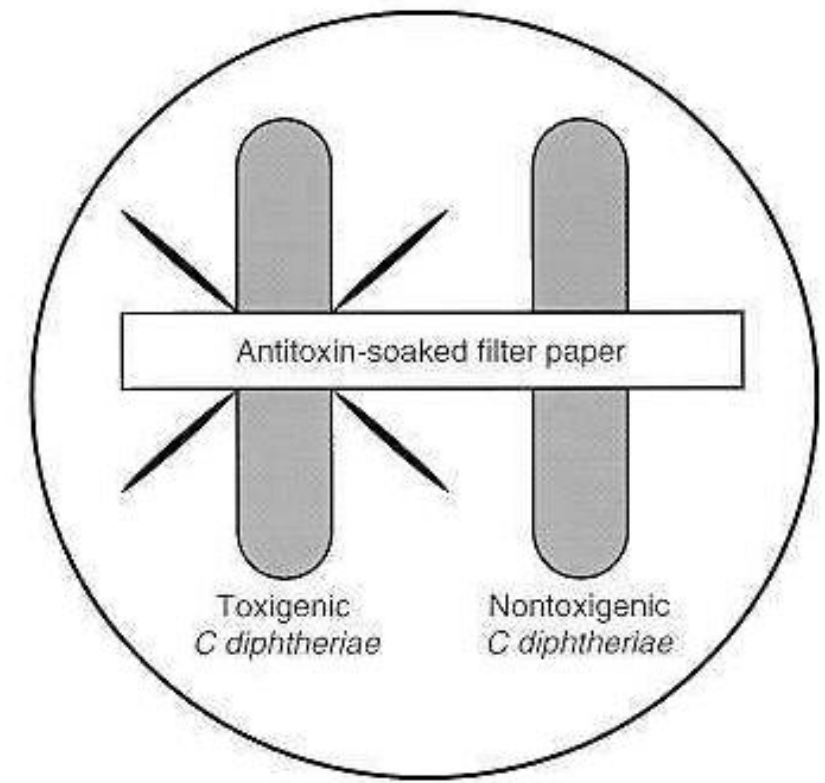
C. diphtheria



❖ 2nd : *In vitro* (Elek's Test)

• Principle :

- It is toxin/antitoxin reaction.
- Toxin production by *C.diphtheriae* can be demonstrated by a precipitation between exotoxin and diphtheria antitoxin.



Method and Results

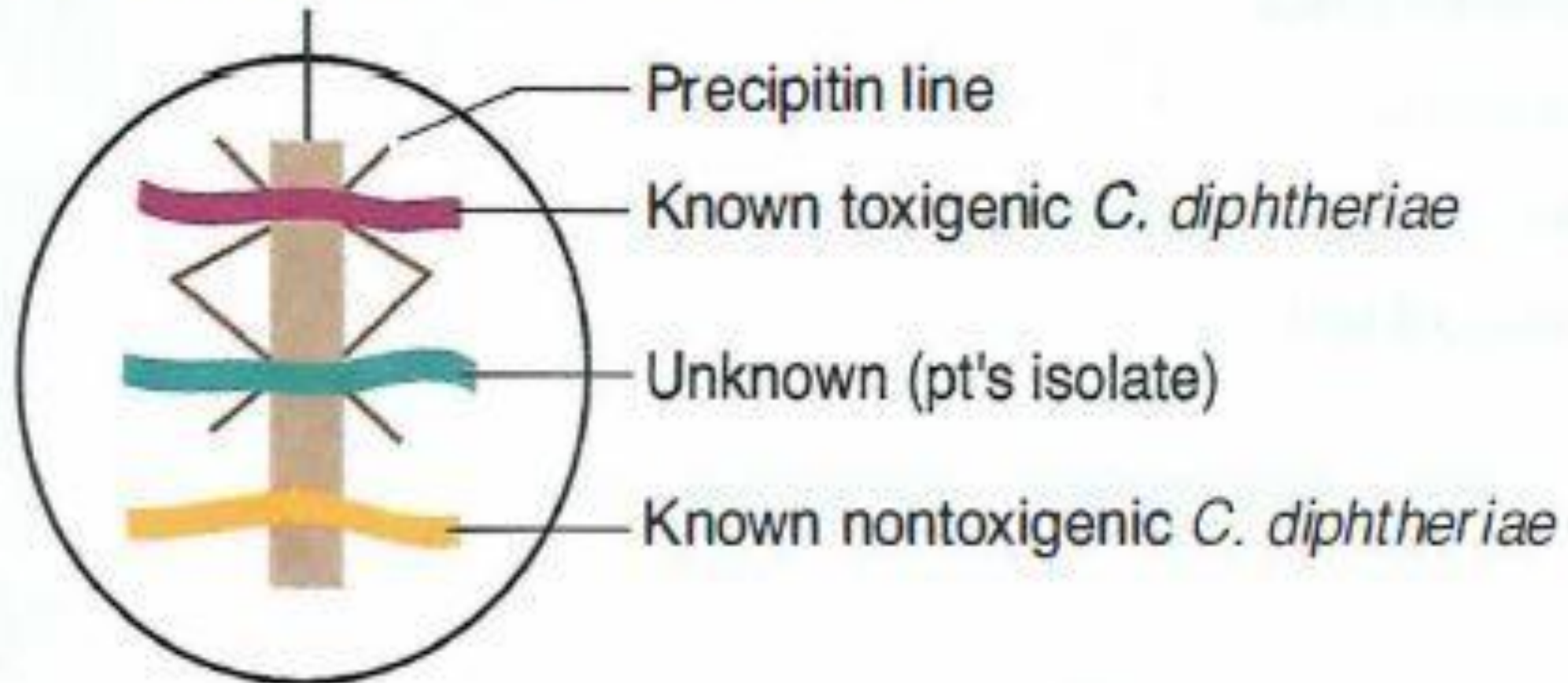
Procedure

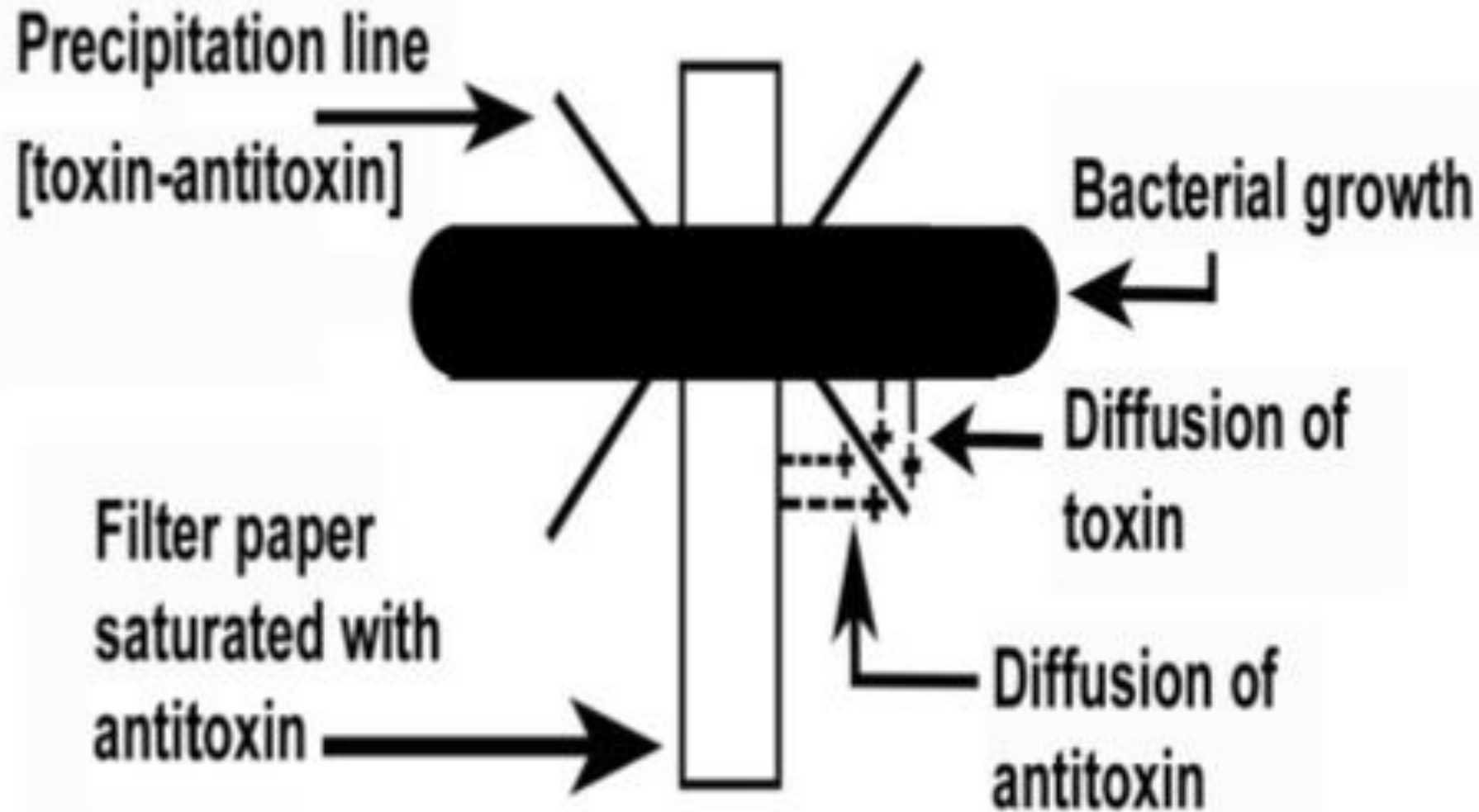
- A strip of filter paper impregnated with diphtheria antitoxin is placed on the surface of serum agar.
- The test organism is streaked at right angles to the filter paper.
- Incubate the plate at 37 °C for 24 hrs.

Result

- The antitoxin diffusing from filter paper strip and the toxigenic strains produce exotoxin, which diffuses and resulted in lines four precipitation lines radiating from intersection of the strip and the growth of organism.

Filter paper strip with *C. diphtheriae* antitoxin







Any Questions

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