METHODS OF DIRECT TESTING

PRACTICAL PART

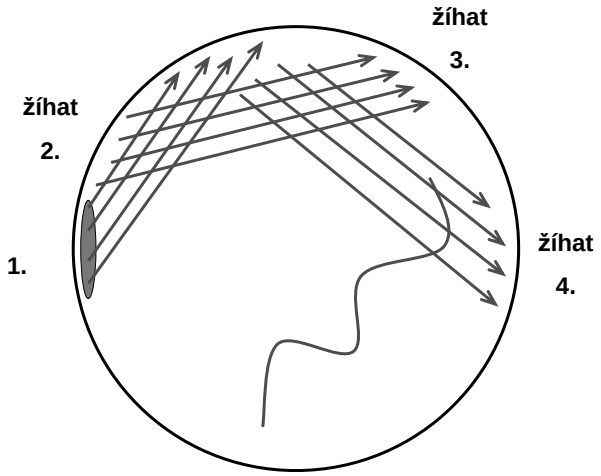
1. DIRECT IDENTIFICATION - CULTIVATION

Cultivation

- **Principle:** Bacterial culture is based on the ability of microorganisms to multiply on inanimate media
- The aim of cultivation is to demonstrate the presence of microorganisms and isolation of individual colonies of bacteria in order to identify and test antibiotic susceptibility

Cultivation – practical performance

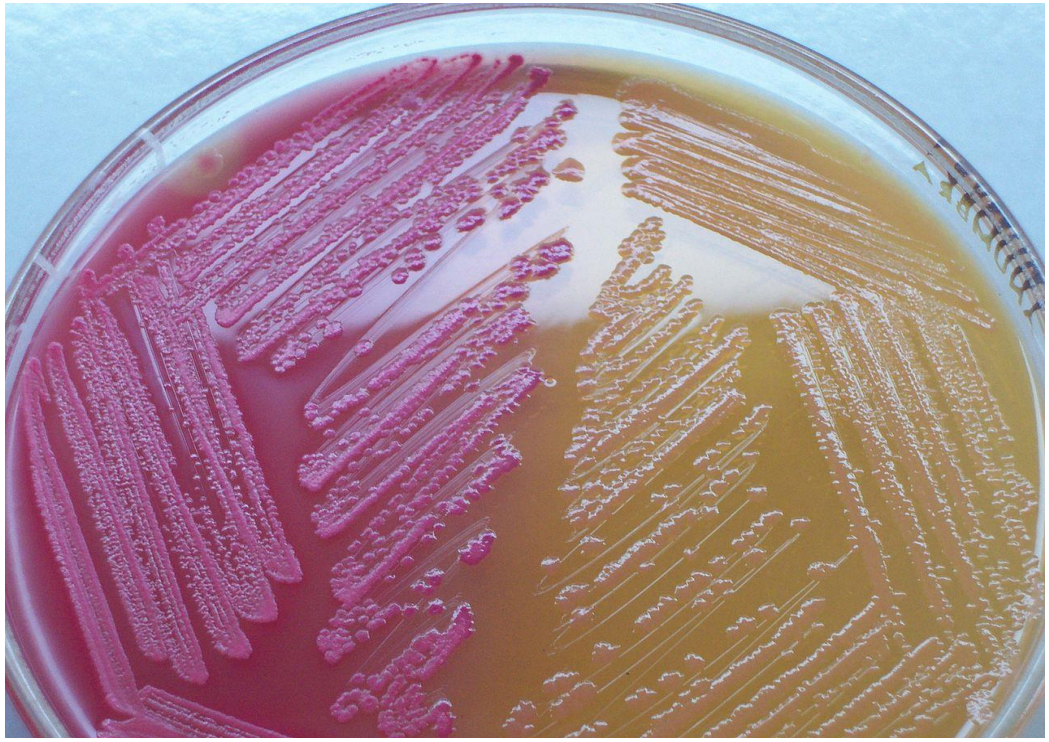
- **Tools:** Blood agar, McConkey agar, bacterial cultures, loops, markers, disinfection



McConkey agar – selective diagnostic medium

Gram negative bacteria

lactose positive lactose negative

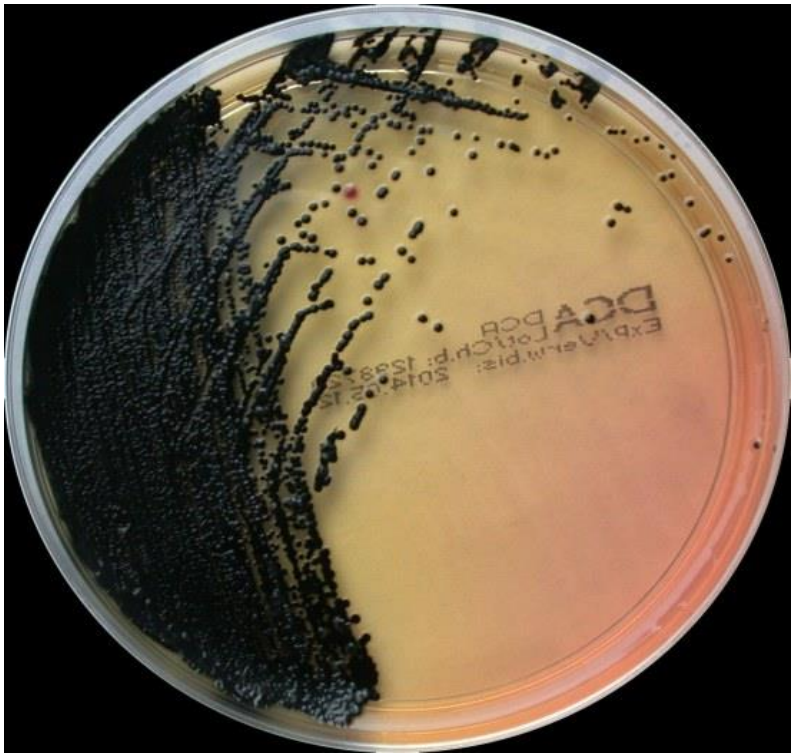


Desoxycholate agar– selective diagnostic medium

Gram negative bacteria

lactose positive/lactose negative

Production H_2S



Blood agar - basic/diagnostic medium

- hemolysis

PRACTICAL PART

2. COUNTING OF COLONIES OF MICROBES

Counting of colonies

- **Principle:** one colony will grow from one microbe cell if the cells on the agar surface are well separated
- To calculate the microbial concentration, the material must be diluted so that the cells are separated after inoculation
- The procedure is mainly used for the determination of bacteriuria

Counting of colonies – practical performance

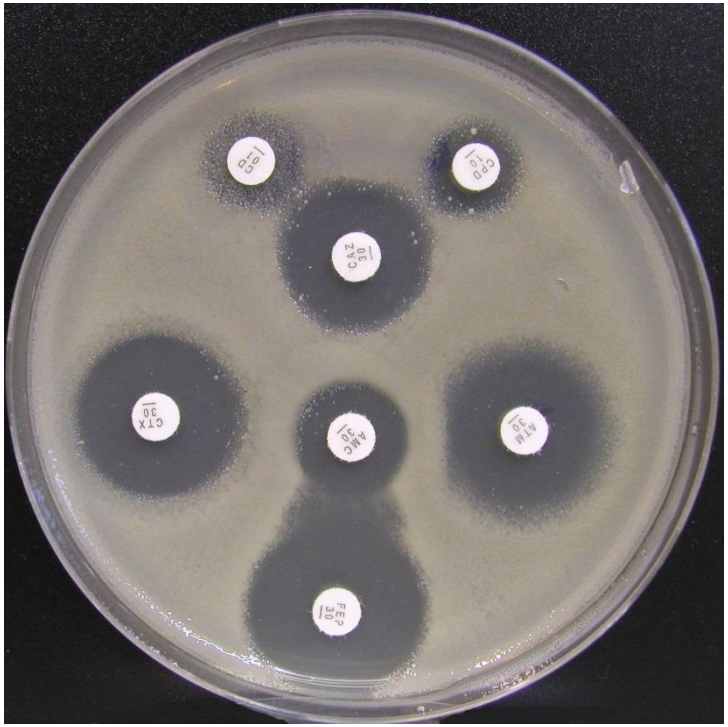
- **Tools:** bacterial suspension, phys. saline solution(3 tubes à 9.9 ml), pipette, calibrated loop (10µl), nutrient agar, marker, disinfection
- **Procedure:** Add 0.1 bacterial suspension to tube 1. After mixing, transfer 0.1 ml from tube 1 to tube 2; From tubes 2 and 3, inoculate a volume (V) of 0,1 ml over the entire surface of the agar using a calibrated loop, and note the dilution of the suspension on the agar.
- Incubate for 18 hours at 37°C
- **Evaluation:** in the 2nd pract. exercises

Dilution (D)	**(9,9ml phys. saline solution + 0,1ml suspension)**
Tube 1	10^{-2}
Tube 2	10^{-4}
Tube 3	10^{-6}

Microbial concentration (K) – calculation

Volume (V), dilution(D), and number of colonies (N)

K = N/V x D



PRACTICAL PART

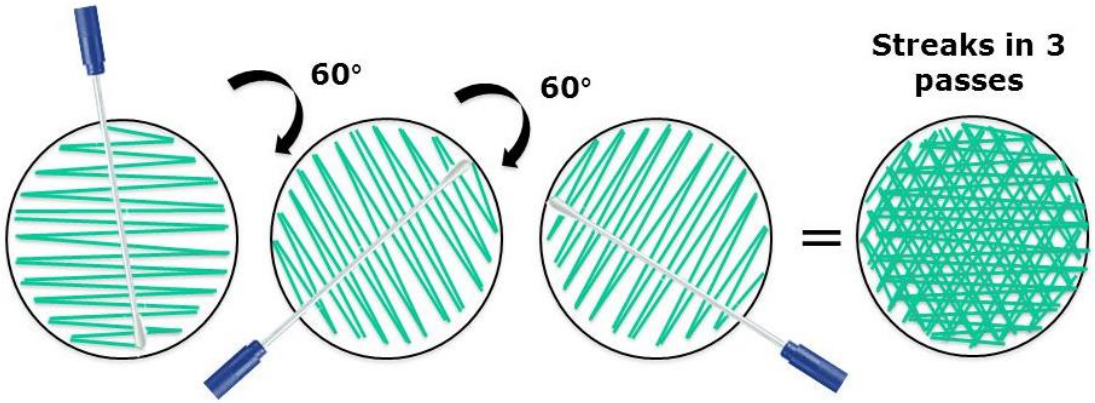
3. ANTIBIOTIC SUSCEPTIBILITY TESTING – DISC DIFFUSION METHOD

Disc diffusion method – practical test

- **Principle:** determining the susceptibility of rapidly growing non-fastidious bacteria to antibiotics using discs with antibiotics
- Agar plate is inoculated with the bacterial suspension and discs containing specific antibiotics are dispense onto plate
- After incubation the size of the inhibition zone that produces an antibiotic diffusing into agar is measured
- The diameter of inhibition zone is compared with the diameter of inhibition zone set for the susceptible and resistant strains (**B**reak **P**oint)
- Inhibition zone diameter directly depends on the susceptibility of the microbe to antibiotic, and on content of antibiotic in the disc
- Indirectly is affected by pH of agar, the deep of the agar, by concentration of inoculum

Disc diffusion method – practical test

- **Materials:** bacterial cultures, MH agar, test tube with 2 ml of saline, turbidity standard 0.5 McFarland, cotton swab, Pasteur pipettes, loops, ATB discs
- **Procedure:** Using sterile loop 1-2 bacterial colonies are taken and carefully suspended in the saline. Turbidity is adjusted according to the turbidity standard (0.5 McFarland)
- **Inoculation:** Using swab suspension is carefully streaked over the whole surface of agar



Disc diffusion method – practical test

- Using an Antibiotic Disc Dispenser discs containing specific antibiotics are dispensed onto the plate. Plates should be incubated overnight (18h) at 37°C
- **Reading:** after incubation zones of inhibition are measured around the disks and compared with limit zones of tested antibiotics.
  - **The zone is greater than limit - strain is susceptible**
  - **The zone is smaller than limit - strain is resistant**

PRACTICAL PART

4. COUNTING OF COLONIES OF MICROBES - EVALUATION

Counting of colonies - evaluation

- **Evaluation:** after incubation, colonies are counted on the dilution plates with 10-200 colonies. The average number of colonies is counted and the microbial concentration is calculated.

Dilution (D)

Tube 1	10^{-2}
Tube 2	10^{-4}
Tube 3	10^{-6}

Microbial concentration (K) – calculation

Volume (V), dilution(D), and number of colonies (N)

K = N/V x D

PRACTICAL PART

5. DIRECT IDENTIFICATION – CULTIVATION EVALUATION

Selective diagnostic medium

- **Evaluation:**

- McConkey agar – lactose positive/lactose negative colony

- Blood agar – describe the type of hemolysis

PRACTICAL PART

6. DISC DIFFUSION TEST RESULTS

Disc diffusion test

- **Reading:** Measure the zones of inhibition around the discs and compare the zones with limit zones of tested antibiotics.

The zone is greater than limit - strain is susceptible

The zone is smaller than limit - strain is resistant

PRACTICAL PART

7. DETECTION OF BETA-LACTAMASE

Testing of beta-lactamase production

- **Principle:**

bacteria can produce an enzyme (beta-lactamase) that breaks down the beta-lactam ring to form penicillin acid. The acid reaction can be demonstrated using the iodometric method or a chromogenic substrate (nitrocefin).

Demonstration of beta-lactamase - practical implementation (nitrocefin test)

- **Tools:** strip of filter paper, nitrocefin solution, loops, bacterial cultures, slide, disinfection
- **Design:** The strip is moistened with a solution of nitrocefin. The culture of the test strain is applied to the wet strip.
- **Evaluation:** red color indicates beta-lactamase production. Note whether the test strain produces beta-lactamase.