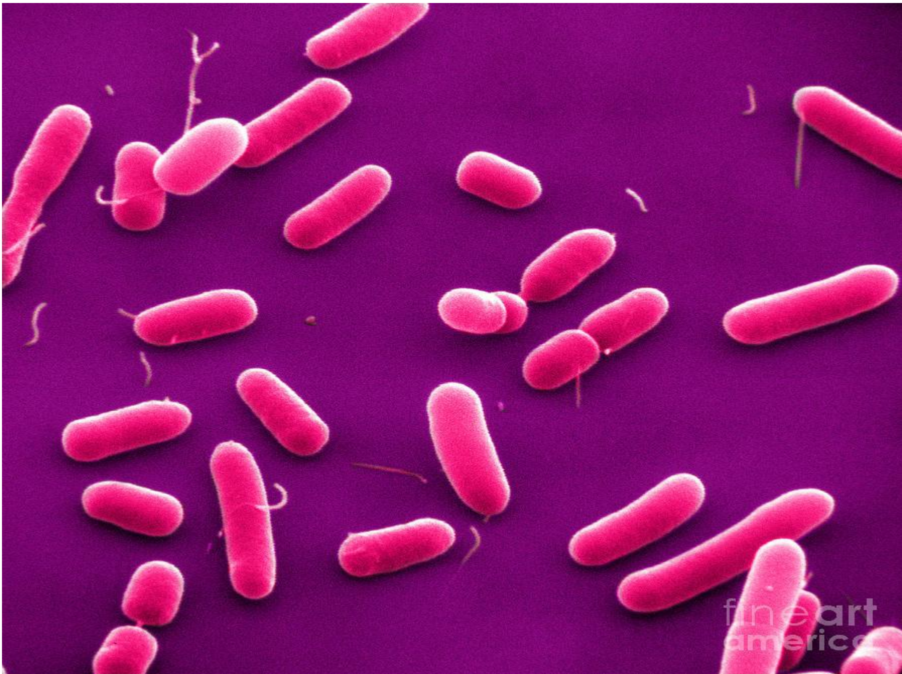
HAEMOPHILUS, NEISSERIA MYCOBACTERIA, CORYNEBACTERIA

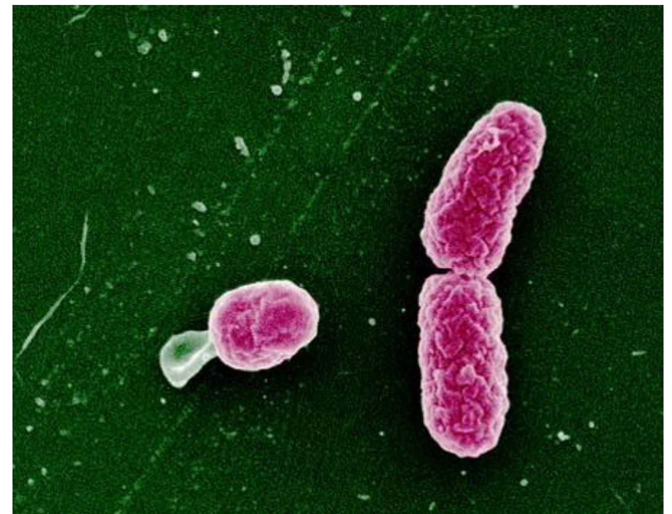
PRACTICAL PART

1. IDENTIFICATION OF HAEMOPHILUS

Haemophilus - Microscopy

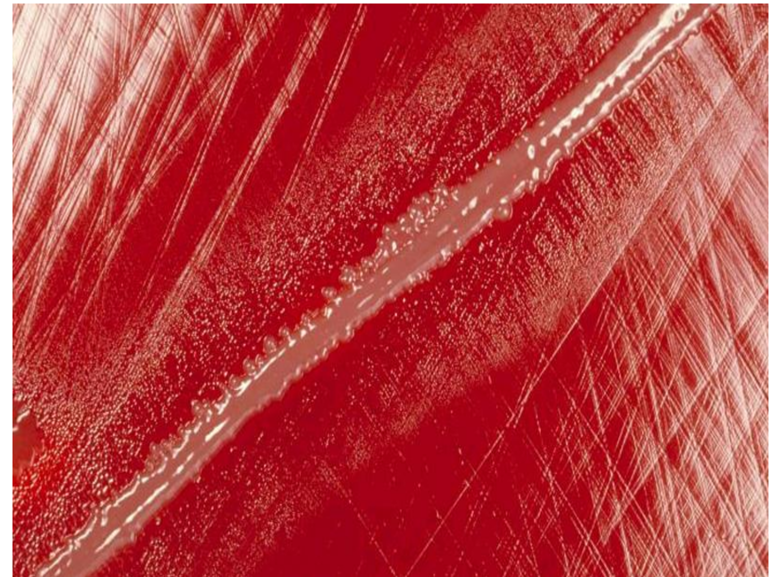
- Gram - negative (red) rods





Haemophilus - cultivation

- Growth on the Chocolate agar, which supplies growth factors such as NAD (nikotinamide-adenine-dinukleotide, factor V) and hemin (factor X)
- Growth on the Blood agar only in presence of the *Staphylococcus aureus* strain – **SATELLITISM** (release of factor X and factor V production)



Satellitism

- **Tools:** *Haemophilus influenzae, Haemophilus parainfluenzae*, *Staphylococcus aureus* strains, culture media, sterile loops

- **Procedure:** Inoculate massively both strains of *Haemophilus* on the blood agar with the sterile loop, each strain on one half of agar. Inoculate a line of the *Staphylococcus aureus* strain in the middle, throuhg boths sides of the agar. Culture in 37°C, 24 hours, in 5-10% CO_2.

- **Evaluation:** *Haemophilus* grows in small colonies along staphylococcal line.

X- and V- factor discs for *Haemophilus* identification

The principle: Particular *Haemophilus* species grows on nutrient agar only around the disc containing the required growth factor.

- ***H. influenzae* – factor X a V**
- ***H. parainfluenzae* – factor V**

X- and V- factor discs for *Haemophilus* identification

- **Tools:**

  Haemophilus influenzae, Haemophilus parainfluenzae strains, discs containing factors X, V and X+V, culture media, sterile loops and needles, markers, disinfection

- **Procedure:**

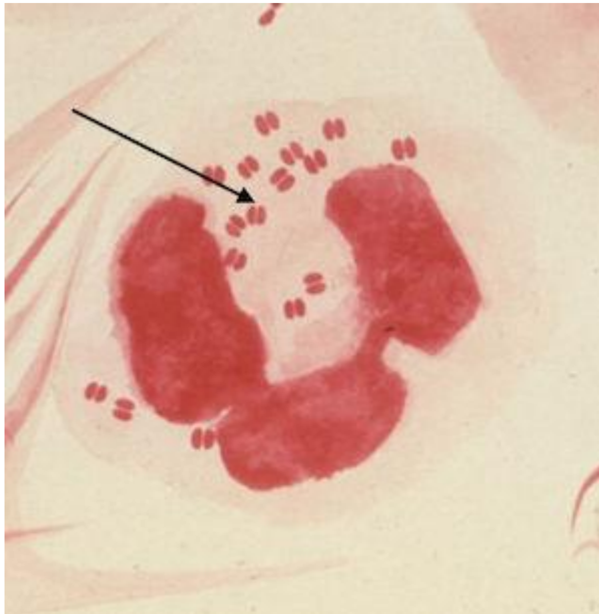
  Inoculate massively both strains on the Müeller-Hinton agar, each on its half. Put one disc containing growth factor X, V and X+V on each half of agar with inoculated strains. Culture in 37°C, 24 hours, in 5-10% CO2.

PRACTICAL PART

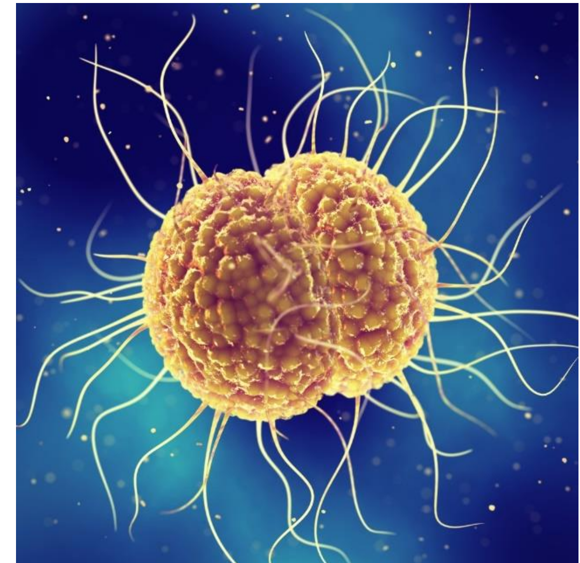
2. IDENTIFICATION OF NEISSERIA

Neisseria – Microscopy
Neisseria gonorrhoeae

- Gram - negative (red) diplococci

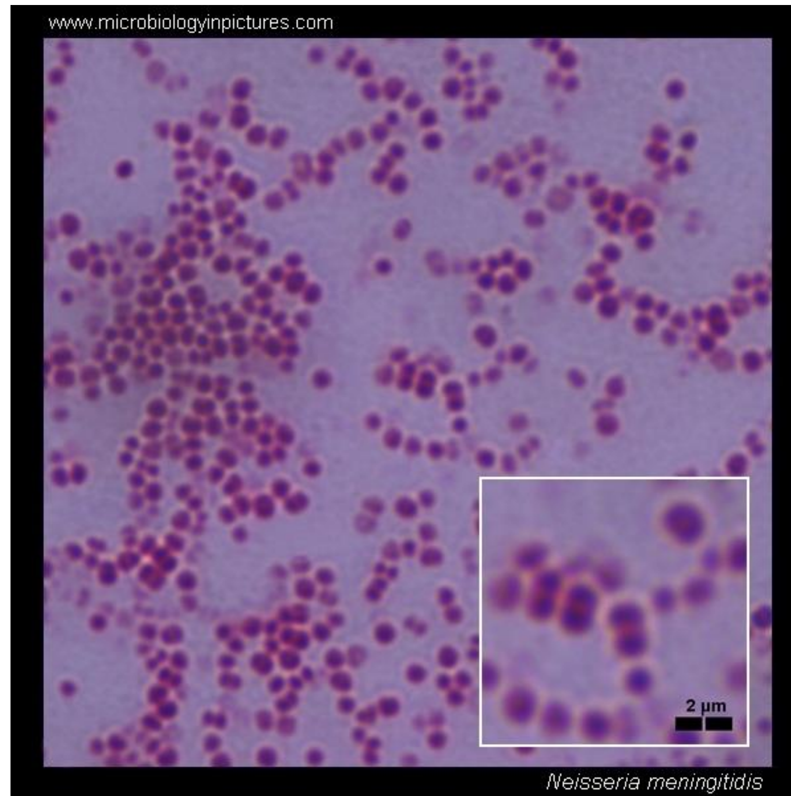


Fimbriae (pili)



Neisseria – Microscopy
Neisseia meningitidis

- Gram - negative diplococci, coffee bean -shaped

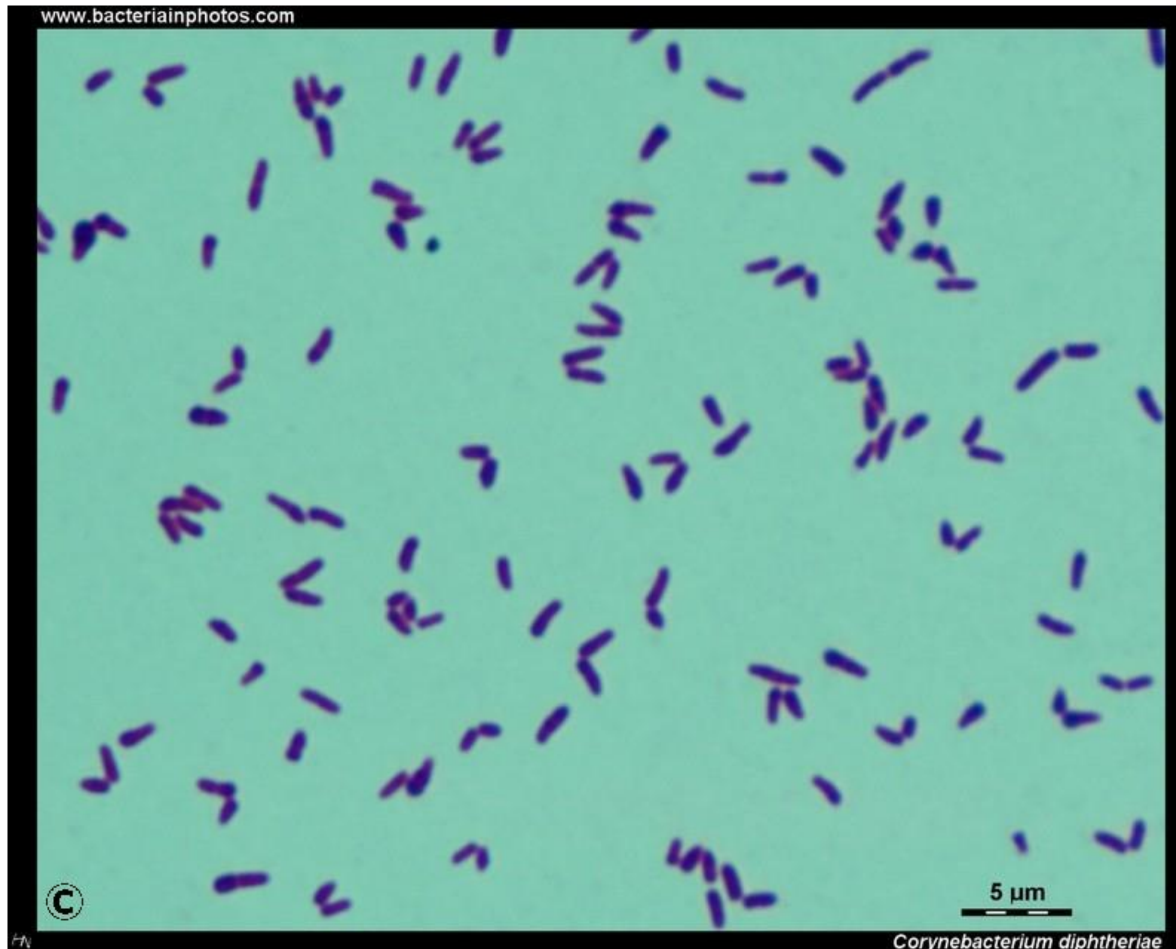


PRACTICAL PART

3. IDENTIFICATION OF CORYNEBACTERIA

Microscopy of Corynebateria

- Gram positive (blue) rods



Microscopy of Neisseria, Corynebacteria – Gram stain

- **Tools:** cultures of *Neisseria sp., Corynebacterium sp.*
- **Procedure:** Put 1 drop of phys. saline solution in the centre of the microscopic slide. Transfer a small amount of bacterial culture with the sterile bacteriologic loop and resuspend (stir) it in the solution. Make the oval inoculum of about 2 cm in diameter;
- After drying fix the slide in the methanol for 5 minutes.;
- Wash with the tap water, than put the slide on the rack in the dyeing basin and cover the smear with the solution of crystal violet for 15-20 sec;
- Wash with the tap water and cover the smear with Lugol solution for 20 sec and wash with tap water;
- Decolorize the smear with alcohol for as long as the dye is flowing away and then dye the smear with carbolfuchsin for 20 sec. Wash with the tap water and let the slide dry;
- Put 1 drop of immersion oil on dry smear and focuse it (without the cover slip!) using **immersion objective** (magnification 10×100).

Reverse CAMP test

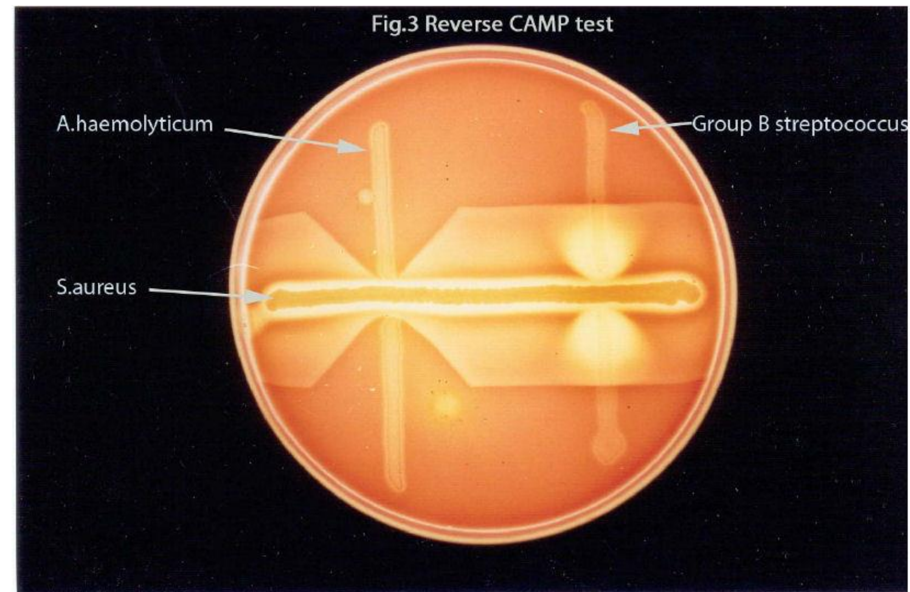
- **Principle:**

  Phospholipase D producing strain inhibits staphylococcal β-hemolysin and reduces or completely cancels the influence of β-hemolysin on blood cells.

- Reverse CAMP test is positive in *Arcanobacterium haemolyticum, Corynebacterium ulcerans* and *C. pseudotuberculosis* strains

- **Procedure:**

  Inoculate a line of tested strain on blood agar. Inoculate a perpendicular line of *Staphylococcus aureus.*



PRACTICAL PART

1. IDENTIFICATION OF HAEMOPHILUS - EVALUATION

Haemophilus – cultivation, evaluation

Evaluation:

- assess the growth of both *Haemophilu*s strains on MH agar around discs with growth factors
(HAIN - requires factor X and V, HAPA - factor V)

- assess growth of *Haemophilus* near the *Staphylococcus* line

- Note all results into the protocol.

PRACTICAL PART

2. IDENTIFICATION OF NEISSERIA
- EVALUATION

Microscopy of Neisseria/Corynebacteria – evaluation

Evaluation: Fill the protocol and describe the microscopy markers of stained microorganism, e.g. shape, Gram positivity, Gram negativity, arrangements of the cells

Oxidase test

- **Principle:**

  Test demonstrates the presence of Cytochrome oxidase as a color reaction of N, N-dimethyl - 1,4 - phenylenediamine with α-naphthol, which forms indolphenole blue.

- **Tools:** *Neisseria* culture, OXI test strips

- **Procedure:** stamp the test zone of the strip directly on tested colonies

- **Evaluation:** assess a color reaction

  <u>positive</u>: intensively blue in 30 sec.

  <u>lately positive:</u> intensively blue in 2 min.

  <u>negative</u>: no reaction, or blue after 2 min.

PRACTICAL PART

3. IDENTIFICATION OF CORYNEBACTERIA

- **EVALUATION**

Reverse CAMP test, evaluation

- **Evaluation:**

  Positive test will show suppression of β-hemolysis by tested strain at the intersection point of a tested strain line and *Staphylococcus aureus* line

- Note all results a drawings into the protocol.

Arcanobacterium haemolyticum – positive rCAMP

Corynebacterium ulcerans – positive rCAMP

Corynebacterium diphtheriae – negative rCAMP

PRACTICAL PART

4. IDENTIFICATION OF LISTERIA MONOCYTOGENES

Listeria monocytogenes

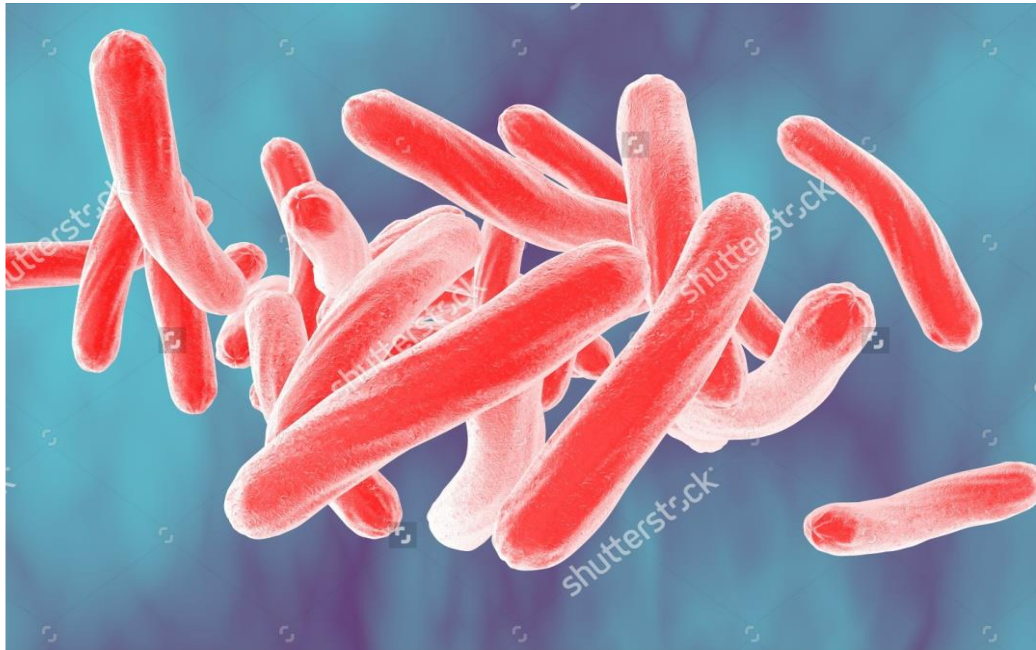
- **Principle**: *L. monocytogenes* is mobile when cultivated up to 25 ° C. A typical somersault movement can be observed in the native specimen.
- **Tools**: broth culture of *Listeria*, slides and coverslips, microscope
- **Procedure**: On the slide, we put a drop of culture broth, cover with a coverslip, observe at 40x magnification

PRACTICAL PART

4. IDENTIFICATION OF MYCOBACTERIA

Mycobacteria - Microscopy

- Ziehl-Neelsen stain
- Acid resistant rods stain



Ziehl-Neelsen stain
(the hot technique)

Acid resistant rods stain

Procedure:

- Concentrated **carbol fuchsine**
- Heating the stain with the flame 3x till vapor outlet
- Pour stain, discoloration with **acidic alcohol!**
- Rinse with water
- **Malachite green**
- Rinse with water, drying

Mycobacteria - cultivation

Culture media:

- Solid, egg – based medium in the tube
- Intended for long-term cultivation, 6 – 9 weeks

Lôwenstein – Jensen medium

Ogawa medium