Ministry of Public Health of Ukraine

Bogomolets National Medical University

Department of microbiology, virology and immunology

SHYROBOKOV V.P., PONIATOVSKYI V.A., MELNYK V.V., RUSALOV V.L., DIUZHYKOVA O.M., NASTENKO V.B., YEHOROV D.P.

STUDY GUIDE

OF THE PRACTICAL CLASSES COURSE

Part V

Specialties:

221 "Dentistry"

222 "Medicine"

225 "Medical Psychology"

226 "Pharmacy, industrial pharmacy"

228 "Pediatrics"

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Authors:

SHYROBOKOV V.P., PONIATOVSKYI V.A., MELNYK V.V., RUSALOV V.L., DIUZHYKOVA O.M., NASTENKO V.B., YEHOROV D.P.

Edited by the Head of the Department of Microbiology, Virology and Immunology, Academician of the NAS and NAMS of Ukraine, Professor **V.P. Shyrobokov**.

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CONTENTS:

Topic: "Clinical microbiology. Microbiological diagnosis of nosocomial infections"

Topic relevance.

Nosocomial infections or healthcare associated infections occur in patients under medical care. These infections occur worldwide both in developed and developing countries. Nosocomial infections accounts for 7% in developed and 10% in developing countries. As these infections occur during hospital stay, they cause prolonged stay, disability, and economic burden. Frequently prevalent infections include central line-associated bloodstream infections, catheter-associated urinary tract infections, surgical site infections and ventilator-associated pneumonia. Nosocomial pathogens include bacteria, viruses and fungal parasites. According to WHO estimates, approximately 15% of all hospitalized patients suffer from these infections. During hospitalization, patient is exposed to pathogens through different sources environment, healthcare staff, and other infected patients. Transmission of these infections should be restricted for prevention. Hospital waste serves as potential source of pathogens and about 20%–25% of hospital waste is termed as hazardous. Nosocomial infections can be controlled by practicing infection control programs, keep check on antimicrobial use and its resistance, adopting antibiotic control policy. Efficient surveillance system can play its part at national and international level. Efforts are required by all stakeholders to prevent and control nosocomial infections.

The clinical microbiology laboratory is the primary site for the diagnosis of human infectious disease; it is often found in a hospital, although it may also be a stand-alone facility. The clinical microbiology lab also supports public health infrastructure, acting as a sentinel in the rapid detection of naturally occurring outbreaks of infection and bioterrorism. The major goals of the clinical microbiologist are (1) rapid and accurate identification of disease-causing microorganisms from clinical specimens, and (2) accurate antimicrobial susceptibility testing of those isolated organisms. Clinical microbiology is interdisciplinary, and the clinical microbiologist must have a working knowledge of microbial morphology, metabolism, growth, reproduction, biochemistry, and physiology. Additionally, the clinical microbiologist needs to understand the principles of immunology, molecular biology, genomics, aseptic technique, sterilization, disinfection, and the dynamics of host-parasite relationships. Importantly, tests developed to exploit (1) the antigen-antibody binding capabilities (the focus of clinical immunology) and (2) nucleic acid sequencing and amplification techniques (genomics) can often detect microorganisms in specimens by identifying microbial antigens and genes or gene products, respectively

Objectives:
- Identify the subject and tasks of clinical microbiology. To study the role of conditionally pathogenic microorganisms in human pathology, peculiarities of etiology, clinical picture and diagnosis of the diseases they cause.

- Familiarize yourself with the problem of dysbiosis and hospital infections. To study biological properties of hospital strains, ways of infection and detection of sources of hospital infections.
- To acquire methods of microbiological diagnostics of opportunistic infections and criteria for evaluation of the etiological role of conditionally pathogenic microorganisms in purulent-inflammatory and hospital infections.

Basic knowledge, skills, skills needed to study topics (interdisciplinary integration).

Names of previous disciplines	Skills
Human anatomy	To analyze information about the structure of body, constituents of his systems, organs and tissues.
Histology, cytology, embryology	To interpret the microscopic and submicroscopic structure of cells
Medical and biological physics	To interpret general physical and biophysical regularities, making the basis of biological processes.
Medical biology	To explain processes regularities at molecular-biological and cellular level
Medical chemistry	To interpret general physical and chemical regularities, that underlay the basis of cells' development processes

List of basic terms, parameters, descriptions that a student must master to prepare for the class:

Terms	Definition
Clinical microbiology	Clinical microbiology is a section of medical microbiology that studies the etiology, pathogenesis, immunology of non-epidemic microbial diseases and develops methods for their microbiological diagnosis, specific therapy and prevention. The objects of clinical microbiology research are mainly pathogenic microbes and the diseases they cause. Nowadays, the number of such opportunistic infections is increasing and they are found in patients of non-infectious clinics of different profiles. In addition, the tasks of clinical microbiology include such common issues as hospital or hospital infections, normal microflora, dysbiosis, sensitivity of the microbes to antibiotics, antiseptics and disinfectants, methods of clinical microbiological research.
Hospital-acquired infection (HAI)	Hospital-acquired infection (HAI) (nosocomial infection) is an infection that is contracted from the environment or staff of a healthcare facility. It can be spread in the hospital environment, nursing home environment, rehabilitation facility, clinic, or other clinical settings. Infection is spread to the susceptible patient in the

	clinical setting by a number of means. Health care staff can spread infection, in addition to contaminated equipment, bed linens, or air droplets. The infection can originate from the outside environment, another infected patient, staff that may be infected, or in some cases, the source of the infection cannot be determined. In some cases, the microorganism originates from the patient's own skin microbiota, becoming opportunistic after surgery or other procedures that compromise the protective skin barrier. Though the patient may have contracted the infection from their own skin, the infection is still considered nosocomial since it develops in the health care setting.
Hospital strains	Hospital strains are variants of microorganisms adapted to life in hospital hospitals (ecosystems). They are characterized by multiple antibiotic resistance, increased resistance to antiseptics, disinfectants, higher virulence, antagonistic activity, intra-population variability, more intensive exchange of genetic material and increased migration under medical conditions. The sources of the hospital strains are staff and patients, especially those who are in hospital for long periods of time. Compared to infections caused by susceptible strains, infections caused by antibiotic-resistant organisms are more likely to prolong hospitalization, to increase the risk of death, and to require treatment with more toxic or more expensive antibiotics.
Opportunistic pathogens	Opportunistic pathogens cause disease when the host's defenses are compromised or when they become established in a part of the body that is not natural to them.
Opportunistic infections	Opportunistic infections are defined as infection caused by non-pathogenic microorganisms which become pathogenic when the immune system is impaired by an unrelated disease.

Theoretical questions to studies.
- What are the issues of clinical microbiology?
- The microflora of the human body and its role in norm and pathology.
- Dysbacteriosis. Causes of development and principles of diagnosis of dysbiosis.
- Biological properties of opportunistic microorganisms. The epidemiology, pathogenesis and microbiological diagnosis of opportunistic infections.
- Hospital infections. Properties of hospital bacterial strains. Etiology of iatrogenic infections. Identification of sources of infections.
- Methods for microbiological diagnosis of opportunistic infections and criteria for evaluating the results of the diagnosis of purulent-inflammatory and hospital-acquired infections.

Students' practical activities:
- To create scheme of diagnosis the pyoinflammatory and sepsis process, caused by opportunistic microorganisms.

- To start a diagnosis of pyoinflammatory process, caused by opportunistic microorganisms.
- To determine the sensitivity to antibiotics of pathogens of purulent-inflammatory processes.

Topic content:

On the class, students study the role of different species of microorganism in hospital infections; they start a diagnosis of pyoinflammatory process, caused by opportunistic microorganisms; create the scheme of diagnosis the purulent-inflammatory and sepsis process, caused by opportunistic microorganisms. Completed tasks students write in the protocol and sign it with a teacher.

Recommendations for design of the protocol

Factors which predispose patients to hospital infection

Age	Aged patients are paticularly susceptibility
Specific immunity	Patient may lack protective antibodies to e.g. measles, chicken-pox, whooping cough
Underlining disease	Other (noninfectious) diseases tend to lead to enhanced susceptibility to susceptibility, e.g. hepatic disease, diabetes, cancer, skin disorders, renal failure, neutropenia(either as a result of disease or treatment)
Other infections	HIV and other immunosuppressing viral infections, patient with influenza prone to secondary bacterial pneumonia, herpes virus lesions may become secondarily infected by staphylococci
Specific medicaments	Cytotoxic drugs (including post-transplant immunosuppression and steroids both lower host defenses, antibiotic disturb normal flora and predispose to invasion by resistant hospital pathogens
Trauma Accidental Intentional	Burns, stab or gunshort Wounds, road traffic accidents Surgical, intravenous, urinary catheters, peritoneal dilysis ⇩ Disturb natural host defence mechanisms

Commonly occurring microorganisms in hospital infection

Urinary tract infections	*Escherichia coli*, *Klebsiella*, *Serratia*, *Proteus* spp, *Pseudomonas aeroginosa*, Faecal streptococci, *Candida albicans*
Respiratory infections	Haemophilus influenzae, Streptococcus pneumoniae, *Staphylococcus aureus*, *Enterobacteriaceae*, Respiratory viruses
Wound and skin sepsis	*Staphylococcus aureus*, *Escherichia coli*, *Proteus* spp, Anaerodes, Faecal streptococci, coagulase negative staphylococci

Gastro-intestinal infections	*Salmonella* spp., *Shigella sonnei*, viruses

Hospital infection: sources and spread

Route	Source	Example of disease
I. **Aerial** (from persons) *Droplets* *Skin scales*	Mouth Nose Skin exudate	Measles, tuberculosis, lower respiratory tract/pneumonia, Staphylococcal sepsis, streptococcal sepsis
II. **Aerial** (from inanimate sources) *Particles*	Respiratory Equipment Air conditioning plan	Gram negative respiratory infection Legionnaures' disease Fungal infections
III. **Contact** (from persons) *Direct spread* *Indirect via equipment*	Respiratory secretions Faeces/urine Skin and wound exudate	Staphylococcal sepsis, streptococcal sepsis Enterobacterial diarrhoea Pseudomonas aeroginosa sepsis
IV. **Contact** (environmental sources)	Equipment Food Medicaments Fluids	Enterobacterial sepsis (*Klebsiella*, *Serratia*, *Enterobacter* spp.) *Pseudomonas aeroginosa* and other pseudomonas
V. **Direct contact** *Into blood, tissue or body fluid*	Sharp injury Blood products	Hepatitis B, AIDS

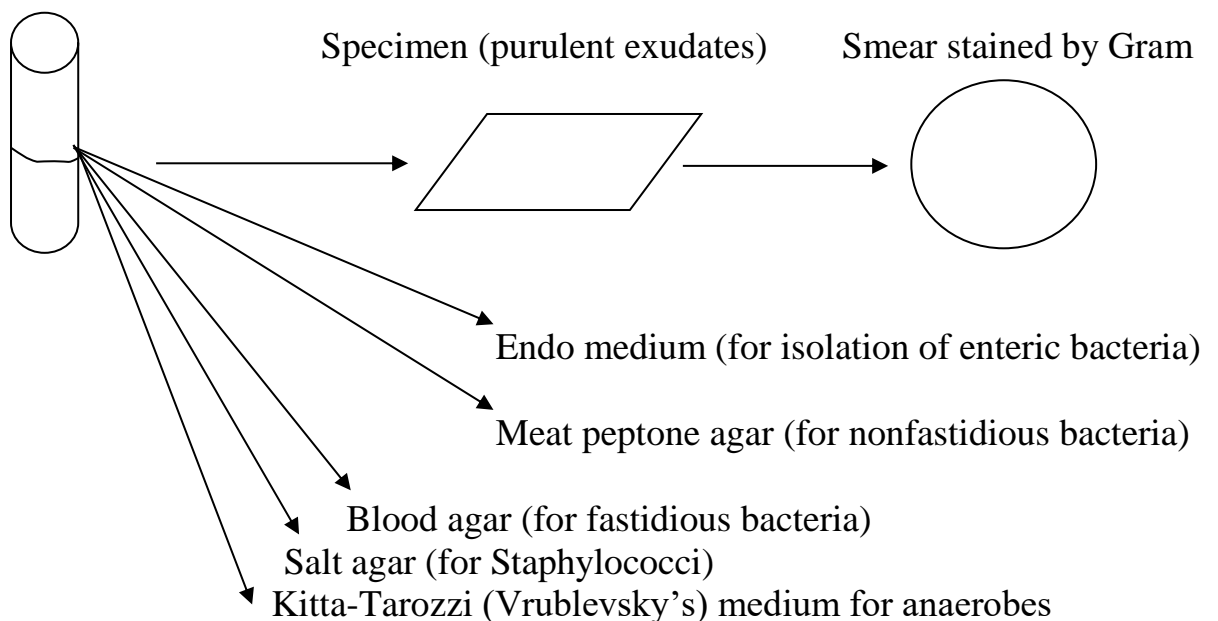## Level of isolation used in clinical settings

Type of isolation*	Protective measure**	To prevent spread of
Enteric precautions	Gowns and gloves must be worn by all persons having direct contact with patient; masks are not required; special precautions taken for disposing of feces and urine	Diarrheal diseases; *Salmonella*, *Shigella* and *Escherichia coli* Gastroenteritis, cholera, hepatitis A, rotavirus and giardiasis
Respiratory precautions	Private room with closed door is necessary; gowns and gloves are not required; masks usually indicated; items contaminated with secretions must be disinfected	Tuberculosis, measles, mumps, meningitis, pertussis, rubella, chickenpox

Drainage and secretion precautions	Gowns and gloves required for all persons; masks not needed; Contaminated instruments and dressing require special requirements	Staphylococcal and streptococcal infections; gas gangrene, herpes zoster, and burn infections
Strict isolation	Private room with closed door required; gowns, masks and gloves must be worn by all persons; contaminated items must be wrapped and sent to central supply for decontamination	Mostly highly virulent of contagious microbes; includes diphtheria, anthrax, some types of pneumonia, extensive skin and burn infection, disseminated herpes simplex and zoster
Reverse isolation (protective isolation)	Same guidelines as for strict isolation; the room may be ventilated by unidirectional or laminar airflow filtered through high efficiency particulate (HEPA) filter that removes most airborne pathogens; infected persons must be barred	Used to protect patients extremely immunocompromised by cancer, therapy, surgery, genetic defect, burns, prematurity or AIDS, and therefore vulnerable to opportunistic pathogens

***Precautions are based upon primary portal of entry and communicability of the pathogen.**

*** In all cases, visitors to the patient room must report to the nurses' station before entering the room, all visitors and personnel must wash their hands upon entering and leaving the room.**

Task 2. To start a diagnosis of purulent-inflammatory process, caused by opportunistic microorganisms.

Specimen (purulent exudates)   Smear stained by Gram

Endo medium (for isolation of enteric bacteria)

Meat peptone agar (for nonfastidious bacteria)

Blood agar (for fastidious bacteria)
Salt agar (for Staphylococci)
Kitta-Tarozzi (Vrublevsky's) medium for anaerobes

Task 2. To create the scheme of diagnosis the pyoinflammatory and sepsis process, caused by opportunistic microorganisms.

Questions for self-control.
- Opportunistic microorganisms, biological properties, etiologic role in development of opportunistic infections. Description of the diseases caused by opportunistic pathogenic microorganisms.
- Hospital infection, conditions of its origin. Properties of hospitals strains of microorganisms. Microbiological diagnostics of purulent-inflammatory infections caused by hospitals strains.
- Clinical microbiology, objects of researches. Methods. Criteria of etiologic role of the microbes isolated from a pathological focuses.

Class №40

Topic: "Sanitary microbiology. Sanitary and microbiological studies in pharmacies"

Relevance of the topic:

Sanitary microbiology - the science that studies the environment microflora (including pathogenic bacteria and viruses) and processes that they cause that can directly or indirectly affect human health or the environment.

In practical classes, students learn the schemes of sanitary-bacteriological analysis of water, air, soil and environmental objects. To carry out results of determining the coli-index of water by membrane filters method. Determining results of the sanitary-indicative microbial number in air. Probe apparatus and nutritional environment that is used in sanitary-bacteriological studies of the environment. Completed tasks students record in the protocol

Concrete objectives:
- To interpret the term sanitary-indicative microorganisms and their role as an indicator in assessing the degree of pathogen contamination of environment objects (water, soil, air).
- Analyze qualitative and quantitative microbial composition of water, soil, air and make conclusions about their safety.

Basic knowledge, skills, needed to study topics (interdisciplinary integration).
See a class № 39

A list of key terms, parameters, characteristics that student should learn in preparation for the lesson:

Terms	Definition
Sanitary microbiology	Sanitary microbiology - the science that studies the environment microflora (including pathogenic bacteria and viruses) and processes that they cause that can directly or indirectly affect human health or the environment.
Tasks of sanitary microbiology	1. The development, improvement, evaluation of microbiological methods for the study of the environment - air, water and soil. 2. Development of state standards and other regulations, guidance. 3. Development of recommendations and measures to improve the health of the environment and monitoring their implementation. 4. Protection of the environment.
The sanitary-indicative microorganisms	Sanitary indicative (or indicator) microorganisms called opportunistic microbes - representatives of normal microflora of

	humans and animals, which detection in the environment objects indicates fecal or airborne contamination.
Requirements for sanitary-indicative microorganisms	1. They must reside in the allocation of human and warm-blooded animals and enter the environment in large quantities. 2. They must not have another natural reservoir in addition to the human body or animals. 3. After entering the environment, they need to remain detectable during the period close to the terms of the pathogenic microbes survival. 4. They should not multiply in the environment. 5. They should not alter their biological properties in the environment objects. 6. They should be fairly typical to their differential diagnosis carried out without much difficulty. 7. Indication, identification and quantitative calculation must be carried out with modern, simple, affordable microbiological methods.

Theoretical questions
- Value of microbiology in activity of the pharmacist
- Objectives and methods of microbiological studies.
- Sanitary-indicative microorganisms of water and environmental objects.
- The main representatives of microflora of soil. The diseases transmitted by soil. Sanitary - exponential species. The significance of soil microflora. Methods of studying of soil microflora.
- The methods of studying of microbial number and coli-index of the water. The specifications of state standard.
- The main representatives and sanitary - exponential species of microflora of the air.
- The diseases transmitted by air.
- The methods of air microflora studying (sedimentation and aspiration methods).

Practical tasks performed in class:
- To make plating of 1 ml of drinking water in melted and cooled agar for determining of total microbial number of water.
- To determine a coli-titer and coli-index of water using membrane filters method.
- To determine the number of bacteria in 1 m^3 of the air by sedimentation method.
- To determine the number of bacteria in 1 m^3 of the air by aspiration method (with Krotov's apparatus).

Recommendations for the design of the protocol:

In the protocol should be made:
- Objects of bacteriological control in pharmacies:
- Purified water and water for injection;

- Medicines manufactured (made) in a pharmacy;
- Pharmaceutical glassware, stoppers and other accessories;
- Tools and equipment used in the areas of production of drugs;
- Hand and clothes of personnel directly involved in the production of drugs;
- Air environment in areas of drugs production.

Distilled water for drugs other than injection solutions and eye drops

Study samples taken from the burette filled with water.

Samples were taken in sterile bottles in 300 ml volume. If the results are unsatisfactory, the samples taken from the distiller receiver. Determine the MAFAM, molds and yeasts. Results estimate the total number of microorganisms those raised by summing the number of colonies of bacteria and fungi. Maximum permissible content of 10-15 CFU in 1 cm3. The presence of coli group bacteria in distilled water is not allowed.

Distilled water for making injection solutions and eye drops.

Sampling was carried out in sterile vials in the amount of 15 ~ 20 mL directly from those vessels, which carry out sterilization.

Research of dry drugs for indications such as in the case of repeated unsatisfactory bacteriological analysis.

Sampling was carried out with sterile spoons in the amount of 30-50 grams, then dissolved with sterile distilled water to a concentration that is used in an appropriate solution injections and eye drops.

Research of pharmaceutical equipment.

Utensils, plugs, gaskets, craters, exploring the cylinders at the time of preparation for pouring injection solutions and eye drops. Research carried out by rinsing equipment 10 mL of sterile water. Then determining the flushing fluid MAFAM and coli index. MAFAM group should not exceed 150 CFU in flushing, three vials, stoppers. The presence of coli group bacteria is not allowed.

Air investigation.

Samples are taken in the aseptic unit, sterilization room, assistance room, packing, material, washing rooms and service hall. The selection is carried out by a pure aspiration prepared to work indoors (no earlier than 30 minutes after wet cleaning the room), closed doors and windows. The level of sampling - desktop height, speed pulling air - 25 l / min. General number of bacteria determined in 100 liters of air, number of *Staphylococcus aureus* - in 250 liters, number of mold and yeast - in 250 liters.

Other objects.

Storage containers for pharmaceutical equipment, mortars, scales, hands personnel, towels, clothing and jobs is also subject for sanitary and microbiological studies. We research it for the coli -index, *Proteus* and *Staphylococcus aureus* (indication). Samples taken by flushing with a cotton ball placed in a test tube with 2 ml of 0.85% NaCl solution

or 0.1% pepton water. In the studied samples presence of coli group bacteria, *Pseudomonas aeruginosa*, *Proteus* and *Staphylococcus aureus* are not allowed.

Criteria for the sanitary-microbiological air quality in pharmacy.

Room name	Time	General number of microbes in 1 m³ of air	Number of *S.aureus* in 1 m³ of air	Nomber of molds and yeasts in 1 m³ of air
Aseptic section, sterilization room	*Before work*	Not more then 500	Absent in 250 l	Absent in 250 l
	After work	Not more then 1000	Absent in 250 l	Absent in 250 l
Assistance, packing, defective rooms	*Before work*	Not more then 750	Absent in 250 l	Absent in 250 l
	After work	Not more then 1000	Absent in 250 l	Absent in 250 l
Washing room	*While working*	Not more then 1000	Absent in 250 l	Less then 12
Service hall	*While working*	Not more then 1500	Less then 100	Less then 20

Methods of sanitary-bacteriological study of air (sedimentation and aspiration)
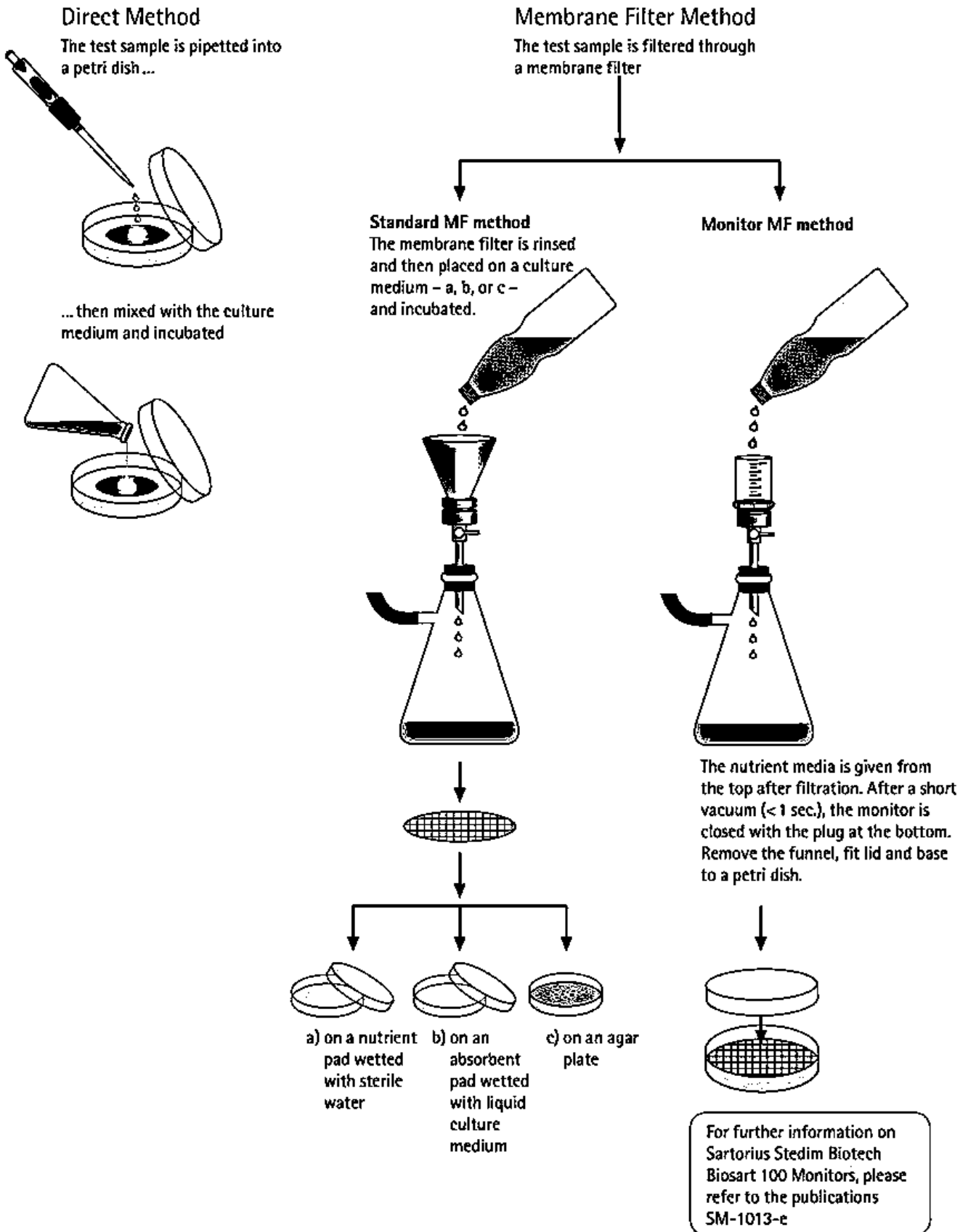
Plate method (sedimentation method)

The Petri's dishes with meat-peptone agar or another special nutrient media for staphylococci and streptococci, for example blood agar, yolk- salt agar are used. They are opened and are stayed in investigated room. Term of exposition depends on prospective quantity of microbes in the air. With a plenty of microorganisms a plate is opened for 5- 10 minutes to detect a total microbial number, with a little - for 20 — 40 minutes for detection of cocci. Then the dishes put into thermostat at 37 °C for 24 hrs. After incubation all colonies are accounted (for determination of total number of microorganisms). Number of grown colonies indicates degree of air conta mi nation. According to Omeliansky's data in 5 minutes on a surface of 100 cm 2 so many microbes sedimentate, as they present in 10 L of air. For example, on the dish surface with MPA after 5 minute exposure 32 colonies have grown. It is necessary to calculate amount of microbes which are present in 1 nr 3 of the air, applying the Omeliansky's formula. The plate has 100 cm 2. 32 colonies of microbes contain in 10 L of the air, and in 1 m 3 (1000 л) there will be (32 • 1000): 10 = 3200.

Aspiration (Krotov's) method

Krotov's apparatus is used for bacteriological air research. It give us the possibility to let pass 50 -100 L of air with a speed of 25 L per minute through clinoid chink in the

special glass above the open dish with MPA. The rotation of Petry's dish (1 rotation/sec) provides uniform dispersion of microorganisms on all surface of a medium. Then dish is incubated in a thermostat at 37 °C for 18-24 hrs. Krotov's apparatus for bacteriological air research

Methods of sanitary-microbiological analysis of water

Direct Method
The test sample is pipetted into a petri dish...

...then mixed with the culture medium and incubated

Membrane Filter Method
The test sample is filtered through a membrane filter

Standard MF method
The membrane filter is rinsed and then placed on a culture medium – a, b, or c – and incubated.

Monitor MF method

a) on a nutrient pad wetted with sterile water

b) on an absorbent pad wetted with liquid culture medium

c) on an agar plate

The nutrient media is given from the top after filtration. After a short vacuum (< 1 sec.), the monitor is closed with the plug at the bottom. Remove the funnel, fit lid and base to a petri dish.

For further information on Sartorius Stedim Biotech Biosart 100 Monitors, please refer to the publications SM-1013-e

Self-control questions

- What sanitary microbiology studies?
- The importance of a sanitary microbiology in the activities of a pharmacist.
- Sanitary-indicative microorganisms of water, environmental objects.
- Methods of sanitary-microbiological analysis of water.
- Determination of bacteria, indicators of fecal pollution: coli index and coli-titer (by membrane filters and fermentation methods).
- Methods of determining the number of microbial in air.
- Methods of sanitary-bacteriological study of air (sedimentation and aspiration).
- Evaluation of the sanitary condition of the overall indoor microbial contamination, the presence of staphylococci and hemolytic streptococci.
- Pathogenic microorganisms in environment.
- Methods of sanitary-microbiological research of environmental objects.

Class №41

Sanitary virology

Topic relevance:
Effective specific prevention measures lack for many diseases with viral etiology. In this regard, the developing of non-specific preventative measures aimed to limitation of circulation of human viral pathogens in environment become more relevant. A new field of medical science Sanitary virology must solve these tasks and problems. Thus, the subject of sanitary virology is to study the patterns of circulation of various human viral pathogens in the environment (air, soil, water), in foodstuffs, etc., the development of methods for their indication in environmental objects and effective measures of their sanitation. Main sections of Sanitary virology: 1) sanitary virology of water; 2) sanitary virology of soil; 3) sanitary virology of air. The doctor of any profile should know how to take the test material, deliver it to the laboratory, perform a virological examination, evaluate its results correctly.

Concrete objectives:
- Interpret the concept of "sanitary-indicated viruses" and their role as an indicator of level of contamination by viral pathogens of environmental objects.
- Analyze the qualitative and quantitative composition of viruses in water, soil, air and make epidemiological conclusions about their safety.
- Interpret the sanitary-virological evaluation criteria of water, soil and air facilities.

Basic knowledge, skills, needed to study topic (interdisciplinary integration).
See a class №39.

A list of key terms, parameters, characteristics that student should learn for the class:

Terms	Definitions
Sanitary virology	Sanitary Virology is a science that studies the patterns of circulation of various human viral pathogens in the environment (air, soil, water), food, etc., develops of methods for their indication in environmental objects and effective measures for their sanitation.
Tasks of sanitary virology	1. Development, improvement, evaluation of virological methods of the study of environmental objects - water, air, soil. 2. Evaluation of ways of human and animal influence to the environment. 3. Development of state standards, methodological guidelines. 4. Development of recommendations and measures for environmental sanitation and monitoring of their implementation.
Sanitary-indicated viruses	Bacteriophages of apathogenic Escherichiae (coli-phages) are used as the most adequate indicator of viral contamination of

aquatic and other environmental objects. Coli-phages are bacterial viruses. They are similar to human viruses in their biological properties and mechanism of replication. Coli-phages present in the human intestine constantly and with faeces enter the sewage in large concentration. Viruses come into the reservoirs with sewage and surface runoff, and as a consequence - into drinking water (water pipes, wells and other sources). Coli-phages detection methods are available to various laboratories, without special equipment, and give results during 6-24 hours.

Theoretical questions:
- Sanitary virology, subject, tasks, importance of sanitary virology for the doctor's work.
- The role of water, soil, air in the transmission of viral infections. Viruses are found in environmental objects most commonly.
- Methods for detection of viral pathogens in soil. Studies of soil for the presence of enteroviruses.
- Sanitary-virological examination of water. Sampling, methods of concentration. Viruses, bacteriophages in drinking and wastewater. Methods of detection.
- The role of the air environment in the spread of respiratory viral infections. Methods for air sampling and indication of respiratory viruses.

Practical activities performed in class:
- Study of algorithms of sanitary-virological research of sewage and drinking water.
- Evaluate the results of coli-phage titration in wastewater using the Grazia's and Appelman's methods (demonstration).
- Get acquainted with the norms of the permissible level of coli-phages in drinking water, water of surface reservoirs, sewage.

Topic content

In the practical class students study the methods of sanitary-virological research of water, air, soil. Determine the coli-phage titer in sewage with the Appelman and Grazia method (demonstration).

Students write down prepared tasks to the protocol and sign it at the teacher

Recommendations for the protocol design

1. **The results of qualitative determination of wastewater's coli-phage titer with Appelman method.**

For this purpose, students receive a stand with 12 test tubes: 10 test tubes (with 10x dilution of coli-phage from 10^{-1} to 10^{-10} in 4.5 ml of MPB + test culture host *E. coli* B) and controls: the 11-th test tube - test culture control (4.5 ml MPB + 0.1 ml *E.co*li B), the 12-th test tube - phage control (only 0.5 ml of phage). Calculate the phage titer: is a dilution with bacterial lysis (transparent contents of the test tube). Make conclusion.
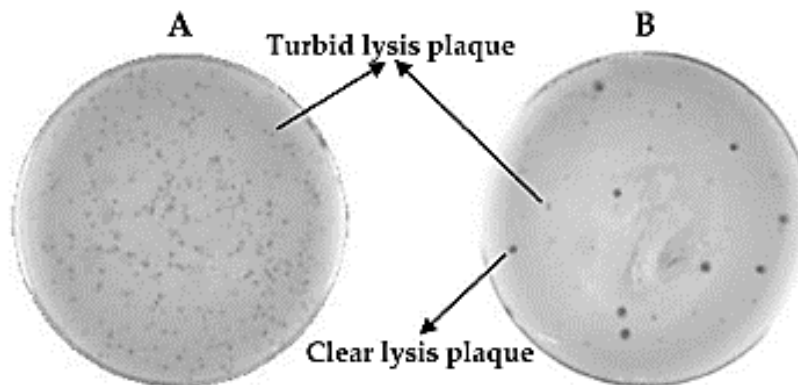
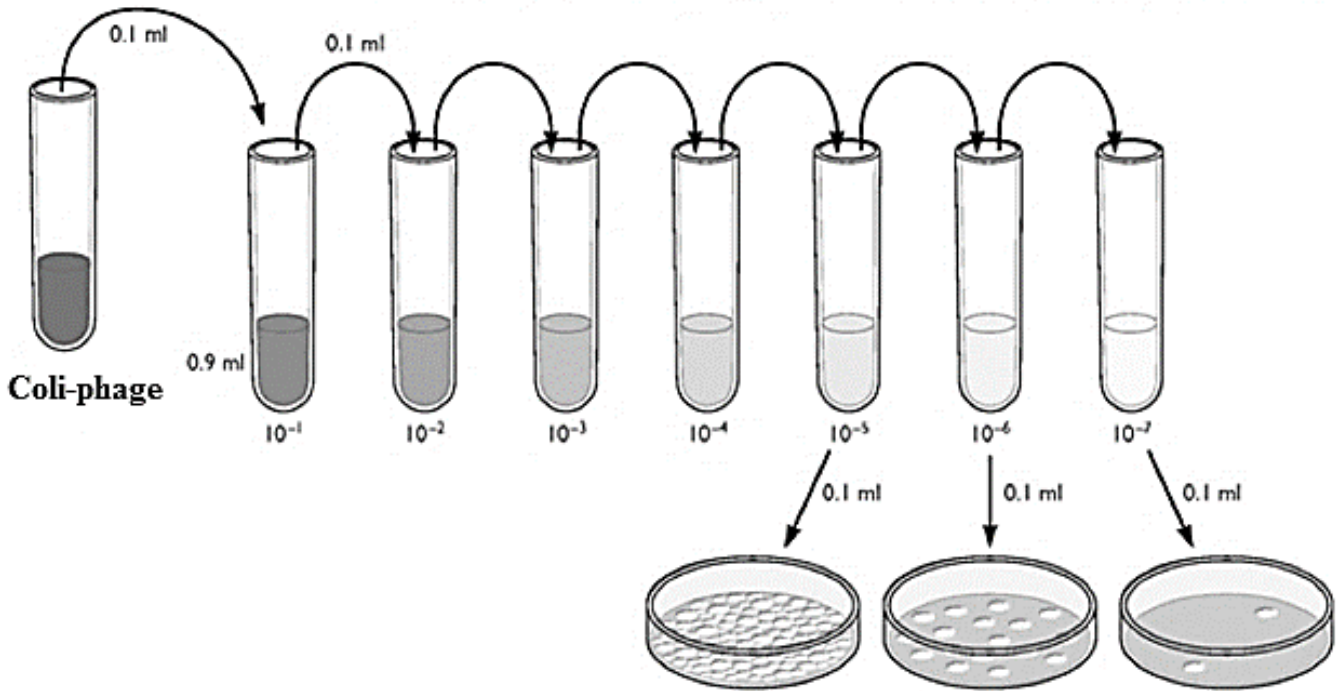**Dilutions of Coli-phage + test culture host E.coli B**

1.
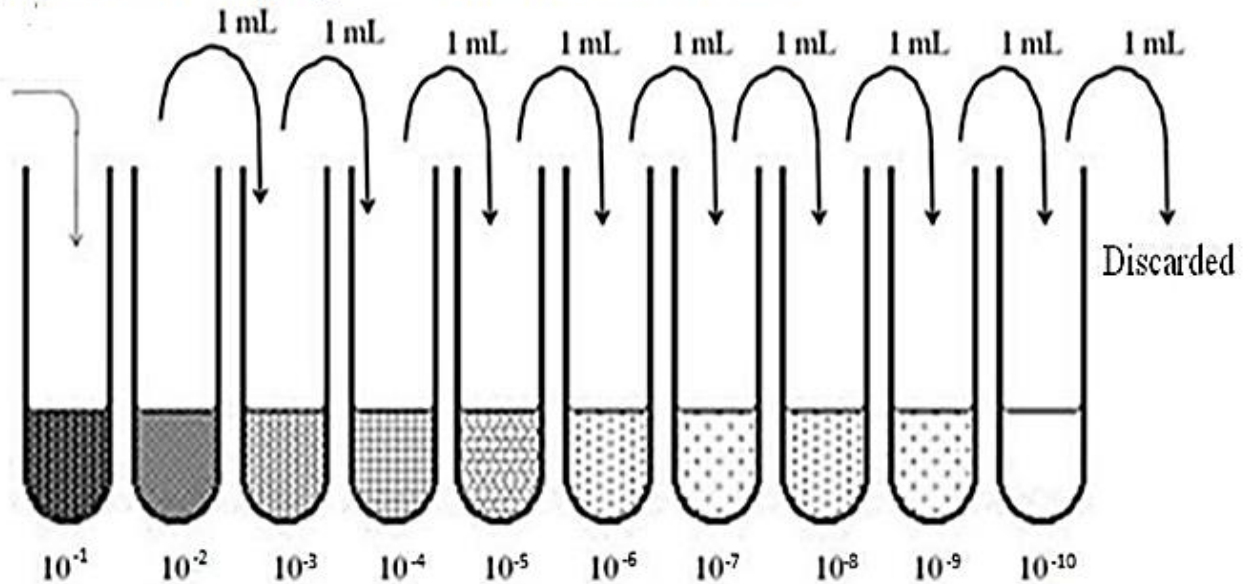
2. The results of the quantitative determination of the coli-phage titer of the waste-water by two-layer Grazia method.
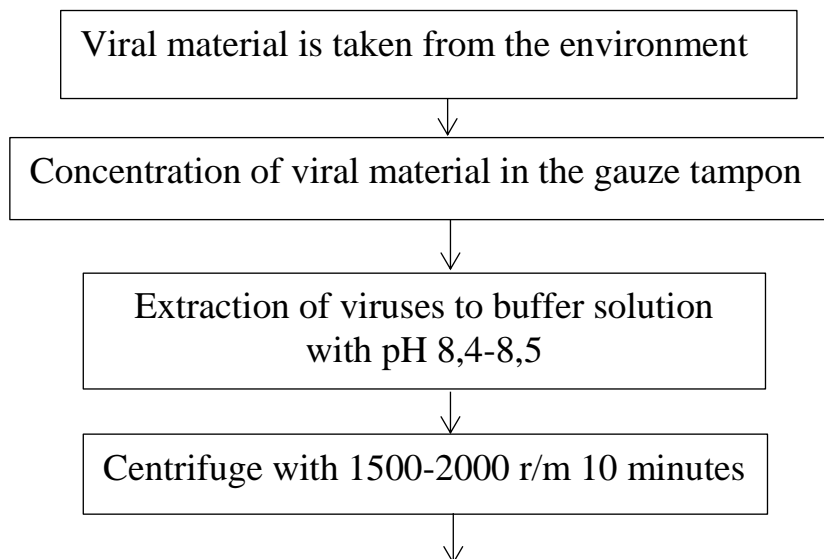
2.0% MPA is melted and poured into Petri dishes with 15-20 ml to each. Flacon with 100 cm^2 of 1.0% MPA is melted and heat to 48° C in a water bath. Prepared 1 ml of every ten-fold dilutions of the wastewater sample from 10^{-2} to 10^{-10} are poured into the tubes, then about 5 ml of "night" broth culture of the host is added, 5 ml of melted 1.0% MPA is added, and all are poured to the prepared layer of 2.0% MPA in Petri dishes. After this add the prepared mixture (top layer) onto the bottom layer of agar and quickly spread over the agar surface, allow to solidify and incubate in a thermostat at 36 ± 10C.

After titration of phages by the Grazia method, after daily incubation, the number of "negative" colonies of bacteriophages in a Petri dish was calculated. The number of these spots corresponds to the number of phages in the prepared suspension. Count the number of lysis plaques of the host culture, is PFU (plaque-forming units) in 1 ml of the original suspension (titer of bacteriophages). The number of colonies is multiplied by the dilution of bacteriophages. For example, at a dilution of 10^{-4} 50 colonies appeared, so the phage titer is 50×10^{-4} or 5×10^{-5} in 1 ml. The result is considered acceptable when positive (sensitivity of the test culture to somatic coli-phages) and negative (phage contamination of the media) controls are obtained. The cleaned wastewater can be drained into the reservoir when level of coli-phages is permissible (must be 1000 PFU / 1 ml).

Turbid lysis plaque

Clear lysis plaque

Segregation of Coli-bacteriophages and Enteroviruses from environment

Viral material is taken from the environment

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Concentration of viral material in the gauze tampon

↓

Extraction of viruses to buffer solution with pH 8,4-8,5

↓

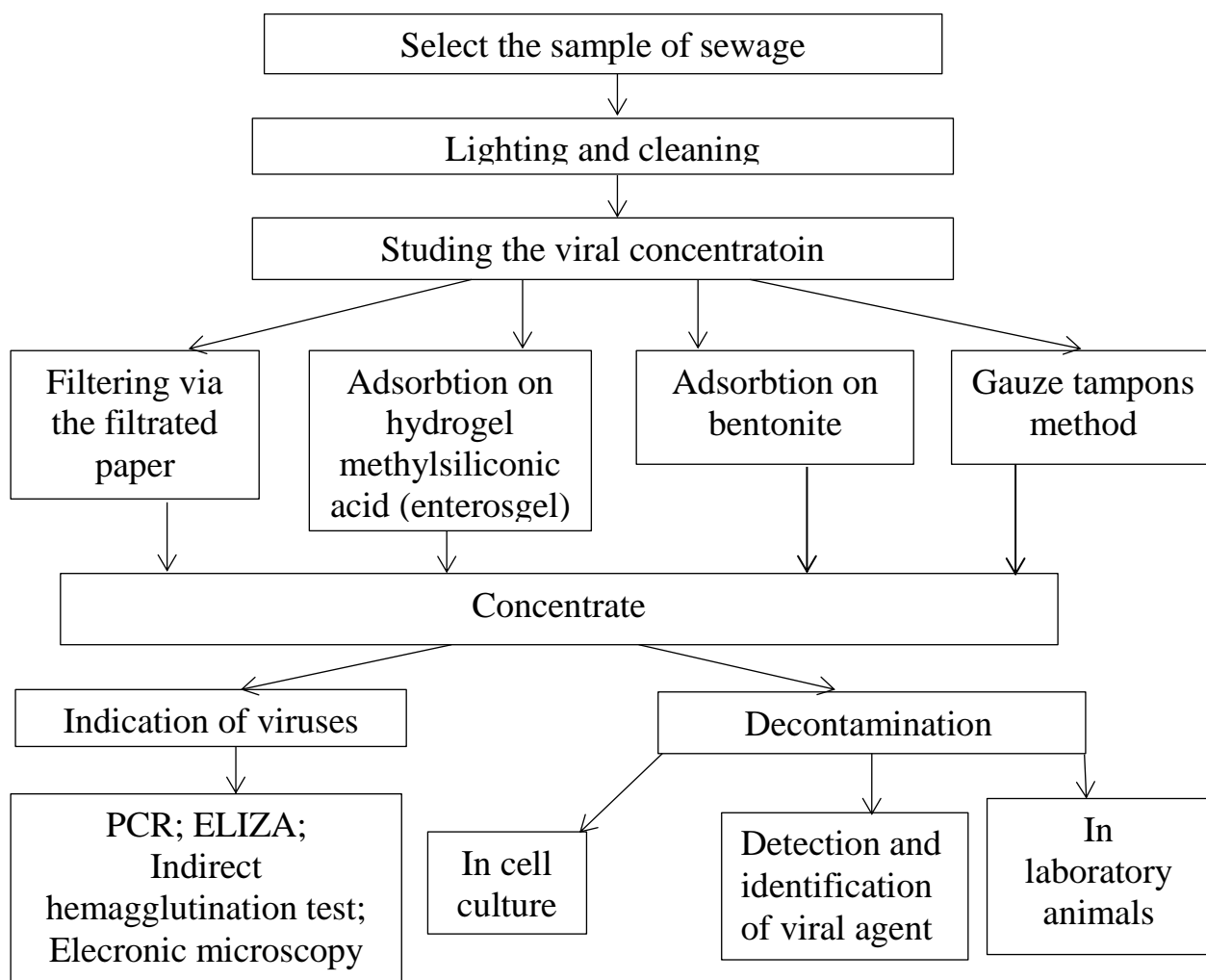| Centrifuge with 1500-2000 r/m 10 minutes |
|---|

↓

Studing of bacteriophages	**Studing of Enteroviruses**
1. 1 ml of over sedimentary fluid take into the melted MPA (42º) with broth culture of *E.coli*. 2. Pour out to Petri dishes with 2% MPA 3. Reveal the plaques of bacterophages	1 ml of over sedimentary fluid + antibiotic introduction to: 1. New-born mause (observe the paralysis and death); 2. Cell culture in the mattress coved by bentonite layer (obsreve plaques of Enteroviruses); 3. Cell culture in the tibes (obsreve the CPE of Enteroviruses).

Scheme of sanitary-virological investigation of sewage

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┌─────────────────────────────────────────────┐
│          Select the sample of sewage         │
└─────────────────────────────────────────────┘
                      │
                      ▼
┌─────────────────────────────────────────────┐
│            Lighting and cleaning             │
└─────────────────────────────────────────────┘
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┌─────────────────────────────────────────────┐
│         Studing the viral concentratoin      │
└─────────────────────────────────────────────┘
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Filtering via the filtrated paper	Adsorbtion on hydrogel methylsiliconic acid (enterosgel)	Adsorbtion on bentonite	Gauze tampons method

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┌─────────────────────────────────────────────┐
│                 Concentrate                  │
└─────────────────────────────────────────────┘
          │                         │
          ▼                         ▼
  Indication of viruses        Decontamination
          │
          ▼
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PCR; ELIZA; Indirect hemagglutination test; Elecronic microscopy	In cell culture	Detection and identification of viral agent	In laboratory animals

Control questions:
- What does medical microbiology study?
- What is structure of bacteriological laboratory and what is assignment of its units?
- Why do we stain microorganisms?
- What properties of microorganisms can be investigated using microscopy of staining smears?
- How staining solution from aniline dyes can be prepared?
- Why do we need to fix a smear?
- What lens is used at the microscopy of bacterial preparations? What signs of such lens?
- How to calculate the magnification of microscope?
- What is resolution of microscope?

Class №42

Topic: «Normal oral microflora. The role of bacteria, fungi, protozoa in the pathology of the oral cavity (1 class)»

Relevance of the topic:

Many different types of microorganisms can be detected in the human mouth because the conditions are favorable for their development. There are sufficient nutrients, optimum temperature and low alkaline response. Many microorganisms enter the oral cavity with food, water and air. Particularly large numbers of bacteria can be detected in the oral cavity at the neck of the teeth and at intervals between them. As in all biological niches, oral microorganisms create a kind of biocenosis, which in recent years has been regarded as a stable biological system in which there are complex relationships between both its constituents and the macroorganism. The main elements of such biocenoses include aerobic and anaerobic bacteria, as well as fungi and protozoa. Among the microorganisms of the oral cavity are dominated by staphylococci, streptococci, lactobacilli, corynebacteria, spirals and spirochetes, protozoa, fungi. Changes in this microbiocenosis develop on the background of impaired specific immune response and nonspecific factors of protection in the tissues of the oral cavity.

Educational purposes:
- To study the composition of normal oral microflora.
- Consider the role of microorganisms in the formation of microbial associations (such as dental plaque).
- To study the morphological and tinctorial features of plaque microflora.
- Determine the role of fungi and protozoa in the normal oral microflora.
- Investigate nonspecific factors for oral protection.

Basic knowledge, skills, skills needed to study topics (interdisciplinary integration). See a practice № 39.

A list of key terms, parameters, characteristics that a student should learn for the lesson:

Terms	Definitions
Resident microflora	Resident (permanent, obligate, autochthonous) microflora is a collection of populations of different microorganisms that have evolved during the course of evolution, is resistant to oral factors and is a mandatory biological system of the oral cavity. Consists of representatives of all groups of microorganisms: bacteria, actinomycetes, fungi, protozoa, spirochetes, viruses (the most represented about 30 species of microorganisms). More than half are streptococci, veylonelles and diphtheroids. Much less - staphylococci, lactobacilli, spirochetes, leptospira, fusobacteria, bacterroids, neisseria, protozoa, yeasts and other fungi. In an adult, a considerable part of the germs of the oral cavity is represented by

	anaerobic species (bacterroids, veylonelles, fusobacteria, spirochetes, etc.).
Transient microflora	Transient (temporary, casual, optional, allochthonous) microflora are microorganisms that are not required for the oral cavity and are microorganisms that have come from other parts of the macroorganism and the environment along with food, water, and air. Their time in the mouth is limited. They can be saprophytic, conditionally pathogenic and pathogenic. Temporary saprophytic and opportunistic bacteria include: gram-negative rods, representatives of the genera Esherichia, Klebsella, Aerobacter, Proteus, Pseudomonas, gram-positive bacilli (Bacillus subtilis), clostridia (C. putrificum, C.perfringens), tetra and others.
Microbiocenosis	Microbiocenosis (microbial association) is a collection of populations of different types of microorganisms that live in a particular biotope (for example, in the oral cavity). In the biotopes of the environment, as well as in healthy humans and animals, microbiocenosis, as a rule, consists of a large number of species related to obligate and optional, autochthonous and allochthonous microorganisms.
Biofilm	A biofilm is a complex (often multiview) layer of microorganisms characterized by the secretion of an extracellular matrix that holds the microorganisms together, performs protective functions and helps to attach to the surfaces. Biofilms are also typically characterized by attachment to a solid surface, structural heterogeneity, significant genetic diversity, complex interactions within a group, and an extracellular matrix composed of polymeric substances.
Pellicle	The pellicle is a derivative of saliva, composed of amino acids and sugars, from which polysaccharides are formed. It is believed that the pellicle is formed on crystals of hydroxyapatites. The role of the pellicle is ambiguous: on the one hand, it performs a protective function, protecting the crystals of enamel from the action of acids entering the oral cavity, on the other - contributes to the attachment of microorganisms and the formation of their colonies - plaque.
Plaque	Plaque - a buildup of bacteria in the form of a film formed on the teeth. Plaque includes both aerobic and optional anaerobic bacteria and begins to form within 1-2 hours after brushing. It is gradually calcified and transformed into a solid substrate called tartar. It is based on plaque. The dental plaque is primarily formed in inaccessible places - in fissures, holes, on contact surfaces. It is found when staining with erythrosine. There is a plaque between the gums, which is formed under the gums, and the gums, which is localized over the gums.

Nonspecific factors of protection	Nonspecific factors of mouth protection - congenital and devoid of selectivity factors of the body's protection against aggressive agents of the external and internal environment. There are mechanical, chemical and physiological mechanisms of action of factors of nonspecific protection of the oral cavity. Mechanical protection performs the barrier function of the intact mucous membrane by washing away the microorganisms with saliva, cleaning the mucous membrane in the process of eating, adhesion on cells of the ciliated epithelium. Saliva, in addition to washing away the microorganisms, also acts bactericidal, due to the presence of biologically active substances (lysozyme) in it. Chemical and physiological mechanisms of protection. Lysozyme (acetyl muramidase enzyme) is a mucolytic enzyme. It is found in all secretory fluids, but most in the mucous membranes, saliva, phlegm. Lysozyme lyses the shell of some microorganisms, primarily gram-positive ones. In addition, it stimulates the phagocytic activity of leukocytes, participates in the regeneration of biological tissues.

Theoretical questions to studies:

- Microbiocenosis of the oral cavity. Resident and transitory microflora. Qualitative and quantitative composition of normal oral microflora.
- Biological properties of fungi and protozoa in the normal oral microflora.
- Nonspecific factors for oral protection.
- The physiological role of microbial associations. Formation of dental plaque.

Practical tasks are performed in class:

- Record the qualitative and quantitative composition of the normal oral microflora.
- Identify nonspecific factors in oral cavity protection and write down the essentials for a lab notebook.
- Consider the mechanism of microbial association (plaque formation) and determine the role of microorganisms in the process.
- Make a dental smear preparation, Gram stain and describe the microflora found and paint.

Contents subject.

In the practical training students should write down the composition of normal oral microflora, consider the system of local immunity of the oral mucosa, determine the role of microorganisms in the formation of microbial associations of the oral cavity, independently conduct bacterioscopic examination of microflora and oral flora. Students complete the tasks in the protocol and sign it with the teacher.

Recommendations for the design of the protocol.

## The composition of the main oral microflora
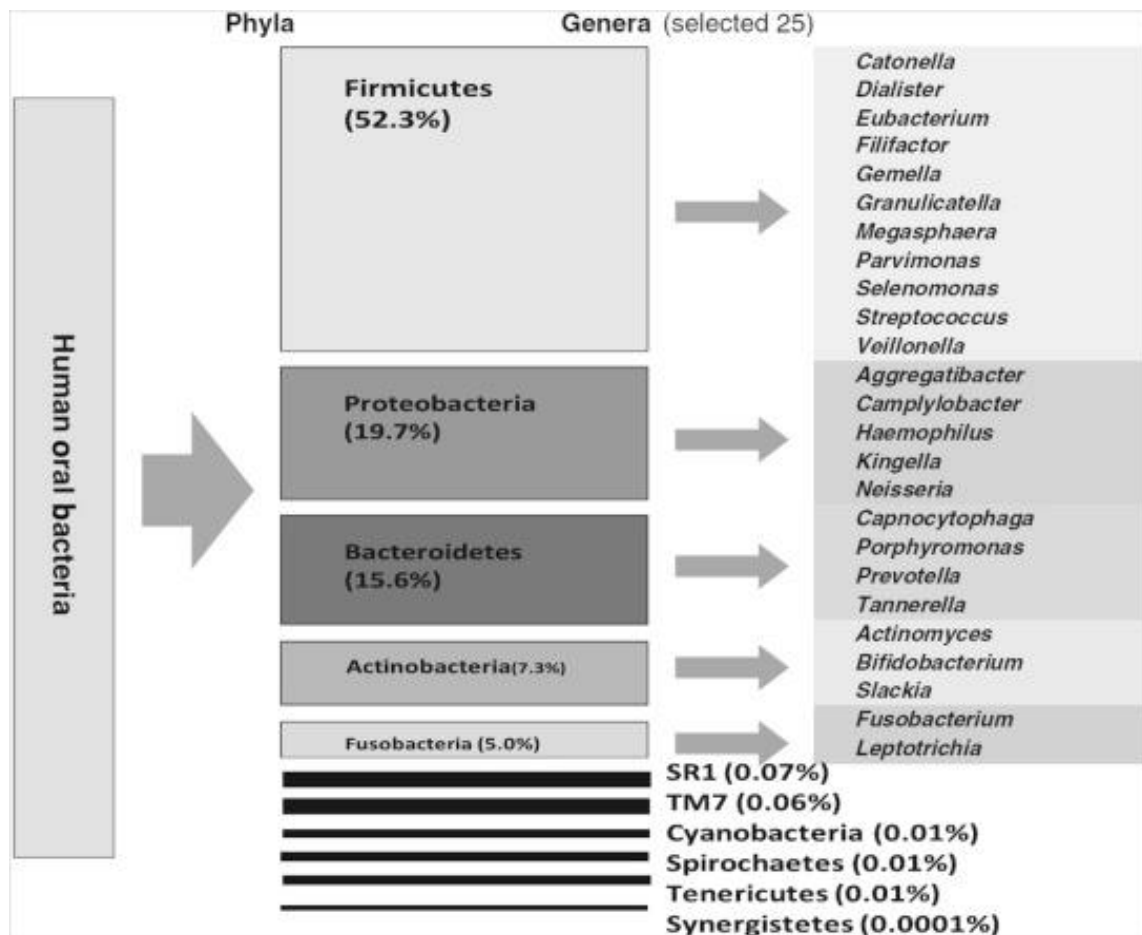
The system of local immunity of the oral mucosa.

The system of organs of local immunity of the oral cavity and oropharynx is represented by tonsils of the system of the Valdeyer-Pirogov lympho-pharyngeal ring, salivary glands, lymph nodes, mucosa-associated lymphoid tissue.

GALT (gut-associated lymphoid tissue) - lymphoid tissue associated with the intestine; BALT (bronchus-associated lymphoid tissue) is a lymphoid tissue associated with the bronchi; SALT (skin-associated lymphoid tissue) is a skin-associated lymphoid tissue.

In the context of biofilm, oral microbiocenosis acquires new properties, namely the ability to "social behavior of microorganisms" ("quorum sensing"). The very existence of this phenomenon explains why a certain level of conditionally pathogenic microflora does not lead to pathological changes in the oral mucosa. The increase in the number of opportunistic bacteria stimulates the appearance of pathogenic properties and damage to the oral cavity. An important issue regarding oral microbiocenosis is the relationship between normal and opportunistic microflora. The ability of the organism to maintain the stability of the microecology of a particular biological niche is realized by the system of colonization resistance.

Bacterial colonization of the oral cavity. The process of biofilm formation in the oral cavity is divided into four stages
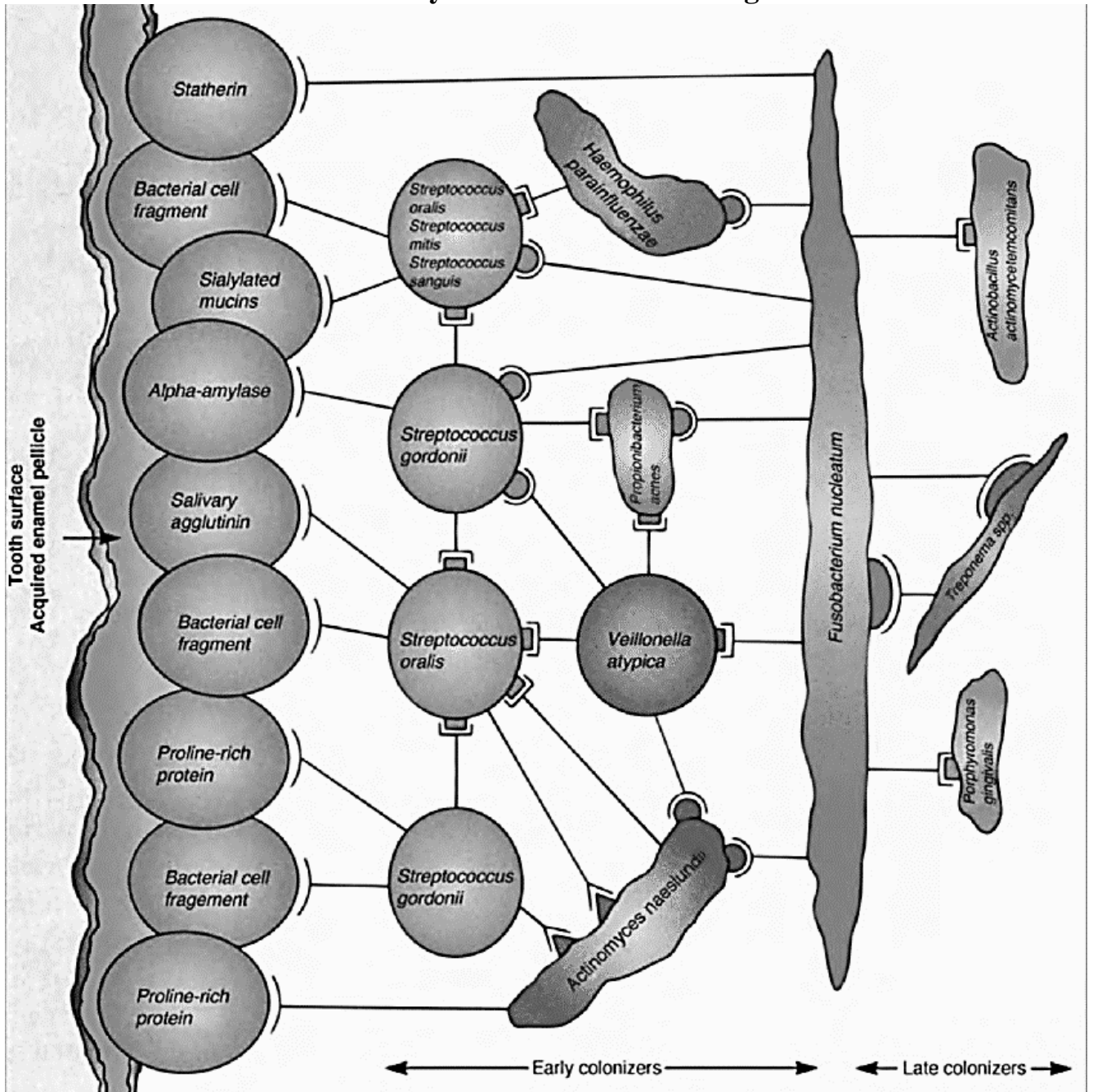
Colonization resistance of the oral cavity its resistance (resistance) to pathological and excess colonization by microflora (bacteria, fungi, viruses, protozoa, etc.).

The system of colonization of oral resistance is represented by:

1. microecological niches (biotopes) - plaque, surfaces of various areas of the mucous membrane (tongue, cheeks, lips, sublingual area, palate, tonsils); oral fluid; biotopes of ash furrows, excretory ducts of salivary glands;

2. microflora of each biotope, with features of qualitative and quantitative composition;

3. mechanisms of activity of bacteria of normal microflora (symbionts) (antagonistic and synergistic);

4. mechanisms of nonspecific oral resistance.

Task 1. To prepare a preparation from your own dental plaque, to paint according to Gram and to make a drawing in the protocol on which different bacteria should be marked.

Plaque microflora (gram)
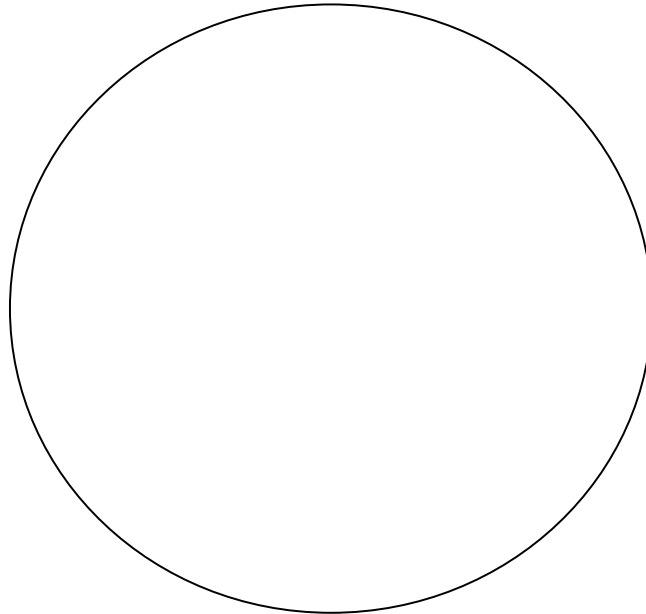
Questions for self-control.
- What is the composition of normal oral microflora?
- What is the role of microorganisms in the formation of microbial associations?
- Nonspecific factors of protection of the oral cavity, their role in the suppression of microbiocenosis of the oral cavity.
- The mechanism of formation of a dental plaque

Class №43

Topic: "Normal oral microflora. Role of bacteria, fungi, protozoa in oral pathology (2 sessions)"

Relevance of the topic:

In the oral cavity are dozens of microorganisms among which there are aerobes and anaerobes, parasites and saprophytes. In violation of immunity, an increase in the number of opportunistic microflora and persistent disturbance of the composition of oral microbiocenoses with the development of teeth and periodontal tissues are noted. The nature of colonization resistance is one of the leading physiologically and pathogenetically significant factors in the formation and progression of dental diseases. The cause of the most common dental diseases are oral microorganisms. Most of them are found in various dental layers, as well as in saliva and on the surface of the mucous membrane of the tongue.

Objective purpose:
• Familiarize yourself with the underlying pathological processes that cause microorganisms in the oral cavity.
• To study the role of microorganisms and microbial associations in the pathogenesis of caries and periodontal diseases.
• Consider methods for microbiological diagnosis of oral diseases.
• Analyze methods of correction and treatment of oral diseases caused by microorganisms.

Basic knowledge, skills, skills needed to study topics (interdisciplinary integration). See a practice № 39.

The list of principal terms, parameters, characteristics, which student should be study for lesson:

Term	Definition
Caries	Caries is a pathological process that occurs after teething, in which demineralization and softening of the hard tissues of the tooth occurs with the subsequent formation of a defect in the form of a cavity.
Periodontitis	Periodontitis is an inflammatory disease of the gums. Its development is usually triggered by microorganisms trapped in the injured soft gum tissue - in this case, doctors are talking about the development of acute periodontitis. In addition, periodontitis can provoke plaque or stone - if they are not removed in time, the pockets of the pocket are filled with plaque containing the products of the life of microorganisms and destroying the tissues of the periodontium.
Gingivitis	Gingivitis is an inflammatory disease of the tissues of the gums that runs in acute or chronic form. Usually, the disease is caused by the microbial flora of the oral cavity. With insufficient care of the oral

	cavity, plaque accumulates on the teeth, in which colonies of microorganisms develop, a microbial film is formed. This plaque can be mineralized, forming a tartar, which is one of the causes of gingivitis.
Stomatitis	Stomatitis - inflammation of the oral mucosa.
Ulcerative necrotic stomatitis of Vincent	Ulcerative necrotic stomatitis Vincent - a mixed infections caused by two pathogens - representatives of autochthonous microflora of the oral cavity - *Fusobacterium*, belonging to the family *Bacterioidaceae*, and *Borrelia*, family members *Spirochaetaceae*.

Theoretical questions to studies:
- Resident oral microflora. Role in pathological conditions.
- The role of microorganisms and their aggression factors in the development of dental caries.
- Impact of local and general factors in the development of periodontitis. Etiological role of microflora, pathogenesis and forms of periodontitis.
- The role of microorganisms in the development of gingivitis. Forms of manifestation of gingivitis.
- Ulcerative necrotic stomatitis of Vincent. Etiology, pathogenesis.
- Methods of microbiological diagnostics of oral diseases.
- Methods for the correction and treatment of oral diseases caused by microorganisms.

Practical tasks are performed in class:
- Record the composition of the oral microflora in pathological conditions.
- Introduce into the protocol basic pathological processes that cause microorganisms in the oral cavity.
- To consider the role of plaque microorganisms in the etiology and pathogenesis of dental caries.
- Investigate demonstration preparations made from the material of patients with periodontitis and ulcerative necrotic stomatitis of Vincent.

Topic content:
In the practical training students should record the composition of the oral microflora in pathological conditions, give some examples, get acquainted with the mechanism of caries development and determine the role of microorganisms in the pathogenesis, to consider and depict demonstration preparations made from the material of patients with periodontitis and with periodontitis. Students complete the tasks in the protocol and sign it with the teacher.

Recommendations for the design of the protocol.

Composition of oral microflora in pathological conditions

AT THE PATHOLOGICAL CONDITION	
Caries	*Str. mitis* biovar 1, *Str. gordonii, Str. sanguis, Str. oralis, Str. mutans*, actinomycetes, lactobacilli
Complicated caries	*Bacteroides, Prevotella, Lactobacillus, Streptococcus spp., Clostridium, Fusobacterium, Peptostreptococcus, Corynebacterium, Bifidobacterium, Capnocytophaga, Actinomyces, Leuconostoc, Porphiromonas, Candida, Veillonella, Gemella, Staphylococcus, Aerococcus, Saccharomyces, Enterococcus, Eubacteria*
Gingivitis	*Actinomyces: naeslundii, viscosus, israelii; Str. sanguis, Str. mitis; Fusobacterium nucleatum, Selenomonas sputigena, Haemophilus parainfluenzae*, peptostreptococci, *Prevotella intermedia, Campylobacter sputorum*, velonelles
Chronic periodontitis	Clon 1025 TM7, *Fusobacterium nucleatum animalis, Atopobium parvulum, Eubacterium spp.* strain PUS9.170*, Abiotrophia adiacens, Dialister pneumonitis, Filifactor alocis, Selemonas spp.* strain GAA14, *Str. Constellatus, Campylobacter rectus, Tannerella forsythia,Wolinella recta, Treponema spp.* strain I:G:T21, *Fusobacterium nucleatum, Atopobium rimae, Megasphaera spp.* clon BB166, *Catonella morbi, Eubacterium saphenum, Gemella haemolysans, Campylobacter gracilis, Haemophilus parainfluenzae, Prevotella tannerae, Porphyromonas gingivalis, Peptostreptococcus micros*
Acute localized periodontitis	*Eikenella corrodens, Capnocytophaga sputigena, Actinobacillus actinomycetemcomitans, Prevotella intermedia*
Stomatitis	Bacteria: autochthonous — S*treptococcus spp., Staphylococcus spp., Micrococcus spp., Fusobacterium spp., Veillonella spp., Bacteroides spp.*, allochthonous — *Enterococcus faecalis, Escherichia coli, Klebsiella spp., Peptostreptococcus spp., Campylobacter spp.*; microflora from the environment — *Trichomonas spp., Mycoplasma spp., Mycobacterium spp., Leptospira spp.* Fungus: *Candida spp., Aspergillus spp., Cryptococcus neoformans, Histoplasma capsulatum.* Viruses: *Picornaviridae* (Enterovirus — virus Coxsackie A type 16, enterovirus type 71), *Herpesviridae* (*Herpes simplex virus 1/2, Herpes zoster virus, Cytomegalovirus, Epstein-Barr virus, Herpes virus* 6, 7, 8), HIV, *Human papillomavirus* (non-oncogenic — 1, 2, 3, 5, oncogenic low risk — 6, 11, 42, 43, 44, oncogenic high risk — 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68). Protozoa: *Trichomonas tenax, Entamoeba gingivalis.*

When considering the mechanisms of tooth decay, attention is drawn to the variety of different factors, the interaction of which leads to the emergence of foci of demineralization.

The main etiological factors of caries development:
1. microflora of the oral cavity;
2. nature and diet, fluorine content in water;

3. quantity and quality of salivation;

4. the general state of the body;

5. extreme effects on the body.

Local factors of caries development:

- dental plaque and plaque isolated by microorganisms;

- violation of the composition and properties of the oral fluid;

- carbohydrate sticky food residues of the oral cavity;

- resistance of dental tissues, due to the complete structure and chemical composition of hard tissues of the tooth;

- deviation in the biochemical composition of hard tooth tissues and defective structure of tooth tissues;

- state of tooth pulp;

- condition of the dento-jaw system during the period of laying, development and eruption of permanent teeth.
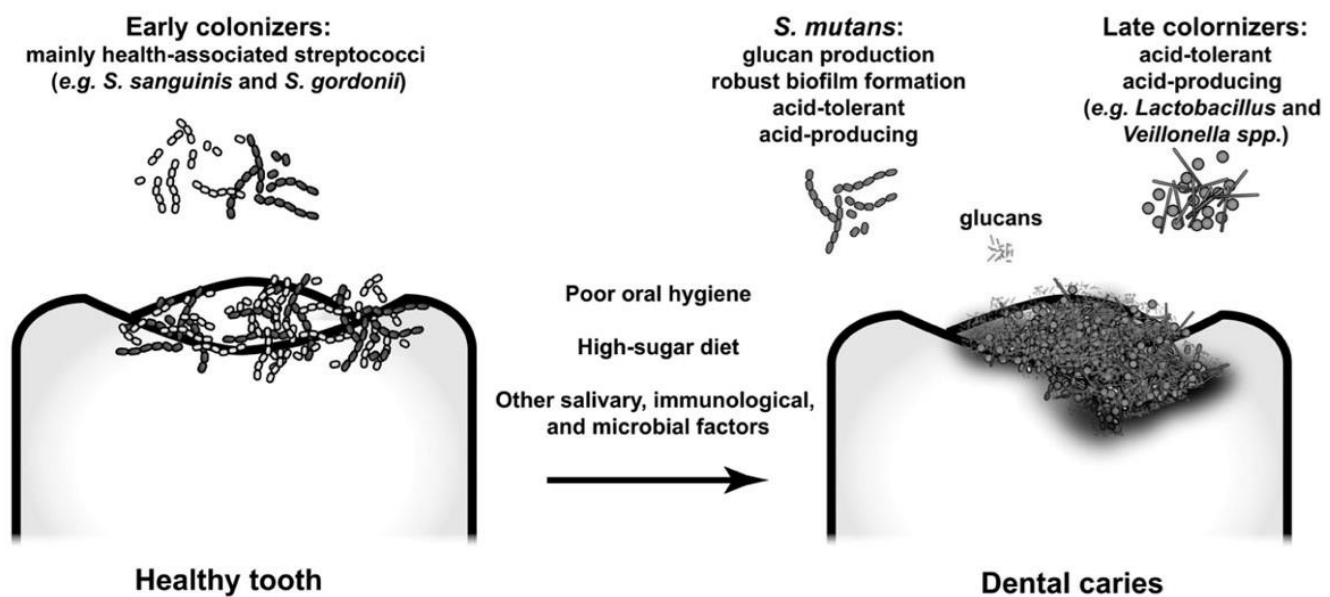
Pathogenesis of caries.

Cariesogenic action of microorganisms is associated with the formation of plaque. Initially, a monolayer of microorganisms (early colonizers, for example, S. sanguinis and S. gordonii) is adsorbed onto the pellicle, to which new layers are gradually applied. The plaque matrix consists of polysaccharides, proteins and a small amount of lipids. An important place in the matrix of plaque belongs to dextran - a polysaccharide that produces streptococci, which promotes their attachment to the tooth surface. Poor oral hygiene, a high-sugar diet, and other salivary, immunological, and microbial factors lead to the development of a pathogenic biofilm (dysbiosis). Among all cariogenic streptococci, a special place is occupied by Streptococcus mutans, first identified by Clark (1924).

Methods of microbiological diagnostics of oral diseases:

1. Microscopy of dyed and native drugs.
2. The method of immunofluorescence.
3. Selection of pure cultures and identification of pathogens (bacteria, spirochetes, mycoplasma, actinomycetes, fungi, protozoa, etc.).
4. Virus isolation and identification.
5. Serological diagnosis.

Ways to restore normal microflora and maintain its functional state:

- antiseptics;
- probiotics, prebiotics and synbiotics;
- immunomodulators of bacterial origin.

Ways to restore and maintain normal function of the mucosal immune system:

- specific immunomodulation with the use of immunomodulators of bacterial origin;
- substitution of function by the use of drugs containing factors of nonspecific immunity;
- normalization of indicators of general immunity through the use of immunomodulators.

Objective 1. To investigate demonstration preparations made from the material of patients with periodontitis and ulcerative necrotic stomatitis of Vincent.
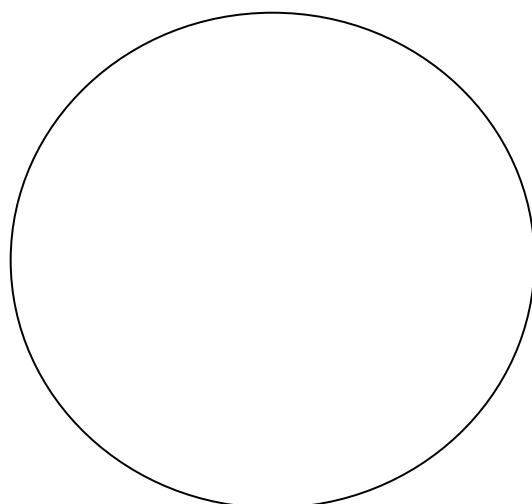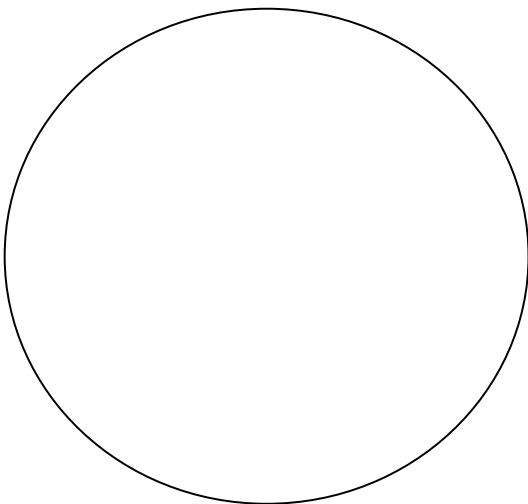
Questions for self-control.

- What is the role of normal human microflora in the norm and pathology?
- What are the causes of dysbiosis?
- What is the role of microorganisms and their factors of aggression in the development of dental caries?
- The influence of local and general factors in the development of periodontitis. Etiological role of microflora, pathogenesis of periodontitis.

- The role of microorganisms in the development of gingivitis.
- Methods of microbiological diagnostics of oral diseases.
- Means of correction and treatment of diseases of the oral cavity.

Class №44

Topic: "Microbiological studies of sterile and non-sterile drugs"

Relevance of the topic:

Sanitary microbiology is a section of medical microbiology that studies environmental microorganisms that can have a negative effect on human health. It develops microbiological indicators of hygienic regulation, methods for monitoring the effectiveness of environmental objects disinfection, and identifies pathogenic, conditionally pathogenic and sanitary-indicative microorganisms in environmental objects.

The pharmacist must know the requirements for the microbiological purity of drugs and the microbiological parameters that are subject to monitoring. To be able to correctly carry out sampling. Own methods for determining these indicators and be able to correctly interpret the results.

Specific objectives:

- To learn the methods of controlling microbial contamination of sterile and non-sterile medical forms.
- Interpret indicators obtained by microbiological examination of non-sterile drugs and medical raw materials.

Basic knowledge, skills, skills needed to study topics (interdisciplinary integration). See a practice № 39.

The list of principal terms, parameters, characteristics, which student should be study for lesson:

Term	Definition
Sanitary microbiology	Sanitary microbiology is a science that studies the microflora of the environment (including pathogenic bacteria and viruses) and the processes they cause that can directly or indirectly affect human health.
Goal of sanitary microbiology	1. Development, improvement, assessment of microbiological methods for the study of environmental objects - water, air, soil. 2. Assessment of the ways in which humans and animals influence the environment. 3. Development of standards, guidelines. 4. Development of recommendations and measures for the improvement of environmental facilities and monitoring their implementation. 5. Environmental protection.

Sanitary indicative microorganisms	Sanitary-indicative (or indicator) microorganisms are some opportunistic microbes - representatives of the normal microflora of humans and animals, the detection of which in objects of the external environment indicates their fecal or airborne contamination by secretions of humans or warm-blooded animals.
Requirements for Sanitary Microorganisms	1. They must constantly be in the secretions of humans and warm-blooded animals and excreted in large quantities. 2. They should not have another natural reservoir other than the humans or animals. 3. Once released into the environment, they must remain viable for periods close to the survival time of pathogenic microbes that are excreted from the body in the same way. 4. They should not multiply in the environment. 5. They should not change their biological properties in environmental objects. 6. They should be typical enough so that their differential diagnosis is carried out without any special difficulties. 7. Indication, identification and quantification should be carried out by modern, simple, accessible microbiological methods.

Theoretical questions for the lesson:
- The importance of sanitary microbiology in the activities of a pharmacist.
- Methods of research of drugs for sterility.
- Research methods for non-sterile drugs.
- Microorganisms, which are determined in non-sterile medicines and raw materials.
- Requirements for microbiological indicators of drugs.

Practical work performed in the lesson:
- Familiarize yourself with the requirements for the microbiological purity of medicines and write down in the protocol the main indicators that are used to evaluate it
- Learn the patterns of sanitary-bacteriological studies of sterile and non-sterile drugs. Research schemes to record in the protocol.
- Record sterility test results

Recommendations for the design of the protocol.

According to the requirements for microbiological purity, 4 categories of drugs are distinguished.

The sterility test is carried out to control substances, finished medicines or products, which, according to the requirements, must be sterile. These drugs belong to the 1st category.

The test is carried out under aseptic conditions that prevent microbial contamination, but do not affect the microorganisms that can be detected as a result of the test. For research, thioglycol medium and soya-casein medium are used. The main methods that are used for control are the membrane filtration method and the direct seeding method; in parallel with them, a control experiment with obviously sterile samples is mandatory. The membrane filtration method is used in all cases where the nature of the drug allows it - to test drugs in the form of aqueous solutions that can be filtered, or drugs that mix or dissolve in aqueous solvents or oils that do not have antimicrobial activity under the conditions of verification. Use membrane filters with a pore diameter of not more than 0.45 microns, capable of effectively retaining microorganisms. The diameter of the filters is about 50 mm. The filtration unit and membrane filters are sterilized and a sample is added in an amount determined for a given form of drug.

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                    ┌─────────────────────────────────────────┐
                    │ Checking the suitability of the drug for use │
                    └─────────────────────────────────────────┘
         ┌──────────────────────────┐      ┌──────────────────────────┐
         │ Drugs do not have        │      │ Drugs have antimicrobial │
         │ antimicrobial properties │      │ properties               │
         └──────────────────────────┘      └──────────────────────────┘
                                                         │
                                           ┌──────────────────────────┐
                                           │ Neutralization of        │
                                           │ antimicrobial action     │
                                           └──────────────────────────┘
   ┌──────────────┐  ┌──────────────┐   ┌──────────────┐  ┌──────────────┐
   │ Membrane     │  │              │   │ Membrane     │  │ Direct       │
   │ filtration   │  │              │   │ filtration   │  │ inoculation  │
   │ and transfer │  │ Direct       │   │ and transfer │  │ into liquid  │
   │ of filters   │  │ inoculation  │   │ of filters   │  │ culture      │
   │ into liquid  │  │              │   │ into liquid  │  │ media.       │
   │ nutrient     │  │              │   │ nutrient     │  │              │
   │ media.       │  │              │   │ media.       │  │              │
   └──────────────┘  └──────────────┘   └──────────────┘  └──────────────┘
         │                 │                   │                 │
         ▼                 ▼                   ▼                 ▼
   ┌───────────────────────────────────────────────────────────────────┐
   │ Incubation, visual assessment of the presence or absence of growth. │
   │ Interpretation of the results.                                     │
   └───────────────────────────────────────────────────────────────────┘
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Crops are incubated for 14 days at a temperature of 30 to 350 ° C for environments that are designed to detect bacteria and 20 - 250 ° C to detect fungi.

When using the direct sowing method, the test drug is introduced directly into the nutrient medium in the amount indicated in the table, the amount of the drug should not be more than 10% by volume of the amount of nutrient medium. In the case when the drug has antimicrobial activity, it is neutralized by the addition of neutralizers or an increase in the

volume of the nutrient medium. The incubation time and temperature are the same as for the filtration method. Crops are reviewed several times during the incubation period, noting the presence or absence of visually visible growth. If the test sample causes clouding of the nutrient medium, which makes it impossible to record, then for 14 days of incubation, a certain amount of medium is transferred to a vessel with the same medium and the incubation of the initial and new crops continues, the total incubation time should be at least $14 + 7$ days from the start of incubation . A medicinal product is considered to have passed the sterility test if the growth of microorganisms is not determined by visual accounting.

Verification of the microbiological purity of non-sterile drugs.

Non-sterile drugs according to DFU belong to the 2, 3, 4 categories.

In non-sterile drugs determine: the total number of mesophilic bacteria and fungi that can grow under aerobic conditions, as well as the total number of viable microorganisms (mesophilic bacteria and fungi); the presence of E. coli and salmonella, S. aureus, Ps. aeruginosa.

The test is carried out under conditions that prevent accidental contamination of the test sample. Determination of the total number of aerobic microorganisms is carried out by the method of membrane filtration or by seeding on Petri dishes. To determine the total microbial number, 3 methods are used (membrane filtration, direct seeding, the most probable number method). For the determination of mesophilic bacteria, meat - peptone agar is used, for the determination of fungi - Saburo medium. The incubation time of crops is 5 days. The incubation temperature for bacteria is 300C, for mushrooms 250-300 C. The most probable number method is used only when it is impossible to use other methods.

They record the results of determining the microbial number and sanitary indicative microorganisms in a non-sterile drug. They study equipment and nutrient media that are used in sanitary-bacteriological studies of drugs. Students write the completed tasks in the protocol and sign it with the teacher.

The student makes the protocol:
- Sterility drug research schedule
- Research schemes for non-sterile drugs by microbiological indicators (total microbial number, the presence of enterobacteria, *S.aureus, Ps. aeruginosa*)
- Tables: drug requirements for microbiological indicators.
- Consideration of the results of a drug test for sterility.
- Taking into account the results of determining the total microbial number of a non-sterile drug.

Question for students' self-control:
- What does sanitary microbiology study?
- The importance of sanitary microbiology in the activities of a pharmacist.

- What is subject to sanitary-microbiological examination in pharmacies, in the pharmaceutical industry?
- Sanitary-indicative microorganisms that are defined in non-sterile drugs.
- Methods of sanitary-microbiological research of medicines.
- Methods for determining the total microbial number in non-sterile drugs (membrane filtration, direct culture, the most probable number method).
- What are the requirements for the microbiological purity of non-sterile drugs, depending on its category.

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