PRACTICALL SKILLS AND RULES OF SAFETY TECHNIQUES ON GENERAL MICROBIOLOGY FOR THE SECOND YEAR STUDENTS

Lesson № 1

THE SUBJECT OF MICROBIOLOGY.

Microscopic Method of Investigation. Simple staining techniques.

Practical work that student has to learn at the lesson and to be able to perform at the concluding session

1. To make a smear using bacteria grown on solid agar medium.
2. To make a smear using bacteria grown in liquid medium (broth).
3. To stain the smear by methylene blue.
4. To stain the smear by aqueous fuchsine.
5. Microscopic investigation of the smears using the immersion microscopy.

TECHNIQUE OF PREPARATION OF A SMEAR USING BACTERIA GROWN ON SOILID AGAR MEDIUM

1. If working from a solid medium (from a slant or plate), place one (**only one**) drop (a loop) of water in the centre of a clean glass slide.
2. Using the bacteriological loop, aseptically (see general aseptic techniques) pick up a ***very small*** amount of bacterial growth and mix the specimen with water completely. Carefully spread the material over the surface of the glass to cover about one third of the total slide area.
3. Allow the slide to air dry at room temperature or dry it keeping a slide high above the flame of burner.
4. After the smear is dry, the next step is fixing the smear by passing the slide several times through the hot portion of the flame of a burner.
5. Let a slide get cool. The smear is now ready for the staining procedure.

TECHNIQUE OF PREPARATION OF A SMEAR USING BACTERIA GROWN IN LIQUID MEDIUM (BROTH)

1. Prepare a smear using the broth culture first of all it is necessary to shake the culture tube and aseptically transfer 1 or 2 loops of bacteria with use of a sterile inoculating loop and place the material on the centre of the glass slide. You don’t need to add water. Spread the material over the surface of the glass to cover about one third of the total slide area.

2. Dry out and fix the smear following the same procedures which have been described for the smear made of bacterial growth on solid media.

STAINING OF THE SMEAR BY METHYLENE BLUE/ AQUEOUS FUCHINE

1. Place your fixed smears on a slide holder over the staining rack.
2. Flood the slide with methylene blue or aqueous fuchsine, leave for 1 minute.
3. Wash the staining solution off the slide with water for a few seconds.
4. To dry, blot the slide with filter paper. Don't rub the smear when drying the slide because this will remove the stained bacteria.
5. Examine the specimen under the oil immersion lens and draw the smear.
6. There is no need to place a coverslip on the stained smear.

MICROSCOPIC INVESTIGATION OF THE SMEARS USING AN IMMERSION MICROSCOPY

1. Put your microscope on the bench in front of you. Turn the knob on the condenser to put the condenser in its topmost position.
2. Place a drop of immersion oil on the stained specimen, put the specimen slide on the stage of the microscope and fix it using stage clips.
3. Switch on the external source of light (lamp). Looking into ocular and using low magnification objective (10x) find the best position of the mirror that helps to get optimal lighting of the field of view. In the case of using electric microscope equipped with the inbuilt light source it is sufficient to switch on the lamp of the microscope. Then locate the stained area.
4. Turn the nosepiece until the oil immersion objective clicks into place. Then lower the observation tube until the tip of the objective touches the immersion oil (the distance between the objective lens and the slide with specimen is about 5 mm). **Don't touch the surface of the glass slide by the objective lens!** Be sure that you lower the tube while looking at the microscope from the side.
5. Looking into the ocular of the microscope slowly raise the observation tube by turning the course focus knob counter clockwise until the specimen appears in the field of view. Then use the fine

focus knob to get the desired (sharpened) image. Draw the microorganisms.

6. **Don't forget to switch the electric microscope off immediately when you have finished your work!**
7. After you have finished your work with the microscope, install the low-power objective by turning the nosepiece until the objective will click into place.
8. Lower the observation tube to its lowest position.
9. Remove any oil from the oil immersion lens with soft lens cleaner and return the microscope to its storage place on the shelf according to its number.

Lesson № 2

MORPHOLOGY AND STRUCTURE OF THE BACTERIAL CELL.

Gram staining technique.

Practical work that student has to learn at the lesson and to be able to perform at concluding session

1. The main steps and the procedure of the Gram staining technique.
2. To identify *Staphylococcus spp.* in a Gram-stained smear.
3. To identify *Streptoccus spp.* in a Gram-stained smear.
4. To identify Gram negative rods stained by aqueous fuchsine or by the Gram staining technique.

MAIN STEPS AND PROCEDURE OF THE GRAM STAIN TECHNIQUE

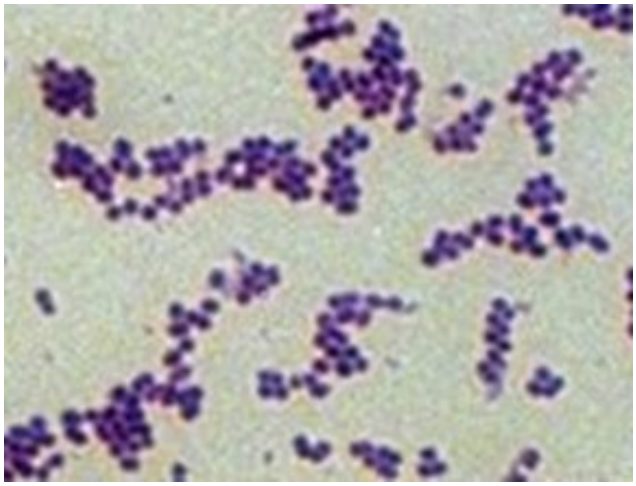
1. Prepare a mixed smear of *E. coli* and staphylococci by mixing one loop of E.coli and one loop of staphylococci in one drop of water placed on the same glass slide and spreading bacteria over the surface of the glass to get the mixed smear.
2. Fix the smear in the flame of burner. Cool the slide with specimen.
3. Place the slide on the slide holder over the staining rack.
4. Place the piece of filter paper (preliminary stained with crystal violet and dried) on the smear and soak it with water from the bottle. Leave for 1 to 2 minutes.
5. Remove the paper with the stain, cover the smear with Gram's iodine solution and wait for 1 minute.

6. Decolourise the smear with 95% ethanol by applying several drops of ethanol on the specimen for about 20 seconds. Add the ethanol drop by drop. **Do not decolourise too long**.
7. Carefully rinse with water for about 5 seconds.
8. Apply the counter stain, aqueous fuchsine (or safranin) onto the smear for 1 to 2 minutes.
9. Rinse with water for 5 seconds.
10. Blot with filter paper to dry. **DO NOT RUB THE SMEAR!**

Gram-positive staphylococci stain blue, gram-negative escherichiae stain pink to red.

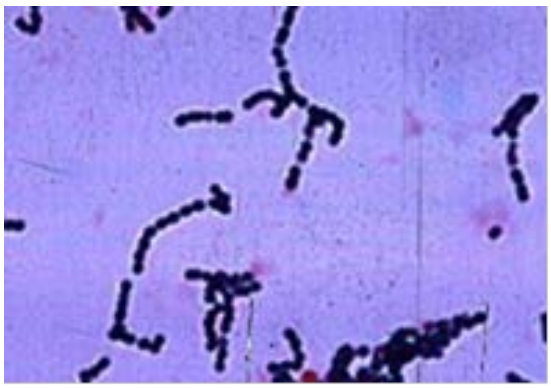
IDENTIFICATION OF STAPHYLOCOCCUS SPP. IN A GRAM-STAINED SMEAR

The student has to know the shape, colour and arrangement of staphylococci in the smear. The cells of these bacteria possess a mathematically ideal spherical shape, they are gram-positive so stain blue when gram stain technique is applied and arranged in the smear forming clusters reminding grape bunches.



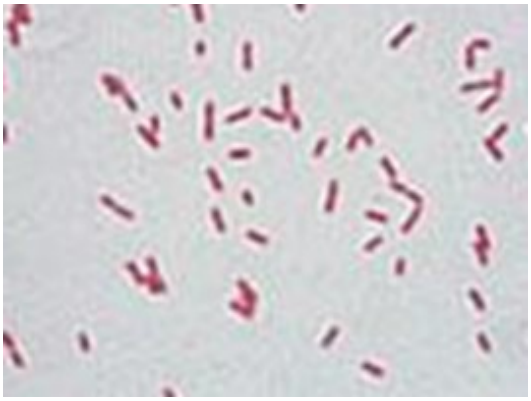
IDENTIFICATION OF STREPTOCOCCUS SPP. IN A GRAM-STAINED SMEAR

The student has to know the shape, colour and arrangement of streptococci in the smear. Like staphylococci they are also gram-positive bacteria so stain blue when gram stain technique is applied. But the shape and arrangement in of streptococci in the smear are different. The shape of the cell of streptococci is oval and they are arranged in the smear in chains.



IDENTIFICATION OF GRAM NEGATIVE RODS STAINED BY FUCHSINE OR BY GRAM STAINING

The student has to know the shape, colour and arrangement of gram negative rods in the smear. Unlike staphylococci and streptococci gram negative rods possess cylindrical shape, stain red by Gram and don't possess special arrangement in the smear.



Lesson № 3

MORPHOLOGY AND STRUCTURE OF THE BACTERIAL CELL
(MORPHOLOGICAL AND ULTRA STRUCTURAL PECULIARITIES OF ACTINOMYCETES, SPIROCHETES, RICKETTSIAE, CHLAMYDIAE, MYCOPLASMAS AND FUNGI
Ziehl-Neelsen staining technique

Practical work that student has to learn during the lesson and to be able to perform at the concluding session

1. Ziehl-Neelsen stain technique.
2. To reveal spores in a *Bacillus spp*. smear using a microscope.
3. To reveal the bacteria which possess capsules in the smear using a microscope.

4. To reveal *Streptomyces spp.* in the smear using a microscope.
5. To reveal fungi in the smear of unstained mycelium using a microscope.
6. To reveal yeasts in the smear stained with methylene blue or fuchsine using a microscope.

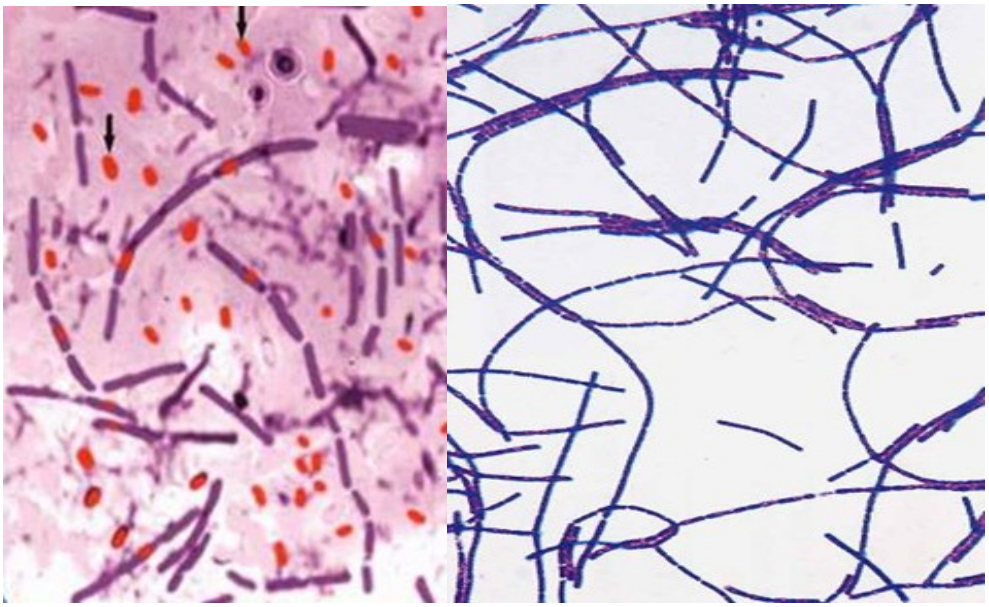
ZIEHL-NEELSEN STAIN TECHNIQUE

1. Prepare a smear of *Bacillus spp.* Fix the smear in the flame of burner. Cool the slide with the specimen.
2. Place the slide with smear you want to stain on the slide holder of the staining rack.
3. Cover the smear with the piece of filter paper.
4. Soak the paper with the carbolfuchsine solution by applying several drops of the staining solution on the paper and press it gently to stick to the surface of the glass slide.
5. Carefully heat the smear by passing the slide through the hot portion of the flame of a burner until the staining solution steams, remove the slide from the flame and repeat the procedure twice (it is necessary to get the smear steaming three times). Do not allow the slide to become dry when heating the smear (add new portions of carbolfuchsine if the paper becomes dry).
6. When you finish heat-staining, remove the paper using forceps, leave the slide to cool, and rinse it with water to remove the residuals of staining solution.
7. Decolorize the smear with 1% sulphuric acid by dipping the specimen into the vessel filled with the solution of acid for about 5 seconds. **Do not decolourise too long**.
8. Carefully rinse with water for about 30 seconds.
9. Counterstain with methylene blue for 2 minutes.
10. Rinse with water for 5 seconds.
11. Blot with filter paper to dry. **DO NOT RUB THE SMEAR!**
12. Examine the specimen under oil immersion.

The spores localised inside of the bacterial cell as well as free spores, stain red; vegetative cells stain blue.

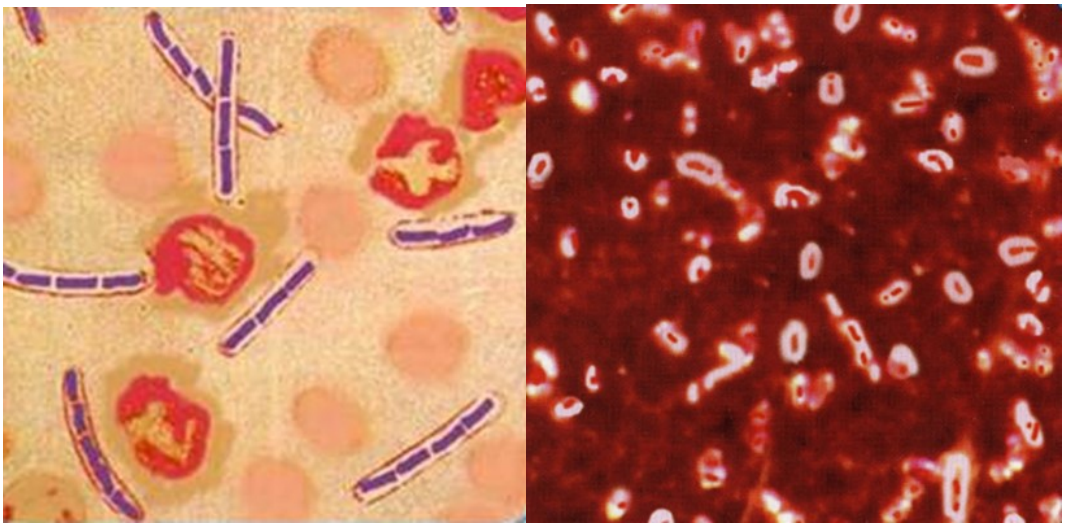
REVEALING OF SPORES IN BACILLUS SPP. SMEAR USING A MICROSCOPE

Student has to know the shape of the cell, colour of the spores and the rest part of the bacterial cell and arrangement of *Bacillus spp.* in the smear. Bacillus cells possess a typical rod shape. The rods have square ends and they are arranged in the smear in long chains. In the smears stained by Ziehl-Neelsen spores get red colour and located centrally inside of the rods which have blue colour.



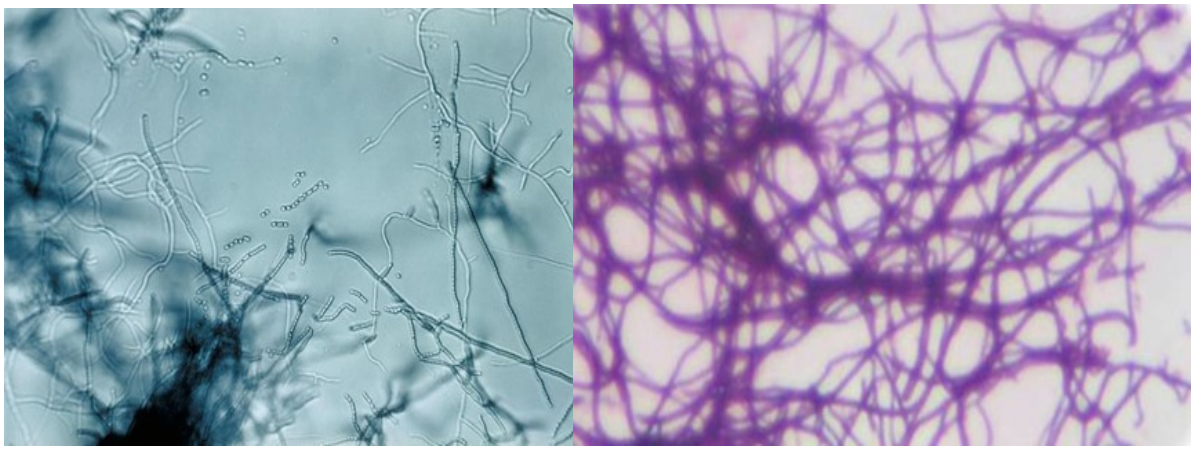
REVEALING OF THE BACTERIA WHICH POSSESS CAPSULE IN THE SMEAR USING A MICROSCOPE

The cells of some bacteria are covered with a polysaccharide covering called a capsule. Unlike the cell, the capsule cannot retain any staining solution. So after staining using a standard techniques it looks like a clear zone around the bacterium.



REVEALING *STREPTOMYCES SPP.* IN THE SMEAR USING A MICROSCOPE

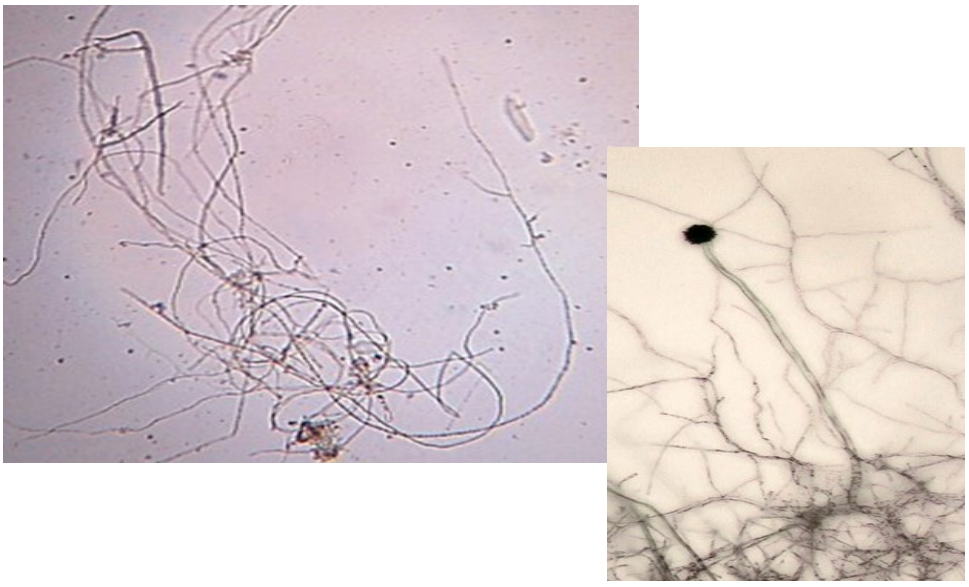
Streptomycetes are gram-positive thin and long branching rods. Student has to remember that if they stained by Gram they will have blue colour but they could be also stained with applying simple staining technique, for example using a aqueous fuchsine in this case they will stain red colour.



REVEALING FUNGI IN THE SMEAR OF UNSTAINED MYCELIUM USING A MICROSCOPE

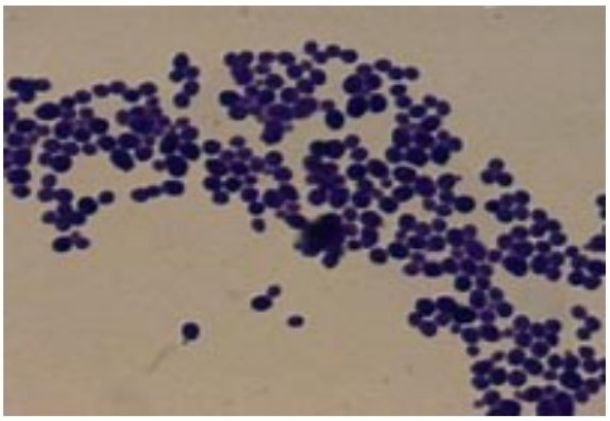
Pathogenic fungi can exist in a mycelial form they called moulds**.** They possess hyphae which are multicellular filamentous structures**.**

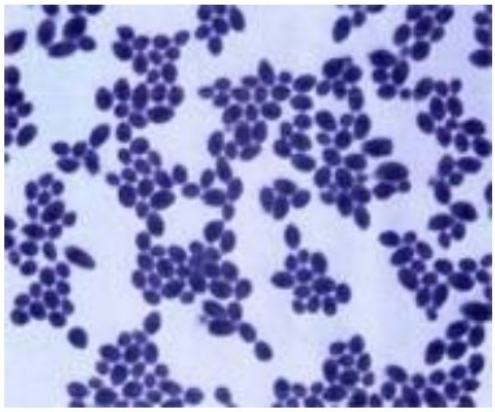
In the unstained smear student has to find wide ribbon-like non-septated mycelium looking like branches and sporangia which contain the dark spores. The smear of unstained fungi is covered with a cover-slip.



REVEALING YEASTS IN THE SMEAR OF STAINED WITH METHYLEN BLUE USING A MICROSCOPE

Pathogenic fungi can exist in the yeast form**.** Yeasts are unicellular organisms, which reproduce by budding. The yeast cells are oval shape, and stain as gram positive microorganisms. Budding yeast cells can remain attached to each other.





Lesson № 4

THE PHYSIOLOGY OF BACTERIA.

Neisser stain

Practical work that student has to learn at the lesson and to be able to perform at concluding session

1. Neisser staining technique.
2. To reveal volutin granules in the smear of Corynebacterium spp. using a microscope.

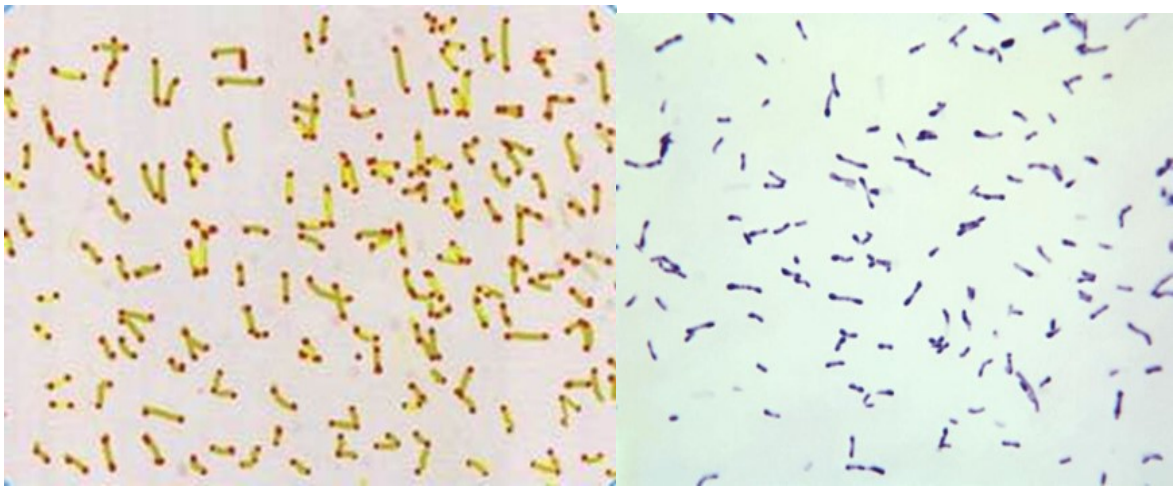
NEISSER STAINING TECHNIQUE

1. Prepare a smear of Corynebacterium spp. Fix the smear in the flame of burner. Cool the slide with specimen.
2. Place the slides with specimen on the slide holder over staining rack.
3. Flood the slide with Neisser blue staining solution leave for 1 to 2 minutes.
4. Remove the stain, rinse the slide with water, flood the smear with iodine solution and wait for 1 minute.
5. Carefully rinse with water for about 5 seconds.
6. Apply the counter stain vesuvin onto the smear for 1 to 2 minutes.
7. Finally rinse with water.
8. Blot with filter paper to dry. **DO NOT RUB THE SMEAR!**

REVEALING VOLUTIN GRANULES IN THE SMEAR OF CORYNEBACTERIUM SPP. USING A MICROSCOPE

Corynebacteria are average size rods having angular arrangement in the smear (two rods under the angle to each other). They have volutin granules which are found in the smears stained by **Neisser** as a brown oval structure inside of the bacterial cells, and the rest of the cytoplasm of the cell is stained yellow. The granules stained with use of **Loeffler's Blue stain** stain dark blue and the cytoplasm stains light blue.

Neisser stain **Loeffler's Blue stain**



Lesson № 5

THE PHYSIOLOGY OF BACTERIA (continuation). METHOD OF CULTIVATION OF BACTERIA. BACTERIOPHAGES.
Bacteria phage typing.

Practical work that student has to learn at the lesson and to be able to perform at concluding session

1. To obtain isolated colonies of bacterial culture by seeding of the pathological material (specimen) using a **streak-plate technique.**
2. To describe the colonies (R- or S-shaped) grown on solid medium (agar plate).
3. To spread the part of the isolated colony making a streak over the surface of an agar slant.
4. To inoculate the Hiss medium with the bacteria grown on an agar slant.
5. To know how to calculate the titre of bacteriophage using a plague assay method.

6. To know the main principles of bacteria phage typing.

OBTAINING OF ISOLATED COLONIES OF BACTERIAL CULTURE BY SEEDING OF THE PATHOLOGICAL MATERIAL (SPECIMEN) USING A STREAK-PLATE TECHNIQUE

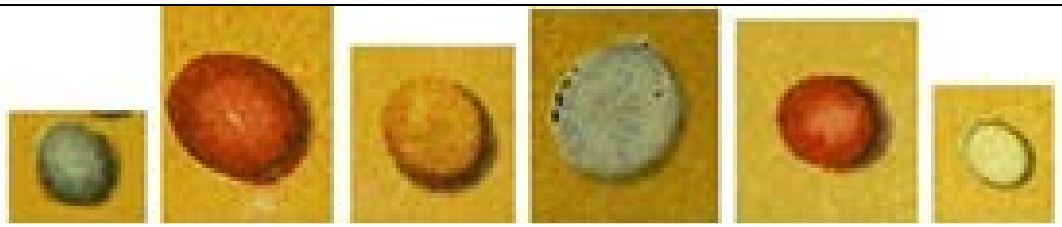
1. Sterilise the inoculating loop by flaming.
2. Remove the cap and sterilise the mouth of the test tube in the flame.
3. Using the sterile loop, take a small amount of the material from the tube.

4. Sterilise the mouth of the test tube after taking the sample, recap it and place it back. **The inoculating loop is not flamed!**
5. Carefully lift the top of the dish containing sterile agar just enough to insert your inoculating loop easily and make a streak over the agar plate spreading out the bacteria.
6. Remove the inoculating loop, close the petri dish and rotate it counter clockwise about 90 degrees.
7. Sterilise the inoculating loop in order to kill any remaining bacteria by flaming them.
8. Open the agar petri dishes again, insert the loop under the lid and cool it at the edge of the agar. Keeping in mind where the initial streaks ended make a streak over the agar plate spreading out bacteria starting from the previous streak.
9. Close the petri dish and turn the petri dish counter clockwise about 90 degrees again.
10. It is necessary to repeat the sequence of the streaking procedures described above twice to make a set of four cross-streaks.
11. After you will finish your work finally sterilise the inoculating loop and put it out.

DESCRIBTION OF THE COLONIES (R- OR S-SHAPED) GROWN ON SOLID MEDIUM (AGAR PLATE)

Description of the colonies

S-shaped
Round, regular margins, ***smooth*** surface, wet and homogenous, small or average size



R-shaped
Irregular shape and margins (curled, wavy, etc.), ***rough*** surface, dry and heterogeneous, big size

SPREADING OF PART OF THE ISOLATED COLONY MAKING A STREAK OVER THE SURFACE OF AN AGAR SLANT

1. Take an inoculating loop, sterilise it by flaming, slightly open the agar plate with grown isolated colonies and sample a small amount of the separate colony.
2. Close the agar plate, take the test tube with pure agar slant, remove the cap and sterilise the mouth of the test tube in a flame.
3. Spread the specimen onto the agar making a streak over the surface of the slant.
4. Sterilise the mouth of the test tube after seeding, recap it and place for cultivation.
5. Sterilise the bacteriological loop and put it away.

INOCULATION OF HISS MEDIUM WITH THE BACTERIA GROWN ON AN AGAR SLANT

1. Place both tubes (one tube with the slant agar bacterial culture and another one with pure Hiss medium) in the palm of one hand to form a V.
2. Flame the inoculating loop along all its length.
3. Remove the caps from the tubes and flame the mouths of the tubes. **Don't place caps on the lab bench!**
4. Cool the loop and pick up a small amount of bacterial growth from the slant and inoculate it into the tube with Hiss medium (moving the loop through the medium from the top up to the bottom).
5. Flame the mouths of the tubes again and recap them and put into thermostated incubator for cultivation.
6. Finally flame the inoculating loop along all its length and put it away.

CALCULATION OF THE TITRE OF BACTERIOPHAGE USING A PLAGUE ASSAY METHOD

The material containing bacteriophage is titrated by making suitable dilutions using a range of test tubes. Then standard volumes of phage-containing material from every tube are mixed in semi-solid agar medium (**top agar**) with a standard volume of young culture of bacteria which are sensitive to the bacteriophage and then poured in a thin layer onto the surface of solid nutrient agar. The method is called the **double-layered culture technique.**

When the top agar jells, the phages and bacteria are found immobilized in its layer. The phage particle present in the agar can infect a bacterial cell and lyse it. The clear areas called **plaques** will appear in the bacterial lawn. In practise one phage will produce one plaque.

The number of plaques will be equal to the number of phages in the sample. To know the starting concentration of bacteriophage particles or **titre of bacteriophage** in undiluted sample we have to calculate it by multiplying the number of plaques got on every plate by the dilution of the phage-containing material in corresponding tube.

MAIN PRINCIPLES OF BACTERIA PHAGE TYPING

Some bacteriophages show quite narrow host range so can only infect very closely related bacteria. Thus, the ability is normally used to identify or type the bacteria.

For this the bottom of petri dish (seeded with a pure culture of the bacteria to be identified) is marked from outside indicating on the glass the number of standard specific phages and separate zones where the suspensions containing corresponding phages will be spotted on the bacterial lawn. Every preparation of the phage has been indicated by specific number and sometimes it could have a number and a letter additionally.

Thus identity of specific phages which lyse the bacterial lawn indicates the bacteria phage type.

Lesson № 6

GENETICS OF BACTERIA.

Microscopic study of live bacteria

Practical work that student has to learn at the lesson and to be able to perform at concluding session

1. To reveal motile bacteria by «plated drop» method.

REVEALING OF MOTILE BACTERIA BY "PLATED DROP" METHOD

1. Put the glass slide with the specimen on the stage of the microscope.
2. First use the low magnification objective (10x) to locate the edge of the drop by focusing on it.
3. Switch to the high magnification (40x) dry objective. In some cases you can also use immersion objective (90x or 100x) with a drop of immersion oil placed on cover slip.
4. In order to see movement of bacteria clearly slightly lower the condenser. The diaphragm should be closed as much as possible for increased contrast.
5. You have to be careful and to be able to distinguish between real motility and Brownian movement.

Lesson № 7

ECOLOGY OF MICROORGANISMS.

Study of micro-flora of the oral cavity

Practical work that student has to learn at the lesson and to be able to perform at concluding session

1. To make the smear using bacteria grown on solid agar medium.
2. To stain the smear using a simple staining technique.

Student has to demonstrate the knowledge and ability to apply both techniques for study of their own normal microflora found in dental deposits.

Making the smear using dental deposit

1. Place one drop (a loop) of water in the centre of a clean glass slide.
2. Using the sterile wooden stick pick up a small amount of dental deposit streaking over the teeth and between them and mix the deposit with water completely. Carefully spread the material over the surface of the glass by stick to cover about one third of the total slide area.
3. Then dry and fix the smear following the steps described in the lesson 1.
4. Stain the smear by methylene blue or aqueous fuchsine applying the simple staining technique (see lesson 1).
5. Investigate the smear using immersion microscopy.

Lesson № 8

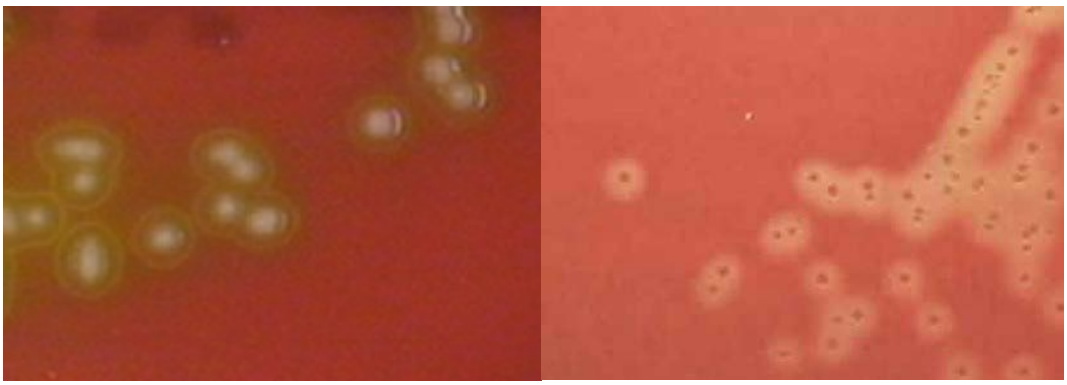
BASICS OF INFECTION.

Practical work that student has to learn at the lesson and to be able to perform at concluding session

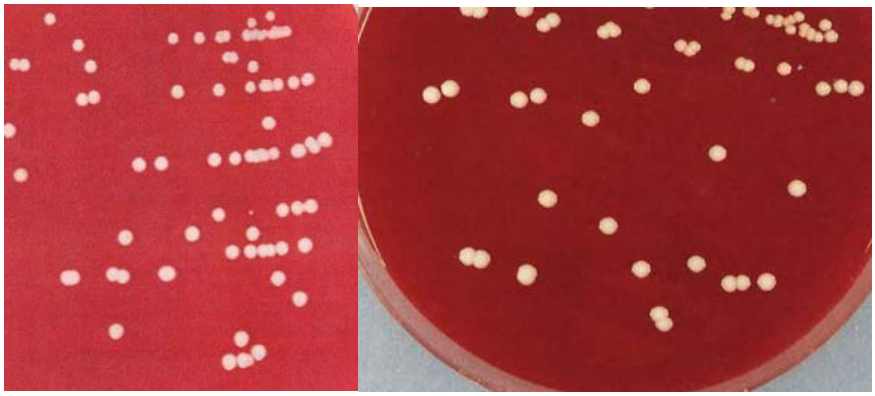
1. Tests revealing virulence of bacteria: haemolytic activity, activity of plasmocoagulase and lecithinase.

TESTS REVEALING VIRULENCE OF BACTERIA: HAEMOLYTIC ACTIVITY, ACTIVITY OF PLASMOCOAGULASE AND LECITHINASE

Haemolysins are bacterial protein enzymes that destroy red blood cells. The production of such toxins can be demonstrated by streaking of the pure culture of pathogenic bacteria on the blood-containing agar plate. When the bacteria are growing they release haemolysins and lyse red blood cells. Haemolytic bacteria can cause **α-haemolysis,** when a zone of greenish coloration forms around colonies growing on blood agar. The colour results from partial decomposition of haemoglobin. **β-haemolysis** is a zone of clear haemolysis with no greenish colour surrounding the colony.



α- haemolysis (greenish zones) β- haemolysis (clear zones)



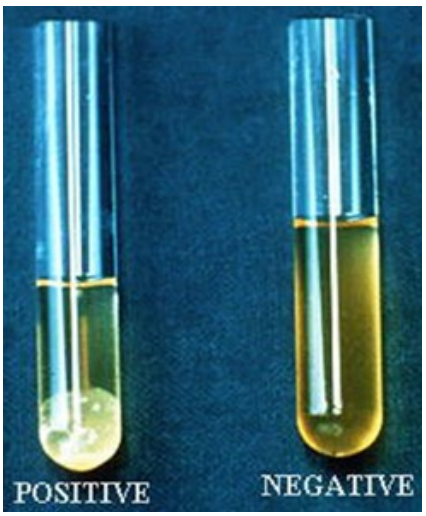
Two examples of the absence of haemolysis (called γ- haemolysis) – no any zones around colonies

Plasmocoagulase (or coagulase) is the enzyme that can coagulate blood plasma.

To set up the test a dense liquid suspension of bacteria is mixed with plasma and incubated. Bacteria having positive coagulase activity cause clotting of blood plasma.

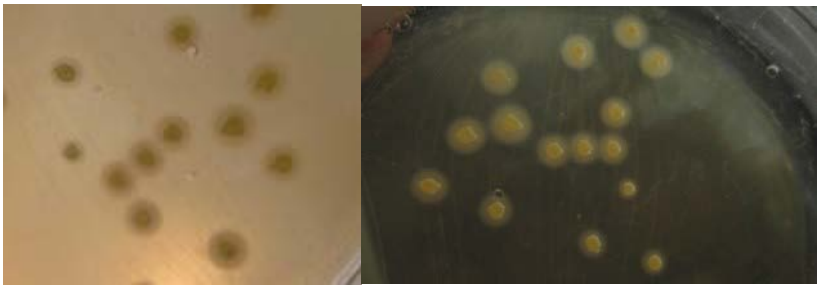
If bacteria when grown in the test tubes containing plasma form a clot these strains are referred to coagulase positive.

Whereas coagulase negative staphylococci do not form a clot.

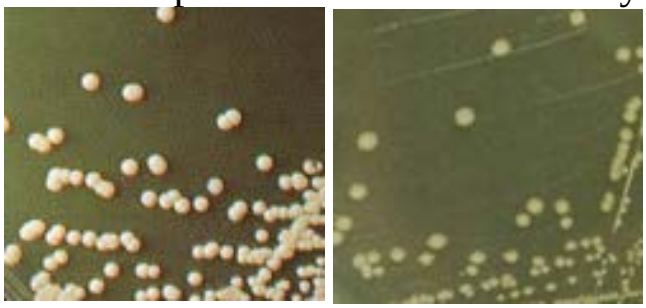


Some protein toxins can attack the lecithin phospholipid constituent of host cell membranes. Such protein enzymes called lecithinases.

The production of lecithinase is usually demonstrated by streaking of the pure culture of pathogenic bacteria on the yolk-salt agar. Egg yolk contains a lot of lecithin. When growing bacteria release lecithinase and split lecithin of egg yolk added to agar medium. The opaque opalescent zones appear around the colonies positive for lecithinase.



Two examples of the positive lecithinase activity.



Two examples of the absence of lecithinase activity

Lesson № 9

MICROBIOLOGICAL BASIS OF CHEMOTHERAPY OF BACTERIAL INFECTIONS.

Antimicrobial susceptibility tests.

Practical work that student has to learn at the lesson and to be able to perform at concluding session

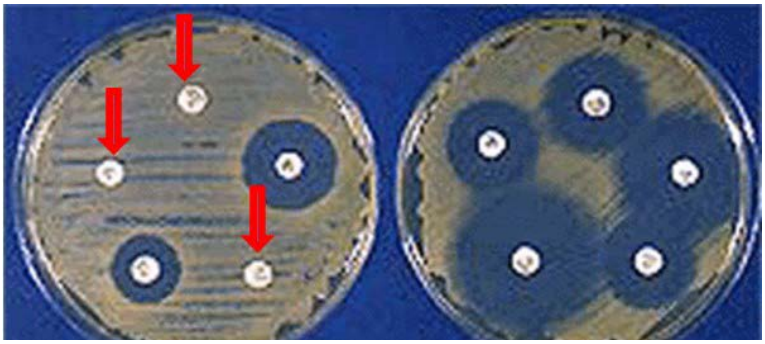
1. To reveal antimicrobial activity of antibiotics using antimicrobial susceptibility tests: disk diffusion technique.
2. To reveal antimicrobial activity of antibiotics using antimicrobial susceptibility tests: broth dilution in tubes technique (to be able to calculate minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of tested antibiotics.

REVEALING ANTIMICROBIAL ACTIVITY OF ANTIBIOTICS USING DISC DIFFUSION TECHNIQUE

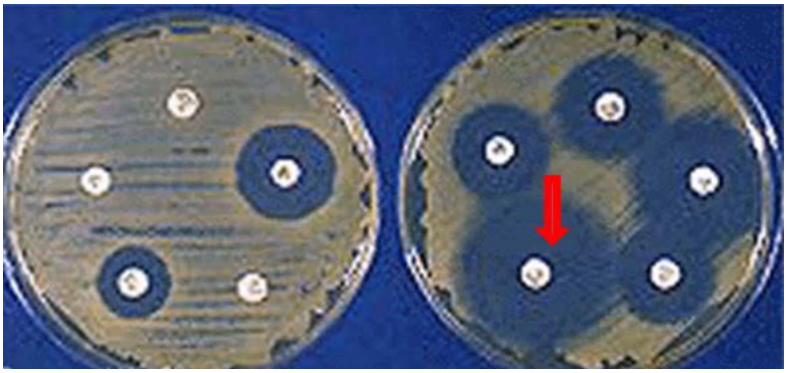
To perform the technique follow these steps:

1. Aseptically take a loopful of broth bacterial culture and streak it to cover all surface of an agar plate, rotating the plate when streaking the material. Don't flame the loop to ensure that the whole surface has been seeded.
2. Allow the culture to dry on the plate for a couple of minutes at room temperature with the top in place.
3. Place the antibiotic disks with antibiotics on the surface of agar plate using alcohol-flamed forceps. Operating by forceps carefully press every disk to the surface of agar. Make sure that good contact is made between them. **Don't push the disks into the agar and avoid moving the disks from the place it was once placed.**
4. Put the plates for incubation. **Don't forget that you should not invert the agar plates bottom up.**
5. After getting the growth of bacteria as a lawn you have to evaluate the inhibition zones which will appear (or not) around the disks and to make the conclusions about susceptibility of test bacteria to antibiotic using the next criteria: 1 – resistant, when the zone of inhibition of bacterial growth is absent; 2 – low susceptible, when the zone of inhibition is small; 3 – susceptible, when the zone of inhibition is wide and 4 – susceptible but some resistant clones are present, when we can see separate colonies of bacteria which appeared in the zone of inhibition.
6. The wider the zone of inhibition means the higher inhibitory power of the antibiotic.
7. But the student has to pay attention on possible appearance of several colonies of resistant bacteria which could be found in the clear zone

of inhibition. **Such antibiotics should not be evaluated as effective ones.**



The results for the antibiotics marked with red arrows: the tested strain is completely resistant



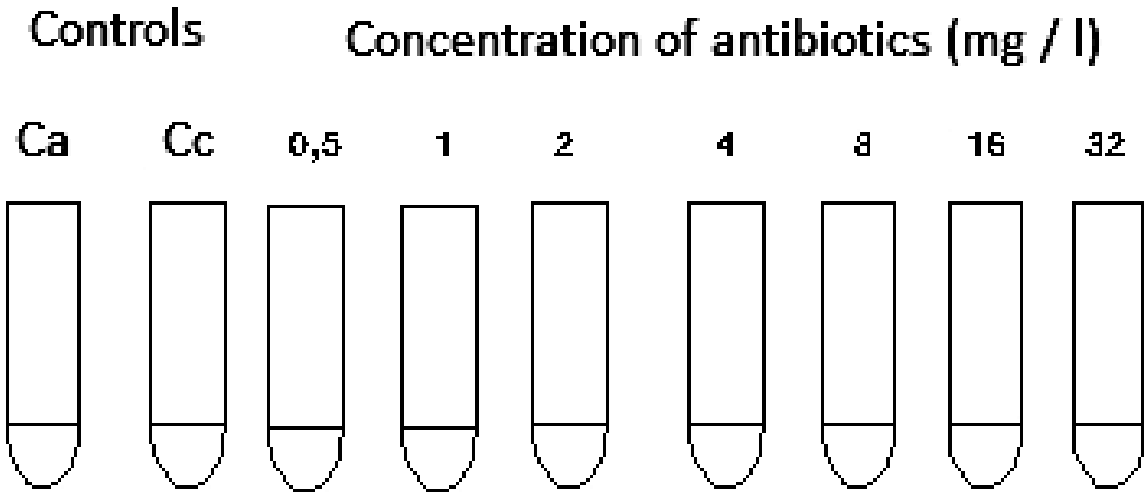
The result for the antibiotic marked with red arrow: the tested strains is **the most sensitive (red arrow)**, for the rest of antibiotics: the strain is **sensitive**.

REVEALING ANTIMICROBIAL ACTIVITY OF ANTIBIOTICS USING BROTH DILUTION TECHNIQUE

Antibiotic sensitivity determined by the dilution method performed in tubes

1. Firstly, it is necessary to prepare **s**erial (double) dilutions of antibiotics in the test tubes: each row of tubes contains different antibiotic diluted in broth. The last tube of every row is control of broth – don't add antibiotic to these wells.
2. Add standard suspension of bacteria under test to every tube. The tube before the last in every row is a control of antibiotic. Don't add bacteria to these tubes.
3. Incubate the tubes in the thermostated incubator.

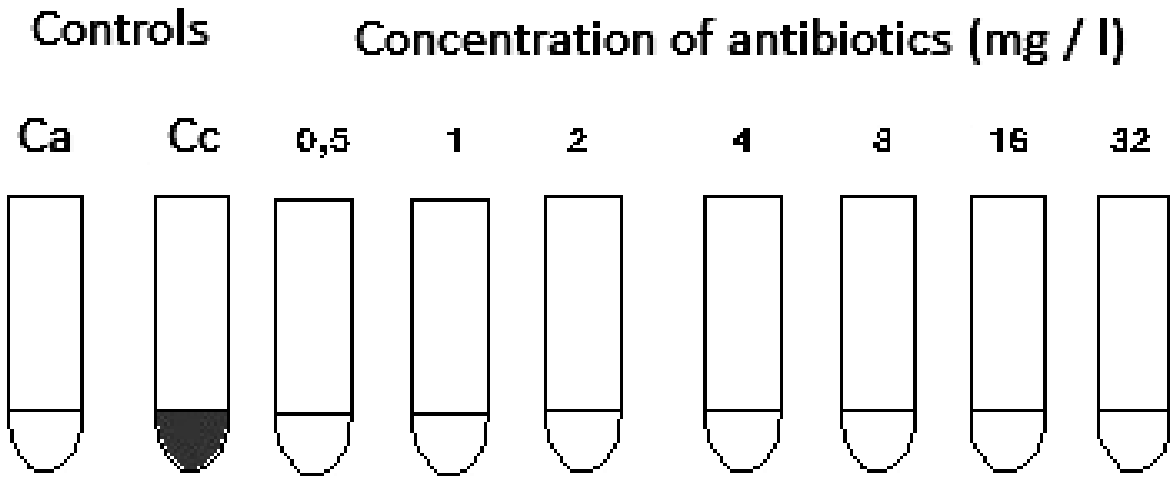
4. Read the results: indicate the MIC (minimal inhibition concentration) for every antibiotic in every raw by the highest dilution of the antibiotic which prevents the growth of bacteria in the tube (there is no turbidity in the broth).
5. To indicate the **MBC** (minimal bactericidal concentration) for the most effective (best) antibiotic student has to seed the Petri dishes containing solid agar medium without antibiotics using the material from the highest dilutions of the antibiotic which prevents the growth of the tested bacterial culture in the tubes.
6. After incubation of the Petri dishes seeded by the material from the tubes the dishes without any growth of bacteria have to be taken into account for the calculation of MBC.
7. The minimal bactericidal concentration of antibiotic will be calculated using the highest dilution of antibiotic which prevents growth of bacteria on the Petri dish (that means all the bacteria were killed in the test tube incubated with this concentration of antibiotic).



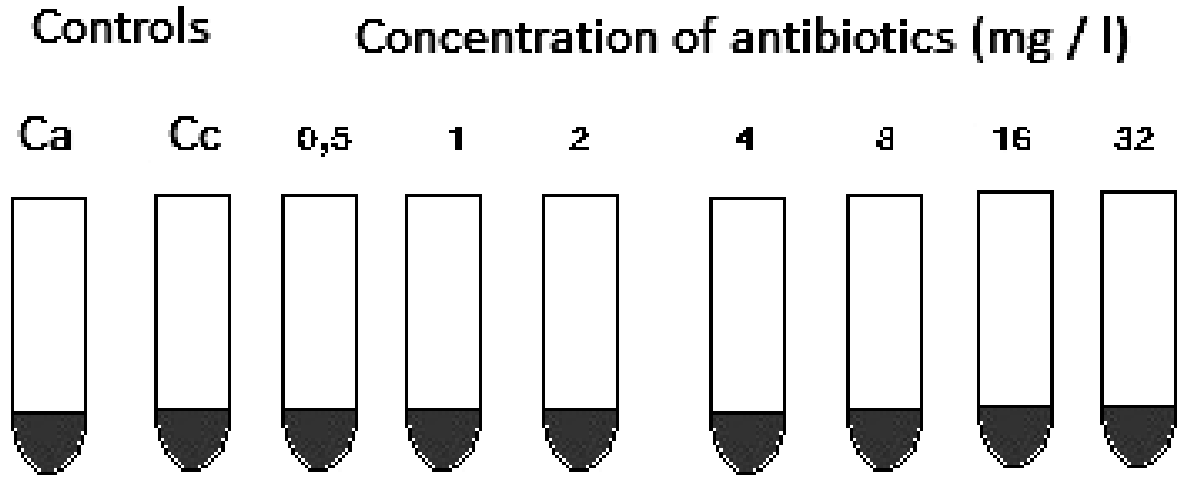
The results shown above **cannot be taken** into account – the tested bacterial strain has lost viability, because even in the control of the bacterial culture and, respectively, in all dilutions of the antibiotic, bacterial growth is not observed.

Viable strain of the tested bacteria should be taken and the test should be repeated.

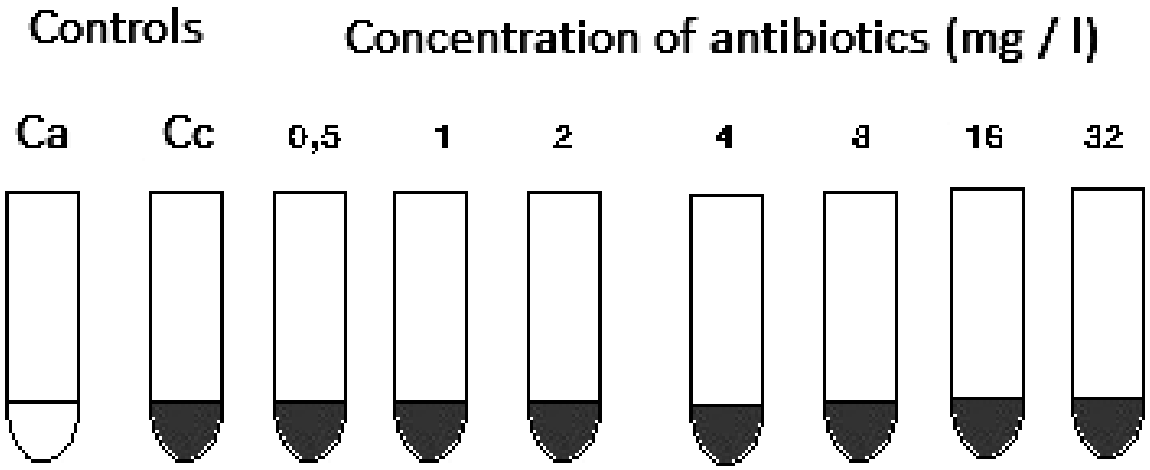
(Above and below: Ca – control of the antibiotic,
Cc – control of the bacterial culture,
white colour of the contents in the tube – no growth,
dark colour of the contents in the tube – bacterial growth is present



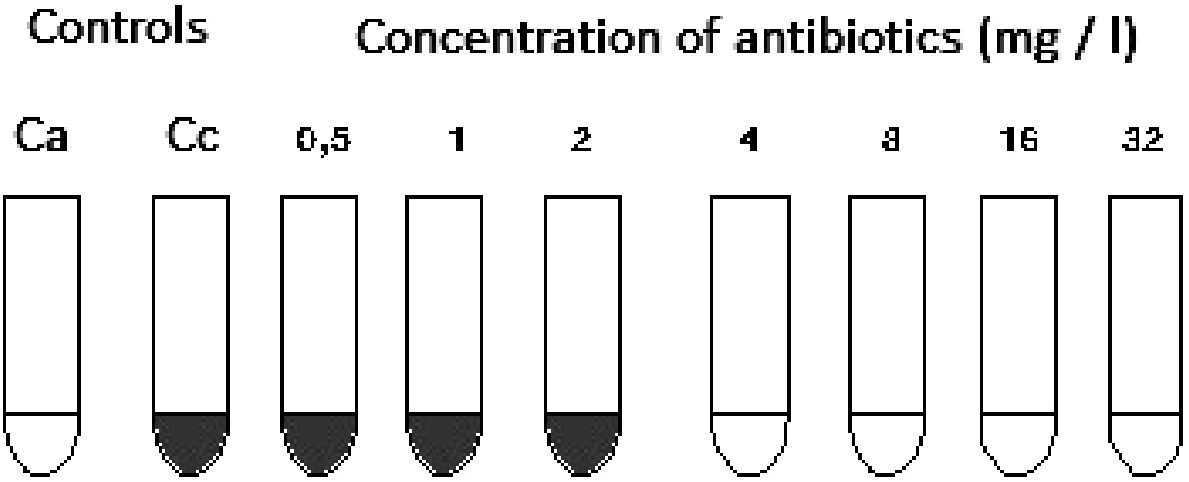
MIC of the antibiotic for the tested strain shown above is less than 0.5 mg/l. **So new dilutions of the antibiotic should be prepared** to get its **concentration less than 0.5 mg/l**, and the test should be repeated



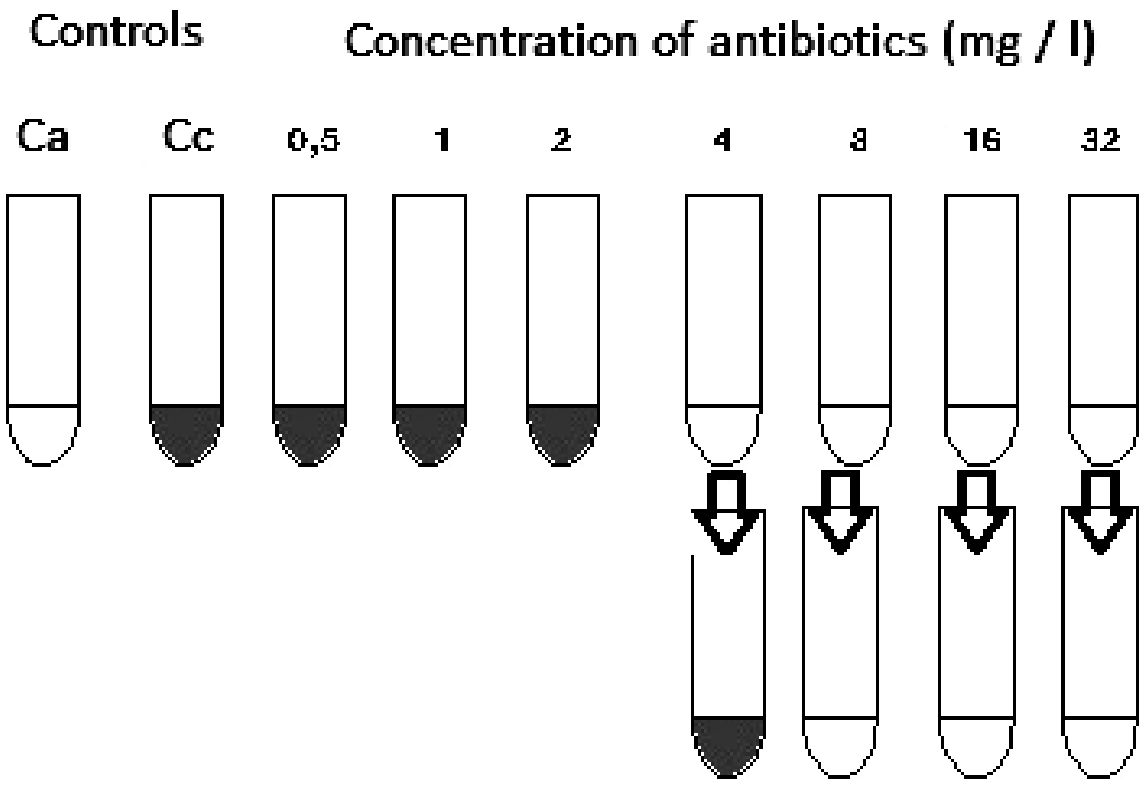
The results shown above cannot be taken into account – **this antibiotic is contaminated with a resistant strain** because bacterial growth is observed in the test tube with control of the antibiotic and, respectively, in all its dilutions. **The new dilutions of the antibiotic should be prepared** using its fresh not contaminated preparation.



MIC of the antibiotic relative to the test strain shown above **is higher than 32 mg/l**; **a new series of antibiotic with concentration greater than 32 mg/l should be prepared**, and the test should be repeated.



MIC of the antibiotic for this tested strain **is = 4 mg/l** (the lowest concentration of the antibiotic that prevents bacterial growth).



MBC of this antibiotic for the tested strain – **8 mg/l**: after seeding the material from the dilution where the concentration of the antibiotic is 4 mg/l into the antibiotic-free medium bacterial growth was present. But for the next **concentration, 8 mg/l, no any visible growth was registered**.