**MINISTRY OF HEALTH SERVISE OF UKRAIN**

**ZAPOROZHYE STATE MEDICAL UNIVERSITY**

***THE CHAIR OF MICROBIOLOGY, VIROLOGY AND IMMUNOLOGY***

# Morphology of microorganisms

## Practicum on Microbiology, Virology and Immunology for English-speaking students II years of the medcal faculty, specialty "Medicine"

**Zaporozhye - 2019**

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*And it is recommended for the use in education process for foreign students.*

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**МІНІСТЕРСТВО ОХОРОНИ ЗДОРОВ'Я УКРАЇНИ**

**Запорізький державний медичний університет**

***Кафедра мікробіології, вірусології та імунології***

# Морфологія мікроорганізмів

## Практикум з мікробіології, вірусології та імунології

**для англомовних студентів**
**II курсу медичного факультету,**
**спеціальність «Медицина»**

**Запоріжжя - 2019**

Практикум з мікробіології, вірусології та імунології для англомовних студентів II курсу медичного факультету, спеціальність «Медицина».

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## *The Subject and Problems of Microbiology.*

Microbiology (Gk. *mikros* small, *bios* life, *logos* science) is the science of minute organisms, invisible to the naked eye, named microbes. It is the study of the laws of the life and development of micro-organisms, and also of the changes which they bring about in animal and plant organisms and in non-living matter.

Modern medical microbiology has become an extensive science. It is subdivided into *bacteriology* — the science of bacteria, the causative agents of a number of infectious diseases; *virology* — the science of viruses, non-cellular living systems capable of causing infectious diseases in man; *immunology* — the science which is concerned with the mechanisms of body protection against pathogenic micro-organisms J and foreign cells and substances; mycology — the study of fungi pathogenie for man, and *protozoology* which deals with pathogenic, unicellular animal organisms. In addition, medical microbiology includes the study of the mechanisms of infection and the methods of specific therapy and prophylaxis of infectious diseases.

## Theme 1. Microbiological laboratory. Safety regulations at work with gas and microorganisms.

### *Rules of work in a laboratory:*

1. Don't be indoors without your special practical clothes (a gown and kb-cap).
2. Don't bring foreign things in a laboratory.
3. Don't leave a laboratory in your special wear.
4. Don't smoke, eat or keep food substances in a laboratory.
5. It is necessary to be carefull while unpacking infectious material: jars, test-tubes should be rubbed dry and put on a tray.
6. If any utensils (jars, test-tubes) containing infectious material break, carry out the procedure for disinfection immediately.

7. Infectious material and needless cultures of bacteria are liable to destruction. Disinfect the instruments used during practice and the table surface after the practicals are completed.
8. After the practicals sponge your hands with soap.
9. Infectious material and cultures of bacteria are put into a safe or a refrigerator after the practicals are completed.

### *Disinfection and sterilization of materials.*

Disinfection means the destruction of all pathogenic organisms or organisms causing infection by using antiseptics. Antiseptics are chemical disinfectants which can be safely applied to skin or mucous membrane surfaces and are used to prevent infection by inhibiting the growth of bacteria. Pathological materials (excrement, urine, phlegm, spinal fluid and blood) are disinfected with 5% Phenol or 3% Lysol before they elimination in sewage system.

Pipettes, glass spatulas and metallic instruments, contaminated by pathological material or cultures of microorganisms, are soaked into jars with disinfectant solutions (3% solution of chloramine).

Object-plates or cover-glasses are disinfected with 3% solution of chloramine because viable microorganisms can preserve in fixed and stained smears, which will be the source of infection.

Laboratory utensils (dishes, test-tubes and bottles) are undergone preliminary disinfection before usage (they are soaked in 3% solution of chloramine for 2-24 hours).

Your hands are disinfected after your work with disinfectious materials. NB! Rub your hands with wool or serviettes soaked in 0.5-1% solution of chloramine, after this wash your hands with warm water and soap.

## 1.1. ORGANIZATION OF THE MICROBOLOGICAL LABORATORY ACTIVITY. MORPHOLOGY OF THE MICROORGANISMS. SIMPLE METHODS OF SPECIMENT STAINING.

**Theme topicality.** Every experienced physician should be able to prepare specimens of any material of a patient or bacterial culture on the glass slide, stain and examine them microscopically in order to determine the morphology of the causative agent for approximate diagnosis of infectious diseases or complications.

**Primary objective**: to be able to carry out microscopic examination of bacterial stained specimens on the specific slide and to distinguish them by morphology.

### QUESTIONS FOR DISCUSSION

1. The rules of practice in microbiology department and in practical bacteriological laboratory.
2. Morphology and classification of microorganisms.
3. Rules of microscopy with the application of oil immersion objective.
4. The rules of preparing specimens of the microorganisms cultures which are cultivated on liquid and solid media.
5. Simple methods of the microorganisms staining.

### PROCEDURE OF PRACTICAL WORK

**Task 1. Learn the safety rules in microbiology laboratory and write them down.**

### GENERAL LABORATORY SAFETY RULES FOR MICROBIOLOGY

1. No smoking, eating, drinking, chewing gum, or applying cosmetics in laboratory area, including laboratory office. No foodstuffs are to be stored in laboratories (including cold rooms, refrigerators, and freezers).

2. Disposable laboratory gloves are not to be worn in communal areas. Door handles, telephones, computer keyboards, and mice (except in clearly defined circumstances), lift buttons, etc. are not to be touched with gloves. If needed, wear one glove and use the ungloved hand to open doors, operate lifts, etc.

3. Rubber or disposable gloves should be worn when handling/working with: human blood or other body fluids, dangerous chemicals, infectious, or potentially infectious materials, ultraviolet light boxes. Select an appropriate glove type.

4. Laboratory gowns or labcoats must always be worn in laboratories, but they should be taken off before entering “clean areas”, e.g., tea room, stores, media, toilets, library, and office rooms.

5. Clothing and footwear must be suitable for laboratory conditions. Flip-flops, sandals or high- heeled shoes are not to be worn in the laboratory. Bare feet are prohibited in the building. Sandals with an enclosed toes and heels are acceptable, but for your own protection, an enclosed shoes are preferable. Long hair must be tied back to avoid contact with microorganisms and equipment.

6. Protective glasses must be worn for all kinds of work involving corrosive or toxic chemicals, radioactivity, and ultraviolet light.

7. The best protection from microorganisms ingestion is avoidance of their penetration in your mouth. Labels and envelopes must not be licked. Pencils and pens must not be laced in the mouth. Biting of fingernails, playing with hair, applying lipstick, eating, drinking, etc., are not allowed. Wash your hands when leaving the laboratory for lunch, etc.

8. Do not pipette by mouth. The use of pipettes with cotton plugs to reduce contamination is preferable. Place pipettes in disinfectant solution to minimize aerosol production. Submerge them for 18–24 hours. Residual volumes from pipettes create aerosols; use mechanical devices that are calibrated to deliver. Fit pipettes to a soft bulb by holding it at the plugged end to avoid the risk of cuts in case the pipette is broken.

9. Minimize the use of “sharps”. Do not bend needles, or try to replace the caps after use. Use syringes fitted with blunt cannulas where possible. Avoid using syringes to mix infectious liquids (if essential, hold the tip of the needle under the surface of the fluid and avoid excessive force). Discard used syringes and needles into an approved sharps container.

10. Hazardous chemical and biological spills and blood spills on the floor, benches or equipment should be cleaned up immediately. Special treatment is required for spills of a biohazardous nature.

11. Hands should be washed after completing each task and always before leaving the laboratory.

12. No running or “horse play”. Report all potential hazards and problems immediately. Try to anticipate potential problems.

13. Any faulty equipment should be removed from service for repair or disposal.

14. When flaming wire loops, draw the loop gradually from the cooler to the hotter part of the flame to minimize spattering, or use electric heaters. Make sure the loop is completely closed and the loop wire is not longer than 6 cm.

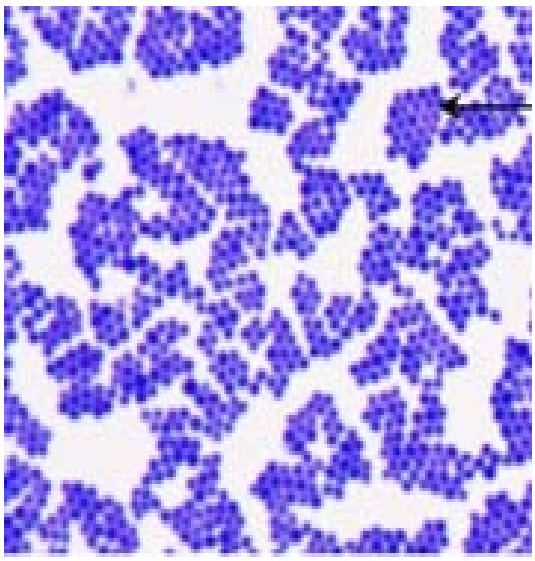
15. Disposable plastic loops must be placed loop-end down in disinfectant for 18–24 hours.

16. Petri dish cultures of fungi should be imprinted and incubated with the lid up permost to prevent the dispersal of fungal spores. Recognize fungi as potential pathogens and be aware of the ability of some species to produce mycotoxins.

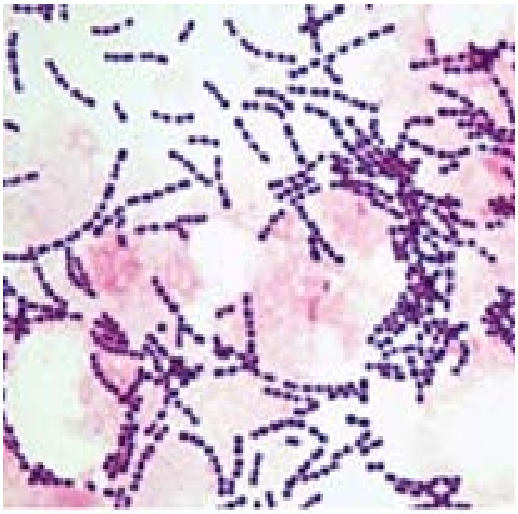
17. Take care when handling Petri dishes that contain condensate. This may contain viable microorganisms that can be spread via droplets or aerosols when the plates are opened or dropped.

18. Open and operate tissue grinders in a biological safety cabinet. Hold glass grinders in a wad of absorbent material and wear gloves. Wait 10 minutes before opening a blender bowl to allow aerosols to settle. Refrigerate to condense aerosols. Use models designed to prevent leakage from rotor bearings and O-ring gaskets or use a “stomacher”.

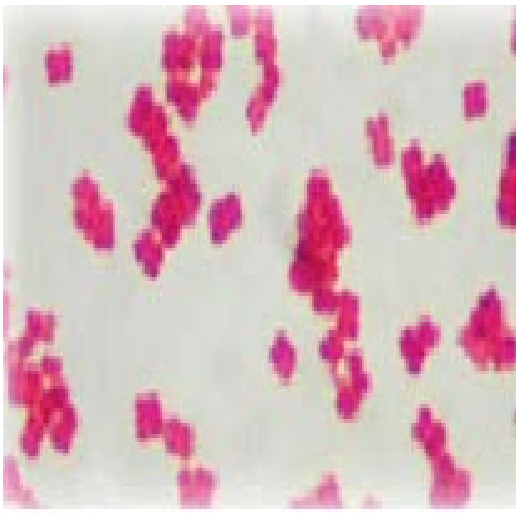
**Task 2. Study products displaying different morphology. Draw (from the demonstration preparations) and name the main coccoid bacterial forms (micrococci, diplococci, tetracocci, sarcina, streptococci, staphylococci).**



All types of *Staphylococcus spp.* have identical staining and morphological properties. *S. aureus* are gram-positive cocci like “bunches of grapes”. Study preparations attentively, put down the results in the protocol.



All streptococci are violet in twisted chains. There are many chains in preparation, because in the result of intensive fission (on a nutrient medium) in one plane streptococci form chains of different length.



***Sarcina spp*. – cocci, located in the form of "packages".**

There are intra- and extracellular diplococci (meningococci) in the spinal fluid. Detection of the causative agent in the spinal fluid is an absolute conformation CNS infectious disease.

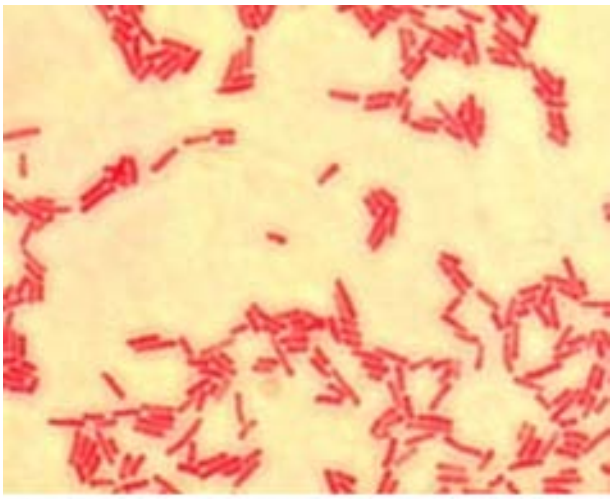
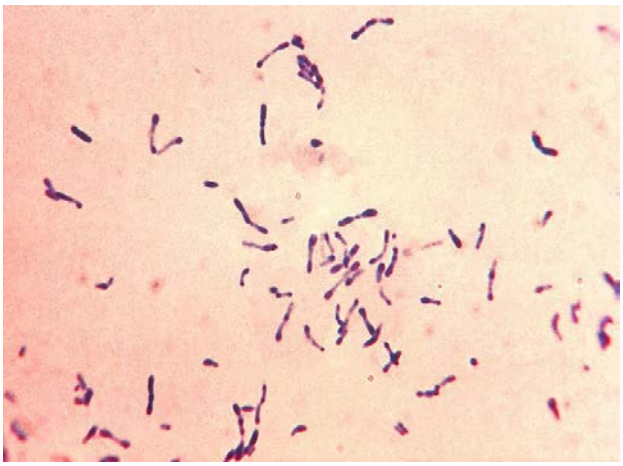


Fig. 5

**Task 3. Perform microscopy and draw the main rod-shaped bacteria (bacteria, bacilli, clostridia). Make the conclusion about their morphology.**

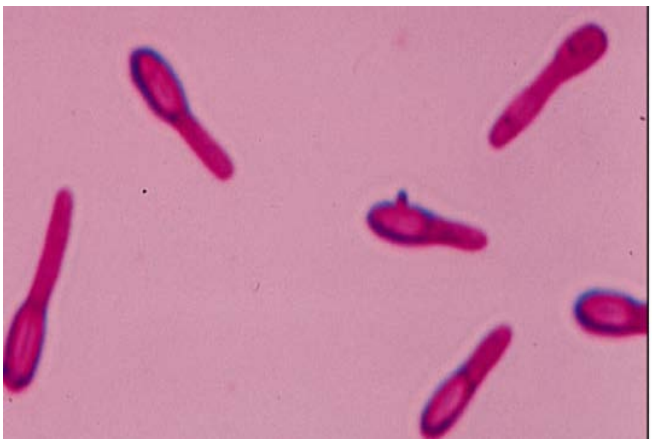
*Escherichia coli* is a short, red, rod-shaped bacterium with rounded ends, 0.5–1.5 μm thick and 24 μm long.

Diphtheria bacteria are violet, pleomorphic, often club-shaped rods. The individual cells tend to group in V, Y, or palisade arrangements.



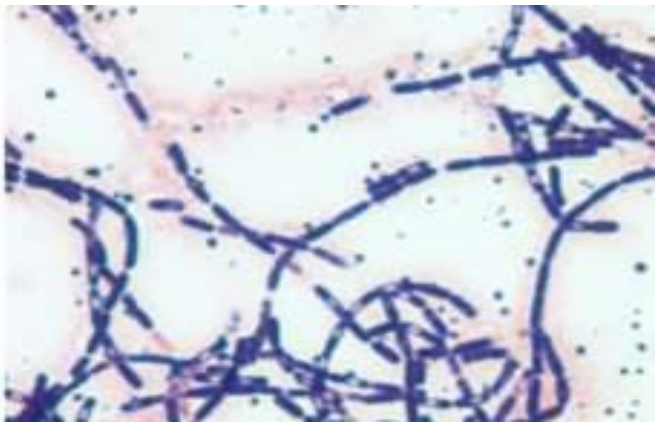
***Corynebacterium diphteriae*,**
**gentian violet staining**

These bacteria are rod-shaped, the length is from 1 to 6 μm. The bacteria are placed isolated and form round terminal spore.



***Clostridium tetani*,**
**water fuchsin staining**

*Bacilli* are rod-shaped bacteria with central spore. After simple staining vegetative part is violet, spore is colourless.



***Bacillus cereus*,**
**gentian violet staining**

**Task 4. Learn the course of rays in the dry and oil immersion system.**

Work with a microscope consists of two main stages:

1) Proper installation of visual field illuminance of the specimen.
2) Microscopy of specimens with different lenses.

Stained bacteria specimens are microscopically examined only by means of immersion lens (x90).

**Rules of work with immersion system:**

1. Lift condenser up to the level of the objective table, open the diaphragm of the microscope.

2. By means of eyepiece 15×, objective 8×, and flat mirror, achieve the maximal visual field illuminance.

3. Put a preparation on the objective table, fix its plugs, by means of the macroscrew find the contours of the image, and by means of the microscrew – obtain precise image.

4. Turning a nosepiece of the microscope, place an immersion objective 90× above the slide; dip it in the oil immersion.

5. Turning the microscrew of the microscope, obtain precise image of the preparation, study microorganism morphology, determine the increase, at which the preparation was investigated.

6. After the termination of microscopy lift a tube, take the preparation away, wipe an objective from oil immersion, carefully turn it aside and lower a tube.

**Possible errors when using a microscope:**

1. Wrong choice of mirrors.
2. Incorrect installation of condenser and iris diaphragm, which leads to inadequate illumination of the object.
3. Wrong choice of the lens for investigation.
4. Incorrect installation of the nosepiece, etc.

Morphological and cytological features of the microorganisms cells can be studied using various methods of microscopy and some methods of staining. The choice of methods and means of microscopic investigation of stain is determined by specific study purpose. So, if we want to detect motion, we should prepare wet-mount preparation or hanging drop; capsules are detected by means of Burri-Gins staining, etc.

**Microscope maintenance**

The microscope should be kept clean and in prevention from mechanical damage. At transportation it is taken by the column and supported by the other hand at the bottom of the rack.

Before work there should be microscope health check. Particular attention should be paid to optical nurity. Do not touch the lens surface – fingerprints are always produsedon it. Eyepiece lens pollution can be detected by moving it under direct vision. Do not disassemble lenses and adjust mechanical parts

.

After completion of work the microscope should be brought in order. Oil should be wiped carefully from the imersion lens with the help of special cloth. Nosepiece is transferred to a small dry lens 8x. Aperture is fully open, condenser is slightly lower. The cloth is put on the objective table. The microscope tube is lowered down as much as possible. Microscope is covered with polyethylene cover.

**Task 5. Prepare 2 smears of staphylococcal culture on nutrient agar, stain the smears with gentian violet, perform the microscopy, and draw it.**

**Technique of cultures smear preparation grown on the solid nutrient medium**

**Manipulation 1. Smear preparation of the culture grown on the solid nutrient medium.**

For smear preparation take a clean slide. On the opposite part of the glass prepare smear test. In the left hand take a test tube with a physiological solution (NaCl), and in the right – a bacteriological loop.

The loop is calcinated in a flame of a spirit-lamp (sterilized) for destruction of extraneous bacteria. By rotary movements take a wadded fuse out of the test tube, pressing it by the 5th and 4th fingers of the right hand to a palm, and burn the edge of

the test tube. A loop is cautiously entered into a test tube, cooled about an internal surface of the glass.

Then put 1–2 drops of the sodum chloride on the glass slide, burn the edge of the test tube again and close its fuse. Then take a test tube with the grown up culture in the left hand, similarly open it, sterilize the edge of the test tube, enter the loop into the test tube cautiously, grasp a material with a sliding movement, take out a loop from the test tube, burn the edge of the test tube again and close its fuse.

The material on the bacteriological loop is brought in the drop of the sodum chloride and prepared for a suspension, not falling outside the limits of the circle (at correct distribution of the material in smear test at microscopy isolated bacterial cells are visible). After preparation of smear test, bacteriological loop is carefully burned in a flame of the spirit-lamp.

**Manipulation 2. Smear test drying**

Smear test is dried up on air or in warm air stream above a flame of the spirit-lamp, not allowing a drop to begin to boil.

**Manipulation 3. Smear test fixation**

Dried up smear tests are physically or chemically fixed for speciments obtaining. During fixation bacteria die, they are better stained and are densely attached to the surface of the glass. Fixe the smear by spent it three times (in 3–5 seconds) through the flame of the spirit-lamp (smear test upwards), carrying out physical fixation. Smear blood tests and smear-prints from organs are fixed, by immersing them for 5–20 minutes in methyl or ethyl spirit (chemical fixation).

**Manipulation 4. Smear staining by simple method**

The prepared specimen is stained by the certain dye, for example, methylene blue (for 3–5 minutes), solution of Pfeiffer's fuchsine (for 1–2 minutes), and gentian violet (for 1–2 minutes). The simple method of staining allows to reveal microorganisms in a material, to define their quantity, shapes, and arrangement.

**Task 6. Prepare 2 smears of colibacterial culture on nutrient broth, stain the smear with using water solution of fuchsin, perform the microscopy, and draw it.**

**Technique of cultures smear preparation grown on the liquid nutrient medium**

**Manipulation 1. Smear test preparation of the culture grown on the liquid nutrient medium.**

On the prepared glass slide a drop of the liquid nutrient medium with microorganisms is put with a bacteriological loop and in regular intervals it is distributed on the slide.

**Manipulation 2. Smear test drying**

**Manipulation 3. Smear test fixation**

**Manipulation 4.Smear staining by simple method** – this stage of smear preparation takes the same as in the task 5.

**Table 1. – Organization of microbiological laboratory activity. Morphology of microorganisms. Simple methods of staining preparations**

| Notion | Definition/ explanation |
|---|---|
| Microbiology | Microbiology is the study (*logy*) of very small (*micro*) living (*bio*) organisms. Often, people are scared by the topic's name of the theme and by science itself |
| Bacteria | Bacteria are relatively simple in structure. They are procaryotic simple unicellular organism with no nuclear membrane, mitochondria, |

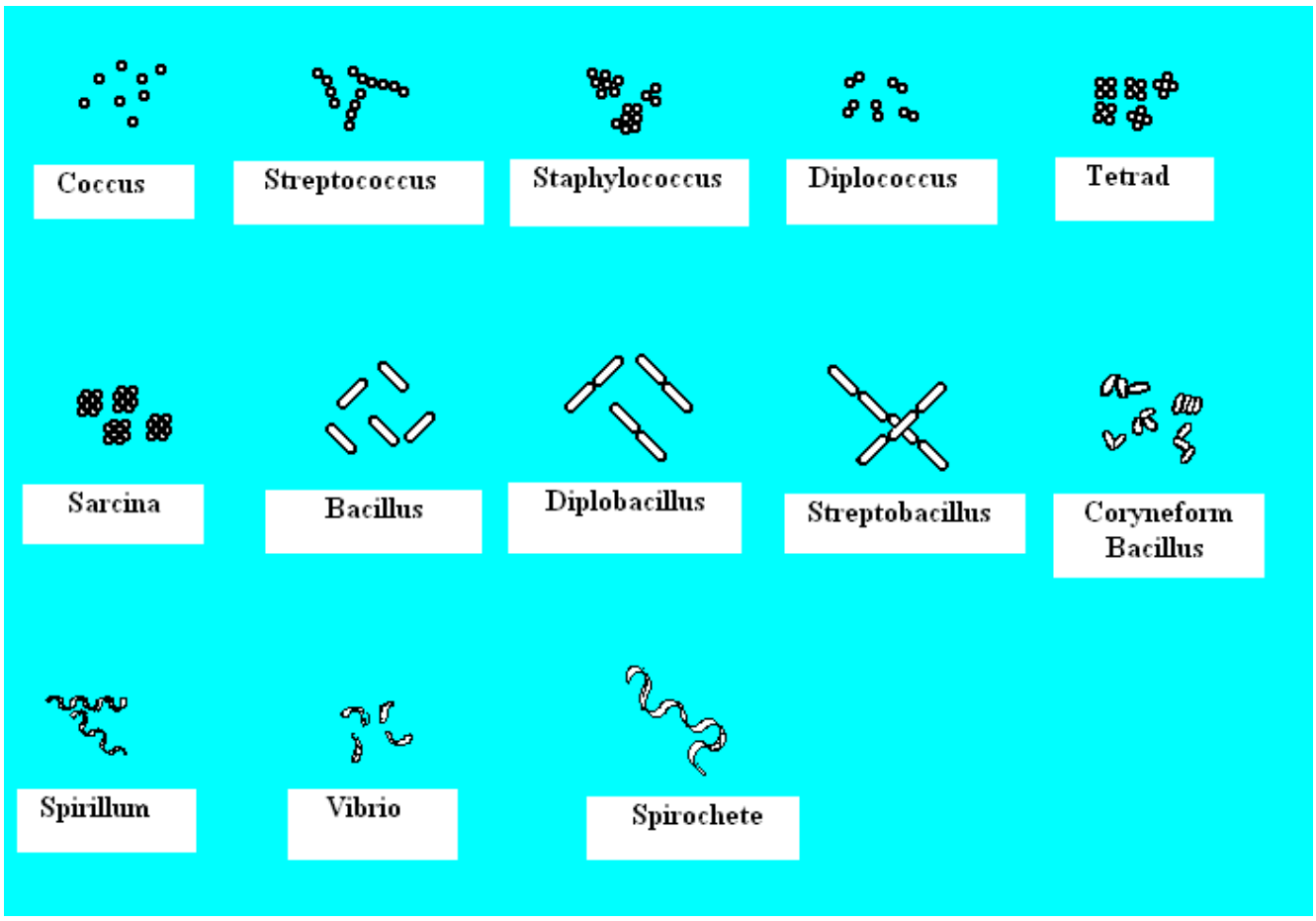
| | |
|---|---|
| | Golgi bodies, or endoplasmic reticulum that reproduces by asexual division.<br>Although the cell wall encircling bacteria is itself complex, |

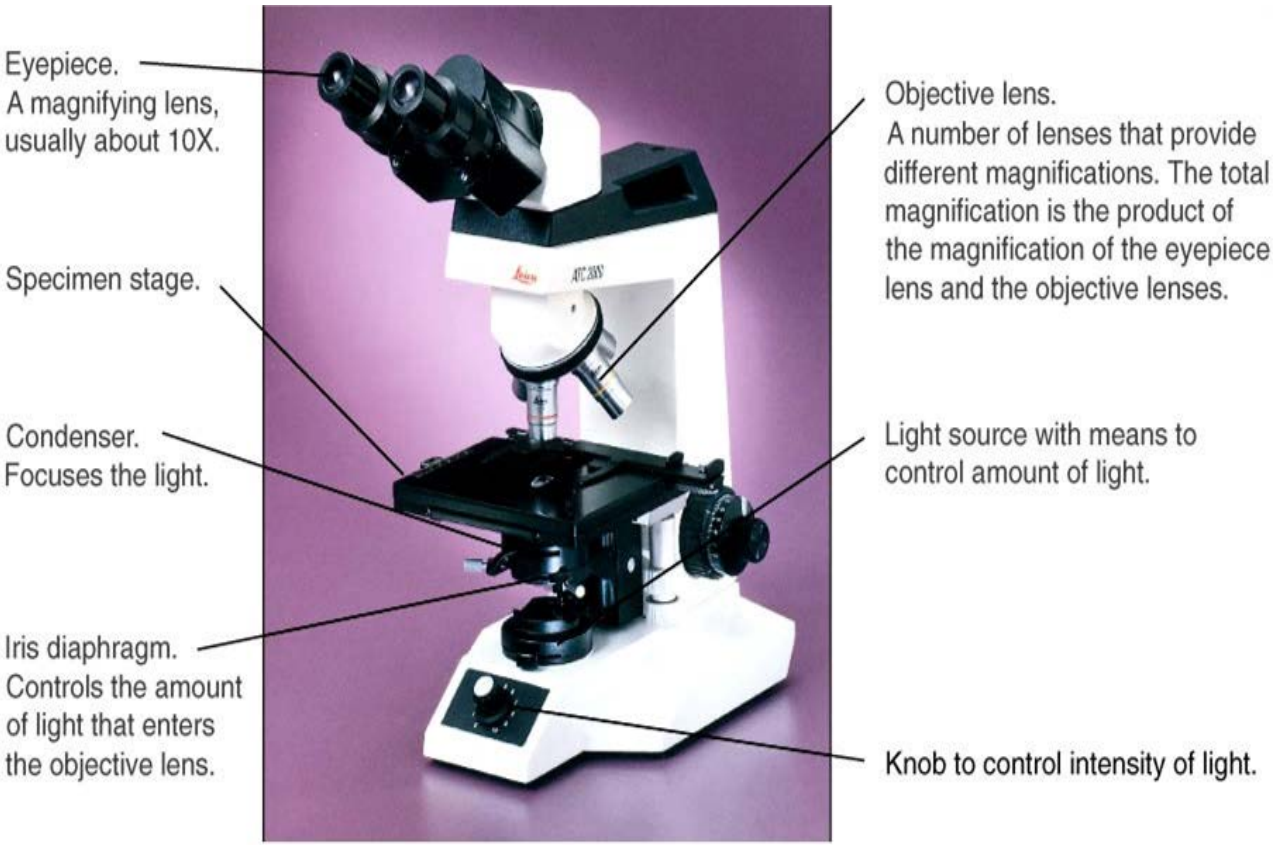
| | |
|---|---|
| | there are two basic forms: gram-positive cell wall with a thick peptidoglycan layer and gram-negative cell wall with a thin peptidoglycan layer and an overlaying outer membrane. Some bacteria lack this cell wall structure and compensate them by surviving only inside host cells or in a hypertonic medium.<br>The human body is initiated by thousands of different bacterial species. Some of them are living transiently, others in a permanent parasitic relationship.<br>Likewise, the environment that surrounds us, including the air we breathe, water we drink, and food we eat, is inhabitated by bacteria, many of which are relatively avirulent and some of which are capable of producing life-threatening diseases |
| Prokaryote / procariote | Prokaryotes are organisms whose cells lack a nucleus and other organelles.<br>Prokaryotes are divided into two distinct groups: the bacteria and the archaea, which scientists believe have unique evolutionary lineages |
| Differences between eukaryotic and prokaryotic cells | 1.a.i.1. Prokaryotic cell is simpler than eukaryotic cell at every level, with one exception: the cell wall may be more complex.<br>1.a.i.2. Prokaryotic cell is smaller than eukaryotic cell.<br>1.a.i.3. Cytoplasm is enclosed within a lipoprotein cell membrane, similar to the prokaryotic cell membrane.<br>1.a.i.4. Eucaryotic cell has a membrane-enclosed nucleus. Despite on eukaryotes, prokaryotes lack a membrane-delimited nucleus. They nave a nucleoid. The bacterial nucleoid contains the DNA fibrils and is not separated from the surrounding cytoplasm by membrane.<br>1.a.i.5. Prokaryotic cells lack autonomous plastids, such as mitochondria, Golgi apparatus and chloroplasts.<br>1.a.i.6. Microtubular structures distinguishing for eukaryotic cells are generally absent in prokaryotes |
| The main | Its nucleus appears as a simple, homogeneous body, |

| | |
|---|---|
| distinguishing features of the prokaryotic cell | containing a single chromosome, not possessing a nuclear membrane separating it from the cytoplasm, nor a nucleolus, nor a spindle, nor a number of separate non-identical chromosomes.<br>It reproduces by binary fission, not by mitotic division |
| The main distinguishing features of the prokaryotic cell | Its rigid cell wall contains as its main strengthening element a specific peptidoglycan (substance not found in eukaryotic organisms).<br>Steroids are absent in the procaryotic cell wall (with the exception of mycoplasmas) |
| Principle of the microscopy with oil immersion | Placing a drop of oil with the same refractive index as glass between the cover slip and objective lens eliminates two refractive surfaces, so that magnifications of 1000x or greater can be achieved while still preserving good resolution |
| Structure of the light microscope | 1. Mechanical part.<br>2. Optical part |
| Optical parts of the microscope | Lighting system, objective, ocular |
| One of the important characteristics of the objective lens | The resolution, which eventually leads to resolution of the microscope as a whole. It defines the smallest distance between two points on the specimens, in which their |
| | image will be separate. The resolution of lens depends on its numerical aperture and wavelength of light, where the investigated object is |
| The main methods of bacteria staining | 1. Simple.<br>2. Complex |
| Features of the simple staining method | Dyes are used for the simple staining method. This method is called tentative because it is used only for detection of bacteria in the investigated materials, |

| | |
|---|---|
| | determination of their number, shape, and mutual arrangement |
| Methods of smears fixation | 1. Physical.<br>2. Chemical |
| The purpose of smears fixation | 1. Fixation of the material on the slide.<br>2. Sterilization.<br>3.Staining is the most appropriate, because killed bacteria perceive aniline dyes better |
| Basic properties of bacteria classification | 1. Morphological characteristics.<br>2. Staining properties.<br>3. Cultural properties.<br>4. Motility of bacteria.<br>5. Spore formation.<br>6. Physiological properties.<br>7. Biochemical properties.<br>8. Sensitivity to specific bacteriophages.<br>9. Antigenic properties.<br>10. Chemistry of cell walls.<br>11. Lipid and fat composition.<br>12. Protein spectra.<br>13. Genetic properties<br>2. |
| Morphological features | Shape, size, and nature of the mutual arrangement of cells spore formation, presence of capsules |
| Staining properties | The features of different cells to stain in different dyes |
| Types of bacteria | Bacteria have three basic shapes: spherical (round), rod-shaped, and spiraled. A round bacterium is called a coccus (plural, cocci). A rod-shaped organism is called a bacillus (plural, bacilli) or simply a rod.<br>A spiraled bacterium with at least two or three curves in its body is called a spirillum (plural, spirilla).<br>Long sinuous organisms with many loose or tight coils are called spirochaetes |

| | |
|---|---|
| Cocci | The patterns formed by bacterial cells grouping together as they multiply are often characteristic for individual bacterial genera or species.<br>Cocci may occur in pairs (diplococci), chains (streptococci), clusters (staphylococci), or packets of four (tetrads), and are seldom found singly |
| Rod-shaped bacteria (bacilli) | Rod-shaped bacteria (bacilli) generally occur as individual cells, but they may appear as end-to-end pairs (diplobacilli) or line up in chains (streptobacilli).<br>Some species tend to palisade, that is, line up in bundles of parallel bacilli, others may form V, X, or Y figures as they divide and split. Some may show great variation in their size and length (pleomorphism) |
| Spiral forms of bacteria | Vibrio, spirochaete, spirilla |


Coccus
Streptococcus
Staphylococcus
Diplococcus
Tetrad
Sarcina
Bacillus
Diplobacillus
Streptobacillus
Coryneform Bacillus
Spirillum
Vibrio
Spirochete


Eyepiece.
A magnifying lens, usually about 10X.
Specimen stage.
Condenser.
Focuses the light.
Iris diaphragm.
Controls the amount of light that enters the objective lens.
Objective lens.
A number of lenses that provide different magnifications. The total magnification is the product of the magnification of the eyepiece lens and the objective lenses.
Light source with means to control amount of light.
Knob to control intensity of light.

## 1.2 STRUCTURE OF THE BACTERIAL CELLS. COMPLEX METHOD OF SPECIMENS STAINING. MICROSCOPIC METHOD OF DIAGNOSIS

**Theme topicality.** Each microorganism has a specific morphological structure and tinctorial properties; knowledge of these properties helps to identify the causative agent.

Complex staining methods allow to differentiate bacteria and to study the structural characteristics of bacterial cells.

Therefore, for causative agent identification it is important to be able to stain specimens and to distinguish different forms of the major groups of microorganisms.

**Primary objective**: to be able to detect and identify different structures of the microorganisms.

## QUESTIONS FOR DISCUSSION

1. Cell wall of gram-positive and gram-negative bacteria: structure, chemical composition, and thickness of the cell wall in gram-positive and gram-negative bacteria. Cell wall of acid-fast bacteria.

2. Complex methods of the specimens staining – Gram staining: principle and staining procedure of the method. Purpose of each step in the Gram staining procedure. Example of gram-positive and gram-negative bacteria (in Latin).

3. Features of the morphological organization of protoplasts, spheroplasts, and L-forms of bacteria.

4. Acid-fast bacteria. The method of acid-fast bacteria staining: Ziehl-Neelsen staining.

5. Cytoplasmic membrane, mesosome, cytoplasm, nucleoid, ribosomes, inclusion: structure and functions.

6. Bacterial capsule: structure, chemical composition, and functions. Complex methods of the specimens staining. Burri-Gins staining, principles and stain procedure.

7. Spores: structure, and functions. Formation and germination of spore.

8. Spore staining: Anjesky`s method.

9. Flagella: the structure, chemical composition, functions. Methods of examination motility: hanging drop and wet-mount techniques.

10. Microscopic method for diagnostic infection diseases.

## PROCEDURE OF PRACTICAL WORK

**Task 1. Study the structure of gram-positive and gram-negative bacteria and characteristics of their cell wall.**

**Table 1.2.1 – The structure of gram-positive and gram-negative bacteria and characteristics of their cell wall**

| **Characteristic of the cell wall** | |
|---|---|
| **Criterion** | **Gram-positive bacterium** |
| Thickness | 20–60 nm |
| Lipids | 1–6% |

| | |
|---|---|
| Peptidoglycan | 40–90% |
| Teichoic acids | Present |

**Task 2. Prepare the smears of mixed (staphylococcal and colibacillar) cultures, stain them by Gram's method, perform microscopy and draw it.**

Gram staining is the most useful and cost-effective method applied in the clinical microbiology laboratory.

Differential stain is the most commonly used for direct microscopic examination of specimens and bacterial colonies because it has a broad staining spectrum.

First devised by Hans Christian Joachim Gram late in the 19th century, it has remained the same procedure and serves in dividing bacteria into 2 groups: gram-positive organisms, which retain the primary gentian violet dye and appear deep blue or purple, and gram-negative organisms, which can be decolourized, thereby losing the primary stain and subsequent fuchsin counterstaining and appearing red or pink.

## Gram staining procedure

1. Smear the material to be stained on a slide. Allow it to dry in air. Fix by gentle heating, which kills
bacteria and allows the material to be attached to the slide.
2. Apply an appropriate solution of gentian violet. Stain it for about 1 minute. Wash carefully.
3. Apply iodine solution (a mordant, which strengthens the bond between dye and substrate) for 1 minute. Wash carefully.

4. Apply 95% ethyl alcohol until all but the thickest parts of the smear are decolourized, or for not more than 10 to 15 seconds. Wash.

5. Counterstain with fuchsin for 1 minute. Wash. Dry.

6. Examine the smear using the oil-immersion lens of the light microscope.

7. Gram-positive organisms are blue-purple and gram-negative bacteria are pink-red. If the spesimen is stained correctly, ***gram-positive*** (violet) and ***gram-negative*** (red) bacteria will be visible on microscopy. At the wrong staining all bacteria are the same colour (violet or red).

**Task 3. Prepare the smears of spore-forming culture, stain them by Ziehl-Neelsen method, perform the microscopy and draw it.**

Causative agents of tuberculosis and leprosy of *Mycobacterium* genus have distinctive character called acid fastness, due to the presence of lipids (waterline mycolic acid) in the cell wall.

These organisms quickly absorb red carbolic fuchsin dye in the presence of a detergent or when wanned and retain dye; after washing with an acidified alcohol solution.

All non-acid-fast bacteria, pus, cells, etc. lose carbolic fuchsin when treated with addition of alcohol and take a contrasting counter stain (e.g., methylene blue, brilliant green).

Tubercle bacilli are more strongly acid-fast than other members of the acid-fast group and have a characteristic beaded appesance.

Both Gram staining and acid-fast staining depend on the integrity of the cell wall. Broken or disintegrated bacilli or their parts are neither gram-positive nor acid-fast.

**Ziehl-Neelsen staining procedure**

1. Fix the smear.

2. Flood the slide with carbolic fuchsin, steam gently for 5 minutes over low flame, do not allow to dry and add more stain if necessary. Cool.
Alternatively, carbolic fuchsin-containing phenol and alcohol (cool) may be used without heating.
3. Apply 90% alcohol containing 3% to 5% HC1 until all but the thickest parts of the smear cease to give off colour (approximately 1 to 3 minutes). Wash.
4. Counterstain of 1 minute with methylene blue. Wash.
5. Examine smear using the oil-immersion lens of the light microscope.

**Sources of error:**

1. Overheating (burning) during fixation can be avoided by just touching the back of the slide to the back of the hand each time the slide has been passed though the flame.
2. Do not stain smears which have only been air dried. Smears must also be “fixed”.
3. Smears should not be too thick. After air drying, examine them under the microscope. If there are no areas of bacteria separation, more water should be added to dilute the smear.
4. After staining it is essential that the back surface of the slide is wiped clean.
5. If washing with distilled water is not done adequately, crystallization of the stain may appear on the slide.

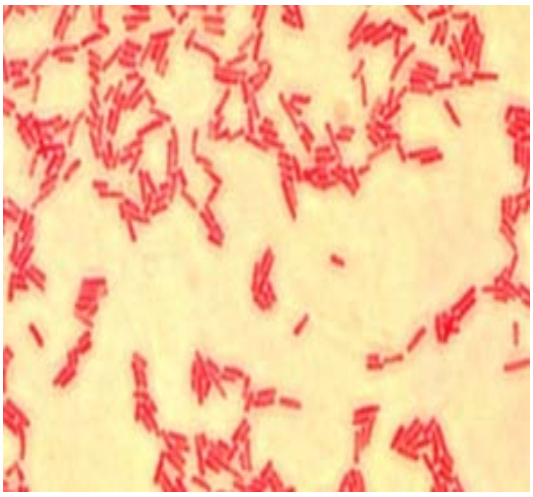
**Task 4. Prepare the smears of spore-forming culture, stain them by Anjesky's method, perform the microscopy and draw it.**

**Anjesky's staining:**
1. A thick smear is dried in the air, treated with 0.5% sulphuric acid, and heated until it steams.

2. The preparation is washed with water, dried, fixed above the flame.

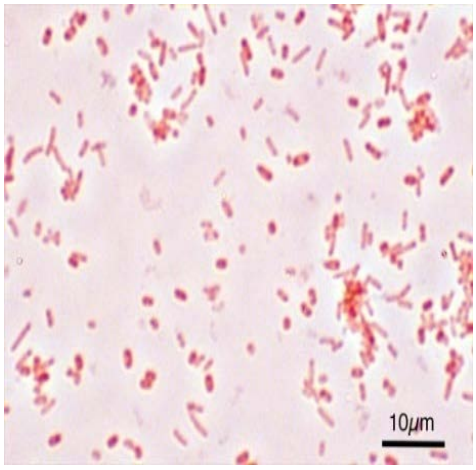
3. The smear is stained by the Ziehl-Neelsen's technique as it is prescribed later. Spores stain pink-red, the cell appears blue.

**Task 5. Study under the microscope slides demonstration and draw them in the protocol.**



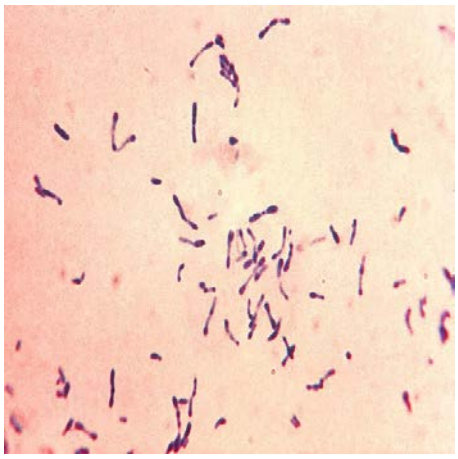
After Gram staining, *Escherichia coli* is short, red, rod-shaped bacterium with rounded ends,
0.5–1.5 μm thick and 24 μm long.

***Escherichia coli,* Gram staining**



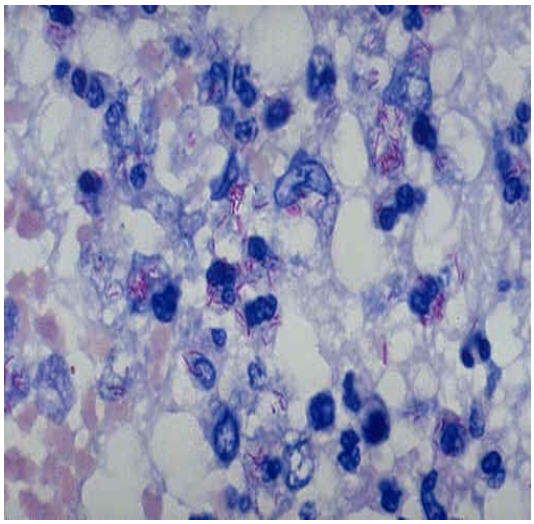

***Salmonella typhi,* Gram staining**

After Gram staining *Salmonella typhi* are short, red rods with rounded ends, 0.5–1.5 μm thick, and 24 μm long.



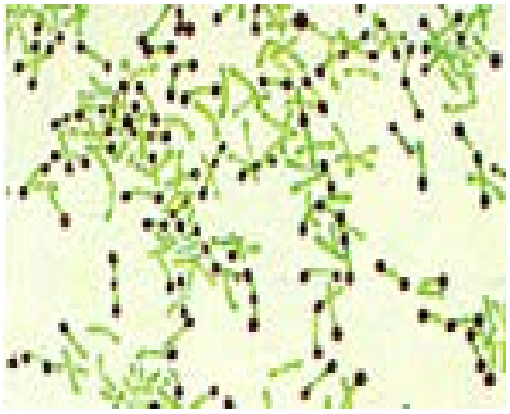
Diphtheria bacteria are violet, pleomorphic, often club-shaped rods. The individual cells tend to group in V, Y, or palisade arrangements.

***Corynebacterium diphteriae*** **in pure culture, Gram staining**



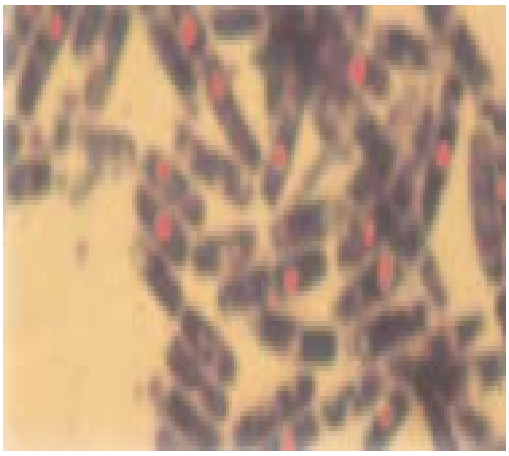
Acid fast stain is used to diagnose the presence of mycobacteria in tissue and cytologic preparations.

***Mycobacterium tuberculosis*** **from patient's sputum (stained by Ziehl-Neelsen method)**



They have the characteristic of forming irregular shaped, club-shaped or V-shaped arrangements.

***Corynebacterium diphteriae* in pure culture, Neisser staining**

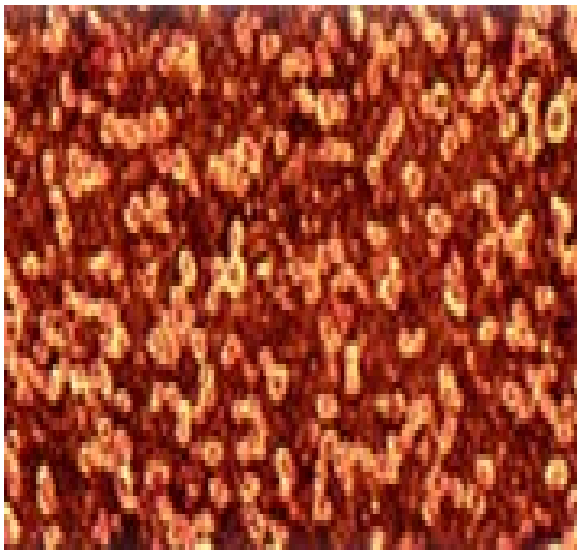


Vegetative part of bacilli is stained red, spore becomes blue.

***Pure Bacillus cereus culture, Ziehl-Neelsen staining***

According with Burri's method living bacteria remain unstained against a dark background. In a drop of Indian ink diluted with distilled water 1 to 10 the culture to be tested is inoculated and spread uniformly with a loop or the edge of the glass slide.

The smear is air dried. Nigrosin, Congo red and other dyes may occasionally be used instead of Indian ink.

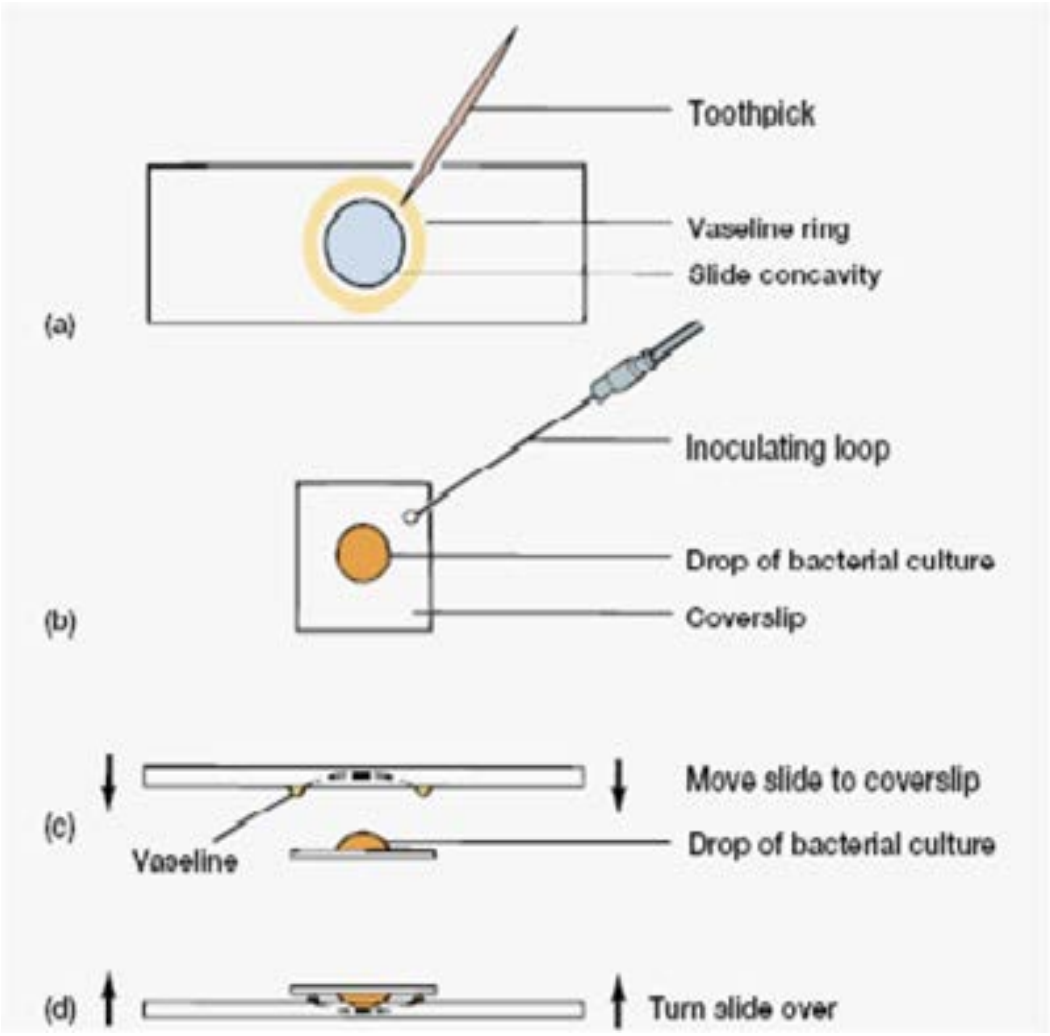


**Klebsiella pneumoniae, Burri staining**

**Gins staining.** Fix an air-dried specimen stained by Burri's method and pipette 2–3 drops of alcohol on it and burn it. Pour Preiser's fuchsin on the cooled slide to act for 3–5 minutes, wash the smear with water and dry it.

In this case the bacteria are stained red, whereas unstained capsules are distinctly outlined against the dark background of the preparation.

Sometimes, small colourless zones can be observed around those stained bacteria that do not form capsules. They are called false capsules and are due to improper drying or fixation of the smear.




**Task 6. Study motile microorganisms by wet-mount and hanging drop technique.**

1. **Wet-mount technique**

1. A drop of the test material, usually 24-hour broth culture of microorganisms, is placed into the centre of the glass slide.

1. The drop is covered with a cover slip in a manner; the fluid should fill the entire space without overflowing.

2. **Hanging drop technique.**

To prepare this kind of specimen, special glass slides with an impression (well) in the centre are used.

1. A small drop of the test material is put in the middle of the cover slip.
2. The edges of the well are ringed with petrolatum.
3. The slide is placed onto the cover slip so that the drop is in the centre of the well.
4. The slide is carefully inverted and the drop hangs in the centre of the impinted, which prevents it from drying.

The prepared specimens are examined microscopically, slightly darkening the microscopic field by lowering the condenser and regulating the entrance of light with a concave mirror.

At first low power magnification is used (objective 8x) to detect the edge of the drop, after which a 40x or an oil-immersion objective is mounted.

Occasionally, molecular (Brownian) motility is mistaken for the microorganisms motility.

To avoid this error, it should be borne in mind that microorganisms propelled by flagella may traverse the entire microscopic field and make circular and rotatory movements.

After the examination the wet-mount and hanging-drop preparations should be immersed in the separate bath with disinfectant solution to kill the microorganisms investigated.

**Task 7. Study the principles of microbiological methods and draw the scheme.**

**Table 1.2.2 – Structure of the bacterial cells.**
**Complex method of preparations staining.**

**Microscopic diagnostic method**

| Notion | Definition/explanation |
|---|---|
| Cell wall | Outer covering of most cells that protects the bacterial cell and gives shape to it |
| The main functions of the cell wall | 1. Cell wall is responsible for the characteristic shape of the cell (rod, coccus, or spiral).<br>2. The strength of the wall is responsible for keeping the cell from bursting when the intracellular osmolarity is much greater than the extracellular osmolarity.<br>3. It has got receptors for chemicals and for bacteriophages (reception function).<br>4. The chemical components of bacterial cell are antigens.<br>5. The cell envelope of the Gram-negative bacteria includes endotoxin.<br>6. It is a rigid platform for surface appendages- flagella, fimbriae, and pili |

| | |
|---|---|
| Peptidoglycan | Peptidoglycan is a huge polymer composed of interlocking chains of identical monomers, the backbone composed of two derivatives of glucose: N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM). The NAG and NAM strands are connected by interpeptide bridges.<br>The rigid peptidoglycan polymer gives the cell its shape and provides protection from the external environment.<br>From the peptidoglycan inwards all bacteria are very similar. Going further out, the bacterial world divides into two major classes: gram-positive and gram-negative cells |
| Gram staining | The Danish bacteriologist J. M. C. Gram (1853–1938) devised a method of bacteria staining using a dye called crystal (gentian) violet. Gram's method helps distinguish different types of bacteria.<br>The characteristics of bacteria Gram staining are denoted as positive or negative, depending upon whether the bacteria take up and retain the crystal violet stain or not.<br>Gram-positive bacteria retain the colour of the crystal violet stain in the Gram stain.<br>This is characteristic of bacteria that have a cell wall composed of a thick layer of a particular substance (specifically, peptidologlycan containing teichoic and lipoteichoic acid complexed to the peptidoglycan).<br>Gram-positive bacteria include staphylococci (“staph”), streptococci (“strep”), pneumococci, and the bacterium responsible for diphtheria (*Corynebacterium diphtheriae*) and anthrax (*Bacillus anthracis*).<br>Gram-negative bacteria lose the crystal violet stain (and take the colour of the red counterstain) in Gram's method.<br>This is characteristic of bacteria that have a cell wall composed of a thin layer of a particular substance (specifically, peptidoglycan is covered by an outer membrane of lipoprotein and lipopolysaccharide - containing endotoxin).<br>Gram-negative bacteria include most of the bacteria |

| | |
|---|---|
| | normally found in the gastrointestinal tract that can be responsible for disease as well as gonococci (venereal disease) and meningococci (bacterial meningitis).<br>The organisms responsible for cholera and bubonic plague are gram-negative |
| Gram-positive bacteria | Gram-positive cells, peptidoglycan makes up as much as 90% of the thick, compact cell wall, and is the outermost layer of the cell.<br>The location and thickness of peptidoglycan is the important factor that results in gram-positive cells staining differently than gram-negative cells |
| Gram-negative bacteria | The cell walls of gram-negative bacteria are more chemically complex, thinner and less compact, with peptidoglycan comprising only 5 to 20% of the cell wall. In gram-negative cells, peptidoglycan is not the outermost |

Table 1.2.2 continuation

| **Notion** | **Definition/explanation** |
|---|---|
| | layer, but is located between the plasma membrane and an outer lipopolysaccharide (LPS) membrane |
| Cytoplasm | A gel-like substance composed mainly of water that also contains enzymes, salts, cell components, and various organic molecules |
| Cytoplasmic structures | Prokaryotic cells lack autonomous plastids, such as mitochondria and chloroplasts; the electron transport enzymes are localized instead in the cytoplasmic membrane.<br>The photosynthetic pigments (carotenoids, bacteriochlorophylls) of photosynthetic bacteria are integrated into internal membrane systems formed by invagination of the cytoplasmic membrane, the cytoplasmic membrane itself, or specialized non-unit membrane-enclosed structures called chlorosomes |

| | |
|---|---|
| Cell membrane or plasma membrane | Surrounds the cell's cytoplasm and regulates the flow of substances in and out of the cell |
| Nucleoid | Prokaryotic nucleoid, the equivalent of the eukaryotic nucleus, can be seen with the light microscope in stained material. It is Feulgen-positive, indicating the presence of DNA |
| Pili | Hair-like structures on the surface of the cell that attach to other bacterial cells. Shorter pili called fimbriae help bacteria attach to surfaces |
| Lipopolysaccharide membrane | The outer membrane of Gram negative bacteria is similar to the plasma membrane, but less permeable and composed of lipopolysaccharides (LPS), a harmful substance classified as an endotoxin. |
| Periplasmic space | The space between the cell wall and the plasma membrane is called the periplasm. Periplasm controls molecular traffic entering and leaving the cell. |
| Flagella | Flagella are composed entirely of a single protein subunit called flagellin, which differs in primary structure among different bacterial speciese, and are responsible for bacterial motility that may inhance bacterial invasion.<br>The surface of flagella is made up of protein antigens with diverse epitopes useful in the identification and classification of organisms.<br>They can be single (monotrichous) or multiple (peritrichous). Spirochaetes contain similar motility apparatus, protein in nature, which leis in the periplasmic space between the cytoplasmic membrane and the outer membrane |
| Three parts of flagellum | 1. Filament. 2. Hook. 3. Basal body. Flagella are made up of proteins (flagellin) similar to keratin or myosin |

| | |
|---|---|
| Number and arrangement of flagella on bacterial body | 1. Monotrichate: one flagellum at one end of the organism, e.g., vibrio, pseudomonas, spirillum, etc.<br>2. Amphitrichate: one flageilum at both poles, e.g., *Alcaligenes fecalis*.<br>3. Lophotrichate: a tuft of flagella at the end, e.g., pseudomonas.<br>4. Peritrichous: several flagella are present all over the surface of bacterium, e.g. *Escherichia coli*, *Salmonella spp.* |
| Function of flagella | It is responsible for bacterial motility. Motility may be observed microscopically (hanging drop preparation) or detecting the spreading growth in semisolid agar medium.<br>It has been suggested that heat formed as a result of metabolism is given off to the environment through the flagella, while ATP serves as an energy source.<br>The created difference in temperature causes a stream of water along the flagella, and the bacterium moves in the opposite direction |
| Capsules, slime layers | Some bacteria have a layer of material lying outside the cell wall. When the layer is well organized and not easily washed off, it is called a capsule.<br>A slime is a zone of diffuse, unorganized material that is removed easily.<br>A glycocalyx network of polysaccharides extending from the surface of bacteria and other cells (in this sense it could encompass both capsules and slime layers). |

Table 1.2.2 continuation

| **Notion** | **Definition/explanation** |
|---|---|
| | Capsules and slime layers usually are composed of polysaccharides, but they may be constructed of other materials. For example, *Bacillus anthracis* has a capsule of polyD-glutamic acid. |

| | |
|---|---|
| | Capsules contain a great deal of water and can protect bacteria against desiccation |
| Two categories of capsules | Capsules may be divided into two categories, macro capsules and microcapsules.<br>Macrocapsules are at least 0.2 μm thick, and can be seen under the light microscope.<br>Microcapsules cannot be seen under the light microscope but can be demonstrated immunologically.<br>In some aerobic bacteria the capsules form a raft in which are found the actively growing cells |
| Function of the capsule in bacteria | Attachment to surfaces; protection against phagocytic engulfment, occasionally killing or digestion; reserve of nutrients or protection against desiccation |
| Spores | Bacteria in genera such as *Bacillus* and *Clostridium* produce quite a resistant structure capable of surviving for long periods in an unfavourable environment and then giving rise to a new bacterial cell.<br>This structure is called an endospore since it develops within the bacterial cell.<br>Endospores are spherical to elliptical in shape and may be either smaller or larger than the parent bacterial cell. Endospore position within the cell is characteristic and may be central, subterminal, or terminal |
| Bacterial endospore | An endospore is not a reproductive structure but rather a resistant, dormant survival form of the organism. It enables bacteria to resist harsh environment.<br>Endospores are quite resistant to high temperatures (including boiling), most disinfectants, low energy radiation, drying, etc.<br>The endospore can survive possibly thousands of years until |

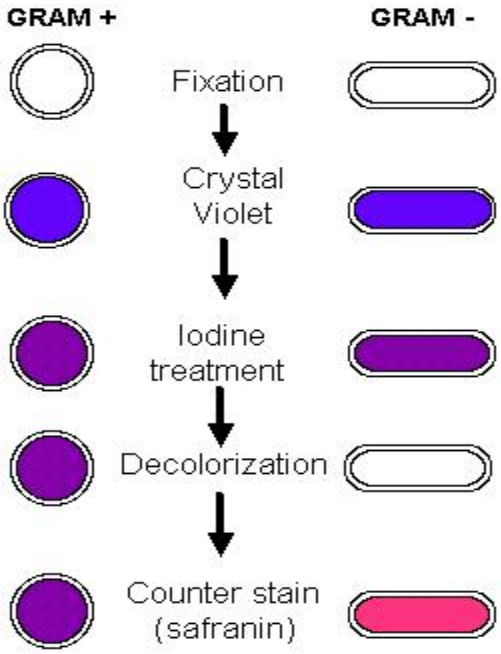
| | |
|---|---|
| | a variety of environmental stimuli trigger germination, allowing outgrowth of a single vegetative bacterium. Spore formation follows a very complex multistage process |
| Bacterial spores | A few species of bacteria have the ability to produce highly resistant structures known as endospores (or simply spores). These resist a range of hazardous environments, and protect against heat, radiation, and desiccation.<br>Endospores form within (hence endo-) special vegetative cells known as sporangia (singular sporangium). Diseases caused by sporing bacteria include botulism (*Clostridium botulinum*), gas gangrene (*Clostridium perfringens*), tetanus (*Clostridium tetani*) and acute food poisoning (*Clostridium perfringens*, again). All these bacteria are anaerobic.<br>The aerobic sporing bacteria can also cause disease. Anthrax is caused by *Bacillus anthracis*. Bacillus cereus causes two types of food poisoning. *Bacillus thuringiensis* produces a toxin that kills insects.<br>This bacterium has gained notoriety by its exploitation in insect-resistant GM plants, but the bacterium itself is used in some organic crop production |
| Three stages of transformation of dormant spores into active vegetative cells | 1. Activation.<br>2. Germination.<br>3. Outgrowth |
| Cycle of spore formation and germination | At the beginning of spore formation, a septum forms, separating the nascent spore from the rest of the cell and all of the genetic material of the cell is copied into the newly-forming cell. The spore contents are dehydrated and the protective outer |

Table 1.2.2 continuation

| **Notion** | **Definition/explanation** |
|---|---|

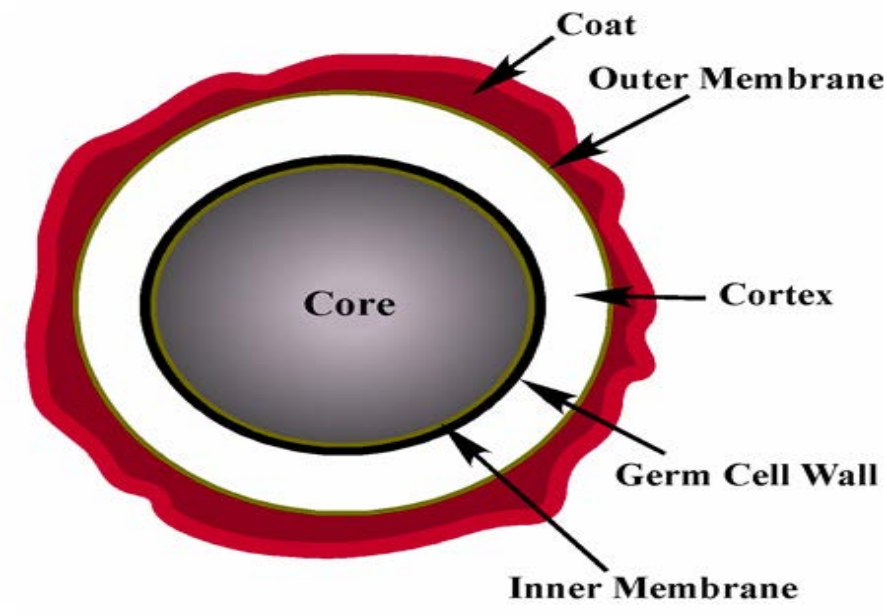
| | |
|---|---|
| | coatings are laid down. Once the spore is matured it is released from the cell.<br>On germination, the spore contents rehydrate and a new bacterium emerges and multiplies |
| Function of the bacterial spore | Bacterial spores are the highly resistant spores. In fact the spores indicate the resting phase of some types of bacteria,which help to tide over the unfavourable conditions. These spores can also be called as endospores.<br>These endospores contain calcium and dipicolenic acid and hence they are very resistant. They are resistant to temperature, UV light, disinfectants, etc. and thus they protect the bacterium. In the favourable conditions these develop into a new bacterium |
| Intracytoplasmatic inclusions | Granular inclusions randomly distributed in the cytoplasm of various species include metabolic reserve particles such as poly-b-hydroxybutyrate, polysaccharide and glycogen-like granules, and polymetaphosphate or metachromatic granules (volutin granules or Babes Ernst granules).<br>They possess high electron density.<br>A characteristic feature of the granules of volutin is their metachromatic stain.<br>Due to high concentrations of metaphosphates and other phosphorous compounds volutin granules (inclusions in the cytoplasm) are characterized by metachromasia.<br>"Upon staining with alkaline methylene blue and acetic-acidic methylene violet, their colour is more intensive as compared to that of the cytoplasm.<br>They are stained reddish-purple with methylene blue while the cytoplasm is stained blue (by Löffler`s staining).<br>Spesial staining techniques such as Neisser`s demonstrate the granules more clearly. They are stained dark-blue or black while the cytoplasm is stained yellow or brownish |
| Principle of microscopic | Microscopy can be done quickly, but accuracy depends on the experience of the microscopist and quality of equipment. |

| | |
|---|---|
| diagnosis of patient’s material | Regulations often limit physicians' use of microscopy for diagnostic purposes outside a certified laboratory.<br>Most specimens are treated with stains that stain pathogens, causing them to stand out from the background, although wet mounts of unstained samples can be used to detect fungi, parasites (including helminth eggs and larvae), vaginal clue cells, motile organisms (eg, Trichomonas), and syphilis (via darkfield microscopy).<br>Visibility of fungi can be increased by applying 10% potassium hydroxide (KOH) to dissolve surrounding tissues and nonfungal organisms |

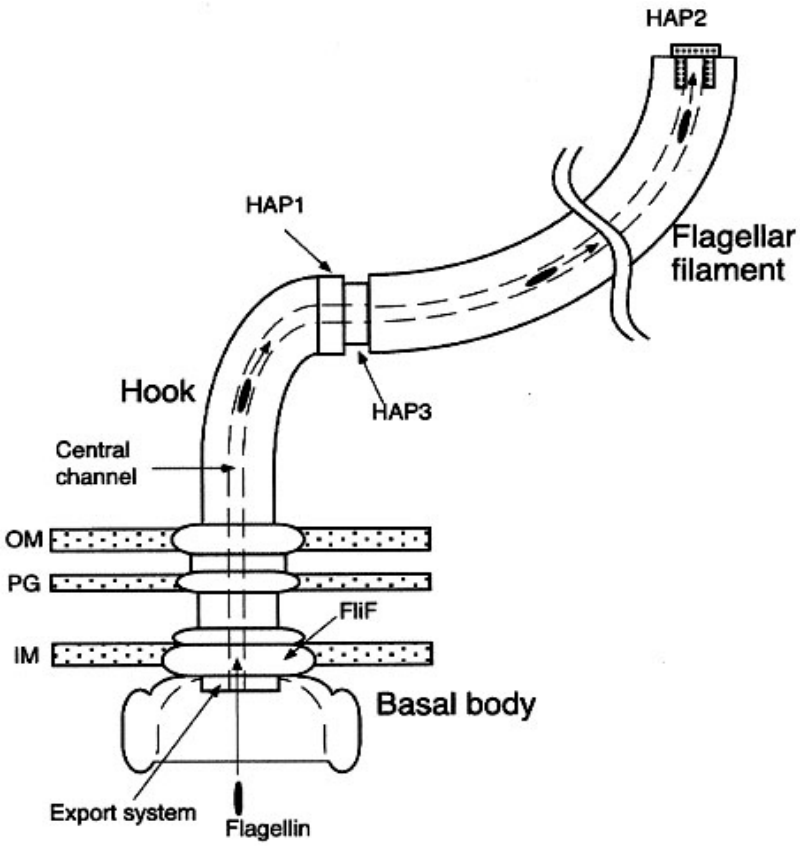



***Staphylococcus aureus*** ***Salmonella typhi***

**Gram staining technique**




**Bacterial spore**



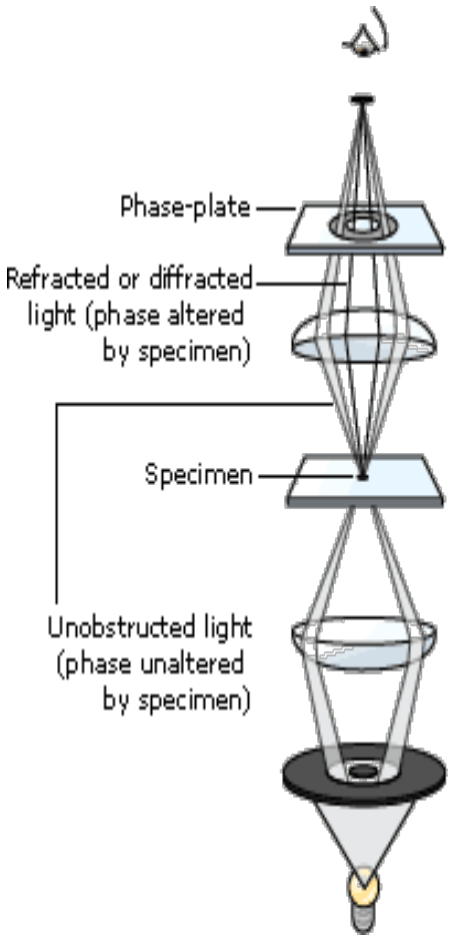

**Structure of flagella**

### 1.3 FEATURES OF CHLAMYDIAE, SPIROCHAETES, RICKETTSIA, AND MYCOPLASMAS ULTRASTRUCTURE. MODERN METHODS OF MICROSCOPIC EXAMINATION

**Theme topicality.** Microscopic method is used for various infectious diseases diagnosis. Such bacteria as spirochaetes, rickettsia, chlamydia, and mycoplasma have special features in the cells structure. These features must be considered during identification. Therefore, every experienced physician must know the peculiarities of the different bacteria structures and be familiar with modern methods of microscopic investigation.

**Primary objective:** to know the structure of spirochaetes, rickettsia, chlamydia, mycoplasma; to be able to conduct microscopic examination of native and fixed specimens using different methods of microscopy.

## QUESTIONS FOR DISCUSSION




1. Morphology of spirochaetes. Peculiarities of ultrastructure of spirochaetes. Differences between structure of *Treponema spp.*, *Borrelia spp.*, and *Leptospira spp.*
2. Morphology of *Ricketsia spp.*, *Chlamidia spp.*, *Mycoplasma spp.* Features of these bacteria ultrastructure. Examples.
3. Life cycle of chlamydia.
4. Modern methods of microscopic examinations: purpose of use, principles.

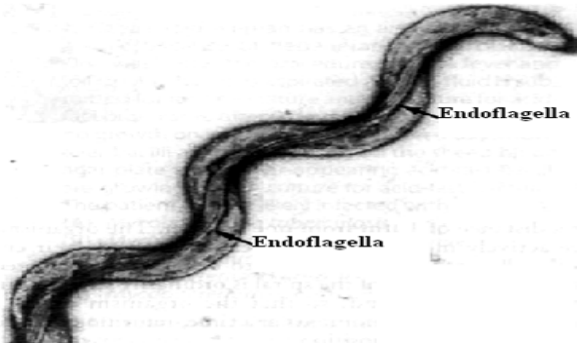
## PROCEDURE OF PRACTICAL WORK

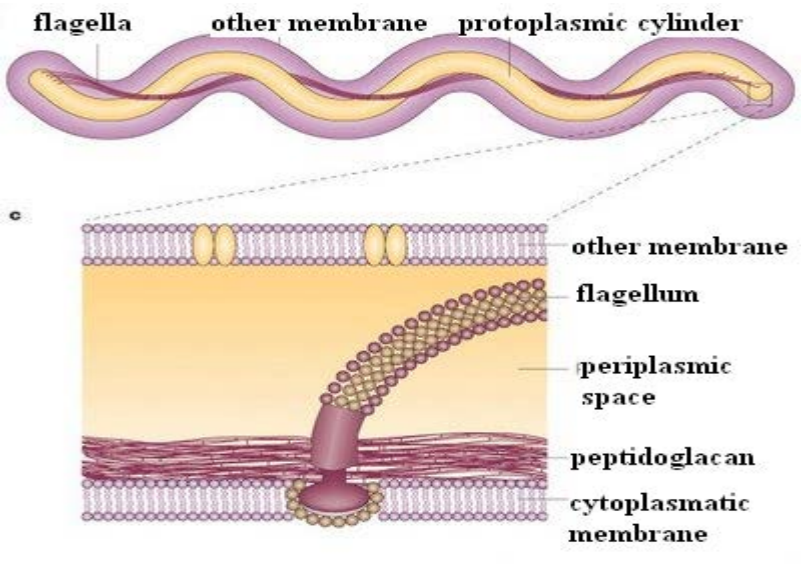
**Task 1. Study the electron micrograph of whole-mounted *Treponema pallidum* subspecies *pallidum*.**

*T. pallidum* has the endoflagella in the periplasmic space between the inner membrane and the outer membrane.

**Electron micrograph of thin-sectioned *T. pallidum***

**Task 2. Study the fine structures of *Treponema pallidum*.**






**Fine structures of *Treponema pallidum***

**Task 3. Study the basic method of microscopic examination of spirochaetes. Dark-field examination, phase-contrast light microscope, and fluorescence microscope.**

Living, unstained cells and organisms can be observed by simply changing the way in which they are illuminated. A hollow cone of light is focused on the specimen in such a way that unreflected and unrefracted rays do not enter the objective. Only light that has been reflected or refracted by the specimen forms an image.

The simplest way to convert a microscope to dark-field microscopy is to place (a) a dark-field stop underneath (b) the condenser lens system.

The condenser then produces a hollow cone of light so that the only light entering the objective comes from the specimen.

The field surrounding a specimen appears black, while the object itself is brightly illuminated; because the background is dark, this type of microscopy is called dark-field microscopy. Considerable internal structure is often visible in larger eucaryotic microorganisms.

The dark-field microscope is used to identify bacteria, like the thin and distinctively shaped *Treponema pallidum* – the causative agent of syphilis.

## Phase-contrast microscope

Unpigmented living cells are not clearly visible in the brightfield microscope because there is little difference in contrast between the cells and water. Thus microorganisms often must be fixed and stained before observation to increase contrast and create variations in colour between cell structures.

A phase-contrast microscope converts slight differences in refractive index and cell density into easily detected variations in light intensity and is an excellent way to observe living cells.

The condenser of a phase-contrast microscope has an annular stop, an opaque disk with a thin transparent ring, which produces a hollow cone of light.

As this cone passes through a cell, some light rays are bent due to variations in density and refractive index within the specimen and are retarded by about ¼ wavelength.

The deviated light is focused to form an image of the object. Undeviated light rays strike a phase ring in the phase plate, a special optical disk located in the objective, while the deviated rays miss the ring and pass through the rest of the plate. If the phase ring is constructed in such a way that the undeviated light passing through it is advanced by ¼ wavelength, the deviated and undeviated waves will be

about ½ wavelength out of phase and will cancel each other when they come together to form an image.

The background, formed by undeviated light, is bright, while the unstained object appears dark and well-defined.

This type of microscopy is called dark-phase-contrast microscopy. Colour filters often are used to improve the image. Phase-contrast microscopy is especially useful for studying microbial motility, determining the shape of living cells, and detecting bacterial components such as endospores and inclusion bodies that contain polyhydroxybutyrate, polymetaphosphate, sulphur, or other substances.

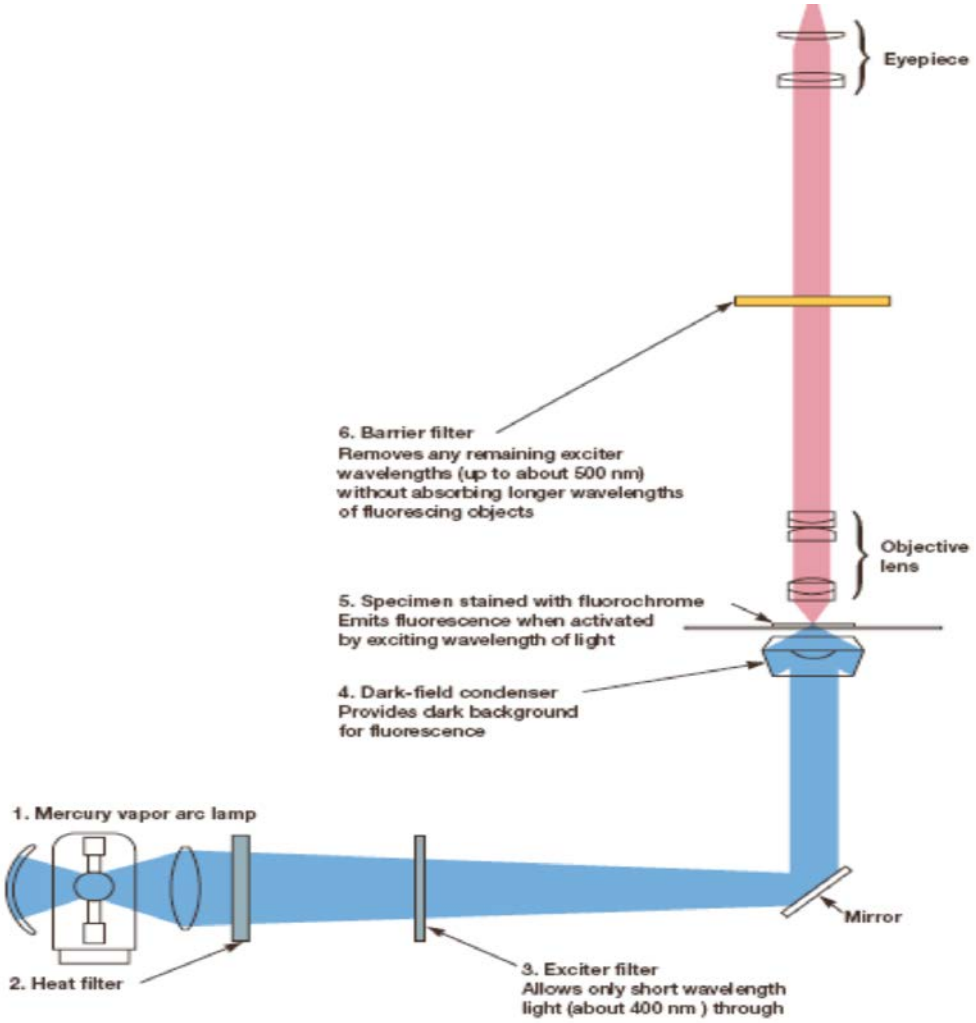
These are clearly visible because they have refractive indexes markedly different from that of water. Phasecontrast microscopes also are widely used in studying eucaryotic cells.

## The Fluorescence Microscope

The microscopes thus far considered produce an image from light that passes through a specimen. An object also can be seen because it actually emits light, and this is the basis of fluorescence microscopy. When some molecules absorb radiant energy, they become excited and later release much of their trapped energy as light. Any light emitted by an excited molecule will have a longer wavelength (or be of lower energy) than the radiation originally absorbed. Fluorescent light is emitted very quickly by the excited molecule as it gives up its trapped energy and returns to a more stable state.

The fluorescence microscope exposes a specimen to ultraviolet, violet, or blue light and forms an image of the object with the resulting fluorescent light. A mercury vapor arc lamp or other source produces an intense beam, and heat transfer is limited by a special infrared filter. The light passes through an exciter filter that transmits only the desired wavelength.

A darkfield condenser provides a black background against which the fluorescent objects glow. Usually the specimens have been stained with dye molecules, called fluorochromes**.** It fluoresces brightly upon exposure to light of a specific wavelength, but some microorganisms are autofluorescing. The microscope forms an image of the fluorochrome-labeled microorganisms from the light emitted when they fluoresce. A barrier filter positioned after the objective lenses removes any remaining ultraviolet light, which could damage the viewer's eyes, or blue and violet light, which would reduce the image's contrast.




**Fluorescence Microscope**

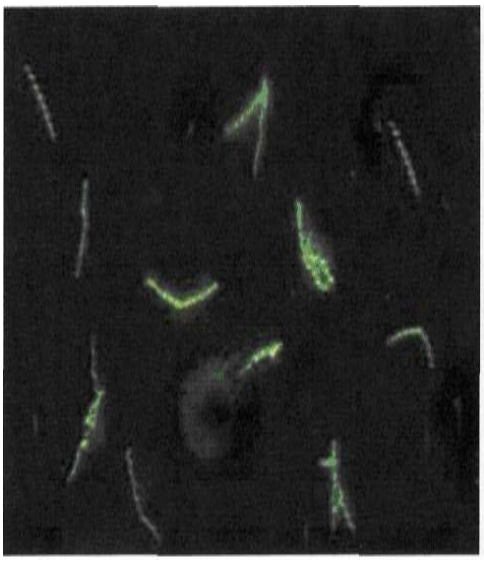
The fluorescence microscope has become an essential tool in medical microbiology and microbial ecology.

Bacterial pathogens (e.g., *Mycobacterium tuberculosis,* the cause of tuberculosis) can be identified after their staining with fluorochromes or specifically labeling with fluorescent antibodies using immunofluorescence procedures. In ecological studies the fluorescence microscope is used to observe microorganisms stained with fluorochrome-labeled probes or fluorochromes such as acridine orange and DAPI (diamidino-2-phenylindole, a DNA-specific stain).

The stained organisms will fluoresce orange or green and can be detected even in the midst of other particulate material.

It is even possible to distinguish live bacteria from dead bacteria by the colour they fluoresce after treatment with a special mixture of stains. Thus the microorganisms can be viewed and directly counted in a relatively undisturbed ecological niche.

**Task 4. Study preparations demonstration.**



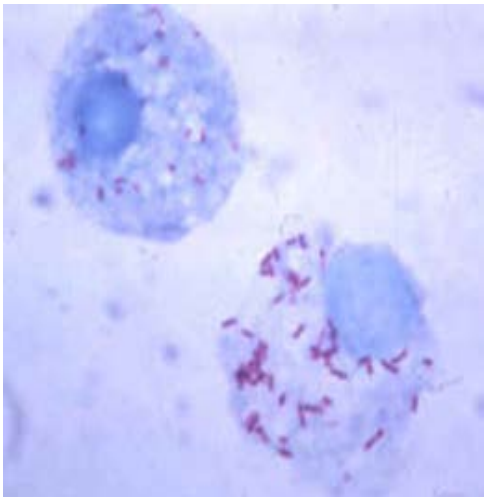
The spirochaetes and *Treponema pallidum* are long, slender, helically coiled, motile gram-negative bacteria.
*Treponema pallidum* has 8–12 delicate regular coils, about 0.2 μm in width and 5–15 μm in length.

***Treponema pallidum*** **by indirect immunofluorescense**

This kind of microscopy is performed using a special device, a dark field. In sidelight, at this microscopy living objects with the size of 0.02–0.06 microns can be observed.

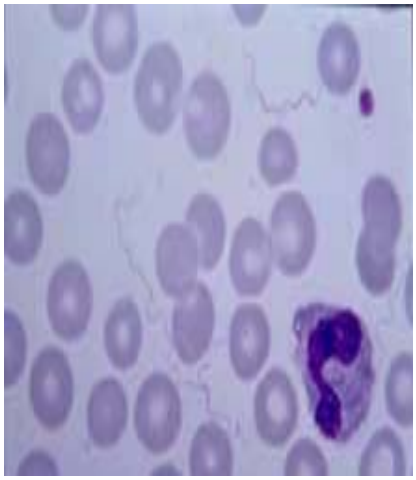
At sighting and sketching specimens pay attention to the form of bacteria. These organisms in the specimen are light, clearly visible spiral-form bacteria on the dark field.

***Treponema pallidum*, dark-field illumination**



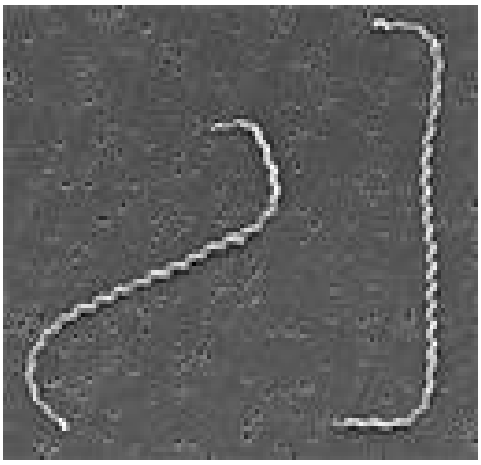
Rickettsia are related to intracellular parasites. Therefore, the investigation shows red rods inside inside cytoplasm of infected cell.

***Rickettsia spp*., Zdrodovsky's staining**



Borrelial cells average 0.2 to 0.5 μm by 4 to 18 μm and have 3–8 coils. Seven to twenty periplasmic flagella originate at each end and overlap at the centre of the cell. Basal bodies of periplasmic flagella of borreliae resemble those in gram-positive bacteria. Because of their larger diameter, borreliae are more readily stained with aniline dyes than other spirochaetes are.

**Romanovsky-Giemsa staining of *Borrelia* in blood**



*Leptospira interrogans* are tightly coiled (approximally 20 coils), thin, flexible spirochaetes 5–15 μm long with very fine spirals 0.1-1.2 μm wide, one or both ends are bent, forming hooks.

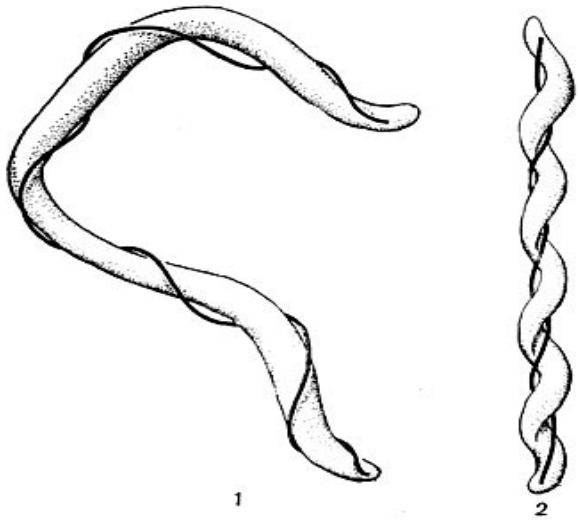
***Leptospira interrogans*, dark-field microscopy**

**Table 1.3.1 – Features of chlamydias, spirochaetes, rickettsia, and mycoplasmas ultrastructure. Modern methods of microscopic examination**

| Notion | Definition/explanation |
|---|---|
| Spiral forms of bacteria | A helical or corkscrew-shaped bacterium. Spirals come in 1 of 3 forms:<br>1. **Vibrio**: curved or comma-shaped rod.<br>2. **Spirillum**: thick, rigid spiral.<br>3. **Spirochaete**: thin, flexible spiral |
| Spirochaetes taxonomy | Order: *Spirochaetales.*<br>Family: *Spirochaetaceae.*<br>Genus: *Treponema.*<br>Species:<br>– *T. pallidum* (causes syphilis);<br>– *T. pertenue* (causes jars);<br>– *T. carateum* (causes pinta);<br>– *T. microdenticum* and *T.macrodenticum* are nonpathogenic species found in oral cavity.<br>Family: *Spirochaetaceae.*<br>Genus: *Borrelia.*<br>Species:<br>o *B. recurrentis* (causes relapsing fever);<br>– *B. burgdorferi* (causes Lyme disease)<br>Family: *Leptospiraceae.*<br>Genus : *Leptospira.*<br>– Species : *L. interrogans* (causes leptospirosis) |
| Morphology and staining properties of spirochaetes | Spirochaetes are long, slender, helically coiled, motile gram-negative bacteria.<br>*Treponema pallidum* has 8–12 delicate regular coils, about 0.2 μm in width and 5–15 μm in length.<br>*Borrelia recurrentis* forms irregular 3–8 spirals 10–30 μm long and 0.3 μm wide. |

| | |
|---|---|
| | *Leptospira interrogans* are tightly coiled (approximately 20 coils), thin, flexible spirochaetes 5–15 μm long with very fine spirals 0.1–1.2 μm wide, one or both ends are bent, forming hooks |
| Ultrastructure of spirochaetes | The outmost structure of the helical cell is multilayed outer membrane, which completely surrounds the protoplasmic cylinder.<br>Protoplasmic cylinder consists of the cytoplasm with nucleiod and other cytoplasmic structures, which is covered by cytoplasmic membrane and peptidoglycan.<br>Endoflagella (axial filaments) are flagella-like organellas in the periplasmic space encased by the outer membrane, thus, endoflagella are located between this membrane and the protoplasmic cylinder.<br>The endoflagella begin at each end of the organism and wind around it, extending to and overlapping at the midpoint |

Table 1.3.1 continuation

| **Notion** | **Definition/explanation** |
|---|---|
| Movement of spirochaetes | The same number of flagella is inserted at each end (e.g., for spirochaetes with two flagella, one flagella at each end).<br>  <br><br>The endoflagella are components of the motility |

| | |
|---|---|
| | apparatus and perform a function of locomotion.<br>Spirochaetes have three main types of movements in liquid environments: locomotion, rotation about their longitudinal axis and flexing motions |
| Methods of staining spirochaetes for microscopical examining | 1. Romanowsky-Giemsa staining. Some species stain blue, others blue-violet, and still others – pink.<br>2. Impregnation with silver by Morozov's method. Spirochaetes are revealed brown above the yellow background.<br>3. Negative Burri staining.<br>4. Gram's method is used for staining saprophytic species (gram-negative). Pathogenic representatives are not stained well by Gram's method.<br><br>For investigation of spirochaetes morphology one use a native microscopy (hanging drop). This method lets to examine morphology and motility |
| Rickettsia taxonomy | Order: *Rickettsiales.*<br>Family :*Rickettsiaceae.*<br>Genus: *Rickettsia.*<br>Species:<br>– *R. prowazekii* (causes epidemic (louse-borne) typhus and Brill-Zinsser disease);<br>– *R. typhi* (causes endemic (flea-borne) typhus);<br>– *R. rickettsii* (causes Rocky Mountain spotted fever).<br>Family: *Rickettsiaceae.*<br>Genus: *Coxiella.*<br>Species: *C. burnetii* (causes Q fever**)** |

| | |
|---|---|
| Properties and structure of rickettsiae | Rickettsia are obligate intracellular parasites that can grow only inside eukaryotic cells.<br>Together with chlamydiae, the rickettsiae are the principal medically important bacteria.<br>Rickettsia are classified into four morphological forms by Zdrodovsky classification: cocci (0.3 μm in diameter), rods (0.3 x 1–2 μm), bacilli (0.3 x 5–10 μm) and filamentous (0.3 x 40 μm).<br>Rickettsia are considered to be gram-negative organisms because they have an outer membrane and thin murein layer. But they do not stain well with Gram stain and are readily visible under the light microscope when stained by Zdrodovsky's method (weak acid fast stain) |
| Properties of rickettsia | Rickettsiae propagate by binary fission with generation time of 8–10 hours. Rickettsiae thrive in the high potassium environment of the eukaryotic cytosole and have specific transport systems for acquiring ATP, amino acids and other metabolites from the host cell. Unlike chlamydiae, rickettsiae are not strict energy parasites as they also are able to synthesize ATP.<br>Rickettsiae are also capable of independent metabolism and use their own biosynthetic machinery to make proteins and other complex components.<br>They cannot be cultivated on artificial medium and in the laboratory they must be grown in the animals, embryonated eggs or cell culture |
| Chlamydiae taxonomy | Order: *Chlamydiales* |

Table 1.3.1 continuation

| **Notion** | **Definition/explanation** |
|---|---|
| | Family: *Chlamydiaceae.*<br>Genus: *Chlamydia.*<br>Species: |

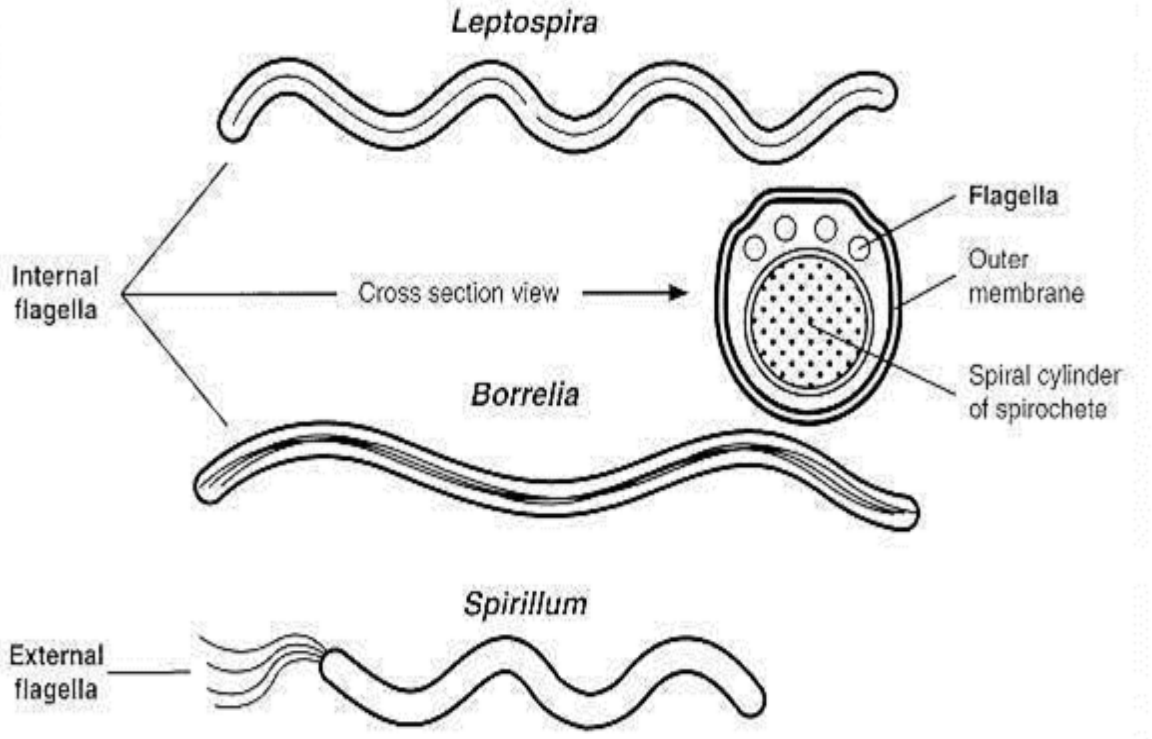
| | |
|---|---|
| | –*C. pneumoniae* (causes pneumonia and respiratory infections);<br>–*C. psittaci* (causes psittacosis);<br>*C.trachomatis* (causes trachoma and urogenital infections (nongonococcal urethritis, epidemitis, cervicitis, pelvic inflammatory disease, leading to sterility and ectopic pregnancy) |
| Ultrastructure of chlamydiae | Chlamydiae are small (0.2 - 0.8 μm in diameter) bacteria. Cell wall of chlamydiae resembles the cell wall of gram-negative bacteria.<br>It has relatively high lipid concentration. It is rigid but does not contain a typical peptidoglycan.<br>But its structure is analogous to murein. The chlamydial genome contains the genes responsible for peptidoglycan synthesis |
| Developmental cycle of chlamydiae | Chlamydiae have the unique developmental cycle. The environmentally stable infectious particle is a small elementary body (EB) about 0.3 μm in diameter with an electron-dense nucleoid.<br><br>The EB membrane proteins have highly cross-linked membrane proteins. The EBs have a high affinity for host epithelial cells and rapidly enters them.<br>The mechanisms thought to mediate entry into the host cells are varied: receptor mediated endocytosis via clathrin-coated pits and pinocytosis via noncoated pits. Lysosomal fusion is inhibited by an unknown mechanisms.<br>Shortly after entry into the host cell, the disulfide bonds of the EB membrane proteins are no longer cross-linked and the EB id reorganized into a large one called a reticulate body (RB) measuring about 0.5 - 1 μm and devoid of an electron-dense nucleoid.<br>Within the membrane-bound vacuole, the RB grows in size and divides repeatedly by binary fission.<br>The entire vacuole becomes filled with EBs derived from |

| | |
|---|---|
| | RBs to form a cytoplasmic inclusions.<br>The newly formed EBs may exit from the host cell and infect new cells. The developmental cycle takes 24–48 hours |
| Mycoplasmas taxonomy | Class : *Mollicutes* Order: *Mycoplasmatales.*Family: *Mycoplasmataceae.*<br>Genus: *Mycoplasma*<br>Species:<br>–*M. pneumoniae* (causes respiratory diseases and infections of joints);<br>–*M. hominis and M. genitalium* (cause urogenital infections).<br>Genus: *Ureaplasma.*<br>Species: *U. urealyticum* (cause nongonococcal urethritis) |
| Ultrasructure and properties of mycoplasmas | There are more than 150 species in the class of cell wall-free bacteria. 15 species can infect humans.<br>They are classified with the bacteria because, in general, they have the structure and composition of the prokaryotes. The main properties of mycoplasmas:<br>1. The smallest mycoplasmas are 125–250 nm in size; the average size is 0.3–0.8 μm.<br>2. They are highly pleomorphic in size because they lack a rigid cell wall (lack murein) and instead are bound by triple-layered “unit membrane” that contains a sterol (mycoplasmas require the addition of serum or cholesterol to the medium to produce sterols for growth).<br>3. Mycoplasmas are completely resistant to penicillin and other antibiotics targeting bacterial cell wall because they lack the cell wall structures. |

Table 1.3.1 continuation

| **Notion** | **Definition/explanation** |
|---|---|
| | But tetracycline or erythromycin are effective for treatment of mycoplasmal infections.<br>4. Mycoplasmas can reproduce in cell free media; on agar, they form small colonies resembling fried eggs during 4 |

| | |
|---|---|
| | weeks (very slow growth).<br>5. Mycoplasmas are sensitive to lysis by osmotic shock, detergents, alcohols and specific antibodies plus complemen<br>6. Mycoplasmas cannot be stained by Gram's method because they lack cell wall. They can be visualized by dark field microscopy, immunofluorescent method and Romanovsky-Giemsa stain.<br>7. Mycoplasmas reproduce by budding, binary fission and division into chains of beads.<br>8. Mycoplasmas can pass through bacterial filters with 450 μm, thus, serum cannot be purified from mycoplasmas, contaminating it.<br>9. Mycoplasmas have unique affinity to mammalian cell membranes |



**Structure of *Leptospira spp.***

## Abreviation

ABS – antigen-binding site
BGEC – Bacteria Group of *Escherichia coli*
CFT – complement fixation test
CNS – central nervous system
DLM – doses letalis minima
DTH – delayed type of the hypersensitivity
ELISA – enzyme-linked immunosorbent assay
EMB – Eosin methylen blue
EPEC – enteropathogenic *E. coli*
EIEC – enteroinvasive *E. coli*
ETEC – enterotoxigenic *E. coli*
EHEC – enterohaemorrhagic *E. coli*
EYA – egg yolk agar
FA – fagocytic activity
FI – fagocytic index
FACS – fluorescence-activated cell sorter
GAS – group A streptococci
IFT – immunofluorescent test
IU – international units
MBT – *Mycobacterium tuberculosis*
MIC – minimal inhibition concentration
MHC – major histocompatibility complex
MPA – meat pepton agar

MRSA – methicillin-resistant Staphylococcus aureus
NBT – nitrat blue tetrasolium
NT – neutralization test
PHAT – indirect (passive) hemagglutination test
PMNL – polimorphonuclear leucocyte
RPR – rapid plasma reagin
RIA – radioimmunoassay
STSS – Streptococcal toxic shock syndrome
TCBS – thiosulfate-citrate-bile salts-sucrose
VDRL – venereal disease research laboratory

## QUIZZES

## MORPHOLOGY OF THE MICROORGANISMS

**1. A bacteriologist has revealed the big bacilli of blue colour with terminally situated spore of red coloring in a slider prepared from wound discharge. What method of colorings did the bacteriologist use?**
A.Gram
B.Neisser
C.Burry
D.Anjesko
E.Ziel – Neelsen

**2. The slider from the patient's sputum is the coloured according to Gram. On microscopy the red bacilli and violet cocci were revealed. Why is the coloring of microorganisms different?**
A.The proportion of DNA and RNA
B.The peculiarities of nucleus substance
C.Isoelectric point
D.The presents of magnasian salt of RNA
E.Flagellae

**3. The patient sputum was presented for confirming the diagnosis of tuberculosis to bacteriological laboratory.**

**The coloring of slider was carried out by complex differential - diagnostic method to reveal the Mycobacteria of tuberculosis. What method of coloring was used?**

A.Gram
B.Neisser
C.Simple
D.Anjehko
E.Ziehl – Neelsen

**4. During microscopy of slider prepared from the discharge of tonsils of a patient with lacunal quinsy and coloured according to Neisser the yellow bacilli situated in V form and with blue bipolar - situated including were revealed. What element of the bacteriological cell did a doctor reveal?**

A.Flagella
B.Capsule
C.Cytoplasm
D.Mesosomes
E. Grains of volutine

**5. Gram-positive cocci situated as grape's bunch were revealed in the slider prepared from pus. How can such situation of microorganisms be explained?**

A. The localization of purulent process
B. The technique of a slider preparation
C. The technique of coloring
D. The peculiarities of a bacterial cell's division
E. The effect on bacteria dye

**6. The white spots were revealed on the mucous membrane of cheeks of the mouth cavity of eight - month-old child. The big blue cells of oval form were revealed in the slider from discharge. What are these microorganisms?**

A. Candida
B. Staphylococci
C. Streptococci
D. Spirochetes
E. Actinomycetes

**7. A patient with fever lasting two weeks was admitted to infectious clinic. The spiral microorganisms with acute ends of blue - violet colour were revealed in**

**the blood slider coloured according to Romanovskiy-Gimza. What microorganisms caused this infection?**

A. Candida
B. Staphylococci
C. Streptococci
D. Spirochetes
E. Actinomycetes

**8. A bacteriologist used the standard set of ingredients in coloring according to Gram. Which of the dyes is used on the first stage of staining to this method?**

A.Crystal violet
B.Iodine solution
C.Alcohol
D.Fuchsin
E.Methylen blue

**9. The intracellular parasites of cocci form were revealed in the sliders coloured according to Zdradovsky. What group of microorganisms is an agent referred?**

A.Cocci
B. Mycoplasma
C.Spirochetes
D.Bacilli
E. Rickettsiae

**10. For the identification of the microorganisms it is necessary to determine their mobility. What method can be used for it?**

A. Gram
B. Neisser
C. Occurring during their lifetime colour
D. The method of luminescent microscopy
E. The isolation of pure culture

**11. Many bacteria on the surface of cell wall form a protective layer badly coloured with dyes. What is the reason of bad coloring of this layer?**

A. Stability to an acid
B. Sensitivity to enzymic action
C. Sensitivity to alcohol
D. High content of lipids and waxes

E. Chemical composition of cell wall

**12. The coloured smears are studied under the microscope with the help of objective which is called:**
A. Dark field
B. Phase contrast
C. Immersion
D. Electronic
E. Dry

**13. Colorless formations of right form with red coloured body of microbic cell were revealed in the sputum of the patient with pneumonia in dark field. It may be a colorless capsule. What method was the preparation coloured with?**
A.Burry
B.Neisser
C.Burry-Ginse
D.Anjesko
E.Ziehl – Neelsen

**14. Under unfavorable conditions of environment, the microorganisms, for the preserving their type form the special structures. To reveal them, a bacteriologist used the method of coloring according to Anjesko. For revealing of what structural elements is it used?**
A. Capsules
B.Spores
C.Flagellae
D.Grains of valutin
E.Nucleus

**15. The spirochetes with 8-14 equal eddies performing different forms of movements were revealed in the smears while studying the ulcer content. In what way was the agent discovered?**
A.Colouring according to Gram
B.Silvering according to Morosov
C.Colouring according to Neisser
D.Colouring according to Romanowsky-Giemsa
E.Hanging drop technique

**16. Bacteria whose flagella are located on the whole surface of a cell are called:**
A.Monotricheal
B.Lophotricheal
C.Amphitricheal
D.Polytricheal
E.Peritricheal

**17. The physical method of fixation of a glass slider is included in?**
A. Moving the smear in the gas
B.Immersion of the smear into formalin
C.Drying the smear in the air
D.Immersion of the smear into alcohol
E.Treatment of the smear with 5 % sulfuric acid

**18. There is a method of coloring which gives the possibility to paint in different colour the spirochetes of the genus Borrelia, Leptospira, Treponema. How do we call it?**
A.Colouring according to Gram
B.Silvering according to Morosov
C.Colouring according to Neisser
D.Colouring according to Romanowsky-Giemsa
E.Colouring according to Burry

**19. One of the complex methods is used for coloring the acid-fast bacteria. When coloured they become red under the influence of the main due. How do we call this method?**
A.Colouring according to Gram
B.Silvering according to Morosov
C.Coloring according to Neisser
D.Coloring according to Romanowsky-Giemsa's
E.Colouring according to Ziehl-Neelsen

**20. The microorganisms which are painted according to Gram in violet colour are called:**
A. Gram-positive
B.Gramnegative
C.Acid-fast

D.Cocci
E.Fungi

**21. Acid fast microbes are resistant to acid because they contain in cell wall:**
**A.** Lipopolysaccharides
**B.** Fatty waxes, fatty acid
**C.** Acetylglucosamine
**D.** Diaminopimelic acid
**E.** Polyphosphates

**22. Cell wall of gram-negative bacteria contain all, except:**
**A.** Lain monolayer peptidoglycan
**B.** Lipoproteins
**C.** Polilayer peptidoglycan
**D.** Lipopolysaccharide
**E.** Outer membrane

**23. In course of practical training students studied a stained blood smear of a mouse with bacteria phagocyted by leukocytes. What cell organella completes digestion of these bacteria?**
A. Golgi apparatus
B. Lisosomes
C. Ribosomes
D. Mytochondrions
E. Granular endoplasmic reticulum

**24. Gram-negative comma-shaped bacteria are revealed in the feces of a patient with diarrhea. What properties should be studied with a microscope first of all to receive additional information about the revealed microbes?**
A. Presence of spores
B. Presence of capsule
C. Motility
D. Presence of volutin inclusions
E. Presence of glycogen inclusions

**25.The patient's sputum was presented for confirming the diagnosis of tuberculosis to bacteriological laboratory. The staining of smear was carried out by complex differential - diagnostic method to reveal the Mycobacteria tuberculosis. What method of coloring was used?**

A.Gram
B.Neisser
C.Simple
D. Anjehko
E. Ziehl – Neelsen

**26. The smear from the patient's sputum is coloured according to Gram. On microscopy the red bacilli and violet cocci were revealed. Why is the colouring of microorganisms different?**
A. The proportion of DNA and RNA
B. The peculiarities of nucleus substance
C. Isoelectric point
D. The presence of magnasian salt of RNA
E. Flagellae

**27. During microscopy of smear prepared from the discharge of tonsils of a patient with lacunal quinsy and stained according to Naisser the yellow bacilli situated in V form and with blue bipolar - situated includings were revealed. What element of the bacteriological cell did a doctor reveal?**
A.Flagella
B.Capsule
C.Cytoplasm
D.Mesosomes
E.Grains of volutin

**28. A bacteriologist used the standard set of ingredients in coloring according to Ziehl-Neelsen. Which of the dyes is used on the first stage of staining with this method?**
A.Crystal violet
B.Iodine solution
C.Alcohol
D.Carbol fuchsin
E.Methylene blue

**29. In some diseases the infection results from getting the spores of bacilli into the wound. What diseases may develop in such case?**
A. Tetanus

B. Tuberculosis
C. Plague
D. Cholera
E. Dysentery

**30. Many bacteria on the surface of cell wall form a protective layer badly stained with dyes. What is the reason of bad colouring of this layer?**
A. Stability to an acid
B. Sensitivity to enzymic action
C. Sensitivity to alcohol
D. High content of lipids and waxes
E. Chemical composition of cell wall

**31. Bacteria whose flagella are located on the whole surface of a cell are called:**
A. Monotricheal
B. Lophotricheal
C. Amphitricheal
D. Polytricheal
E. Peritricheal

**32. Colorless formations of right form with red colored body of microbic cell were revealed in the sputum of the patient with pneumonia in dark field. It may be a colorless capsule. What method was the preparation stained with?**
A. Burry
B. Neisser
C. Burry-Ginse
D. Anjesko
E. Ziehl-Neelsen

**33. The physical method of fixation of a smear is included in:**
A. Immersion of the smear into formalin
B. Drying the smear in the air
C. Immersion of the smear into alcohol
D. Treatment of the smear with 5% sulfuric acid
E. Moving the smear in the flame

**34. The spirochetes with 8-14 equal eddies performing different forms of movements were revealed in the smears while studying the ulcer content. In what way was the agent discovered?**
A.Colouring according to Gram
B.Silvering according to Morosov
C.Colouring according to Neisser
D.Colouring according to Romanowsky-Giemsa
E.Hanging drop technique

**35. There is a method of colouring which gives the possibility to paint in different colour the spirochetes of the genus Borrelia, Leptospira, Treponema. How do we call it?**
A.Colouring according to Gram
B.Silvering according to Morosov
C.Colouring according to Neisser
D.Colouring according to Romanowsky-Giemsa
E.Colouring according to Burry

**36. Under microscopic examination of blood micropreparation coloured according to Romanovsky-Giemsa, a bacteriologist revealed a microorganism in the form of a thin thread of blue violet color with several gross curls and acute ends. The agent of which infectious disease looks like that under the microscope?**
A. Syphilis
B. Toxoplasmosis
C. Relapsing fever
D. Gonorrhea
E. Diphtheria

**37. The intracellular parasites of cocci form were revealed in the smear coloured according to Zdradovskiy. What group of microorganisms is an agent referred to?**
A.Cocci
B.Mycoplasmas
C.Spirochetes
D.Bacilli
E.Rickettsiae

**38. For the identification of the spirochetes it is necessary to determine their mobility. What method can be used for it?**
A.Gram
B.Neisser
C.Occuring during their lifetime colour
D.The method of luminescent microscopy
E.The isolation of pure culture

**39. The pink spirochetes with 8-14 equal eddies were revealed in the smears while studying the ulcer content. In what way was the agent discovered?**
A.Colouring according to Gram
B.Silvering according to Morosov
C.Colouring according to Neisser
D.Colouring according to Romanowsky-Giemsa
E.Hanging drop technique

**40. A case of syphilis took place in the hospital. What spirochete causes this infection?**
A. Treponema carateum
B. Treponema pallidum
C. Leptospira interorgans
D. Borrelia recurrentis
E. Borrelia vincentii

**41. A doctor diagnosed that the patient had leptospirosis. What microorganism caused this infection?**
A. Leptospira icterohaemorrhagiae
B. Treponema pallidum
C. Leptospira interorgans
D. Borrelia recurrentis
E. Borrelia vincentii

**42. Spiral organisms with numerous closely set coils and hooked ends were revealed in the smear stained according to Romanowsky-Giemsa. What group of microorganisms is an agent referred to?**
A.Cocci
B.Mycoplasmas
C.Spirochetes

D.Bacilli
E.Rickettsiae

**43. A special method of staining is used for revealing rickettsiae. What is this method?**
A. Gram
B. Morosov
C. Neisser
D. Romanowsky-Giemsa
E. Zdradovskiy

**44. A component of the cell membrane of most fungi is:**
A. Cholesterol
B. Chitin
C. Ergosterol
D. Peptidoglycan
E. Keratin

**45. The asexual spores of many fungi are called:**
A. Endospores
B. Conidiospores
C. Ascospores
D. Zygospores
E. Basidiospores

**46. The protozoal trophosoite phase is characterized by:**
A. Metabolic dormancy
B. Toxin production
C. Active feeding and reproduction
D. Flagellar locomotion
E. Residence in the intermediate host

**47. Espundia and kala-asar are local names for two manifestations of the disease:**
A. Giardiasis
B. Toxoplasmosis
C. Trichomoniasis

D. Malaria
E. Leishmaniasis

**48. Plasmodium falciparum, which causes malaria, is an example of:**
A. An ameboid protozoan
B. A sporosoan
C. A flagellate
D. A ciliate
E. A schizont

**49. A United States businessman who has recently returned home from Haiti suddenly develops a periodic high fever followed by orthostatic hypotension. What is the likely preliminary diagnosis?**
A. Giardiasis
B. Syphilis
C. Malaria
D. Toxoplasmosis
E. Leishmaniasis

**50. A bacteriologist prepared a smear using “hand drop” technique. What property of protozoon did he want to study?**
A. Motility
B. Spore formation
C. Acid fast
D. Capsule formation
E. Composition of cell wall

**51. Obligate intracellular parasite of semilunar form was revealed in tissue liquid. Its nucleus stained in red and cytoplasm stained in blue according to Romanowsky-Giemza. Which protozoon has got such morphology?**
A. Toxoplasma gondii
B. Leishmania donovani
C. Trichomonas vaginalis
D. Leishmania tropica
E. Trypanosoma cruzi

**52. Special method of staining is used to study the morphology of Protozoa. What is this method?**

A.Anjesko
B.Neisser
C.Gram
D.Romanowsky-Giemza
E.Ziehl-Neelsen

**53. Protozoon having a prune form and 4 pairs of symmetrical flagella was revealed in study of smear prepared of duodenal content. What is this protozoon?**
A. Toxoplasma gondii
B. Lamblia intestinalis (Giardia lamblia)
C. Trichomonas vaginalis
D. Leishmania tropica
E. Trypanosoma cruzi

## Recommended reading list

**Main literature**

1. Ananthanarayan R. Textbook of Microbiology / R. Ananthanarayana, Jayaram CK. Paniker ; ed. by.: A. Kapil. - 9th ed. - India : Universities Press (Verlag), 2015. - 710 p.
2. Gaidash I. Microbiology, Virology and Immunology. Vol. 1 / I. Gaidash, V. Flegontova; Ed. N. K. Kasimirko. - Lugansk : S. N., 2004. - 213 p.

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4. Jawetz, Melnik & Adelberg's Medical Microbiology : учебное пособие. - 22 Edition. - New York : Lange Medical Books/McGraw-Hill, 2001.
- 695 p.
5. Medical Microbiology : textbook / D. Greenwood [et al.]. - 17th ed. - Toronto : Churchill Livingstone, 2007. - 738 p.

**Further Reading**

1. Talaro K. Faundations in microbiology. Basic principles. - Talaro K., Talaro A. - Pasadena, 2005, by TMHE group.
2. Microbiology. A human perspective / M. T. Nester, E. V. Nester, C. E.Roberts. - 1995.
3. Levenson W. E. Medical microbiology and immunology / W. E. Levenson, E. Javetz. – Norwalk, 1994,
4. Krivoshein Yu. S. Handbook on microbiology / Yu. S. Krivoshein– Moscow : Mir Publishers,.1989
5. Tropical Diseases : A Practical Guide for Medical Practitioners and Students / Y.A. Meunier, M. Hole, T. Shumba, B. J. Swanner. - OUP USA, 2013.

## *Informational resourses:*

1. American Society for Microbiology — http:// asm.org.;
2. http://journals.asm.org; (American Society for Microbiology) — http:// asm.org.;
3. http://www.news-medical.net/health/Virus-Microbiology-(Russian).aspx;
4. http://www.rusmedserv.com/microbiology; http://www. rusmedserv.com/
5. http://rji.ru/immweb.htm; http://www.rji.ru/ruimmr;

6. http://www. infections.ru/rus/all/mvb journals.shtml;
7. http://dronel.genebee.msu.su/journals/ microb-r.html.
8. http://commons.wikimedia.org/wiki/Category:Medical_illustrations_by_Patrick_ Lynch.
9. http://www.nejm.org/doi/pdf/10.1056/nejmra064142
10. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3438653/
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