460 MBIO
Medical Bacteriology

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2017

Lab 2
Gram Positive Group
(Cocci – Streptococci)

❖ General Characters of Streptococci :

- Arrange in chains or pairs, 1 µm in diameter.
- Non motile and non spore forming.
- Usually capsulated.
- Facultative anaerobic.
- Fastidious.

Streptococci can be classified according to :

▪ **Oxygen requirements**	▪ Anaerobic → Peptostreptococcus ▪ Aerobic or facultative anaerobic → Streptococcus
▪ **Serology**	Lancefield classification
▪ **Hemolysis on blood agar**	Divided into three groups (β, α and γ)

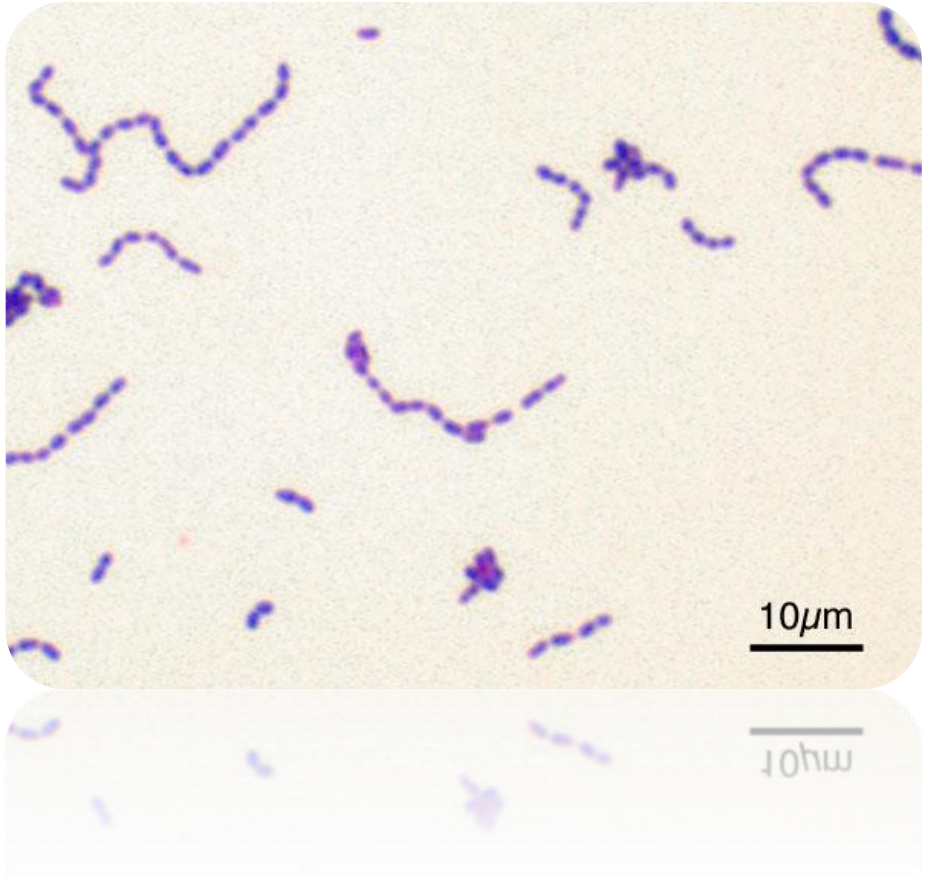
❖ Laboratory diagnosis of *Streptococcus* sp. :

- **Gram Stain :**

  Gram positive cocci arranged in chain or pairs.

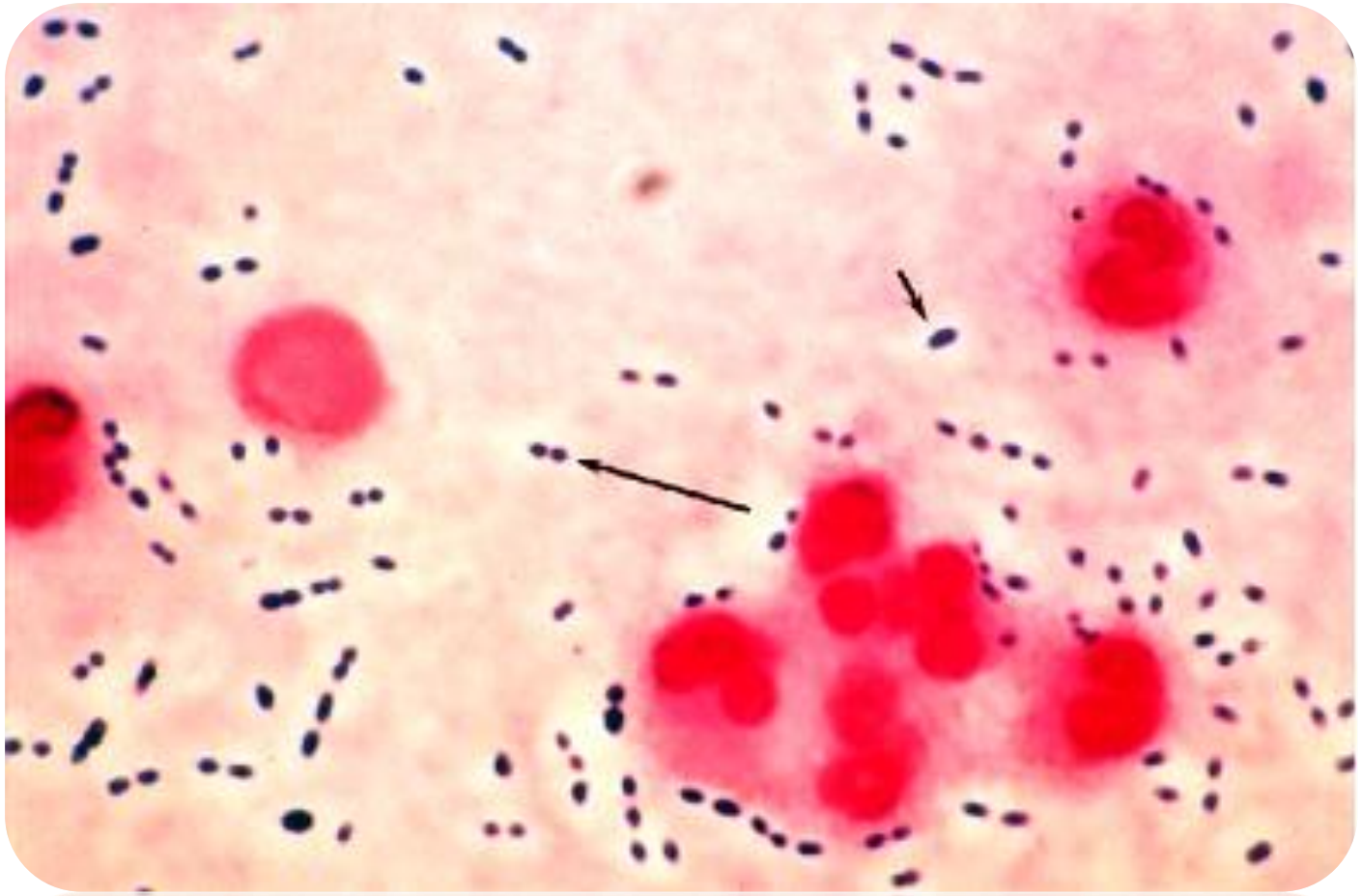
- **Microscopical Examination:**

  Transparent pin point colonies.



Streptococcus pneumonia

(Pairs)



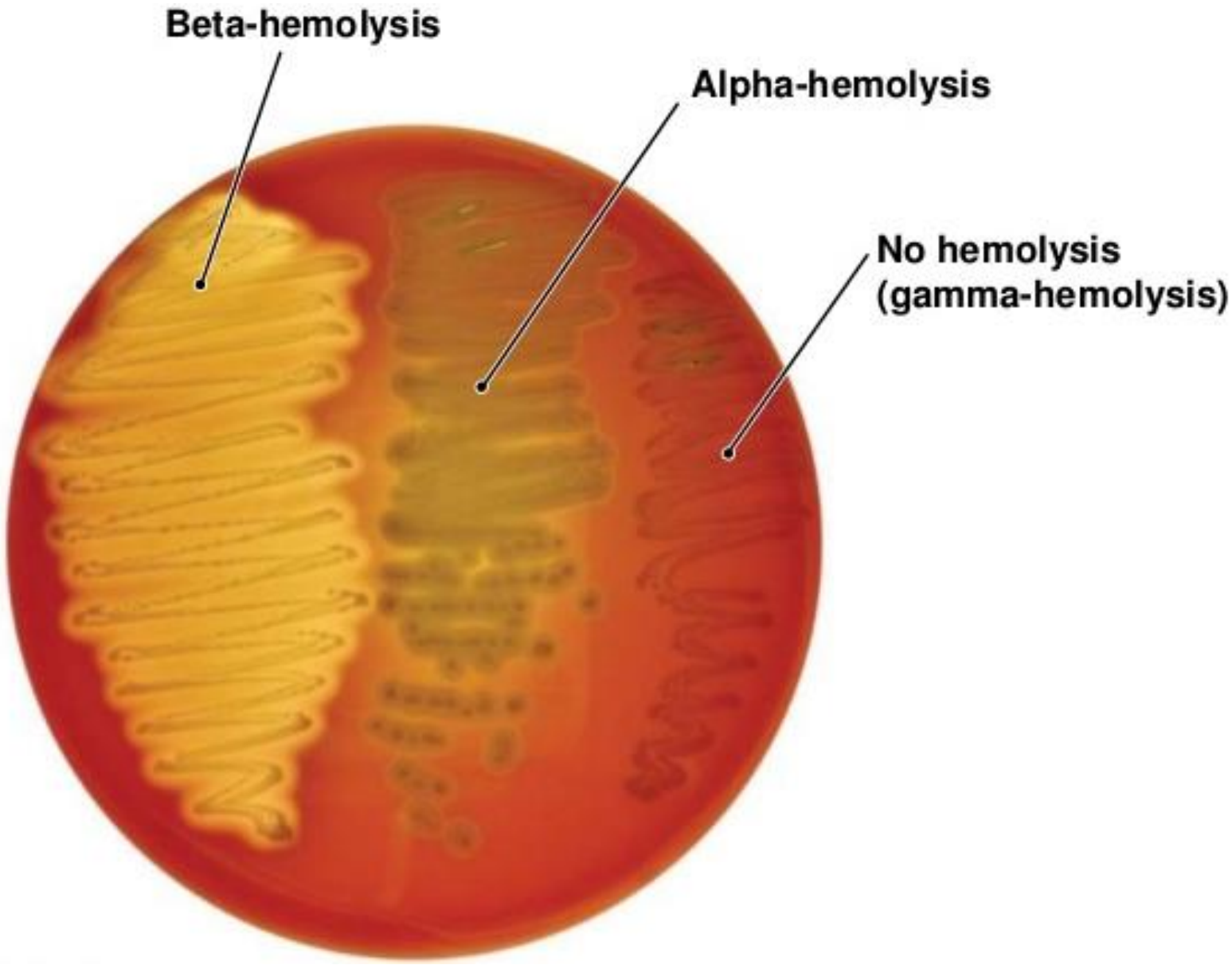
❖ Growth on Blood Agar :

- Streptococci are divided into <u>three</u> main groups according to it's action on erythrocytes :

1. **β**-hemolytic streptococci.
2. **α**-hemolytic streptococci.
3. **γ**- hemolytic streptococci.

Growth of Streptococci sp. on Blood Agar

	Description	Example
β	It causes complete hemolysis to RBCs leading to formation of clear zone around the colonies.	▪ *Strep. Pyogenes* (Group A) ▪ *Strep. agalactiae* (Group B)
α	It causes partial hemolysis by the act of enzyme leading a green discoloration around the colonies.	▪ *Strep. Pneumonia* ▪ *Strep. viridans*
γ	It has no effect on RBCs.	*Enterococcus faecalis*


Beta-hemolysis
Alpha-hemolysis
No hemolysis
(gamma-hemolysis)

Each group of streptococci has a unique test to differentiate between species

- **β-hemolytic**
  - Bacitracin sensitivity test
  - CAMP test
- **α-hemolytic**
  - Optochin sensitivity test
  - Bile solubility test
- **γ-hemolytic**
  - Growth on MacConkey's agar

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❖ First : β – hemolytic Streptococci

- There are two definitive test to differentiate between group A (*Strep. pyogenes*) and group B (*Strep. agalactiae*), which are :

1. **Bacitracin sensitivity test.**
2. **CAMP test.**

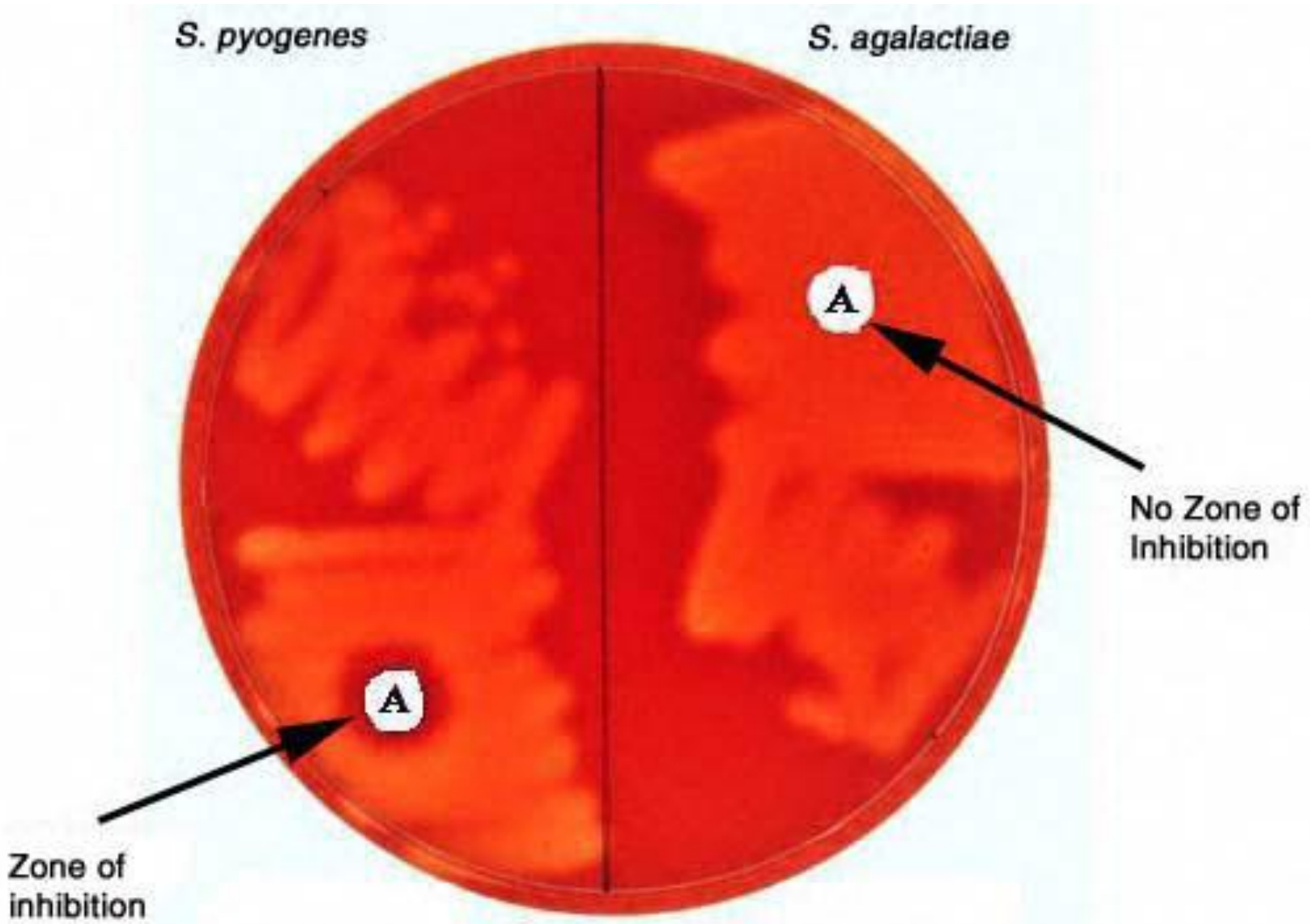
❖ 1st : Bacitracin Sensitivity Test :

- **Principle :**
  - A low concentration of Bacitracin (0.04 units) will selectively inhibit the growth of *Strep. pyogenes* giving a zone of inhibition around the disc.

beta-hemolysis
Streptococcus pyogenes

Method and Results

Procedure	▪ Inoculate blood agar plate with the test organism. ▪ Aseptically apply Bacitracin disc onto the center of the streaked area. ▪ Incubate the plate at 35℃ for 18 hrs.
Result	▪ Any zone of inhibition around the antibiotic disc considered as a positive result (sensitive). ▪ Positive → *Strep. pyogenes* (sensitive) – Group A ▪ Negative → *Strep. agalactiae* (resistance) – Group B



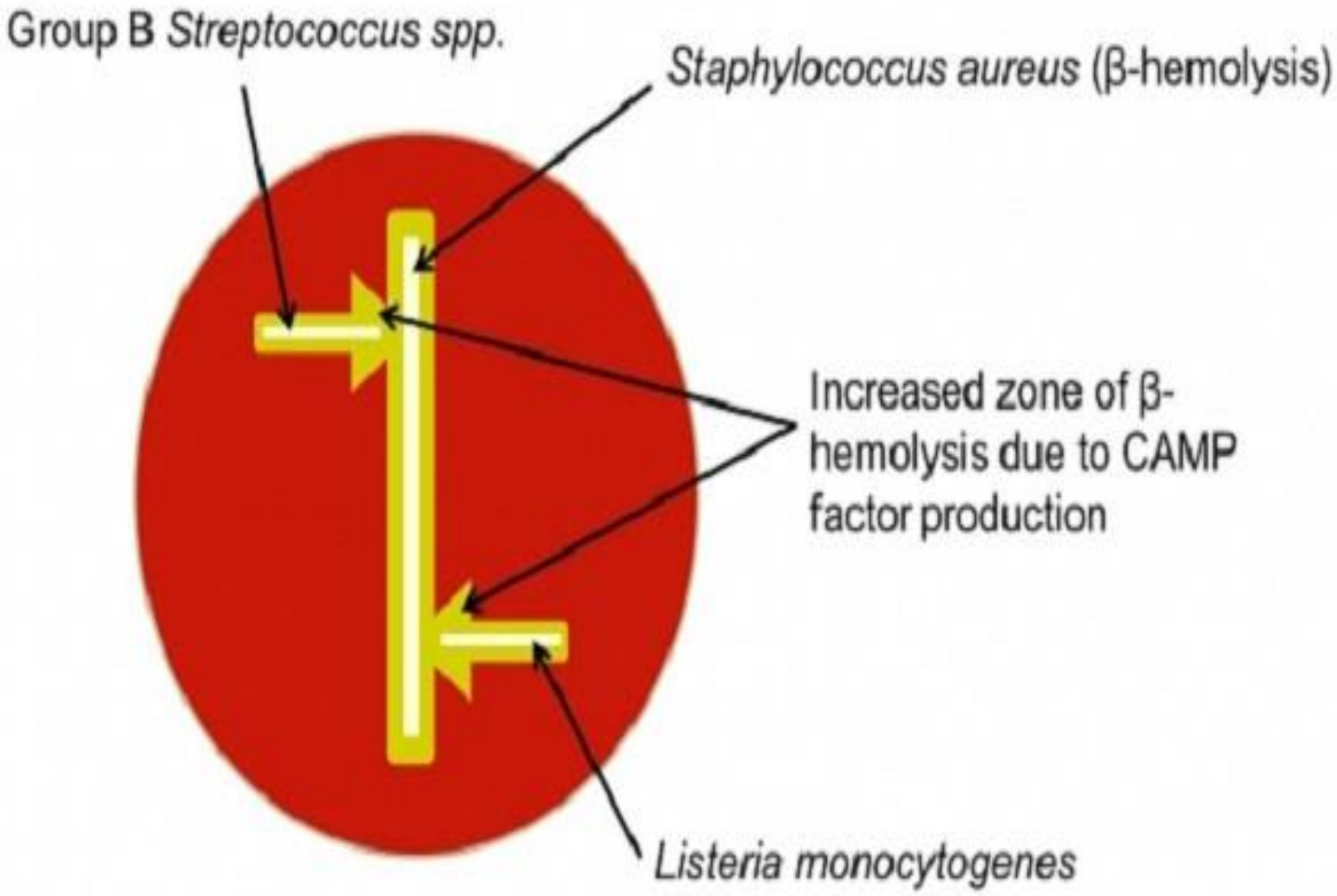
5% Sheep Blood Agar Plate

❖ 2nd : CAMP test :

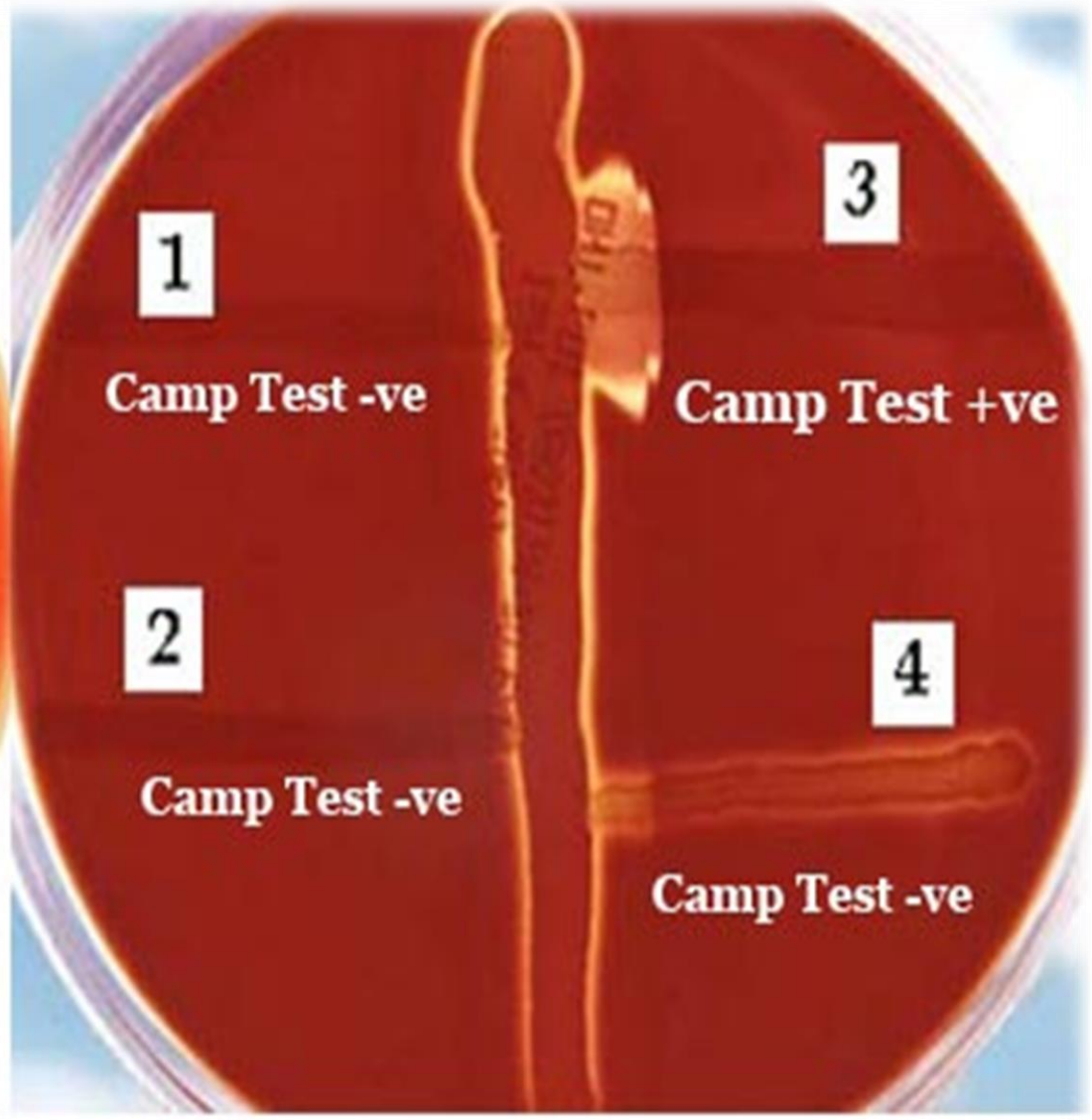
- **Principle :**
  - Group B streptococci produce extracellular protein (**CAMP** factor)
  - CAMP act synergistically with *Staph. aureus* β-lysin to cause extremely lysis of RBCs.

Method and Results

Procedure	▪ Single streak of *Streptococcus* to be tested and a *Staph. aureus* are made perpendicular to each other. ▪ 3 – 5 mm distance was left between two streaks. ▪ After incubation, a positive result appear as an arrowhead shaped zone of complete hemolysis.
Result	▪ Positive → *Strep. agalactiae* ▪ Negative → *Strep. pyogenes*


Group B *Streptococcus spp.*
Staphylococcus aureus (β-hemolysis)
Increased zone of β-hemolysis due to CAMP factor production
Listeria monocytogenes

Camp Test +ve
Camp Test -ve


1
Camp Test -ve
3
Camp Test +ve
2
Camp Test -ve
4
Camp Test -ve

Each group of streptococci has a unique test to differentiate between species

β-hemolytic

- Bacitracin sensitivity test
- CAMP test

α-hemolytic

- Optochin sensitivity test
- Bile solubility test

γ-hemolytic

- Growth on MacConkey's agar

❖ Second : α – hemolytic Streptococci

- There are two definitive test to differentiate between *Strep. pneumonia* and *Strep. viridans*, which are :

1. **Optochin sensitivity test.**
2. **Bile solubility test.**

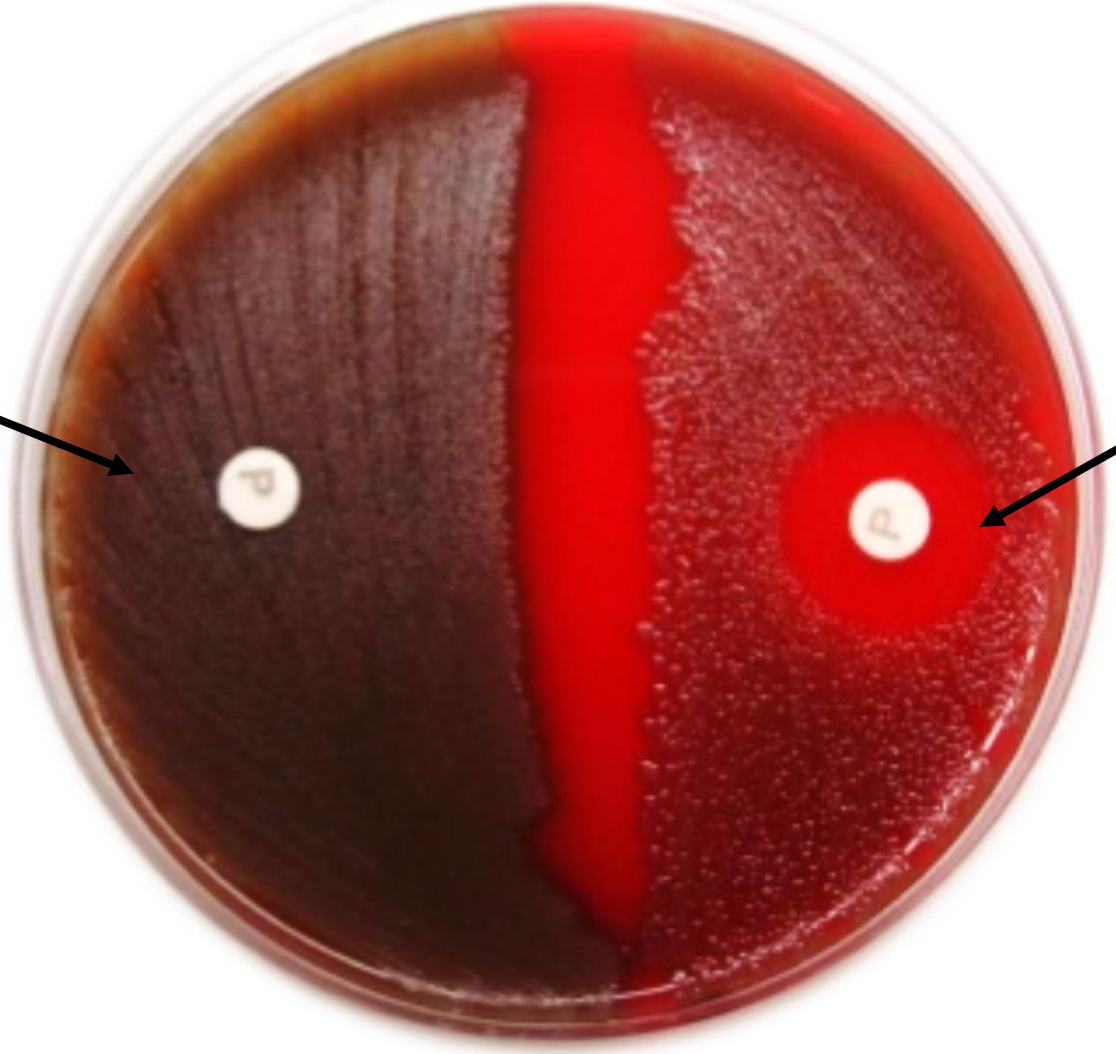
❖ 1st : Optochin Sensitivity Test :

- **Principle :**
  - A low concentration of Optochin (less than 5 µg/ml) will inhibit the growth of *Strep. pneumonia* giving a zone of inhibition around the disc.

Method and Results

Procedure	▪ Inoculate blood agar plate with the test organism. ▪ Aseptically apply Optochin disc onto the center of the streaked area. ▪ Incubate the plate at 37℃ for 18 hrs. ▪ Accurately measure the diameter of the inhibition zone around the disc.
Result	▪ The positive result should have more than 15 mm in diameter of zone inhibition, less than 15 mm considering as negative result. ▪ Positive → *Strep. pneumonia* (sensitive) ▪ Negative → *Strep. viridans* (resistance)

Strep. viridans
(resistant)



Strep. pneumonia
(sensitive)

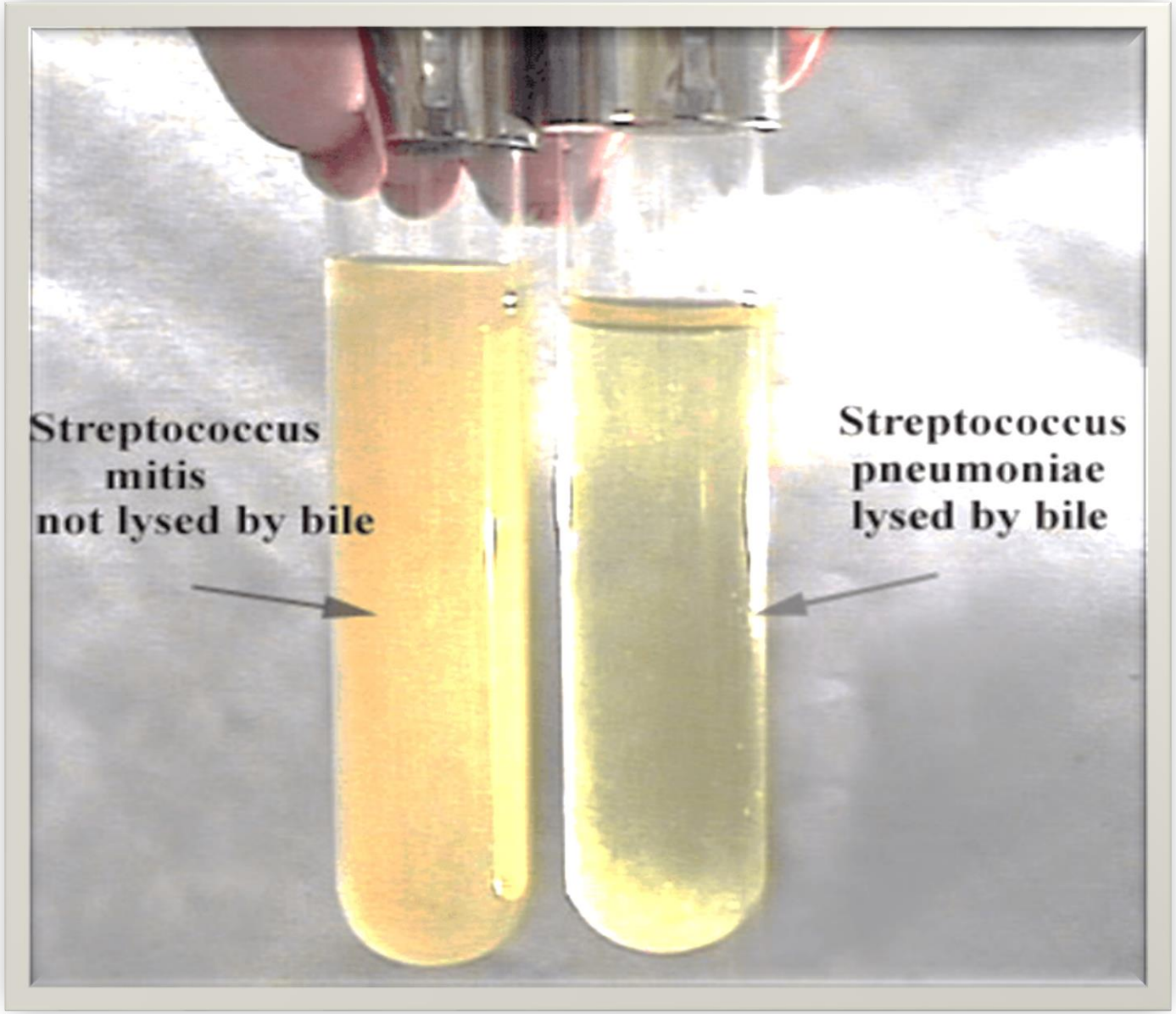
❖ 2nd : Bile Solubility Test :

- **Principle :**

▪ *Strep. pneumoniae* produce a self-lysing enzyme to depress the growth of old colonies. The presence of bile salt accelerate this process.

Method and Results

Procedure	▪ Add 10 ml of sample cultured in broth. ▪ Add 1 ml of 10% bile salt solution. ▪ Incubate at 37℃ for 15 minutes. ▪ Observe the visible clearing of the turbid culture.
Result	▪ Visible clearance of the turbid culture considered as positive result. ▪ Positive → *Strep. pneumonia* (visible clearance) ▪ Negative → *Strep. viridans* (remain turbid)


Streptococcus
mitis
not lysed by bile
Streptococcus
pneumoniae
lysed by bile

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- **β-hemolytic**
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  - CAMP test
- **α-hemolytic**
  - Optochin sensitivity test
  - Bile solubility test
- **γ-hemolytic**
  - Growth on MacConkey's agar

❖ 3rd :Growth on MacConkey's agar :

- **Principle :**

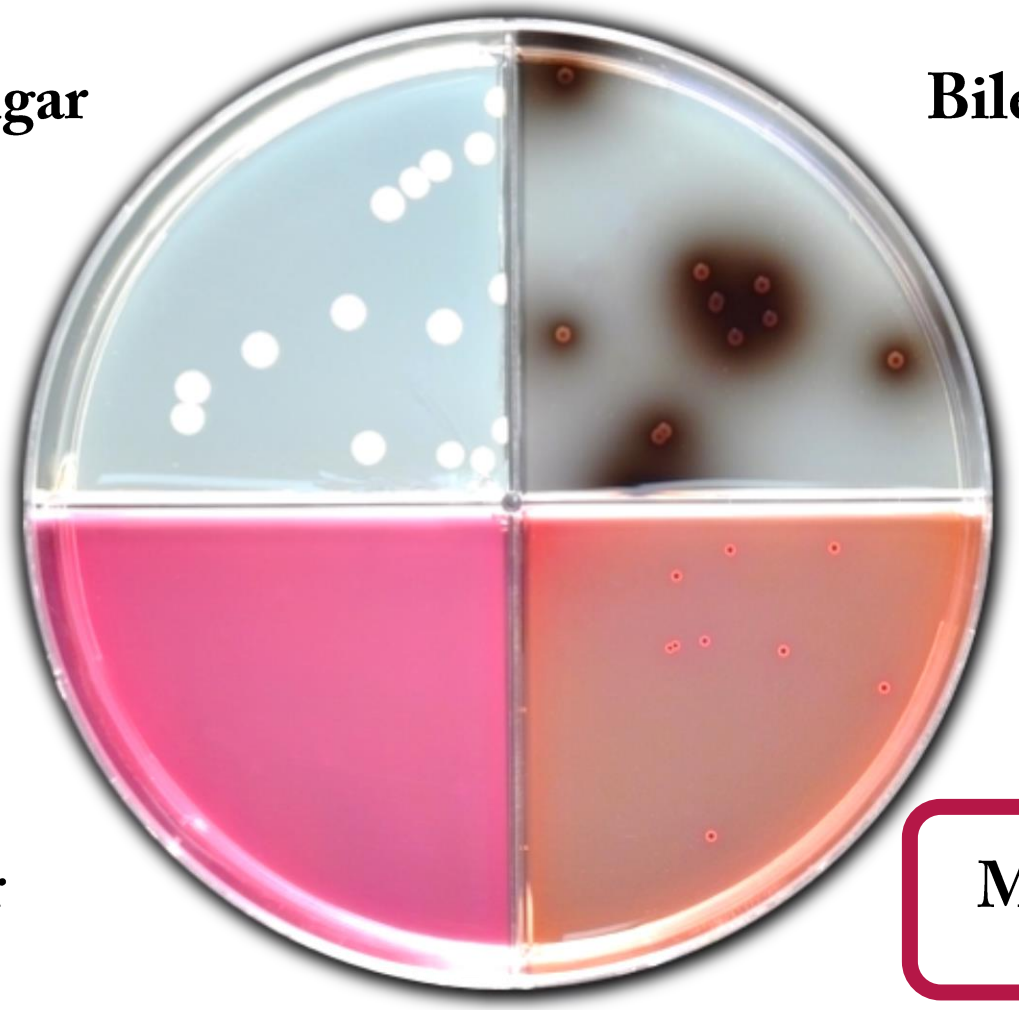
- MacConkey's agar is a selective medium for Gram's **–ve** bacteria.
- It contains **bile salt** and **crystal violet** to inhibit the growth of Gram's +ve bacteria.
- ***Enterococcus faecalis*** is the only Streptococci species which can grow on MacConkey's agar giving pink colonies.

- **Results :**

  - **Positive** → growth of pink colonies (*Enterococcus faecalis*)
  - **Negative** → No growth of bacteria (other stain of γ-hemolytic streptococci)

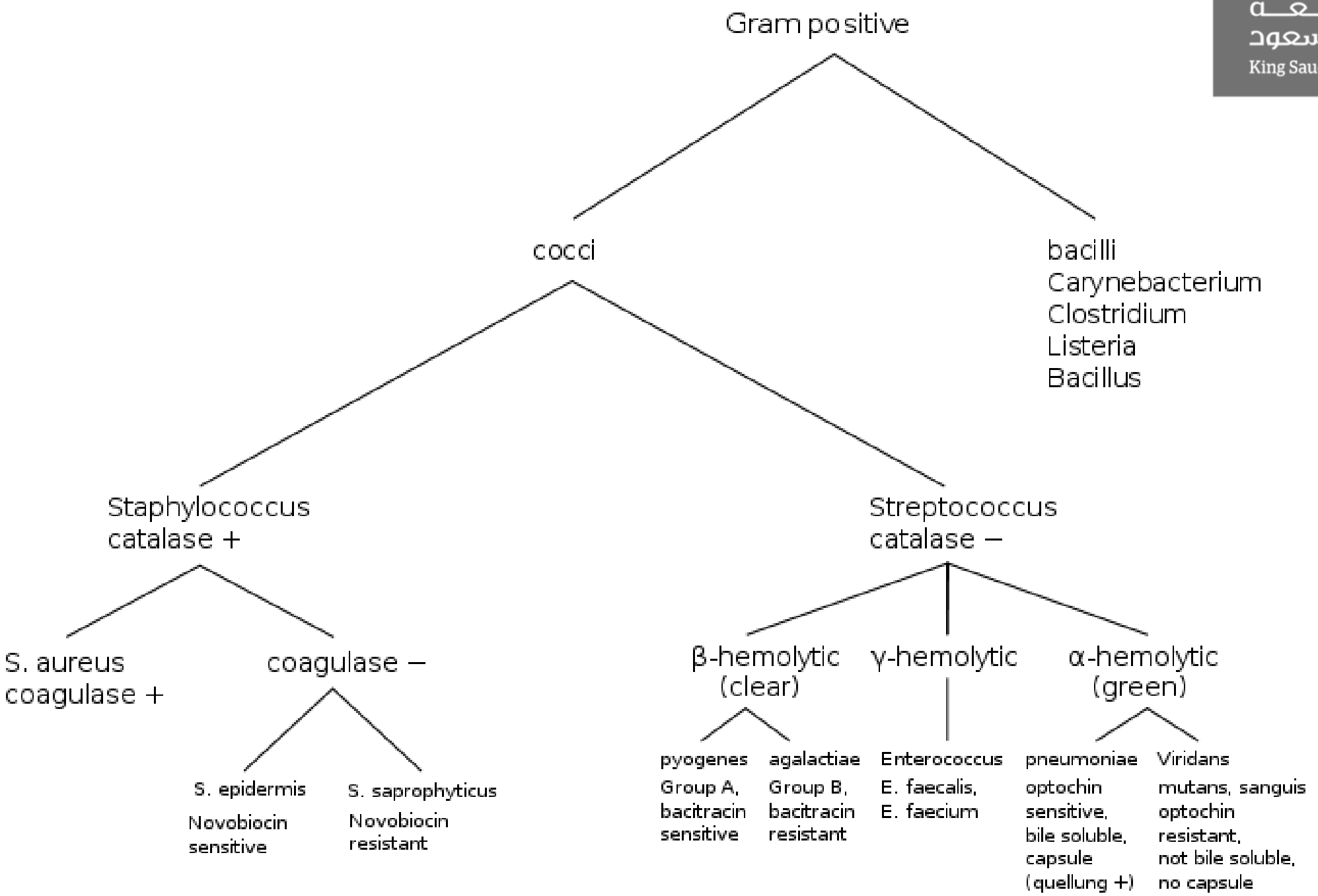
Tryptic Soy Agar

Bile Esculin Azid Agar



Endo Agar

MacConkey's Agar


Gram positive
cocci
bacilli
Carynebacterium
Clostridium
Listeria
Bacillus
Staphylococcus
catalase +
Streptococcus
catalase −
S. aureus
coagulase +
coagulase −
S. epidermis
Novobiocin
sensitive
S. saprophyticus
Novobiocin
resistant
β-hemolytic
(clear)
γ-hemolytic
α-hemolytic
(green)
pyogenes
Group A,
bacitracin
sensitive
agalactiae
Group B,
bacitracin
resistant
Enterococcus
E. faecalis,
E. faecium
pneumoniae
optochin
sensitive,
bile soluble,
capsule
(quellung +)
Viridans
mutans, sanguis
optochin
resistant,
not bile soluble,
no capsule

Any Questions

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