MINISTRY OF HEALTH OF UKRAINE
POLTAVA STATE MEDICAL UNIVERSITY

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MEDICAL MICROBIOLOGY.
GUIDE FOR PREPARING FOR THE LICENSED INTEGRATED EXAM "KROK 1"

Poltava – 2023

МІНІСТЕРСТВО ОХОРОНИ ЗДОРОВ'Я УКРАЇНИ
ПОЛТАВСЬКИЙ ДЕРЖАВНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ

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МЕДИЧНА МІКРОБІОЛОГІЯ.
ПОСІБНИК З ПІДГОТОВКИ ДО ЛІЦЕНЗІЙНОГО ІНТЕГРОВАНОГО ІСПИТУ «КРОК 1»

Полтава - 2023

УДК 578+579+577.27(075.8)

Рекомендовано до видання Вченою радою Полтавського державного медичного університету (протокол № 3 від 30 листопада 2022 року).

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Навчальний посібник Medical microbiolodgy. Guide for preparing for the licensed integrated exam «Krok 1» (Медична мікробіологія. Посібник для підготовки до ліцензійного інтегрованого іспиту «Крок 1») /В.І. Федорченко, І.М. Звягольська, В.П. Полянська. – Полтава : 2023. – 168 с.

У начальному посібнику розглянуто питання, пов'язані з теоретичним обґрунтуванням та алгоритмом застосування мікробіологічних методів діагностики інфекційних захворювань, і вміщено тестові завдання для складання тестових компонентів етапу 1 ЕДКІ із субтесту «Мікробіологія, вірусологія та імунологія», проілюстровані відповідними схемами, рисунками, мікрофотографіями.

Навчальний посібник призначений для студентів закладів вищої медичної освіти, які навчаються за спеціальностями 221 «Стоматологія», 222 «Медицина», 228«Педіатрія».

УДК 578+579+577.27(075.8)

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PREFACE

Study guide "Medical microbiology. Guide for preparing for the licensed integrated exam "Krok 1" was created for students of medical and stomatological faculties of the higher medical educational institutions of Ukraine. The tutorial's main purpose is to optimise students' independent preparation for the "Step 1" licensing exam in microbiology, virology and immunology for students of the higher medical educational institutions of Ukraine in the field of knowledge 22 "Health care", speciality 222 "Medicine" and specialities 221 "Dentistry" and 228 "Pediatrics".

Following the discipline's curriculum, the manual contains the main methods of microbiological research. The chapters are dedicated to general and special microbiology, virology, and immunology. The tutorial is supplemented with tests from the Testing Center under the Ministry of Health of Ukraine database for the last 5 years. In addition, the manual contains diagrams, drawings and photomicrographs. The illustrations contribute to the conscious assimilation of the microbiology, virology and immunology concepts.

Illustrative materials are used from open Internet resources.

The authors hope that the proposed study guide will improve students' preparation for the "Step 1" licensing exam in microbiology, virology, and immunology, as well as in preparation for practical classes and final module tests.

Chapter I. METHODS OF LABORATORY RESEARCH

Different microbiological methods are used in modern laboratories to establish infectious diseases aetiology. There are:

1. Microscopical method (in bacteriology, virology, protozoology, mycology);

2. Cultural method (bacteriological, virological, protozoological, mycological);

3. Serological method;

4. Biological (experimental) method;

5. Allergic method;

6. Express methods;

7. Molecular genetic methods.

Microscopical methods

Microscopy (bacterioscopy, viroscopy, protozoscopy and mycoscopy) is based on determining a pathogen's morphological and structural features in the studied material taken from the patient.

The basis of the method is light-optical microscopy with all its variants (in particular, light-field, dark-field, phase-contrast, and luminescent) and electron microscopy.

Light-field immersion microscopy is the most common and accessible research method in the microbiological diagnostics of infectious diseases. The detected microorganisms are identified in the stained slides in transmitted light by the morphology, structure, and tinctorial properties. An immersion lens x90 must be immersed in oil (cedar or peach). The refractive index of the oils is close to the refractive index of glass. Therefore, the beam of light, which passes by the slide, is not scattered. Instead, the rays fall into the lens immediately without changing their direction. The resolution of the immersion lens is within 0.02 μm.

A smear of sputum, taken from a patient Yu., 41 years old, was made. The microscopy was performed using an immersion microscope. What exactly is affected by the immersion oil applied to the smear? *

A. The direction of the rays

B. The transparency of the smear

C.The size of objects in the field of view

D.Neutralises microorganisms

E.The colour of objects in the field of view

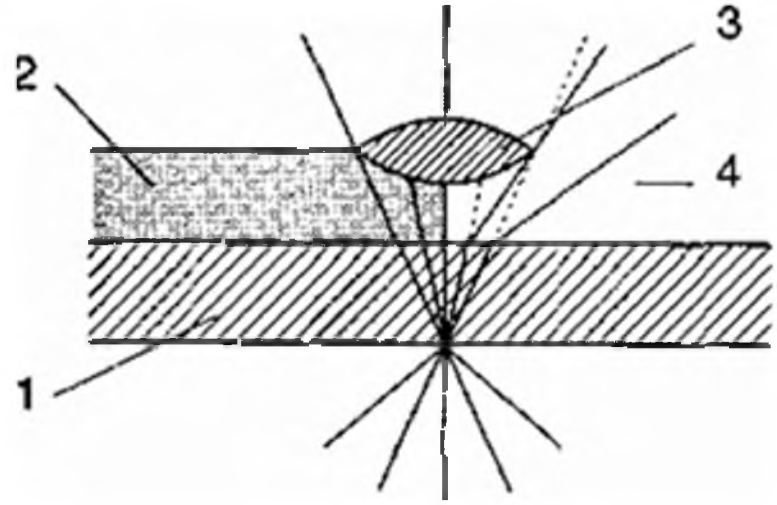

Figure 1 – The direction of the rays when passing through the immersion oil and air: 1. Slide. 2. Immersion oil. 3. Immersion lens. 4. Air

The study of preparations containing bacteria conducts with an immersion microscope in a bacteriological laboratory. What should be done to establish the optimal field of view lighting?

A.Raise the condenser to the uppermost position

B.Lower the condenser to the lowest position

C.Set the condenser to the middle position

D.Close the diaphragm as much as possible

E.Set the diaphragm to the middle position

Darkfield, phase contrast and anoptral microscopy are used to study living (native) objects (bacteria, spirochetes, fungi, protozoa, etc.) in the unstained state to detect their mobility in the smears («hanging» or « crushed « drop).

The vomiting mass of a suspected cholera patient was delivered to the bacteriological laboratory. The preparation «hanging drop» is prepared from the material. What microscopy method will be used to detect the pathogen by its motility?

A.Phase-contrast

B.Electronic

C.Immune electronic

D.Luminescent

E.Immersion

Figure 2 – Phase-contrast microscope

The material was taken from the chancre of patient M., 34-year- old, for microscopic examination in an unstained state. The microscope used was equipped with a paraboloid condenser. What type of microscopy was used?

A.Darkfield

B.Luminescent

C.Phase-contrast

D.Electronic

E.Immersion

Fluorescent microscopy allows us to observe the primary or secondary glow (luminescence) of microorganisms, cells, tissues, and their structures in ultraviolet rays. The source of rays is a quartz or mercury lamp.

Electron microscopy allows studying the structure of microorganisms at the subcellular and molecular levels.

Figure 3 - Schematic structure of a fluorescent microscope

Figure 4 - Appearance of the electron microscope

Chapter II. MORPHOLOGY AND STRUCTURE OF BACTERIA. SIMPLE AND COMPLEX METHODS OF STAINING

Making smears and methods of their staining

The main stages of smears preparation for light field immersion microscopy are:

1) making smear preparations from the studied material (sometimes it can be smears-imprints from human or animal corpses, foodstuffs of dense consistency);

2) drying of the smear;

3) fixation of the smear;

4) staining of the smear.

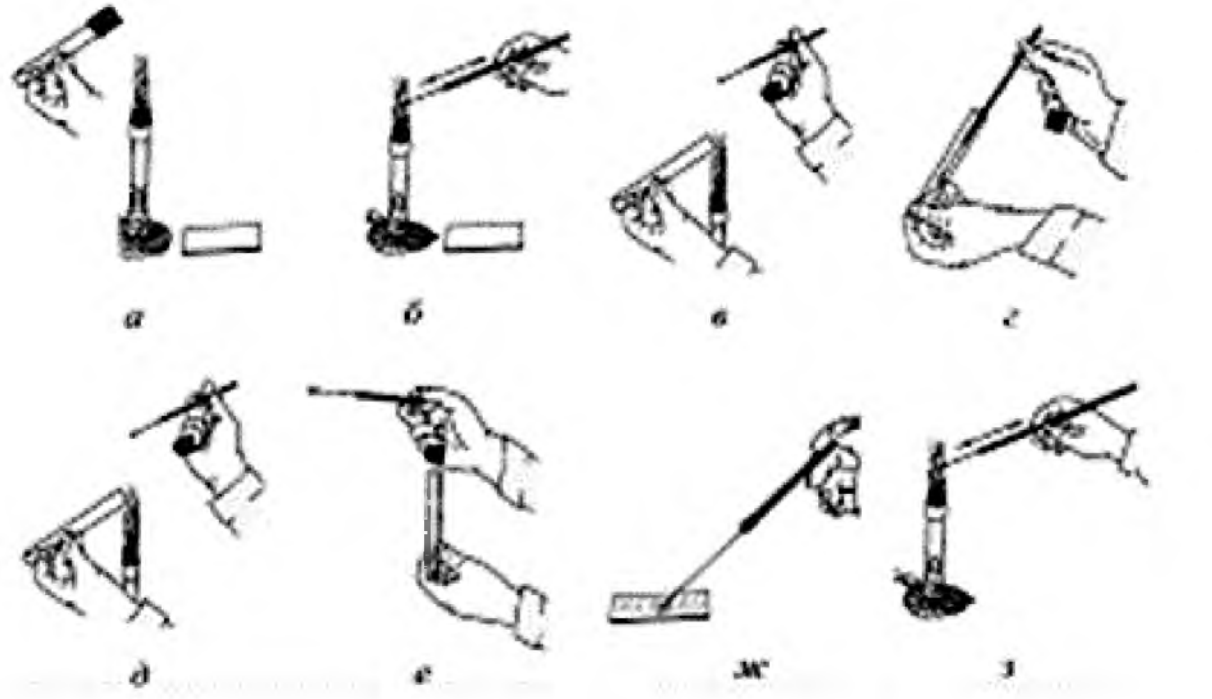

Figure 5 - Stages of making smears

Simple and complex staining methods are used in light microscopy to study microorganisms, namely, their morphological, tinctorial properties and individual structures.

Morphological properties of bacteria, fungi, and protozoa characterise the diversity of their forms and structural organisation that determine their functional activity.

Tinctorial properties of bacteria, fungi, and protozoa characterise their ability to react with dyes and be stained in a certain way.

Simple methods of staining the microorganisms

Only one dye is used for these methods. Most often, it is a) diluted Pfeiffer's dye - fuchsin or safranin - (microorganisms stained 1-2 minutes is red); b) alkaline methylene blue Loeffler (microorganisms stained 3-5 minutes is blue or dark blue).

Simple methods include the negative Burri and Hins' method - the India ink creates a dark background, and microorganisms remain unstained. India ink suspension (usually black) is used for the study. A thin preparation should be prepared, as well as a blood smear. It is dried but not fixed then. The smear is studied using an immersion dark-field, phase-contrast or anoptral system. Microscopic picture: microbial cells are unstained and look white (transparent) in the field of view on a dark background.

Simple staining methods provide an opportunity to investigate: the morphological properties of microorganisms, namely the shape, size, relative position of microbial cells, their number, and so on.

A simple staining method was used to stain the patient's sputum smear. It means that:

A.The smear was stained with one dye

B.The method does not use acids

C.The Gram method was used

D.The method does not use volatile, highly toxic substances

E.The method does not use organic solvents

A plaque smear on a patient's tonsils with suspected diphtheria revealed blue rods with thickenings on the poles. What method of staining was used?

A.Leffler

B.Burri and Hins'

C.Gram

D.Neisser

The shape of bacteria (prokaryotic kingdom representatives) is determined by the structure of the cell wall and the mutual location (the orientation in space and the degree of relationship of bacterial cells during reproduction, taking into account the

synchronicity of their divisions during this process). The forms of bacteria detected by microscopy of stained smears are:

1. Cocci are spherical, elliptical, bean-shaped, or lanceolate form bacteria. By location, these are:
2. diplococci (paired cocci);
3. streptococci (chains of cocci);
4. staphylococci (irregular clusters of cocci, often in the form of grapes' bunches);
5. micrococci (single or randomly arranged cocci).
6. tetracocci

Spherical microbes, located as a "bunch" of grapes, were found in the inoculation of manure from the boil. What microbes were found?

A.Staphylococci

B.Diplococci

C.Micrococci

D.Streptococci

E.Tetracocci

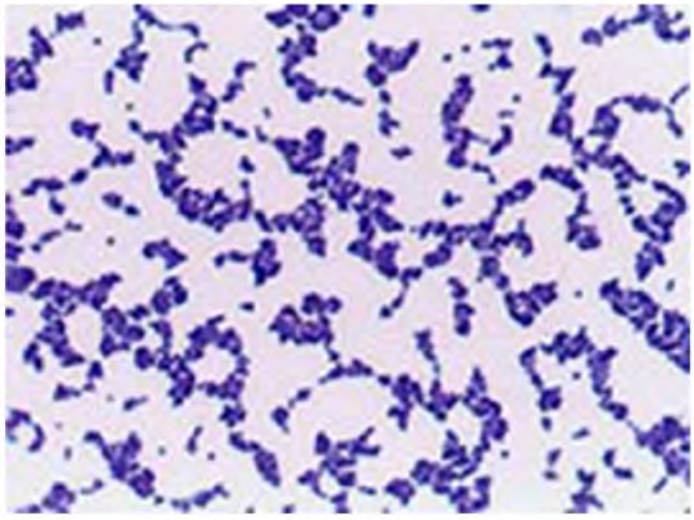

Figure 6 - Morphology of Staphylococcus genus bacteria

A culture was isolated from the oropharynx of the boy suffering from chronic tonsillitis. Coccal bacteria were arranged in chains. What can these bacteria be?

A.Streptococci

B.Staphylococci

C.Escherichia

D.Clostridia

C.Vibrio

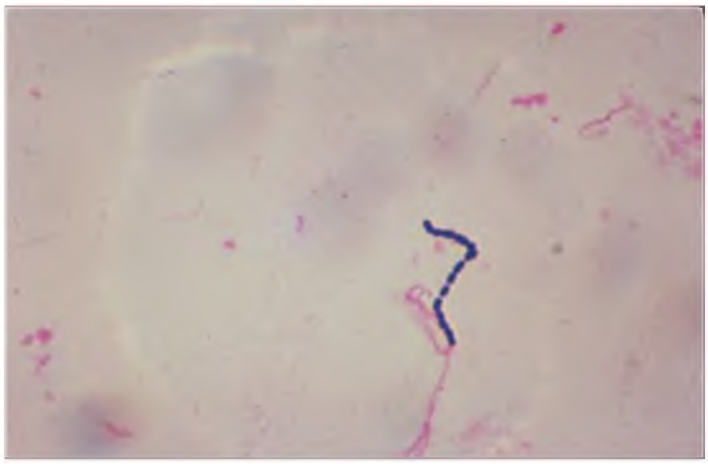

Figure 7 - Morphology of Streptococcus genus bacteria

7. **Rod-shaped bacteria** are non-spore-forming bacteria, spore-forming bacteria (bacilli and clostridia); by location, it is: single, diplo- and streptobacteria, located at an angle; in shape, it is: straight rods of different sizes, bent rods (in particular, vibrios), rods with thickenings (in particular, corynebacteria), ovoid form (in particular, plague bacteria).

Catgut, which is used in surgical interventions, was sent to the bacteriological laboratory to check sterility. Bacilli were found. What sign allowed to name of the selected bacteria as the bacilli?

A.The presence of spores

B.The presence of capsules

C.The presence of metachromatic granules

D.The presence of flagella

E.Gram-positive colour

Figure 8 – Morphology of bacteria. Streptobacilli

Pure vibrio culture was isolated from the faeces of a patient with infectious intestinal disease. To which group of bacteria should morphological characteristics classify these microorganisms?

A.Bent rods

B.Clostridia

C.Cocci

D.Bacteria

E.Bacilli

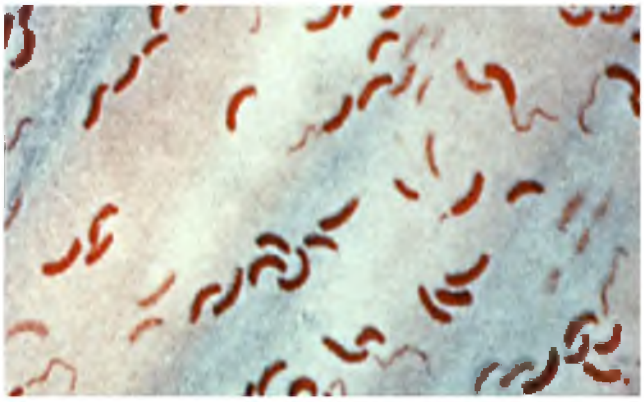

Figure 9 –Morphology of genus Vibrio bacteria

3. Spirochetes (coiled microbes) are spiral bacteria or corkscrew-shaped (Borrelia, Treponema, Leptospira).

Spirochetes were found during dark-field microscopic examination of the chancre material from the patient's vaginal mucosa. To which group of bacteria should morphological characteristics classify these microorganisms?

A.Coiled

B.Clostridia

C.Cocci

D.Bacteria

E.Bacilli

Figure 10 –Morphology of bacteria of Spirochetes order

4. Filamentous bacteria form long, thin structures (actinomycetes).

A smear was made from the purulent contents of the lesion localised in the patient's neck. Actinomycetes were detected. To which group of bacteria should morphological characteristics classify these microorganisms?

A.Filamentous

B.Cocci

C.Bacteria

D.Coiled

E.Clostridia

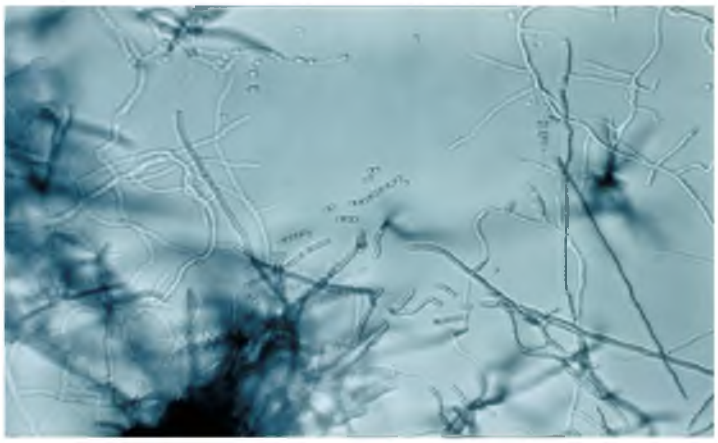

Figure 11 - Morphology of bacteria of Actinomyces order. Staining with methylene blue by Leffler

Complex methods of microorganisms' staining

Complex staining methods are carried out mainly in several stages and use, as a rule, two dyes and auxiliary chemical compounds. It is possible to study the structure of the microbial cells in detail and differentiate some microorganisms from others with the complex staining methods and morphological features (shape, size, and location of cells). Therefore, compared to simple staining methods, these methods are more informative and have more critical diagnostic value.

The structure of bacteria. Bacteria are unicellular prokaryotic haploid microorganisms. As prokaryotes, they do not contain a differentiated nucleus, and a nucleoid is a functional equivalent of it.

The internal environment of the bacterial cell is the cytoplasm, which contains the genetic apparatus (nucleoid, plasmids, transposons, Is-elements), ribosomes, mesosomes, and inclusions. A three-component shell surrounds the cytoplasm. It includes a cytoplasmic membrane, cell wall, and capsule. Surface structures are flagella and cilia (fimbriae or pilli). In addition, some species of bacteria can form spores, and the shape, size and location characteristic of the species used to identify bacteria. The genetic apparatus, ribosomes, mesosomes, cytoplasmic membrane, and cell wall are constant components of the bacterial cell (in the composition of all bacteria); inclusions, capsules, flagella, cilia, spores are non-permanent components (present or absent depending on the type of bacteria).

Nucleoids, plasmids (R-, Col-, Ent-, etc.), transposons and Is-sequences functionally determine the heredity and variability of microorganisms.

Ribosomes are organelles responsible for protein synthesis, in particular at the stage of translation.

Mesosomes are functional analogues of eukaryotic mitochondria.

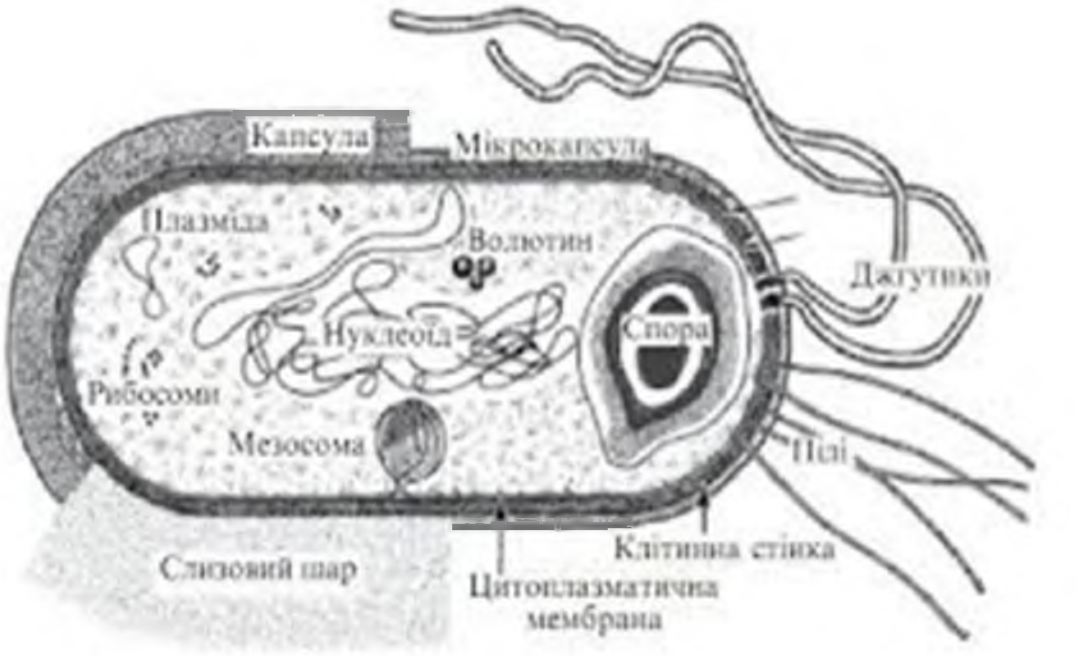

Figure 12 - The structure of the bacterial cell

Inclusions, bodies, or granules are primarily products of microbial cell metabolism and have different origins, locations in the cytoplasm of cells, and functional purposes. The diagnostic value of inclusions, such as Metachromatic bodies, is significant. Metachromatic bodies are intracytoplasmic granules consisting of inorganic polyphosphates. They are called metachromatic. The microscopy of stained smears shows the phenomenon of metachromasia (inclusions are stained in a different colour than other microbial cell components). Detection of metachromatic bodies is used in the microbiological diagnosis of diphtheria.

The cytoplasmic membrane is a chemical composition similar to eukaryotic plasmalemma by structure and functions.

The cell wall has a layered structure, the primary material of which is peptidoglycan (murein); its presence provides a specific shape of the bacterial cell and resistance to osmotic pressure. It is absent only in mycoplasmas. Disruption of

cell wall synthesis can lead to the transformation of bacteria into L-forms. The presence of the cell wall of microorganisms is detected by the Gram method.

The capsule is a mucous layer that covers the bacterial cell from the outside and consists of polysaccharides and a significant amount of water in most bacteria. Capsules as pathogenic factors prevent phagocytosis and participate in microorganisms' adhesion to human tissues.

Flagella are locomotor organelles of bacteria and other microorganisms, consisting of a contractile protein (flagellin). These are responsible for chemotaxis. The number and location of flagella differentiate motile bacteria.

Pili (fimbriae, or pili) are the tubular formations that cover the body of a bacterial cell and consist of a specialised protein (pilin). According to their functions, pili are divided into pili of the general type (provide specific metabolic processes of the bacterial cell) and F-pili (sexual or sex pili responsible for the combination of genetic material in bacteria during conjugation).

A spore (endospore) is a bacteria's stable, resting life form. Due to the presence of a keratinised layered shell (cortex) and suppressed metabolic processes. Bacterial spores are more stable than their vegetative form and can remain viable for long (years, decades) in environmental conditions. Endospores can form bacteria - members of the genus Bacillus and Clostridium. In bacilli, the size of the spores does not exceed the diameter of the vegetative cell. It is tipical for clostridia. The location of the spores in the bacterial cell can be central (Bacillus anthracis - the causative agent of anthrax), subterminal (Clostridium botulinum - the causative agent of botulism), or terminal (Clostridium tetani - the causative agent of tetanus).

Gram staining

The Gram method is the universal differential diagnostic method of staining. It divides bacteria into gram-positive and gram-negative. The Gram method Syniov's modification is widely used in bacteriological laboratories. In this case, instead of a solution of gentian violet, use filter paper, which is pre-impregnated with this solution, and dried out then. At the first staining stage, the smear preparation is covered with a strip of prepared filter paper, and a few drops of water are applied

with a pipette. The following steps coincide with the stages of staining by the Gram method: a) the paper is removed in 1-2 minutes, and the excess dye is drained; b) Lugol's solution is applied for 1-2 minutes (until intense darkening of the smear); c) the smear is decolourised with 96° alcohol for 30-40 sec until the extraction of greyish-purple streams of paint; d) the smear is washed with water and Pfeiffer's magenta is applied for 1-2 minutes; e) the smear is thoroughly washed with water and dried out. Microscopy should be performed using the immersion light microscope system.

Dark purple bacteria are gram-positive microorganisms, and red (pink) bacteria are gram-negative microorganisms.

Staphylococci, streptococci, bacilli, clostridia, corynebacteria, and mycobacteria are gram-positive. Neisseria (gonococci, meningococci), enterobacteria, vibrios, spirilla, spirochetes, mycoplasmas, rickettsiae gram-negative.

Students were asked to stain a mixture of bacteria by the gram method at the practical lesson in the microbiology department. What morphological structures of bacteria determine gram-negative and gram-positive staining of bacteria?

A.Cell wall

B.CPM

C.Capsule

D.Flagella

E.Cytoplasm

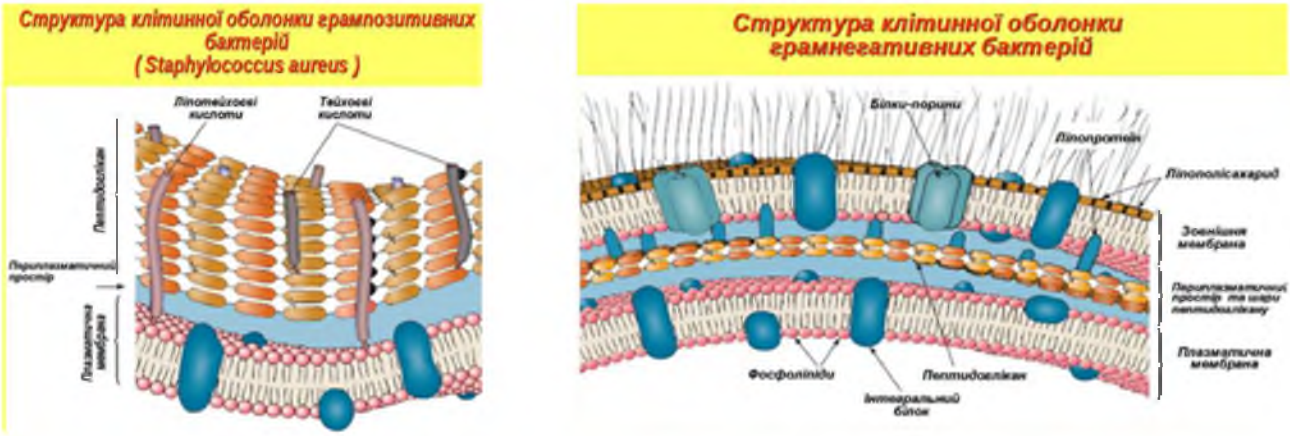

Figure 13 - Structure of cell walls of gram-positive and gram-negative bacteria

Gram-positive diplococci were found in the sputum of a patient with suspected pneumonia. They are slightly elongated with slightly pointed opposite ends. What microorganisms were found in sputum?

A.Streptococcus pneumoniae

B.Staphylococcus aureus

C.Klebsiella pneumoniae

D.Neisseria meningitidis

E.Neisseria gonorrhoeae

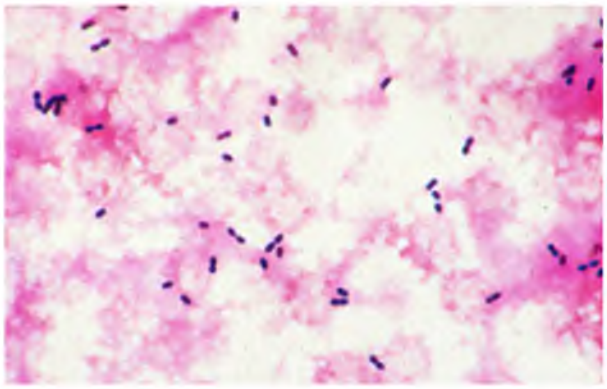

Figure 14 – Morphology of bacteria S. pneumoniae. Gram staining

Bean-shaped diplococci were revealed by bacterioscopy of purulent secretions from the cervix. Microorganisms are Gram-negative bean-shaped and located inside and outside the leukocytes. What is the causative agent of purulent inflammation of the cervix?

A.Neisseria gonorroeae

B.Chlamydia trachomatis

C.Haemophilus vaginalis

D.Trichomonas vaginalis

E.Calymmatobacterium granulomatis

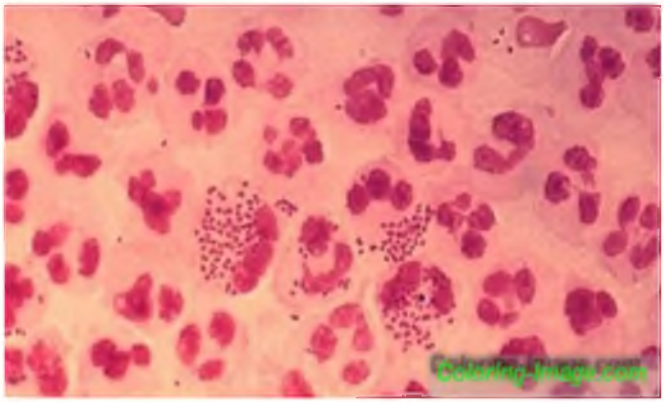

Figure 15 – Morphology of Neisseria meningitidis. Gram staining

A bacteriologist made a bacterioscopic examination of the pathogens taken from the patient's nasopharyngeal mucus. Gram-negative cocci, resembling coffee beans arranged in pairs or tetrads, were revealed. Name the revealed pathogen:

A.Neisseria meningitidis

B.Staphylococcus aureus

C.Neisseria gonorrhoeae

D.Moraxella lacunata

E.Acinetobacter calcoaceticus

Culture from the nasopharynx of a patient with a previous diagnosis of «ozena» was obtained. There were Gram-negative rods in a capsule on a nutrient medium. What microorganisms caused the disease?

A.Klebsiella

B.Mycoplasmas

C.Salmonella

D.Chlamydia

E.Shigella

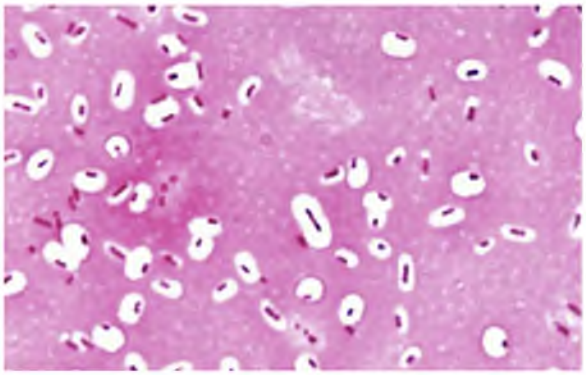

Figure 16 – Morphology of bacteria Klebsiella ozaena. Burri and Hins' staining

Methods for detecting other structures of the microbial cells

Various complex methods (Leffler, Gray, Morozov, etc.) are used for staining **flagella**. Their essence is: increasing the sorbent surface for dyes due to artificial swelling of these organelles. Therefore, the smears are treated with mordant for 10-15 minutes before staining. Microscopic picture: flagella (regardless of the dye used) look lighter than the bacterium's body. Monotrichous, peritrichous, lofotrichous, amphitrichous are distinguished according to the number and location of locomotor organelles.

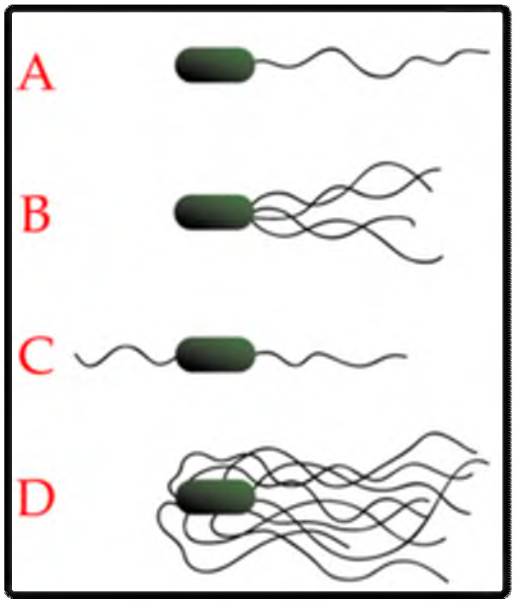

Figure 17 – Placement of flagella in bacteria:

A – monotrichous; B – lofotrichous; C – amphitrichous; D – peritrichous

The capsule of most bacteria consists of polysaccharides, sometimes polypeptides. The capsule has a jelly-like consistency and practically can not be stained. Therefore, when using most staining methods to detect it under a microscope, the capsule looks like an unstained area between the stained body of the bacterial cell and the background of the smear. The most common is the Burri and Hins' method. Firstly, a negative Burri and Hins' smear is prepared from the test material. Bacterial culture should be dropped by Pasteur pipette on a slide with diluted 1:10 India ink (usually black). Next, a thin smear should be made with the edge of polished glass, like a blood smear, and then fixed chemically, washed and stained for 3-5 minutes with a solution of alkaline magenta, washed then with water and dried out. Microscopic picture in immersion system is: the background of the smear is black, capsules in the form of a colourless halo around the bright red body of the bacterial cell. In microbiological practice, it is possible to use other methods of detecting capsules in fixed and native preparations.

A microscopic examination of pathological material from a patient with the suspected plague was conducted in the laboratory of opportunistic infection. The smear was stained by Burri and Hins' method. What property of the pathogen does this method allow to determine?

A. Capsule formation

B. Spore formation

C. Alkali resistance

D. Acid resistance

E. The presence of metachromatic bodies

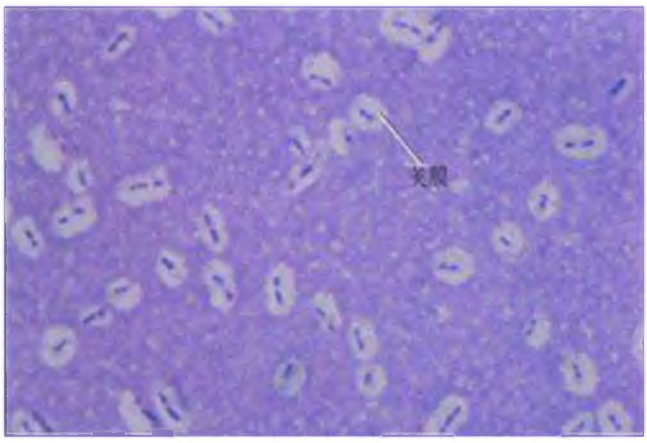

Figure 18 – Morphology of Yersinia pestis. Burri and Hins'staining

Spores perceive poorly the aniline dyes due to their structural and chemical features. Methods of staining with mordant, heating the smears in the burner's flame, and concentrated carbolic or alkaline dyes are used. The shell of the spores loosens up under these conditions. It facilitates the penetration of certain dyes into its middle. Microscopic picture: a) when stained by the method of Ziehl-Neelsen (Ozheshko), the spore is red, and the cytoplasm of bacterial cells is blue; b) by the method of Peshkov – the spore is blue colour, and the cytoplasm of the bacterial cell is pink or pink-brown; c) by the Gram method – the spore is unstained.

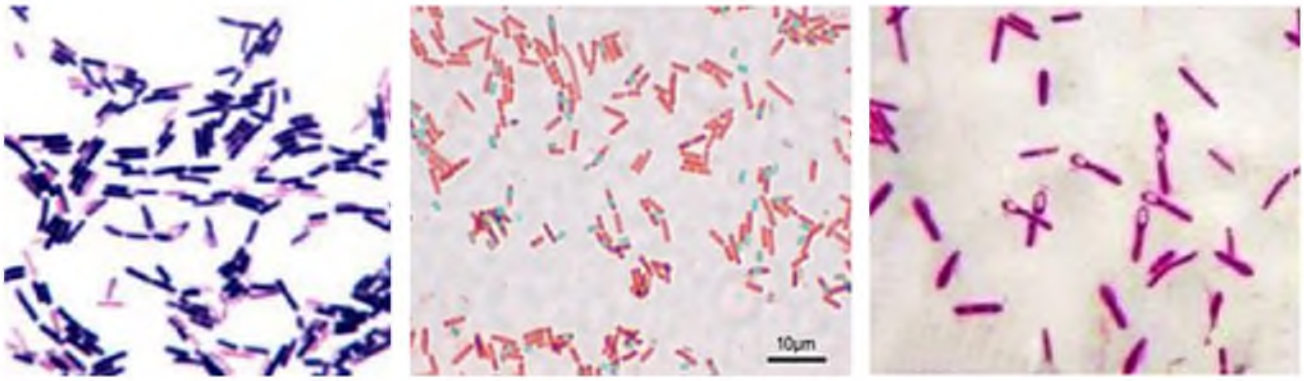

Figure 19 – Staining of spores by the Ziehl-Neelsen (Ozheshko), Peshkov, Gram method

Microscopy of the microbial culture revealed spore-forming microorganisms that are fusiform and coloured blue-violet by Gram. What are these microorganisms?

A.Clostridia

B.Streptococci

C.Spirochetes

D.Actinomycetes

E.Diplococci

You can see blue rod-shaped microorganisms and red terminal components of a round shape in the preparation stained by the Ziehl-Neelsen (Ozheshko) method. What are these components called?

A.Spores

B.Cilia

C.Flagella

D.Capsules

E.Mesosomes

Detection of **Volutin granules (metachromatic bodies)** has differential diagnostic value during laboratory diagnosis of diphtheria. The causative agent of diphtheria (Corynebacterium diphteriae) usually has one pair of metachromatic bodies at the poles of the bacterial cell. It causes club-shaped thickening of its ends. The most commonly used staining methods are the Neisser and Loeffler. Microscopic picture: a) by the Neisser method – the cytoplasm of the bacterial cell has a straw-yellow colour, and metachromatic bodies are dark blue, almost black; b) according to the Loeffler method – the cytoplasm of a bacterial cell is pale blue colour, and Metachromatic bodies are dark blue.

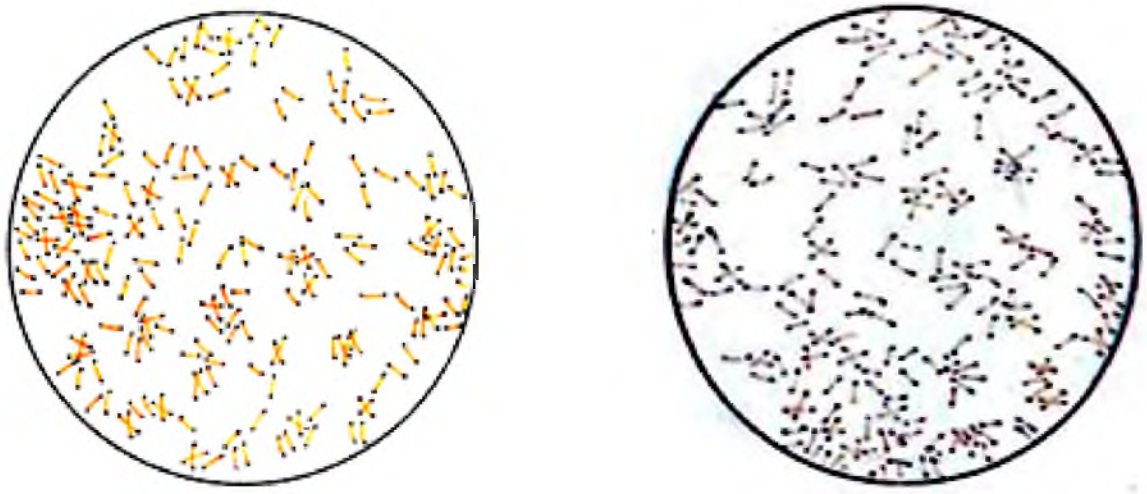

Figure 20 – Detection of metachromatic bodies by Neisser and Loeffler's methods.

A specimen of the affected pharyngeal mucosa of a sick child with suspected diphtheria was examined. A smear was prepared and stained. Yellow rods with dark blue thickenings at the ends were revealed microscopically. What is the structural element of a microbial cell determined in the detected microorganisms?

A.Metachromatic bodies

B.Plasmids

C.Capsule

D.Spores

E.Flagella

In a smear of material taken from a patient with suspected diphtheria, yellow rods with blue grains at the ends were found. What method of staining is used in this case?

A.Neisser

B.Loeffler

C.Neisser

D.Kozlovsky

E.Romanovsky

There are cases of sore throat among children of the boarding school. At microscopy of smears from the tonsils stained by the Neisser method, thin yellow rods with dark brown grains at the ends were revealed in the form of the Roman numeral five. What infection can be suspected in this case?

A.Diphtheria

B.Infectious mononucleosis

C.Listeriosis

D.Tonsillitis

E.Scarlet fever

A smear from the plaque on a patient's tonsils with suspected diphtheria revealed blue rods with thickenings on the poles. What method of staining was used?

A.Loeffler

B.Burri

C.Neisser

D.Hins'

E.Gram

Detection of acid-fast microorganisms. Some microorganisms contain high molecular weight lipids and mycolic acid. There are Mycobacterium tuberculosis, Leprosy, Actinomycetes, Nocardia, etc. It makes them acid-fast and resistant to the environment. Due to this feature, acid-fast microorganisms are poorly stained with aniline dyes, and the Ziehl-Neelsen method is usually used for staining. Concentrated carbolic magenta, followed by heating the smear, is used in the first staining stage. The sequence of the staining stages of smears by the method of Ziehl-Neelsen are:

1) a fixed smear is covered with a square or strip of filter paper and applied with Ziehl-Neelsen magenta. Next, the smear is heated over the burner's flame to the appearance of vapours and cooled. After 1-2 times heating, the cooled smear is washed with water;

2) the smear is decolourized by 5% solution of sulfuric or hydrochloric acid (approximately 30 s) and then washed several times with water;

3) additionally, the smear is stained with an aqueous-alcoholic solution of methylene blue (3-5 minutes), washed with water, dried out and studied microscopically.

Microscopic picture: red-ruby colour acid-fast microorganisms are located on a blue background (blue background due to other microbes, cells of the studied material).

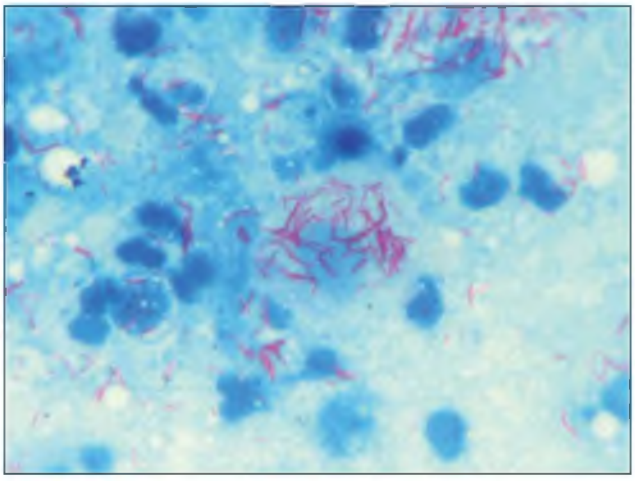

Figure 21 - Detection of acid-fast bacteria by the method of Ziehl-Neelsen

A patient has been treated for a long time for chronic pneumonia. Microscopic examination of sputum in smears stained by the Ziehl-Neelsen method

revealed red rods measuring 0.25 × 4 μm, located singly, sometimes in small clusters. What is the patient's disease?

A.Pulmonary tuberculosis

B.Pneumococcal pneumonia

C.Actinomycosis of the lungs

D.Influenza pneumonia

E.Pulmonary candidiasis

Red rods were found in the laboratory during microscopy of smears from the sputum of a patient with chronic lung disease, stained by* Ziehl-Neelsen. *What property of tuberculosis causative agent is found in this case?

A.Acidfastnes

B.Alcohol resistance

C.Spore formation

D.Capsule formation

E.Alkali resistance

The complex method of staining by Romanowsky-Giemsa produces smears stained polychromatic. It is widely used in laboratory practice to study microorganisms of different taxonomic affiliations, cellular structures, and certain types of tissues (in particular, blood) with the help of light field microscopy. Microscopic picture: cytoplasm of eukaryotic cells is blue; cell nuclei, flagella, and other structures, as well as the bodies of bacteria, are red-purple.

A doctor found thin microorganisms with 12-14 uniform coils with sharp ends 10-13 microns long, pale pink in the micro preparation made from the punctate of the patient's regional lymph node, stained by Romanowsky-Giemsa. So what infectious disease pathogen can we talk about in this case?

A.Syphilis

B.Trypanosomiasis

C..Leptospirosis

D.Typhoid fever

E.Leishmaniasis

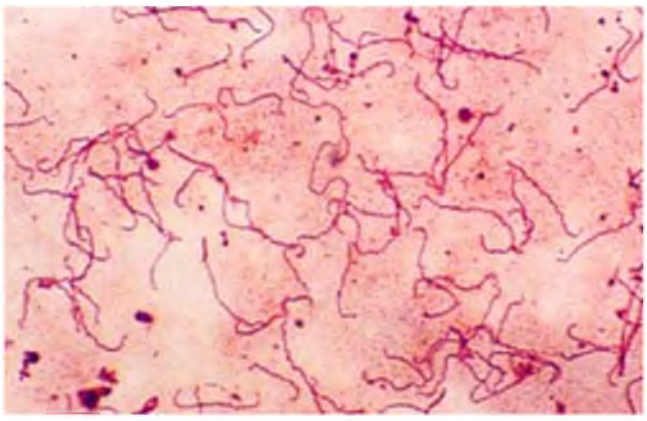

Figure 22 - Morphology of Treponema pallidum. Staining by Romanowsky-Giemsa

Microscopic examination of living microbes

"Hanging" or wet mount drop is used to study living microorganisms by dark-field, phase-contrast, or anoptral microscopy. Such methods make it possible to detect the motility of microbial cells, differentiate bacteria by the type of movement, or study the fine structure of sufficiently large microorganisms (fungi, protozoa). It is advisable to use in vivo staining of microorganisms with low-toxic dyes in large dilutions (methylene blue, neutral red, Congo, etc.). When microscopy of vitally coloured smears is made by "hanging" or wet mount drops, the contrast of the studied objects is increased significantly. It is possible to use fluorescent microscopy when staining smears with fluorochromes.

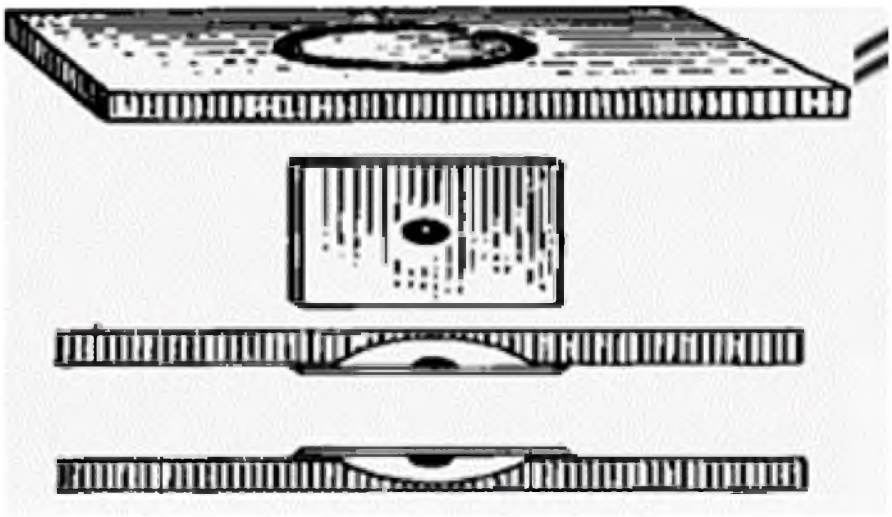

Figure 23 - "Hanging" drop preparation

Microscopic examination of the native smear from the patient's stools, which have a blood-mucous character, revealed microorganisms of round shape, the cytoplasm of which contains erythrocytes, as well as small cysts with four nuclei. What pathogen is it?

A.Entamoeba histolitica

B.Entamoeba coli

C.Lamblia intestinalis

D.Trichomonas intestinalis

E.Leichmania donovani

A patient who returned from one of the southern countries showed clinical symptoms of parasitic lesions of the colon. Microscopic examination of faeces revealed rounded shape microbes of about 10 μm in size with 4 nuclei identified as pathogen cysts. What diagnosis can be made based on laboratory data?

A.Amoebic dysentery

B.Balantidiasis

C.Enterobiosis

D.Enterocolitis

E.Teniosis

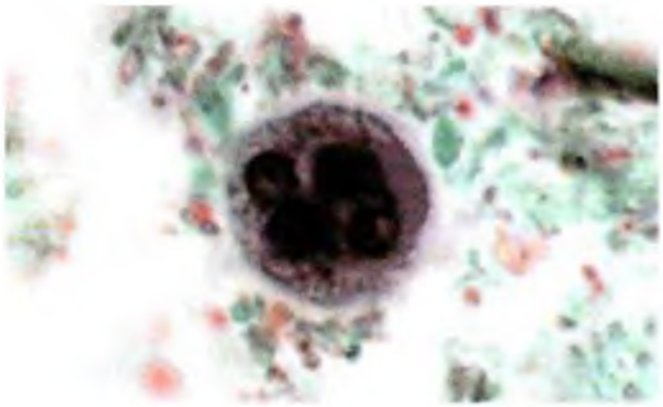

Figure 24 – Cyst of Entamoeba histolytica

Pathological material was obtained from the chancre of patient M., 28- year-old. Dark-field microscopy was performed in the laboratory of the dermatovenereological hospital. What property of syphilis treponema is observed, first of all, in an unfixed preparation?

A.Active motility

B.Spiral shape

C.Number of coils

D.The primary nature of coils

E.The secondary nature of coils

Features of the morphology of other groups of microorganisms

Spirochetes are thin, long, coiled microorganisms. Unlike bacteria, they have a protoplasmic cylinder surrounded by an outer membrane. An axial thread (axostil) is turned around the protoplasmic cylinder with periplasmic fibrils. It forms primary coils and causes the spirochetes' rotational, bending, and translational motion. Pathogens for humans are representatives of 3 genera: Treponema, Borrelia, and Leptospira. They differ in the number, nature, origin of coils, as well as features of movement. Fixed smears for light field microscopy are usually stained by Romanowsky-Giemsa and with silver by Morozov. Microscopic picture:

a) by Romanowsky-Giemsa treponema has a pale pink colour and 8-12 small, uniform primary coils; purple Borrelia, rather long and has 3-8 different large primary and secondary coils and pointed ends; Leptospira is pale pink and has 12–18 shallow tiny frequent primary coils, the ends of microbial cells are bent in the form of hooks with thickenings due to the formation of secondary coils, which make Leptospira S- or C-shaped;

b) spirochetes stained by Morozov are dark brown with individually distinct morphological features on a yellow background of the smear.

Microscopic detection of spirochetes contrasted by Burry is possible: coiled unstained microbial cells are located on a black background of the smear (under conditions of black India ink).

The mobility of spirochetes is detected in native smears «hanging» or «crushed» drop by dark-field, phase-contrast, or anoptral microscopy.

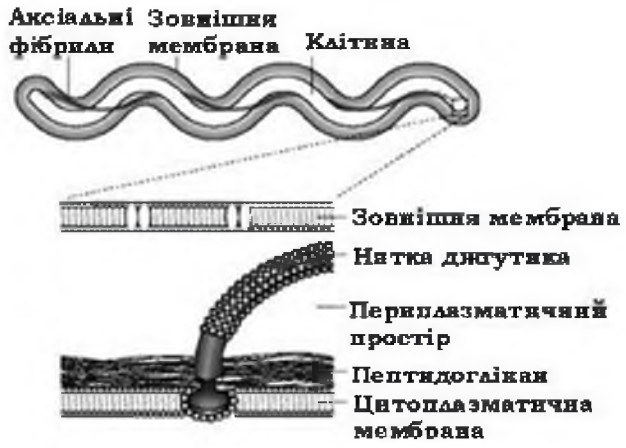

Figure 25 – Scheme of the spirochetes' structure

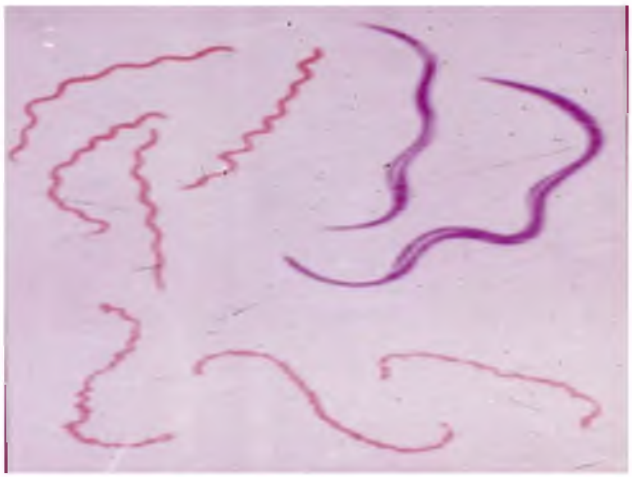

Figure 26 – Morphology of spirochetes: distinctive features of Treponema, Borrelia and Leptospira

In the smear made from the punctate of the patient's regional lymph node, stained by Romanowsky-Giemsa, the doctor found thin microorganisms with 12-14 uniform coils with sharp ends 10-13 microns long, pale pink. So what infectious disease pathogen can we talk about in this case?

A.Syphilis

B.Trypanosomiasis

C.Leptospirosis

D.Typhoid fever

E.Leishmaniasis

A patient with recurrent fever was admitted to the infectious disease hospital. Spiral-like microorganisms with sharp blue-violet ends were found in a blood preparation (thick drop) stained by the Romanowski-Giemza method. What pathogen was detected?

A.Relapsing fever

B.Leptospirosis

C.Spotted fever

D.Typhoid fever

E.Malaria

The man died of acute infectious disease, accompanied by fever, jaundice, hemorrhagic rash on the skin and mucous membranes, and acute renal failure. Histological examination of kidney tissue (Romanowski-Gimza staining) revealed coiled bacteria appearing in letters C and S. Which bacteria were detected?

A.Leptospira

B.Spirilla

C.Borrelia

D.Campylobacteria

E.Treponema

Actinomycetes are gram-positive acid-fast bacteria, elongated cells capable of branching. Long or short branched cells resemble hyphae of fungi, the accumulation of which in fungi is called mycelium. This term is still used for actinomycetes in textbooks and scientific sources. According to current scientific data, they have no spore and are non-motile. They reproduce only asexually, mainly by fragmentation with the formation of rod-shaped or coccal cells. In the affected tissues, actinomycetes form «sulfur granules». The granules are formed by a close plexus of thin filamentous cells, departing from the centre in the form of rays and ending in flask-shaped thickenings. Microscopically, actinomycetes are detected:

1) by preparing native (unstained) smears from the affected tissues, use the method of «crushed» drop with preliminary preparation of the studied material. Microscopy is usually phase-contrast or anoptral. Microscopic picture: there are «sulfur granules» - aggregates of elongated cells, from which radially (like the sun's rays) depart filamentous cells with thickenings at the ends);

2) by preparing the fixed, stained preparations with their studying in a light field microscope. Microscopic picture: a) by Gram: bacterial cells are rod-shaped or coccal-shaped; rod-shaped bacteria often have thickened ends; bacteria are located singly, in pairs, like a palisade or V- or Y-letters (reminiscent of hieroglyphs). Bacteria are usually purple, but they do not hold Gram dyes well, so actinomycetes are considered Gram-variable; b) by Ziehl-Neelsen: elongated red colour cells of a certain shape.

Actinomycetes were detected at preparation made from the purulent contents of the lesion localized in the patient's neck. To which group of bacteria should these microorganisms be classified by morphological characteristics?

A.Filamentous

B.Cocci

C.Bacteria

D.Coiled

E.Clostridia

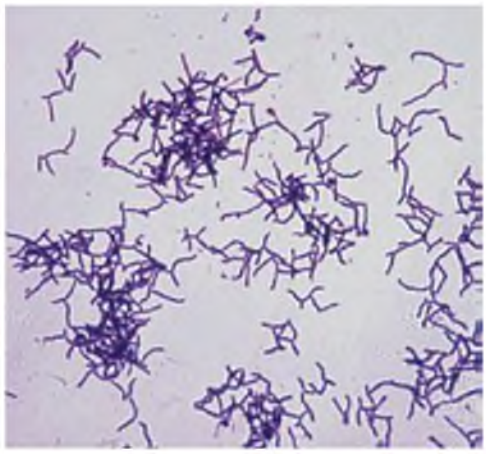

Figure 27 - Morphology of the Actinomyces order. Gram staining

A solid phlegmon-like infiltrate of blue-purple colour with numerous fistulas was founded in a sick man's cervical and maxillary region. Pus with an unpleasant odour. To confirm the diagnosis of actinomycosis by microscopic examination of pus bacteriologist must identify:

A.«Sulfur granules»

B.Gram-positive streptococci

C.Gram-negative diplobacteria

D.Acid-fast rods

E.Gram-negative diplococci

Figure 28 - «Sulfur granules» of actinomycetes

Pus was taken from the fistulous canal of the mandible, stained by Gram and investigated **microscopically. «Sulfur granules»** ***were revealed. It was stained gram-positive in the centre and gram-negative in the peripheral flask-shaped formations. The causative agent of which disease resembles this morphology?***

A.Actinomycosis

B.Candidiasis

C.Anaerobic infection

D.Staphylococcal osteomyelitis

E.Fusobacteriosis

Rickettsia. Small polymorphic prokaryotic microorganisms are close to gram-negative bacteria in their cell structure. According to morphological features, there are several types of rickettsiae: coccoid, rod-shaped, bacillary, and filamentous. All types have a microcapsular layer and fimbriae. Do not have spores, capsules, and flagella. Unlike bacteria, they are obligate intracellular parasites of animals, including arthropods and humans. However, they are not entirely energy dependent on the metabolic processes of the infected cell and have their systems and enzymes of macromolecular synthesis. Rickettsia reproduces asexually: by transverse division or fragmentation of microbial cells. Rickettsia cells are poorly receptive to dyes, so contrast stainings methods are used to detect them in the test material. Microscopic picture:

1) by Zdrodovsky - ruby-red rickettsiae located in the cytoplasm and the nucleus of an infected cell; the cytoplasm of the infected cell is blue, and its nucleus is blue;
2)) by Romanowsky-Giemsa - Rickettsia of bluish-purple (purple) colour in the cytoplasm and the nucleus of the infected cell; the cytoplasm of the infected cell is blue, and its nucleus is reddish-purple.

Representatives of the genus Rickettsia are the causative agents of typhus and spotted fever. On the other hand, Coxiella (pathogens of Q fever), due to differences from rickettsiae in structure and development according to modern classification, are classified as a separate family and genus.

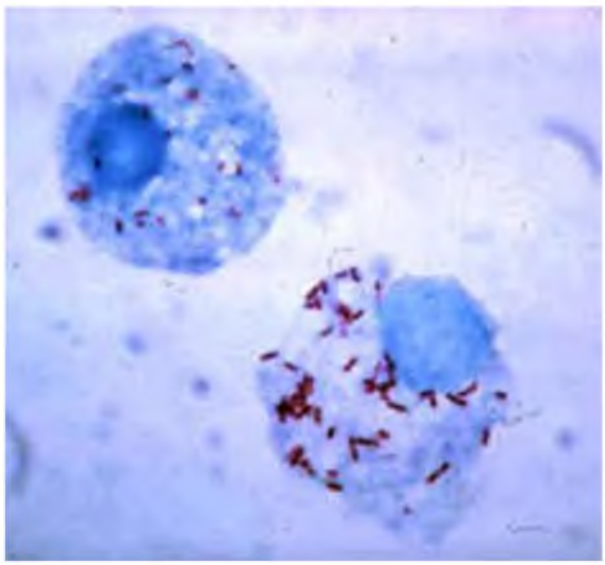

Figure 29 – Morphology of microbial cells of the genus Rickettsia. Staining by Zdrodowski

Chlamydia. Small gram-negative bacteria of spherical or ovoid shape. Do not form spores and do not have capsules and flagella. The cell wall does not contain peptidoglycan typical of bacteria. There are obligate intracellular parasites according to physiological processes. The life cycle of chlamydia, unlike other groups of bacteria, is accompanied by the formation of elementary and reticular bodies. Elementary bodies are the extracellular form of existence of these bacteria and have infectious properties; reticular bodies are the intracellular form of their existence and have no infectious properties. Reticular bodies form clusters (microcolonies) in the cytoplasm of affected cells. Chlamydia multiply by repeated binary division. Such clusters in microscopic examination of smears are called CPI (cytoplasmic inclusions). Due to intracellular parasitism, the Romanowsky-Giemsa staining method is used for detecting chlamydia in micro preparations. Microscopic picture:

a) elementary bodies of pink or red-violet colour are located extracellularly or sorbed on the plasmalemma of host cells;

b) reticular bodies of blue colour in the form of clusters (CPI) are located around the nucleus of the affected cell and are shaped like "gendarme caps". They can be located in the cytoplasm diffusely; the cytoplasm of the affected cells is purple, and their nucleus is burgundy-purple. It is possible to detect CPI chlamydia in acridine-stained micro preparations using fluorescence microscopy. Microscopic picture: CPIs

have a bright green colour on a reddish-brown background of the affected cells. Chlamydia is the causative agent of trachoma, urogenital infections, pneumonia, ornithosis, etc.

Cytoplasmic inclusions were revealed in epithelial cells by microscopy of smears from the urethra. These had the form of characteristic "caps" over the cell nucleus. What disease should be assumed?

A.Chlamydia

B.Gonorrhoea

C.AIDS

D.Syphilis

E.Genital herpes

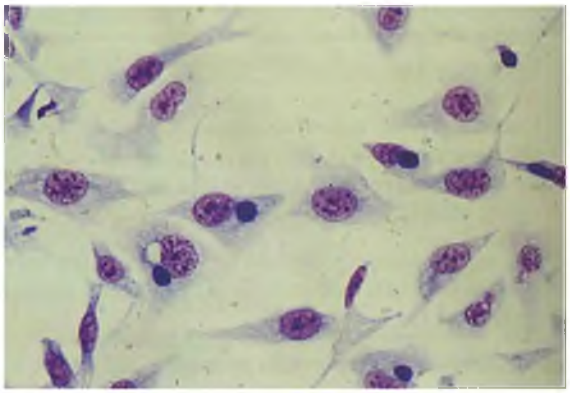

Figure 30 - Cytoplasmic inclusions of chlamydia (genus Chlamydia). Staining by Romanowsky-Giemsa

Epithelial cells containing reticular and elementary bodies were found during the microscopic examination of the scraping of the mucous membrane from a patient with a urogenital infection. The Romanowsky-Giemsa staining method was used. What pathogenic microorganism is it?

A.Chlamydia

B.Mycoplasmas

C.Rickettsia

D.Viruses

E.Fungi

Mycoplasmas have peculiarities in structural and biological properties. These are the smallest self-replicating prokaryotes among non-nuclear microorganisms with minimal organelles. The cytoplasmic membrane is similar to eukaryotic in composition. Unlike bacteria, they do not have a cell wall and its precursors. So, they

are osmotically unstable, polymorphic, and resistant to beta-lactamase antibiotics. They can be coccoid, pear-shaped, filamentous forms, and long filamentous branched forms. Coccoid forms can form a ring. Mycoplasmas need sterols (cholesterol and its derivatives) and fatty acids to ensure life processes and growth on nutrient media. They reproduce asexually: by binary division, fragmentation, and budding of microbial cells. Mycoplasmas are similar to viruses in genome size and susceptibility to intracellular parasitism. The microscopic method of detection and study of mycoplasmas for diagnosing infectious diseases in routine practice is not used. Mycoplasmas are the causative agents of respiratory diseases and lesions of the urinary and genital systems.

Protozoa are eukaryotes. The body consists of a single cell that performs all the functions of a whole organism.

In the cytoplasm, one or more nuclei and organelles of general purpose are inherent in the cells of the body of multicellular organisms. In addition, they contain organelles specific only to this group of organisms: organelles of movement (pseudopods, flagella, cilia), the kinetoplast, contractile and digestive vacuoles, defence organelles (trichocysts), supporting formations (axostyle), and light-sensitive eyes (stigmas) in free-living forms. Under favourable conditions, they can form cysts and are mainly heterotrophs. They have sexual (by gamete fusion, conjugation) and asexual (mitotic and amitotic cell division, schizogony) reproduction. Many species of protozoa have complex developmental cycles. Parasitic protozoa cause malaria, toxoplasmosis, trypanosomiasis, leishmaniasis, trichomanosis, giardiasis, amoebiasis in humans. For the detection of protozoan cells (vegetative forms), their certain stages of development, and cysts in the light field microscope, the most commonly used contrast staining method is by Romanowski-Giemsa.

Microscopic picture: cytoplasm of blue protozoan cells; nucleus, undulating membrane, the kinetoplast, flagella, if any have, red or pink-purple. Other methods of staining the protozoa are possible: methylene blue, neutral red, hematoxylin by Heidenhain, Lugol's solution, Gram's method, etc. Native preparations («hanging» or «crushed» drop) are studied using dark-field or phase-contrast microscopy.

Microscopic examination of the smears of the cerebrospinal fluid, stained by Romanowski-Giemsa, revealed protozoa, crescent-shaped with pointed ends, blue cytoplasm, and ruby-red nucleus. What pathogen is it?

A.Toxoplasmosis

B.Malaria

C.Leishmaniasis

D.Trypanosomiasis

E.Amoebiasis

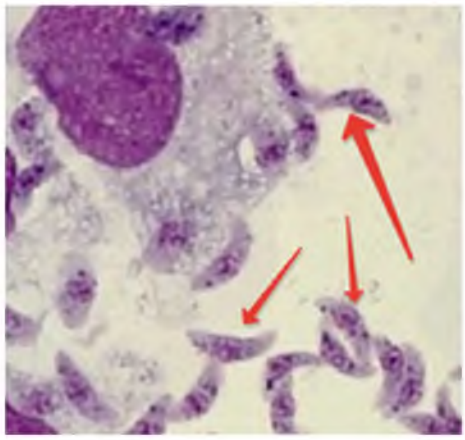

Figure 31 – Morphology of protozoa genus Toxoplasma. Staining by Romanowski-Giemsa

During microscopy of a blood smear stained by the method of Romanowski-Giemsa, the doctor found the protozoa in the form of a crescent moon, the protoplasm stained in blue colour, and the nucleus in – red. What are the protozoa most likely to be found?

A.Toxoplasma

B.Trypanosomes

C.Leishmania

D.Giardia

E.Balantidia

Patients have similar complaints: weakness, intestinal pain, and gastrointestinal disorder. After the faeces investigation, it became clear that one of the patients, who had cysts with four nuclei, needed urgent hospitalization. Which protozoa is characterized by such cysts?

A.Dysenteric amoeba

B.Giardia

C.Trichomonas

D.Balantidius

E.Intestinal amoeba

A patient with biliary tract inflammation was admitted to the gastroenterology department. Mobile protozoa pear-shaped, dinuclear, with a support rod-axostyle, were found in portions of bile. What protozoan disease is diagnosed in a patient?

A.Lambliasis

B.Intestinal amoebiasis

C.Intestinal balantidiasis

D.Amoebic dysentery

E.Trichomoniasis

Protozoa size of 10-18 microns is founded in the smear of duodenal content of a patient with dyspepsia. Pear-shaped body, 4 pairs of flagella, two nuclei which are placed symmetrically in the extended front of the body. What kind of protozoa is it most likely?

A.Giardia

B.Balantidius

C.Intestinal amoeba

D.Dysenteric amoeba

E.Trichomonas

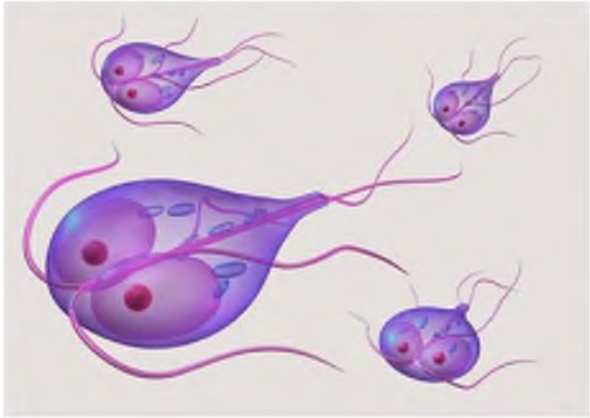

Figure 32 –Morphology of protozoa genus Giardia. Vegetative form

During the examination of a patient, the gynaecologist noted symptoms of inflammation of the genital tract. In addition, pear-shaped protozoa with a thorn and flagella departed from the front, with an undulating membrane found in a smear taken from the vagina. What disease does the doctor suspect in the patient?

A.Urogenital trichomoniasis

B.Balantidiosis

C.Giardiasis

D.Toxoplasmosis

E.Intestinal trichomoniasis

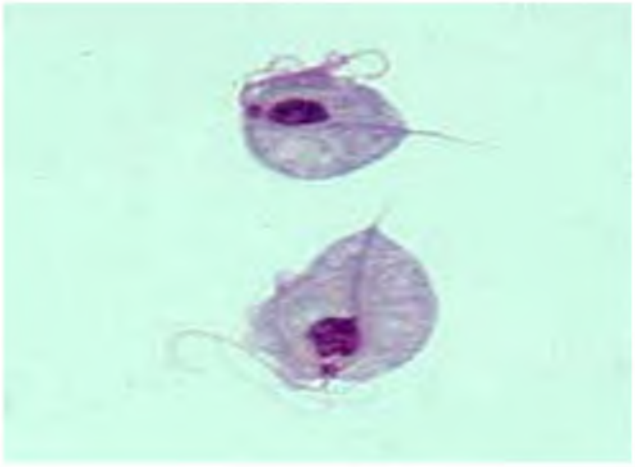

Figure 33 – Morphology protozoa Trichomonas genus

Skin ulcers in a person after being bitten by a mosquito. Analysis of the contents of the ulcer revealed flagellate unicellular organisms inside human cells. What is the previous diagnosis?

A.Dermatotropic leishmaniasis

B.Toxoplasmosis

C.Visceral leishmaniasis

D.Trypanosomiasis

E.Balantidiosis

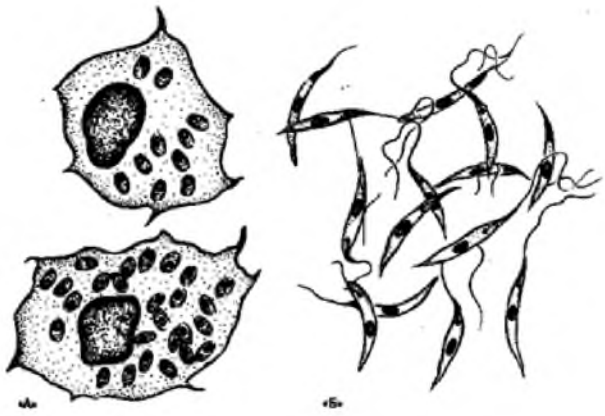

Figure 34 – Morphology protozoa Leischmania genus

Fungi are a heterogeneous group of unicellular or multicellular eukaryotic organisms. A fungal cell usually has a single nucleus and cytoplasm with organelles surrounded by a cytoplasmic membrane (CPM) and a cell wall. CPM is similar in composition to that of mammals; the presence of ergosterol is distinctive (in

mammals – cholesterol). The cell wall is multilayered, rigid, and consists of several types of polysaccharides, including chitin. By type of nutrition, they are heterotrophs; some are saprophytes, parasites, and symbionts. They reproduce asexually or asexually and sexually, specific to each taxon within the kingdom of Fungi.

There are two main types of fungi: hyphae and yeast. Hyphae (in moulds) form branched thin threads (hyphae), the interweaving of which forms mycelium. Hyphae can be septated by membranes – in higher fungi (Penicillium, Aspergillus) or non-septate (lack of membranes) in lower fungi (mucor). Hyphae that grow into the nutrient substrate form the vegetative mycelium (nourishes the fungus), and those above the substrate surface form the aerial or reproductive mycelium (involved in asexual reproduction). Asexual reproduction occurs by budding, fragmentation of hyphae, and asexual spores. There are endospores (mature inside spherical structures named sporangia) and exospores (conidia), formed at the tips of reproductive hyphae, the so-called conidiophores. Conidia (spores) are of several types depending on their formation: arthroconidia, taloconidia, blastoconidia, and chlamydoconidia. Large multicellular conidia are called macroconidia, and the small one is named microconidia. Sexual reproduction of fungi occurs with the formation of gametes, sexual spores, and other sexual forms. In the vast majority, yeast fungi have the form of individual oval cells. They reproduce asexually and sexually. Those that reproduce only asexually are called yeasts, represented by fungi of the genus Candida. Reproduction of yeast-like fungi of the genus Candida occurs by budding or division, possibly forming false mycelium (pseudomycelium), which consists of chains of elongated cells. Fungi are pathogens of mucormycosis, penicillosis, aspergillosis, candidiasis, dermatophytes, and deep mycoses. Microscopic study of fungi that cause mycoses in humans is carried out in 2 directions: 1) the study of native micro preparations; 2) the study of fixed, stained micro preparations. Native preparations are usually prepared from hair, nail scrubs, skin flakes, and other dense materials pre-clarified in 10-30% solution of sodium hydroxide or potassium or other solutions when heated. The treated material is placed on a glass slide in a drop of glycerin or

saline solution, covered with a cover glass and examined in a dark-field or phase-contrast microscope. Microscopic picture:

fungi of the genus Mucor have unseptated mycelium, spherical sporangia with endospores in it, located on reproductive hyphae;

fungi of the genus Penicillium have septated mycelium, reproductive hyphae (conidiophores) cone-shaped, from which endospores are untied in the form of tassels (brushes);

fungi of the genus Aspergillus have septated mycelium, reproductive hyphae (conidiophores) with a spherical thickening at the apex, and radially arranged exospores (in the form of a funnel);

fungi of the genus Candida have round or oval budding cells, possibly pseudomycelium.

In preparations from the affected hair, septated or unseptated mycelium, spores of a certain shape and location are either/and in the middle of the hair (endothrix type) and outside the hair (ectothrix type) is present.

Also, in the affected hair, it is possible to detect air bubbles and different morphological fragments of hyphae (branched, round or square); the presence of a cover that envelops the hair is possible.

Septated or non-septated mycelium and characteristic location of fungal spores are visible in preparations from nail scrubs and skin scales.

Stained preparations are usually prepared from a material of liquid or viscous consistency. Gram, Ziehl-Neelsen, Romanowski-Giemsa, and other methods are used for staining, followed by the study of smears in a light-field immersion microscope. Microscopic picture: fungi of the genus Candida stained by Gram have dark purple round or oval buds. Pseudomycelium is possible;

by Ziehl-Neelsen – round or oval cells of blue colour with pinkish-yellowish inclusions of lipids;

by Romanowsky-Giemsa, there are rounded or oval cells of pink-yellow colour with dark purple inclusions of volutin and red chromatin substance.

Dermatophytes and mould fungi usually are not stained. Both native and stained preparations are used for the microscopic study of deep mycosis pathogens.

A child has whitish spots on the mucous membrane of the cheeks and tongue, resembling boiled milk. In addition, gram-positive oval yeast-like cells were found in the prepared smear. What are these pathogens?

A.Fungi of the Candida genus

B.Staphylococci

C.Diphtheria rods

D.Actinomycetes

E.Fusobacteria

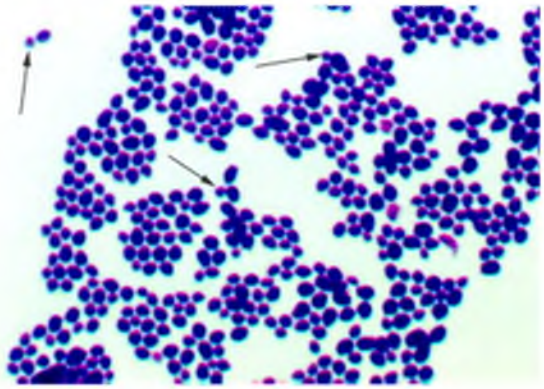

Figure 35 – Morphology of fungi Candida genus. Gram staining

Big gram-positive oval budding and elongated cells arranged in chains were isolated from a patient's mucous membranes and sputum. The patient had been receiving immunosuppressants for a long time. Which pathogen is isolated?

A.Candida

B.Streptobacteria

C.Actinomycetes

D.Streptococci

E.Yersinia

During microscopy of the micro preparation from the secretions of a patient with chronic colpo-vaginitis, the doctor found rounded and elliptical budding cells, 3-6 μm in size. The causative agent of which fungal disease can we talk about in this case?

A.Candidiasis

B.Coccidiosis

C.Microsporia

D.Cryptococcosis

E.Epidermophytia

Viruses. They do not have a cellular structure. These are obligate molecular genetic parasites of cells of lower and higher plants, animals, including humans; they do not have their protein-synthesizing and energy-supplying systems, incapable of growth and binary separation. The elementary unit that retains the properties of the virus is the virion. Viruses can be simple or complex in structure. Simple viruses consist of two chemical compounds – nucleic acid and proteins- structurally forming a nucleocapsid. Complex viruses have an additional shell (supercapsid or envelope), which is a phospholipid fragment of the cell membrane of host cells (target cells), enriched with viral proteins. Nucleic acids make up the genome of virions, but unlike cellular organisms, the carrier of genetic information can be either DNA molecules or RNA molecules. According to the topography of structural proteins of virions, there are nucleocapsid and supercapsid proteins. Protein nucleocapsid consists of capsid surrounding the virion genome.

Capsomeres are monomeric capsid structures. The spatial arrangement of capsomers has 3 types of symmetry (spiral, cubic, and combined). It determines the shape of virions.

Supercapsid proteins are located in the lipoprotein shell of complex virions and are typical intramembrane proteins.

Matrix protein (tegument) may be located between the capsid and supercapsid in certain complex viruses. Glycoprotein spikes are located superficially on many virions. Spikes, as well as other surface proteins, play a significant role in the specific interaction of virions with the receptors of target cells, which determines the ability of viruses to infect sensitive cells of living organisms. There are three types of interaction of virions with the host cell: productive, integrative, and abortive. Non-structural proteins provide viral reproduction. Bacterial viruses (bacteriophages) are common in nature. Bacteriophages interact specifically with bacteria. They are used in medical practice.

It can be phage therapy, phage prophylaxis, phage diagnostics and phage typing.

Microscopic detection of viruses

Different types of electron microscopy are often used in modern methods of microbiological diagnosis of viral infections to detect and study the structure of viruses in preparations from the test material or infected cell cultures. Less often,

fluorescent microscopy is used. Fluorochrome-stained preparations are examined in the UV spectrum of a fluorescent microscope.

Microscopic picture: there are green spots for DNA-containing viruses or brick-orange spots for RNA-containing viruses on a dark background.

Some viruses can be detected by intracellular inclusions, clusters of viral particles, their components, and metabolites, and located in the nucleus and/or cytoplasm of infected cells. Smears for this purpose are stained by a certain method and studied in a light field immersion microscope.

Thus, in cells infected with the smallpox virus and stained by the Morozov method of silvering, perinuclear inclusions are detected (Guarneri bodies in cells of pathogenic material from the patient; Paschen in corneal cells of rabbits infected with this virus).

Babes-Negri bodies are cytoplasmic inclusions under rabies conditions stained by Turevich's method. Inclusions of red colour, yellow cytoplasm of nerve cells, brown nuclei of nerve cells. Cowdry bodies are herpes simplex virus intranuclear inclusions. It should be stained by Romanowsky-Giemsa. Chickenpox virus intranuclear inclusions (Lipschutz bodies) also are stained by Romanowsky-Giemsa.

The vesicle contents from a smallpox patient's mucous membrane were sent to the virology laboratory. Which of the following changes will be detected by smear microscopy?

A.Guarneri bodies

B.Babes-Negri bodies

C.Pashen bodies

D.Babes-Ernst bodies

E.Syncytium

Figure 36 – Guarneri bodies

A rabbit was infected with the contents of the vesicles of a patient with suspected smallpox. A smear imprint was prepared from the rabbit cornea. Microscopy of the smear, stained by Romanovsky, found bodies of different sizes and shapes located in the cytoplasm of the infected cells. Name these bodies.

A.Pashen

B.Aragao

C.Guarneri

D.Lipschutz

E.Babes-Negri

Specific to rabies is the detection of bodies:

A.Babes-Negri

B.Babes-Ernst

C.Provachek

D.Guarneri

E.Paschen

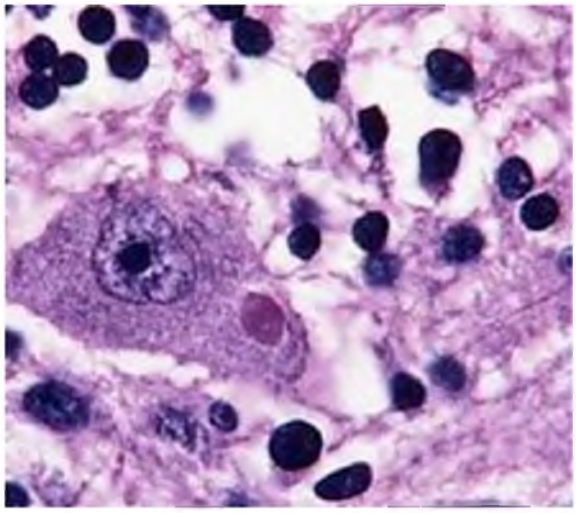

Figure 37 - Babes-Negri bodies

The staining method used to detect Babes-Negri bodies by the histological method:

A.By Turevich

B.By Gins

C.By Morozov

D.By Neisser

E.By Peshkov

Chapter III. PHYSIOLOGY OF BACTERIA. CULTURAL METHOD

The cultural method (bacteriological, mycological, protozoological, virological methods) provides:

1. Isolation of the pathogen culture from the studied material.
2. Identifying the isolated microorganism determines its taxonomic position (in particular, belonging to a family, genus, species, or other taxonomic categories).

Children who ate cheese in kindergarten developed a disease characterised by acute onset, nausea, vomiting, and diarrhoea. Microscopy of smears made of cheese and vomit revealed gram-positive microorganisms located in the smears in the form of clusters resembling bunches of grapes. What will be the following steps to establish the aetiology of the food poisoning outbreak?

A.Conducting a bacteriological method of research

B.To conclude, the cause of the disease was Staphylococcus

C.Additionally, put an allergy test

D.Additionally, determining the antibodies in the serum

E.Additionally, determining the staphylococcal phagotype

A patient with suspected dysentery was admitted to the infectious department. Which of the following methods of laboratory diagnosis should be prescribed?

A.Bacteriological

B.Serological

C.Allergic

D.Biological

E.Microscopic

In the admission department of the hospital, the material is selected for bacteriological examination. For what purpose should the material be taken from a patient with purulent lesions of the deep tissues of the lower extremity?

A.To establish the aetiology of the purulent process and determine antibiotic susceptibility.

B.To detect pathogenic Staphylococcus and determine the antibiotic susceptibility pattern.

C.To detect the pathogen to prevent nosocomial infection

D.To confirm anaerobic infection

E.To detect the toxicity of the pathogen

Media for the cultivation of microorganisms

The bacteriological method is carried out in several stages using particular nutrient media in each of them.

Requirements for nutrient media:

1) must contain an optimally balanced complex of organic and inorganic compounds and growth factors;

2) must be isotonic;

3) it must be sterile;

4) must have a specific pH of the media;

5) be as transparent as possible;

6) must have sufficient humidity.

Classification of nutrient media:

by origin: 1) natural; 2) artificial; 3) synthetic and semi-synthetic;

by composition: simple (meat peptone broth - MPB, meat peptone agar - MPA, Hottinger broth, Hottinger agar) and complex (in addition to simple media, certain nutrients are added);

by density:

Liquid, semi-solid (0.15-0.7% agar-agar) and solid (1.5-2% agar-agar); for thickening, it is possible to make gelatin or silica gel;

by appointment:

a) Basic (universal) are suitable for the cultivation of most species of bacteria (MPB, MPA, peptone water).

b) Special are suitable for cultivating those species of bacteria that do not grow on simple nutrient media or are suitable for studying specific properties of bacteria (sugar or blood MPB, sugar, ascetic or blood MPA, Kitt-Tarozzi medium).

A 55-year-old patient was hospitalised in a surgical clinic with suspected sepsis. What should material for research be taken from the patient, and in what media should it be inoculated?

A.Blood, sugar broth

B.Urine, meat-peptone broth

C.Lymph node puncture, cysteine agar

D.Cerebrospinal fluid, serum agar

E.Pus, yolk-salt agar

The air in the operating room was checked before the operation. The sedimentation method revealed 5 small round colonies, around which the hemolysis zone was visible. In what media was the culture made?

A.Blood MPA

B.MPA

C.Endo (McConkey agar)

D.YSA

E.Levin-Eosin-Methylene Blue Agar

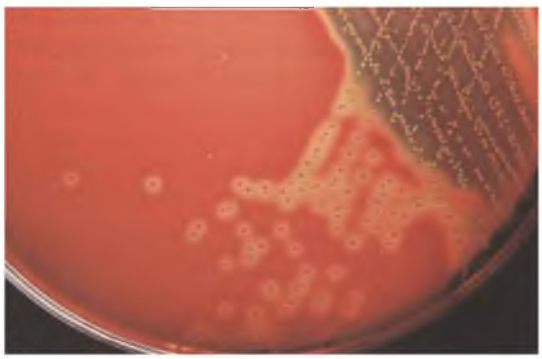

Figure 38 - Hemolytic properties of bacteria on blood agar

Eight days after the operation, the patient developed tetanus. The doctor suspected the cause was contaminated suture material delivered to the bacteriological laboratory. What nutrient medium should be used for primary inoculation of suture material?

A.Kitt-Tarozzi

B.Endo (McConkey agar)

C.Sabouraud's medium

D.YSA

E.Hiss's agar

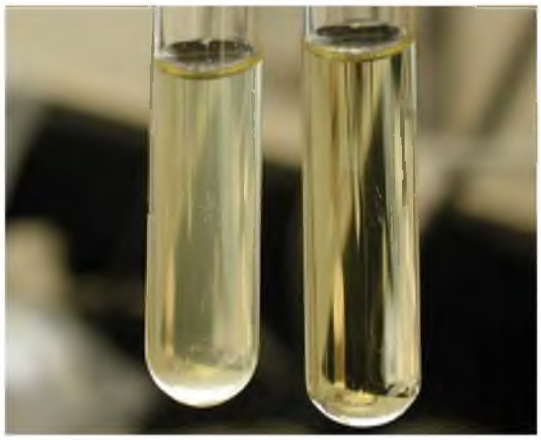

Figure 39 - Kitt-Tarozzi Medium

c) Elective (selective, enriched) are suitable for the cultivation of bacteria of one genus or species (Mueller-Hinton medium, Rapoport, selenite cysteine media, 1% alkaline peptone water):

From the faeces of a patient with acute gastroenteritis, a pure culture of motile small, slightly curved gram-negative rods was isolated, which for 6 hours gave growth in alkaline 1% peptone water in the form of a thin bluish film. Which microorganisms have such properties?

A.Vibrios

B.Spirochetes

C.Clostridia

D.Bacilli

E.Spirilla

1% alkaline peptone water after inoculation of the test material (faeces) and 8 hours of incubation on a thermostat at 37°C showed growth in the form of a thin blue film. Which pathogen is characterised by such cultural properties?

A.Cholera

B.Plague

C.Typhoid fever

D.Paratyphoids A, B

E.Dysentery

A gentle film on the surface of the media is revealed at primary inoculation of water in 1% of peptone water in 6 hours of growth. Which pathogen is characterised by such cultural properties?

A.The causative agent of cholera

B.The causative agent of the plague

C.The causative agent of tuberculosis

D.The causative agent of dysentery

E.The causative agent of pseudotuberculosis

d) Differential-diagnostic. It allows us to determine bacteria's biochemical properties and distinguish some species from others. For example, the growth of bacteria is accompanied by a change in the colour of the medium, its density and structure, or a change in the indicator's colour (Endo (McConkey agar), Levin-Eosin-Methylene Blue Agar, Ploskirev, Hiss's agar, etc.) media. Thus, in Endo (McConkey agar), Levin-Eosin-Methylene Blue Agar and Ploskirev media, depending on the indicator, Escherichia coli colonies acquire red or crimson colour with a metallic sheen, blue-black colour with a metallic sheen, pink colour (these media include lactose, which is fermenting by *Escherichia coli*, changing the pH of the medium to acidic). In contrast, the colonies of other members of the *Enterobacteriaceae* family are colourless, as they cannot ferment lactose and the pH of the medium remains unchanged.

When inoculation the faeces of a patient with typhoid fever on the Endo (McConkey agar) media, grown colonies have different colours and sizes: some are large red, and others are colourless with medium size. Which group of the nutrient media by the purpose is the nutrient medium specified?

A.Differential diagnostic

B.Elective

C.Special

D.Selective

E.Enrichment

A 7-year-old boy has a cholera-like illness (vomiting, profuse diarrhoea). When inoculation, the patient's faeces on the media Endo (McConkey agar) grew the same type of colonies: crimson with a metallic sheen. Which microorganism is the most likely pathogen?

A.Enterotoxigenic Escherichia coli

B.Salmonella enteritidis

C.Yersinia enterocolitica

D.Shigella sonnei

E.NAG vibrio

Bacteriological research was done on the washing waters of the patient with food poisoning. A pure culture of bacteria with such properties was isolated: gram-negative mobile rods. On the Endo (McConkey agar), media grows in the form of colourless colonies. **What genus of bacteria the representative that caused the disease is?**

A.Salmonella

B.Yersinia

C.Escherichia

D.Citrobacter

E.Shigella

Figure 40 - Endo (McConkey agar) medium

Hiss's agar medium with an indicator under the conditions of fermentation of a carbohydrate becomes blue (acid formation) or blue with bubbles in the thickness of the medium (acid and gas formation).

Pure culture of bacteria was isolated from the studied material of a patient. On what nutrient media is the pathogen identified by enzymatic properties?

A.Hiss's media

B.Ploskirev's media

C.Endo (McConkey agar)

Levin-Eosin-Methylene D.Blue Agar

E.Wilson-Blair medium

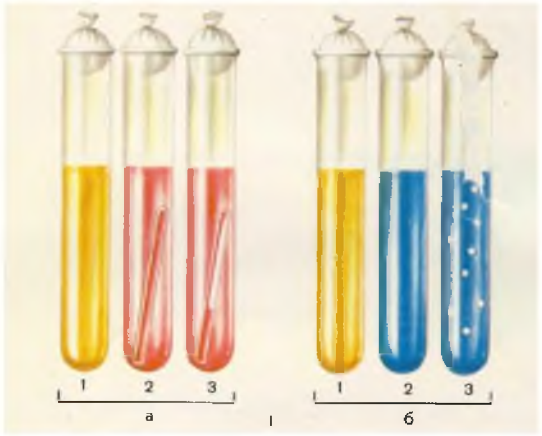

Figure 41 – Determining saccharolytic activity of bacteria on Hiss's media

At the preparatory stage of microbiological research, nutrient media, laboratory utensils, tools, and other auxiliary materials must be sterilised.

Basic sterilisation methods

Sterilisation is a set of tools aimed at destroying all the vegetative and spore forms of microorganisms (pathogenic, opportunistic, and non-pathogenic) and neutralising their toxins and metabolites. Different methods are used for sterilisation:

1) physical (thermal sterilisation - exposure to high temperatures; cold sterilisation - filtration through fine-grained antibacterial or antiviral filters; radiation sterilisation - the use of ultraviolet and gamma rays;

2) chemical (use of various antiseptics);

3) biological (use of antibiotics).

The use of the sterilisation method is due to the characteristics of the material, its physical and chemical properties, weight, and volume.

Thermal sterilisation is a kind of physical method of sterilisation that is divided into:

1) calcination or flambering in the flame of the burner is used for sterilisation of the glass pipettes, bacteriological loops, slides, edges of test tubes and flasks, cotton plugs, dissecting needles, and tweezers;

2) boiling is used for 40 minutes to sterilise reusable syringes, needles, surgical instruments, glass and metal utensils, and rubber tubes;

3) dry heat sterilisation is carried out in an oven, usually at a 160-170°C temperature, for 2 hours. It is used for sterilisation of heat-resistant glass and metal tools, utensils, heat-resistant powders, and other substances;

4) steam sterilisation under pressure (autoclaving) is carried out in stationary autoclaves, in which the simultaneous action of temperature and steam provides the highest sterilisation efficiency when using different process modes. Simple nutrient media (MPB, MPA), isotonic solutions, laboratory glassware, dressings, items that deteriorate from dry heat, sterilised in an autoclave at 120°C for 20 minutes; neutralisation of infectious material, spent cultures of microorganisms at 134°C for 40 minutes;

5) fluid steam sterilisation (fractional sterilisation) - is carried out at a temperature of 100°C, fractionally for three days with fluid steam for 20-30 minutes in an autoclave with an unscrewed lid. It is used for neutralisation of vegetative and spore microorganisms, sterilisation of media, which under the action of temperatures above 100°C lose their qualities, in particular, nutrient media with carbohydrates and protein substrates;

6) pasteurisation is carried out by single heating of the material at 90°C. It destroys asporogenic microorganisms, mainly pathogenic and opportunistic pathogens. The method proposed by L. Pasteur is used in the food industry.

Control of sterilisation efficiency and reliability of sterilisers is carried out using:

1) biotests (specially made tests containing dried spores of known strains of bacteria). At the end of sterilisation, biotests are subject to bacteriological examination.

2) chemicals (sealed glass tubes containing certain chemicals with a known melting point with the addition of aniline dye). The change in colour of the dye indicates the proper mode of sterilisation in the autoclave.

Meat-peptone broth was prepared for sterilisation in the bacteriological laboratory. Which of the sterilisation methods should be used?

A.Autoclaving at 121°C for 30 min

B.Dry heat at 160°C for 2 hours

C.Boiling for 1 hour

D.Filtering

E.Tyndalization

In the bacteriological laboratory, it is necessary to sterilise nutrient media with substances that change properties at temperatures above 100°C* (urea, carbohydrates*). Which method of sterilisation should the laboratory assistant choose?

A.Liquid steam, fractionally

B.Steam under pressure in an autoclave

C.Boiling

D.Tyndalization

E.Pasteurisation

The biological method was decided to use in a hospital to control the instrument's sterilisation quality in autoclave use. Which microorganisms are most appropriate to use as test cultures?

A.Spor forming

B.Capsulated

C.Acid-fast

D.Pathogenic

E.Thermophilic

Disinfection is a set of tools aimed at the destruction of pathogenic microorganisms and their toxins in contaminated medical objects (patients' excretion, utensils for excretion and food, linen, clothing, premises, laboratory equipment, furniture, utensils) in order to prevent the transmission of a pathogen from a source of infection to a susceptible organism. Disinfection measures are specific and aimed at destroying pathogens of certain infectious diseases. To carry out disinfection, you need to know:

1) what you need to disinfect;

2) when it is necessary to carry out the disinfection;

3) which substance should be used;

4) how to do it.

There are two types of disinfection: focal (final and current) and preventive.

Physical and chemical disinfectants are used for disinfection. Physical means include mechanical, thermal and radiation disinfectants.

Chemical disinfectants are chemicals that have a bactericidal or bacteriostatic effect on microorganisms. These are chlorine-containing substances, alcohols, oxidants, phenols and their derivatives, aldehydes, iodine, and bromine. Also, there are detergents, including chloramines, chlorine, methyl and ethyl alcohol, hydrogen peroxide, potassium permanganate, phenol, formaldehyde, Zulema, soap, etc.

The properties of the pathogen determine the choice, the properties of the media pathogens are located (consistency, number of secretions), and the properties of objects to be disinfected.

Upon completing work in the laboratory, the student must disinfect the workplace. What chemical should he use for this?

A.Alcohol

B.Hydrochloric acid

C.Antibiotics

D.Chloroform

E.Ether

After finishing work in the laboratory, the student must tidy up his workplace and disinfect the table and tools. What chemicals should he use for this?

A.Chloramine

B.Hydrochloric acid

C.Formalin

D.Chloroform

E.Ether

Antisepsis is a set of measures aimed at destroying microbes or suppressing the activity of microorganisms already present in the human body (in the trauma of skin, mucous membranes, and body cavities). It can cause an infectious process that is dangerous to human health (purulent wounds, purulent-inflammatory skin formations, soft tissues, and mucous membranes). Antisepsis includes methods: mechanical (removal of infected necrotic tissues, foreign bodies, etc.), physical (drainage of wounds, tampons, hygroscopic dressings), chemical (use of various antiseptics), biological (use of proteolytic enzymes for lysis of non-viable cells, the use of bacteriophages, antibiotics). Usually, a set of these methods is used.

Asepsis is a set of measures, the implementation of which prevents the entry of pathogenic microorganisms into the human body during surgery and certain medical and diagnostic procedures. For example, it is possible in the uncontaminated air of operating and treatment rooms using sterile surgical instruments, medical equipment, dressings, sutures and other operating material, and overalls of surgeons and other medical workers. Aseptic methods are used to combat exogenous infection, the sources of which are patients and carriers. Asepsis measures also include planning operating rooms (floor, boxing, ventilation, air conditioning, etc.). In addition, aseptic methods have been introduced in microbiological production and the food industry.

Isolation and identification of pure bacterial culture by bacteriological examination

A pure culture is a population of microorganisms of the same species that grew on a solid or liquid nutrient medium. The pattern of microbial growth on nutrient media is called cultural properties.

1st stage.

Microscopy of the smear made of the test material; determining of morphology and tinctorial properties of detected microorganisms; the inoculation of the studied material on solid nutrient media to obtain isolated colonies. Inoculation is usually carried out by the Gold method (sector method), which involves the mechanical separation of bacterial cells. The culture is kept on a thermostat at 37°C for 18-24 hours.

2nd stage.

Macro- and microscopic study of isolated colonies. Isolated colonies are the separated clusters of microorganisms on a solid nutrient medium, and each species of microorganism have genetically inherent traits that are important in identifying the pure isolated culture.

The size, shape, and degree of transparency are studied in transmitted light by examining Petri dishes from the bottom;

colour, nature of the surface, and position on the media are studied in reflected light from the side of the cups' lid;

edges and structure are studied at low magnification of the light microscope;

the consistency is determined by a bacteriological loop touching the studied colony with a half-open Petri dish.

According to the nature of the surface and edges of the colonies, there are so-called R-, S-, M, and other forms of bacterial dissociation, which in most cases coincide with certain of their biological properties. After microscopic confirmation of the purity of the studied colony, it is reinoculated on slope nutrient agar to accumulate the pure culture. The culture is incubated on a thermostat at 37°C for 18-24 hours.

3rd stage.

Macro- and microscopic confirmation of the purity of the selected culture; study the selected pure culture for its subsequent identification. Identifying the pure culture is based on studying many genetically determined traits to determine the selected microorganism's taxonomic position. Such features are:

1) morphology, structure, tinctorial properties, and mobility;

2) cultural properties (the pattern of growth on nutrient media);

3) biochemical properties (the ability to ferment specific substrates in the presence of proteolytic, saccharolytic, lipolytic, and other enzymes in differential diagnostic media);

4) antigenic properties (seroidentification by serological reactions with standard immune sera);

5) specific biological properties, in particular, virulence (by experimental infecting of susceptible laboratory animals);

6) susceptibility to antibiotics by Kirby-Bauer disk diffusion test (the second name "method of standard paper disks), or the method of serial dilutions;

7) susceptibility to phages (phage identification, phagotyping with standard sets of bacteriophages) and others.

Isolated from a specific source and studied, the culture of the microorganism is called a **strain**.

4th stage.

Accounting for the results of bacteriological research;

identification of the pure isolated culture of the microorganism when, according to the studied features, its taxonomic position is determined - family, genus, species, variant (type), and subvariant (subtype).

The principles of identification are universal for all types of bacteria.

Depending on the origin of the test material (blood and other biological fluids) at the beginning of the bacteriological study, the "growth" of the culture is carried out in a liquid nutrient medium.

When cultivating bacteria, the type of their respiration is taken into account. There are obligate (mandatory) aerobes, obligate anaerobes, and facultative anaerobes concerning molecular oxygen. Culture with obligate aerobes and facultative anaerobes are usually kept on a thermostat at 37°C.

Oxygen is toxic for obligate anaerobes (Clostridium botulism, tetanus, anaerobic wound infection, bacteroids, etc.). So for their cultivation, the special oxygen-free media with low reduction potential (Kitt-Tarozzi, Wilson-Blair media, anaerobic blood agar) are used. Anaerobic conditions are achieved by physical, chemical, biological, or combined methods. In bacteriological laboratories, special anaerobic jars are used

to cultivate anaerobes.

Automated systems with computer software are being introduced into microbiological diagnostics of infectious diseases.

Features of isolation and identification of virus culture in virological research

Algorithm of virus cultivation main stages

1st stage.

Treatment of material samples with antibiotics penicillin and streptomycin to kill bacteria. Infecting of biological objects with the prepared research material:

1) susceptible laboratory animals (orally, airways, skin, intradermally, subcutaneously, intramuscularly, etc.), taking into account the tropism of viruses, previous diagnosis of the disease, etc.;

2) 6–12-day-old chicken embryos open or closed in certain parts (chorioallantoic membrane, allantoic cavity, amnion cavity, yolk sac);

3) incubation of infected chicken embryos on a thermostat for several days at a temperature of 36-38°C;

4) cell cultures are highly susceptible to many viruses: primary trypsinised, continuous-culture (passaged), and diploid cell culture. Therefore, synthetic nutrient media 199, Eagle's Minimum Essential Medium (MEM), Hanks' solution, and others are used to maintain the viability and growth of cells.

Monolayer cell cultures are commonly used for infection. Matrasses with infected cell cultures are kept on a thermostat at a temperature of 37°C.

The cultivation of viruses is planned in a laboratory. What media are needed to cultivate cell cultures in the form of a monolayer?

A.Eagle's Minimum Essential Medium

B.Endo (McConkey agar) Media

C.Yolk-salt agar

D.Blood meat peptone agar

E.Bile broth

2nd stage.

Indication of viruses, or detection of virus reproduction in infected biological objects:

1) disease clinical signs appearance, infected animals slaughtered, and their organs examination to reveal viruses;

2) in various structures of the chicken embryo:

after revealing the presence of cytopathogenic action of viruses in the form of whitish plaques, and nodules, the presence of haemorrhages is possible;

in the absence of visible lesions, a hemagglutination reaction is used to determine its titer. Granular red precipitate in the wells of the plastic plate in the form of an inverted umbrella is visible in the test material from the chicken embryo. Hemadsorption reaction can be used too.

Material from a patient with a previous diagnosis of "Influenza" was sent to the laboratory. A hemadsorption reaction was used in the virological study. For which viruses can this reaction be used to detect?

A.Viruses with hemagglutinins

B.All simple viruses

C.All complex viruses

D.DNA genomic viruses

E.Any viruses

3) in cell culture, the reproduction of viruses is detected by:

a) their cytopathic action (CPA), the degree and time of manifestation determined by the type of virus, their infecting dose, and morpho-physiological properties of cells. It can be complete degeneration, round cell degeneration, symplast formation, syncytia, or proliferative changes.

The laboratory doctor diagnosed respiratory syncytial viral infection during microscopy of a cellular monolayer infected with infectious material. What changes does this virus cause in cell culture?

A.Formation of multinucleated cells

B.Round-cell degeneration

C.Destruction of the cell monolayer

D.The presence of Babes-Negri bodies

E.Exfoliation of the monolayer

b) the phenomenon of plaque formation (formation of focal cell degeneration in the form of plaques under bentonite nutrient medium);

c) according to the formation of inclusions in infected cells, their shape, size, and location in the cell (specific features of viruses). Light or fluorescence microscopy is used to detect them.

An imprint smear was prepared from the cornea of a rabbit infected with the contents of a patient's vesicles with suspected smallpox. Microscopy of the smear, stained by Romanowsky-Giemsa, found bodies of different sizes in the cytoplasm. Name these bodies.

A.Guarneri

B.Aragao

C.Pasture

D.Lipschutz

E.Babes-Negri

3rd stage.

Identification of virus cultures involves determining their antigenic properties.

It is carried out by serological reactions with immune (diagnostic) antiviral sera. The most commonly used are the following serological reactions: hemagglutination inhibition test (HIT), hemadsorption inhibition test (HADIT), neutralisation reaction (NR or colour test), plaque neutralisation reaction, complement fixation test (CFT), immunoelectrophoresis reaction, immune electron microscopy (IEM), fluorescent antibody (FAT), enzyme-linked immunosorbent assay (ELISA), passive hemagglutination test (PHAT), and others. In addition, the molecular genetic method is widely used to identify viruses using polymerase chain reaction (PCR), DNA and RNA probes, biochips, etc.

The patient's faeces with suspected intestinal viral infection was treated with antibiotics for one day at 40°C. Primary and continuous cell cultures were infected

then with the suspension. After 2-3 days, cytopathic action was detected in infected cells. How are enteroviruses identified?

A.By the reaction of the cytopathic action neutralisation with type-specific enterovirus sera;

B.By immunofluorescence reaction;

C.By hemagglutination inhibition reaction;

D.By agglutination reaction;

E.By precipitation reaction.

Features of selection and identification of fungal culture in the mycological method of research

Mycological research aims to isolate pure cultures of fungi, studying their micro- and macroscopic structure and genus and species identification. The algorithm for performing the main stages of isolation and identification of fungal culture is similar to the bacteriological for bacteria. First, the pathological material is inoculated in selective liquid (Sabouraud's medium, beer-wort, meat peptone glucose broth) and solid media (Sabouraud's agar, beer-wort agar, glucose-blood agar, Chapek and Francis media, potato and corn agar). Then, isolated cultures of fungi are identified mainly by the appearance and shape of the colonies, their consistency, colour and microstructure. In addition, for some species of fungi, their enzymatic properties are determined.

The material of the whitish layer from the mucous membrane of the oral cavity was sent to the laboratory. The material was inoculated on Sabouraud's medium, and the growth of creamy colonies was noted. Short budding threads were revealed by bacterioscopy. Which type of pathogens are isolated microorganisms?

A.Mycosis

B.Chlamydiosis

C.Mycoplasmosis

D.Rickettsiosis

E.Spirochetosis

Chapter IV. CHEMOTHERAPEUTIC DRUGS. ANTIBIOTICS. DETERMINATION OF BACTERIAL SENSITIVITY TO ANTIBIOTICS

General characteristics of chemotherapeutic drugs

Antimicrobial chemotherapeutic drugs have a selective effect on pathogenic microorganisms in the macroorganism. It can be used in the etiotropic therapy of infectious diseases. The term **"selectivity"** characterises the varying degree of the chemotherapeutic agent toxicity to pathogens and cells of the host. The antimicrobial drug must have a destructive effect on the target, which is present in the microbe but is absent in the host organism's cells. The chemotherapeutic index is a qualitative indicator of compliance with chemotherapeutic drugs' requirements for antimicrobials. It is the ratio of the maximum tolerated dose of a chemical agent used in chemotherapy to its minimum effective dose. It must be **equal to or greater than 3**.

Antibiotics are one of the essential classes of chemotherapeutic drugs. The basis for their production and use in medical practice is the phenomenon of antagonism discovered in the world of microorganisms (when natural substances produced by some microorganisms inhibit the growth and development of others).

Antibiotics are chemotherapeutic preparations of natural origin chemical compounds and their semi-synthetic derivatives and synthetic analogues, which in low concentrations have a selective damaging or destructive effect on microorganisms and tumours.

A 12-year-old boy developed rheumatic heart disease after suffering from a sore throat. Each subsequent streptococcal infection worsens the patient's condition. Which drug should be used to prevent complications?

A.Penicillin

B.Streptococcal toxoid

C.Streptococcal bacteriophage

D.Donor gamma globulin

E.Autovaccine

The primary sources of antibiotics are:

actinomycetes - 80% of all antibiotics (streptomycin, tetracycline, etc.);

fungi (penicillin and other beta-lactams);

typical bacteria (polymyxins, bacitracin);

plants (phytoncides);

animals (interferons, ecmoline).

Methods of antibiotic obtaining:

1) biological synthesis is obtaining antibiotics from live producers in the process of their life;

2) biosynthesis with the following chemical modifications is obtaining semi-synthetic antibiotics (first, for example, get a natural antibiotic, and then attach certain radicals to its initial molecule);

3) chemical synthesis is obtaining synthetic analogues of natural antibiotics.

According to the direction of action, antibiotics are divided into antibacterial, antifungal, antiprotozoal, antiviral, and antitumor.

By the number of susceptible microorganisms, there are broad-spectrum antibiotics (3rd generation cephalosporins, macrolides) and narrow-spectrum antibiotics (cycloserine, lincomycin, benzylpenicillin).

The antibacterial action of antibiotics can be bactericidal. So it causes the death of bacteria (penicillins, cephalosporins). The action can be bacteriostatic (delay the growth and development of bacteria (tetracyclines, chloramphenicol)).

According to the mechanism of action, there are:

inhibitors of cell wall synthesis (beta-lactams);

inhibitors of protein synthesis (aminoglycosides, tetracyclines, macrolides, chloramphenicol);

inhibitors of CPM functions (polyenes, polymyxins);

inhibitors of nucleic acid synthesis (rubomycin and rifampicin).

The biological activity of antibiotics is measured in International Units of action (IU). 1 IU corresponds to 1 μg of a chemically pure drug for most antibiotics.

Benzylpenicillin sodium was prescribed to treat bacterial pneumonia. What is the mechanism of antimicrobial action of the drug?

A.Inhibition of microorganisms' cell wall synthesis

B.Inhibition of intracellular protein synthesis

C.Inhibition of cholinesterase activity

D.Inhibition of SH-groups of microbial enzymes

E.Antagonism with para-aminobenzoic acid

Complications are possible in antibiotic therapy. There are two possible complications: macroorganism and microorganisms.

The first group includes:

1) allergic reactions (the development of allergic reactions of delayed and immediate type from mild to severe manifestations: urticaria, oedema of the eyelids, lips, nose, dermatitis, anaphylactic shock);

2) toxic reactions (hemato-, hepato-, cardio-, ototoxicity, nephrotoxicity, teratogenic effect) depending on the properties of the drug, its dose, method of administration, the patient's condition;

3) endotoxic reactions developing under conditions of mass destruction of gram-negative bacteria under the antibiotic's action, accompanied by the release and entry into the blood of their endotoxin and the endotoxic shock occurrence;

4) dysbiosis (the violation of the qualitative and quantitative composition of the normal microflora);

5) immunosuppressive effect (a negative effect on various parts of the immune system (i.g., inhibition of antibody synthesis, certain T lymphocyte subpopulations).

A 50-year-old patient, chloramphenicol, was prescribed to treat typhoid fever, but the next day the patient's condition worsened, and the temperature rose to 39.6°C. How do you explain the deterioration of the patient's condition?

A.The action of the endotoxins of the pathogen

B.Allergic reaction

C.Insusceptibility to chloramphenicol

D.Joining a secondary infection

E.Re-infection

The patient developed intestinal dysbacteriosis after long-term use of antibiotics. What drugs should be prescribed to restore the normal microflora?

A.Eubiotics (probiotics)

B.Sulfanilamides

C.Interferon

D.Antıfungal drugs

E.Nitrofurans

A 37-year-old patient developed intestinal dysbacteriosis as a result of long-term antibiotic therapy. What kind of drugs should be used to normalise intestinal microflora?

A.Eubiotics

B.Sulfanilamides

C.Bacteriophages

D.Autovaccines

E.Vitamins

The second group includes the emergence of changes in microorganisms undesirable for humans:

1) changes in morphological, biochemical and other properties (in particular, the formation of L-forms of bacteria);

2) development of antibiotic resistance in microorganisms to different groups of chemotherapeutic drugs. Chromosomal and extrachromosomal mutations, migration and different modes of transmission in populations of microorganisms R-plasmids, and genetic recombination of hereditary material are known.

R-plasmids encode the synthesis of·

A.Enzymes that cause the inactivation of antibiotics and reduce the permeability of the cell wall for antibiotics

B.Sexual pili for the transfer of genetic information

C.Constitutive enzymes

D.Endotoxins

E.Aggressines

Bacteria have a conjugation process in which a cytoplasmic bridge is formed between the bacteria, through which plasmids and fragments of the DNA molecule arrive from the donor cell to the recipient cell. What is the significance of the process?

A.Provides exchange of genetic material

B.Provides metabolism between cells

C.Eliminates unwanted mutations

D.Increases heterozygosity

E.Promotes the activation of the mutation process

The strain of Staphylococcus caused an outbreak of nosocomial infection and showed high resistance to penicillin. Which of the following factors is associated with antibiotic resistance?

A.With the synthesis of beta-lactamase

B.With the synthesis of adenyltransferase

C.With changes in cell wall components

D.With changes in ribosomal proteins

E.With the synthesis of phosphotransferase

Nosocomial staphylococcal infections were revealed in the surgical department of a dental clinic. The strains are multiple drug-resistant. The substance that determines this feature is:

A.R-plasmid

B.F-plasmid

C.Moderate bacteriophages

D.Exotoxins

E.Virulent bacteriophages

The resistance of bacterial cells to antibiotics is due to an autonomous genetic structure, the transfer of which is conjugative. What is the name of this structure?

A.R-plasmids

B.Ent-plasmids

C.Col-plasmids

D.Transposons

E.Prions

It is known that bacterial cells contain additional genetic structures which give additional properties and can exist independently of the bacterial chromosome as a separate element or integrate with it. What are these additional genetic elements called?

A.Plasmids

B.Prophages

C.Nucleotides

D.Is-sequence

E.Wandering genes

Methods for the susceptibility of bacteria to antibiotics determining

The susceptibility of bacteria isolated cultures from patients to various antibiotics and other chemotherapeutic drugs used for treatment is determined To increase the effectiveness of antibiotic therapy. Unified methods in microbiological research are the Kirby-Bauer disk diffusion test (the method of standard paper disks) and serial dilutions. Standard disks are used for the Kirby-Bauer disk diffusion test. Disks are applied with sterile tweezers to the surface of a solid nutrient medium inoculated with a pure culture of the pathogen isolated from the patient (not more than 6 disks per 1

Petri dish). The plates are placed on the thermostat upside down and incubated at 37°C for 18-20 hours. The effect of antibiotics is assessed by the growth retardation of microorganisms around the disc. Accounting for results: use a ruler to measure the diameters of the growth retardation zones around the disks, including the diameter of the disks, to the nearest 1 mm. According to the degree of susceptibility to antibiotics following the instructions for unified methods in clinical practice, there are 2 groups of microorganisms: susceptible and resistant. The results are evaluated according to a standard table, which shows the respective diameters of growth retardation zones and susceptibility groups to those antibiotics recommended for practical medicine.

The susceptibility of Staphylococcus to antibiotics was determined in the laboratory. The diameter of the growth retardation zones is equal for penicillin - 8 mm, oxacillin - 8 mm, ampicillin - 25 mm, and gentamicin - 22 mm. What research method was used?

A.The method of paper disks

B.Method of serial dilutions

C.Biochemical

D.Bacterioscopic

E.Biometric

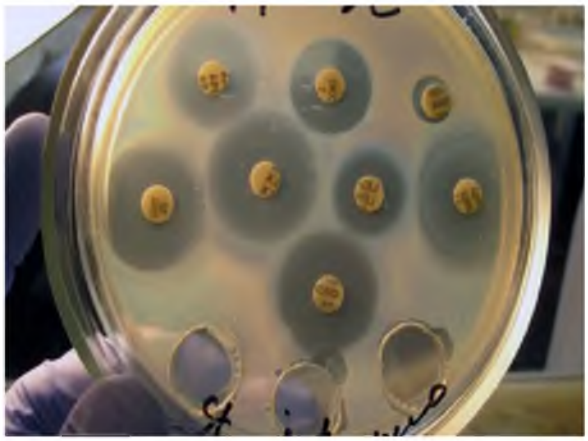

Figure 42 - Determination of antibiotic susceptibility by the disk diffusion test

Pathogenic Staphylococcus was isolated from the purulent wound of the patient, and Hiss's agar susceptibility to antibiotics was determined: penicillin - growth retardation zone of 8 mm; oxacillin - 9 mm; ampicillin - 10 mm; gentamicin - 22 mm; lincomycin - 11 mm. Which antibiotic should be chosen to treat the patient?

A.Gentamicin

B.Oxacillin

C.Ampicillin

D.Penicillin

E.Lincomycin

The **Kirby-Bauer disk diffusion test** is regarded as a qualitative method for determining the susceptibility of microorganisms to antibiotics.

The **serial dilution method** is an accurate quantitative method. It allows determining the minimum inhibitory (inhibitory) concentration (MIC or IPC) of an antibiotic or another chemotherapeutic agent in μg/ml, which will be considered in therapeutic dosing. The serial dilutions method is used to determine the susceptibility of microorganisms to antibiotics.

A liquid nutrient medium in several tubes (8-11) with double dilutions of the basic antibiotic solution is used. Two control tubes are present. The first control is culture growth control, and the second is meat peptone sterility broth. Except for the second control, all test tubes drop a suspension of the studied culture. First, the culture is incubated at 37°C for 18-24 hours on a thermostat. Then the last test tube with the complete delay of microbial growth is noted. The antibiotic concentration in the tube is the minimum inhibitory concentration for the studied strain of the microorganism (MIC in μg/ml). It determines the degree of its susceptibility to the antibiotic.

Klebsiella pneumoniae, highly resistant to antibiotics, was isolated from a patient with pneumonia. Which of the following methods can be used to determine the minimal inhibitory concentration of antibiotics planned to be prescribed for the treatment of the patient?

A.Method of serial dilutions
B.Cylinder method
C.Hole method
D.Disk method
E.Enzyme-linked immunosorbent assay

Figure 43 - Determining the minimum inhibitory concentration of the antibiotic by the serial dilutions method

Chapter V. INFECTION. BIOLOGICAL METHOD

Infectious process

The infectious process is one of the dynamic forms of interaction of pathogenic or opportunistic microorganisms and susceptible macroorganisms in certain medical conditions. The macroorganism develops a set of adaptive-reparative reactions to maintain the infected organism's homeostasis. An invasion is a similar process caused by protozoa, helminths, and certain arthropods. The infectious process is parasitism, in which one species (parasite) uses another species of a living organism (host) as a source of food and habitat and harms it. The microorganisms are obligate (mandatory), opportunistic and random parasites. The infectious process has various manifestations: from asymptomatic carriers to infectious disease (with recovery or death). **Infectious disease is the extreme manifestation of the infectious process**.

The origin, development, and completion of the infectious process and infectious disease are determined by three groups of factors:

1) the presence of a living microorganism (pathogenic, opportunistic, or saprophytic);

2) susceptibility of the macroorganism, which depends on age, sex, immune profile, anatomical and physiological characteristics of the organism, its state of health, employment, etc.;

3) environmental conditions in which the interaction of micro- and macroorganisms (including climatic-geographical, seasonal, and social conditions with all their components).

Pathogenicity is the potential ability of a microorganism to cause an infectious process, i.e. penetrate the macroorganism (infectivity) and multiply in it (invasiveness), causing a violation of homeostasis with the formation of a complex of pathological processes and the development of macroorganism's reactions in response to the pathogen. Pathogenicity is a genetically determined species trait of a microorganism.

The measure of pathogenicity is **virulence.** In contrast to pathogenicity, virulence is a dynamic individual property of a particular microbe strain to cause an infectious process. Quantitatively, the virulence of microorganisms and the strength of their toxins are determined using special units - **the lethal and infectious dose**.

A **lethal dose** (LD) is the lowest amount of live pathogen or toxin that causes a certain number (%) of experimental laboratory animals to die within a certain period.

An infectious dose (ID) is the smallest number of living microorganisms that can cause an infectious disease in a certain number (%) of experimental laboratory animals. There are:

Dlm (Dosis letalis minima) (the minimum number of living microorganisms or their toxins, which for a certain time causes the death of 90-95% of experimental laboratory animals;

Dcl (Dosis certa letalis) is the minimum number of living microorganisms or their toxins, which over some time causes the death of 100% of experimental laboratory animals;

LD_{50} is the minimum amount of living microorganisms or their toxins, which over some time causes the death of 50% of experimental laboratory animals.

A pathogenic microorganism must enter the macroorganism through the entrance gate for an infectious process or disease to occur. The entrance gates of infection are tissues that do not have physiological protection against a particular type of microorganism. Instead, it is the place of overcoming protective barriers, and the subsequent penetration into a macroorganism for microbes are tissues whose cells have specific receptors with which microorganisms can infect.

Mechanisms of transmission of infectious agents:

1) faecal-oral;

2) aerogenic;

3) contact;

4) vector-borne;

5) vertical;

6) parenteral.

Each of them is carried out using certain ways of transferring the pathogen.

Factors of pathogenicity and virulence

Pathogenicity and **virulence** are phenotypically realised by a complex of genetically determined pathogenicity factors.

Adhesion and colonisation.

Adhesion is the ability of microorganisms to attach or adhere to specific cells of the host organism that are susceptible to a given microbe. On the one hand, it is due to the operation of nonspecific physicochemical mechanisms (hydrophobicity of microbial cells, the amount of energy of repulsion and attraction), resulting in contact between pathogen cells and tissue cells of the macroorganism. On the other hand, the ability to adhere is determined by the specific chemical groups (ligands) on the surface of microorganisms that correspond to the receptors of the macroorganisms' cells. Different types of pili play the role of adhesins in gram-negative bacteria. Teichoic and lipoteichoic acids of the cell wall, components of the capsule provide the process in gram-positive bacteria.

Colonisation (insemination) is the reproduction process of microbes at the adhesion site or on the cell surface.

Invasion and penetration.

Invasion is the ability of pathogenic microorganisms to penetrate through mucous and connective tissue barriers into underlying tissues, other organs, and tissues due to their production of enzymes such as:

hyaluronidase (which breaks down a hyaluronic acid intercellular substance that promotes microbial penetration into tissues);

neuraminidase (cleaves sialic acid from cell membranes, which allows microbes to penetrate the cells and spread in the intercellular space).

Penetration is the ability of pathogenic microorganisms to penetrate the cells of a macroorganism (in particular, into epithelial cells, leukocytes, and lymphocytes), where they either multiply or are inactive for some time. For example, some bacteria, viruses, rickettsiae, and chlamydia can penetrate.

Aggression is carried out by virulence factors (aggressins) of microorganisms that suppress the host's innate and adaptive immune defence.

Aggression is carried out:

1) by **substances** that are part of the surface structures of bacterial cells: **capsules** (polysaccharides, proteins), **cell wall** (staphylococcal protein A, streptococcal protein M, gram-negative bacteria's lipopolysaccharides); most of them inhibit the migration of leukocytes, prevent phagocytosis;

2) **enzymes** that inhibit the protective functions of the macroorganism - **proteases** (destroy antibodies), **coagulase** (coagulates blood plasma), **fibrinolysin** (involved in the dissolution of fibrin clots), **lecithinase** (acts on lecithin), etc.;

3) **toxins** (**exo-** and **endotoxins**).

Exotoxins are protein toxins.

Their main characteristics are:

produced mainly by gram-positive bacteria;

most are thermolabile;

have two centres (one fixes the toxin molecule on the corresponding cell receptor, and the other is a toxic fragment that penetrates the cell, where it blocks vital metabolic reactions);

the effect on the host cell is direct and specific;

high immunogenicity (formation of antitoxic antibodies - antitoxins);

possible transition to toxoid under the action of formalin.

According to the mechanism of action, exotoxins are divided into 4 groups:

1) **cytotoxins** (block protein synthesis at the subcellular level);

2) **membrane toxins** (increase the permeability of the surface membrane of erythrocytes (hemolysins) and leukocytes (leukocidins), destroying them);

3) **functional blockers** (block the functions of specific cellular and tissue systems (**enterotoxins, neurotoxins**));

4) **exfoliatins and erythrogenins** (formed by some strains of Staphylococcus aureus and scarlet fever Streptococcusand affect the interaction of cells with each other and with intercellular substances.

Toxicity is measured in the same units as virulence - Dlm, LD_{50}.

Endotoxins are lipopolysaccharides (LPS). Their main characteristics:

are the product of autolysis of the cell wall of gram-negative bacteria;

moderately resistant to high temperature;

the whole LPS molecule determines toxic properties;

the effect on the host cell is nonspecific and mediated by activated cytokines (IL-1, TNF, etc.);

immunogenicity is relatively weak;

there is no transition to toxoid.

The interaction of viral and cellular genomes is the basis of viral infections.

Features that are characteristic only of viral infections are:

1) the ability to cause integrative infection (virogeny, when the nucleic acid of the virus is embedded in the chromosome of the host cell);

2) the presence of viremia (virus circulates in the blood). The exception is for viruses spread by neurogenic pathways (rabies, herpes simplex viruses, etc.);

3) the affection of lymphocytes (human immune system cells). Influenza, measles, herpes, polio, rotavirus and other viruses suppress T-lymphocyte immune responses. On the other hand, chickenpox and cytomegalovirus viruses increase the absolute number of T lymphocytes. Obligatory-lymphotropic is relatively viruses of HIV infection, HTLV-1, HTLV-2 to T-lymphocytes. The Epstein-Barr virus is the causative agent of infectious mononucleosis to B-lymphocytes;

4) formation of intranuclear or intracytoplasmic inclusions (bodies).

Forms of infection

Exogenous infection is an infectious process when a person is infected with a pathogen that has entered the organism from the environment with food, water, soil, secretions from a sick person, convalescent, or microbiocarrier.

Endogenous infection is an infectious process caused by representatives of the microflora (opportunistic pathogens of the individual). It often occurs in the immunodeficiency background.

Autoinfection is an endogenous infection that occurs by transferring the pathogen from one habitat to another.

Depending on the localisation, there are:

1) focal infection is localised in the local foci and does not spread outside it (in particular, under conditions of sore throat, furunculosis, conjunctivitis);

2) generalised form is characterised by the spread of microorganisms in the human body in a lymphogenous manner, hematogenously, and perineurally. It causes multiple lesions of human organs and tissues.

Forms of generalised infection are:

a) **bacteremia or viremia**. Pathogens circulate in the blood but do not multiply, so blood is their mechanical carrier (for example, one of the stages in the pathogenesis of typhoid fever). Toxaemia is the circulation of the pathogen's toxins in the blood;

b) **sepsis.** Pathogens multiply in the blood with strong suppression of the main mechanisms of immunity; the main feature of sepsis is that the clinical picture does not depend on the type of pathogen;

c) **septicemia**. The emergence of secondary purulent foci in the internal organs of the macroorganism;

d) **bacterial or toxic-septic shock** is a massive entry of bacteria and their toxins into the blood.

Depending on the quantity, there are:

Monoinfection is caused by one type of microorganism;

Mixed infection is the infection with 2-3 different pathogens (e.g., diphtheria and streptococcus, mycoplasmas in various combinations with viruses).

A bacteriologist isolated the causative agent of Flexner's dysentery - type 2, Sonnei - type I, and enteropathogenic Escherichia coli - 055 / B5 in a sick child. What is the name of this type of infection in the child?

A.Mixed infection

B.Secondary infection

C.Carrier of pathogenic bacteria

D.Superinfection

E.Re-infection

Depending on the other peculiarities, there are:

A secondary infection is a joining infection to an existing one in the body. Secondary infection can be caused by another (new) pathogen (for example, typhoid fever can develop into pneumonia caused by other bacteria or viruses; against the background, measles, pneumonia caused by opportunistic cocci joins).

A child recovering from measles developed pneumonia caused by opportunistic bacteria. What is the most likely form of the infection?

A.Secondary infection

B.Re-infection

C.Superinfection

D.Persistent infection

E.Nosocomial infection

The same type of microorganism causes re-infection after the disease due to a lack of high-grade immunity after their completion (e.g., re-infection in dysentery or gonorrhoea).

A patient who recovered from Sonnei dysentery was re-infected with the same pathogen. What is the name of the infection form?

A.Re-infection

B.Relapse

C.Superinfection

D.Persistent infection

E.Chronic infection

A young man, who had an anamnesis of gonorrhoea and was wholly cured, fell ill with gonorrhoea again. In this case, we can talk about

A.Re-infection

B.Mixed infection

C.Relapse

D.Superinfection

E.Secondary infection

Superinfection is a re-infection with the same pathogen before the recovery of the macroorganism (e.g., tuberculosis)

Relapse is the return of clinical manifestations of the disease without re-infection due to microorganisms that remain in the macroorganism. Examples are erysipelas, relapsing fever, and osteomyelitis.

A person living in an endemic region with 3-day malaria has recovered from the disease. Then, after moving to another area, 1.5 years after moving, she contracted malaria again. What is the most likely form of this disease?

A.Relapse

B.Re-infection

C.Superinfection

D.Persistent infection

E.Secondary infection

A patient was diagnosed with acute gonorrhoea. It is known from the anamnesis that he had previously suffered from gonorrhoea, and recovery was complete. To which category of infections can this new disease be attributed?

A.Re-infection

B.Superinfection

C.Secondary infection

D.Relapse

E.Autoinfection

Depending on the time of interaction of the pathogen with the macroorganism, there are:

1) **acute infections** have a relatively short course (from 1 week to 1 month) and are characterised by specific pathogenesis and clinical symptoms;

2) **chronic infections** have a long course (from several months to years). They are characterised by **prolonged exposure** to the pathogen in the body or **persistence**, particularly in malaria, syphilis, tuberculosis, brucellosis, leprosy, hepatitis B, herpes infections and others. The pathogen is released into the environment is possible.

Depending on the manifestation of symptoms, there are:

The **manifest form** is a course of the disease with a full range of symptoms characteristic of this disease;

The **inapparent (asymptomatic)** form is a course of the disease without severe or mild symptoms.

The **abortive form** is a disease course with an incomplete set of symptoms.

The condition where the pathogen continues to be isolated after clinical recovery is called microcarrier (bacteriocarrier, virus carrier).

Periods of infectious disease:

1) incubation period is the time from the moment the pathogen enters the body until the first precursors of the disease; the duration varies (from several hours to several weeks;

2) **prodromal period (prodrome)** is the time of manifestation of the first clinical symptoms of the general plan, uncharacteristic of the disease (weakness, loss of appetite, insomnia, fatigue, etc.); exception is the appearance of characteristic spots on the mucous membrane of the cheeks (Koplyk spots) and the tongue bark;

3) the period of **acute manifestations** of the disease (**the height of the disease**) is the time characterised by specific to this disease symptoms manifestation (temperature curve, rash, local lesions). Specific antibodies appear in the patient's blood, their titer increases, and the pathogen continues to multiply intensively and accumulate a significant amount of toxins and enzymes;

4) the period of **convalescence (recovery)** - the period of extinction and disappearance of typical symptoms, the time of clinical recovery, and the time of gradual restoration of physiological functions of affected cells, tissues, organs, and

the body as a whole. During this period, the pathogen is excreted from the body in large quantities in many diseases.

Ways of the pathogen excretion from the body depend on the location of the infectious process, and the exception is the blood.

Basic epidemiological concepts

Epidemiology studies the patterns of occurrence and spread of infectious diseases, their prevention, and elimination in human society.

The epidemic process is the interaction of micro- and macro-organisms at the population level. It is a continuous chain of infectious conditions among humans, supported by three links, which ensures the preservation of the pathogen in nature as a species.

The epidemic process links:

1) **source of infection** (sick person, animal, or bacterial carrier);

2) **mechanisms and ways of transmission** of the pathogen;

3**) the susceptibility of the population**.

According to the source of infection, there are **anthroponoses, zoonoses, zooanthroponoses, and sapronoses**.

Mechanisms and ways of transmission are:

1) **faecal-oral** when alimentary, water and contact-household ways carry out a pathogen;

2) **aerogenic (respiratory)**, when droplets and airborne dust routes carry out a pathogen;

3) blood, when transmissible, parenteral, sexual routes carry out a pathogen;

4) **contact** when a pathogen is carried out by wound and contact-sexual ways;

5) **vertical** when a pathogen is carried out by transplacental and other pathways of transmission of the pathogen from mother to embryo or newborn.

Examples of specific human parasites are Plasmodium falciparum, pinworm, and others. The source of invasion of such parasites is always a person. Such specific human parasites cause diseases are called:

A.Anthropogenic

B.Multifactorial

C.Infectious

D.Zoonotic

E.Anthropozoonic

Rodents are a reservoir of pathogens of many diseases. So what is it connected with first?

A.Biological features of rodents that promote the exchange of parasites and pathogens with humans

B.Belonging rodents to essential components of terrestrial biocenoses

C.The property of rodents to multiply rapidly

D.Living in conditions where ectoparasites use rodents as a food source

E.Belonging rodents to the most numerous class of mammals

Manifestations of the intensity of the epidemic process:

1. **Sporadic morbidity** is the usual level of morbidity of a particular nosological form in a given area in a given historical period;
2. **An epidemic** is a level of morbidity of a certain nosological form in a given area in a specific period, which sharply exceeds sporadic morbidity;
3. **A pandemic** is a level of incidence of a certain nosological form that sharply exceeds the epidemic level (rapid spread with the capture of more and more geographical regions such as countries and continents);
4. **Endemic** is the relative incidence of a certain nosological form in a given geographical area. There is a natural-focal endemic (associated with natural conditions, the range of vectors, and the reservoir of infection) and a statistical endemic (due to a complex of climatic, geographical, and socio-economic factors).

Relapsing fever caused by B. caucasica occurs only in certain areas where there is a vector of ticks of the genus Alectorobius. So what can you call such an infection?

A.Endemic

B.Exotic

C.Sporadic

D.Pandemic

E.Epidemic

5. **Quarantine diseases** are the most dangerous diseases that spread quickly (particularly cholera, plague, and anthrax).

Biological research method

The biological method of research involves experimental infection of susceptible laboratory animals, in some cases wild animals and birds.

Purpose:

1) reproduction of infectious diseases to study the mechanism of their development, the immunity formation, specific and nonspecific treatment, and prevention;

2) establishing the aetiology of infectious disease;

3) isolation of pathogens;

3) determining of virulence, toxigenicity, or toxicity of isolated cultures of pathogens;

4) testing of new drugs;

5) control of immunogenicity, toxicity, sterility, and the safety of medical and biological drugs;

6) diagnostic and therapeutic immune sera obtaining;

7) studying the dynamics of normal microflora formation and its role in physiological and pathological processes. It is the solution of such science as gnotobiology. The research is conducted on germ-free animals (gnotobionts). Animal species, methods of infection, animal care, observation and direction of their study depend on the purpose of the experiment.

Chapter VI. IMMUNOLOGY. TYPES AND FORMS OF IMMUNITY

Immunity is the defence of an organism against genetically foreign substances (antigens) of exogenous or endogenous origin to preserve homeostasis at the cellular level, its antigenic individuality, and thus the preservation of species.

Types of immunity:

1) **innate** (hereditary, constitutional, species) immunity;

2) **adaptive** (acquired, specific, individual).

Innate immunity

Innate immunity is phylogenetically older, genetically determined, and with the help of non-specialised defence mechanisms. It determines the immunity of representatives of this species to all existing foreign factors.

Mechanisms of nonspecific protection function in the body constantly, and the appearance of foreign substrates determines the development of an inflammatory reaction, which is the same for any pathogen.

The factors of nonspecific protection of the organism are:

1. **External barriers** (normal microflora, skin, mucous membranes). The defence is due to their functional components and histological features, etc.
2. **Internal barriers** (lymph nodes, tissue, and cell barriers). The protection is due to histo-hematic barriers and the properties of cell membranes.
3. **Cellular factors** (phagocytes and natural killers).

Phagocytes are cells capable of phagocytosis. They engulf foreign material, destroy, and excrete it from the cell:

a) all phagocytic cells **in tissue** (tissue macrophages) are united in the system of **mononuclear phagocytes** (**MPS**);

b) **mononuclear cells in the blood**;

c) **polymorphonuclear cells (neutrophils** in the blood).

Stages of complete phagocytosis:

1) **activation** of the phagocytic cells;

2) **chemotaxis** (migration of the phagocyte to the object that activated it);

3) **adhesion** (attachment to this object);

4) **absorption** of this object;

5) **processing** (digestion) of the absorbed object due to oxygen-dependent or oxygen-independent mechanisms.

The absence or violation of the last stage of phagocytosis is considered incomplete. It is observed in gonococcal infection, certain rickettsiosis, tuberculosis, and the first stages of brucellosis.

The course of phagocytosis events is due to the interaction of receptor-ligand structures. The interaction between phagocytes' biologically active substances and objects undergo phagocytosis. In particular, the presence of capsules in bacteria helps them avoid phagocytosis. Opsonins facilitate the recognition and disposal of foreign objects by phagocytosis. Opsonins (IgG, complement component C3b, fibronectin) are produced in the macroorganism.

The patient went to the dermatologist's office. The doctor prepared a Gram-stained smear from this patient's purulent discharge of the urethra. Microscopy of the smear revealed a mass of leukocytes, and many gram-negative bean-shaped diplococci were revealed in the cytoplasm. The results of which process are observed in the smear?

A.Phagocytosis

B.Metabolism

C.Capsule formation

D.Spore formation

E.Malignancies

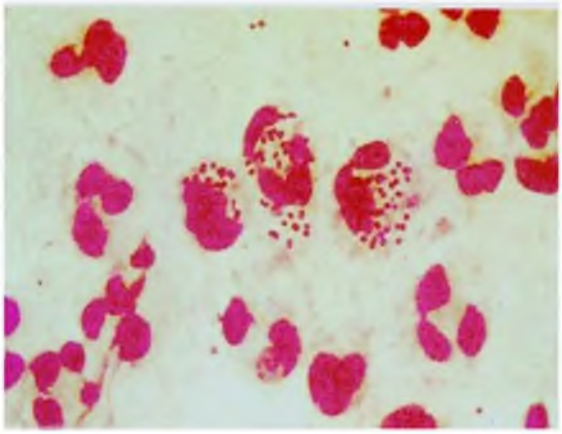

Figure 44 - The phagocytosis phenomenon

***Natural killer cells (*NK)** can destroy target cells (tumour cells, cells infected with viruses, and other intracellular parasites). The killing effect is due to secretion by the NK cells of perforins and granzymes or other damaging structures and substances.

3. **Humoral factors**. Lysozyme, complement, cytokines, acute phase proteins, etc.).

Lysozyme is the thermostable protein of the mucolytic enzyme type. It destroys the chemical bonds of the bacterial cell wall peptidoglycan layer. It has an apparent lytic effect against many saprophytic bacteria, is less effective against many pathogenic microorganisms, and is entirely inactive against viruses. Lysozyme is found in biological fluids of animals and humans (in tears, saliva, peritoneal fluid, plasma and serum, leukocytes, and breast milk). Blood monocytes and tissue macrophages produce it.

A thermostable protein found in humans in tears, saliva, and breast milk, and can be found in freshly laid chicken eggs, was prescribed to accelerate the healing of the mucous membrane wound in the oral cavity. It is known that it is a factor of natural resistance of the organism and is called:

A.Lysozyme

B.Complement

C.Interferon

D.Interleukin

E.Imanin

Complement is a complex of serum proteins interacting in a specific sequence and providing the course of humoral and cellular immune responses. The complement system consists of more than 20 fractions of different physicochemical properties, denoted by the "C". 9 significant fractions (C1, C2, C3, ..., C9) and 3 inhibitors. Complement proteins are globulins or glycoproteins produced by macrophages, neutrophils, and liver cells. The main functions of the complement system are:

a) opsonising (increased phagocytosis);

b) participation in inflammatory and anaphylactic reactions (stimulation of this process, as some activated fractions, promote the release of histamine and other pro-inflammatory biologically active substances from tissue basophils and basophilic granulocytes);

c) cytotoxic, or lytic action (at the final stage of complement activation, a membrane-attacking complex is formed – MAC. It is embedded in the membrane of different origins cells and their destruction due to osmotic);

d) direct antiviral action (certain components of the system prevent the penetration of certain viruses in target cells);

e) increasing immunoglobulins production (due to binding individual fractions of the complement system with a specific receptor for B-lymphocytes).

The system of complement proteins is activated by the type of enzymatic-cascade reaction with the formation of soluble and insoluble enzymes. Complexes are capable of causing various biological phenomena. Activation of the complement system is carried out in three pathways: classical (immune), alternative (properdin), and lectin.

Activation of the complement system in the **classical pathway** begins with the C1 fraction. The activation factor is the mandatory formation of the immune complex antigen-antibody (antibodies of only two classes - Ig G or Ig M). Antibodies of the formed immune complex with their Fc fragment interact with the C1 complement, resulting in cascade activation of early complement components. It leads to activation of late (terminal) components of complement C5-C9, which attach to the target cell membrane and form a lytic complex named "membrane attacking complex" MAC).

As a result of the attachment of the MAC to the target cell membrane, cylindrical or funnel-shaped pores are formed in it. Electrolytes and water pass through it into the cell. Osmotic lysis occurs (in particular, bacteriolysis and hemolysis of erythrocytes).

Differences of an **alternative pathway** of the complement activation:

1) an immune complex antigen-antibody formation is not needed for initiation of it;

2) the whole process begins with the activation of component C3 due to the direct action of the antigen (in particular, polysaccharides and lipopolysaccharides of the cell wall of gram-negative bacteria, surface structures of viruses, tumour cells, parasites - protozoa or helminths);

3) the necessary participation of properdin (a serum protein that is active only in the presence of Mg_2+ ions);

4) is carried out immediately after the invasion of the antigen, before the production of antibodies in the body.

Similarities of alternative and classical pathways of complement activation:

1) is a cascade of enzymatic reactions;
2) the critical point of both pathways of complement activation is the formation of C3-convertase, although its formation for the classical and alternative pathways is different;
3) the final stage of both processes is forming a membrane-attacking lytic complex.

The **lectin pathway** of complement activation is also carried out without antibodies and initiated by a unique **mannose-binding protein**.

Cytokines are peptides or glycopeptides. These are hormone-like mediators of intercellular interactions involved in the cell division, differentiation and involvement in the course of innate and acquired immune responses. There are two main areas of biological action of cytokines: **protection against infectious agents** and **reparation of damaged tissues**. More than 200 types of cytokines are currently known,

classified according to their biochemical and biological properties and the type of receptors through which cytokines perform their functions.

The following families are distinguished according to structural and functional characteristics:

1) **interferons** (antiviral, regulatory and immunomodulatory action);

2) **haemopoietins** (growth factors of hematopoietic cells);

3) **interleukins** (interaction between leukocytes, macrophages, and other immunocompetent cells);

4) **TNF family** - a **factor of tumour necrosis** (regulation of growth and differentiation of many cells);

5) **chemokines** (determine the migration of different cell types);

6) family of **epidermal growth factors**.

Interferons (IFN) are glycopeptides that differ in physicochemical properties and source. In addition, they have species specificity and carry out antiviral, antitumor, and immunoregulatory action within the species whose representatives are their producers. There are currently three classes of interferons, each consisting of some subclasses.

Class 1 - **leukocyte, or alpha-interferon (IFN-α)**, is produced by macrophages, monocytes, and B-lymphocytes. It has antiviral, antiproliferative, and antitumor effects.

Class 2 - **fibroblast or interferon beta (IFN-β)** is produced by fibroblasts of human cell cultures infected with the virus and has mainly antitumor and antiviral action.

Class 3 - **immune or lymphocytic, or gamma-interferon (IFN-γ)** is produced by activated T-helpers and natural killer cells.

Interferon does not directly affect the virus outside the cell but disrupts its reproduction in the infected cell at the stage of viral protein synthesis. Inducers of interferon production are so-called interferonogens (they can be RNA, DNA, and complex polymers). Interferons prevent many acute viral infections (including influenza) and treat chronic viral infections (including parenteral hepatitis B, C, and

D), malignant tumours, and certain immunodeficiency conditions. In addition, leukocyte and recombinant genetically engineered interferons are used in practical medicine.

There is a flu epidemic in the city. Which remedy should be used for nonspecific disease prevention?

A.Leukocyte interferon

B.Penicillin

C.Anti-influenza immunoglobulin

D.Anti-influenza serum

E.Influenza vaccine

The acute phase proteins include **C-reactive protein, a pro-inflammatory protein, and other proteins** synthesised by liver cells in response to cell and tissue damage.

C-reactive protein promotes opsonisation of bacteria, participates in complement activation in the classical pathway and is considered an **indicator of inflammation**.

Adaptive immunity

Adaptive immunity is the immunity of the human or animal body to the antigen, formed during ontogenesis due to the interaction of a sensitive organism with this antigen due to specific immune system factors. In addition, there is **natural** and **artificial** acquired (adaptive) immunity, which can be **active** and **passive**.

Natural active immunity occurs after an infectious disease (diphtheria, anthrax, brucellosis, measles, polio, Etc.) or another contact with the antigen.

Natural passive immunity is caused by antibodies transmitted from the immune mother to the child (transplacentally, with breast milk).

A 1.5-year-old boy who did not receive routine vaccinations came into contact with a measles patient. Donor gammaglobulin was administered to the child for emergency-specific prophylaxis. What kind of immunity was created in this case?

A.Passive

B.Natural

C.Antitoxic

D.Post-vaccination

E.Local

A paediatrician, talking to parents about the prevention of measles, noted that a specific category of children has a natural passive immunity to this disease. What kind of children did the doctor mean?

A.Newborns

B.Over 14 years

C.Those who suffered from measles in the first year of life

D.Those who received routine vaccinations

E.Those whose parents did not have measles

Antibodies to the measles virus were detected in the serum of the newborn. What immunity can this indicate?

A.Natural passive

B.Natural active

C.Artificial passive

D.Artificial active

E.Hereditary, species

Artificial active immunity occurs during vaccination (post-vaccination immunity).

The following preparations are offered for work: 1. Brucellosis skin vaccine. 2. Leptospirosis vaccine. 3. BCG vaccine. 4. Adsorbed pertussis-diphtheria-tetanus vaccine DPT. 5. Adsorbed tetanus toxoid. What immunity do these drugs create?

A.Artificial active

B.Non-sterile (infectious)

C.Antibacterial

D.Artificial passive

E.Antitoxic

Artificial passive immunity occurs after immune serum injection and compatible immune cell transplanting.

Active immunity can be:

humoral (due to antibodies);

cellular (due to immunocompetent cells);

cellular-humoral (due to both antibodies and immunocompetent cells).

There is also **sterile immunity** (preserved in the absence of microorganisms - post-infectious, post-vaccination immunity) and **non-sterile or infectious immunity** (in the presence of the pathogen in the body only).

There is a separate **local immunity** as protection of certain body parts (including mucous membranes) from pathogens. It formed with the participation of the secretory Ig A.

Antigens, classification of antigens

Antigens are genetically foreign substances of any origin, which can cause a specific immune response in the body and participate in its implementation.

Properties of antigens are:

a) antigenicity

antigens are specific stimuli to immunocompetent cells, and interaction with them occurs due to some regions of the antigen, called "antigenic determinant" or "epitope";

b) foreignness

the antigen has structures that are absent in this organism;

c) immunogenicity

the ability of the antigen to induce an immune response.

Classification of antigens

1. **By origin:** exogenous (formed outside the body) and endogenous (originated in the body are auto- and neoantigens);

2. **By nature:** protein biopolymers and non-protein biopolymers (polysaccharides, lipids, lipopolysaccharides, nucleic acids);

3. **By molecular structure:** globular (spherical molecules) and fibrillar (thread-shaped molecules).

4. **By degree of immunogenicity**:

a) **complete antigens** have a sufficiently large molecular weight and large molecule size. The immune system responds to them with a specific reaction during which the antigen interacts with its components. The conductor of antigenicity in such antigens are usually protein biopolymers;

b) defective antigens or **haptens** are low molecular weight compounds that cannot induce an immune response but can interact specifically with ready-made immune factors (antibodies, lymphocytes). The hapten molecule is enlarged with a carrier protein called a schlepper to be transformed into a complete antigen.

5. According to the degree of foreignness:

a) **xenogenic or heterologous antigens** are common to organisms of different taxonomic groups (in particular, the causative agent of plague and erythrocytes of human blood group 0 have common antigens);

b) **allogeneic or group antigens** are different antigens within one species (in particular, blood groups according to the AB0 system, rhesus blood groups);

c) **isogenic or individual antigens**. There are, for example, histocompatibility antigens in the human population and type antigens in bacteria.

6. According to the direction of activation and provision of the immune response, there are:

a) **immunogens** induce a productive reaction of the immune system, which ends with the production of antibodies, antigen-reactive lymphocyte clones;

b) **tolerogens** lead to the formation of immunological tolerance (lack of immune response to epitopes of this tolerogen);

c) **allergens** are antigens that affect the acquired immune system by forming a pathological reaction of the body in the form of immediate or delayed hypersensitivity.

Antigens of the human body

Antigens of human blood groups are antigens of the AB0 system, other blood groups, and rhesus groups (Rh).

Histocompatibility antigens are the glycoproteins of almost all body cells' cytoplasmic membranes. They are named **major histocompatibility complex** (**MHC,** abbreviation synonym for human is **HLA**). HLA determines individual antigenic properties. It is encoded in the chromosome 6th genes group.

Role in the immune response:

1) induces acquired immune responses to any antigen;

2) induces transplant reactions in allograft transplants;

3) determines the possibility of the immunocompetent cells' interaction in the immune response formation.

An immune response formation is carried out by immunocompetent cells. The cells should be genotypically identical by MHC expressed on their membranes. It is the genetic restriction by the haplotype phenomenon.

There are two main classes of MHC molecules: MHC class I and MHC class II. These are polymorphic glycoprotein heterodimers localised on the cell membrane and have a furrow for binding to a specific peptide (peptide-binding furrow). It is known that MHC class I induces mainly cellular immunity and MHC class II - humoral.

HLA, class I molecules, provide antigen presentation to cytotoxic CD8 lymphocytes. This complex is located on the surface of almost all cells except erythrocytes and ciliated trophoblast cells. Cells that differ by class I are neutralised as foreign.

MHC class II antigens are found mainly on antigen-presenting cells - dendritic cells, macrophages, monocytes, and B-lymphocytes. The structure of the MHC class II with the included peptide triggers the mechanism of the specific immune response, the final link of which is the proliferation and differentiation of antigen-specific lymphocyte clones and the formation of immunological memory. Thus, the **helper** T-lymphocyte subpopulation (CD4+ cells) recognises a foreign peptide represented by HLA class II.

The **cytotoxic** or killer/suppressor lymphocyte/suppressor subpopulation (CD8+ cells) recognises the peptide represented by HLA class I molecules.

CD antigens (clusters of cell differentiation) are antigenic structures that determine the characteristics of the immune system cells. They are the so-called marker molecules that can detect differences in groups of cells) and CD19-22 - for B-lymphocytes. There are more than 200 variants of cell CD markers, the expression of which depends on the stage of cell differentiation and its functional state.

Tumour-associated antigens are classified by location and genesis.

According to the location, there are serum (secreted by tumour cells into the intercellular space) and membrane antigens; **by origin,** there are viral, embryonic, normal hyperexpressible, and mutant antigens.

A bone marrow transplantation was performed on the liquidator of the Chornobyl accident, who received a hefty radiation dose. Later after the operation, the patient was diagnosed with graft-versus-host disease. What antigens were the trigger for this reaction?

A.Antigens of the HLA system of the liquidator body cells

B.Antigens of the Rh erythrocyte liquidator system

C.Antigens HBs, HBc, HBe

D.Antigens of the ABO erythrocyte liquidator system

E.Antigens of the HLA system of donor cells

Bacterial antigens

Flagella (**H-antigens**) are present in bacteria's locomotor system (flagella). They consist of the contractile thermolabile protein flagellin.

Somatic (**O-antigen**) is present on the cell wall of bacteria. It is based on thermostable lipopolysaccharides (LPS).

Capsule (**K-antigens**) is located on the surface of the cell wall. They consist of acidic polysaccharides or polypeptides with different sensitivity to high temperatures.

Virulence antigen (**Vi-antigen**) is a variant of capsule antigen. It is present on the surface of the causative agent of typhoid fever and other virulent enterobacteria.

Antigenic properties are inherent in **bacterial protein toxins** (particularly the causative agents of diphtheria and botulism), enzymes, and other proteins (including tuberculin).

Protective antigens are bacterial antigens with strong immunogenicity. Those are mostly subunits of protein toxins.

Viral antigens

The following groups of viral antigens are core, capsid, and supercapsid (envelop). One part of the supercapsid is virus-specific (nucleic acids, capsid proteins, spikes), and the other is the components of the host cell (carbohydrates, lipids). The virion's antigenic composition depends on the viral particle's structure. Thus, some antigenic components in complex viruses are associated with the nucleocapsid and others with the supercapsid glycoproteins. Many simple and complex viruses have surface antigens, such as hemagglutinin and the enzyme neuraminidase. Viral antigens can be group-specific (present in different species within a genus or family) and type-specific (present in individual strains of the same species). Antigens of many viruses differ in a high degree of variability (influenza viruses, HIV).

Immune system. Immune response

Adaptive (specific, acquired) immunity is provided by the central and peripheral organs of the immune system, which have their inherent anatomical features and corresponding functions. Immunocytes are formed and mature in the central organs (red bone marrow, thymus, or Fabricius sac in birds). Immune cells directly carry out immune surveillance and protect the body from microorganisms. They are localised in peripheral organs (spleen, lymph nodes, lymphopharyngeal ring, lymphoid tissue of mucous membranes, cell of circulating blood and lymph, liver, appendix, etc.)

All cells of the immune system (as well as all blood cells) are derived from a single precursor, hematopoietic polypotent stem cells. As a result of the proliferation and

differentiation of stem cells, two main groups of lymphocytes are formed - T- and B-lymphocytes.

T lymphocytes are up to 80% of all lymphoid populations. Their maturation and differentiation in two populations occur in the thymus in the thymic microenvironment and thymic hormones. The populations are different by markers: CD4 and CD8.

All T lymphocytes have a smooth surface, a common marker CD3, and a receptor for sheep erythrocytes on the electrogram. T-lymphocytes perform two main functions: **regulatory** (performed by T-helpers) and **effector** (**cytotoxic T**-killers and natural killers). **T-helpers** are a heterogeneous subpopulation of T4 lymphocytes with the CD4 marker, perform a regulatory function, and account for up to 75% of all T lymphocytes. The T-helper uses a specific receptor to analyse the information provided by **antigen-presenting cells (APCs**). Activated CD4+ T-lymphocyte (T0-helper) is differentiated into T1- or T2-helpers, which differ only in the spectrum of their cytokines. T1-helper produces IL-2, -3, -γ-IFN, tumour necrosis factor (TNF), and others necessary for developing a cellular immune response, delayed hypersensitivity, and immune inflammation. T2-helper produces IL-4, -6, -9 and others needed for creating a humoral immune response and immediate hypersensitivity.

T-killers are a subpopulation of T-lymphocytes with the CD8 marker and perform effector functions. Target cells for T-killer are altered and mutant cells of the body, cells affected by the virus, and allogeneic graft cells. The target cell neutralising is carried out by cell-mediated cytotoxicity. For this purpose, T-killers synthesise toxic substances (perforin, granzyme, and granulysin). Also, T-killers are involved in T-cell memory and delayed hypersensitivity.

In contrast to T lymphocytes, natural or normal killers (NK) do not undergo differentiation and selection in the thymus and are found in the blood, liver, spleen, and uterine mucosa. Target cells for NK are cells of the body infected with intracellular parasites (bacteria, viruses, and protozoa) allogeneic graft cells, the destruction of which occurs due to antibody-independent cell-mediated cytotoxicity.

Natural killers can produce cytokines that initiate the macrophage-phagocytic link, the development of the immune response, and immune inflammation before adaptive immunity activates.

γ-, λ-T-lymphocytes are small subpopulations of T-lymphocytes. They are cytotoxic effector cells and immunoreactivity regulators. Local immunity and inflammatory response are activated, and T2-helper formation increases due to the synthesis of specific cytokines.

Thus, in the body, T lymphocytes provide cellular forms of immune response (delayed-type hypersensitivity, transplantation, antitumor, antiviral, antifungal immunity, etc.), determining the strength and duration of the immune response. In addition, cytokines control their maturation, differentiation, and activity.

A patient with significant burns underwent a donor skin graft. However, on the 8th day, the graft swelled, changed its colour, and began to be rejected on the 11th day. What cells are involved?

A.T lymphocytes

B.Erythrocytes

C.B-lymphocytes

D.Basophils

E.Eosinophils

After 1.5 months of liver transplantation, the patient worsened due to the onset of graft rejection. What factor of the immune system plays a crucial role in this reaction?

A.T-killers

B.Interleukin-1

C.Natural killers

D.B-lymphocytes

E.T-helpers

Many mutant cells appear in humans under the influence of mutagenic factors. However, most of them were recognised and destroyed by cells:

A.T-lymphocytes killers

B.Plasmablasts

C.B-lymphocytes

D.Stem cells

E.T-lymphocyte suppressors

B-lymphocytes are about 15% of all lymphocyte immunocompetent effector cells and are professional antigen-presenting cells (APC). The cells have a rough surface on electrogram, markers CD 19-22, and others. Mature B-lymphocytes and their progeny (plasma cells) produce antibodies, ensuring the formation of humoral immunity. Also, B-lymphocytes form B-cell memory and immediate hypersensitivity.

In the second year of life, the boy frequently suffered from respiratory diseases, stomatitis, and pustular skin lesions. Even minor gums and mucous membranes damage is complicated by inflammation that lasts a long time. It is established that almost no immunoglobulins of all classes are in the child's blood. Decreased functional activity of which cell population underlies the described syndrome?

A.B-lymphocytes

B.T lymphocytes

C.NK lymphocytes

D.Macrophages

E.Neutrophils

Insufficiency of immunoglobulin quantity was detected during the examination of the patient. Which of the cells of the immune system produce them?

A.Plasma cells

B.T-helpers

C.T-suppressors

D.T-killers

E.Plasmablasts

Other cells of the immune system

Macrophages provide processing and presentation of antigens to T-helpers.

Eosinophils (granular blood leukocytes) - when activated, emit toxic substances that have a detrimental effect on helminths.

Mast cells and basophils synthesise similar biologically active substances (human histamine). The volley into the intercellular space develops an immediate hypersensitivity reaction (type I allergic reactions). Allergens activate these cells, anaphylatoxins, mediators of activated neutrophils, and norepinephrine. Immune complexes can inhibit the reaction.

Dendritic cells. Process the antigen presentation to T-helpers in combination with MHC class II is the most active among the APC, particularly Langerhans cells (in the epidermis) and interdigital (in the lymph nodes).

Tricellular cooperation of immunocompetent cells

Immunocompetent cells include T- and B-lymphocytes, NK-cells, and antigen-presenting cells (APC - macrophages, monocytes, and dendritic cells). These cells can specifically recognise a foreign antigen and respond to it with an immune response. Immunocompetent cells interact due to the receptors located in the membrane of interacting cells and the stimulating effect of different types of cytokines. The cooperation of immunocompetent cells begins with the presentation of the antigen. Macrophages and other APCs carry out this process. Then, they engulf the antigen with followed intracellular enzymatic cleavage (limited proteolysis or processing)

. The formed antigenic peptides bind to MHC molecules; antigenic peptides are "loaded" into the furrows of intrinsic class MHC I, II class.

The resulting complex goes to the outer surface of the cytoplasmic membrane for presentation to T0-helper cells.

T0-helpers contact APC through their receptors and, with their proteins MHC I or MHC class II, analyse the presented antigen-MHC I or MHC II class antigen. In case of its foreignness, the T0-lymphocyte-helper is activated.

IL-12, which is produced by antigen-presenting cells, contributes to it. The result of T0-helper activation is its reproduction and differentiation in one of the offspring - TI- or T2-helper, which has a different cytokine profile and is responsible for forming a specific immune response.

Thus, T-helper 2-nd type lymphocytes are responsible for developing the humoral response. They activate B-lymphocytes and help them transform into plasma cells, producing different classes of immunoglobulins. While type 1 helper T lymphocytes are responsible for developing a specific cellular response because they help convert CD 8+ lymphocytes into mature cytotoxic T cells.

For the activation of T and B lymphocytes and their interaction with the APC, other stimuli are possible, both individual and total, different in origin.

Antibodies

Antibodies are gamma globulins synthesised in response to the penetration of the antigen and can bind specifically to it and participate in many immunological reactions. Therefore, antibodies are also called immunoglobulins and are denoted by the Ig symbol. Immunoglobulins exist in circulating form, in receptor molecules on immunocompetent cells and myeloma proteins.

Immunoglobulin producers are B-lymphocytes and their derivatives plasma cells. Antibodies produced in the body after immunisation or due to an infectious process are called immune. Antibodies that are not associated with immunisation or infection are called normal. Antibodies of a certain specificity produced by one clone of antibody-producing cells are called monoclonal.

There are five classes of immunoglobulins - IgA, IgM, IgG, IgD, and IgE- different in structure, antigenic composition, and functions, but all are glycoproteins and have a universal structure. In the structure of each immunoglobulin, the carbohydrate component stabilises and protects the protein component from the action of enzymes and other biologically active substances.

The protein component of the monomeric structure of each of the five classes of immunoglobulins consists of 4 polypeptide chains. There are two identical heavy H-

chains and two identical L-chains (Light chains), which differ in their molecular weight. Heavy and light chains are connected by disulfide bridges (-SS-). Each of the H- and L-chains has a variable (V) and constant (C) region, so the monomeric unit of Ig resembles the letter Y.

Both chains' variable regions are antigen-binding fragments. Both heavy chains' constant regions are parts of the constant fragment (Fc fragment) located in the lower part. Therefore, the monomeric Ig molecule has two Fab fragments and one C fragment. Free distal ends of the variable parts of the H and L chains (Fab fragment) form a region providing specific binding of the immunoglobulin to the antigen. The regions are considered the active site of the Ig molecule **(paratope**).

The exact coincidence of the spatial configuration of the active centre of the immunoglobulin with the configuration of the antigenic determinant leads to the formation of the immune complex (IC) antigen-antibody. So, not the whole Ig molecule takes part in the immune complex formation process, but only its limited area at the antigen-binding centre (active centre, paratope). The number of active centres determines the valence of antibodies. Antibodies with two or more valencies are called complete, and monovalent antibodies are called incomplete (blocking).

This property of antibodies is used in serological reactions to confirm the previous clinical diagnosis. The specific interaction of antibodies with antigen forms macromolecular structures of giant immune complexes. That can be visually assessed in agglutination and precipitation reactions. It is impossible for monovalent antibodies. However, the Coombs test detects such antibodies.

The constant regions of 2 heavy chains are part of the constant immunoglobulin fragment (Fc fragment). In contrast to the Fab fragment, it is nonspecific. It contains the centres for fixing Ig on lymphocytes and phagocytes, binding the first component of the complement system and transporting antibodies across the placenta and other biological membranes.

Two Fab fragments and one C fragment in an immunoglobulin molecule are connected by a hinge region, which gives flexibility to this molecular structure.

Antigenicity of antibodies:

1) **species antigenic determinants** are characteristic of immunoglobulins of all individuals of this species (in particular, dogs, monkeys, and humans); they can be determined as the species antibodies;
2) **isotype antigenic determinants** are groups differentiated Ig into 5 isotypes (classes) and subclasses;
3) **allotypic antigenic determinants** are individual, so they are available for each specific organism; they can distinguish persons within one species;
4) **idiotypic antigenic determinants** that reflect the structure of the active centre of the immunoglobulin molecule.

Classes of immunoglobulins

Immunoglobulins of class M (Ig M)

There are subclasses M1 and M2. They are "early" antibodies, as they are first synthesised in the fetus, make up the bulk of antibodies produced by newborns during infection or vaccination, first appear in the blood after immunisation with most antigens, and are a marker of acute viral infectious diseases.

At 4–6 days after immunisation, antibody biosynthesis "switches" to Ig G. Prolonged synthesis of Ig M alone indicates a violation of the regulatory function of helper T lymphocytes.

Class M immunoglobulins activate the complement in a classical pathway. They protect the body from viruses and bacteria. Also, they have an opsonising effect (activation of phagocytosis). IgM does not cross the placenta. They make up 5-10% of all serum immunoglobulins. Its structure is a pentameter, which consists of 5 monomeric units connected by a special bond into a single structure, has 10 active centres, and is accordingly 10-valent. Simultaneous binding of this immunoglobulin to five antigen molecules leads to the formation of large immune complexes, which promotes faster removal of antigens from the circulation, prevents their attachment to cells and initiates the development of the pathological process.

Immunoglobulins of class G (Ig G)

There are subclasses G1, G2, G3, and G4, which in total make up to 70-80% of all serum immunoglobulins formed at the height of the primary immune response and re-entry of the antigen (secondary immune response). IgG is a highly specific antibody. It has a high binding rate to the antigen, especially bacterial origin, activates complement in the classical pathway, and has an opsonising effect. IgG is similar to IgE. It has an affinity for mast cells and basophils and is involved in developing type I allergic reactions. In addition, IgG is the only class of antibodies that crosses the placenta into the fetus. Structurally, it is a monomeric molecule with two active sites and is a divalent antibody.

Class A immunoglobulins (Ig A)

Ig A exists in serum and secretory forms.

Serum Ig A can neutralise microorganisms and toxins circulating in the blood. Still, its action is inferior to secretory Ig A. It is a monomer with two active centres, i.e. is a 2-valent synthesised by plasma cells in the spleen, lymph nodes, and mucous membranes. It does not pass, as well as secretory immunoglobulin A through a placental barrier.

Secretory Ig A (sIg A) is characterised by an additional secretory component (S), which is synthesised by epithelial cells of mucous membranes and joins it at the time of passage of the immunoglobulin molecule through the epithelial cells. The S-component increases the resistance of the immunoglobulin structure to the proteolytic enzymes. Secretory Ig A is an element of local immunity. It synergises with nonspecific protection mechanisms (complement, lysozyme, and phagocytic cells). It protects mucous membranes from the settlement of pathogenic microorganisms and penetration of pathogens into the body's internal environment. Secretory Ig A predominates in saliva, tears, secretions of the stomach and intestines, vaginal secretions and amniotic fluid, bronchial contents, and the urinary tract. Unlike serum, sIg A is a di- or trimeric structure with 4 or 6 active sites, respectively (i.e., it is a 4- or 6-valent immunoglobulin).

Immunoglobulins of class E (Ig E)

Another name is "reagins". It is synthesised by mature B-lymphocytes and plasma cells, mainly in the respiratory and gastrointestinal tract lymphoid tissue. Ig E does not bind complement, does not cross the placental barrier, but quickly and strongly binds to tissue basophils and mast cells; participates in the development of allergic reactions such as hypersensitivity of the immediate type (type I reactions), and the formation of immunity against helminths. It is a monomer with two active centres, i.e. 2-valent.

Immunoglobulins of class D (Ig D)

The biological function of Ig D has yet to be studied. However, immunoglobulin of this class does not bind complement, does not cross the placental barrier, has no tropism to tissues, but is a receptor for B-lymphocyte precursors. It is a monomer by the molecular structure.

A patient with viral hepatitis A was admitted to an infectious disease hospital. Which antibodies will be synthesised first in response to the pathogen?

A.IgM

B.IgG

C.IgA

D.IgD

E.IgE

A pregnant woman was comprehensively studied for many infections when registered in a women's clinic. IgM to rubella virus was detected in blood serum. What does this examination show?

A.The initial infection of a woman

B.The chronic process

C.The woman is healthy

D.Exacerbation of the chronic process

C.Re-infection with rubella virus

Skin allergy sensitisation of poplar down allergen in patients with bronchial asthma was established. What factor of the immune system plays a crucial role in this immunopathological condition development?

A.IgE

B.IgD

C.IgM

D.Sensitised T lymphocytes

E.IgG

In a 34-year-old patient, disease symptoms disappeared after the intestinal infection caused by Salmonella. Which class of immunoglobulins can be detected in the patient's blood during convalescence?

A.IgG

B.IgE

C.IgM

D.IgA

E.IgD

A 54-year-old woman, D., complained to a doctor about her recent chicken egg intolerance. Antihistamines prescribed led to some improvement in the patient's condition. What antibodies contributed to this reaction?

A.Ig E

B.Ig A

C.Ig D

D.Ig G

E.Ig M

The patient was diagnosed with SARS. Class M immunoglobulins were found in the serum. What is the period of the infectious process in this case?

A.Acute period

B.Prodromal period

C.Incubation period

D.Convalescence

E.Carryarity of microbs

The production of antibodies in response to antigenic stimuli has characteristic dynamics and consists of the following phases:

1) the **latent** or inductive phase is the processing and presentation of antigen to immunocompetent cells, maturation of B-lymphocytes, and formation of plasma cells. Antibodies are not observed;

2) **logarithmic** phase is an intensive increase in the number of specific antibodies;

3) **stationary** phase - in it, the number of specific antibodies and cells synthesising them reaches a maximum and stabilises;

4) **reduction** phase is a phase with a gradual decrease in the number of antibody-producing cell clones and the number of corresponding antibodies.

There is a **primary** and **secondary** immune response.

Primary immune response: contact of the body's immune system with the antigen occurs first. As a result, numerous clones of antigen-specific B-lymphocytes are formed (cells are producers of antibodies and B-lymphocytes of immunological memory).

Secondary immune response: contact of the body's immune system with the antigen occurs a second time. In the process, clones of antigen-specific B-lymphocytes left after the primary immune response begin to multiply rapidly and synthesise the appropriate antibodies. The primary and secondary immune response phases coincide but differ in the course time. At the secondary meeting with the antigen, the body's immune response is more active and faster due to immunological memory. The preservation of immunological memory takes place in T- and B-lymphocytes after initial activation and transformation into small resting cells (immunological memory cells). The immune memory persists for many years.

Immunological tolerance is a lack of a specific immune response to the antigen (tolerogen). It is the counterbalance of the immune response and immunological memory.

There are:

a) **innate immunological tolerance** (in particular, the lack of response of the body's immune system to its antigens);

b) **acquired immunological tolerance** (in particular, after the introduction of the antigen in the embryonic period or the first days of life of the newborn individual).

Immunological tolerance is specific, i.e. is strictly directed to a specific antigen. The mechanisms of immunological tolerance are diverse and still need to be fully understood.

Chapter VII. IMMUNODIAGNOSTICS

Immunodiagnostics involves immune reactions (antigen interaction with antibodies) to diagnose infectious and non-infectious diseases. Immune reactions (also called serological reactions) are highly sensitive (they can detect even single molecules of antigen or antibody) and specific (only the corresponding antigen and antibody react).

Based on the principle of specificity, the presence of one known component of the reaction (antigen or antibody) can determine the second unknown component (antibody or antigen). The result of the immunological reaction is considered positive if the antigen and antibody match. The result is negative in the absence of such a match. An immunological reaction result can be registered subjectively (visually) or objectively (using various hardware).

All immune reactions are divided into two major groups:

Group 1 - serodiagnosis: determination of unknown antibodies in the serum of sick or examined people using known antigens.

Substances that contain known standard antigens are called "diagnostics". These are usually commercial substances, live-attenuated or inactivated microorganisms (bacteria, fungi, viruses, protozoa) or only their antigens. Antigens of microorganisms can be sorbed on certain carriers. The carriers are erythrocytes (erythrocyte diagnostic antigen) or latex particles (latex diagnostic antigen).

Group 2 - seroidentification (serotyping, immunoidentification): determination of the nature of the antigen using standard antibodies. Substances that contain known antibodies are called "diagnostic immune sera". The standard immune serum is obtained at specialised enterprises by immunising animals with certain antigens. Antibodies and antigens can be sorbed on erythrocytes, latex particles, on the surface of Staphylococcus aureus and others. The corresponding names of such substances are antibody erythrocyte, latex, and staphylococcal diagnostic antigens (diagnostic).

There are:

A) **Simple serological reactions** (direct, two-component or sedimentary). These reactions' results are visible so that they can be assessed visually (subjectively). In particular, these are agglutination and precipitation reactions. Staging, accounting, and evaluation of results are usually performed in vitro.

B) **Complex serological reactions (indirect, multi-component)**. An indicator system is used to assess the result. The indicators are erythrocytes, fluorochromes, enzymes, radioactive labels, laboratory animals, and tissue cultures. Examples are CFT, CHLT, RIA, ELISA, Etc. The results' implementation, estimation, and evaluation are carried out in vitro or in vivo.

At the initial stage of a serological reaction, the antigen interacts with the antibody, forming an immune complex. It is the immunological phase. The antigen (soluble, corpuscular) and the reaction (simple or complex) nature determines the result and the possibility of its evaluation. Thus, the immune complex formed in the first phase is enlarged and precipitated when direct sedimentary reactions are formed.

In the second phase (nonspecific or physicochemical phase), the precipitate formed is visible, and its features can be assessed visually. A necessary condition for this type of reaction is the presence of an electrolyte (saline). At the same time, when setting complex multicomponent immune reactions, the indicator systems that are their components allow detecting the formed complex of antigen-antibody in the first phase.

Serological reactions are performed in test tubes, in the wells of plastic plates, on slides, on plastic or glass trays, and on other laboratory equipment (in vitro experiments) and in experimental conditions using laboratory animals (in vivo experiments).

In a family with two children, a child under one year had an attack of spastic cough on the background of a fever. A similar pattern was observed in an older preschool child a month ago. The doctor suspected a pertussis infection. What is the method of retrospective diagnosis of this disease?

A.Serological

B.Bacteriological

C.Biological

D.Microscopic

E.Molecular biological

A pure culture of the pathogen was isolated from a patient with suspected typhoid fever. The culture was identified by morphological, cultural, and biochemical properties as Salmonella typhi. What research should be used for the final identification of the pathogen?

A.Seroidentification

B.Serodiagnostics

C.Allergy diagnostics

D.Antibiotic susceptibility pattern

E.Phagotyping

An intestinal infection outbreak with signs of colitis has been registered in children of the younger group of the orphanage. What research should be conducted for the final identification of the isolated pathogen?

A.To study the antigenic properties of the pathogen

B.To determine sensitivity to antibiotics

C.To examine susceptibility to bacteriophages

D.To study the biochemical properties of the pathogen

E.To study the virulence of the pathogen

Scheduled vaccinations against measles were carried out in the kindergarten. What method can be used to check the formation of post-vaccination immunity?

A.Serological

B.Virological

C.Bacteriological

D.Allergic

E.Bacterioscopic

Serological reactions

Precipitation reaction (PR) is the reaction of formation and precipitation of a complex of soluble molecular antigens (toxins, enzymes, globulins, polysaccharides, extracts of microorganisms) with antibodies in the form of turbidity (precipitate). In a liquid, a precipitate has the form of a ring; in a dense medium - in the form of delicate white lines ("tendrils", "arrows").

The formation of a precipitate is possible only with an equivalent ratio of antigens and antibodies.

There are several methods of setting the precipitation reaction: **ring precipitation, gel precipitation (agar), immunophoresis, and counter immunoelectrophoresis**.

In laboratory practice, depending on the tasks, conditions, and carrier of antigenicity, it is possible to use modifications (varieties) of any of these methods.

The ring precipitation reaction is carried out in narrow tubes with immune serum, on which the soluble antigen is carefully layered. The results are recorded in a few minutes. A cloudy ring-like precipitate is formed at the optimal ratio of antigens and corresponding antibodies at the boundary of the two solutions. If boiled and filtered, extract from the organs or tissues of the test object is used as the antigen. Such a reaction is called **Ascoli's thermo-precipitation test** (performed to confirm the presence of anthrax, plague, or tularemia antigens in the studied material of animal origin).

The Ouchterlony double immunodiffusion reaction is one of the most sensitive gel precipitation reactions, the results of which are evaluated by the nature of the location of the precipitation lines (merger or intersection of these lines). The reaction is used to reveal the toxigenicity of diphtheria bacteria. It is the specific interaction of the pathogen's exotoxin (antigen) with diphtheria antitoxin (antibody). The precipitate has the form of white lines at the optimal ratio of antigens.

The reaction is carried out in a Petri dish with a freshly prepared solid medium. A paper strip or standard paper discs impregnated with diphtheria antitoxic serum is

imposed on the medium surface. The selected and standard cultures of diphtheria corynebacteria should be inoculated at a certain distance. Evaluation of results is carried out in 18-24, 48 hours. If the test culture's precipitation lines merge with the control strain's precipitation lines, such culture is considered toxigenic. However, if the precipitation lines intersect, the culture under study is not toxigenic.

Simple radial immunodiffusion by Mancini determines the number of immunoglobulins in the serum. To evaluate the results, the diameter of the precipitation rings formed between a particular antiserum - anti-IgG, IgM, or IgA and a well with test serum should be measured; for the final result, the data of the calibration curve should be taken into account).

Counter immunoelectrophoresis is used to diagnose many bacterial and viral infections. It is a combination of two methods such as electrophoresis and immunoprecipitation. Precipitation lines are formed in the case of antibodies' specificity to antigens at their "meeting" place; evaluation of results after 1-2 hours).

A flocculation reaction determines the activity of the antitoxic serum or toxoid. These are toxin-antitoxin or toxoid-antitoxin reactions)

The test is evaluated by the appearance of opalescence or small flakes (microprecipitate) in the test tubes.

Immune electron microscopy is based on precipitation. It is used to detect microorganisms (most often viruses).

In general, reactions based on precipitation are widely used in infectious disease diagnostics and sanitary microbiology.

It is possible to determine the falsification of meat, flour, cereals, and other food products. Likewise, the species of blood can be determined in forensic examination.

The State Sanitary Inspectorate confiscated a consignment of sausages called "pork homemade" at the bazaar on suspicion of falsification. What can the serological immune test be used to identify a food product?

A.Precipitation

B.CFT

C.Agglutination

D.Immunofluorescence

E.IHAT

Ascoli precipitation reaction was used during the examination of animal leather. A whitish ring was formed a few minutes after combining immune serum and leather extract. What does this result indicate?

A.The presence of anthrax antigens

B.Presence of anaerobic infection toxin

C.The presence of the brucellosis causative agent

D.Escherichia coli surface antigen

E.The virulent antigen of Salmonella

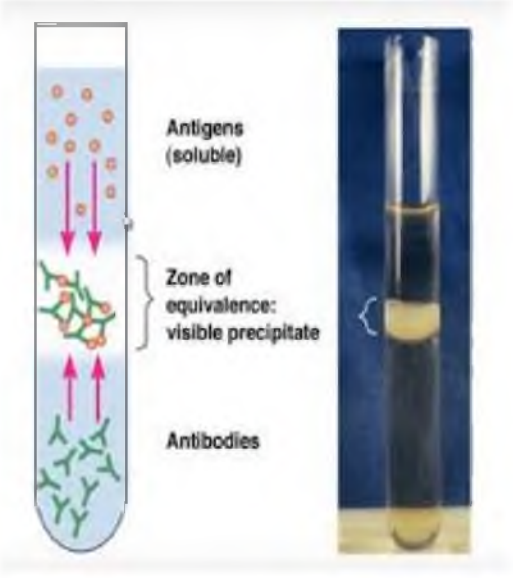

Figure 45 – Ring precipitation reaction

Cultures of diphtheria pathogens were isolated from patients. They were inoculated on nutrient plate agar in a Petri dish on both sides of a central filter paper strip. The strip is impregned by diphtheria antitoxic serum. After incubation of bacteria, striped areas of the medium turbidity were detected in agar between individual cultures and a strip of filter paper. What immunological reaction was performed?

A.Gel precipitation reaction

B.Coombs test

C.Agglutination reaction

D.Ring precipitation reaction

E.Opsonisation reaction

In the village of K., mass deaths of rats were found on several farms. There is a suspicion that the cause may be the plague. What should postmortem animal studies be performed to identify an infectious agent?

A.Ring precipitation reaction

B.Agglutination reaction

C.Passive agglutination reaction

D.Complement fixation test

E.Neutralisation reaction

The laboratory received material (extract of livestock raw materials) from the area where cases of anthrax among animals have been reported. What serological reaction should be used to detect antigens of a pathogen in the test material?

A.Thermoprecipitation reaction

B.Complement fixation test

C.Indirect hemagglutination reaction

D.Radioimmune assay

E.The agar precipitation reaction

A strip of filter paper impregnated with antitoxic diphtheria serum was placed on a solid nutrient medium. The investigated microbial culture and toxigenic strain were inoculated as plaques. The toxigenicity of diphtheria bacilli is determined. If the studied culture of microbes produces an exotoxin, the following are formed:

A.Lines of precipitation

B.Zones of hemolysis

C.Zones of diffuse turbidity

D.Zones of lecithovetylase activity

E.Ring of precipitation

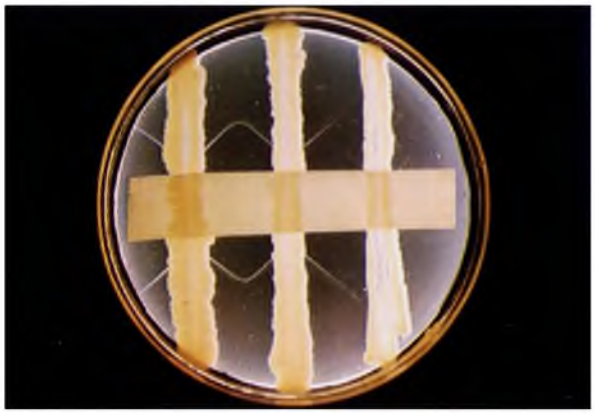

Figure 46 - Agar gel precipitation reaction (determination of bacterial toxigenicity)

Agglutination reactions (AR) are characterised by bonding (binding) and deposition of corpuscular antigens interacting with specific antibodies in the electrolyte solution. The antigens are bacteria, rickettsiae, erythrocytes or other cells, insoluble particles with adsorbed antigens, etc. The formation of flakes or a characteristic precipitate (agglutinate) is considered a positive result. Only the surface antigenic structures of cells are involved in the immune complex formation because the internal antigenic structures are inaccessible to antibodies. Various agglutination reactions (direct, indirect, slide, tub, etc.) are used, on glass, in test tubes, and the wells of polystyrene trays.

The purpose of AR in the microbiological diagnosis of infectious diseases:

1) **detection of specific antibodies in the serum of patients (serodiagnosis);**

2) **determination of the pathogen (its antigenic properties)** isolated from the patient (seroidentification).

Tube agglutination reaction

Suspension of microorganisms or their antigens should be added to the dilutions of a patient's sera (in tubes or wells of a polystyrene tray).

The serum titer is the highest dilution of serum at which agglutination occurred after several hours of incubation at 37°C sediment formed.

It can be **visually or microscopically assessed**. The determined serum titer is compared with the diagnostic one, which is important for confirmation or refutation of the previous clinical diagnosis. The nature and rate of agglutination depend on the characteristics of the antigen and antibodies.

The agglutination reaction ends in the formation of:

with **bacterial O-diagnostic antigen fine-grained sediment**;

with **bacterial H-diagnostic antigen, there are large flakes faster than with O-diagnostic.**

Tube AR is used for the serodiagnosis of many infectious diseases. Many of them have a specific name. For brucellosis, these are Wright's andHedelson reactions. For typhoid fever and paratyphoids, it is Widal's test.

Agglutination reaction on glass *(approximate reaction)* is carried out on a glass slide. A drop of diagnostic serum at a dilution of 1:10 or 1:20 is applied. Pure culture of a pathogen isolated from the patient is added. Controls are present at the same time, on the same slide.

Sedimented flakes appear in the experimental drop for a positive result. Therefore, the control drop should be without flakes in the negative result.

The obtained result is confirmed by the tube AR, in which diagnostic adsorbed serum is desirable to be used. The reaction is considered positive if, compared with controls, the determined titer is close to the diagnostic one (at least ½ of diagnostic). The agglutination reaction on glass is used for seroidentification and serotyping of bacterial pathogens (Escherichia, Salmonella, Shigella, vibrio, etc.).

Indirect (passive) hemagglutination reaction (IHAR or PHAR), latex agglutination reaction (LAT), co-agglutination (COAR)

In these serological reactions, one of the components (antigen or antibody) is adsorbed on certain carriers. In IHAR, a known component is adsorbed on erythrocytes, latex particles, and staphylococcal cells (co-agglutination reaction). Options for visual evaluation of the results of reactions to positive results are:

fine-grained red sediment with a scalloped border resembling an "inverted umbrella" is observed at the bottom of the tube or well of a polystyrene tray in **IHAR**;

fine-grained bottom agglutination of staphylococci in LAT;

coarse-grained sediment in COAR.

The result is negative in the absence of immune complex formation. Compact precipitate with smooth edges in the form of a button is observed at the bottom of the test tube or well of a plastic tray in IHAR, LAT, and COAR.

Usually, with the IHAR, antibodies are detected in the serum of patients with bacterial, fungal, parasitic and viral infections. Then, the appropriate erythrocyte diagnostic antigen is applied to dilutions of serum from the patient (in the wells of the polystyrene tray). The type of erythrocyte sediment is controlled after 2-3 hours of incubation at 37°C.

If a positive result is obtained, the titer of IHAR is determined, which will be necessary for confirming or refuting the previous clinical diagnosis. In addition, antibody erythrocyte diagnostics are sometimes used, in particular for serological identification of isolated pathogens and detection of botulinum toxin in the test material. Such a reaction is called the **reaction of reverse indirect hemagglutination (RIHAR)**.

Some viral causative agents can cause agglutination of various animals' erythrocytes. It is tipical for influenza, parainfluenza, measles, rubella, tick-borne encephalitis, adenoviruses, etc.). In these cases, the hemagglutination inhibition reaction (HAIR) is used for diagnostics.

HAIR is a particular reaction where specific antiviral antibodies, interacting with viral antigens, block (neutralise) it. Viruses lose the ability to agglutinate erythrocytes then (inhibit the hemagglutination reaction). The result is positive in forming compact red colour sediment with smooth edges ("button") in the experimental wells of the polystyrene tray. The result is negative in the formation of fine-grained with the scalloped border of the red colour sediment, resembling an "inverted umbrella".

Latex agglutination reaction (LAR) is an analogue of indirect hemagglutination reaction. Differences are:

1) a known component is adsorbed on polystyrene latex particles;

2) test is made on glass or dark plastic plate or using special commercial kits;

3) sediment is another colour and character;

4) the result is in a few minutes (2-7 minutes), so the reaction is used as an express method.

LARs are now used to identify pathogens of viral infections (rotaviruses, herpesviruses), some pathogens of bacterial infections (Salmonella, Streptococcus), and to detect specific antibodies in sera from patients with influenza, measles, scarlet fever and others.

In the co-agglutination reaction (CoAR), Staphylococcus aureus of a certain strain is used as a sorbent for a known component. Usually, CoAR is performed on glass trays. The results are taken into account on dark background 2-5 minutes after combining the ingredients. It is used for seroidentification of influenza viruses, hepatitis B viruses, para- and rotaviruses and others.

A patient consulted a doctor in the second week of the disease, which according to clinical and epidemiological data, resembled typhoid-paratyphoid disease. The doctor decided to confirm the diagnosis by detecting specific antibodies. What drug should be used for this purpose?

A.Diagnostic antigen

B.Diagnostic sera

C.Labelled serum

D.Monoclonal antibodies

E.Adsorbed monoreceptor sera

In a patient with the typical clinical picture of dysentery, due to the early use of antibiotics during a bacteriological examination, Shigella was not detected in the

stool. The titer of antischigella antibodies in IHAR with this patient paired sera increased 4 times. What does this indicate?

A.Confirms the diagnosis of dysentery

B.Excludes the diagnosis of dysentery

C.He suffered from dysentery earlier

D.Nonspecific reaction

E.Vaccine reaction

Vibrio cultures were isolated from the faeces and vomit of a patient with suspected cholera. Which reaction will determine the type of microbe that caused this disease?

A.Agglutination with sera containing O-antibodies

B.Agglutination with sera containing H-antibodies

C.Passive hemagglutination with erythrocyte antigenic diagnostic antigen

D.Vidal agglutination

E.Precipitation

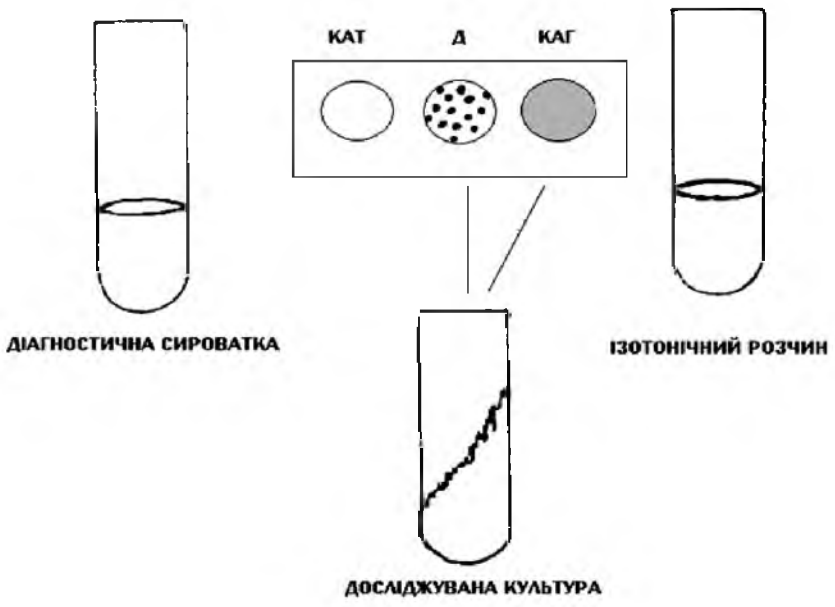

Figure 47 - Agglutination reaction on glass

Serological diagnostics of infectious diseases is based on the specific interaction of antibodies with antigens. What is the name of the serological reaction of microorganisms adhesion exposed to specific antibodies in the presence of electrolytes?

A.Agglutination reaction

B.Precipitation reaction

C.Complement fixation test

D.Hemadsorption reaction

T.Neutralisation reaction

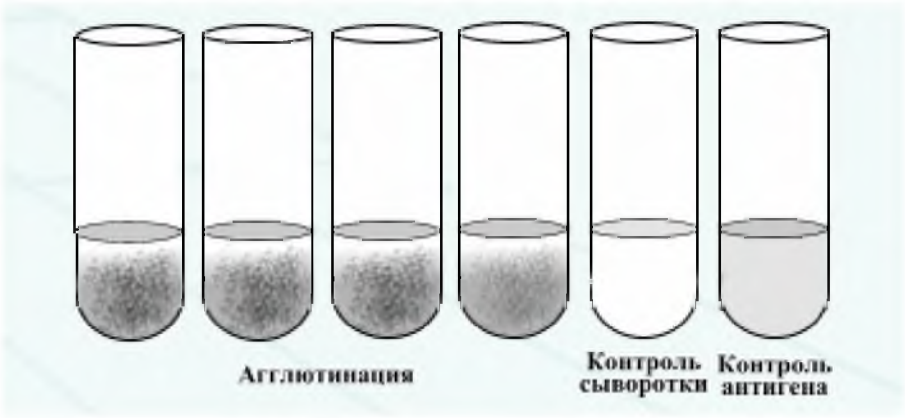

Figure 48 - Tube agglutination reaction

Diagnostic antigen was used for serological diagnosis of typhoid fever. These were tannin-treated sheep erythrocytes adsorbed Salmonella typhi Vi-antigen. In what reaction will this diagnostic antigen be used?

A.IHAR

B.HAIR

C.HAR

D.PR

E.CFT

Figure 49 - Passive hemagglutination reaction

A patient with clinical signs of encephalitis was admitted to the infectious disease hospital. A tick bite is present in the anamnesis. Antibodies against the causative agent of tick-borne encephalitis were detected at a dilution of 1:20 by hemagglutination inhibition reaction.The titer is not diagnostic. Indicate the following decision of the doctor after receiving the result.

A.Repeat the study with sera taken after 10 days

B.Examine the same sera again

C.Use a more sensitive reaction

D.Repeat the study with another diagnostic antigen

E.Reject the diagnosis of tick-borne encephalitis

The annotation to the diagnostic substance states that it contains antigens of the causative agent of typhoid fever, adsorbed on stabilised erythrocytes of sheep. What is the purpose the drug use?

A.To detect antibodies in the indirect hemagglutination reaction

B.To detect antibodies in the complement fixation test

C.To detect antibodies in the Widal's test

D.To detect antibodies in the hemagglutination delay reaction

E.For serological identification of the causative agent of typhoid fever

Shigella sonnei was isolated during a bacteriological examination of the faeces of a patient with an intestinal infection. Which serological reactions were used to identify the isolated pure culture?

A.Agglutination reaction

B.Precipitation reaction

C.Complement fixation reaction

D.Neutralisation reaction

E.Lysis reaction

A pure culture of bacteria was isolated in a patient with signs of colitis. The culture belongs to the genus Shigella according to morphological, cultural and biochemical properties. Which reactions should be used for serological identification of the culture?

A.Agglutination with diagnostic sera

B.Complement fixation

C.Indirect hemagglutination

D.Precipitation

E.Delayed hemagglutination

A 4-year-old child has clinical signs of pertussis. A tube agglutination reaction with pertussis and parapertussis diagnostics was performed for serological diagnosis. A granular precipitate at the bottom of the tubes with Bordetella parapertussis was formed. What antibodies did this reaction detect?

A.Agglutinins

B.Precipitins

C.Opsonins

D.Bacteriolysins

Serological testing of sera was performed to determine the titer of antibodies to Shigella in the retrospective diagnosis of bacterial dysentery. Which of the following reactions should be used for this?

A.Passive hemagglutination

B.Complement fixation

C.Precipitation

D.Hemolysis

E.Bacteriolysis

When Widal's test was repeated, the antibody titer increased to S.typhi O-antigens in the patient's serum from 1: 100 to 1: 400. How can the obtained results be interpreted?

A.Suffers from typhoid fever

B.Is an acute carrier of typhoid pathogen

C.Is a chronic carrier of typhoid germs

D.Previously relapsed with typhoid fever

E.He is previously vaccinated against typhoid fever

Effective diagnostics of intestinal pathogens carriers are based on detecting antibodies to certain bacterial antigens in the indirect hemagglutination reaction. What should standard diagnostic antigen be used in this reaction?

A.Erythrocyte diagnostic antigens with adsorbed bacterial antigens

B.Monoreceptor diagnostic sera

C.Antibodies against immunoglobulins of the main classes

D.Ram erythrocytes and hemolytic sera

E.Monoclonal antibodies

Serological diagnosis of infectious diseases is based on the specific interaction of antibodies with antigens. What is a serological reaction in which highly dispersed antigens are adsorbed on erythrocytes?

A.Indirect (passive) hemagglutination reaction

B.Complement fixation reaction

C.Hemadsorption reaction

D.Neutralisation reaction

E.Precipitation reaction

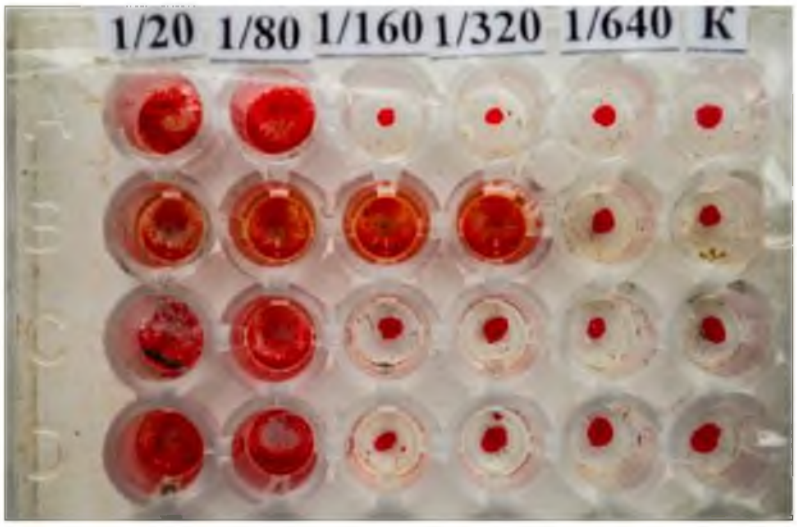

Figure 50 - The result of the indirect hemagglutination reaction (passive)

Neutralisation reaction (NR)

Antibodies of immune sera interact with microbial antigens and, in forming an antigen-antibody complex, neutralise the damaging effect of the pathogen or its toxin on sensitive cells and tissues.

Neutralisation reaction options:

A) **The reaction of virus neutralisation**

- viruses lose the ability to penetrate and reproduce in sensitive cells and tissues during their reaction due to the strong binding of specific antibodies to antigens of viruses.

Compounds of the reactions are serum, viruses, and biological objects as indicators of the neutralisation process (laboratory animals, chicken embryos, cell cultures).

The setting of the reaction

The mixture of sera and viruses is injected into the laboratory animal body or chicken embryo, or a suspension of cell cultures is added to the mixture. Evaluation of the results of the reaction:

- no signs of infection in laboratory animals or chicken embryos, no cytopathogenic action of viruses in cell culture is the negative result;
- in the presence of certain changes is a positive result.

Evaluation of the **colour test** results as a neutralisation reaction is carried out after adding to the main ingredients of the cell culture suspension.

With the formation of an immune complex, the virus is blocked, and the cells of the added suspension remain viable. As a result, acidic products of their metabolism accumulate in the reaction mixture, the pH of the medium changes, and its colour turns yellow.

Conversely, in the absence of the immune complex formation, unblocked viruses infect cells of the added suspension, inhibit their metabolic processes, lose viability, the pH of the medium does not change, and it remains pink.

The NR of viruses is used for the serodiagnosis of viral infections and the seroidentification of viruses isolated from diseased cultures (for example, in the laboratory diagnosis of polio).

B) **The reaction of the toxin neutralisation with antitoxin**

The test is used to detect bacterial toxins and determine their type (in particular, the exotoxin of Clostridium botulism, anaerobic wound infection, and tetanus). After incubation in a thermostat, the mixture of prepared material and corresponding antitoxic sera was injected into laboratory animals (in vivo) and observed for 3-4 days. The experimental animals remain alive when the toxin is neutralised with antitoxic sera. When determining the type of toxin, the animals remain alive under the conditions of compliance between the types of toxin and antitoxin. The neutralisation reaction is carried out in vitro in some cases.

The doctor suspected scarlet fever in a 2-year-old child with catarrhal symptoms and a skin rash. Standard serum to the erythrogenic toxin of streptococcus was injected intradermally. The rash disappeared at the injection site. What does the result of the reaction mean?

A. The clinical diagnosis was confirmed

B. The child is hypersensitive to erythrogenic toxin

C. The disease was caused by non-hemolytic streptococcus

D. The entire dose of serum can be administered intravenously

E. The child's immune system is significantly weakened

Canned meat is studied for botulinum toxin. An experimental group of mice was injected with an extract of the test material and antitoxic anti-botulinum serum types A, B, and E. The control group of mice was injected with the extract without anti-botulinum sera. What serological reaction was used?

A.Neutralisation

B.Precipitation

C.Opsonophagocytic

D.Complement fixation

E.Double immune diffusion

The patient developed symptoms of bulbar paralysis after eating canned mushrooms: ptosis, diplopia, aphonia, and swallowing disorders. The preliminary diagnosis is botulism. What can reaction be used to determine the type of toxin?

A.Neutralisation reaction

B.Agglutination reaction

C.Precipitation reaction

D.Complement fixation reaction

E.Immunofluorescence test

Immune lysis reaction. Complement fixation test (CFT)

These serological reactions involve complement (a nonspecific component of reactions), antigen, and antibody (specific components of the reaction).

The principle of the immune lysis reaction: when the antibodies match the antigen, an immune complex is formed, which activates complement in the classical pathway, destroying the antigen. Bacteria, spirochetes, erythrocytes and other cells can be used as antigens. Serum from patients or specific diagnostic serum is used as an antibody. Lyophilised preparation of guinea pig sera or freshly made (native) sera of this animal is used as a compliment. Antibodies in immune lysis reactions are called «lysins» (bacteriolysins, spirochetolysins, hemolysins, cytolysins).

The results of the immune hemolysis reaction are well amenable to visual assessment. Compact red sediment appears at the bottom of the test tube in the absence of hemolysis. The supernatant is transparent and colourless.

No precipitate is visible in the presence of hemolysis, and the content of the tube is transparent and red («laky blood»). Therefore, the immune hemolysis reaction is used as an indicator system in the complement fixation test (CFT).

CFT is a two-component indirect serological reaction. It is widely used for serodiagnostics of bacterial, viral, fungal, and protozoan infections. Examples are syphilis, leptospirosis, tularemia, brucellosis, ozena, ornithosis, influenza, mumps, etc. It is also used for the seroidentification of many pathogens.

Two systems take part in CFT:

1) the investigated system consists of antigen, antibodies (one of them is unknown) and complement;

2) indicator (haemolytic) system consists of sheep erythrocytes and haemolytic serum with antibodies to these erythrocytes.

There is a reaction in two phases.

If the antigen-antibody complex is formed in the 1st phase of CFT, it binds complement. Then, in the 2nd phase, hemolysis of antibody-sensitised erythrocytes, as components of the indicator system, does not occur. The precipitate is compact and red, and the supernatant is transparent and colourless). The result of the reaction is considered positive.

If the antigen and the antibody of the experimental system do not correspond to each other, the immune complex does not form in the CFT 1st phase. The complement remains free. It joins with the erythrocyte-haemolysin complex (components of the indicator system), resulting in hemolysis of erythrocytes (the test tube liquid is red and transparent). The result of the reaction is considered negative.

Before setting up the CFT in the microbiological laboratory, each reaction reagent is prepared correctly. Thus, hemolytic sera and complement are titrated according to certain schemes. The serum from patients for serodiagnostics of infectious diseases was preheated for 30 min at 56°C to inactivate their complement. Serial dilutions are

used to determine the titer of antibodies in the test sera. Standard diagnostic sera of a certain titer are used for seroidentification of a pathogen, Etc.

Figure 51 – Scheme of setting the complement fixation test

For serological diagnosis of syphilis in Wasserman reaction, the laboratory assistant prepared the following reagents: cardiolipin antigen (alcohol extract of lipids from bull heart muscle with cholesterol), treponemal antigen destroyed by the ultrasound, hemolytic system, saline solution, and investigated serum. What other component is needed to set the reaction?

A.Complement

B.Living treponema

C.Sheep erythrocytes

D.Diagnostic precipitating sera

E.Antiglobulin sera

Serological diagnosis of infectious diseases is based on the specific interaction of antibodies with antigens. One of the reactions requires 5 ingredients. There are antigen, antibody, and complement (first system) and sheep erythrocytes with haemolytic serum (second system). What is the name of the serological test?

A.Complement fixation test

B.Passive (indirect) hemagglutination reaction

C.Precipitation reaction

D.Hemagglutination inhibition reaction

E.Neutralisation reaction

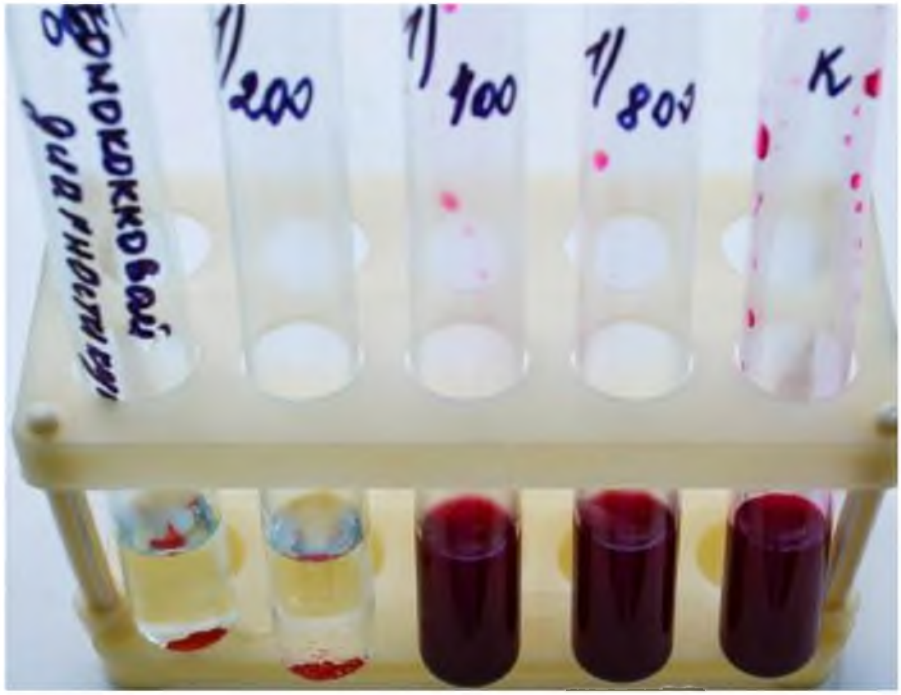

Figure 52 – The result of the complement fixation test

The complement fixation test examines a patient's serum for ornithosis serologic diagnostics. However, due to the device's malfunction, the studied sera needed to be heated more, and its complement needed to be inactivated. The result of the reaction is negative (hemolysis of erythrocytes). Why can't the result of this reaction be taken into account?

A.An excess compliment from the serum causes hemolysis

B.There is a decrease in antibody titer due to the complement action

C.Own complement blocks the antigen

D.Own complement blocks the reaction

E.Complement binding does not occur

Haemolytic serum against sheep erythrocytes is necessary for serological diagnosis of infectious diseases. What is it used for?

A.As a component of the hemolytic system in the complement fixation reaction

B.For indirect hemagglutination reaction

C.For the diagnosis of hemolytic disease in infants with rhesus conflict

D.For hemagglutination delay reaction

E.To establish the species of erythrocytes in forensic examination

In order to diagnose an infectious disease serologically, the doctor must perform a complement fixation test. What should be used in this reaction besides the patient's serum?

A.Diagnostic antigen, complement, haemolytic system

B.Diagnostic serum

C.Interferon

D.Erythrocyte diagnostic antigen

E.Anatoxin

Reactions using 128iarrhe antigens or antibodies

1. ***The chemiluminescent test (CHLT or Coon's technique)*** is based on detecting the labelled immune complexes. The label is a (fluorochrome), and the luminescent microscope should be used. A special dye (fluorochrome isocyanate or fluorescein) is used as a label. It gives a glow in the UV rays of a fluorescent microscope and is pre-conjugated to a known antigen or antibody. There are direct and indirect methods of CHLT.

Antibodies to immune serum are 128iarrhe with fluorochrome in the direct method, and in case they comply with the antigen, an antigen-antibody complex is formed. It can be detected in a fluorescent microscope by 128iarrhea.

The indirect method's essence is detecting antigen-antibody complex using 129iarrhe fluorochrome antiglobulin serum. When antibodies to the antigen coincide, the formed immune complex (antigen-antibody-anti/antibody) is detected by fluorescent microscopy under the characteristic glow in the UV rays of the microscope.

Option of indirect CHLT: diagnostic rabbit serum with antimicrobial antibodies is applied to the prepared smear from the suspension of any microorganisms. Antibodies that did not bind to the antigen are then washed out with saline, and the smear is treated with fluorochrome-labelled antiglobulin (anti-rabbit) serum and washed again. The formed immune complex microorganism-antimicrobial antibodies rabbit-anti-rabbit antibodies 129iarrhe with fluorochrome are detected by fluorescent microscopy by the characteristic glow in the UV rays of the microscope. Chemiluminescent tests are widely used in laboratory practice as an express method for the seroidentification of infectious diseases pathogens, identifying individual cells in cell cultures, and sometimes for serodiagnostics.

A patient with complaints of repeated 129iarrhea and vomiting, leg muscle pain, weakness, and dizziness was hospitalised in the infectious department. After the examination, the doctor made a preliminary diagnosis of «cholera». How is it necessary to examine the material from the patient for rapid diagnosis?

A.Direct and indirect CHLT

B.AR

C.Bacteriological method

D.Serological method

E.Biological method

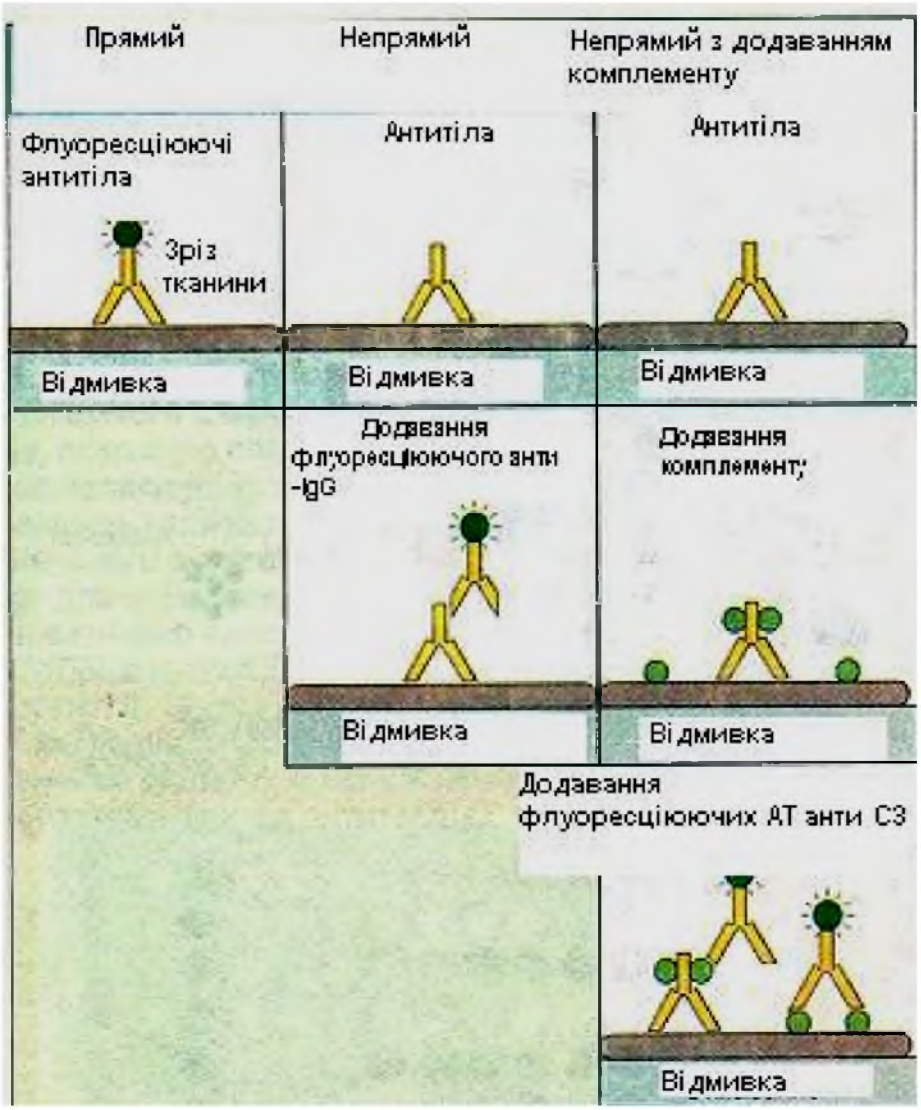

Figure 53 – Scheme of the chemiluminescent test

A man who, he said, received an envelope with suspicious powder in the mail went to the reception room of the infectious disease hospital. The man was hospitalised in quarantine. The powder from the envelope was sent to the laboratory to investigate the presence of anthrax spores. What research method makes it possible to identify a possible pathogen as soon as possible?

A.Chemiluminescent test

B.Complement fixation test

C.Gel precipitation reaction

D.Selection of pure culture

E.Bioassay on mice

Pathological material (secretion of the mucous membrane of the nasal passages) taken from a patient with a previous diagnosis of «flu» was delivered to the virology laboratory. Which rapid method will detect a specific viral antigen in the test material?

A.Direct and indirect CHLT

B.Direct and indirect ELISA

C.HAIR

D.IHAR

E.RIA

In the event of an outbreak of an acute respiratory infection, a rapid diagnosis based on the detection of a specific viral antigen in the test material (nasopharyngeal lavage) is made to diagnose influenza. What serological reaction is used for this?

A.Chemiluminescent test

B.Complement fixation test

C.Agglutination reaction

D.Precipitation reaction

E.Opsonisation reaction

A disadvantage of the microscopic method of diagnosing infections is its lack of information due to the morphological similarity of many species of microorganisms. What immunological reaction can significantly increase the informativeness of this method?

A.Chemiluminescent test

B.Coombs' reaction

C.Enzyme-linked immunosorbent assay

D.Opsonisation reaction

E.Radioimmunoassay

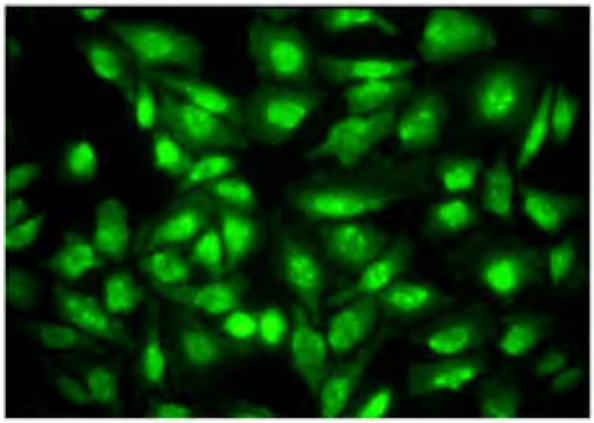

Figure 54 – The result of the chemiluminescent test

The immunofluorescence test is widely used to diagnose many bacterial and viral infections rapidly. First, choose a condition without which it is impossible to determine the result of the reaction.

A.The presence of a fluorescent microscope

B.The presence of an electron microscope

C.The presence of an immersion microscope

D.Isolated pure culture of the pathogen

E.Serum of the patient

2. ***Enzyme-linked immunosorbent assay or ELISA***

The test is based on immune complexes detecting when an enzyme (horseradish peroxidase, alkaline phosphatase or beta-galactosidase) is used as a label. The substrate/chromogen mixture is used in the reaction. (hydrogen peroxide or orthophenylene diphamine for horseradish peroxidase)

The principle of the reaction: in the formation of an immune complex, an enzyme that has been conjugated with one of the known reagents (antibody or antigen) breaks down the substrate, resulting in a change in the colour of the reaction product (reaction mixture turns yellow, light brown, or orange). The intensity of the colour is directly proportional to the number of antigen molecules and antibodies that interact with each other. There are several options for ELISA, depending on the purpose of the study and the characteristics of the media used

(direct and indirect methods; solid-phase ELISA, competitive solid-phase ELISA, etc.).

In solid-phase ELISA, as one of the most commonly used immunological tests, one of the components of the immune response (antigen or antibody) is sorbed on the surface of a solid carrier, in particular on the wells.

3. Filter paper or nitrocellulose can be used as a solid carrier. Laboratories usually use standard (commercial) test systems. The sequence of stages of solid-phase ELISA for the determination of antibodies:

1) antigen was adsorbed in the wells of the tray, and serum from a patient was added consistently;

2) antiglobulin serum 133abeled with the enzyme (usually horseradish peroxidase) is added after incubation of the reagents;

3) An enzyme substrate (chromogen) is added after incubation.

At each step, excess unbound reagents are thoroughly removed by washing.

With a positive result, the enzyme-substrate reaction occurs, which is registered by the change in colour of the reacting mixture. The reaction result is assessed with special, susceptible equipment.

To determine the antigens, in the wells of the test tray with sorbed antibodies, the test antigen should be added, then the immune serum against the antigen 133abeled with the enzyme, and then the substrate for the enzyme. Evaluation for results is similar.

Competitive ELISA variants:

a) test antigen and enzyme-labelled known antigen compete with each other for a limited amount of immune serum antibodies;

b) test antibodies and enzyme-labelled antibodies compete with each other for antigens.

With a positive result, the activity of the enzyme will be negligible, and the colour of the reaction mixture in the well almost does not change; with a negative result, the

activity of the enzyme is high, and the colour of the reaction mixture in the well changes significantly.

ELISA is used to diagnose many bacterial, viral and parasitic infections and identify various biologically active substances (hormones, enzymes, drugs, etc.).

A patient was hospitalised with a previous diagnosis of hepatitis B. A serological reaction was performed to diagnose the disease. The reaction is based on the interaction of the antigen with chemically bound to peroxidase or alkaline phosphatase antibody. What is the name of the serological reaction used?

A.Enzyme-linked immunosorbent assay

B.Radioimmunological method

C.Chemiluminescent test

D.Complement fixation test

E.Immobilisation reaction

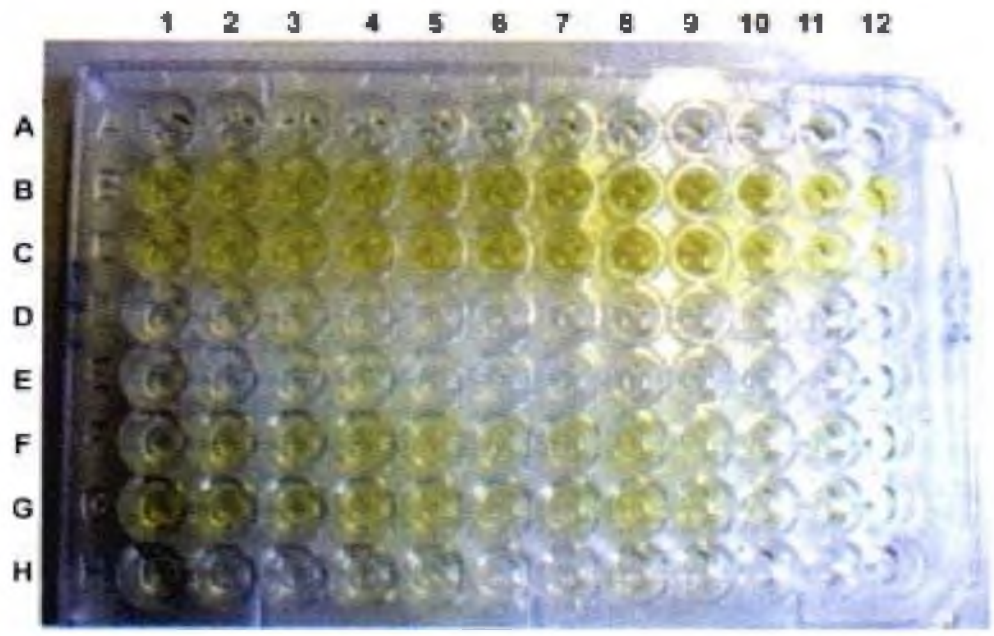

Figure 55 - The result of enzyme-linked immunosorbent assay

4. **Radioimmune method or analysis (RIA)**

It is a highly sensitive method where radionuclides (isotopes of iodine, carbon, hydrogen, chromium, etc.) are used as a label that binds to the antigen or antibody. In the formation of the immune complex, its beta or gamma radiation is determined using special equipment. The radiation intensity is directly proportional to the

number of antigen molecules and interacting antibodies. According to the technic, there are solid-phase and competitive RIA. RIA is used to determine the antigens of microorganisms and biologically active substances, but this method has environmental hazards.

5. **Immunoblotting (IB)** is a highly sensitive modern method based on electrophoresis and ELISA or RIA.

   The essence of the stages of IB:

1) electrophoresis of antigens of the test material in polyacrylamide gel in the presence of ionic detergent sodium dodecyl sulfate;

2) the separated fractions of antigens are transferred by capillary forces or by additional electrophoresis on a porous.

Separated biopolymers of antigens (imprints) are fixed on the porous membrane surface in the sequence in which they were located in the gel and are called "blot". The process of transferring separated molecules of biopolymers (antigens) from the electrophoretic gel on the surface of the porous membrane is named "blotting";

3) the antigens (blots) located on the membrane are treated with standard immune sera and washed out. The results are detected by ELISA or RIA, followed by their interpretation.

Options for immunoblotting in the laboratory are:

a) Hybridisation blotting is based on the principle of complementarity in nucleic acid construction. DNA or RNA probes are used to identify nucleic acids that cause infectious diseases.

Under the conditions of the nucleotides complementarity of a certain genetic probe and nucleotides of the studied DNA or RNA, a hybridised probe (hybrid molecule) is formed, the nucleotides of which can be detected by a certain label (radioactive isotope, biotin);

b) Western immunoblotting is based on the detection of immune complexes antigen-antibody is used to detect specific antibodies in the serum of patients, particularly HIV, herpes infections, syphilis, Lyme disease, etc.

The core of the procedure:

The patient's serum should be applied to the branded strips with "blots" and washed out. The serum against human immunoglobulins was labelled with the enzyme added and washed out again.

The substrate/chromogen should be applied. The formed complex of antigen + antibody of the patient + antibody against human Ig is detected by colour change (visible arcs are formed in the places of the interaction of antibodies with the corresponding antigens). It is possible to use other markers (isotopic labels, biotin).

Western immunoblotting can be used for determining different classes of immunoglobulins and antibodies against them during the development of an infectious disease.

Chapter VIII. IMMUNOPROPHYLAXIS AND IMMUNOTHERAPY

Vaccines and immune sera

Vaccines are immunobiological drugs to form artificially acquired active specific individual or collective adaptive immunity.

The active substance of all vaccines is a specific antigen (in particular, live attenuated microorganisms, inactivated microorganisms, subcellular antigenic complexes, microbial toxins - toxoids, chemically or biologically derived molecular antigens). There are four generations of vaccines:

1. **First-generation vaccines** (whole microorganisms).

2. These include living attenuated and heterologous vaccines. Examples are tuberculosis - BCG, plague, tularemia, anthrax, measles, rabies, polio Sebina, smallpox vaccine, etc. These are also inactivated vaccines (leptospirosis, pertussis, brucellosis, influenza, etc.).

After the BCG vaccine is given to infants, immunity to tuberculosis continues as long as there are live bacteria of the vaccine strain that remain in the body. What is the correct name for this type of immunity?

A.Non-sterile

B.Humoral

C.Type-specific

D.Congenital

E.Cross

A significant role in the prevention of tuberculosis is played by the planned mass vaccination against tuberculosis of newborns aged 5-7 days of life. The vaccine is used:

A.BCG

B.DPT

C.DP

D.DPT

A group of dentists has to go on a business trip to one of the African countries. However, it is known that in this country, on average, several hundred people get the plague every year. Which of the following vaccines should be used to prevent plague?

A.Live EV vaccine

B.Plague toxoid

C.STI live vaccine

D.Combined vaccine

E.Chemical vaccine

3. **Second-generation vaccines** (purified microbial components). These include chemical vaccines (meningococcal, cholera, typhoid, influenza) and toxoids (staphylococcal, diphtheria, tetanus, gangrenous, cholera).

Toxoid is a bacterial exotoxin after a certain treatment of it. As a result, the exotoxin loses its toxic properties but retains antigenic and immunogenic properties. Therefore, only antitoxic immunity is formed after the toxoid injection in humans.

Due to the approaching flu epidemy, the district epidemiologist is applying for prophylactic drugs. Which of them will promote the formation of active specific immunity and is the least reactogenic?

A.Subunit vaccine

B.Live vaccine

C.Killed vaccine

D.Donor gamma globulin

E.Leukocyte interferon

Due to the case of diphtheria, there was a need for preventive vaccination in the student group. What vaccine should be used to create artificial active immunity?

A.Diphtheria toxoid

B.Antidiphtheria serum

C.Specific immunoglobulin

D.DPT vaccine

E.Killed bacteria

4. **Third-generation vaccines.** These include genetically engineered or recombinant vaccines (hepatitis A, B, influenza). The basis for obtaining them is fundamental knowledge in molecular biology and genetics in combination with biotechnological processes.

5. **Fourth-generation vaccines**. These include vaccines still under development or only implemented in medical practice (in particular, anti-idiotypic vaccines, vaccines with artificial adjuvants, DNA, RNA vaccines, etc.).

Associated vaccines consist of a balanced amount of antigens from inactivated or live vaccines.

Suppose the vaccine contains homogeneous antigens, such as an associated vaccine. In that case, it is called a **polyvaccine** (in particular, live polio vaccine contains I, II, and III types of attenuated strains of the virus, or polytoxoid contains tetanus, gas gangrene toxoids).

If the associated drug consists of different antigens, it is called a **combined vaccine** (DPT-vaccine, TABte).

All vaccines as chemical, combined, polyvaccines and toxoids are enhanced by **adjuvants**. Adjuvants are substances of various origins with sorbing properties. They enhance the immunogenicity of vaccine components, create a "depot" of vaccine components, and activate the cellular component of non-specific immunity. The duration of artificially acquired immunity is usually from one to several years—the longest immunity under conditions of vaccination with a live vaccine (for years and decades).

Vaccines are usually used **to prevent** infectious diseases specifically. Carrying out **vaccine therapy** is possible at chronic or sluggishly proceeding infectious processes. Inactivated vaccines, toxoids, or autovaccines are usually used for vaccine therapy. **Autovaccines** are vaccine made in microbiological laboratories from microorganisms isolated from the patient and then used to treat this patient.

A doctor prescribed a vaccine made from a strain of bacteria isolated from the patient to treat pyoderma. What type of vaccine does this drug belong to?

A.Autovaccine

B.Attenuated vaccine

C.Genetically engineered vaccine

D.Associated vaccine

E.Chemical vaccine

Immune sera. These sera contain ready-made specific antibodies (immunoglobulins), so artificially acquired passive immunity is formed after their injection in humans. Depending on the nature of the antibodies, such immunity may be antitoxic, antibacterial, or antiviral, which develops immediately after serum administration and lasts 3-4 weeks. Homologous and heterologous immune sera are distinguished by the method of production. Homologous sera are obtained from biological fluids of the same species. Homologous sera for humans is obtained from donated blood, placental or abortion blood. Such sera contain a complex of various specific antibodies. It is possible to obtain homologous sera from pre-immunised volunteers, preferably containing antibodies of targeted specificity. Heterologous sera are obtained from the blood of animals (particularly horses) that have previously been immunised with toxoid or other specific vaccines according to a specific regimen. Such sera contain high concentrations of specific target antibodies. All obtained sera contain ballast substances, which are then removed by various methods (washing, fermentation, ultrafiltration, affinity chromatography, etc., respectively, carry out dialysis, diaferm, or 100% purification). Purified substances contain **immunoglobulins.**

Usually, immune sera are used for treating infectious diseases (serotherapy).

A specific emergency in prevention (**seroprophylaxis**) can be carried out by immune sera in certain circumstances. After contact with diphtheria or chickenpox patients, an unvaccinated person is administered anti-diphtheria antitoxic serum in the first situation and an immunoglobulin drug in the second.

A 1.5-year-old boy who did not receive routine vaccinations came into contact with a measles patient. Donor gammaglobulin was administered to the child for emergency-specific prophylaxis. What kind of immunity was created in this case?

A.Passive

B.Natural

C.Antitoxic

D.Post-vaccination

E.Local

A patient with a stab wound to the foot, which he received during mowing, was admitted to the hospital's surgical department. What specific drug should be used for emergency passive immunoprophylaxis of tetanus?

A.Antitoxic serum

B.Tetanus vaccine

C.DPT vaccine

D.Antibiotics

E.Toxoid

In a child with diphtheria 10 days after administration of antitoxic antidiphtheritic serum, a rash appeared on the skin, accompanied by severe itching, body temperature rose to 38°C, and there was a pain in the joints. What is the reason for these phenomena?

A.Serum sickness

B.Anaphylactic reaction

C.Atopy

D.Hypersensitivity delayed-type allergy

E.Contact allergy

Shortness of breath, rapid pulse, and blood pressure decrease appeared in a few minutes after administering the drug to the patient with tetanus. What drug could be the most likely cause of the complication?

A.Antitoxic serum

B.Sulfanilamide

C.Antibiotic

D.Toxoid

E.Donor gamma globulin

A patient injured in a car accident had the first symptoms of tetanus 7 days later. He was prescribed a course of anti-tetanus serum, and the patient recovered. However, two weeks later, the patient had a fever, swollen lymph nodes, swollen joints, rash, itching, and cardiovascular system disorders. What is the name of the condition that occurred in the patient?

A.Serum sickness

B.Urticaria

C.Anaphylactic shock

D.Dysbacteriosis

E.Quincke's oedema

Immune status assessment

Immune status is a structural and functional state of the human immune system, which is determined by a set of clinical and laboratory immunological parameters.

Immunodeficiency is a disorder of the immune system, the ability to respond to antigens and perform other immunological functions.

Immunodeficiency conditions are divided into congenital (**primary immunological insufficiency**) and acquired (**secondary immunological insufficiency**).

Congenital immunodeficiency conditions are associated with the genetic block of the immune system in ontogenesis.

Acquired immunodeficiency states are formed under the influence of the environment at the phenotype level and are caused by dysfunction of the immune system due to various diseases or adverse environmental factors.

Congenital immunodeficiency conditions can be divided into specific and non-specific immune deficiencies.

Forms of specific immune deficiency

1) defects of the **B-arm of the immune system** (hypo- or agammaglobulinemia of all or some classes of Ig);

2) defects of the **T-arm of the immune system** (aplasia of the thymus gland);

3) **combined defects** of humoral and cellular immunity.

Forms of non-specific immune deficiency

1) **complement system** defects;

2) phagocytes defects.

At the acquired immunodeficiency status, there can be a defection of T- and B-systems of immunity and factors of non-specific resistance. Their combinations are possible.

Acquired immune deficiency occurs after infections and invasions, burns, uremia, dysbiosis, severe injuries, major surgery, cancer, radiation, etc.

The two-stage principle is used to assess a person's immune status.

Level 1 tests (approximate) is the determination of the:

number of T- and B-lymphocytes in the blood;

the absolute and relative number of lymphocytes in the peripheral blood;

serum Ig M, Ig G, and Ig A concentration;

leukocytes phagocytic activity.

Level 2 tests (analytical) is the determining of the:

subpopulations of regulatory T-lymphocytes;

spontaneous leukocyte migration and leukocyte migration inhibition test;

skin allergy testing;

activating markers of T-lymphocytes;

Ig synthesis evaluation;

assessment of the killer lymphocytes activity;

evaluation of the T- and B-lymphocytes proliferative activity;

complement components testing;

assessment of different stages of phagocytosis.

A 1.5-year-old boy frequently suffers from respiratory diseases, stomatitis, and pustular skin lesions. Even minimal gums and mucous membranes damage is complicated by long-term inflammation. It is established that there are almost no immunoglobulins of all classes in the child's blood. Decreased functional activity of which cell population underlies the described syndrome?

A.B-lymphocytes

B.T-lymphocytes

C.Neutrophils

D.Macrophages

E.NK lymphocytes

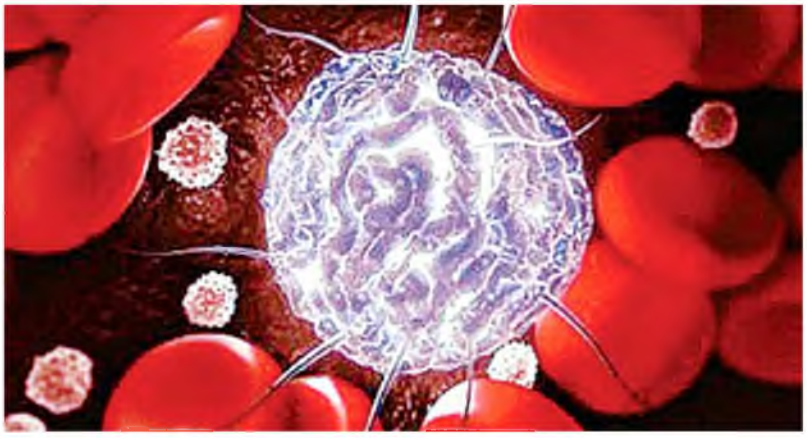

Figure 56 – B-lymphocytes

A young man of 20 years injured his right testicle. What is the danger for the left (healthy) testicle?

A.Antigen unmasking and antibody damage

B.Does not threaten anything

C.Development of atrophy

D.Development of the infectious process

E.Development of hypertrophy

A 5-year-old child was diagnosed with Bruton disease, which manifests in severe bacterial infections caused by lacking B-lymphocytes and plasma cells. What changes in the content of immunoglobulins will be observed in the serum of this child?

A.Reduction of IgA, IgM

B.Increase Ig D, IgE

C.There will be no changes

D.Increase IgA, IgM

E.Decrease IgD, IgE

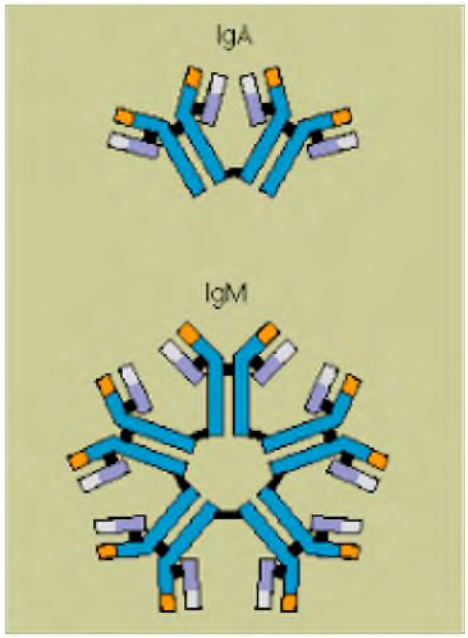

Figure 57 – Schematic structure of the IgA and IgM

Low levels of immunoglobulins were detected during the examination of the patient. Which of the cells of the immune system produce them?

A.Plasma

B.T-helpers

C.T-suppressors

D.T-killers

E.Plasmablasts

A 25-year-old man was diagnosed with acute diffuse glomerulonephritis. From the anamnesis: 18 days before the onset of the disease, suffering from sore throat. What mechanism of glomerulus damage is observed in this case?

A.Immune

B.Medicinal

C.Nephrotoxic

D.Ischemic

The number of immunoglobulins of different classes should be determined in studying human immune status. Which of the following reactions is used for this?

A.Radial immunodiffusion

B.Inverted indirect hemagglutination

C.Chain-polymerase

D.Double immunodiffusion

E.Blast transformation

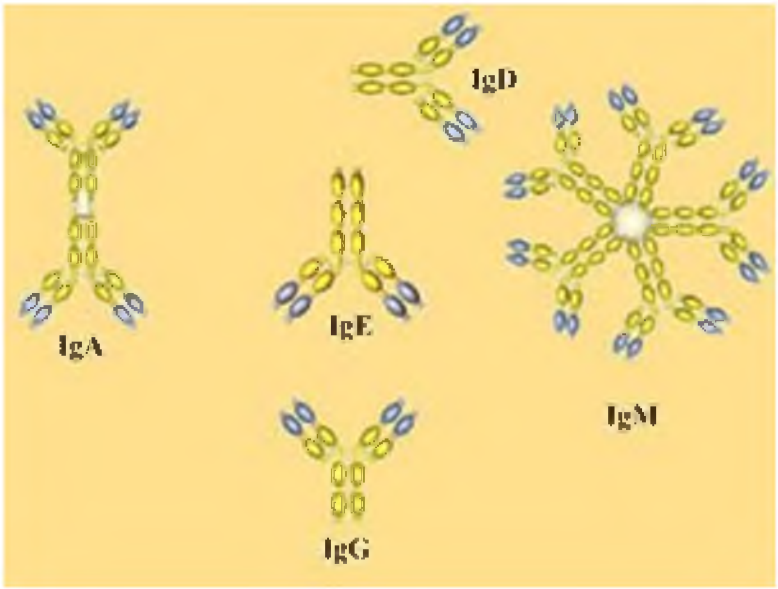

Figure 58 - Schematic structure of immunoglobulins

In a patient with clinical signs of immunodeficiency, the number and functional activity of T- and B-lymphocytes are normal. However, examination revealed a defect at the molecular level with the impaired function of antigen presentation to immunocompetent cells. Defect of which cell structures is possible?

A.Macrophages, monocytes

B.T-lymphocytes, B-lymphocytes

C.NK cells

D.Fibroblasts, T-lymphocytes, B-lymphocytes

E.0-lymphocytes

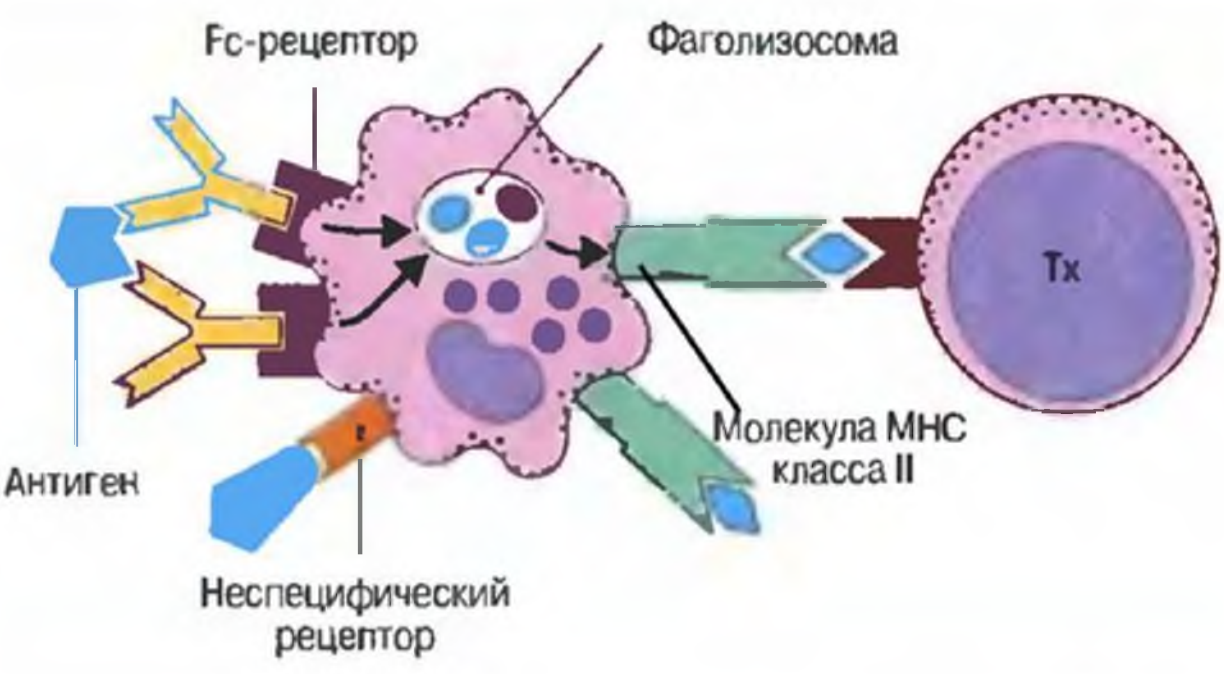

Figure 59 - Cooperation of the immune system cells

Chapter IX. ALLERGY, ALLERGIC METHOD (DIAGNOSTIC ALLERGIC TESTS)

The allergic method involves the detection of hypersensitivity in response to a specific antigen injected into the body. According to modern notions, allergy (allergic reaction, hypersensitivity reaction) is an increased "perverted" altered specific (immunological) reaction of the organism, which develops when the macroorganism re-contacts the antigen (allergen). Inducers of hypersensitivity reactions can be both foreign antigens and their antigens. It results in the development of many pathological processes and diseases. However, in some cases, cellular hypersensitivity reactions are components of immune protection against pathogens (including tuberculosis, syphilis, candidiasis, etc.).

Allergic reactions are carried out in three stages:

1) **immunological stage** (formation of antigen-sensitive cells, specific antibodies and immune complexes in response to the allergen);

2) **pathochemical stage** (synthesis of inflammatory mediators and biologically active amines as the main components in the mechanism of allergic reaction);

3) **pathophysiological stage** (manifestation of clinical signs of allergic reaction).

Usually, allergic reactions to foreign antigens-allergens are divided into immediate-type hypersensitivity reactions (ITH) and delayed-type hypersensitivity (DTH), which differ in the occurrence time and mechanism.

Brief description of ITH and DTH

1. The ITH reactions appear after the second contact with the allergen due to the activation of the B-arm of immunity and synthesis of specific antibodies, particularly Ig E. These reactions are mediated by the humoral part of the immune system. Specific desensitisation is possible. According to current data, ITH includes hypersensitivity types of I, II, and III (respectively:

Immediate type reactions or anaphylactic reactions (appear after 20–30 min), cytotoxic allergic reactions, immunocomplex allergic reactions).

Clinical manifestations are:

anaphylaxis;

Arthus phenomenon (local manifestations after repeated administration of the allergen intradermally or subcutaneously);

serum sickness (after administration of large doses of serum or other protein preparations);

atopic reactions (bronchial asthma, allergic rhinitis, allergic dermatitis, food and drug allergies, etc.);

immune complex diseases (including autoimmune disease), etc.

1. DTH reactions appear after secondary contact of the organism with the allergen in 6-8 hours and later. The factor is almost any low-immunogenic antigen; due to the activation of the T-arm immunity. These reactions are mediated by the cellular component of the immune system. Desensitisation is not possible. According to current data, hypersensitivity is type IV (delayed-type or DTH). Forms of manifestation: tuberculin reaction, contact allergy, delayed reaction to proteins.

The reaction of hypersensitivity to microorganisms and products of their vital activity occurs in many infectious diseases, and an allergic test can detect its presence. Moreover, allergy tests are specific and have significant diagnostic value. Currently, allergy testing is used to diagnose tuberculosis (Mantoux test), brucellosis (Bürne's allergic test), tularemia, anthrax, actinomycosis, dermatomycoses, toxoplasmosis and others. These tests are based on the development of type IV hypersensitivity (delayed-type), so they are usually taken into account after 24-48-72 hours. Allergens are usually administered intradermally, sometimes dermally. As a result, skin redness and swelling appear at the injection site in infected persons. The result is evaluated by the diameter of the formed infiltrate (papules). The limiting point of this method is that allergic tests can be positive in patients who have had the disease or are vaccinated.

Examples of the allergic tests

1. **Tuberculosis diagnostics** is carried out using a skin-allergic reaction (Mantoux test) to detect the hypersensitivity of people infected with *Mycobacterium tuberculosis*. It is used to assess the course of the tuberculosis process in patients, monitor the effectiveness of BCG vaccination, and select persons for revaccination. The allergen is named **tuberculin**. The substance contains purified protein derivative (PPD) of human or bovine tuberculosis mycobacteria or vaccine strain BCG. It is administered intradermally after standard dilution at a volume of 0.1 ml. The test's accounting and evaluation are carried out in 48-72 hours. A positive result with a papule diameter of 5 mm or more indicates infection and, in a particular situation, may be considered a contraindication for vaccination or revaccination with BCG vaccine.

2. **Brucellosis diagnostics**. The skin allergic reaction (Bürne's test) is performed to detect hypersensitivity (DTH) in patients with brucellosis from 15-20 days of illness. 0.1 ml of allergen (brucella) is injected intradermally into the dorsal surface of the forearm. The reaction's result is considered in 24-48 hours. A positive reaction is characterised by swelling and redness of 4x6 cm. The allergic reaction has been positive for many years in patients with brucellosis and vaccinated with the live brucellosis vaccine. Therefore, for reliable confirmation of the diagnosis of "brucellosis", serodiagnosis is also performed.

3. For **early diagnostics of tularemia**, skin allergic reaction (test) is used from the fifth day of illness. Two types of tularin are used as allergens, and depending on the composition of the drug, it is administered dermally (outer shoulder surface) or intradermally (on the back of the forearm). The results are taken into account in 24-36-48 hours. A positive result is considered to be the appearance of redness and infiltration of at least 5 mm at the site of tularin injection. Allergy tests remained positive for several years in patients with tularemia and vaccinated with live anti-tularemia vaccine. Therefore, serodiagnostic is performed to confirm the diagnosis of tularemia.

4. For **retrospective diagnosis of anthrax**, if necessary, an epidemiological study of the subjects put skin allergy tests with the anthraxin. The drug is administered intradermally in a volume of 0.1 ml. The results are taken into account in 24-48 hours. The reaction is positive at the injection site of anthrax antigen if redness with a diameter of more than 16 mm and infiltrate is revealed.

5. To diagnose **chronic gonorrhoea,** a skin allergy test with the gonococcal allergen is used. The drug is administered intradermally. The results are taken into account after 24 hours. It is considered positive in the presence of redness and swelling at the injection site.

Novocaine was used for analgesia during surgical procedures. After 10 minutes, the patient had pale skin, shortness of breath, and hypotension. What allergic can reaction be expected?

A.Anaphylactic

B.Immunocomplex

C.Cytotoxic

D.Cell-mediated

E.Stimulating

A 10-year-old child was tested for Mantoux (with tuberculin). After 48 hours, a papule up to 8 mm in diameter appeared at the site of tuberculin injection. What type of hypersensitivity reaction developed after tuberculin administration?

A.Type IV hypersensitivity reaction

B.Arthus-type reaction

C.Serum-type reaction

D.Atopic reaction

E.Type II hypersensitivity reaction

Figure 60 - Positive Mantoux test

A patient with periodic attacks of asthma, which occur when inhaling various aromatic substances, was diagnosed with atopic bronchial asthma. An increase in IgE was diagnosed. For what type of reactions is it characteristic:

A.Anaphylactic reactions

B.Cytotoxic reactions

C.Immunocomplex reactions

D.DTH

E.Autoimmune reactions

Figure 61 - Manifestations of anaphylactic reaction

Patient M. has a local reaction to a bee sting that occurred in the first minutes after the sting. What type of hypersensitivity reaction is it?

A.Anaphylactic

B.Cytotoxic

C.Immunocomplex

D.Delayed type

E.Idiotype-antiidiotype

A patient injured in a car accident had the first symptoms of tetanus 7 days later. He was prescribed a course of anti-tetanus serum, and the patient began to recover. However, two weeks later, the patient had a fever, swollen lymph nodes, swollen joints, rash, itching, and cardiovascular system disorders. What is the name of the condition that occurred in the patient?

A.Serum sickness

B.Urticaria

C.Anaphylactic shock

D.Dysbacteriosis

E.Quincke's oedema

In a patient with bronchial asthma, skin allergy sensitisation of poplar down allergen was established. What factor of the immune system plays a crucial role in developing this immunopathological condition?

A.IgE

B.IgD

C.IgM

D.Sensitised T lymphocytes

E.IgG

In a child with diphtheria, 10 days after the injection of antitoxic diphtheria serum, skin rashes appeared, accompanied by severe itching, fever up to 38°C, and joint pain. What is the reason for these phenomena?

A.Serum sickness

B.Anaphylactic reaction

C.Atopy

D.Delayed type hypersensitivity

E.Contact allergy

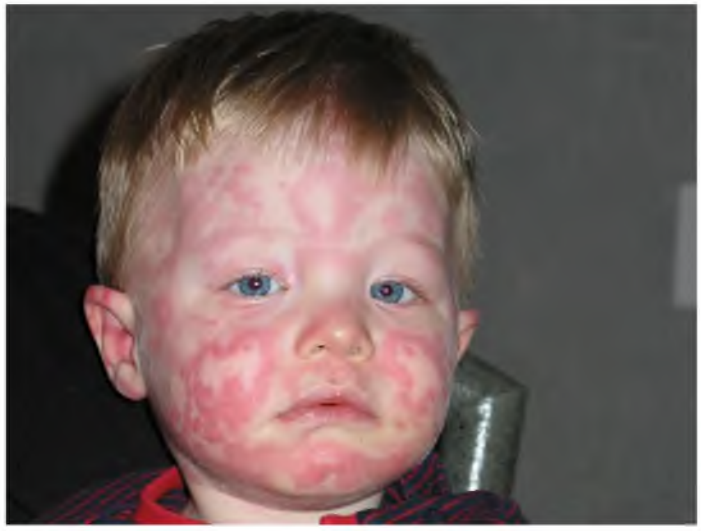

Figure 62 - Manifestations of serum sickness

At the heart of the development of immune and allergic reactions, the body uses the exact mechanisms of the immune system's response to the antigen. Identify the main difference between allergic and immune reactions:

A.Development of tissue damage

B.Ways of getting antigens into the body

C.The amount of antigen that enters

D.Hereditary predisposition

E.A peculiarity of antigen structure

What condition can develop 15-30 minutes after re-administration of the antigen due to elevated levels of antibodies, mainly IgE, which are adsorbed on the surface of target tissue basophils (mast cells) and blood basophils?

A.Anaphylaxis

B.Serum sickness

C.Delayed type hypersensitivity

D.Immune complex hypersensitivity

E.Antibody-dependent cytotoxicity

A 22-year-old woman developed small itchy papules on the skin of the torso and distal extremities 5 hours after eating seafood. Some of the papules merged. A day later, the rash disappeared spontaneously. Name the mechanism of hypersensitivity that underlies these changes:

A.Atopy (local anaphylaxis)

B.Cellular cytotoxicity

C.Systemic anaphylaxis

D.Immunocomplex hypersensitivity

E.Antibody-mediated cellular cytolysis

Chapter X. METHODS OF EXPRESS DIAGNOSIS OF INFECTIOUS DISEASES

Advantages of express methods:

1) high sensitivity;

2) high specificity;

3) universality;

4) high-speed analysis;

5) the possibility of quantitative analysis;

6) the ability to diagnose both acute and latent infections;

7) the possibility of practical use in the diagnosis of infectious diseases, the causative agents of which are difficult, either not cultivated at all, or are in persistent form;

8) high capacity, availability of production and reproduction of results.

Furthermore, rapid methods quickly detect the pathogen in the test material or specific antibodies in the serum.

Methods of rapid diagnosis are a complex group of diagnostic methods and are currently considered as such: CHLT, ELISA, RIA, PCR, and immunoblotting.

The chemiluminescent test is widely used for many bacterial and viral infections express diagnostics. Choose a condition without which it is impossible to determine the result of the reaction.

A. The presence of a fluorescent microscope

B. The presence of an electron microscope

C. The presence of an immersion microscope

D. Dedicated pure culture of the pathogen

E. Serum of the patient

Figure 63 - Assessment of the chemiluminescent test

In an acute respiratory infection outbreak, a rapid test based on detecting a specific viral antigen in the material (nasopharyngeal lavage) is made to diagnose influenza. What serological reaction is used for this?

A.Chemiluminescent test

B.Complement fixation test

C.Agglutination reaction

D.Precipitation reaction

E.Opsonisation reaction

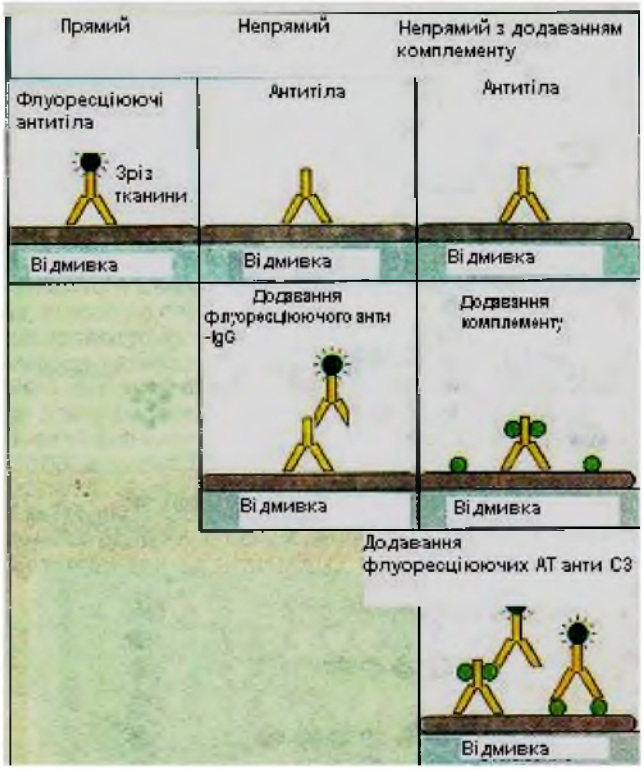

Figure 64 - The scheme of the chemiluminescent test

The reliability of bacteriological research in the diagnostics of plague increases with the chemiluminescent test. Describe the resulting microscopic picture.

A.Small ovoid sticks with a bright green glow

B.Tiny coccoid bacteria of pink colour

C.Large rods with trimmed edges

D.Small pink rods with rounded edges

E.Slightly bent red sticks at an angle

A man who, he said, received an envelope with suspicious powder in the mail was delivered to the reception room of the infectious disease hospital. The man was hospitalised in an isolator. The powder from the envelope was sent to the laboratory to investigate the presence of anthrax spores. What research method makes it possible to identify the pathogen as soon as possible?

A.Chemiluminescent test

B.Complement fixation test

C.Gel precipitation reaction

D.Selection of pure culture

E.Bioassay on mice

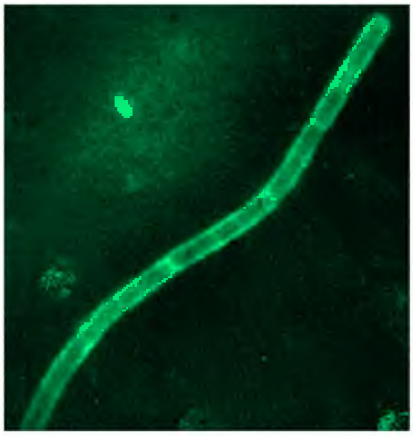

Figure 65 - Microscopic picture of the chemiluminescent test

A patient was hospitalised with a previous diagnosis of hepatitis B. A serological reaction based on the antigen interaction with an antibody chemically bound to peroxidase or alkaline phosphatase was performed to diagnose the disease. What is the name of the serological reaction used?

A.Enzyme-linked immunosorbent assay

B.Radioimmunological method

C.Chemiluminescent test

D.Complement fixation test

E.Immobilisation reaction

A patient with signs of pneumonia, which developed on the sixth day of influenza, was admitted to the infectious disease hospital. Which method most reliably confirms the influenza aetiology of pneumonia?

A.Detection of influenza virus antigens in sputum by ELISA

B.Study of paired sera

C.Infecting of chicken embryos

D.Immunoluminescent examination of smears-imprints from the nasal passages

E.Detection of antibodies against hemagglutinins of influenza virus

Sensitive methods should be used to test donor blood for hepatitis B antigens. Which of the following reactions should be used for this purpose?

A.Solid-phase enzyme-linked immunosorbent assay

B.Complement fixation test

C.Indirect hemagglutination reaction

D.Immunoelectrophoresis

E.Indirect chemiluminescent test

Figure 66 - Scheme of enzyme-linked immunosorbent assay

Chapter XI. MOLECULAR NUCLEIC ACID HYBRIDIZATION TECHNIQUES

Molecular genetic methods diagnose infectious diseases based on the detection of nucleic acids (DNA or RNA) of microorganisms in the investigated material. Molecular genetic research methods have many advantages over traditional ones: they are susceptible, specific, fast and accessible in production, fast in evaluating results, and most importantly, identifying detected microorganisms does not require the selection of pure cultures. The most commonly used genetic methods for diagnosing infectious diseases are molecular hybridisation (DNA hybridisation, genetic probes) and polymerase chain reaction (PCR).

The method of genetic probes is used in the identification of microorganisms to determine their taxonomic position. The method is based on comparing and establishing the similarity of DNA or RNA fragments of the studied microorganism with DNA or RNA probes of pathogenic bacteria and viruses, the systematic position of which is already known. Genetic probes (DNA or RNA probes) are single-stranded nucleic acid molecules of a specific microorganism produced in the laboratory and conjugated to a label. Either a radioactive isotope or an enzyme is used as a label. A hybrid molecule is formed in the case of complementarity of the probe's nucleotides and the studied microorganism's nucleic acid fragment.

When a probe has a radioactive label, the results are considered using a gamma counter.

When a probe is labelled with an enzyme, the substrate is added at the last stage of the study. A positive result is a change in the used substrate's colour. The positive results generally indicate correspondence between the genetic probe's nucleotide sequence and the studied microorganism's nucleic acid fragment. They, therefore, make it possible to determine the systematic affiliation of the pathogen.

Polymerase chain reaction (PCR) is a method of directed accumulation (amplification) of microbial DNA. This method detects even tiny fragments of the nucleotide sequence of the genetic information carrier (one or more genes) of any pathogenic microorganism in the investigated material (clinical material from the

patient, water samples, food, etc.) and carries out identification at the final stage. Thus, identification occurs without isolating the culture of the microorganism.

Sets of primers (fragments of DNA) as markers of the suspected pathogen are used.

Combining the primer and the test material with the prepared single-stranded DNA of the test microorganism forms a double-stranded DNA of the complementary region. It can be reproduced by the polymerase enzyme included in the PCR kit. The synthesised DNA fragments are templated for the synthesis of new nucleotide sequences complementary to this fragment in the following amplification cycle, and this process occurs sequentially (cycle after cycle). This is a chain of reactions. After a few cycles, the number of copies of the DNA fragment increases 10^6-10^8 times, which are already usually identified by agarose gel electrophoresis.

It is possible to carry out identification by hybridisation method using oligonucleotide labelled with fluorochrome or enzyme probes. A new direction in the registration of PCR results is the use of optical biosensors, which detect even picogram amounts of nucleic acid fragments.

PCR algorithm

1) isolation and extraction of DNA from the studied microorganism;
2) amplification of the studied nucleotide sequences (genes) of DNA;
3) registration of amplification results.

PCR processes are carried out in a thermocycler (according to a given program in a programmable thermostat).

Scheme of the polymerase chain reaction cycle

1. Native double-stranded DNA of the studied microorganism.
2. Denaturation of microbe DNA at 94–95°C.
3. Hybridisation at 55°C (attachment of primers to the template DNA under the conditions of complementarity of their nucleotide sequences at 55°C.

4. Synthesis of complementary DNA strand at 72°C, formation of double-stranded DNA.

Like other molecular genetic methods, PCR has numerous advantages over traditional methods of microbiological diagnostic of infectious diseases (high sensitivity, specificity, the ability to detect multiple pathogens in one bioassay simultaneously, the ability to detect pathogens with high antigenic variability, the ability to detect intracellular parasite, Etc.). However, its limited capabilities in diagnosing opportunistic infections and the high sensitivity of PCR pose a risk of obtaining pseudo-positive results due to minimal DNA contamination of the laboratories that constantly perform research in this area.

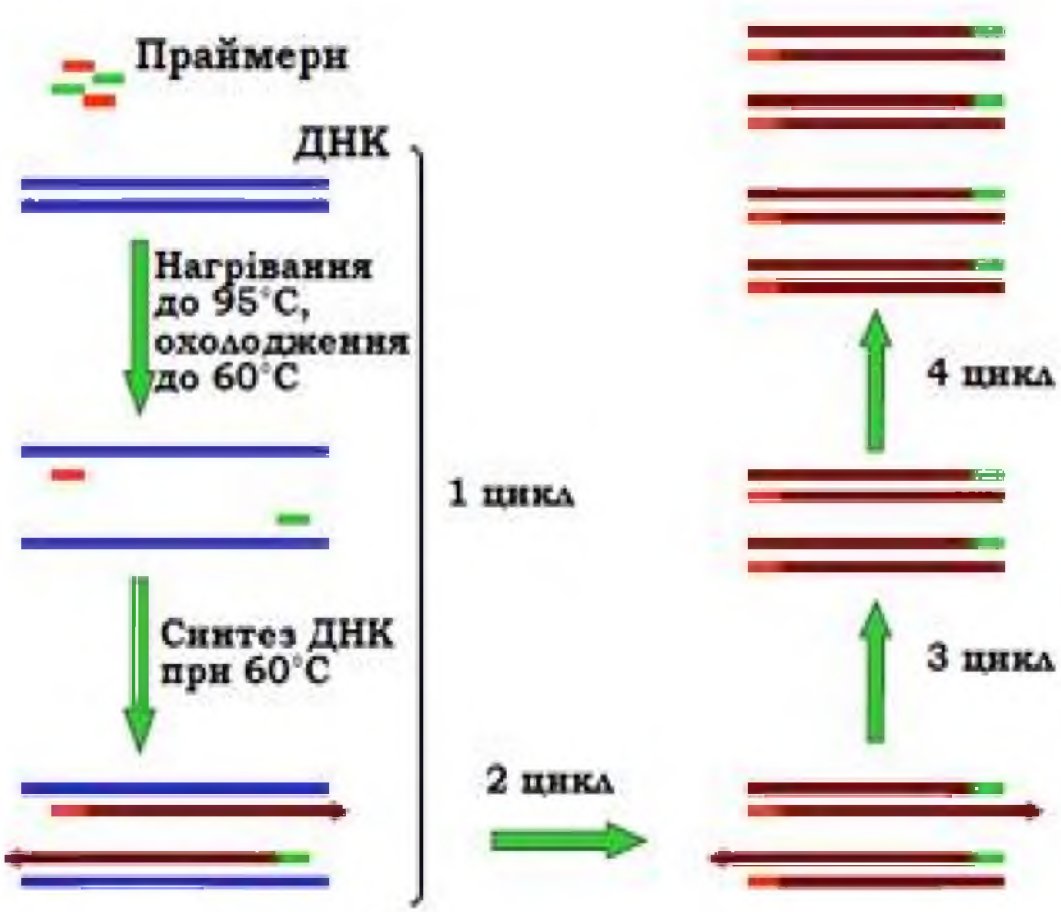

Figure 67 - Scheme of polymerase chain reaction

In** recent **years, the method of genetic indication of pathogens has been used, which allows the detection of pathogen nucleic acid fragments in the studied samples. Therefore, choose the one suitable for this purpose from the following reactions.

A.Polymerase chain reaction

B.Phage titer

C.Radioimmune analysis

D.Precipitation reaction

E.Enzyme-linked immunosorbent assay

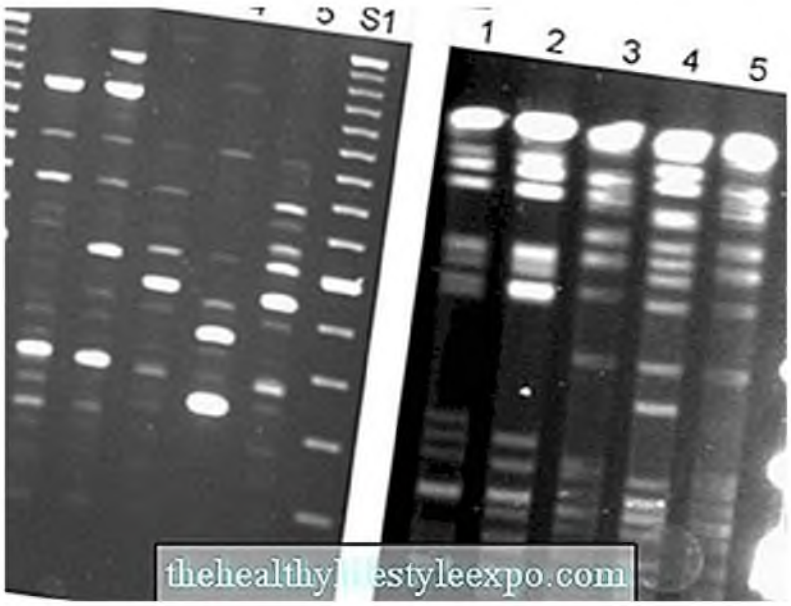

Figure 68 - The results of the polymerase chain reaction

In recent years, the laboratory diagnosis of hepatitis B determines the presence of viral DNA in the patient's blood. Using which of the listed reactions is it established?

A.Polymerase Chain reaction

B.Indirect hemagglutination reaction

C.Complement fixation test

D.Enzyme-linked immunosorbent assay

E.Hemagglutination inhibition reaction

APPENDIX

Open electronic image resources are used in the training manual [Electronic resource]. – URL:

Figure 1. http://ep3.nuwm.edu.ua/8646/1/05-02-27.pdf

Figure 2. https://prom.ua/Fazovo-kontrastnye-mikroskopy.html

Figure 3. https://www.foramed.com.ua/uk/laboratorna-diagnostika /mikroskopi/mikroskop-lyuminescentniy-micromed-xs-8530.html

Figure 4. https://present5.com/metodi-molekulyarno%D1%97-biologi%D1%97-mikroskopiya-interferencijna-mikroskopiya/

Figure 5. https://studopedia.org/4-41875.html

Figure 6. https://poznayka.org/s97768t1.html

Figure 7. https://ru.wikipedia.org/wiki/%D0%A1%D1%82%D1%80% D0%B5%D0%BF%D1%82%D0%BE%D0%BA%D0%BE%D0%BA%D0%BA%D 0%B8

Figure 8. https://uk.carolchanning.net/obrazovanie/89704-bacilla-eto-palochkovidnaya-bakteriya.html

Figure 9. https://www.corriere.it/salute/dizionario/vibrione/index.shtml

Figure 10. https://ua.depositphotos.com/stock-photos/%D1%81%D0% BF%D1%96%D1%80%D0%BE%D1%85%D0%B5%D1%82%D0%B8.html

Figure 11. https://uk.wikipedia.org/wiki/%D0%90%D0%BA%D1% 82%D0%B8%D0%BD%D0%BE%D0%B1%D0%B0%D0%BA%D1%82%D0%B5 %D1%80%D1%96%D1%97

Figure 12. http://www.d-learn.pu.if.ua/data/users/10128/%D0%A2%D 0%B5%D0%BC%D0%B0_1_%D0%9C%D0%BE%D1%80%D1%84%D0%BE%D 0%BB%D0%BE%D0%B3%D1%96%D1%8F.pdf.

Figure 13. https://svitppt.com.ua/biologiya/morfologiya-ta-ultrastruktura-bakteriy.html

Figure 14. https://slide-share.ru/mikrobiologicheskaya-kharakteristika-i-laboratornaya-diagnostika-stafilokokkovikh-i-9000

https://studopedia.org/13-112868.html
Figure 15. https://uk.coloring-image.com/11243-diplococci-in-smear-in-women-and-men
Figure 16. http://www.syntacon.spb.ru/CATALOG/HEMATOL/HCITO/CITO0067.HTM
Figure 17. https://ru.wikipedia.org/wiki/%D0%96%D0%B3%D1%83%D1%82%D0%B8%D0%BA
Figure 18. http://mediku.com.ua/metodichni-vkazivki-dlya-studentiv-specialenosti-laboratorna-d.html?page
Figure 19. https://en.ppt-online.org/366911
Figure 20. http://uchika.in.ua/metodichni-vkazivki-dlya-studentiv-specialenosti-laboratorna-d.html?page=5
Figure 21. http://www.atmph.org/viewimage.asp?img=AnnTropMedPublicHealth_2011_4_2_110_85763_f3.jpg
Figure 22. https://moezdorovia.com.ua/pozitivniy-analz-na-sifls-prichini-scho-oznachaye
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Figure 24. https://subject.com.ua/biology/medical/235.html
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Figure 27. https://lektsii.org/12-22168.html
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Figure 31. http://www.tarifi.info/imasstutz-%D0%A2%D0%BE%D0%
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Figure32. https://ukrhealth.net/lyamblii-chi-tak-voni-nebezpechni/
Figure33. http://www.afclinic.ru/pages/trihomonoz-trihomoniaz
Figure 34. https://studfile.net/preview/546622/page:44/
Figure 35. https://goicb.by/news/%D0%BA%D0%B0%D0%BD%
D0%B4%D0%B8%D0%B4%D0%BE%D0%B7.html
Figure 36. https://www.kelechek.ru/detskie_infekcionnye_bolezni/
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Figure 47. https://medlec.org/lek-63317.html
Figure 48. https://studfile.net/preview/5602954/page:39/
Figure 49.https://studfile.net/preview/5602954/page:40/
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Figure 53. https://docplayer.net/41850033-V-p-polishchuk-i-g-budzanivska-t-p-shevchenko-posibnik-z-praktichnih-zanyat-do-kursu-zagalna-virusologiya.html
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Підписано до друку 20. 12. 2022 р. Формат 60x84/16. Папір офсетний.
Друк офсетний. Ум. друк. арк. 9,66
Тираж 50 пр. Зам. № 3.
Редакційно-видавничий відділ
закладу вищої освіти Полтавського державного медичного університету
36011, м. Полтава, вул. Шевченка, 23