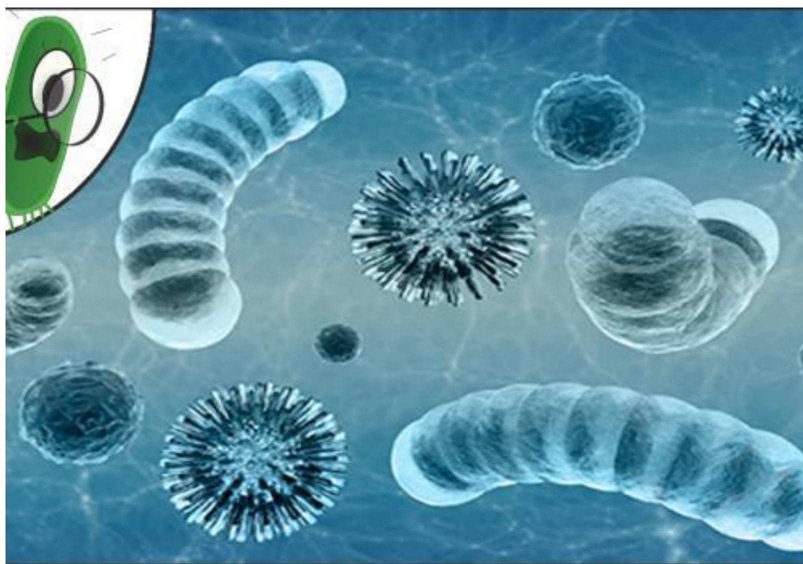

SANITARY MICROBIOLOGY

Learning guide for the 2nd and 3rd year English media students of the Faculty of Medicine and the Faculty of Dentistry (Microbiology, virology and immunology)

САНІТАРНА МІКРОБІОЛОГІЯ

Методичні вказівки з дисципліни «Мікробіологія, вірусологія та імунологія» для студентів ІІ і ІІІ курсів медичного та стоматологічного факультетів з англійською мовою викладання

МІНІСТЕРСТВО ОХОРОНИ ЗДОРОВ’Я УКРАЇНИ
Харківський національний медичний університет

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Compilers N. I. Kovalenko,
T.M. Zamaziy

Learning guide is related to the program of Ministry of Health of Ukraine and is recommended to students of the Faculty of Medicine and the Faculty of Dentistry of high medical schools of III-IV level accreditation.

Learning guide includes sections of medical microbiology that studies the microflora of the environment and its impact on human health and the state of the environment. Characteristics of the main groups of sanitary indicative microorganisms of environment, food and medical products as well as the most modern information on methods of sanitary and microbiological research are represented.

Санітарна мікробіологія : метод. вказ. з дисципліни "Мікробіологія, вірусологія та імунологія" для студентів II–III курсів медичного та стоматологічного факультетів з англійською мовою викладання / упоряд. Н. І. Коваленко, Т. М. Замазій. – Харків : ХНМУ, 2021. – 48 с.

Упорядники Н. І.Коваленко
Т. М. Замазій

Theme: Sanitary microbiology

Relevance of the theme

Germs spread throughout the environment. They are found in soil, air, water, in plants, animals, food, various vessels, in the human body and on the surface of the human body. The relationship of microorganisms with the environment is called ecology (from Grek *Oikos* home, home country, logo idea, science). The study of the ecology of microorganisms is the basis for understanding parasitism, infectious diseases, and for developing measures to combat them.

In the biosphere of the Earth there are practically no environments devoid of microorganisms. Their vital activity makes a significant contribution to the cycle of the basic vital elements - carbon, nitrogen, sulfur, phosphorus, etc., and also maintains a dynamic equilibrium in the biosphere.

Sanitary inspection of environment, data analysis and interpretation are the most important elements that characterize microbiological safety. It allows detection of sources of infection, and the data of microbiological quality of water, air, medicines, food, soil, etc., confirm the presence of danger.

Increasingly, hospital-acquired infections are caused by pathogenic and opportunistic microorganisms. All of this requires the development of new and improvement of existing methods of laboratory study of microflora of water, air, soil, other objects of environment, food. It is very important to determine human dysbacteriosis, the carrier of *Staphylococcus aureus*, meningococci, causative agents of diphtheria, cholera, typhoid, dysentery. The doctor of any profile must know how to properly take the test material, to deliver it to the profile laboratory, to conduct microbiological study, to evaluate its results correctly.

Goals:

– general: master the methods of microbiological study of environmental objects;

– specific:

a) to know:

1. safety rules when working with biological material (State Sanitary Rules).

b) be able to:

1. Prepare a workplace.
2. Observe the rules of operation and safety with infected material, cultures of microorganisms, equipment.
3. Select and transport research material.
4. Prepare material for microbiological research.
5. Investigate the material using methods designed to detect sanitary and pathogenic microorganisms.
6. Evaluate the results of the study and complete relevant laboratory documentation.

Equipment: slides, immersion microscope, biological preparations, tables, atlas

SUBJECT MATTER AND TASKS OF SANITARY MICROBIOLOGY

Sanitary Microbiology (SM) is a section of medical microbiology that studies the microflora of the environment and its impact on human health and the state of the environment. It should be borne in mind that humans and warm-blooded animals are the main reservoir of pathogens of most infectious diseases and the overwhelming number of pathogens are transmitted through air-droplet and fecal-oral mechanisms.

The objects of study of sanitary microbiology are:

• potentially pathogenic and sanitary microorganisms of the environment,

• physical, chemical and biological factors of the environment that promote or interfere with the existence of these groups of microorganisms in the environment and their penetration into the human body.

Sanitary and microbiological control of environmental objects is the most important section of state sanitary control. Its main purpose is the prevention of infectious diseases of man, since various objects of the external environment - water, soil, air, inventory, clothing, etc. contaminated with pathogens can contribute to the transmission of pathogens from patients to a healthy person.

The objectives of the SM:

1. early detection of pathogenic microflora in the external environment;
2. study of the activity of microorganisms in the external environment;
3. study of biocenoses in which there are microorganisms that are pathogenic to humans, patterns of circulation of microorganisms potentially harmful to humans between populations of humans, animals, and environmental objects;
4. assessment of ways of human and animal impact on the environment. Contamination of environmental objects by pathogenic microorganisms occurs as a result of industrial and individual activity of people. Special attention is paid to the study of violations of the processes of self-purification of water, soil;
5. development of methods of microbiological studies of environmental objects and microbiological standards, which determine the conformity of the qualitative and quantitative composition of the microflora of specific environmental objects with hygienic requirements;
6. making recommendations to improve the environment through sanitation and assess their effectiveness.

PRINCIPLES OF THE SANITARY AND MICROBIOLOGICAL INVESTIGATION

1. Proper sampling. It is carried out in compliance with all the necessary conditions, regulated for each investigated object. Sampling is carried out in accordance with the rules of sterility; optimal conditions must be created during transportation to avoid distortion of results. Research needs to be done quickly; if it is impossible to carry out the analysis immediately, the material is stored in the refrigerator for no longer than 6-8 hours.

2. The sequence of analyzes. Most of the studied objects contain a variety of microorganisms, distributed extremely unevenly and permanently in antagonistic relationships. Accordingly, in order to obtain adequate results, a series of samples are collected from different sites of the object. In the laboratory, the samples are mixed then accurately measured the required amount of material (usually average relative to the material under study as a whole).

3. Repeat sampling. As a rule, in the studied objects the composition of the microflora changes quite quickly, in addition, pathogenic microorganisms are distributed unevenly in them. Accordingly, re-selection allows for more adequate information on substrate contamination.

4. Apply only standard and unified research methods. The use of standards-approved approaches and guidelines, which enables to obtain comparative results across laboratories.

5. Use of a set of tests. More adequate information can be obtained when direct (which will detect pathogens) and indirect (indicate contamination of environmental objects by human and animal secretions and its extent) methods are involved.

6. Assessment of objects based on the totality of the obtained results. The interpretation of the results of sanitary and microbiological studies should be made taking into account other hygienic parameters (organoleptic, chemical, physical, etc.).

MAIN GROUPS OF SANITARY MICROORGANISMS

The objects of study of SM are, on the one hand, potentially pathogenic and sanitary microorganisms of the environment, on the other hand - physical, chemical and biological factors of the environment that contribute to or interfere with the existence of these groups of microorganisms in the environment and their penetration into the human body.

Sanitory indicative microorganisms (SIM) are microorganisms that can indirectly confirm the possible presence of pathogens in the external environment (Table 1). That is, when determining them, they proceed from the assumption that the more the object is contaminated with human and animal extracts, the more sanitory indicative microorganisms there and the more likely the presence of pathogens. Sanitary microorganisms include representatives of obligate microflora of humans and warm-blooded animals, for which the intestines or airways are site of location. All SIM are regarded as indicators of biological contamination.

Table 1. Sanitary indicative microorganisms of environment and food

Object	The nature of the pollution	Sanitory indicative microoranisms
Water	Fecal	Coliform bacteria: *Escherichia coli, Citrobacter spp., Enterobacter spp., Enterococcus faecalis*
Soil	Fecal Industrial (decomposable waste)	Coliform bacteria and clostridia (*Clostridium perfringens*) Thermophylic bacteria and *Proteus vulgaris*
Air	Respiratory droplets	*Staphylococcus aureus, Streptococcus pyogenes*
Food	Fecal Respiratory droplets	Coliform bacteria: *Escherichia coli, Citrobacter spp., Enterobacter spp., E.faecalis, P.vulgaris* *Staphylococcus aureus*
Houseware	Fecal Respiratory droplets	Coliform bacteria: *Escherichia coli, Citrobacter spp., Enterobacter spp., P.vulgaris* i *E.faecalis* *Staphylococcus aureus*

The main features of the SIM are:

1). The microorganism must constantly live in the cavities of humans and animals and is constantly excreted in large quantities with feces or droplets of mucus from the respiratory tract into the external environment.

2). The microbe must not reproduce in the external environment (excluding food), or its reproduction is of minor or short-lived nature.

3). SIM should be stored in the environment for the same time as pathogenic bacteria that parasitize the intestine and respiratory tract.

4). The resistance of SIM in the external environment should be similar to or greater than that of pathogenic microorganisms.

5). The microbe must not change its properties in the environment, in any case in terms of the survival of pathogens.

SIM are conditionally classified into 3 groups:

1. Group A contains residents of the intestine of humans and animals. These microorganisms are regarded as indicators of fecal polution. It includes so-called bacteria of the group of *Escherichia coli* (coliform bacteria), which belong to different genera of the family Enterobacteriaceae (*Escherichia, Salmonella, Proteus*), as well as enterococci, clostridia, thermophiles, bacteriophages, bacterioids, *Pseudomonas aeruginosa*.

Detection of E. coli in various environmental objects, food is considered to be the most reliable indicator of fresh faecal polution. The presence of bacteria of the genera Citrobacter and Enterobacter in the same objects indicates a relatively long fecal polution.

The presence of *C.perfringens, C.sporogenes* and other clostridia in the soil indicates its fecal contamination, both fresh and ancient, since these bacteria form spores, allowing them to survive for a long time in the environment (particularly in the soil).

Detection of *Enterococcus faecalis* in environmental objects also indicates their fresh faecal contamination.

Thermophilic bacteria include groups of different bacteria (*Lactobacillus lactis, Streptococcus thermophylus*, etc.), which reproduce at 60 °C and above. They are not permanent residents of the human intestine and do not serve as a criterion for fecal pollution. Sharp increase in the amount of thermophylic bacteria in the compost can indicate soil contamination by decomposing waste.

Bacteria of the genus *Proteus* belong to the family *Enterobacteriaceae* and their detection in food indicates a putrefactive process.

2. Group B includes residents of the upper respiratory tract and nasopharynx. Microorganisms are regarded as indicators of oral contamination. It consists of green, α- and β-streptococci, staphylococci (plasma-coagulating, lecithinase-positive, hemolytic).

The hemolytic *Streptococcus pyogenes*, being transit inhabitants of the nasopharynx and the pharynx, are excreted orally by droplets of mucus. There survival times in the environment are almost indistinguishable from those of most other pathogens. Detection of hemolytic streptococci in the air of the premises indicates its possible contamination by microorganisms contained in the upper respiratory tract of the person and that are agents of airborne infections.

S. aureus is an opportunistic resident of the nasopharynx, pharynx, and skin. Its presence in the air of the premises or on objects therein is an indication of pollution by oral droplets.

Simultaneous detection of *Staphylococcus aureus* and hemolytic streptococci indicates a high degree of air pollution.

3. Group C includes saprophytic microorganisms resident in the external environment. They are regarded as indicators of self-purification processes. It includes bacteria-proteolites, bacteria-ammonifiers and bacteria-nitrifiers, some spore-forming bacteria, fungi, actinomycetes, cellulose bacteria and blue-green algae.

METHODS OF SANITARY AND MICROBIOLOGICAL INVESTIGATION

Basic sanitary-microbiological methods are aimed at detection of total microbial contamination (total microbial number), detection and titration of SIM, detection of pathogenic microorganisms and their metabolites in the

investigated objects, detection of the degree of poor quality of the investigated objects or products caused by activity of microorganisms. *Practical sanitary microbiology uses two basic methods for assessing the sanitary-epidemic state of the environment: the direct detection of pathogenic microorganisms and the detection of indirect signs of pathogens in the external environment.*

Methods of direct detection of pathogenic microorganisms.

The methods used in sanitary microbiology can be divided into 2 groups: direct and indirect.

Direct methods involve the direct detection of infectious agents or their toxins in environmental objects.

The following methods can be used to identify pathogens:

• direct seeding of the test material on nutrient media;

• pre-concentration of pathogenic microorganisms by passing the test object (liquid consistency) through membrane filters or seeding into the accumulation media;

• detection of pathogens by the infection of susceptible animals (bioassay);

• application of express-methods: serological, immunoluminescent and radioimmunoassay.

Direct detection methods are the most accurate and reliable criteria for assessing the epidemiological risk of the environment. The most commonly carried out seeding of the test material on nutrient media. However, the expected results are influenced by the following factors:

– presence of pathogenic microorganisms in the external environment is relatively low, since they make up only 1/30000 of the entire species composition of the microbiota of the external environment. In this case, the pathogenic microflora is unevenly distributed in the external environment, which makes it necessary to conduct serial studies in the dynamics of a certain period.

– Isolation of one species of pathogen is not always indicative of the presence of other pathogenic species. This makes it necessary to conduct multidirectional research, which is practically difficult to carry out. The situation is significantly exacerbated by the growing role of opportunistic microorganisms capable of causing epidemic outbreaks of disease.

Despite the fact that methods for the direct, rapid and quantitative detection of potentially pathogenic microbes are currently being developed, this method has a number of disadvantages. These include:

• pathogenic microorganisms are not constantly in the environment, they can be relatively easily detected during an epidemic of a particular infection, but very difficult in an inter-epidemic period. The main activity of sanitary microbiologists is aimed at preventing epidemics and therefore all work is carried out in the inter-epidemic period;

• concentration of pathogenic microorganisms in the environment is significantly inferior to non-pathogenic and their spread in objects is uneven;

• when pathogenic microorganisms are isolated by cultivation methods on nutrient media, even inhibitory ones, they inevitably suffer from the competition of the saprophytic flora.

In view of the above, the negative results of the direct detection of pathogenic microorganisms in environmental objects do not yet confirm certainty their absence.

Indirect identification methods.

Detection of pathogenic microorganisms in objects of the external environment is an indicator of epidemiological danger. Since the direct detection of them is associated with a number of difficulties associated primarily with the low concentration of these microorganisms, in sanitary and microbiological practice indirect methods are used that based on the determination of microbial contamination of a particular object and the detection in it sanitary-indicative microorganisms.

There are 2 criteria that can be used to indirectly confirm the possible presence of the pathogen in the external environment: total microbial number and content of sanitary-indicative microorganisms.

Microbial insemination is confirmed by **the total microbial number** (TMN) - the total number of microorganisms contained in a unit of volume or mass of the test object (1 cm^3 of water, 1 g of soil, 1 m^3 of air). Using this criterion, it is usually assumed that the more the object is contaminated with organic matter, the higher the TMN and the more likely the presence of pathogens. For example, the TMN for water should not exceed 100. However, this is not always the case: the TMN may be greater at the expense of saprophytes and pathogens may be absent, or the predominance of saprophytes significantly shortens the survival time of pathogens in the external environment. Therefore, it is more appropriate to regard the TMN as an indicator of the intensity of environmental pollution by organic substances.

The content of SIM is determined by two methods.

The direct count of bacteria is carried out with the help of special cameras or special electronic counters. The method is used in urgent cases, if necessary, an urgent answer about the quantitative content of bacteria (for example, in accidents in the water supply system, in assessing the effectiveness of treatment facilities, etc.).

Seeding on nutrient media. A less accurate method, as it detects only groups of microorganisms that grow at a given regime (on certain nutrient media and at a certain temperature).

The content of the SIM is evaluated by two indicators - the titre and the index. The **SIM titre** is that minimum volume of material under study (in milliliters) or weighted quantity (in grams) in which at least one individual SIM can be found. **SIM Index** is the number of SIM individuals contained in a given volume (amount) of the test material (1 liter of fluid, 1 g of dense substance, 1 m^3 of air). Index is value, inverse of the titre; knowing one metric, you can calculate another.

SANITARY AND MICROBIOLOGICAL INVESTIGATION OF AIR

The main task of the sanitary-bacteriological study of air is hygienic and epidemiological assessment of the air environment, as well as the development of a set of measures aimed at preventing the aerogenic transmission of causative agents of infectious diseases. In assessing the sanitary condition of enclosed premises, depending on the objectives of the study the TMN, the presence of SIM (staphylococci, α- and β-hemolytic streptococci), which are indicators of contamination by the microflora of the nasopharynx, are detected.

The microbial polution of the air is non-permanent, ie the microflora of the air depends on the place and time of sampling. In summer, the air pollution is several times higher than in winter. Atmospheric air is espessialy saturated by microorganisms over large cities. When considering the qualitative composition of the microflora of air, it is necessary to distinguish between the microflora of atmospheric air and the air of enclosed premises. Atmospheric air and indoor air differ greatly in quantitative and qualitative composition of microflora. The bacterial contamination of enclosed premises always exceeds the pollution of the atmospheric air, including pathogenic microorganisms that get into the air from sick people, animals and bacterial carriers.

Microflora of atmospheric air. In the atmospheric air, SIM (staphylococci and streptococci) are detected only in 3.7 % of samples taken at large clusters of people. Microorganisms are dominated by species that inhabit the soil. Three groups of microorganisms are mainly found in atmospheric air:

– pigment-forming cocci on sunny days make up to 70–80 % of the whole flora (the pigment protects the bacteria from insolation).

– Soil spore-forming and putrefactive microorganisms. Their content increases dramatically in dry and windy weather.

– Mold and yeast. Their content increases with increasing humidity.

The microflora of air are conditionally divided into resident (detected more often) and temporary, less resistant to the influence of various factors (detected sporadically).

The constant microflora of air is formed by soil microorganisms. *Micrococcus roseus, M.flavus, M.candicans, Sarcina flava, S.alba, S.rosea, Bacillus subtilis, B.mycoides, B.mesentericus, Actinomyces, Penicillium, Aspergillus, Mucor* and others belong to it regularly.

Temporary microflora of air is also formed mainly due to soil microorganisms, as well as due to species coming from the surface of water. Microorganisms circulating in the atmospheric air are permanently exposed to the sun's rays, temperature fluctuations, changes in wind speed and direction, precipitation, etc. Therefore, the microflora of the air is very dynamic and continuously updating.

In contrast to indoor air, self-cleaning processes are constantly taking place in the ambient air. This process is due to precipitation, insolation, temperature influences and other factors. In turn, atmospheric air itself is a factor in the purification of indoor air.

The microflora of the indoor air is more uniform and relatively stable. Inhabitants of the human nasopharynx are dominated among microorganisms, including pathogens that enter the air when coughing, sneezing, or talking. The main source of air pollution by pathogenic species is bacterial carriers. The level of microbial contamination depends mainly on the density of the population, the activity of the movement of people, the sanitary condition of the room, including dust pollution, ventilation, the frequency of ventilation, the degree of light and other conditions. Self-cleaning of indoor air does not occur.

Staphylococci (*S. aureus*), as well as greenery and hemolytic streptococci are sanitary indicative microorganisms of indoor air pollution. Sources of contamination with pathogenic streptococci and staphylococci are sick people suffering from chronic infection and healthy human carriers. In the external environment, streptococci remain viable for about the same time as diphtheria pathogens, and staphylococci – even longer. The greater the number of streptococci is found in the air, the more likely the possibility of human infection with airborne infections. The increase of S. aureus in air pollution and its frequent detection testify to sanitary and epidemiological problems. In medical institutions bedding, linen, from which these microorganisms get into the air, can be a secondary source of air contamination with *S. aureus*. The most comprehensive analysis of air pollution by airborne droplets is obtained by the definition of both streptococci and staphylococci. However, given that it is difficult to cultivate streptococci, it is limited to the release of *S. aureus* in laboratory practice.

Circulation conditions of microorganisms in the air. Air contamination by pathogenic microorganisms occurs mainly by droplets in the composition of the aerosol formed during talking, coughing, sneezing. Less often germs get into the air with the epithelium of the skin, with the dust of contaminated bedding and contaminated soil. An aerosol is a colloidal system consisting of air, liquid droplets, or solids containing various microorganisms. The size of the aerosol particles may be different (from 10–100 to 2000 nm). Up to 40,000 drops can be formed when sneezing. Depending on the size of the droplets, their electric charge, the speed of movement in the air, there are three main phases of bacterial contamination.

The droplet or coarse-grained phase consists of bacterial cells surrounded by a water-salt shell. The particle diameter is about 0.1 mm or more. The particles settle fast enough: the duration of stay in the air is a few seconds, and the maximum speed of movement is an average of 30 cm/s.

The small-nucleus phase is formed during the drying of the particles and consists of bacterial cells that retain only chemically bound water on its surface and free water inside the cells. In this phase, the particles have the smallest size, are easily moved by air flows, and are in a suspended state for a long time. This is the most stable phase, since the diameter of most particles does not exceed 0.05 mm, and the settling velocity of the particles averages 0.013 cm/s. The speed of their movement exceeds 30 cm/s, so they can be scattered over long distances. This phase is the most epidemiologically dangerous, as it is composed of most causative agents of airborne infections, especially those resistant to external influences (eg, whooping cough).

The "bacterial dust" phase. From the first two phases, the bacteria can form larger particles, which settle in the form of dust on different objects, forming the so-called "bacterial dust". An important feature is the ability to easily disperse under the influence of even small airflows. The particle size varies from 0.01 to 1 mm. Depending on the particle size and the velocity of the air currents, the velocity of their movement is within 0.5–30 cm/s. Due to the long stay in the suspended state and the ability of the particles to penetrate into the distal lungs, fine "bacterial dust" also poses an epidemiological risk. This phase of bacterial aerosol prevails in indoor air and disperses pathogenic microorganisms that are resistant to drying (mycobacteria, clostridia, staphylococci, streptococci, fungi).

Sanitary indicative microorganisms of indoorair. The SIM of the air include *S. aureus*, streptococci, gram-negative bacteria, fungi. Sanitary and microbiological indicators of air are normalized depending on the type and purpose of the premises (*Table 2*).

Table 2. Acceptable levels of microbial air contamination in the premises of some inpatient units

Place of sampling	Sampling time	Results of the research (CFU/m^3)		
		The total number of microorganisms	1 m^3 of air	
			S. aureus	Gram negative bacteria
Operating unit	Before work	Not more 100	Absent	Absent
	After work	Not more 1000	Absent	Absent
Resuscitati on wards	Prepared for work	Not more 1000	Not more 4	Absent
Procedural room	Before work	Not more 50	Absent	Absent
	While working	Not more 1000	Not more 1–2	Not more 1–2

S. aureus and streptococci are dominant in the air in hospital rooms. Thus they should be absent in 1 m^3 of air of operating rooms, postoperative wards, dressing rooms, resuscitation departments, maternity rooms.

Due to the increasing frequency of diseases caused by gram-negative bacteria, the standard includes determining their amount in 1 m^3 of air in the premises of medical establishments.

A special case is the air of drugstores, where, due to the presence of antimicrobial drugs, bacteria can quickly die, but fungi are stored, so they must be detected in the study of drugstore air.

Additional criteria. The presence of spore-forming rods is estimated as an indicator of dustiness and lack of wet cleaning and molds are the indicator of humidity. The indicator of poor light is the absence of pigment-forming bacteria (sometimes this indicator can be determined by the task of phthisiologists).

Sanitary-bacteriological examination of air includes:

1) detection of total bacterial air contamination (total number of bacteria in 1 m^3);

2) detection of sanitary indicative microorganisms;

3) isolation of viruses and pathogenic bacteria from indoor air according to epidemic indications;

4) in the study of atmospheric air additional determination of the qualitative composition of the microflora, taking into account the presence of spore-forming aerobes and anaerobes, which serve as an indicator of air pollution by soil microorganisms.

In assessing the sanitary condition of closed premises, depending on the objectives of the study, the total microbial number, the presence of sanitary indicative microorganisms (staphylococci, α- and β-hemolytic streptococci) is determined, as well as pathogenic microorganisms (depending on the nature of the premises: *M. tuberculosis*, yeast and molds, etc.). For example, in the study of air in medical institutions the presence of microorganisms related to pathogenic flora (*P. aeruginosa*, *Proteus* spp. and other gram-negative rods) that cause nosocomial infections is determined.

Sanitary and microbiological study of air can be divided into 4 stages:

1) sampling;

2) processing, transportation, storage of samples, obtaining microorganism concentrate (if necessary);

3) cultivation of microorganisms;

4) identification of the isolated culture (detection of pathogenic and sanitary indicative microorganisms).

Proper sampling guarantees the accuracy of the study. Indoors, sampling points are set one sample air for every 20 m^2 of space, by the type of envelope: 4 points at the corners of the room (at a distance of 0.5 m from the walls) and point 5 – in the center. Air samples are collected at a height of 1.6–1.8 m from the floor at the level of breathing in closed permises. Samples should be taken during the day (during the period of active human activity), after wet cleaning

and ventilation of the premises. Atmospheric air is investigated in a residential area at 0.5–2 m from the ground near pollution sources, as well as in green areas (parks, gardens, etc.) to assess their impact on the air microflora.

It should be noted that in many cases, when sampling air it is seeded on a nutrient medium.

Methods of sampling air for bacteriological research are divided into:

1) aspiration, based on the active pumping of air with the help of various devices;

2) sedimentation, based on the principle of mechanical sedimentation of germs.

Methods of isolation of microorganisms from the air. There are two methods of sampling for bacteriological analysis of air. The simplest one that does not require special equipment is the Koch sedimentation method. The level of microbial conamination is detected by the number of colonies grown on the MPA in a Petri dish after keeping 2 open Petri dushes in air for 30 min, then the cultures are incubated in a thermostat at 37 °C and the species of microorganisms is identified. Most often the TMN (on MPA), staphylococcus content (on yolk-salt agar) are detected in the air. According to epidemic indications the content of streptococci is detected on blood agar. When inspecting air pharmacies, the content of fungi is detected using Saburoud medium. The results are estimated by the total number of colonies grown on both plates. While calm state of air on an area of 100 sm^2 as many microorganisms deposit as there are contained in 0.01 m^3 of air. This method is used as a presumptive.

The aspiration method using the Krotov apparatus is more perfect (*Fig. 1*).

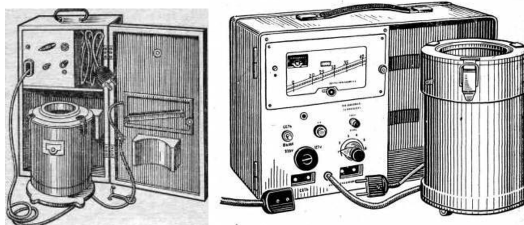

Fig. 1. Krotov device for the bacteriological study of air

It is based on the forced deposition of microorganisms from the air onto the surface of a solid nutrient medium or into a liquid (meat-peptone broth, buffer solution, isotonic solution of sodium chloride, etc.). The principle of operation of the device is based on the mechanical pumping of air through a wedge-shaped slit in the lid, which is located above the surface of the nutrient medium in a rotating Petri dish. In 1 min, 25 liters of air is pumped through the narrow gap of the plexiglass plate, the germs from which settle on the surface

of the nutrient medium in the Petri dish, and because of the constant rotation of the Petri dish under the inlet, microorganisms are uniformly fixed on all surfaces of the medium. MPA is used as a nutrient medium to determine microbial numbers and blood agar is used to determine SIM. The great advantage of this method is the ability to seed a certain volume of air.

After incubation of the culture in the thermostat, the number of colonies is calculated and the microbial number is calculated by the formula:

$$x = \frac{a \times 1000}{V},$$

where ***a*** is the number of colonies grown on the Petri dish;

V is the volume of air passed through the device, dm^3;

1000 – investigated air volume, dm^3.

The main indicators of sanitary air condition are as follows:

Total microbial number (TMN) of air is the number of colony forming units (CFU) in 1 m^3 of air (colonies that grow on the surface of nutrient agar when seeding a certain volume of air and incubation for 24 h at 37 °C and 24 h at room temperature are calculated).

Detection of staphylococci. Air sampling is performed using Krotov apparatus in the amount of 250 liters on 2–3 Petri dishes with yolk-salt agar and on Petri dishes with blood agar. The cultures are incubated at 37 °C for 48 hours. One studys the cultural characteristics of all kinds of colonies, smears are prepared and stained afer Gram.

One counts the number of staphylococci colonies and determines the number of germs in 1 m^3 of air.

In the case of nosocomial infections with staphylococcal etiology, studies are conducted to identify the sources and routes of spreading the infection: by phagotyping one determines the identity of staphylococci isolated from environmental objects, as well as from patients and staff.

Detection of streptococci. Air sampling in the study for the presence of α- and β-hemolytic streptococci is made using a Krotov apparatus on Petri dishes with blood agar. One takes 200–250 liters of air, the Petri dishes with cultures are held in a thermostat for 18–24 hours and then for another 48 hours at room temperature (after preview and accounting). The calculation of the number of colonies that have grown is carried out per 1 m^3 with subsequent control microscopy and selective seeding of colonies on blood agar or sugar broth.

Detection of pathogenic microorganisms. Given the low concentrations of pathogens in indoor air, their isolation is a difficult task. According to epidemiological indicators the presence of mycobacteria, viruses and others is determined in the air.

In the study of nosocomial infections, the presence of staphylococci, streptococci, *P. aeruginosa*, etc. is determined in the air. Sampling of air is

carried out in a volume not less than 1000 l. Seeding is done on appropriate selective media. If a liquid medium is used, the liquid test tube is placed in a thermostat per day for growing (to obtain a cumulative culture) and then one seeds on an selective medium.

In the study of air for the presence of *Mycobacterium tuberculosis* the medium of Levenstein-Jensen is used, *Corinebacterium diphtheria* – Clauberg agar.

Detection of fungi. One determines the presence of yeast and molds in the air – one counts the number of colonies grown on Saburoud medium for 96 h at 22–28 °C.

SANITARY AND MICROBIOLOGICAL INVESTIGATION OF WATER

Water is a natural habitat for a variety of microorganisms. In the water of rivers, open reservoirs, seas, oceans one finds representatives of all taxonomic groups of bacteria, as well as fungi, algae and protozoa. The aggregate of all microorganisms that inhabit the reservoirs is referred to as "microbial plankton".

According to the current normative documents, to sanitary and microbiological supervision there are subject:

– drinking water (centralized and local water supply, ie from water supply, springs and wells);

– water of swimming pools;

– water of open reservoirs (rivers, reservoirs);

– sewage (domestic-fecal, industrial, melt and drain);

- water purified for preparation of medicines;

– medical and commersial ice;

Current sanitary supervision is carried out:

– when assessing the quality of drinking water supply in populated areas;

– when assessing the sanitary status of surface waters to determine the degree of influence of biological contamination on the ability of water to self-purification;

– when controlling wastewater disposal;

– according to epidemic indications to find out a possible way of transmission of infectious diseases (one carries out the detection of SIMs and pathogens).

Autochthonous and allochthonous microflora of open reservoirs. The microflora of reservoirs are formed by two groups of microorganisms: autochthonous (constantly living and breeding in the reservoir) and allochthonous (falling from the outside when contaminated by different sources).

As a rule, the microbial composition of water is reminiscent of the soil microflora from which water flows. That is, most aquatic microorganisms are also common soil dwellers. Microorganisms that have adapted to the conditions of existence in water and are regularly detected in it can be considered as a water-specific flora. These include aerobic cocci: micrococci, sarcines, *Serratia marcescens, Bacillus cereus, Bacillus mycoides*, bacteria of the genus *Pseudomonas, Proteus, Leptospira*. There are few anaerobic bacteria in

uncomplicated waters; most often in them clostridia appear. The number of microorganisms in open reservoirs varies widely: from several tens, hundreds to millions in 1 ml, depending on the type of reservoir, the degree of its contamination, seasons, etc. The microbiota of reservoirs depends on the substances contained in them and on the biocenosis, ie on the species composition and abundance of other living creatures. So, phages and protozoa found in water kill bacteria. Microorganisms capable of producing antibiotics cause the death of other bacteria sensitive to these substances.

Water microorganisms play a significant role in the circulation of substances in nature. They break down organic substances to form substrates that are used in nutrition by other aquatic microorganisms. Biological activity in reservoirs is maximum in summer and autumn.

Surface waters (rivers, streams, lakes, reservoirs) are opened to all kinds of contamination. Contamination with microorganisms in contact with sewage, storm, meltwater, dramatically changes the microbial landscape and sanitary regime of the reservoir. The main way of microbial polution of reservoirs is the entry of untreated urban waste and wastewater. The number of microorganisms in the water of surface run off in the spring and flood period increases to 2.8–3 million in 1 ml. Secondary contamination of the water supply network may occur during the flood. The microflora of economic-fecal wastewater consists of microorganisms released from the intestines of humans and animals, among which there are representatives of normal and opportunistic flora (*Escherichia*, enterococci, klebsiella, clostridia, fungi of the genus *Candida*, protozoa, etc.) and pathogenic – causative agants of intestinal infections (salmonella, shigella, vibrios, yersinia, leptospira, polioviruses, hepatitis viruses A, E, etc.). The risk of contamination by the latter is especially great if insufficiently disinfected wastewaters of infectious hospitals enter the reservoirs.

Self-purification of open reservoirs. Water is not an environment conducive to the propagation of pathogenic microbes for which natural biotopes are human and animal bodies. The viability of pathogenic bacteria is affected by the accompanying competitive flora (microbes-antagonists, phages, protozoa, algae), as well as temperature, insolation, various chemicals, etc. Self-purification of open reservoirs from contaminating microorganisms is observed after organic contamination. The main factor of purification is the competitive activation of saprophytic autochthonous microflora, which leads to rapid decomposition of organic matter and to decrease in bacterial numbers. The ability of the reservoir to self-purification is related to the presence of autochthonous microorganisms belonging to a specific biocenosis. However, quantitative and qualitative ratios in biocenoses are unstable and vary under the influence of various factors, that is, they change in saprobability. The term "saprobity" (from the Greek. *Sapros*, rotten) is refered to a complex of features of the reservoir, including the composition and number of microorganisms in

water containing organic and inorganic substances in certain concentrations. The processes of self-purification of water in reservoirs occur sequentially and continuously, with a gradual change in biocenoses. Polysaprobic, mesosaprobic and oligosaprobic zones are distinguished.

Polysaprobic zones (zones of high contamination) contain a large amount of easily decomposable organic substances and are almost completely devoid of oxygen. The microbial biocenosis of such zones is especially abundant, but the species composition is limited by anaerobic bacteria, fungi, actinomycetes. The number of bacteria in 1 ml of water in the polysaprobic zone reaches one million or more.

Mesosaprobic zones (zones of moderate pollution) are characterized by the dominance of oxidation and nitrification processes. The qualitative composition is diverse. It is mainly nitrifying, obligate aerobic bacteria, as well as species of *Clostridium, Pseudomonas, Mycobacterium, Streptomyces, Candida* and others. The total number of microorganisms is hundreds thousands per 1 ml.

Oligosaprobic zones (zones of pure water) are characterized by a complete process of self-purification, low content of organic compounds and completion of the process of mineralization. Water has a high degree of purity. The number of bacteria is from 10 to 1 000 per 1 ml of water.

Pathogenic microorganisms that enter the reservoirs are abundant in polysaprobic zones, gradually die off in mesosaprobic zones and are virtually undetectable in oligosaprobic zones.

When determining the water contamination, a coli-titer and a coli-index are calculated. The coli-titer of water is measured by the minimum amount of water (ml) in which the coliform bacteria are detected, the coli-index by the amount of coliform bacteria contained in 1 liter of test water. These parameters are determined by ***the titration (fermentation) method or the method of membrane filters***. In the titration method, the isolation and identification of bacteria are performed in nutrient media. In the method of membrane filters, the water is first filtered using a Bunsen flask with a Seitz funnel built into it with a membrane filter and by means of a vacuum pump, the water is passed through the filters. Filters are then placed on the surface of the nutrient medium in Petri dishes and after incubation in a thermostat number of colonies typical for coliform bacteria are counted and identified by using biochemical tests.

Sanitary and microbiological control of drinking water quality. Sanitary and microbiological study of drinking water consists of the determination of SIMs, the number of enterobacteria, spores of sulfite-reducing clostridia and coliphages.

Detection of TMN in drinking water quality assessment. ***The total microbial number*** is the number of mesophilic aerobic and facultative anaerobic bacteria in 1 cm^3 (1 ml) of water, which is determined in all types of water. The test water is added to 1 ml in two sterile Petri dishes and filled with molten and cooled nutrient agar (deep seeding method), incubated at 37^0C for

24 hours, then at room temperature for another 24 hours. The number of colonies on 2 Petri dishes (on the surface and at the depth of nutrient agar) and the arithmetic mean are calculated. To detect mold and yeast fungi, the test water is seeded on 0.5 ml of Saburoud medium and incubated at room temperature for 3–4 days. The number of colonies and the arithmetic mean is calculated. The result (TMN) is calculated by summing the mean of bacteria, yeast and molds and expressed in colony forming units (CFU/ml).

The TMN allows estimating the level of microbial polution of water, supplementing the faecal polution indicators, while also detecting contamination from other sources (eg industrial discharges). An unexpected increase in TMN (even within the standard), detected again, serves as a signal for finding the cause of contamination. Also, this indicator is indispensable for the urgent detection of massive microbial contamination of unknown origin in drinking water.

Detection of the amount of enterobacteria. The presence of enterobacteria (coliform bacteria) is also determined in all types of water. The term coliform bacteria includes bacteria of the *Enterobacteriaceae* family and thermotolerant coliform bacteria.

The bacteria of the Enterobacteriaceae family include gram-negative asporogenic rods that do not have oxidase activity and ferment lactose with acid and gas at 37 ° C for 24–48 h (or ferment glucose with acid and gas at 37 °C for 24 h). The detection of bacteria of the family *Enterobacteriaceae* in drinking water indicates a potential epidemic risk of water use. The detection of bacteria of the genus *Escherichia* in food, water, soil, on the equipment indicates fresh faecal polution, which is of great sanitary and epidemiological importance. The bacteria of the genus *Citrobacter* and *Enterobacter* are believed to be indicators of earlier (several weeks) fecal polution and therefore have less health value than bacteria of the genus *Escherichia*.

Thermotolerant coliform bacteria have the same characteristics but additionally ferment lactose with the formation of acid and gas at 44.5 °C in 24 hours. Thermotolerant coliform bacteria die quickly in the environment, so their detection indicates fresh faecal polution of water.

Method of membrane filtration is used to detect enterobacteria in drinking water. The test water (3 samples by 100 ml) is passed through 3 bacterial nitrocellulose filters, which are then placed on Endo medium and incubated at 37 °C for 24 h. One calculates the number of lactose-positive colonies grown on the filters that are identified as coliform bacteria. Smears are prepared and stained after Gram from 2–3 red colonies, then oxidase activity is determined. For this purpose, the filter with bacterial colonies grown on it is transferred with tweezers, without turning over, on a piece of filter paper moistened with dimethyl-n-phenyldiamine. In the presence of oxidase, the indicator turns the colony blue. 2–3 colonies that did not change the original color were seeded in a semi-liquid medium with a 0.5 % glucose solution. Cultures are incubated at

37 °C for 24 h. In the presence of gas formation, the number of red colonies on the filter is counted and the coli-index is determined.

Bacteria of the genus Enterococcus are normal inhabitants of the intestine, but they are released into the environment in smaller quantities than *E. coli*. Enterococci die more quickly in water and soil. As a rule, they do not reproduce in these sites, which allows to considere them as an indicator of fresh faecal polution.

The presence of enterococci is considered an additional indicator of fecal contamination of water and other objects. However, their isolation requires more complex preparation environment and they grow more slowly.

Bacteria of the genus Proteus are found in both the gut of humans and animals (*P. mirabilis*) and in rotting residues (*P. vulgaris*). The presence of *Proteus* in environmental objects indicates their contamination with degradable substrates and an extremely unfavorable sanitary condition.

Detection of spores of sulfide-reducing clostridia. Spores of sulphite-reducing clostridia are more resistant to decontamination and the effects of unfavorable environmental factors than other indicator bacteria. Based on this property, the indicator is recommended to evaluate the effectiveness of technological processes of water treatment. This indicator is of particular importance when evaluating primary chlorination, since it inactivates virtually all indicator bacteria. Detection of clostridia in water before entering the distribution network indicates insufficient purification and that the pathogen-resistant microorganisms are likely not killed during the purification.

To determine the clostridia, the investigated water is introduced into the molten and cooled Wilson-Blair medium. The medium contains thiosulphate (hyposulphite) and a colorless iron salt. As a result of germination of spores, reproduction of clostridia and their restoration of sulfite, iron sulfide is formed, which gives the medium a black color.

Detection of the number of coliphages. The presence of coliphages (bacteriophages that are parasitic on *E. coli*) is determined in the surface water sources and the drinking water prepared from it, as well as in wastewater. They are indicators of the efficiency of groundwater protection and drinking water treatment. The research is carried out by the Grace’s method of agar layers. For titration of the coliphages, the test water is mixed with the molten and cooled nutrient agar, which also contains indicator strain *E.coli*. The resulting mixture was poured into a second layer on a nutrient agar in a Petri dish and after hardening the medium was incubated at 37 °C for 24 hours. As a result, indicator culture forms a uniform continuous growth, and in the presence of coliphages in this "lawn" transparent plaques ("negative colonies" of bacteriophages) are formed. The result of the study is expressed in plaque-forming units (PFU/ml).

Drinking water standards: The number of microorganisms in 1 ml of water is not more than 50, the coli index is not more than 3 bacteria in 1 liter of water. Bacteria of the *Enterobacteriaceae* family and thermophilic bacteria should be absent in 300 ml of drinking water, spores of sulphite-reducing clostridia should be absent in 20 ml of water, and coliphages should be absent in 100 ml of water.

SANITARY-MICROBIOLOGICAL INVESTIGATION OF SOIL

Soil is the most rich in microorganisms biotope, characterized by the stability of the composition of the microflora. The formation of microbial soil biocenoses, including sanitary significance, their qualitative and quantitative composition is influenced by many factors.

• *Type of soil and degree of cultivation.* The higher it is, the greater the microbial contamination. Respectively, the initial "normal" TMN for different soils is different.

• *Physico-chemical properties of soil:* structure, aeration, humidity, water permeability, presence of free and bound oxygen.

• *Age, geographical location of soils.* In the direction from south to north the content of organic matter in it, and accordingly, of microorganisms decreases.

• *Climatic conditions and seasonality.* In the spring, anaerobic bacteria prevails in the soil, and spore-forming bacteria in the summer. By the end of the summer, the content of actinomycetes, which absorb organic matter that has not been disposed by bacteria, increases. The biological activity of all soil microorganisms increases in the fall and decreases markedly in the winter.

• *Depth of the soil layer.* In the soil thickness there are three main zones: A (0–10 cm), B (10–20 cm) and C (20–30 cm). On the surface and in zone A, there are low microorganisms due to the low humidity and microbicidal effects of direct sunlight. In the untreated soil of zone A, their content is greatest at a depth of 5–10 cm (ie in the area boundary with zone B). In the treated soil, there are particularly abundant microorganisms at the boundary of zones B and C. At a depth of 1 m, single microorganisms are isolated. Species released at depths of 4 m or more are considered not as soil but as having geological significance.

Groups of soil microorganisms. Soil microorganisms have a direct effect on their own formation and formation of soil, on the mineralization (decomposition) of organic residues and the formation of humus. Therefore, without understanding of the basic ecological, physiological, morphological groups of soil microflora it is impossible to objectively assess the sanitary condition of the soil, the activity of its processes of self-purification from pathogenic microorganisms. In carrying out sanitary and microbiological researches special attention is paid to physiological groups of soil microorganisms.

Physiological groups of soil microorganisms contain species that are involved in the cycle of nitrogen, carbon, sulfur and phosphorus. However, for a full-scale assessment of the sanitary condition of the soil and the processes of its self-purification, it is necessary to determine the presence not only of species involved in the circulation of substances, but also of individual groups of microorganisms that contribute to the rapid decomposition of organic substances: spore-forming bacteria (primarily bacilli), actinomycetes, fungi (especially penicillium and candida).

Groups of soil microorganisms pathogenic to humans (*table 3*). As a rule, pathogenic microorganisms do not survive in the soil long. However, some species are part of the soil biocenoses, becoming permanent residents. Such microorganisms are divided into three groups:

• Microorganisms for which soil serves as a natural biotope – causative gents of botulism, actinomycetes, pathogens of deep mycosis aspergilum, that produces mycotoxin.

• Microorganisms that get into the soil with secretions of humans, animals, and are stored there for a long time (years and decades) – *B. anthracis*, a causative agent of tetanus, and gas gangrene.

• Microorganisms that get into the soil with excretions of humans, animals, but remain in it for a relatively short time (weeks and months) – *E. coli* (up to 8 months), salmonella (up to a year at sub-zero temperature), shigella (up to 100 days), *V. cholera* (2 months).

Table 3. Pathogenic microorganisms found in soil

Microbes for which soil is a natural biotope	Microbs that enter the soil with secretions of humans and animals	
	They are stored for a long time	They are stored relatively long
Causative agents of subcutaneous mycosis, *C.botulinum*, *Actinomyces spp.*	*Clostridium tetani*, *Clostridium spp.*, that cause anaerobic infections, *Bacillus anthracis*	Species of *Salmonella, Shigella, Vibrio, Francisella, Brucella, Mycobacterium, Pseudomonas*, *Leptospira,* enteroviruses

Non-spore forming pathogenic bacteria due to lack of essential nutrients, as well as due to lethal activity of light, drying, antagonistic germs and phages do not live in the soil long time (from several days to several months). Usually soil is an unfavorable habitat for most pathogenic species of bacteria, rickettsia, viruses, fungi and protozoa. The survival time of some pathogenic bacteria is shown in Table 4. However, soil as a transmission factor for a number of infectious agents is a very complex substrate. So, for example, B. anthracis, after being in the soil, form spores that can remain viable for many years. Under favorable conditions (in dark brown soil and black soil), they undergo the entire cycle of development: in the summer months, the spores germinate into vegetative forms, after which this cycle repeats.

Table 4. Survival period of some pathogenic microorganisms in soil

Species of bacteria	Average survival period, weeks	Maximum survival period, months
Salmonella typhi	2–3	12
Shigella	1,5–5	9
Vibrio cholerae	1–2	4
Vibrio cholerae El Tor	4	6
Mycobacterium tuberculosis	13	7
Brucella	0,5–3	2
Yersnia pestis	0,5	1
Francisella tularensis	1,5	2,5

It is known that spores of clostridia that cause tetanus, anaerobic infections and botulism, and many soil microbes survive long in the soil. Soil is a habitat for various animals (rodents) that are carriers of plague, tularemia, hemorrhagic fever viruses, encephalitis, leishmaniasis, etc. Intestinal protozoa cysts (amoeba, balantidium, etc.) conduct a certain stage in the soil. Soil plays an important role in the transmission of helminth infestations (ascaris, nematode worms, etc.). Some fungi live in the soil. Once in the body, they cause fusariotoxicosis, aspergillosis, mucormycosis, penicilliosis, etc.

Given the epidemiological role that soil plays in the spread of certain infectious diseases of animals and humans, sanitary and epidemiological practice provides for measures to protect the soil from contamination and infection by pathogens.

Indicator microorganisms for assessment of sanitary condition of soil. Sanitary monitoring of the soil condition of settlements is important in the prevention of intestinal infections and other diseases. Therefore, the selection of indicator microorganisms is of particular importance for adequate soil assessment. SIMs indicating faecal contamination of soil include: coliform bacteria, *C. perfringens*, thermophilic bacteria and nitrifying bacteria (*Table 5*).

Table 5. Requirements for microbiological soil purity

Soil category	Coliform bacteria titer	Nitrifiers titer	Clostridia titer	Thermophilic bacteia index**
Clean	≥ 1,0	≥ 0,1	≥ 0, 01	10^{2}–10^{3}
Contaminated	0,9–0,01	0,01–0,001	0,009–0,0001	10^{3}–10^{5}
Very dirty	≤ 0,009	≤ 0,0009	≤ 0,00009	1×10^{5}–4×10^{6}

Note. * – is the smallest amount of material (g) in which these SIMs are still found; ** – number of SIMs per 1 g of the soil under study.

Of all enterobacteria *E. coli* is the longest stored in the soil, so the presence of other enterobacteria is confirmed by its content in the soil. Thermophilic bacteria enter the soil with manure that is overgrown or composted, so it is

advisable to detect them to determine the nature and duration of organic soil contamination. Fresh manure, wastewater usually contains a lot of coliform bacteria but few thermophilic bacteria. During the decomposition of organic matter, the number of thermophiles increases. The appearance of nitrifying bacteria (nitrifiers) indicates the development of a self-purification process, as they complete the decomposition cycle of nitrogen-containing compounds, converting ammonia into nitrogen. With fresh fecal polution there will be no nitrifiers, since there is no substrate for their development. During the life of microorganisms that decompose organic matter, ammonia is formed, which will lead to the development of nitrifiers.

Fresh faecal polution of soil is indicated by the detection of enterococci, high titers of coliform bacteria at low titers of nitrifiers and thermophiles, as well as the relatively high content of vegetative forms of *C.perfringens*.

Detection of enterococci always indicates fresh faecal polution, which would not be other indicators.

Detection of thermophilic bacteria helps to assess soil polution with manure, compost or wastewater and the stage of decomposition of their organic substrate.

The appearance of nitrifying bacteria indicates the development of self-purification processes. For a more complete evaluation of the process of self-purification groups of microorganisms that rapidly destroy the organic substrate (bacilli, actinomycetes, fungi) are also determined. The high content of all SIM indicates the completion of soil self-purification and its release from enterobacteria and organic matter.

Objectives of sanitary and microbiological soil research. Knowledge of the composition and importance of soil microflora for humans, their impact on different conditions are necessary for correct sanitary and microbiological assessment of soils (in terms of their epidemic risk). Sanitary and microbiological study of soil is governed by the instructions on preventive and current sanitary supervision.

The warning supervision is carried out:

a) during the planning, construction and reconstruction of newly populated areas and settlements;

b) when choosing sites for the construction of medical and preventive and pharmacy establishments, sanatoriums, children's institutions;

c) in solving the issues of water supply and sewerage of populated areas;

d) in the sanitary evaluation of beaches, places of collective rest, etc.

Current sanitary supervision is carried out:

a) when assessing the sanitary condition of the soil and its ability to self-purify after contamination (for example, the soil of kindergartens, hospitals, recreation areas are examined twice a year);

b) when controlling groundwater and biothermal waters, wastewater and waste disinfection methods (1-4 times a month);

c) according to epidemic indications to find out a possible way of transmission of infectious diseases.

Sanitary and microbiological study of the soil, depending on the purpose of the study provides a short and complete analysis.

Short sanitary-microbiological analysis is carried out during the current sanitary surveillance. It consists of the detection of TMN, titer of coliform bacteria, enterococci, *C.perfringens*, thermophilic bacteria, nitrifying bacteria. The obtained data indicate the presence and degree of fecal polution. It can also determine the state of the processes of soil self-purification from pathogenic enterobacteria and organic contamination.

Complete sanitary-microbiological analysis consists of the detection of all indicators of a short analysis, as well as the total number of saprophytes; TMN and percentage of spore-forming microorganisms; aerobic bacteria that destroy cellulose; actinomycetes and ammonifiers, major soil microbiocenosis groups and a number of additional studies (eg soil toxicity for microorganisms). According to epidemic indications, pathogenic microorganisms are detected during the research. Full analysis is carried out in the course of preventive sanitary supervision, initial inspection in the selection of the territory for the placement of individual objects etc.

Frequency of control. To control soil polution of kindergartens, health centers, recreation areas, the sampling is carried out at least twice a year - in spring and autumn. At other monitored sites, soil analysis is performed at regular intervals, but at least once a year. When studying the dynamics of soil self-purification in contaminated territories, samples are taken during the first month after pre-contamination – weekly, in the following months – once a month during the growing season until the active phase of self-purification is completed.

Sampling is carried out from a square area (not less than 5×5 m) of 5 points ("envelope method"). In aseptic conditions, samples are taken from a depth of 20–30 cm from each corner and center of the square to prepare a mixed sample. Sample volume is 1 kg.

Research methodology. TMN of the soil is determined by deep seeding (in solid medium) with 10-fold dilutions or by direct microscopy (after Perfilev). If a low degree of fecal polution is assumed coliform bacteria in the soil is determined by fermentation method or membrane filtration method (see above); at high degree – direct seeding of soil suspension (1 : 10) on Endo medium. An important criterion for the sanitary condition of the soil and its ability to self-purify is the soil perfringence titer (the minimum amount of soil in which *C.perfringens* is still determined). When fecal polution of soil, *Escherichia* disappear after 4–5 months and clostridia are detected in the titer of 0.01 g. Perfringence titer is determined by deep seeding on Wilson-Blair medium with 10-fold dilutions of soil suspension. The cultures are incubated at 43 °C for

24–48 h, after which the results on the formation of black colonies of *C. perfringens* in an agar stab are taken into account. Smears are made from the colonies, stained after Gram, examine in a microscope and calculate the perfringence-titer. The number of thermophilic bacteria is determined by deep seeding on solid media with incubation at 60 °C for 24 hours. The titer of nitrifying bacteria is determined by seeding from 10-fold dilutions of soil suspension in a liquid nutrient medium.

SANITARY AND MICROBIOLOGICAL INVESTIGATION OF FOOD PRODUCTS

Foodstuffs are the most complex objects in sanitary microbiology. This is due not only to the diversity and abundance of microflora in them, but also to the use of microorganisms in the production of many products and, unfortunately, the lack of complete methods of detection of germs. Pathogens of many infectious diseases can be transmitted through food products - typhoid and paratyphoid, salmonellosis, dysentery, escherichiosis, botulism, cholera, brucellosis, tuberculosis, anthrax, some rickettsioses (Qu-fever) and viral infections (poliomyelitis, vesicular stomatitis, ets). Food toxic infections caused by numerous opportunistic microorganisms occur after the consumption of contaminated food. Germination can take place at all stages of procurement, storage and preparation. Food products are usually impossible to completely free from the presence of microorganisms without the risk of changing their taste.

The presence of many different growth factors and vitamins in food contributes to the growth of microorganisms. This fact is the main difference between the study of food and other sanitary and microbiological investigationes, since neither in water or in the soil, nor more so in the air so rapid breeding of germs does not occur. The knoleedge of food microflora can give qualitative and quantitative study of its population. Specific and non-specific microflora are distinguished in food.

Specific microflora of foodstuffs are represented by "cultural" microorganisms that are used for the preparation of different products and are a necessary link in the technology of their preparation. Specific microorganisms are used in the preparation of all fermented milk products, bread, beer, wine, in pickling vegetables, etc. *Lactococcus lactis* (lactic acid streptococcus) are used in the preparation of kefir, sour milk, kumis, cheese, sour cream, butter. For the fermentation of kefir so-called kefir grains, consisting of casein, which contain associations of microorganisms (lactic acid streptococci, lactobacilli, and yeast) are used. Lactose-hydrolyzed cocci and rods supply the fungi with the acid they need for their vital activity, and fungi, by causing alcoholic fermentation, saturate the product with carbon dioxide, which gives the kefir a distinct taste. In the preparation of some fermented milk products *Lactobacillus bulgaricus* (Bulgarian rod) and *Lactobacillus acidophilus* (acidophilus rod) are used.

Non-specific microflora of foodstuffs contains microorganisms that accidentally get into foodstuffs from the environment. It consists of saprophytes, pathogenic and opportunistic microorganisms, as well as species that cause food spoilage. The presence of some saprophytes contributes to the development of biochemical processes that are natural for a food product, on which its quality and often its preservation as a result of antagonistic resistance to pathogenic bacteria that enter the products depend. The degree of contamination by foreign microflora depends on many factors: the correct preparation of the food product itself, its transportation, storage, technology of further processing and, at all stages, the observance of the sanitary regime.

The reproduction of certain microorganisms may cause deterioration of food which become unusable for consumption. In some cases, foods may be inoculated with salmonella and shigella, staphylococci, *E. coli*, *B. cereus*, *C. botulinum, C. perfringens*, and other microbes that cause foodborne toxicity and other human diseases.

Milk can be infected with *Mycobacterium bovis, Brucellae, Coxiella burnetti*, pathogenic streptococci and encephalitis virus from diseased animals. During transport or during bottling or processing, milk can be infected with salmonella and shigella, pathogenic streptococci and staphylococci, *C. diphtheriae, V. cholera.*

Meat may be infected by diseased animals or poultry, or during slaughter, cutting or improper storage and transportation of the carcass. *C. perfringens, B. cereus, E. coli, E. faecalis, Proteus* and other bacteria are usually found in meat. Meat and meat products, including minced meat, are most often contaminated during cooking when pathogenic microbes are found on the surface of the meat grinder, on the hands and on kitchen utensils (cutting board, etc.).

The flesh of fish is contaminated with a large number of microbial species found in water, scales and guts of fish, on the hands of persons involved in the processing of fishery products, and on various objects (knives, tables, boards used for cooking, fishing boat deck, etc.). The most dangerous microorganisms are *C. botulinum*, which produces exotoxin in canned fish and *Vibrio parahaemolytica*. When sanitary regimes are not adhered to, *S. typhi, S. flexneri*, and in some cases El Tor vibrios are detected in fish and oyster meat.

Vegetables and fruits can be contaminated with shigella and salmonella, *V. cholera* and microflora found in the soil and in the hands of the persons involved in their harvesting, packaging, transportation and those who sell them. Improperly canned vegetables (tomatoes, mushrooms, etc.) can sometimes cause botulism.

Different microflora, pathogenic species (salmonella, fungi, actinomycetes) often penetrate eggs; egg powder can be contaminated with staphylococci.

Sanitary and microbiological analysis of food quality has three goals.

1. Quality control of raw materials used in food production and assessment of sanitary and hygienic conditions of their production.

2. Control of food storage regimes and assessment of sanitary and hygienic conditions for their transportation and sale.

3. Control of epidemic safety of food.

The nature of microbial contamination is influenced by the physicochemical properties of the products. Most microorganisms survive poorly in products with very low and high pH values. They are especially abundant in products with liquid and semi-liquid consistency. In dense, especially dry or powdered products, the conditions for the multiplication of germs are difficult and they are located "nests" in them. Contamination of foodstuffs is influenced by some features of the technology of their production and storage.

Mechanical processing (production of minced meat, mashed potatoes, etc.) increases the likelihood of contamination and promotes the homogeneous spread of microorganisms throughout the product.

Chemical treatment (pickling) contributes to a sharp reduction in the number of microorganisms.

The growth of microorganisms is significantly influenced by the temperature regime of their production and storage. Temperature rise has a more adverse effect on germs than decrease, so high temperature action is widely used for food processing.

Hygienic standards for microbiological parameters include control of 4 groups of microorganisms:

• SIMs, which include mesophilic aerobic and facultative anaerobic microorganisms – MAFAM (give growth after incubation at 30 °C for 72 h with deep seeding method) and colifom bacteria.

• Opportunistic microorganisms include *E. coli, S.aureus, Bacillus cereus*, proteus and sulphite-reducing clostridia.

• Pathogenic microorganisms, primarily salmonella.

• Microorganisms that cause spoilage of products, first of all yeast and molds.

There are specific standards for different food raw materials and food groups. In the absence of a standard, hygienic requirements for the quality and safety of food raw materials and food are used. The regulation of microbiological quality and safety of food raw materials and foodstuffs for most groups of microorganisms is carried out on the alternative principle, that is, the mass of the product is established, in which it is not allowed the content of coliform bacteria, most opportunistic microorganisms, as well as pathogenic microorganisms. In other cases, the standard indicates the allowable amount of CFU in 1 g (ml) of product.

Feature of sanitary-microbiological control of foodstuffs is used not only of direct, but also indirect methods of detection of pathogenic microbes. Microorganisms and their toxins are isolated by direct methods. Indirect methods are used to determine sanitary hazard of food products by indirect characteristics, for example, by general microbial contamination and SIM content.

Commonly accepted sanitary and microbiological indicators of food quality are the total microbial number (TMN), the presence of SIM and some species of opportunistic bacteria.

TMN is the number of mesophilic aerobic and facultative anaerobic microorganisms in 1 g or 1 ml of product. Sanitary assessment of the indicator: the increased number of microorganisms in prepared foods indicates a violation of the technological mode of production of the product or conditions of its storage.

The classic SIMs that indicate contamination of the environment with feces are bacteria from the group of *Escherichia coli*. Three genera of the family *Enterobacteriaceae* are of sanitary importance: the genus *Escherichia*, the genus *Citrobacter* and the genus *Enterobacter*. Sanitary evaluation of the indicator: the presence of an increased amount of coliform bacteria in the finished product indicates poor sanitary conditions for processing and storage of the product.

Opportunistic microorganisms presenting a sanitary hazard when found in food are the golden coagulase-positive staphylococcus, bacteria of the genus *Proteus*, salmonella, and spore-forming anaerobic sulphite-reducing rods.

S.aureus is a danger because it is able to produce enterotoxin and cause food intoxication. Increased amounts of coagulase-positive staphylococcus in products, as a rule, indicate secondary contamination of the product through contact with contaminated equipment, personnel hands, and airborne droplets.

Bacteria of the genus *Proteus*, found in finished products, indicate contamination with decomposing substrates, as well as the risk of rotting processes.

The presence of salmonella in food is not allowed, as these microorganisms are able to cause not only toxic infections with massive reproduction in products, but also infectious diseases.

Spore-forming sulphite-reducing rods (genus *Clostridium*) are limited, as they can be intensively propagated in food in the presence of anaerobic conditions and at a concentration of 10^6 or more in 1 ml/g cause food poisoning.

Sampling. To determine the total number of microorganisms per unit of volume or mass of the product, a so-called average sample is examined. The average sample is the amount of tested material, taken as the sum of samples from different sites and that represents the average content of microorganisms in the material.

The sampling rules vary depending on the nature and consistency of the sample being tested, as well as the purpose of the analysis.

Liquid products (water, milk, sour cream, sauces) are mixed with a sterile spoon (or pipette), and then colected in a sterile container from 50 to 300 ml, depending on the type of product.

From the products of semi-liquid consistency (for example, ice cream) the top layer up to 2,5 cm thick is removed with a sterile spoon, mixed the product and selected for research 50 ml.

Samples of solid products (meat, fish, sausages, culinary products), taken from several places in the thickness of the product, are crushed, mixed and selected for the study of 15–20 g of the obtained mass. For sampling from the surface of products (sausages without shells, some prepared culinary products, etc.), methods of washing and scraping are used. The washing of a certain surface is carried out with a sterile moistened swab or cloth, limiting the area with a metal stencil. One removes a thin (about 1 cm) surface layer of the material under study with an area of 1 cm^2 and transfers it to sterile saline.

Bulk products are selected with a sterile spoon – about 50 g of product from different places. For the analysis of portioned, pre-packaged, animal and vegetable products, a small number of units are selected.

Samples are taken, strictly adhering to the conditions of sterility, and transported in a cooled state, analysis is begun no later than 2 hours from the time of sampling.

Research methodology. Before conducting the study, samples were homogenized and serial dilutions were prepared (1 : 10, 1 : 100, 1 : 1000, etc.).

Detection of total microbial contamination. TMN is determined for those products and dishes that should not contain specific microorganisms (fermented milk products, souring, etc. are not investigated). Methods of the study of liquid and solid products are the same as for water and soil, respectively, but the cultures are incubated at 30 °C for 72 hours under natural aeration. These conditions of cultivation allow the detection of mesophilic aerobic and facultative anaerobic microorganisms in the substrate. The essence of the method is to seed a well-defined volume of the test substance into a solid medium in Petri dishes and count the grown colonies.

The bacterial content of group of Escherichia coli is determined using liquid nutrient media (Kessler medium), called fermenters, as the detection of bacteria is based on their ability to digest carbohydrates to form gaseous products. A number of dilutions of the product is seeded into test tubes with Kessler medium with floats and kept in a thermostat at a temperature of 43 °C for 24 h, and then inspected. The fermentation titer is determined for gas production (bubble in the float). The fermentation titer is the smallest amount of product, expressed in g or ml, which showed signs of fermentation characteristic of coliform bacteria. A product is considered unpolluted if there is no gas in the Kessler medium. Detection of the titer requires *E. coli* identification. For this purpose, the tubes in which the gas is detected, are seeded on differential diagnostic media (Endo medium), followed by the identification of the culture by biochemical properties.

Detection of S. aureus. A certain amount of the test material is seeded for the accumulation of staphylococci in salt broth and incubated at 37 °C for 24 hours. In the presence of growth (turbidity of the medium) seeding is performed on yolk-salt agar to obtain isolated colonies, which are then

identified by cultural, morphological, biochemical (fermentation of glucose and mannitol) properties and the presence of plasma coagulase.

Detection of other opportunistic microorganisms is carried out by microbiological method using appropriate storage and differential diagnostic media.

Detection of Salmonella. The homogenized sample of the product (usually 25 g) is introduced into the storage medium (selenite broth) and incubated at 37 °C. Seeding on solid differentiated media (bismuth-sulfate agar) is carried out a day and suspicious cultures are identified by biochemical and other characteristics.

FOOD POISONS OF MICROBIAL ETIOLOGY

When pathogenic microorganisms enter foodstuffs and multiply in them, the conditions for the occurrence of food poisoning of bacterial nature appear. Reproduction of microorganisms and the accumulation of their toxins in the food product becomes possible as a result of violation of standards of their procurement, the process of manufacturing semi-finished products or finished products, with improper transportation, storage, exceeding the product sales time.

Food poisoning is an acute systemic disease that results from the ingestion of foods that are massively seeded with germs or contain substances that are poisonous to humans.

Distinctive features of food poisoning are:

• the massive ("explosive") nature of the disease;

• short incubation period (2–48 hours);

• acute onset and duration of disease (1–3 days);

• presence of "common" food in the history of patients;

• stopping the outbreak of disease after the causal product has been removed;

• absence of the so-called epidemic "tail" (a chain of sequential transmission from human to human);

• the similarity of the clinical picture in patients and the dependence of its severity on the amount of product used.

Food poisonings of a bacterial nature are divided into food toxicosis and microbial toxicosis.

Food toxic infections are acute intestinal diseases with infectious inflammation of bowel and general intoxication resulting from the consumption of products massively contaminatted by some bacteria.

Food toxic infections are caused by: enterobacteria (representatives of the genera *Proteus, Morganella, Citrobacter, Klebsiella, Hafnia, Yersinia*), vibrios (*Vibrio parahaemolyticus, Aeromonas hydrophila, Plesiomonas shigeloides*), streptococci (*Streptococcus pyogenes*), bacilli (*Bacillus cereus, Pseudomonas aeruginosa*), clostridia (*Clostridium perfringens*), etc. The pathogen multiplies rapidly in the small intestine, penetrates into the lymphoid apparatus, where there is its mass death, accompanied by the release of endotoxin. Endotoxin

causes damage to the intramural nerve apparatus of the intestine, small vessels and cells of the CNS, and bacteria – producers of toxin – the inflammatory process of the intestinal wall. The disease begins suddenly, with nausea, vomiting, diarrhea, abdominal pain. Local signs are often associated with common signs: headache, sometimes fever, falling cardiovascular activity, until collapse. Recovery is rapid, but weakness and excretion of the causative agent may be delayed.

Microbial toxicosis are acute diseases resulting from the consumption of products containing microbial toxins. Microbial toxicosis include botulism (*C. botulinum*) and toxicosis caused by staphylococcal enterotoxin (*S. aureus*). The latter is usually associated with the consumption of dairy and cream products, canned food, meat and vegetable salads, etc. Foods that contain enterotoxin do not alter their organoleptic properties. Warming it up does not save it from the toxin. Food poisoning caused by staphylococcal enterotoxin is manifested by vomiting, abdominal pain, and watery diarrhea within 2–6 hours after eating infected products. The pathogenesis of lesions is due to the ability of enterotoxin to induce excessive formation of interleukin-2 (with manifestations of common symptoms of intoxication and impaired intestinal smooth muscle).

Botulism is a serious toxinemic disease of humans from the group of enteral clostridioses. It results from ingestion of food containing the exotoxin of *C. botulinum*. Bacteria can produce exotoxins of 7 serovars: A, B, C1-2, D, E, F, G. The toxin has an extremely high toxicity to humans, heat resistance (10–15 min at 100 °C), acid reaction, digestive enzymes, it is absorbed through the intestinal wall into the lymph and blood in the unaltered state and causes prolonged toxinemia. The toxin binds to nerve cells and blocks impulse transmission through neuromuscular synapses. The clinic in the initial stage, to irreversible fixation of the toxin, is polymorphic. Specific signs are caused by a disorder of the activity of the nerve nuclei of the medulla oblongata.

All cases of food toxicosis and toxicoinfection are subject to mandatory investigation using bacteriological methods.

Bacteriological examination. Isolation, identification and counting of food poisoning agents in the study material is the main method of diagnosis. Appropriate nutrient media and tests are used to isolate and identify the bacteria. The amount of bacteria detected in the test material is expressed as CFU/g or CFU/ml. In addition to the fact of the detection of bacteria and the degree of contamination of the material, epidemiological markers (serovar, phagovar, etc.) are revealed in isolated cultures. Such markers help to identify identity of the cultures isolated from patients and from suspected foods. In some cases, the pathogenicity factors of pathogens are determined in isolated cultures or directly in the material under study. Different models of infection of laboratory animals (botulinum neurotoxin, staphylococcal enterotoxin, yersinia toxin, etc.), infection of tissue cultures ("vomiting" *B. cereus* toxin). In addition, immunological methods for the detection of toxins (gel precipitation,

immunodiffusion, PHAT, etc.), as well as gene indication of specific nucleotide sequences encoding toxin synthesis (PCR) are used for this purpose.

The main method of diagnosis of botulism is to determine the presence and type of toxin in the food (or raw material from which they are made), which are associated with the disease, in serum and plasma, in the vomit, gastric lavage, feces, urine. The products (20-30 g) are triturated in a mortar with 20-30 ml saline or gelatin-phosphate buffer. The product suspension as well as the feces, vomiting masses and other test material are centrifuged and supernatant is aspirated. The presence of the toxin in such material and serum and its type is established in the neutralization reaction in white mice. The bioassay technique is that each of the types of test material (1.4 ml) is mixed with 0.6 ml of polyvalent (A, B, E) diagnostic antitoxic serum, incubated at room temperature for 5 min, and then 0.7-1 ml of the mixture is injected intraperitonealy or intravenously in two white mice. The two control mice were injected with the same dose of material mixed with 0.6 ml of saline. There is another control - heated at 80–90 °C for 20 min the material injected into two mice. Each material is injected with a separate syringe. In the presence of toxin in material control animals die, as a rule, after 6-24 h (observation is up to 4 days) with the phenomena of muscle paralysis, decline of the abdominal wall muscles ("aspen waist"), respiratory failure. Mice that were treated with a mixture of material with serum survive. Similarly, the type of toxin is determined. In the presence of typical antitoxic erythrocyte diagnostics, the serotype of the toxin is determined by PHAT.

In the diagnosis of staphylococcal toxicoinfection, isolation and identification of staphylococci are performed using a bacteriological method on yolk-salt and blood agar, with subsequent determination of plasma-coagulase and lecithinase activity, ability to ferment mannitol under anaerobic conditions. To detect enterotoxin in a isolated staphylococcus culture, PHAT and biological sample – intravenous infection of cats with filtrate broth culture (2–3 ml/kg) are used. If toxins enter the bloodstream in cats, vomiting and diarrhea develop.

Diseases caused by some pathogenic bacteria (salmonella, shigella, yersinia, Escherichia, *V. cholera*, etc.) can occur by type of food poisoning. When isolating these bacteria from a food product and / or clinical (section) material instead of the diagnosis of food poisoning, a diagnosis of the corresponding infection is made.

It should be borne in mind that certain foods can be both a substrate for the propagation of food poisoning agents and a factor in the transmission of intestinal infections.

MICROFLORA OF MEDICINAL PRODUCTS

Sources of drug contamination. The modern drug arsenal has a large assortment of drugs. The technology of their production does not always guarantee complete microbial purity.

The main sources of contamination of drugs: raw materials, process water, production equipment, air of working premises, working personnel, packaging of finished products. Microbial contamination of the drug can cause disease in human, in addition, it violates its stability. Propagation of germs changes the consistency of ointment bases, the appearance of unpleasant odors, etc. In liquid forms, metabolites of microorganisms can alter its chemical composition and lead to the formation of toxic products. In solid dosage forms, the risk of microbial spoilage is minimal, as there are no conditions for the reproduction of germs.

Injectable and ophthalmic preparations should be sterile, for other drugs contamination is undesirable, but it is possible, since the regulation of their manufacture does not require strict sterility.

Microflora of medicinal herbs. Microbes living on medicinal plant materials may contain representatives of normal epiphytic and phytopathogenic microflora. The microbial contamination of herbal medicinal products depends on the initial contamination, but may increase during the stages of primary treatment, grinding, bringing to a standard state. The deterioration of raw materials occurs mainly at high humidity, which promotes the propagation of putrefactive microorganisms.

Epiphytic microflora (from the Greek. *Epi* – on + *phyton*, a plant) is represented by microorganisms living on the surface of plants. Epiphyte microorganisms do not cause harm to the plant, and in some cases compete with phytopathogenic microbes. The main representatives of the epiphytic microflora – *Erwinia herbicola, Pseudomonas fluorescens, Bacillus mesentericus.*

Phytopathogenic microflora. Various viruses, bacteria, fungi and viroids have the ability to cause plant diseases. Once inside the cell, the microorganisms disrupt the normal course of physiological processes, especially photosynthesis and respiration, thereby causing its death. Many plant diseases are caused by bacteria of the genus *Pseudomonas, Agrobacter, Acetobacter, Erwinia, Xanthomonas*. In violation of the rules of storage of raw materials and drugs microbes are not only persistently stored, but also develop, causing significant changes in medicinal raw materials. Signs of microbial spoilage of raw materials: discoloration and change of consistency, the appearance of an uncharacteristic odor, mold, rotting. The affected raw material is unsuitable because the content of active substances it reduced and it loses its pharmacological properties.

Microbial contamination of finished dosage forms. Bacteria of the family *Enterobacteriacea, P. aeruginosa, S. aureus, Enterococcus*, as well as

some types of spores, yeast and molds are most frequent detected in the drugs that caused the disease. In this regard, the World Health Organization (WHO) has introduced pharmacopoeial standards that limit the microbial contamination of non-sterile finished dosage forms. According to WHO and pharmacopoeia, no more than 1000 bacteria and 100 molds and yeasts should be present in 1 g (ml) of the oral preparation. Drugs that do not require sterility should not contain bacteria of the family *Enterobacteriaceae, P. aeruginosa, S. aureus.*

In the manufacture of medicinal products, its microbiological purity is ensured by observing the rules of aseptic – a set of measures aimed at preventing the entry of microorganisms in drugs.

Aseptic conditions are achieved through the use of distilled water, sterile utensils, tools, disinfection of the air of the premises with the help of bactericidal lamps, wet cleaning with disinfectants, and personal hygiene of employees.

In chemical and pharmaceutical industries, the quality of medicines, including microbial purity, is guaranteed by the observance of mandatory principles, rules and regulations, called Good Manufacturing Practice (GMP). The basic idea behind GMP is to provide an appropriate manufacturing and control system to ensure that quality medicines are obtained.

The algorithm ***"Detection of the total microbial number of water".***

The essence of the method is detection the total content of mesophilic aerobes and facultative anaerobes in 1 cm^3 water, capable of growing on a nutrient agar at a temperature of 37 °C for 24 h, forming colonies, visible when increasing in 2–5 times.

Detection of the total microbial number of water can be carried out by the method of serial tenfold dilutions with seeding on meat-peptone agar (MPA) and by the method of direct microscopic counting of microorganisms in the test water.

When determined by the first method, the seeding of water samples is carried out on nutrient media, followed by the calculation of the grown colonies.

The volume of water for seeding is chosen in such a way that 30 to 300 colonies have grown on the Petri dish. Tap water is inoculated in 1 ml volume, water of opened reservoirs in volumes 1; 0.1 and 0.01 ml. For seeding 0.1 cm^3 and more little volumes of water dilution of the analyzed water is used. For this purpose in a test tube with 9 cm^3 of sterile water 1 cm^3 of analyzed water is added. Blowing air thoroughly one mixes the contents of the tube by another sterile pipette, selects from it 1 cm^3 and transfers into a Petri dish that will correspond to the seeding of 0.1 cm^3 of analyzed water. If smaller volumes of water are needed, 1 cm^3 of the contents of the first tube is transferred by the same pipette to the next tube of 9 cm^3 of sterile water. The seeding 1 cm^3 from the second tube will correspond to the seeding 0.01 cm^3 of analyzed water, etc.

The paper caps are removed from water sample bottles, the caps are removed, the hole is flambered and the water is thoroughly mixed with gentle air purging through a sterile pipette.

Seeding each dilution is carried out in a deep way. To do this, use a sterile pipette to select 1 ml of water and make in 2 sterile Petri dishes, slightly opening the lid. After adding water to the Petri dishes, it is filled with 10–12 cm^3 of cooled nutrient agar. Water is quickly mixed with agar, gently tilting or rotating the cup across the table surface. After that, the Petri dishes are left on a horizontal surface until freezing. After solidification of the agar, the Petri dishes are placed in a thermostat and grown at 37 °C for 24 hours.

Water from open reservoirs is seeded in parallel on two series of Petri dishes, one of which is incubated at 37 °C for 24 h and the other at 20°22 °C for 48 h. At a temperature of 20 °C more saprophytes grow and they are the most active participants in the process of self-purification of the reservoir. In places of high pollution by sewage the numerical value of both groups of saprophytes is close, therefore the dynamics of the number of this indicator is considered as a sensitive indicator of pollution of reservoirs, especiallyby organic substances.

To detect molds and yeasts, 0.5 ml of the test water is seeded on Saburoud medium and incubated at room temperature for 3–4 days. The number of colonies and the arithmetic mean are calculated. The result (TMN) is calculated by summing the mean of bacteria, yeast and molds and expressed in CFU/ml.

Accounting results. Colonies grown both on the surface and in the depths of the agar were counted using a magnifying glass with a 2–5 fold magnification or a colony counting instrument. To do this, put the Petri dish upside down on a black background. Only those dilutions are evaluated, when they are seeded on a Petri dish, 30 to 300 colonies grow. When sowing 1 cm^3 of undiluted sample, any number of colonies are taken into account, but not exceeding 300. If the Petri dish with the highest dilution has grown more than 300 colonies and the analysis can not be repeated, then it is allowed to count the colonies using a plate with a grid and magnifying glass in strong lateral light . At least 20 squares with an area of 1 cm^2 each in different places of the Petri dish are calculated, then the average arithmetic number of colonies is calculated in 1 cm^2, the value of which is multiplied by the area of the Petry dish in cm^2, which is calculated by the formula: S = πr2.

The result of counting the colonies in each Petri dish is expressed in the number of bacteria per cm^3 of analyzed water, taking into account the volume that was seeded. For the final number of bacteria the arithmetic mean of counting on two parallel Petri dishes or different dilutions is estimated. The results are rounded up as follows: if the result is in the range of numbers from 1 to 100, then record the numbers that are obtained; if the result is in the range of numbers from 101 to 1000, then the result is rounded to 10; if the result is in the range from 1001 to 10000, then the result is rounded to 100, etc.

The number of colonies is taken into account, focusing on one Petri dish, in cases: if less than 20 colonies have grown on the other Petri dish when diluted; the sweeming growth of bacteria that spread to the entire surface of the Petri dish; with colonies over 300.

It is recommended to use the counting plate when calculating the number of colonies when sweeming growth is noted on both plates. At the same time, the squares are calculated on the free growth spots of the Petri dish.

A direct microscopic method for determining the total number of microorganisms is the concentration of bacteria on the membrane filters (if water is passed through them), followed by erythrosine staining and microscopy.

The direct method is convenient in that the result, ie the number of microorganisms in 1 ml of water, can be obtained in a few hours. Therefore, it is recommended to use the direct method if it is necessary to give a quick sanitary assessment of water: when assessing the process of natural self-cleaning of reservoirs, when evaluating the efficiency of the treatment facilities at all stages, etc. Membrane filters, Seitz's filter or a special Dolgov-Razumov apparatus are used for filtering water.

The membrane filter with stained microorganisms is dried and placed on a glass slide, pre-adding a drop of immersion oil on the glass and on the filter, which is covered with a thin cover glass. Immersion microscope is used, with mesh micrometer into the eyepiece, divided into small squares. Microorganisms are counted in 20 fields of view (in each field of view in 4 small squares arranged diagonally). The total bacterial count in 1 ml (X) is calculated by the formula:

$$X = S \times N \times 10^6 / (S1 \times V),$$

where S is the filter area of the device (mm^2);

10^6 is the conversion factor of square millimeters to square micrometers;

N is the average number of bacteria in one square;

$S1$ is the square area of an ocular micrometer (μm^2);

V is the volume of filtered water (ml).

The algorithm ***"Detection of coliform bacteria".***

The number of coliform bacteria is expressed as coli-titer or col-index. Coli-index of water is the minimum amount of coliform bacteria in 1 liter of water. Coli-titer is the minimum amount of water that contains 1 cell of coliform bacteria.

To determine these indicators, the method of membrane filters and titration (fermentation) method are used.

Investigation of water by the method of membrane filters. The method is based on filtration of the established volume of water through membrane filters (*Fig. 2*), cultivation of cultures on the differential-diagnostic media and subsequent identification of colonies by cultural and biochemical characteristics.

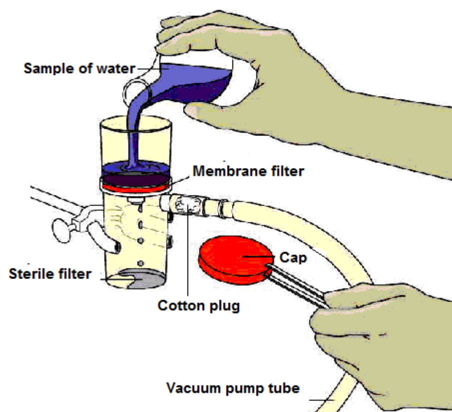

Fig. 2. Filtrating water with Seitz filter

Before using the membrane filters one checks for the absence of cracks, holes, bubbles and one boils in distilled water for 10 minutes (it is not allowed to twist filters). For complete removal of residual solvents manufacture from the filters used in their, boiling should be repeated 3-5 times with the change of distilled water. The prepared filters are stored in jars with distilled water or in dry form. On the day of the experiment, the filters are re-sterilized by boiling in distilled water for 10 minutes.

Filtering is performed using special devices or Seitz filter (*Fig. 3*). On the lower part of the filter apparatus (table) sterile membrane filter is put with a tweezers, pressed with the top of the device (glass, funnel) and secured with the device provided by the design of the device. The outlet of the flask into which the water is filtered is connected to a pump by a rubber tube to create a vacuum in the receiving vessel (about 0.25 atm).

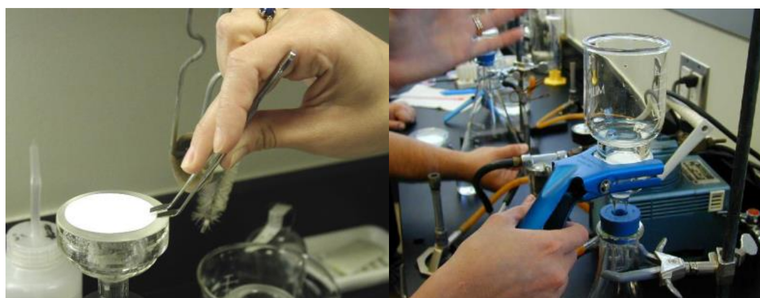

Fig. 3. Preparation of the filter apparatus

The volume of water sample depends on the purpose of the study. In the analysis of water entering the water supply network, and at its most characteristic points, it is necessary to analyze a volume of at least 333 cm^3, filtering this volume through at least two filters.

During the purification stages, at least two ten-fold volumes of water are analyzed, that are selected depending on its quality, so that no more than 30 colonies of colifrm bacteria grow on one of the filters. At the same time, it is necessary to be guided by the results of preliminary analyzes of water in the same points (for example, volumes of 10 and 100 cm^3 can be selected for water after primary chlorination). When analyzing water of unknown quality, 3–4 ten-fold volumes should be seeded (for example, 3; 30; 100; 200 cm^3 of water can be filtered from the water supply network; 0.1; 1; 10; 100 cm^3 of water during the purification stages).

To filter the required volumes of water are poured into a funnel or glass, starting with smaller ones and then large ones, each time changing the filters. The minimum volume of 1 ml of water should be filtered through a filter pre-moistened with sterile water. After filtration, the top of the instrument is removed and the filter (while maintaining the vacuum to remove excess moisture on the filter) is transferred with sterile tweezers to Endo medium in a Petri dish carefully. The filter is applied upward by the surface on which the bacteria have settled, avoiding the appearance of air bubbles between the filter and the medium. Four filters can be placed per one Petri dish.

If the water is turbid, then the filtration is performed through two filters: the previous filter No. 6 (to retain large particles) is placed on filter No. 2. After filtering, both filters are transferred to Endo medium. The Prtri dishes with filters are placed in a thermostat and incubated at 37 °C for 24 h. The final result takes into account the colonies grown on both filters.

When coliform bacteria are present, the growth of colonies typical of these bacteria appears on the filters: dark red with metallic glistening or red, pink with a red center, having a clear imprint on the back of the filter (*Fig. 4*). Bacteria from such colonies are stained by Gram and microscopy is performed. The oxidase test is performed with the culture of gram-negative bacteria from lactose-positive colonies for differentiation of bacteria of the family *Enterobacteriaceae* from *Pseudomonadaceae* (the latter are oxidase-forming bacteria). For this purpose, the filter with the colonies of bacteria is transferred with tweezers, without turning, on a piece of filter paper moistened with dimethyl-p-phenylenediamine. In the presence of oxidase, the reagent turns the colony blue. 2–3 colonies that did not change color (oxidase negative) are seeded in a semi-solid medium with 0,5 % solution of glucose and incubated at 37 °C. When acid and gas appear, the result is considered positive. The number of red colonies is counted on the filter and the coli- index is calculated.

The coli-index is calculated as follows: the number of coliform bacteria that grown in the volume of water is multiplied by 1000 cm^3 and divided by that volume of water.

$$index = \frac{K \times 1000}{V},$$

where K is the number of colonies of coliform bacteria on the filters;

V is the volume of filtered water through the filters on which the record is maintained.

Fig. 4. Colonies of *E. coli* on the filter

Example 1. When seeding three volumes of 100 cm^3 water, three colonies of coliform bacteria grew on one filter, there was not growth on the other two filters; the coli-index is (3 × 1000) : 300 = 10.

Example 2. When seeding 10 and 100 cm^3 of water, one colony grew on one filter, five colonies grew on the other; the coli-index is (6 × 1000) : 110 = 54.

If there is a continuous growth of bacteria on one of the filters and their calculation is not possible, then the volume of water is taken into account that is filtered out of which isolated colonies have grown on the filter.

To translate the index in the titer the formula is used:

1000 / Titre = Index

According to the standard, the drinking water index TMN should be no more than 3, the water of the swimming pool – no more than 10.

The membrane filter method is modern, more accurate, less time consuming and less expensive than the titration method. It is also convenient for concentrating bacteria contained in large volumes of water on a small filter surface. However, one of the most significant disadvantage of the method is that this method detects fewer bacteria than titration. For greater accuracy, it is recommended that study of the water should be performed in parallel by both methods.

The titration method of water testing is based on the accumulation of bacteria after seeding a set volume of water in a liquid nutrient medium, followed by transplanting to a differential diagnostic medium and identifying colonies by culture and biochemical tests.

The volume of seeded water depends on the nature of the object under study, but necessarily seeding is carried out in 2–3, and in some cases – in 5 repetitions. The volume of water is chosen in such a way that at least one negative result can be obtained in one of the dilutions. In the study at the stages of cleaning and disinfection 100; 10; 1 and 0.1 cm^3 of water are seeded; at the outlet of the water supply network and at its most characteristic points, three volumes of 100 cm^3, three volumes of 10 cm^3 and three volumes of 1 cm^3 are seeded.

Seeding water is carried out in the glucose peptone medium (1% peptone water, 0,5 % glucose solution, 0,5 % sodium chloride solution, Andrade indicator, float). For large volumes of water, a concentrated medium containing 10 times the amounts of these substances is used. So, seeding 100 ml of water is done in 10 ml of concentrated medium, 50 ml – in 15 ml of concentrated medium, 10 ml – in 1 ml of concentrated medium; 1 ml and subsequent dilutions in 10 ml of glucose peptone medium of normal concentration. Large volumes of water are seeded into vials or flasks, smaller ones into tubes. The cultures are incubated in a thermostat for 24 hours at 37 °C.

From the tubes with cultures in which there is turbidity (as well as the formation of acid and gas in the float), seeding is performed by streak plate methods on Endo agar, divided into 3-4 sectors. The cultures are maintained in a thermostat at 37 °C for 16-18 hours. In the presence of Endo agar specific colonies for coliform bacteria (red with metallic sheen), all the tests listed above should be performed. A positive result for the presence of coliform bacteria is given if the growth of characteristic colonies formed by oxidase-negative, Gr (-) bacteria that cause fermentation of glucose at 37 °C with the formation of acid and gas is observed. Thus, a positive result is given in 40–42 hours.

The result is expressed as an index (titer) of coliform bacteria, the digital expression of which is determined by the tables: in the analysis of drinking water at the outlet of the water supply network and from it – according to the *table 6*; in the analysis of water at the stages of purification and disinfection – according to the *table 7*.

Table 6. Detection of coli-index of the coliform bacteria in the study of 500 cm^3 of water

Number of positive results in the analysis of water from five bottles of 100 cm^3	Coli-index	Index boundaries (confidence intervals)		Coli-titer
		lower	upper	
0	Less then 2	0	6,0	More than 455
1	2	0,1	12,6	455
2	5	0,5	19,2	196
3	9	1,6	29,4	109
4	16	3,3	52,9	62
5	More then 16	8,0	–	Less then 62

Table 7. Detection of the coli- index of coliform bacteria in the study of water at the stages of purification

The volume of the investigated water, cm^3				Coli-index	Coli-titer
100	10	1,0	0,1		
–	–	–	–	Less then 9	More then 111
–	–	+	–	9	111
–	+	–	–	10	105

The algorithm ***"Detection of thermotolerant coliform bacteria (TCB)"***

TCB are determined by the same methods as coliform bacteria, except for the last identification step, which is carried out by fermentation of lactose on a semi-solid nutrient medium at 44.5 °C. In the case of growth on the Endo medium of typical lactose-positive colonies, Gr (-), oxidase-negative, capable of fermenting lactose at 44,5 °C, they are identified as TCB, index or titer is determined according to table 6.

Algorithm ***"Detection of coliphages".***

The presence of coliphages (bacteriophages that are parasitic on *E. coli*) is determined by the Gracia's agar layer method.

To determine bacteriophages sensitive museum cultures of microorganisms (test organisms) non-pathogenic to human are used: mutant *Salmonella tuphimurium*, strain *E. coli* K-12 Hfr from the corresponding collection of cultures ATCC 23631 or NTCT12486, strain *E. coli* of the genus CN, WG5, and bacteriophages MS2, NCTC12487 or ATSS 15597 to control the sensitivity of test organisms.

Seeding test culture E. coli K12 F+ on slant nutrient agar (MPA) should be made 18–24 hours before analysis. Before performing the analysis, wash with 5 ml of sterile tap water from the slant agar and prepare a suspension of the test organism at a concentration of 10^9 cells/ml according to the standard of turbidity.

Melt and cool to 45 °C 2 % nutrient agar. Add test water in an amount of 100 ml in 5 sterile Petri dishes (20 ml each). Add washed *E. coli* (1 ml per 100 ml agar) to the culture medium and mix well. First an empty Petri dish (control) is filled by the resulting mixture with 30 ml and then all Petri dishes containing the test water. The contents of the Petri dishes are mixed in rotary motion. After solidification of the culture medium, the Petri dishes are turned upside down and incubated in a thermostat at 37 °C for 18–24 h.

As a result, the indicator culture forms a uniform continuous growth, and in the presence of coliphages in this "lawn" transparent plaques ("negative colonies" of bacteriophage) are formed. The results are recorded by counting and summarizing the plaques grown on 5 Petri dishes. The results are expressed in plaque-forming units (PFU) per 100 ml of water. No plaques should be present in the control sample.

Algorithm ***"Detection of spores of sulfide-reducing clostridia".***

The test water is introduced into the molten and cooled Wilson-Blair medium. The medium contains thiosulphate (hyposulphite) and a colorless iron salt. As a result of germination of spores, clostridia reproduction and restoration of sulfite, iron sulfide is formed, which gives the medium a black color.

Quantitatively these microorganisms can be determined in water by membrane filtration method or direct seeding.

Before seeding, the water sample is heated in a water bath at 75 ± 5 °C for 15 min to destroy vegetative forms. In the study of chlorinated water, it can not be heated. Applying a membrane filtration method, a sample of water of a certain volume is passed through a filter, which is then placed in a tube with the prepared molten nutrient medium inward (the test tube with the nutrient medium after seeding must be immediately cooled in cold water to avoid entering air) or on Petri dish on the surface of the nutrient medium, which is then filled with the same nutrient medium in a thick layer.

The method of direct seeding involves seeding into sterile tubes 20 ml of water as follows: 10 ml in 2 tubes (not less than 30 ml) or 5 ml in 4 tubes (not less than 15 ml).

On top of sample of seeded water hot (75–80 °C) iron sulphite agar is poured in an amount that exceeded 2 times the volume of water. The medium is poured on the wall of the tube, trying to prevent the formation of air bubbles. The test tubes are quickly cooled in a cold water glass, incubated at 44 °C for 24 h.

Only seeding where isolated colonies are obtained are to be quantified. Black colonies grown both on the filter and in the culture medium are counted. The result of the analysis is expressed by the number of colony forming units (CFU) of the sulfide-reducing clostridia in a given volume of water (subjected to analysis).

Therminology. Sanitary indicative microorganisms, autochthonous and allochthonous microflora, saprobity, total microbial number, coli-titer, coli-index, sedimentation method, aspiration method, titration (fermentation) method, method of membrane filters.

Theoretical questions for control:

1. The subject, methods and objects of the study of sanitary microbiology.
2. What sanitary microorganisms are used to characterize environmental objects in sanitary and microbiological research? What are the requirements for them? What are their implications for the characterization of environmental objects?
3. What are the principles of sanitary and microbiological studies of environmental objects?
4. Water as a habitat for existence and preservation of microorganisms. Indigenous and allochthonous microflora of open reservoirs. What are saprophytes? What microorganisms are indicators of the process of water self-purification?
5. What is the sanitary and microbiological control of drinking water quality? What methods are used for sanitary and microbiological examination of water?
6. What sanitary microorganisms are used to evaluate drinking water quality? What are the requirements of the State Standard for drinking water?
7. Describe the soil microflora. What is the role of soil in the transmission of infectious diseases? What factors influence the survival of pathogens in soil?
8. What sanitary microorganisms are used in soil contamination assessment?
9. What methods are used for sanitary-microbiological soil research?
10. What is the role of air in the transmission of infectious diseases? Describe the microflora of the air.
11. What are the sanitary indicative microorganisms of indoor air, methods of their detection? What are the criteria for assessing indoor air cleanliness?
12. Give a classification of food poisoning and name the pathogens that cause them.
13. What are the methods of sanitary and microbiological research of food products?
14. What are the sanitary indicative microorganisms used in food quality assessment?
15. How are the sanitary and microbiological investigations of foodstuffs carried out in order to detect botulotoxin, pathogenic staphylococci and salmonella - agents of acute gastroenteritis?

Test tasks for control:

1. The quality of drinking water is carried out in the bacteriological laboratory. Its microbial count was near 100. Which of the following of microorganisms were taken into account?

A. All bacteria that have grown on a nutrient medium

B. Enteropathogenic bacteria and viruses

C. Bacteria pathogenic to humans and animals

D. Opportunistic bacteria

E. Coliform bacteria

2. When determining the microbial number of air in the hospital ward, it turned out that it was 1500 CFU/m^3. Which of the following groups of microorganisms were considered in this process?

A. All bacteria grown on a nutrient medium

B. Staphylococci and hemolytic streptococci

C. Bacteria and viruses – agents of respiratory infections

D. All pathogenic and opportunistic microorganisms

E. Pathogens of hospital infections

3. During sanitary and bacteriological examination of tap water the following results were obtained: total number of bacteria in 1 ml – 80, coli-index – 3. the result of the study can be interpreted in wivh of the following way:

A. Water suitable for consumption

B. Water of dubious quality

C. Water of very dubious quality

D. Water contaminated

E. Water is very polluted

4. During the sanitary and bacteriological study of water with the method of membrane filters, two red colonies were detected on the membrane filter (Endo medium), through which 500 ml of test water was passed. Which of the folowing are coli-index and the coli-titer of the test water.

A. 4 and 250

B. 2 and 500

C. 250 and 4

D. 500 and 2

E. 250 and 2

5. The hospital decided to carry out quality control of sterilization of tools in the autoclave using the biological method. Which of the following microorganisms should be used as test systems?

A. Spore-forming

B. Capsulated

C. Acid resistant

D. Pathogenic

E. Thermophiles

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Навчальне видання

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Упорядники Коваленко Наталія Іллівна
Замазій Тетяна Миколаївна

Відповідальний за випуск Н.І.Коваленко

Комп'ютерний набір Н. І. Коваленко
Комп'ютерна верстка О. Ю. Лавриненко

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Редакційно-видавничий відділ
ХНМУ, пр. Науки, 4, м. Харків, 61022
izdatknmurio@gmail.com

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