# STAPHYLOCOCCI, STREPTOCOCCI

# PRACTICAL PART

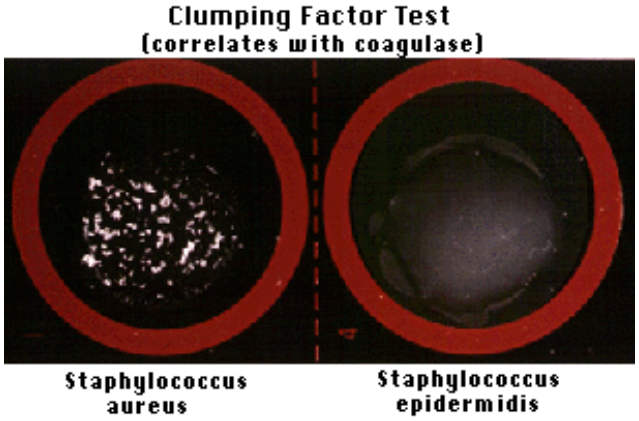
## 1. IDENTIFICATION OF STAPHYLOCOCCI

# Free plasmacoagulase

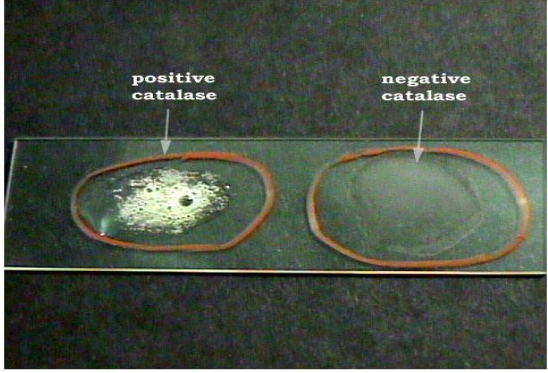
- **Principle:** plasmacoagulase enzyme converts plasma soluble fibrinogen into insoluble fibrin
- **Tools:** test-tube with 0.5 mL of rabbit plasma, fresh staphylococcal culture, loop
- **Procedure:** Inoculate several staphylococcal colonies into the test tube with plasma, incubate 24 hours at 37°C
- **Evaluation:** Observe clot (coagulation) formation after 1 and 24 hours

# Bound coagulase

- **Principle:** bound coagulase (Clumping factor) is part of the surface of coagulase-positive staphylococci – It leads to clumping of staphylococci and their protection against phagocytosis
- **Tools:** staphylococcal culture, plasma, microscopic slide, loops
- **Procedure:** drop rabbit plasma on the slide, add staphylococcal culture. Mix it properly.
- **Evaluation:** observe agglutination in case of *S. aureus*



# Catalase test



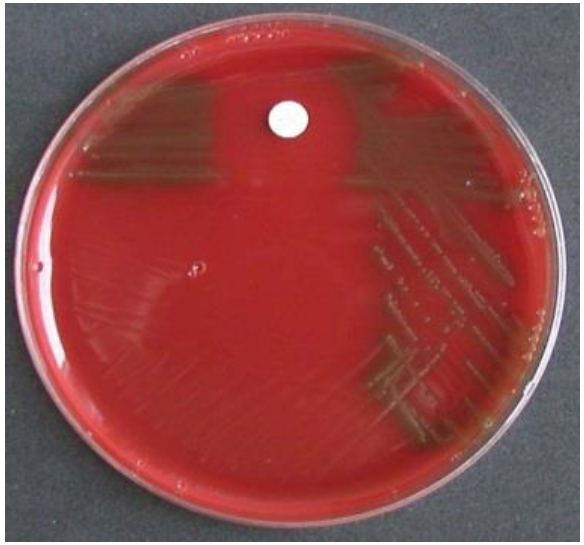

- **Principle:** using for differentiation between staphylococci from streptococci
- Staphylococci produce catalase enzyme (disintegration of hydrogen peroxide – bubbles formation). Typical feature of aerobic or facultative anaerobic bacteria.
- **Tools:** culture of staphylococci and streptococci, hydrogen peroxide, microscopic slide, loops, disinfection
- **Procedure:** Add few collonies of bacterial culture on the slide than drop hydrogen peroxide onto culture. Observe bubbles formation.
- **Evaluation:** observe positivity (bubbles formation) in staphylococci, negativity in streptococci

PRACTICAL PART

# 2. IDENTIFICATION OF STREPTOCOCCI

# Optochin test

- **Principle:** *Streptococcus pneumoniae* is differentiated from other viridans streptococci on the basis of its susceptibility to antibiotic - optochin (Ethyl Hydrocuprein)



# Optochin test – practice

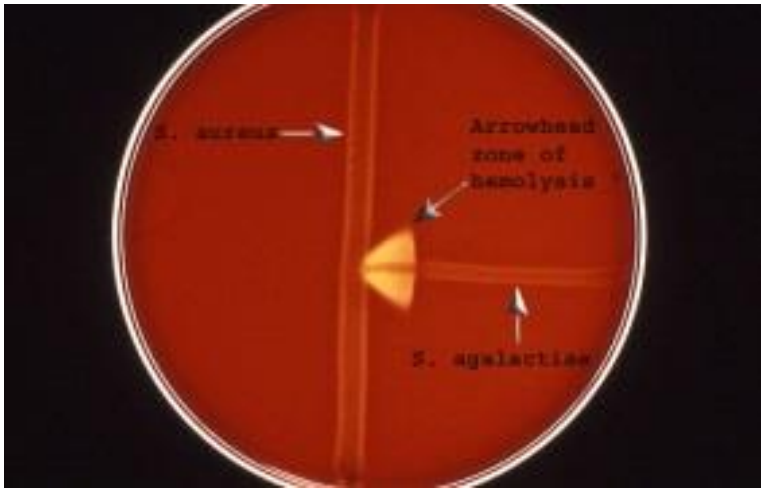
- **Tools:** *Streptococcus pneumoniae* and viridans streptococci cultures, blood agar, optochin discs, loops, markers, disinfection
- **Procedure:** inoculate several colonies of *Str. pneumoniae* on the first half of blood agar, viridans streptococci on the second half. Cultures of both strains must not overlapped!!!
- Put optochin disc on each strains and incubate 18-24 hours at 37°C, in 5% $CO_2$
- **Evaluation:** measure the zone of inhibition, in case of more than 14 mm report as susceptible

PRACTICAL PART

# 3. IDENTIFICATION OF BETA-HEMOLYTIC STREPTOCOCCI – CAMP TEST

# CAMP test

- **Principle:** group B streptococci (*Streptococcus agalactiae*) produce extracellular protein (CAMP factor), which is able potentiate beta-hemolysin of *Staphylococcus aureus*



# CAMP test – practice

- **Tools:** bacterial culture of tested strain, blood agar*, St. aureus* strain, marker, loops, disinfection
- **Procedure:** inoculate the line of tested streptococcal strain on blood agar and perpendicularly to this line inoculate the line of *St. aureus*
- Incubation 24 hours at 37°C, aerobically

PRACTICAL PART

# 4. DIFFERENTIATION OF ENTEROCOCCI FROM STREPTOCOCCI

# Bile - esculin test

**Principle**:

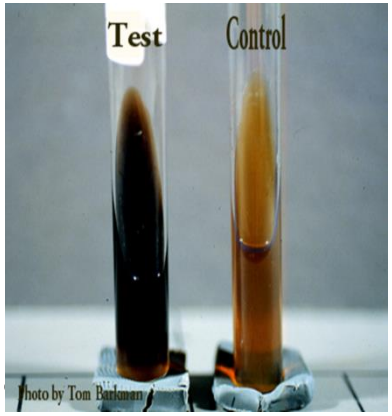
- Differentiation of enterococci from other streptococci using cultivation in highly selective-diagnostic bile – esculin medium
- Bile inhibits the growth of streptococci
- Enterococci hydrolyzes esculin and medium color turns black

# Bile - esculin test

**Tools:** bacterial culture of tested strain, bile-eskulin medium, marker, loops

**Procedure**:

- Pick tested bacterial culture using the loop and inoculate via performing the puncture through the medium to the bottom of tube.
- Incubation 24 hours at 37°C, aerobically




**Evaluation**:

Positive reaction – **blackening of medium**

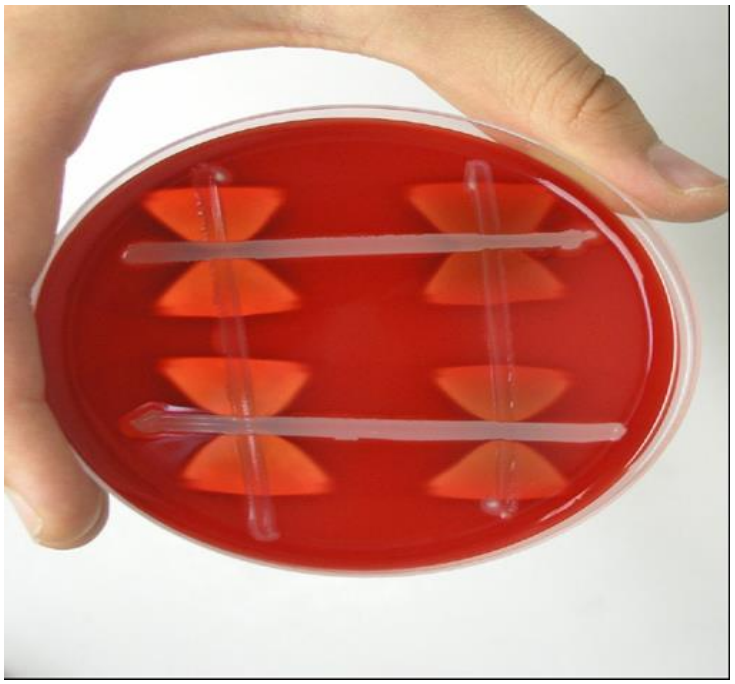
Negative reaction – color of medium stays unch

PRACTICAL PART

# 5. IDENTIFICATION OF STREPTOCOCCI – EVALUATION

# CAMP test

- **Evaluation:** positive test is shown as enhancement of beta-hemolysis of tested strain (butterfly wings like) in case of group B streptococci



# Optochin test

- **Evaluation:** Measure the zones of inhibition around antibiotics after the incubation. Notice the diameter and interpret result of identification according to optochin susceptibility

**Inhibition zone ≥ 14 mm**
**Report as susceptible**
***Streptococcus pneumoniae***

**Inhibition zone < 14 mm**
**Report as resistant**
**viridans streptococci**

# PRACTICAL PART

## 6. IDENTIFICATION OF VIRIDANS STREPTOCOCCI – BILE SOLUBILITY TEST

# Confirmation of identification of *Streptococcus pneumoniae* – bile solubility test

**Principle:**

- Deoxycholate (bile) activates autolysin in cell wall of *Str. pneumoniae*
- *Str. pneumoniae* colonies are dissolved in presence of deoxycholate
- Difference – viridans streptococci colonies show no change in presence of deoxycholate (absence of autolysin in cell wall)

# Bile solubility test - practice

- **Tools**: *Str. pneumoniae* and viridans streptococci colonies, 10% solution of sodium deoxycholate
- **Procedure**: Drop deoxycholate on tested colony (colonies). After 15 min observe and report if colony was dissolved or not.
- **Evaluation**:

Dissolved colonies – report as *Str. pneumoniae*

Colonies are intact – viridans streptococci

PRACTICAL PART

# 7. LATEX AGGLUTINATION OF BETA-HEMOLYTIC STREPTOCOCCI

# Detection of group antigen of beta-hemolytic streptococci

**Principle:**

Typing of beta-hemolytic streptococci according to specific type of polysaccharide C in streptococcal cell wall (Lancefield antigen). Commercial sets – known types of antibodies (against different types of Lancefield antigens) bound on latex particles.

Antigen presence will appear as agglutination with specific antibody

**Tools:** Group specific antisera (antibodies on latex particles), cell wall extract of streptococcal strain, plates, droppers, sticks, disinfection

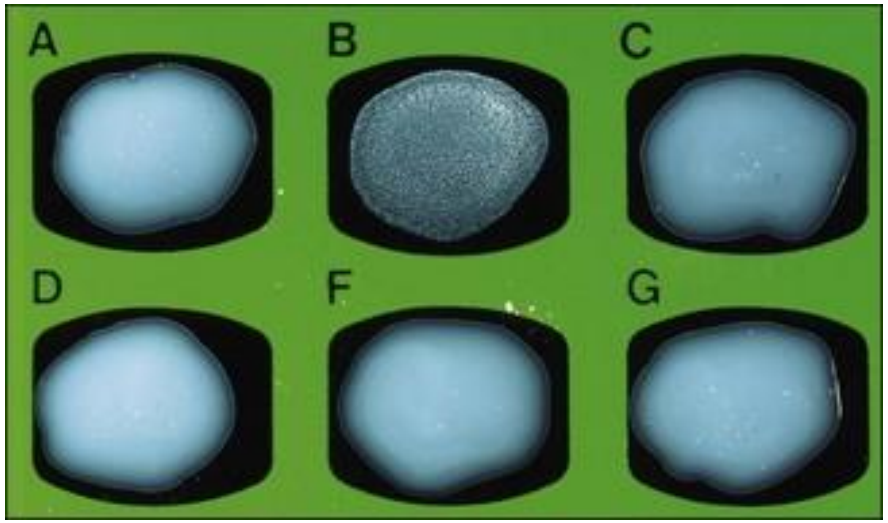
# Latex agglutination of beta-hemolytic streptococci

- **Procedure:** transfer one drop of extract (prepared from 5 colonies of tested strain in advance) to each of the five black circles labeled A, B, C, F, G.
- Shake the bottles with latex particles anti-A, B, C, F and G and add on drop close to the drop of extract according to the label. Mix the contents in each circle in turn with a stick and spread to cover area of the circle
- Rock the card gently for a maximum of 1 minute and read the reaction.

# Latex agglutination of streptococci

**Evaluation:**

•Observe the formation of agglutination during the rotation




•http://www.pro-lab.com/products-strep.php?country=DK

•https://www.youtube.com/watch?v=xicBT3FaSM0

# PRACTICAL PART

## 8. EVALUATION OF BILE-ESCULIN TEST

# Bile - esculin test

After 24 hours incubation at 37°C, aerobically evaluate the colour change o medium

**Evaluation**:

Positive reaction – **blackening of medium**

Negative reaction – color of medium stays unchanged