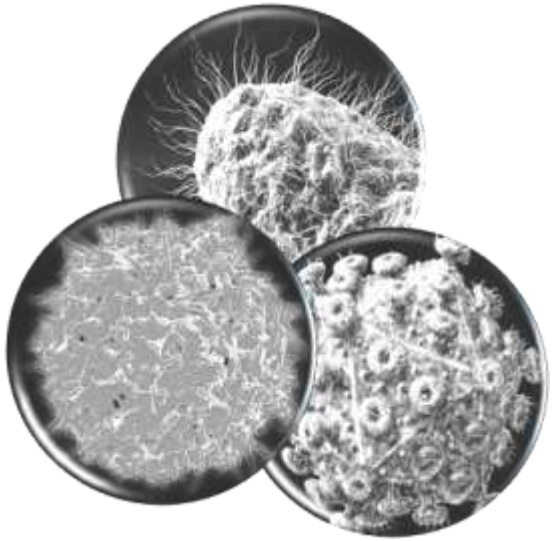
MICROBIOLOGY, VIROLOGY, IMMUNOLOGY

Laboratory workbook



Student's name _____

Faculty_____

Group _____

Minsk BSMU 2025

МИНИСТЕРСТВО ЗДРАВООХРАНЕНИЯ РЕСПУБЛИКИ БЕЛАРУСЬ
БЕЛОРУССКИЙ ГОСУДАРСТВЕННЫЙ МЕДИЦИНСКИЙ УНИВЕРСИТЕТ
КАФЕДРА МИКРОБИОЛОГИИ, ВИРУСОЛОГИИ, ИММУНОЛОГИИ

МИКРОБИОЛОГИЯ, ВИРУСОЛОГИЯ, ИММУНОЛОГИЯ

MICROBIOLOGY, VIROLOGY, IMMUNOLOGY

Практикум

2-е издание

Минск БГМУ 2025

УДК [579+578+612.017](076.5)(075.8)
ББК 52.64:52.63:52.54я73
М59

Рекомендовано Научно-методическим советом университета в качестве практикума 16.10.2024 г., протокол № 2

А в т о р ы: канд. мед. наук, доц. Е. Ю. Кирильчик; канд. мед. наук, доц. Д. А. Черношей; канд. мед. наук, доц. В. В. Кочубинский; канд. мед. наук, доц. В. В. Слизень; канд. мед. наук, доц. Т. А. Канашкова; канд. мед. наук, доц. Ж. Г. Шабан; канд. мед. наук, доц. И. А. Гаврилова; канд. мед. наук, доц. Т. Г. Адамович

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М59 **Микробиология**, вирусология, иммунология = Microbiology, virology, immunology : практикум / Е. Ю. Кирильчик, Д. А. Черношей, В. В. Кочубинский [и др.]. – 2-е изд. – Минск : БГМУ, 2025. – 132 с.

ISBN 978-985-21-1693-0.

Отражены вопросы общей и частной медицинской микробиологии, вирусологии и иммунологии. Даны алгоритмы, схемы, некоторые справочные сведения, методики выполнения лабораторных работ по дисциплине «Микробиология, вирусология, иммунология». Первое издание вышло в 2024 году.

Предназначен для студентов 2–3-го курсов медицинского факультета иностранных учащихся, обучающихся по специальности «Лечебное дело» на английском языке.

УДК [579+578+612.017](076.5)(075.8)
ББК 52.64:52.63:52.54я73

Учебное издание

Кирильчик Елена Юрьевна
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MICROBIOLOGY, VIROLOGY, IMMUNOLOGY

Практикум
На английском языке
2-е издание

Ответственная за выпуск Т. А. Канашкова
Компьютерный набор Е. Ю. Кирильчик
Переводчик Е. Ю. Кирильчик
Компьютерная верстка Н. М. Федорцовой

Подписано в печать 06.12.24. Формат 60×84/8. Бумага писчая «Снегурочка». Ризография. Гарнитура «Times». Усл. печ. л. 15,34. Уч.-изд. л. 9,1. Тираж 131 экз. Заказ 6.

Издатель и полиграфическое исполнение: учреждение образования «Белорусский государственный медицинский университет». Свидетельство о государственной регистрации издателя, изготовителя, распространителя печатных изданий № 1/187 от 24.11.2023. Ул. Ленинградская, 6, 220006, Минск.

ISBN 978-985-21-1693-0

LABORATORY SAFETY PROCEDURES

1. Place all extra clothing, unnecessary books, purses, backpacks, and paraphernalia in an appropriate place. Racks are provided for these materials. The laboratory work area must be kept free of articles not actually in use.
2. Eating, drinking, and smoking are forbidden at all times in the laboratory.
3. Keep your locker or laboratory door clean. Do not allow your locker drawer to become filled with cultures that have no value in your current work.
4. Return all reagents, cultures, and glassware to their appropriate places.
5. Wear a laboratory coat, smock, or lab apron when working in the laboratory. This will protect clothing from contamination or accidental discoloration by staining solutions.
6. Do not place anything in your mouth while in the laboratory. This includes pencils, food, and fingers. Learn to keep your hands away from your mouth and eyes.
7. Avoid contamination of benches, floor, and wastebaskets.
8. Clean your work area (laboratory bench) with a phenolic disinfectant such as 5 % Lysol or 5 % phenol or a quaternary compound such as cetylpyridinium (Ceepyrn) before and after each laboratory period. This standard procedure lessens the chance for accidental infection as well as for contamination of cultures.
9. Special receptacles will be provided for infectious materials and used glass slides. Place all discarded cultures and contaminated glassware into these receptacles. Do not let unwanted and unneeded materials accumulate. Tall jars filled with a solution such as 5 % Lysol or special receptacles will be provided for pipettes.
10. When infectious material is accidentally spilled, cover it immediately with a disinfectant such as 5 % Lysol or 5 % phenol and notify your instructor at once.
11. Flame wire loops and needles before and immediately after transfer of cultures. Do not move through the laboratory with a loop or pipette containing infectious material.
12. Wash your hands thoroughly before and after each experiment, using disinfecting soap if possible.
13. Label all experimental material with your:
    a. Name _____
    b. Date ___/___/2024.
    c. Exercise number: *Ex. 5*
14. Telephone number to call in case of an emergency 101, 103.

Class № 1. METHODS IN DIAGNOSTIC MICROBIOLOGY. MICROSCOPIC METHOD OF EXAMINATION. BASIC MORPHOLOGICAL FORMS OF BACTERIA. SIMPLE METHODS OF STAINING

The subject to study:

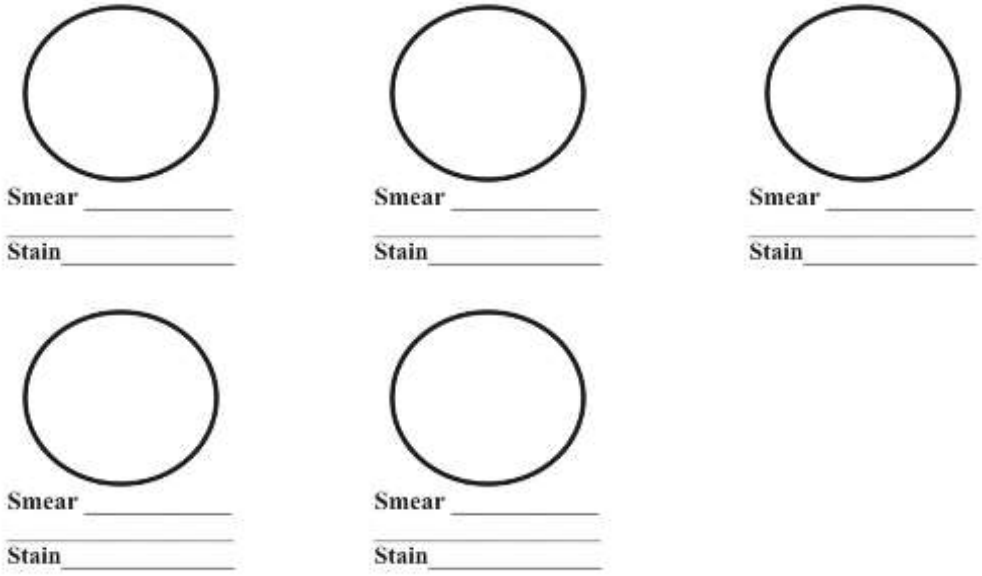
History of the department microbiology, virology, immunology; main spheres of activity and trends in research. Design and equipment of microbiological laboratory, biosafety levels. Basic rules of work in microbiological laboratory (biosafety in work with class I biohazards). Universal precautions in work with burners and electric supplies.

Taxonomy of microorganisms: classification and nomenclature. Modern approaches to taxonomy of microorganisms. Taxonomic ranks. Vars (types), strains, clones, pure cultures.

Basic morphological forms of bacteria. Morphological characteristics of cocci, rods and spiral-shaped bacteria.

Microscopic method of examination: tasks, procedure, evaluation of the method. Bright-field light microscope: components and proper use of the microscope. Smear preparation and fixation. Simple methods of staining. The technique of oil immersion microscopy.

Laboratory work

Laboratory exerscises	**Laboratory report**
1. Prepare heat-fixed slide of *Escherichia coli*, cultured on agar medium, stain with methylene blue, examine under the oil immersion lens and complete the report. 2. Prepare heat-fixed slides of *Staphylococcus spp.*, cultured on liquid medium, stain with basic fuchsin, examine under the oil immersion lens and complete the report. **Demonstration:** 1. *Streptococcus spp.*, pure culture, stained with crystal violet. 2. *Vibrio spp.*, pure culture, stained with basic fuchsin. 3. *Bacillus spp.*, pure culture, stained with crystal violet.	 **Signature of the tutor _____ Date ____/____/2024**

BACTERIOSCOPIC METHOD

Bacterioscopic method is a set of techniques for detecting and studying of morphological and tinctorial (staining) properties of bacteria in the specimens by microscopy.

Specimens: laboratory culture, pathological samples, samples from the environment.

The steps of the method:

1. Specimen collection (pus, sputum, blood, urine, feces, aspirates from the bronchi and stomach, the liquor, the nasal washes, autopsy, etc.).
2. Transporting the material to the laboratory in special containers.
3. Preparation of smear (if necessary, fixation and staining).
4. Microscopy of the slide.
5. Report.

Types of microscopic preparations:

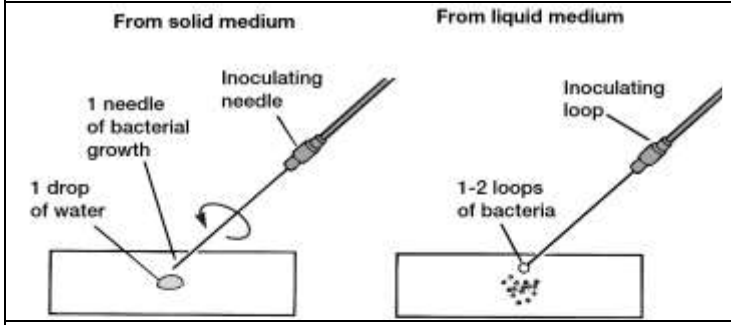
1. **Native preparations** (***wet-mount; hanging drop***) are used to observe living bacteria. The poor contrast of such preparations makes it necessary to amplify this aspect (darkfield and phase-contrast microscopy). They are often used to determine the active motility of bacteria.
2. **Fixed (stained) preparations** (*bacteriological smear; thin smear; thick drop of blood, etc.*) are richer in contrast.

BACTERIAL SMEAR PREPARATION

The main purpose — to establish the etiology of the disease, as well as determination of purity isolated pure culture.

A bacterial smear — a small amount of microorganisms spread in a very thin film on the surface of the slide**. A good smear is one that appears as a *thin whitish layer of film* when dry.**

Smears from broth cultures and cultures from solid media require different preparation techniques:



a) Broth cultures:

1. Using a sterile inoculating loop, apply one to two drops of broth culture to the center of the slide and gently spread the liquid on the surface of the slide (about 1 cm in diameter).
2. Allow the smear to air dry completely.

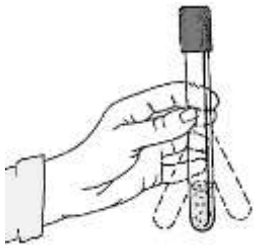
b) Cultures from solid medium:

These cultures must be diluted:

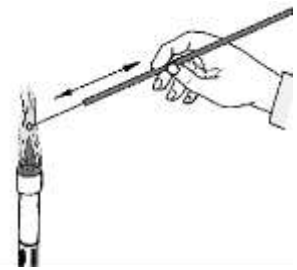
1. Place a drop or two of 0.9 % NaCl solution in the center of a glass slide.
2. Take some of the culture with a sterile bacterial loop and spread it in a drop of saline (about 1 cm in diameter).
3. Allow the smear to dry completely.

Aseptic procedure for bacterial smear preparation:

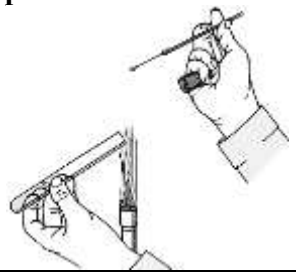
1. Shake the culture from side to side to suspend organisms. **Do not moisten cap on tube.**



2. Heat the loop and wire to red-hot. Flame the handle slightly also.

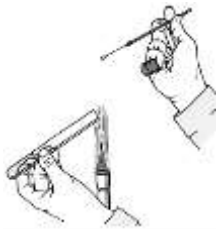


3. Remove the cap and flame the neck of the tube. **Do not place the cap down on the table.**



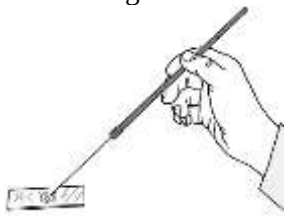
4. After allowing the loop to cool for at least 5 seconds, remove a loopful organisms. **Avoid touching the sides of the tube.**

5. Flame the mouth of the culture tube again.

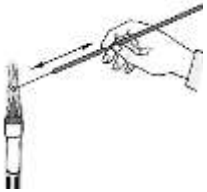


6. Return the cap to the tube and place the tube in a test-tube rack.

7. Place the loopful of organisms in the center of the target circle on the slide.



8. Flame the loop again before removing another loopful from the culture or setting the inoculatig loop aside.

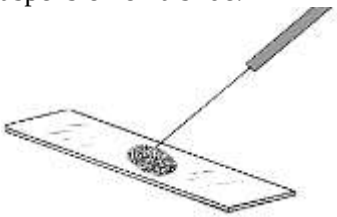


FIXATION

Fixation is necessary to attach microorganisms to the glass slide and preserve their structure in its original state.

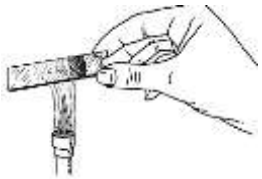
1. Heat fixation is performed by the rapid passage on the air-dried smear two or three times over the flame of the burner:

1. Application of a bacteria suspension on a slide.



2. Air-drying.

3. Fixation. Slide is passed through flame severel times to heat-kill and fix organisms to slide.



2. Chemical fixation used to protect cell morphology as well as cell fine substructures of the more delicate microorganisms.

Chemical fixative gets inside the cells and cross-links components present in cells, such as most proteins and nucleic acid.

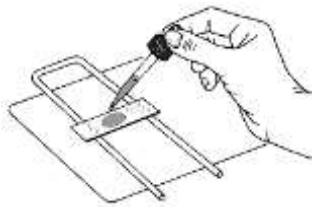
Fixatives are acids and aldehydes such as acetic acid, glutaraldehyde, etc.

SIMPLE STAINS

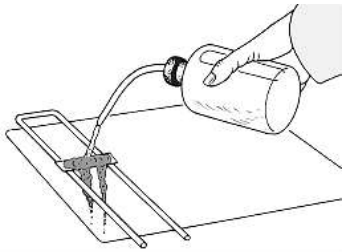
A simple stain is using an aqueous or alcohol solution of **a single dye**. Some of the common simple stains in the lab are *methylene blue (aqueous), crystal violet (an alcohol solution) and basic or carbol fuchsin.*

Simple Stain Technique:

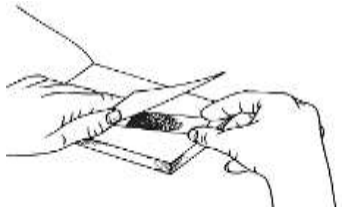
1. Apply dye to the fixed smear for the required time.



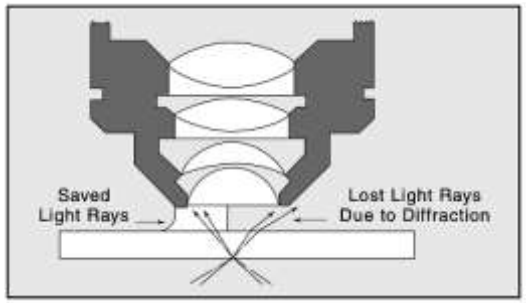
2. Rinse with tap water.



3. Place the smear in an upright position, allow excess water to drip off and allow the smear to dry. Alternatively, you can blot dry.



4. Examine the stained smear under a **100× (oil) immersion microscope objective.**



Immersion oil, having the same refractive index as glass, prevents light loss to diffraction.

Class № 2. BACTERIOSCOPIC RESEARCH METHOD. THE STRUCTURE OF THE BACTERIAL CELL. COMPLEX METHODS OF STAINING. FEATURES OF MORPHOLOGY AND METHODS OF STUDYING SPIROCHETES, RICKETTSIA, CHLAMYDIA, MYCOPLASMAS

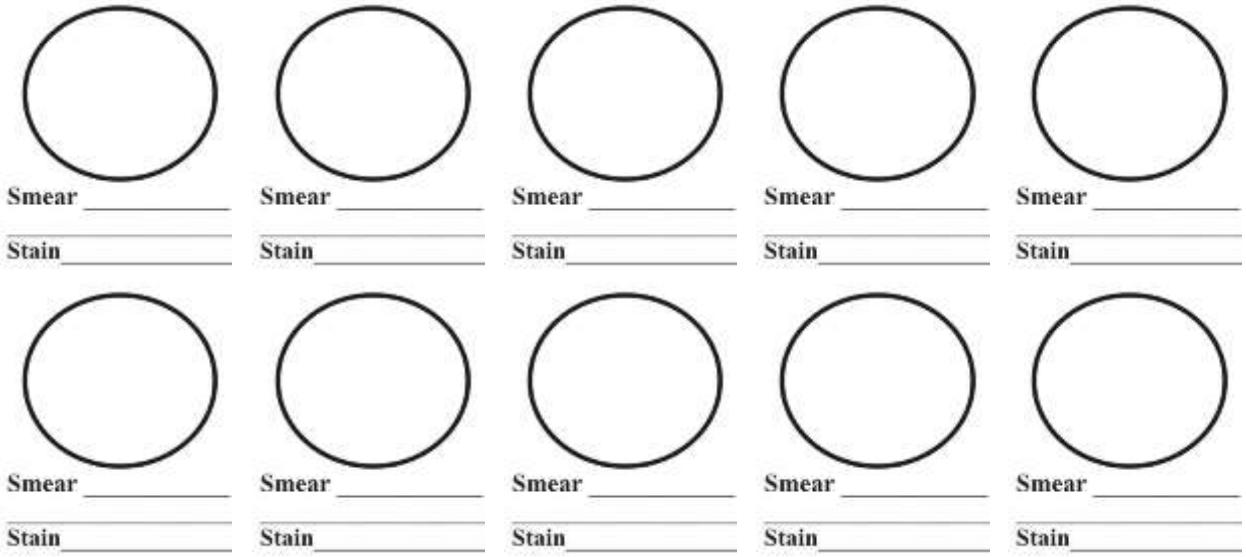
The subject to study:

Distinctive features of prokaryotic and eukaryotic cells. Basic bacterial cell structure. The composition, function, detection methods of bacterial cell wall. Gram stain: medical application, principles, procedure for Gram stain. Bacterial forms with defective cell wall. The composition, function of capsule, flagella, pili (fimbriae) and methods for their detection. The cytoplasmic membrane: structure, function. Bacterial core: cytoplasm, cytoplasmic structures. Inclusion bodies — storage granules. Methods for nucleoid and volutin detection. Loeffler and Neisser stain for volutin granules. Acid-fast bacteria and unique properties of their cell wall. Ziehl–Neelsen acid-fast staining.Resting (dormand) forms of microorganisms. Spore stain using Ozheshko method: principle, procedure.

Taxonomy, morphology, medical significance of the spirochetes. Methods for spirochetes morphology study. Romanowsky–Giemsa stain. Actinomyces: taxonomy, morphology, medical significance. Rickettsiae: taxonomy, morphology, medical significance and methods of examination. Chlamydiae: taxonomy, morphology, reproduction cycle (elementary and reticulate body), medical significance and methods of examination. Mycoplasmas: taxonomy, morphology, medical significance, methods of examination.

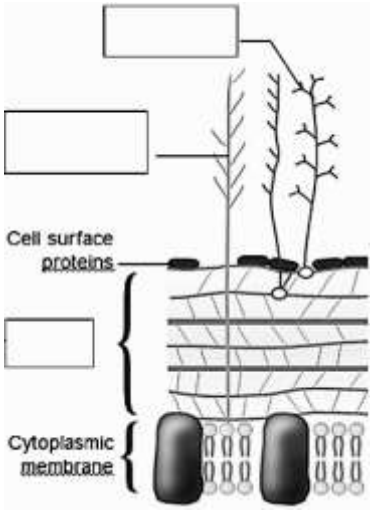
Methods for the motility detection of the living bacteria. Preparation of the hanging-drop and wet-mount slides. Dark-field light microscopy. Phase-contrast light microscopy. Fluorescence microscopy.

Laboratory work

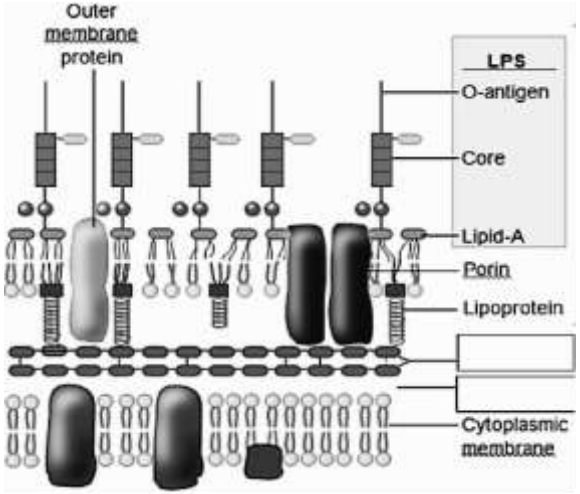
Laboratory exerscises	Laboratory report
1. Prepare heat-fixed slide of the mixed culture of *Escherichia coli (*gram-negative) and *Staphylococcus aureus* (gram-positive), Gram stain, examine under oil immersion, complete the report. 2. Prepare slide of *Rickettsia spp*., stain with fuschin, complete the report. 3. Prepare the hanging-drop slide using motile microorganisms, examine native microorganisms under microscope. **Demonstration:** 1. Slide with capsule of Klebsiella pneumonia, negative staining. 2. Slide with volutin granules of Corynebacterium diphtheria, *Loeffler staining.* 3. Slide with volutin granules of Corynebacterium diphtheria, *Neisser staining*. 4. Slide with spores of Bacillus anthracis, Ozheshko staining. 5. Slide with Treponema denticola in dental plaque, Gram staining. 6. Leptospira spp., dark-field mycroscopy. 7. Slide with Actinomyces spp., pure culture, Gram staining.	 **Signature of the tutor _____ Date ____/____/2024**

Write down the names of the cell wall components of:

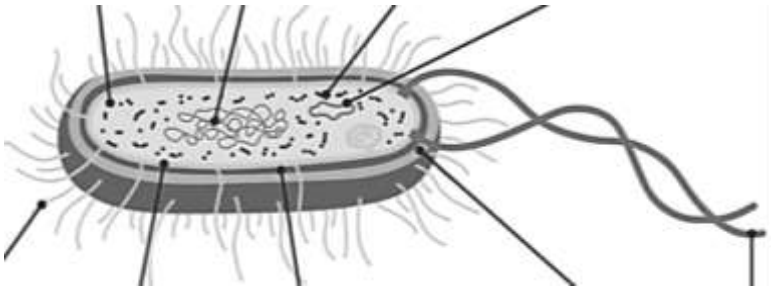
Gram-_____ bacteria



Gram-_____ bacteria



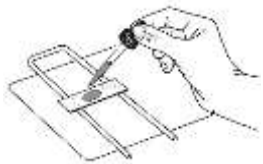
Indicate the components of bacterial cell: structures:



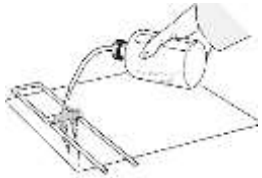
1 — Cell wall
2 — Cell membrane
3 — Cytosol
4 — Capsule
5 — Ribosome
6 — Nucleoid
7 — Plasmid
8 — Pili
9 — Flagellum

GRAM'S STAIN TECHNIQUE

1. CRYSTAL VIOLET
1–2 minutes

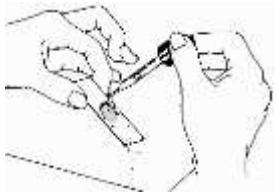


2. WASH

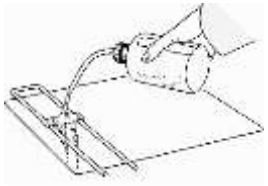


3.GRAM'S IODINE
1 minute

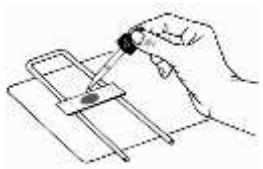
4. DECOLORIZE WITH ALCOHOL
30–45 seconds or until solvent flows colorlessly



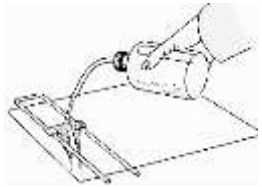
5. WASH



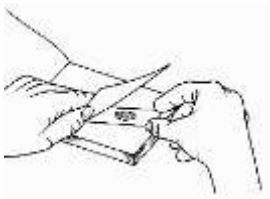
6. FUCHSINE
1–2 minutes



7. WASH



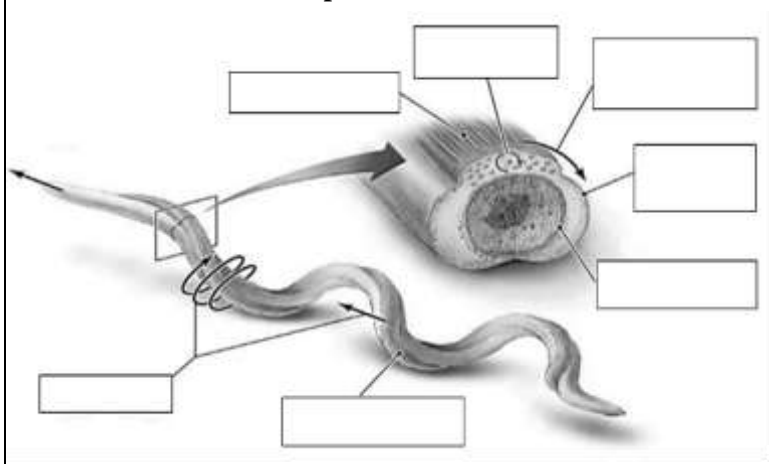
8. BLOT DRY



Specify the color of the smear at each step:

Steps of Gram Staining	Gram-positive	Gram-negative
Smear Preparation / Hit fixation		
Primary Stain (Crystal violet)		
Gram'sIodine		
Alcohol (95 %)		
Secondary Stain (Fuchsin)		
Example	*S. aureus*	*E. coli*

Write the names of the spirochete structures:



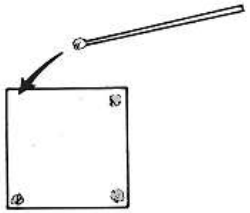
Differentiation of pathogenic spirochetes:

Features		Spirochetes genera		
		Treponema	***Borrelia***	***Leptospira***
Size	Length	5–20	3–20	7–14
	Width	0.09–0.5	0.2–0.5	0.1–0.15
Coils number				
Shape				
Romanovsky–Giemsa staining				

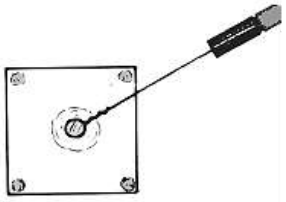
Method	Principle and application
Direct Examination	
Wet mount Handing drop	Unstained preparation is examined by brightfield, darkfield, or phase-contrast microscopy
India ink	*Negative staining* with India Ink is used to detect *capsules*
Differential stains	
Gram stain	Most commonly used stain in microbiology laboratory, forming basis for separating major groups of bacteria (e.g., gram-positive, gram-negative)
Silver impregnation method	Cells and structures that *are too thin* to be visualized by the light microscope can be rendered visible by impregnation of silver on their surface. Silver impregnation method is a common method used for staining *spirochetes and some small bacilli*
Romanowski–Giemsa stain	Used to detect of many bacteria (e.g., spiral-shaped borreliae, treponemas, or leptospira) as well as for protozoans, and mammalian cells (e.g., for blood cell count). Giemsa stain combines methylene blue and eosin. Eosin ions are negatively charged and stain basic components of cells orange to pink, whereas other dyes stain acidic cell structures various shades of blue to purple
Ziehl–Neelsen stain	Acid-fast stain. Used to differentiate acid-fast & non-acid fast bacteria
Ozheshko stain	Endospore stain. Used to detect the *endospore*
Special stains	
Loeffler and Neisser stain	Used to detect the *volutin granules*

Preparation of a hanging-drop slide:

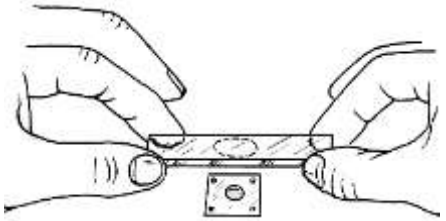
1. A small amount of Vaseline is placed near each corner of the cover glass with a toothpick.



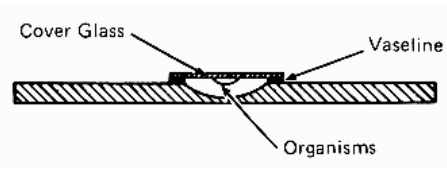
2. Two loopfuls of organisms are placed in center of cover glass.



3. Depression slide is pressed against Vaseline on cover glass and quickly inverted.



The completed preparation can be examined under oil immersion.



Class № 3. ANTIMICROBIAL MEASURES: METHODS OF STERILIZATION AND DISINFECTION, ANTISEPTICS, ASEPSIS. CULTURAL (BACTERIOLOGICAL) RESEARCH METHOD. METHODS FOR ISOLATING PURE CULTURES OF BACTERIA

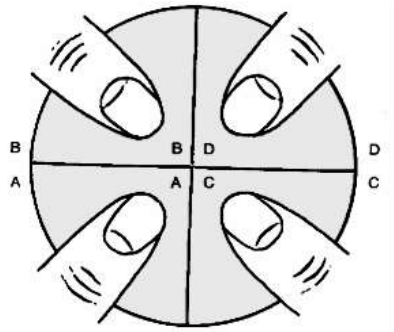
The subject to study:

Definition of terms asepsis, sterilization, disinfection, antisepsis. Methods of sterilization: physical, chemical, mechanical. Differences between sterilization and disinfection. Types and methods of disinfection. Practical disinfection. Types and methods of antisepsis. Practical antisepsis. Biocides. Classification of disinfectants. Classification of antiseptics, origin and characteristics of groups. Mechanisms of action on microorganisms. Sterilization, disinfection and antisepsis quality control. Antimicrobial management in hospitals.

Cultivation of microorganisms. Conditions required for growth. Nutrient media for culturing bacteria: classification and characteristics. Culture media ingredients, procedure of preparation and sterilization. General requirements to bacteriologic nutrient media. Incubator.

Bacteriological method of laboratory diagnosis: tasks, procedure, evaluation of the method. Methods of isolation of aerobic and anaerobic microorganisms in pure culture. Streak-plate technique. Bacterial colony characteristics on agar media (form, size, elevation, margin, consistency, surface, texture, density). Cultivation of anaerobic bacteria: culture media, techniques, equipment.

Laboratory work

Laboratory exerscises	Laboratory report
1. Test the effectiveness of hygienic and surgical hand antisepsis.	Test the effectiveness of hygienic and surgical hand antisepsis: 1. Divide a nutrient agar plate into four sections with a marking pen or pencil. Label each section of the plate with numbers № A, B, C, D. 2. Label each plate with your group number and your name. 3. On the surface of agar medium at section № A make a fingerprint of skin **untreated with any antiseptic (control).** 4. **Wash your finger with soap** and make a fingerprint on the surface the agar medium at section № B. 5. **Treat your other finger with antiseptic** (1 % solution of iodopyron) — 2 minutes, **neutralize iodopyron with neutralizer** (1 % solution of sodium thiosulfate) for 2 minutes and make a fingerprint on the surface of agar medium at section № C. 6. **Wash your hands with soap twice and treat the fingers with antiseptic** (1 % solution of iodopyron) — 2 minutes, **neutralize iodopyron with neutralizer** (1 % solution of sodium thiosulfate) for 2 minutes and make a fingerprint on the surface of agar medium at section № D. 7. Incubate Petri dishes at 37 °C for 24 hours. 

8. After incubation count the amount of colonies grown at each section and fill in the table. Formulate the conclusion regarding effectiveness of hygienic and surgical hand antisepsis.

Results of the experiment:

Section	Experiment description	Quantity of CFU
A	Control	
B	Washing with soap (hygiene)	
C	Antisepsis with antiseptic iodopyron	
D	Surgical hand antisepsis	

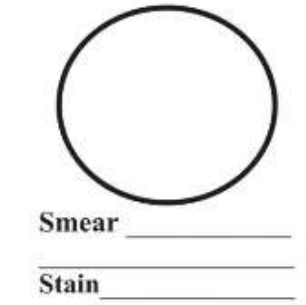
Conclusion _____

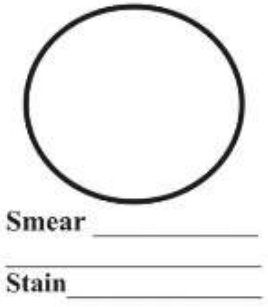
2. Perform 2nd period of bacteriological diagnosis (inspection and accumulation of pure cultures isolation of aerobic microorganisms):

1. Characterize morphology of two different types of colonies present on agar medium.
2. Determine morphology and purity of two different types of colonies using Gram stain.
3. Use aseptic technique and transfer the colony of Gram-negative microorganisms for subculturing on a surface of agar slant for microbial biomass accumulation.

2nd period of bacteriological diagnosis (inspection and accumulation of pure culture)

Colony morphology	№ 1	№ 2
Form		
Size		
Elevation		
Margin		
Color		
Texture		





Signature of the tutor _____ Date ____/____/2024

BACTERIOLOGICAL METHOD

Bacteriological method — the method based on pure culture isolation using bacteriological media and identification of pure culture by detection of morphological, biochemical, antigenic, genetic, biological properties of isolated pure culture.

1. Preliminary analytical step

1. Specimen collection.
2. Transportation to the lab.

2. Analytical step

The method of five I's — inoculation, incubation, isolation, inspection, and identification

First day (1st period)

Second day (2nd period)

Third day (3d period)

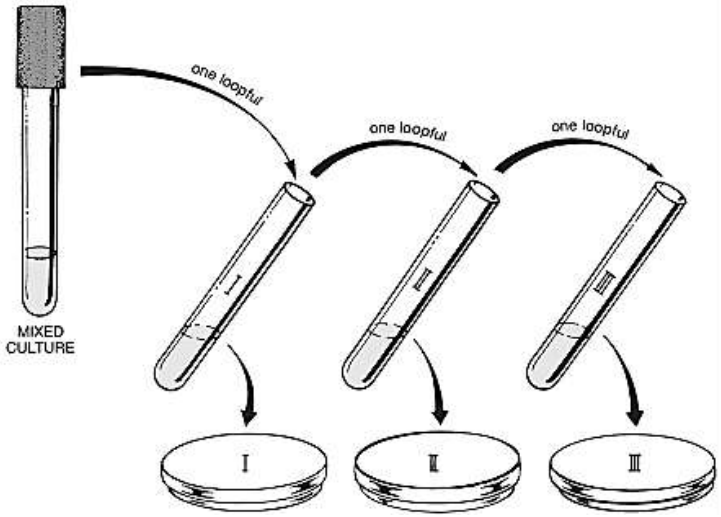
Fourth day

Streak Plate Technique

Forms of nutrient medium:

Slant/ Deep | **Slant** | **Deep** | **Plate** | **Broth** | **Broth + durham tube**

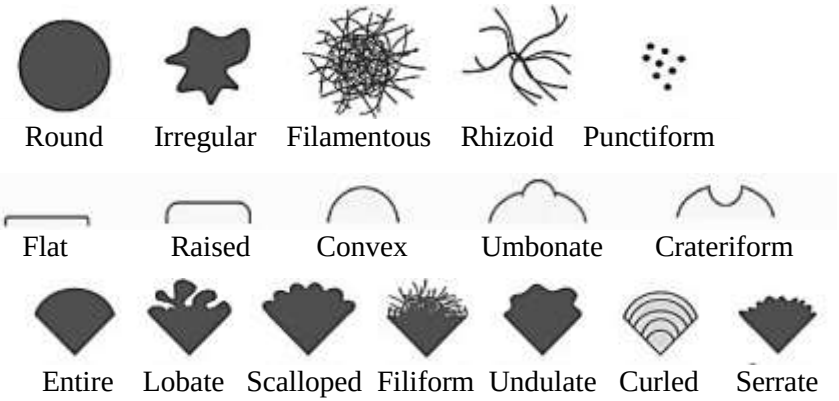
Loop dilution technique for separating out organisms:



Describing colony morphology:

Size:
Punctiform: < 1 mm
Small: 1–2 mm
Moderate: 3–4 mm
Large: > 5 mm

Texture:
Dry
Smooth
Viscid
Mucoid

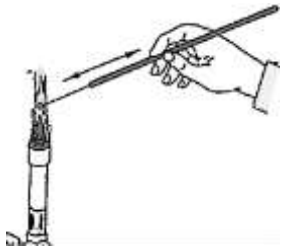


S- and R-colonies:

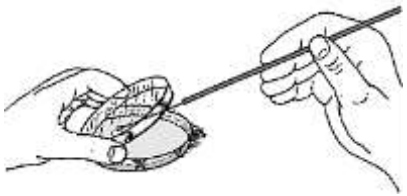
Morphological features	Colony type	
	S	R
Form		
Size		
Margine		
Elevation		
Surface		
Texture		

Technique of seeding by loop from Petri dish to tube:

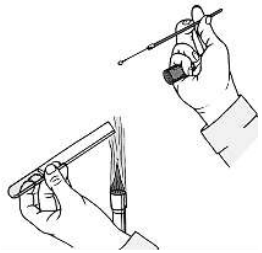
1. Inoculating loop is heated till red-hot.



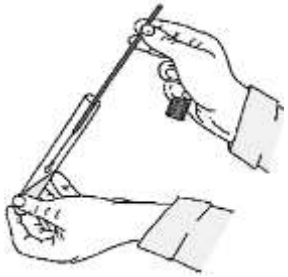
2. With free hand, raise the lid of the Petri plate just enough to access a colony to pick up a loopful of organisms.



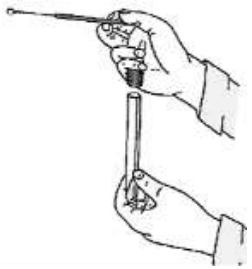
3. Remove the cap and flame the neck of the tube. **Do not place the cap down on the table.**



4. Streak surface of a sterile slant.



5. Flame the mouth of the tube and re-cap the tube.



6. Flame the inoculating loop and return it to receplacle.

Define the terms:

Specimen — _____

Bacterial colony — _____

Pure culture — _____

Cultivation of anaerobic bacteria:

Specimen collection: _____

Anaerobic media: _____

Equipment: _____

Define the terms:

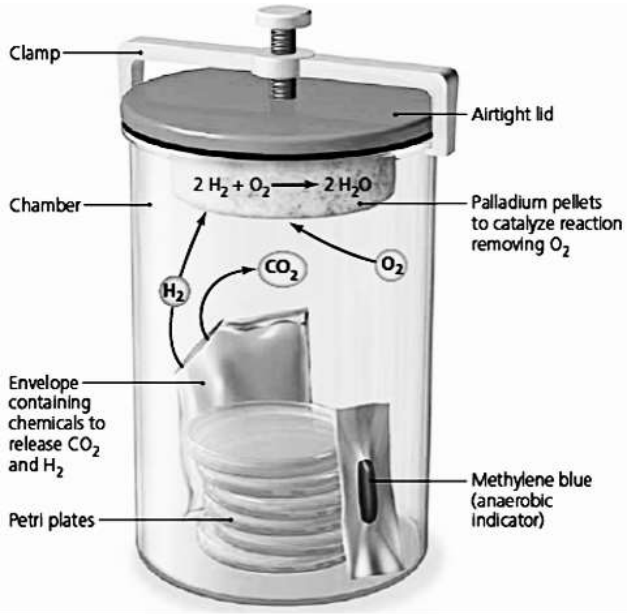
Sterilization — _____

Disinfection — _____

Antisepsis — _____

Asepsis — _____

Anaerobic jar



Enter in cells possible methods of sterilization:

Cotton, bandage	
Plastic products	
Glass products	
Rubber products	
Air (in operating room)	
General-purpose media	
Enriched media with serum or blood	
Solution which is inactivated at above 60 °C	

Modes of action of disinfectants and antiseptics:

Disinfectants/Antiseptics	**Mode of action**

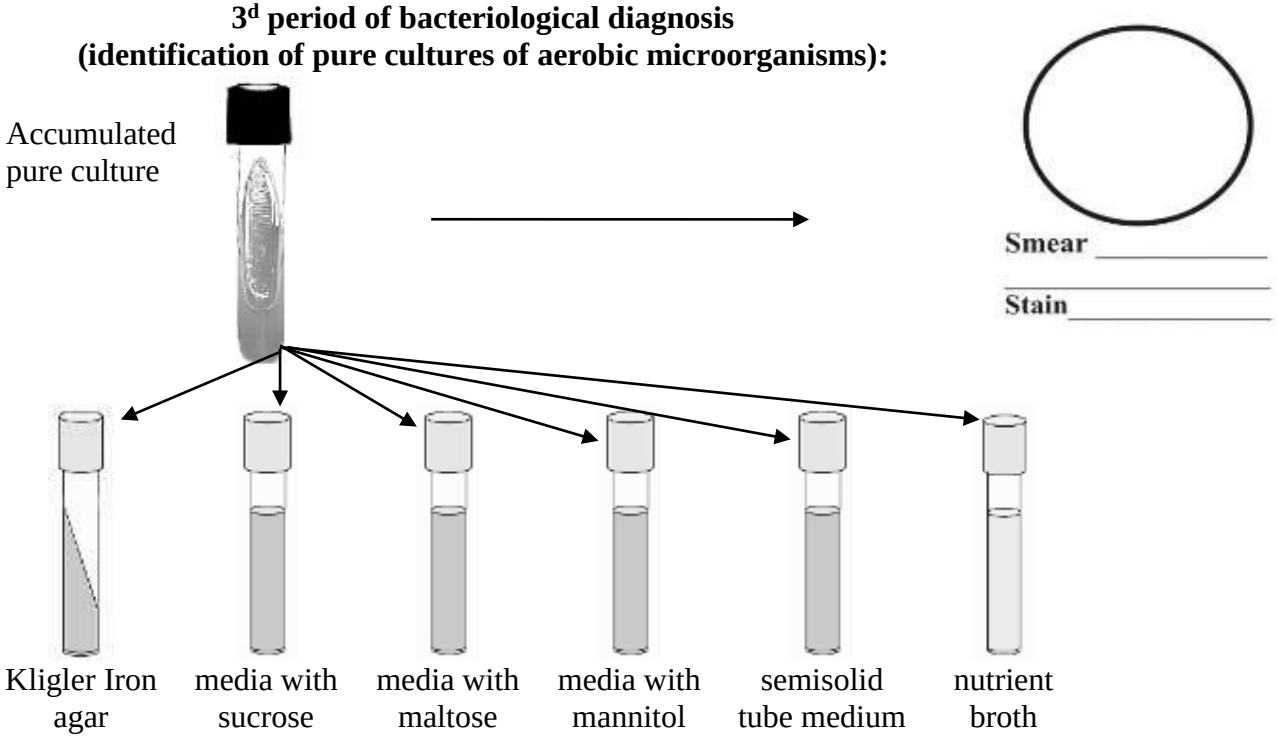
Class № 4. CULTURAL (BACTERIOLOGICAL) RESEARCH METHOD. METHODS FOR IDENTIFICATION OF PURE CULTURES OF BACTERIA

The subject to study:

Identification of microorganisms: approaches and methods. Bacterial species: definition of the term, species criteria and methods for discovering bacterial species. Using Bergey's manual of systematic bacteriology to identify bacteria.

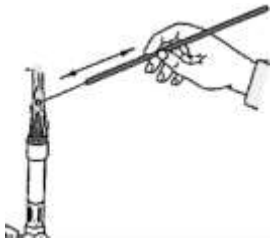
Biochemical activities of bacteria and methods for the biochemical properties' detection of microorganisms. Enzymes of microorganisms: classification, importance for identification: a) proteolytic (proteases, peptidases, decarboxylases, deaminases, cysteine desulfurase, urease, tryptophanase); b) carbohydrate hydrolyses (carbohydrase, amylase); c) lipolytic (lipases, lecithinase); d) oxidative-reductive (dehydrohenase, oxidase, catalase); e) hemolysins; α-, β-, γ-hemolysis. Rapid multitest systems for identification of microorganisms. Automatic bacteriological analyses: structure and principle of bacterial identification.

Laboratory work

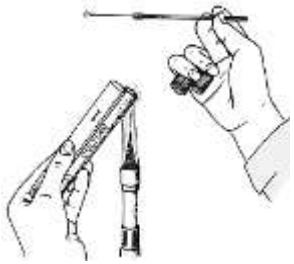
Laboratory exerscises	**Laboratory report**
1. Register the results of experiment on antisepsis (see classes № 3). 2. Perform 3d period of bacteriological diagnosis (identification of pure cultures of aerobic microorganisms). **Demonstration:** 1. Semisolid and liquid Hiss media with different pH indicators. 2. Hemolysis on blood agar medium, lecitinase activity, indol detection. 3. Biochemical activity of different enterobacteria on Kligler Iron agar. 4. Rapid multitest systems for identification of microorganisms.	**3d period of bacteriological diagnosis (identification of pure cultures of aerobic microorganisms):**  **Signature of the tutor _____ Date ____/____/2024**

Technique of seeding by loop from the tube to tube:

1. Inoculating loop is heated till red-hot.

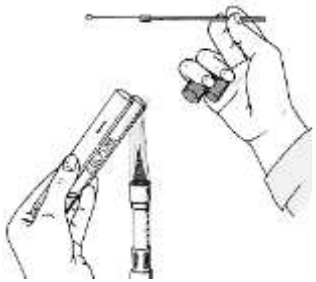


2. Flame necks of the tubes.



3.Transfer one loopful of the culture to tube 1.

4. Flame Flame necks of the tubes.



5. Replace the caps on the tubes and return the culture to the test-tube rack.

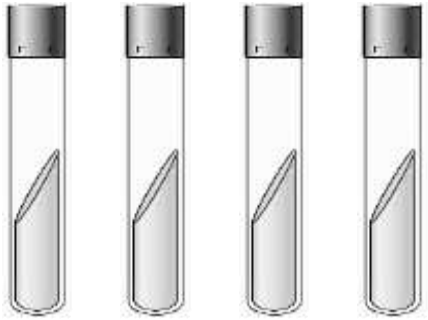
6. Flame the inoculating loop and return it to receplacle.

Detection of biochemical properties of microorganisms:

Enzymes	Media	Positive reactions
Carbohydrases	Differential media with lactose and dyes (Endo, Levin, McConkey agar)	Escherichia coli, Klebsiella oxytoca, K. pneumonia which ferment lactose will grow with colored colonies (purple or violet), Salmonella, Shigella which do not ferment lactose will grow with pale colonies
	Triple Sugar Iron (TSI) Agar is used for the detection of 3 things: (1) carbohydrate fermentation, (2) gas production, and (3) hydrogen sulfide production. Carbohydrate fermentation: Fermentation of the carbohydrates into acid end-products will result in a yellow color. If glucose alone is fermented the slant becomes yellow, the butt stays red. If lactose is fermented the butt becomes yellow. Gas production: Production of gas (primarily CO_2) during fermentation can be determined by observing the tube for bubbles or cracks. Hydrogen sulfide production: If the microorganism produces hydrogen sulfide, it will utilize the sodium thiosulfate as a substrate, producing H2S which reacts with the ferrous sulfate to cause a black precipitate	
	Liquid or semisolid media with one type of sugar and indicator of pH. For detection of gas production a vacuole is added to liquid media	Utilization of sugars results in acid or acid plus gas production. Acidification of media is detected by pH indicator which changes its color. In case of gas production it is accumulated in vacuole and seen as bubbles, in semisolid media gas leads to holes in media or bubbles
Indol production	Indole is a by-product of the metabolic breakdown of the amino acid tryptophan used by some microorganisms. Nutrient bullion or medium with tryptophan and indicator paper with oxalic acid fixed under the cap of the tube	The presence of indole in a culture grown in a medium containing tryptophan can be readily demonstrated by the formation of a red colored indicator paper. If indole is present, it combines with the oxalic acid on the surface of indicator paper to produce a pink-red color
Lecithinase	Egg yolk agar (one chicken egg yolk is added to 300 ml of sterile and melted nutrient agar cooled to 45–50 °C)	Organisms (Staphylococcus aureus, Clostridium spp., Fusobacterium spp.) which produce lecithinase and are able to hydrolyze lecithin will show a zone of cloudiness (opacity) around the growth of the microorganism

Enzymes	Media	Positive reactions
Catalase	A drop of 3 % hydrogen peroxide on a glass slide	The enzyme catalase catalyzes the conversion of hydrogen peroxide to water and oxygen. When a colony is placed in hydrogen peroxide, liberation of oxygen as gas bubbles can be seen. The test is particularly useful in differentiation of staphylococci (positive) from streptococci (negative)
Hemolysines Media: 5–10 % blood agar	α-hemolysis — partial hemolysis — alpha-hemolytic microorganisms will show a greenish zone around the growth of the microorganism meanwhile the rest of medium will stay red; β-hemolysis — complete hemolysis — beta-hemolytic microorganisms will show a zone of clearing around the growth of the microorganism meanwhile the rest of medium will stay red; γ-hemolysis — absence of visible hemolysis	

Draw the indicated biochemical properties of bacteria:



gl+; l+; H_2S –

gl–; l+; H_2S –

gl–; l–; H_2S +

gl+; l–; H_2S –

Lecithinase +

α-hemolysis

β-hemolysis

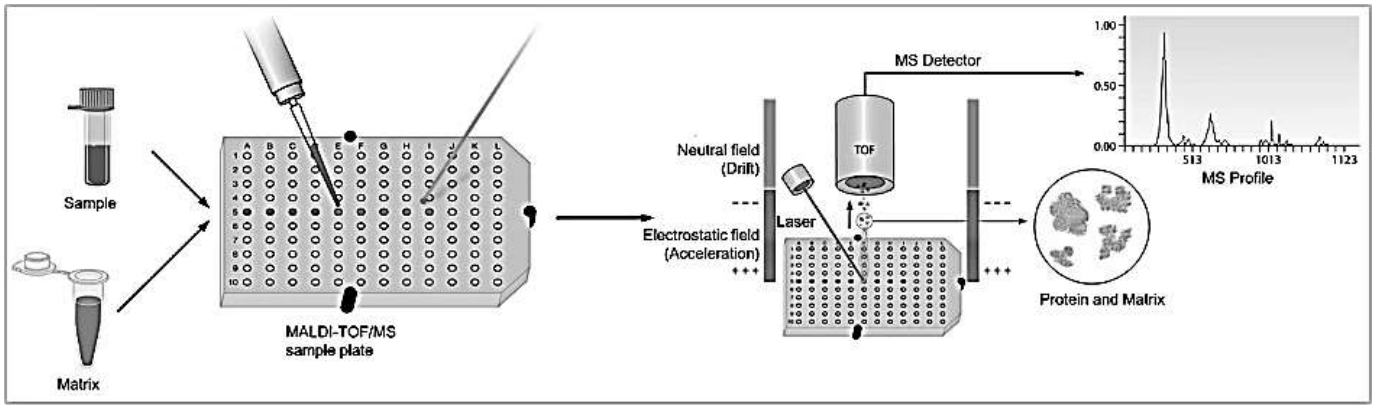
Catalase-positive and Catalase-negative test

Lactose-positive and Lactose-negative colonies grown on Endo agar

Matrix-**A**ssisted **L**aser **D**esorption/**I**onization **T**ime-**O**f-**F**light **M**ass **S**pectrometry **(MALDI-TOF MS)** is a soft ionization technique allowing desorption and ionization of biomolecules, such as proteins and peptides (mainly of ribosomal origin), in a non-destructive manner and couples high sensitivity with accuracy.

The generated ions provide a peptide fingerprint, i.e. mass spectrum, that can be used to characterize and identify bacteria at genus/species-level, provided that the species is represented in the identification database.

This is achieved by comparing the mass spectra generated of unknown bacterial strains to a reference database containing mass spectra generated from well-characterized bacterial strains.



Class № 5. GENETICS OF MICROORGANISMS. METHODS FOR STUDYING THE GENETICS OF BACTERIA. METHODS OF MOLECULAR DIAGNOSTICS

The subject to study:

The structure of bacterial genetic apparatus. Phenotype, genotype, genome, genes. Regulation of gene expression. General properties and varieties of plasmids. Detection of plasmids. Mobile genetic elements: transposons and IS elements.

Bacterial varibility: phenotypic and genetic. Practical significance of bacterial variability. Mechanisms of genetic variability: Mutation and recombination. Classification of mutations. Methods of mutant bacteria selection. Horizontal gene transfer: transformation, transduction, conjugation.

Molecular methods: tasks, specimens for investigation, advantages of the methods. Classification of molecular methods. Molecular hybridization: test materials, DNA extraction, components of DNA hybridization reaction, molecular probes, detection of DNA hybrid duplexes, interpretation of results. Equipment. Practical application of molecular hybridization method. Polymerase chain reaction (PCR): test materials, principle, DNA extraction, components of PCR reaction mixture, primers, PCR termal cycle, detection of amplicons, interpretation of results. Equipment for PCR. Practical application of PCR. Genomics. Bioinformatics. Genetic engineering. Gene Cloning.

Laboratory work

Laboratory exerscises

1. Identify isolated pure culture and complete the final report:

a) Register the biochemical properties of tested pure culture in table.

b) Analyze the results and determine the specie of tested pure culture.

Laboratory report

Species	Morphology	Cultural characterristics	Biochemical characteristics							Motility
			Glucose	Lactose	Maltose	Mannitol	Sucrose	H_2S	Indole	
E. coli	Rods, Gr–	S-colonies	AG	AG	AG	AG	–	–	+	+
S. typhi	Rods, Gr–	S-colonies	A	–	A	A	–	+	–	+
S. paratyphi A	Rods, Gr–	S-colonies	AG	–	AG	AG	–	–	–	+
S. schottmuelleri	Rods, Gr–	S-colonies	AG	–	AG	AG	–	+	–	+
X-microbe										

Report: According to morphological, cultural, biochemical properties X-microbe is attributed to _____

1. Perform a bacterial conjugation experiment:

a) Prepare the mating mixture by aseptically transferring 0.5 ml of an overnight meat-pepton brothculture of donor and recipient *E. coli* into separate tube.

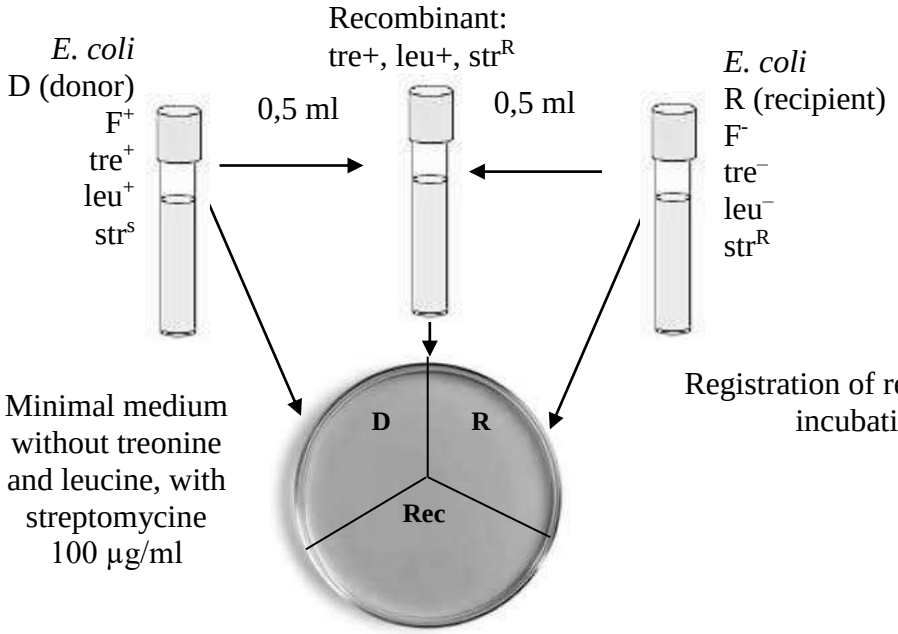
In bacterial conjugation experiment donor *E. coli* is susceptible to streptomycine and synthesize treonine and leucine. Recipient *E. coli* displays complementary properties: resistant to streptomycine and unable to synthesis treonine and leucine. Recombinants of these two strains will have combination of either the donor or recipient strains' characteristics and can be readily detected by using selective minimal media.

b) Mix and incubate at 37 °C for 1 hours.

c) Confirm the resistance status and leucine and treonine production by culturing donor, recipient and recombinant E. coli on minimal medium supplemented with streptomycin.

Demonstration:

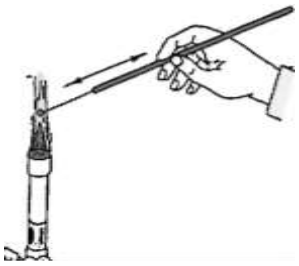
1. Replica method for bacterial mutant forms selection.



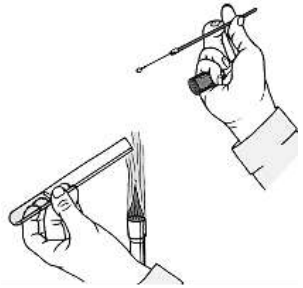
Conclusion _____

Technique of seeding by loop from tube to Petri dish:

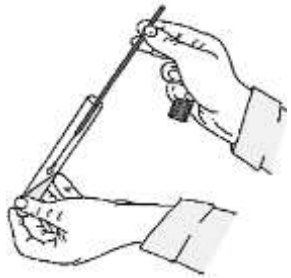
1. Inoculating loop is heated till red-hot.



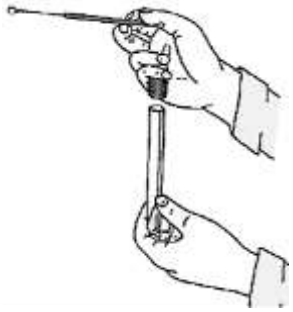
2. Remove the cap and flame the neck of the tube. **Do not place the cap down on the table.**



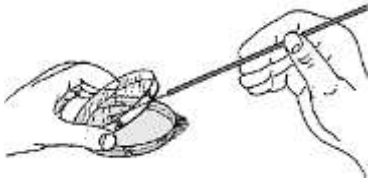
3. Streak surface of a sterile slant.



4. Flame the mouth of the tube and re-cap the tube.



5. With free hand, raise the lid of the Petri plate just enough to access a colony to pick up a loopful of organisms.



6. Flame the inoculating loop and return it to receplacle.

Signature of the tutor _____ **Date** ____/____/**2024**

MOLECULAR GENETIC ANALYSIS

Molecular genetic analysis is highly sensitive and specific method of the investigation of genomic structure and function. Molecular genetic analysis is the most reliable test for detection of presence of pathogenic agents in clinical specimen.

Polymerase Chain Reaction (PCR)

Polymerase chain reaction allows amplification of exactly specific DNA fragments. About one billion copies (amplicons) of tested DNA fragment can be produced in one hour starting from initial single DNA.

PCR is elaborated in several stages:

1. First, DNA is isolated from a cell and heated to approximately 95–97 °C, causing the separation of two DNA strands breaking down the hydrogen bonds between A-T and G-C (DNA melting).

2. In the second step, the temperature is decreased to about 65–70 °C. It allows the attachment of two short specific fragments of DNA, termed primers. The primers (forward and reverse) are complementary to the amplified DNA sequence. They bind to the ends of complementary DNA (primer annealing) and play a role of initiators of DNA polymerization.

3. The third step (amplification) is the synthesis of complementary strands of new DNA molecules on the templates of both parental DNA strands. The process begins from the place of primer attachment. This reaction requires all number of nucleotide substrates and thermostable DNA-polymerase (Taq-polymerase).

After the first cycle of enzyme action the single original DNA is converted into two identical DNA molecules. Thus, the duplication of original genetic material is achieved. Next amplification cycle is stimulated by heating of the reaction mixture again up to 95–97 °C to dissociate all of existing strands of DNA. And the amplification cycle is repeated again. Each cycle of heating, cooling and doubling of tested DNA segment lasts about several minutes.

The registration of PCR results was primarily made by agarose electrophoresis of DNA amplification products (amplicons) followed by their fluorescent stain with DNA-specific fluorescent dyes (e.g., ethydium bromide, propidium iodide, Sytox Green and many others).

Sequencing methods determine the direct order of nucleotides in nucleic acid chains. This clarifies the organization of genes within microbial genome and allows to deduce the structure of corresponding gene products.

Molecular Hybridization of Nucleic Acids

Hybridization method is based on complementary interaction of single-stranded DNA or RNA molecules resulting in specific formation of double-stranded complex.

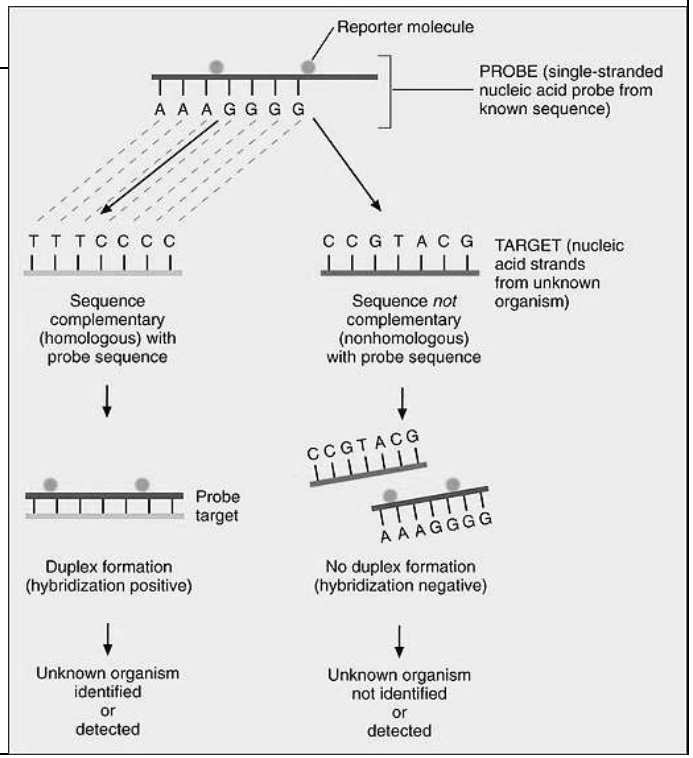
At the initial step of hybridization test (e.g., dot or spot hybridization) the sample, containing unknown nucleic acid sequence is heated to produce single-stranded DNA molecules (DNA melting).

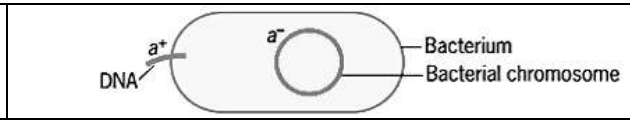
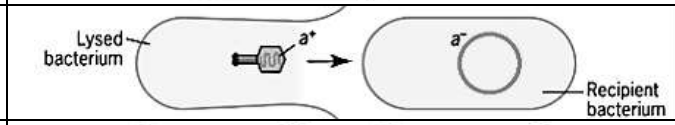
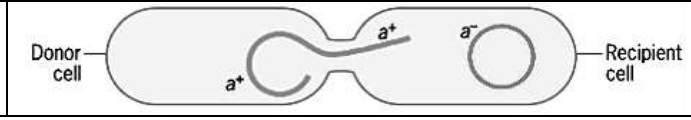
Then single-stranded DNA is adsorbed on some solid phase (e.g., nitrocellulose paper sheet).

Afterwards the sample is treated by specific hybridization probe (The probe is the known short sequence of one-stranded DNA molecule, complementary to investigated nucleic acid sequence and labeled with highly sensitive tag — fluorescent or chromogenic dye, or radiochemical label).

If the investigated specimen contains the nucleic acid of interest, the probe will bind to its complementary sequence.

After thorough wash the specimen fluorescence or radioactivity is analyzed. Positive samples demonstrate the increased levels of activity.



Comparison of transformation, transduction, and conjugation:			
Methods of transfer		**Mechanism**	**Nature of DNA transferred**
Transformation			
Transduction			
Conjugation			

The polymerase chain reaction (PCR):	
Stages	**Amplification**
Evaluation of method	**Practical application**

Class № 6. ECOLOGY OF MICROORGANISMS. METHODS OF HUMAN NORMAL FLORA INVESTIGATION. BASICS OF THE INFECTION DOCTRINE. BIOLOGICAL RESEARCH METHOD

The subject to study:

Ecology of microorganisms. Basic terminology of ecology. Interspecific and intraspecific relations. Symbiosis, its variants. Antagonistic microbial relationships, its background and medical importance. Bacteriocins. Diversity of microorganisms, inhabiting soil, water and air.

Diversity of normal flora at different sites of human body. Origin of the normal flora. Beneficial effects of the normal flora. Methods of normal flora investigation. Gnotobiology. Dysbacteriosis: aetiology, pathogenesis, symptoms, approaches to treatment, prophylaxis.

Basic terminology of infectiology. Definition of infection. Classification of infections.

Bacterial pathogenicity and virulence. Measurements of virulence: ID50, LD50, DLM. The Henle–Koch Postulates. The genetics of bacterial pathogenicity. Pathogenicity islands. Pathogenicity factors: adhesins, invasins, impedins, agressins, modulins. The role of bacterial biofilms. Methods of detection of adhesins, capsule, invasins, toxigenicity. Bacterial endotoxins and exotoxins.

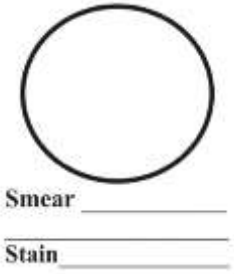
Laboratory work

Laboratory exerscises

1. Perform isolation of normal flora from skin and mucus membrane surfaces to gain an understanding of the diversity of microorganisms at these body locations and exclude/confirm disbacteriosis.
2. Prepare heat-fixed smear from dental plaque, Gram stain, explore under microscope, complete the report.
3. Register the reaults of bacterial conjugation experiment (see class № 5).

Demonstration:

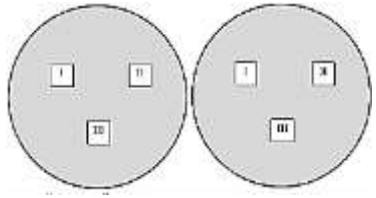
1. Slide with dental plaque, Gram stain.
2. Methods for detection of pathogenicity factors (capsule, hemolysins, lecithinase, cougulase).



Laboratory report

Experiment on normal flora isolation from skin and mucus membrane surfaces:

1. Divide agar plates into four sections with a marking pen or pencil. Label each section with 1, 2, 3, 4. Label each plate with group number and your name.
2. Add sterile isotonic solution to the Petri dish with sterile filter paper squares (1×1 cm).
3. Use flamed forceps to cover with the squares of filter paper for 0.5 min the various body sites which normal flora is to be investigated (hands, lips, forehead, mucus membranes of tong, cheeks).
4. Put the squares of filter paper for 1 minute on the surface of blood and MacConkey agar.
5. Fill in the table with the sites which microbial flora is under study.
6. Incubate the plates at 37 °C for 24–48 hours.



Blood agar Endo medium

Perform registration of results at class № 7:

Register the results of experiment on normal flora isolation from skin and mucus membrane surfaces, Gram stain different types of colonies, explore under microscope, complete the report.

Body site	I:		II:		III:	
Amount of colonies	Blood agar	Endo medium	Blood agar	Endo medium	Blood agar	Endo medium

Signature of the tutor _____ Date ____/____/2024

Beneficial effects of the normal flora:

1. The normal flora acts as nutrients and active metabolites source. Normal flora synthesize vitamins (B, K).
2. The normal flora confer the infection resistance via passive and active antagonism with pathogens:
   a. the normal flora prevent colonization by pathogens by competing for attachment sites or for essential nutrients (passive antagonism).
   b. the normal flora may antagonize other bacteria through the production of substances which inhibit or kill nonindigenous species (active antagonism). The intestinal bacteria produce bacteriocins (antibiotic like molecules, which inhibit the growth of closely related species).
3. The normal flora stimulate the immune system.
4. Detoxification some metabolites — hormones, bile salts etc.

Characteristics of Endotoxins (LPS) and Exotoxins:

Feature	Endotoxin	Exotoxin
Composition		
Effect on host		
Release of toxin		
Tissue affinity		
LD_{50}		

Define the terms:

Infection —	**Pathogen —**
Reinfections —	**Etiologic (causative) agent —**
Inapparent infection —	**Opportunistic pathogen —**
Atypical infection —	**Commensals —**
Latent infection —	**Pathogenicity —**
Infectious Disease —	**Virulence —**
Bacteremia —	**Pathogenicity islands —**
Septicemia —	**LD_{50} (lethal dose) —**
Pyemia —	**ID_{50} (infectious dose) —**

Class № 7. MICROBIOLOGICAL BASES OF CHEMOTHERAPY AND ANTISEPTICS OF BACTERIAL INFECTIONS. METHODS FOR DETERMINING THE SENSITIVITY OF MICROBES TO ANTIBIOTICS

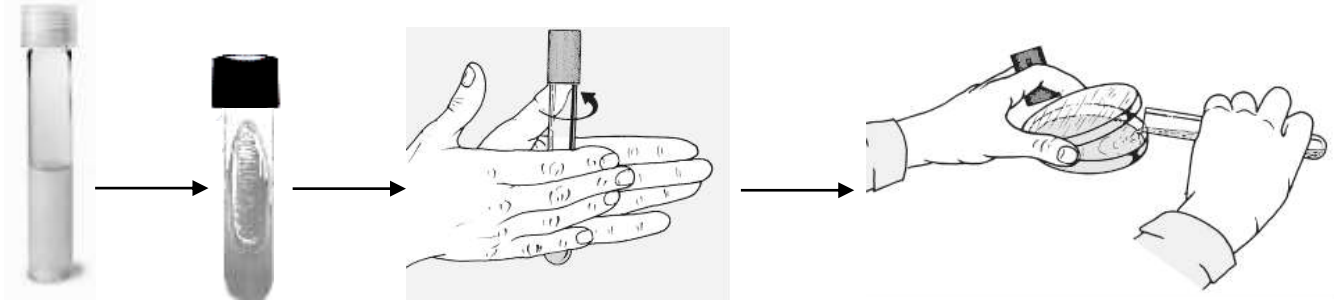
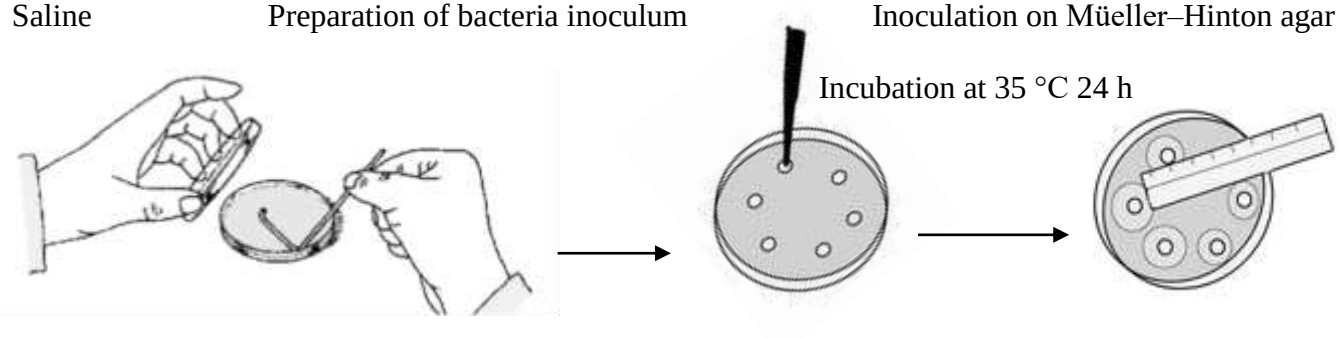
The subject to study:

Definitions: chemoprophylaxis and chemotherapy; antimicrobial chemotherapeutic agents and antibiotics. Sources of antibiotics. Spectrum of action. Chemical classification of antibiotics. Mechanisms of action. Side effects. Principles for rational antimicrobial therapy.

The problem of resistance to antimicrobials: definitions (intrinsic, acquired resistance), incidence, significance. Resistance mechanisms: non-genetic and genetic origin of drug resistance. Biochemical resistance. Beta-lactamases. Evolution of resistance to anti-infective agents. Antibiotic susceptibility testing of microorganisms: methods and principles. Minimal inhibitory concentration. Procedures of agar and broth dilution tests and Kirby–Bauer agar disk diffusion test.

Biological method (application of laboratory animals in microbiology): tasks, phases, evaluation of the method. Animal models for infection diseases. Routs for animal infection. Ethical, humane and legal considerations involved in the use of laboratory animals.

Laboratory work

Laboratory exerscises	Laboratory report
1. Perform the disk diffusion method (Kirby–Bauer) for determination of antibiotic susceptibility of four different microorganisms which often infect humans — *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa,* and *Klebsiella pneumoniae.* Müeller–Hinton agar (composition): meat extract — 2.0 g; Casein hydrolysate — 17.5 g; Corn starch — 1.5 g; Agar — 17.0 g; Aqua distillate — 1 l; pH 7.4 ± 0.2	 Saline — Preparation of bacteria inoculum — Inoculation on Müeller–Hinton agar  Inoculation on Müeller–Hinton agar — Application of antimicrobial discs onto the surface of the inoculated agar plate — Incubation at 35 °C 24 h — Registration of results

2. Determine antibiotic susceptibility of microorganisms by agar dilution test.

Petri dishes with the serial doubled dilutions of amicillin in agar medium:

Control 8 μg/ml 16 μg/ml 32 μg/ml

Antibiotic	MIC, μg/ml		
	resistant	intermediate resistant	susceptible
Ampicillin	≥ 32	16	≤ 8

Results registration and interpretation:

Microbial culture	MIC, μg/ml	Interpretation of results
Culture № 1		
Culture № 2		
Culture № 3		
Culture № 4		

Conclusion about advantage of dilution method _____

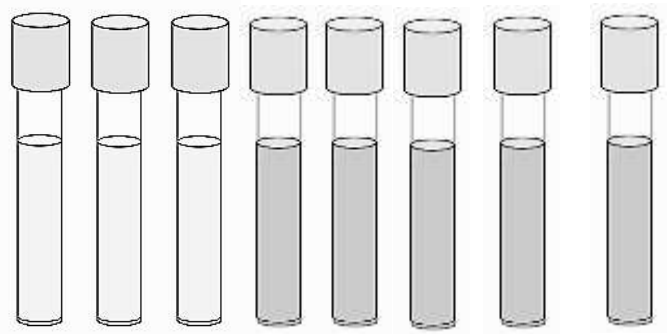
3. Determine antibiotic susceptibility of microorganisms by broth dilution test.

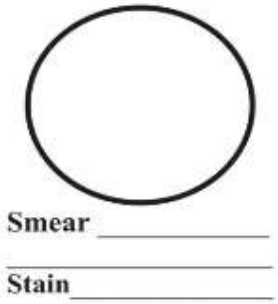
Demonstration:

1. Agar disk diffusion test for antibiotic susceptibility testing of microorganisms.
2. Rapid test for antibiotic susceptibility testing of microorganisms.
3. Slide of *Bacillus anthracis* in tissues of white mouse, Gram stain.

μg/ml

0.5 1.0 2.0 4.0 8.0 16.0 32.0 Control





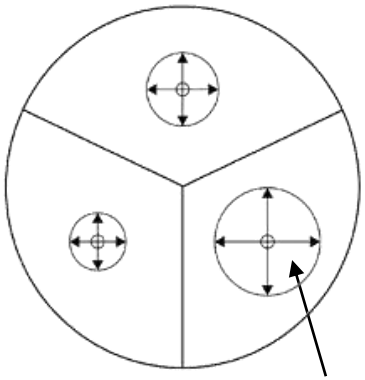
Smear _____

_____

Stain_____

Report: minimal inhibitory concentration of antibiotic is _____ μg/ml.

4. Determine antibiotic susceptibility of microorganisms by disk diffusion method, complete the report (perform it at classes № 8).



Zone of inhibition, d mm

Give the definition of the following terms:

Antibiotic	–
Minimal inhibitory concentration	–
Multiple resistance	–

Interpretation of inhibition zones of test cultures (mm)

Antibiotic	Diameter of inhibition zones (mm)	
	resistant	susceptible
Staphylococcus spp.		
Penicillin	≤28	≥29
Oxacillin		
S.aureus	≤10	≥13
CNS	≤17	≥18
Canamycine	≤13	≥18
Gentamicin	≤12	≥15
Ciprofloxacin	≤15	≥21
Tetracycline	≤14	≥19
Erythromycine	≥23	≥23
Lincomycine	≤13	≥21
Chloramphenicol	<17	≥18
Enterobacteriaceae		
Ampicillin	≤13	≥17
Cefazolin	≤14	≥18
Cefotaxime	≤14	≥23
Canamycine	≤13	≥18
Gentamicin	≤12	≥15
Ciprofloxacin	≤15	≥21
Lomefloxacin	≤18	≥22
Tetracycline	≤14	≥19
Doxicycline	≤12	≥16
Chloramphenicol	≤12	≥18

Results of pure culture _____ testing by disc diffusion method:

Antibiotic	Diameter of inhibition zone, mm	Interpretation of results

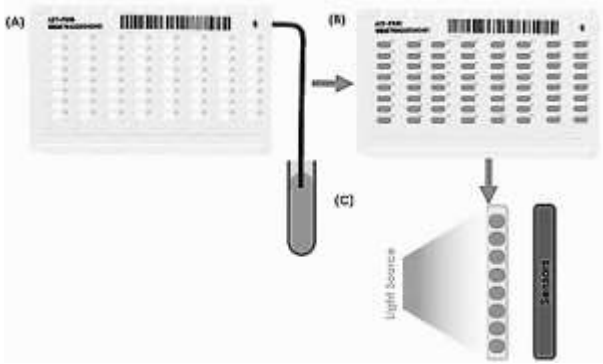
Report (formulate what antibiotics can be recommended for the therapy): _____
_____
_____
_____

Signature of the tutor _____
Date ____/____/202

Pathogenicity factors' groups (write in cells):	Side effects of antimicrobial drugs (write in cells):	Mechanisms of resistance of bacteria to an antimicrobial agents (write in cells):

AUTOMATED TESTS

Workstations are now available that carry out rapid, automated identification and susceptibility testing of microorganisms. In these systems the bacteria are incubated with the antimicrobic in specialized modules that are read automatically every 1–2 hours. The multiple readings and registration of growth by turbidimetric or uorometric analysis make it possible to generate MICs in as little as 4 hours. These methods are no more expensive than manual methods. Computerized results can be used in the interhospital monitoring systems.



METHODS OF THE ANTIBIOTIC SUSCEPTIBILITY TESTING

Antimicrobial susceptibility testing methods are divided into types based on the principle applied in each system:

1. Molecular methods.

2. Phenotypic methods:

a) Diffusion methods, or Kirby–Bauer method.

b) Dillution methods (in broth or agar media) or minimum inhibitory concentration detection tests.

c) Combined methods — diffusion&dilution — E-test.

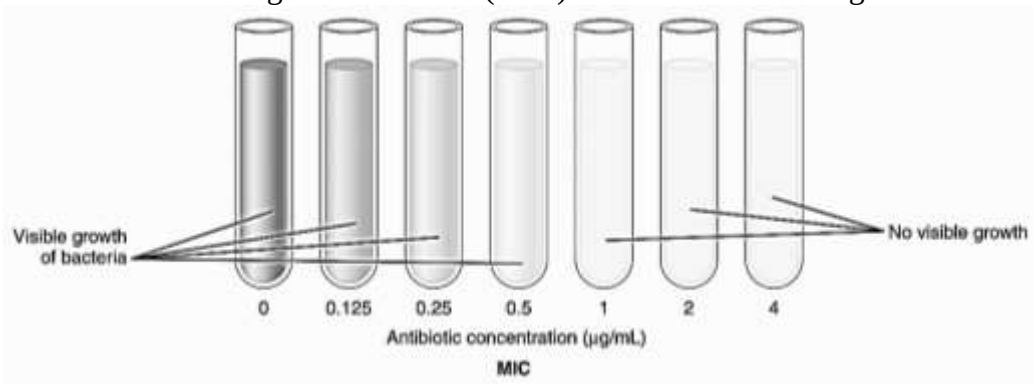
d) Automatic method.

MOLECULAR TESTS

The molecular techniques of nucleic acid hybridization, sequencing, and amplification are applied to the detection and study of resistance. These methods allow to detect resistance genes or mutation in genes associated with the resistance. These methods offer the prospect of automation and rapid results. The system comprises a predefined antibiotic gradient which is used to determine the Minimum Inhibitory Concentration (MIC), in μg/mL, of different antimicrobial agents against microorganisms as tested on agar media using overnight incubation.

DILUTION TESTS

Dilution tests determine the MIC directly by using two-fold serial dilutions of the antimicrobials in broth or agar media. The two-fold serial dilutions of antibiotics are prepared in tubes with broth. The bacterial inoculum is adjusted to a concentration of 10^5 to 10^6 CFU/ml and added to the broth. After incubation overnight, the tubes are examined for turbidity produced by bacterial growth. The first tube in which visible growth is absent (clear) is the MIC for that organism.



In the *agar dilution test* a series of agar plates containing progressively lower concentrations of a given antibiotic (and an antibiotic-free control plate) are each surface inoculated with the test organism and incubated; the MIC is indicated by the lowest concentration of antibiotic at which growth does not occur.

Class № 8. CONCLUDING SESSION «GENERAL MICROBIOLOGY»

List of questions:

1. History of microbiology as a science. Periods. The founders of main routs of microbiology.
2. Microscopic method of examination: tasks, procedure, evaluation of the method.
3. Bright-field light microscope: components and proper use of the microscope. Darkfield light microscopy: the principle behind dark-field microscopy. Phase-contrast light microscope: basic principles behind phase-contrast microscopy. Fluorescence microscopy: principles behind the fluorescence microscopy. The technique of oil immersion microscopy.
4. Type of microscopic preparations. Smear preparation and fixation. Simple methods of staining.
5. Differential stains of microorganisms. Gram stain: medical application, principles, procedure for Gram stain.
6. Basic bacterial cell structure: components of bacterial cell. Morphology of bacteria. Distinctive features of prokaryotic and eukaryotic cells. Basic morphological forms of bacteria. Morphological characteristics of cocci, rods and spiral-shaped bacteria.
7. Structure and function of cell envelope and appendages. Capsule. Detection methods of the capsule.
8. The composition, function, detection methods of bacterial cell wall. The structure of murein (syn. peptidoglycan). The cell wall of gram-positive bacteria. The cell wall of gram-negative bacteria. Bacterial forms with defective cell wall. Factors inducing cell wall removal, medical importance of L-forms.
9. Bacterial core: cytoplasm, cytoplasmic structures; their functions and detection methods. Acid-fast bacteria and unique properties of their cell wall. Methods of acid-fast staining: medical application, principle, procedure.
10. Resting forms of microorganisms. Bacterial endospores: medical importance, properties of endospore, the stages of endospore formation, detection methods (principles, procedures).
11. Motility of bacteria, methods of detection.
12. Taxonomy of microorganisms: classification and nomenclature. Modern approaches to taxonomy of microorganisms. Taxonomic ranks. Vars (types), strains, clones, pure cultures.
13. Taxonomy, morphology, medical significance of the spirochetes. Methods for spirochetes detection.
14. Taxonomy, morphology, medical significance of Actinomyces.
15. Taxonomy, morphology, medical significance of Mycoplasmas. Methods for Mycoplasmas investigations.
16. Taxonomy, morphology, medical significance of Chlamydiae.
17. Nutrition of microorganisms. Source of macro- and micronutrients, growth factors. Nutritional types. Transport mechanisms for nutrient absorption.
18. Energy strategies in microorganisms. aerobic and anaerobic respiration. structures involved in respiration in microorganisms.
19. Reproduction of microorganisms. Mechanisms and phases of bacterial division.
20. Bacteriological method of laboratory diagnosis: tasks, procedure, evaluation of the method.
21. Cultivation of microorganisms. Conditions required for growth. Nutrient media for culturing bacteria: classification and characteristics. Culture media ingredients, procedure of preparation and sterilization. General requirements to bacteriologic nutrient media.
22. Methods of isolation of aerobic microorganisms in pure culture.
23. Methods of isolation of anaerobic microorganisms in pure culture. Cultivation of anaerobic bacteria: culture media, techniques, equipment.
24. Identification of microorganisms: morphological, cultural, serologic, biological, genetic.
25. Biochemical identification of microorganisms. Detection of: a) proteolytic enzymes; b) carbohydrate hydrolyses enzymes; c) lipolytic enzymes; d) oxidative-reductive enzymes; e) hemolysins. Automatic stations for identification of bacteria.
26. The structure of bacterial genetic apparatus. Phenotype, genotype, genome, genes. Regulation of gene expression. General properties and varieties of plasmids. Detection of plasmids. Mobile genetic elements: transposons and IS elements.
27. Bacterial varibility: phenotypic and genetic. Practical significance of bacterial variability. Population variability.

28. Mechanisms of genetic variability: mutations and recombinations. Classification of mutations. Methods of mutant bacteria selection. Horizontal gene transfer: transformation, transduction, conjugation. Genomics. Bioinformatics. Genetic engineering. Gene Cloning.
29. Molecular methods in diagnosis of infection diseases: aims, methods, advantages. Molecular hybridization and polymerase chain reaction: principles of the methods. Equipment for PCR and hybridization. DNA extraction.
30. Doctrine regarding infections. Terms for emergence of infectious disease. Basic terminology of infectology. Classification of infections.
31. Role of microorganisms in infection emergence. Bacterial pathogenicity and virulence. Measurements of virulence: ID50, LD50, DLM. The genetics of bacterial pathogenicity. Pathogenicity islands. Pathogenicity factors: adhesins, invasins, impedins, agressins, modulins. Bacterial toxins.
32. Role of macroorganism, social and physical factors in infection emergence.
33. Evolution of microorganisms and infection diseases.
34. Biological method (application of laboratory animals in microbiology): tasks, phases, evaluation of the method. Animal models for infection diseases. Routs for laboratory animal infection. Ethical, humane and legal considerations involved in the use of laboratory animals.
35. Chemoprophylaxis and chemotherapy; antimicrobial chemotherapeutic agents and antibiotics. Sources of antibiotics. Spectrum of action. Chemical classification of antibiotics.
36. Mechanisms of action of antibiotics.
37. Side effects of antibiotics. Principles for rational antimicrobial therapy.
38. The problem of resistance to antimicrobials: definitions (intrinsic, acquired resistance), incidence, significance. Resistance mechanisms: non-genetic and genetic origin of drug resistance. Biochemical resistance.
39. Evolution of resistance to anti-infective agents. Antibiotic susceptibility testing of microorganisms: methods and principles.
40. Ecology of microorganisms. Basic terminology of ecology. Interspecific and intraspecific relations. Symbiosis, its variants. Antagonistic microbial relationships, its background and medical importance. Bacteriocins.
41. Diversity of normal flora at different sites of human body. Origin of the normal flora. Beneficial effects of the normal flora. Methods of normal flora investigation. Gnotobiology. Dysbacteriosis: aetiology, pathogenesis, symptoms, approaches to treatment, prophylaxis.

42. Sterilization: definition, methods of sterilization (physical, chemical, mechanical), quality control.
43. Disinfection: definition, methods of disinfection.
44. Antisepsis: definition, methods of antisepsis. Disinfectant and antiseptics: classification and modes of action.
45. Asepsis: definition, surgical, medical asepsis, asepsis in microbiological laboratory.

Practical skills:

1. Prepare fixed smears from the broth culture of bacteria and Gram stain.
2. Prepare fixed smears from agar cultures of bacteria and Gram stain.
3. Determine the morphology of staphylococcus, pure culture, Gram stain.
4. Determine the morphology of streptococcus, a pure culture, Gram stain.
5. Determine the morphology of Neisseria gonorrhoeae in pus from urethra, Gram stain.
6. Determine the morphology of Escherichia coli, pure culture, Gram stain.
7. Determine the morphology of the mixture of Staphylococcus aureus and Escherichia coli, Gram stain.
8. Determine the morphology of Bacillus anthracis, a pure culture, Gram stain.
9. Determine the morphology of Vibrio cholerae, pure culture, Gram stain.
10. Determine the morphology of Brucella, a pure culture, Gram stain.
11. Determine the morphology of Corynebacterium diphtheria, pure culture, Loeffler stain.
12. Determine the morphology of Klebsiella, a pure culture, negative staining by Hins-Burri.
13. Determine the morphology of mycobacteria in sputum stain Ziehl–Nielsen.
14. Technique of seeding by loop on Petri dish from tube.
15. Technique of seeding by loop from Petri dish to Petri dish.
16. Technique of seeding by loop from the tube to tube.
17. Evaluate the results of antibiotic resistance detection by disk-diffusion method.
18. Evaluate the biochemical properties of enterobacteria on triple sugar iron agar (Kligler agar).

Signature of the tutor _____
Date ____/____/2024

Class № 9. IMMUNOLOGY. THE IMMUNE SYSTEM. INNATE IMMUNITY

The subject to study:

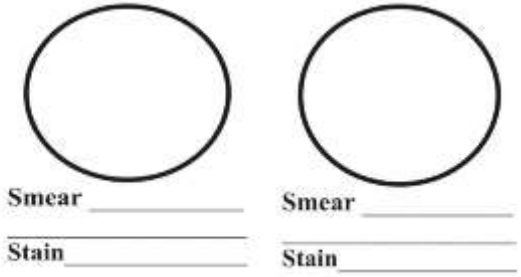
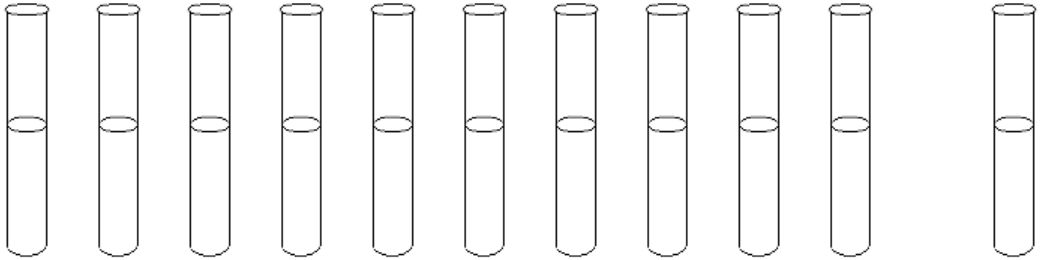
Human immune system: organs, cells, molecules (CD receptors; MHC I, II, III; cytokines, adhesion molecules etc.).

Immunity, types of immunity.

Innate immunity. Immune and not-immune factors. Complement system: composition, way of activation, functions. Lysozyme, b-lysins. Polynuclear and mononuclear phagocytes systems. Phagocytosis: phases, intracellular killing mechanisms, outcomes. Antigen presenting cells. Dendritic cells. Natural killer cells.

Methods for estimation of complement system activity and phagocytosis.

Laboratory work

Laboratory exerscises	Laboratory report
1. Determine phagocytosis parameters in prepared slides stained by Gimza method. **Demonstration:** 1. Incomplete phagocytosis of *N. gonorrhoeae.* 2. Incomplete phagocytosis of *K. rhinoscleromatis.* 2. Register the complement system activity by 50 % hemolysis method. Serum is diluted and added in tubes from 0.05 to 0.5 ml. Then saline solution is added to the final volume of 1.5 ml. 1.5 ml of hemolytic system is added to each tubes. Reaction is incubated at 37 °C for 45 min, cooled at 4 °C and centrifuged at 1500 rpm for 5 min. The tube in which 50 % hemolysis occurred is determined visually. This means the volume of patient serum which contains 1 unit of CH50. Next the CH50 for the whole serum is calculated.	Staphylococci are mixed with leucocytes (50 : 1) and incubated at 37 °C for 15–120 min. Then slides are prepared and stained by Gimza method. Under oil immersion the phagocyting leucocytes and phagocyted staphylococci are counted and phagocytosis parameters calculated. **PI (Phagocytosis index)** = (Number of phagocyting leucocytes / All leucocytes counted) × 100 % N = 40–60 % **PN (Phagocytosis number)** = Number of phagocyted staphylococci / Number of phagocyting leucocytes N = 4–7  Volum of diluted (1 : 10) serum, ml 0.05 0.1 0.15 0.20 0.25 0.30 0.35 0.40 0.45 0.50 (50 % hemolysis)  1 CH_{50} — in _____ ml serum X CH_{50} — in 1 ml serum N 40–60 CH_{50} **Conclusion** _____ **Signature of the tutor _____ Date ____/____/2024**

COMPLEMENT SYSTEM

The complement system consists of serum and cell surface proteins that interact with one another and with other molecules of the immune system in a highly regulated manner to generate products that function to eliminate microbes.

The complement system is activated by microbes and by antibodies that are attached to microbes and other antigens. **Complement focuses immune attack on microbial surfaces.** Activation of complement involves the sequential proteolysis of proteins to generate enzyme complexes with proteolytic activity.

Three different pathways of activation are distinguished, triggered by either target-bound antibody or immune complex (*the classical pathway*), by microbial repetitive polysaccharide structures (*the lectin pathway*), or by recognition of other foreign surface structures (*the alternative pathway*). All three merge in the pivotal activation of C3 and, subsequently, of C5 by highly specific enzymatic complexes, so-called ***convertases.*** In the common terminal pathway, downstream of C5 further complement components are activated and assembled into the ***membrane attack complex (MAC).***

1. Based on the figure, write down the scheme of complement activation;

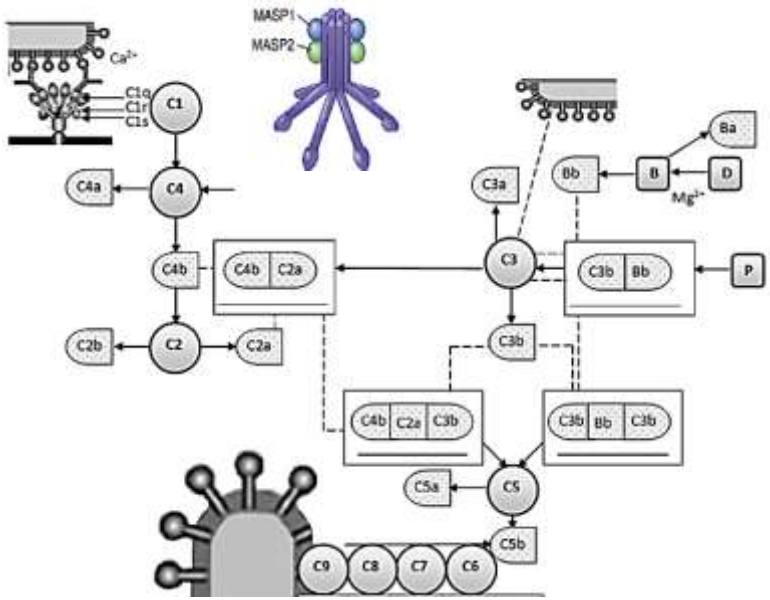
2. Indicate C3-, C5-convertases, anaphylatoxins and MAC development:

Classical Pathway	1. *Immune complex* → *C1q+C1r+C1s* → *C1qrs* → *C4* → *^{C4a}C4b* →			
	2. C3-convertase:	C5-convertase:	Anaphylatoxins:	MAC development:
Lectin (MBL) Pathway				
	2. C3-convertase:	C5-convertase:	Anaphylatoxins:	MAC development:
Alternative Pathway				
	2. C3-convertase:	C5-convertase:	Anaphylatoxins:	MAC development:

Classical Pathway — IgG/IgM bound to antigen

Lectin (MBL) Pathway — MBL binds mannose on patogens

Alternative Pathway — Patogens, injurtd tissue



Mediators	**Receptors**	**Cofactors**	**Regulators**
C1(q,r,s)	CR1	Factor B	C1INH
C2 (a,b)	CR2	Factor D	Factor H
C4(a,b)	CR3	Factor I	CPN1
C3(a,b,Bb)	CR4		CD55 (DAF)
C5 (a,b)	C3aR		CD46 (MCP)
C6, C7, C8, C9	C5aR		CD59 (protectin)

Acute phase proteins

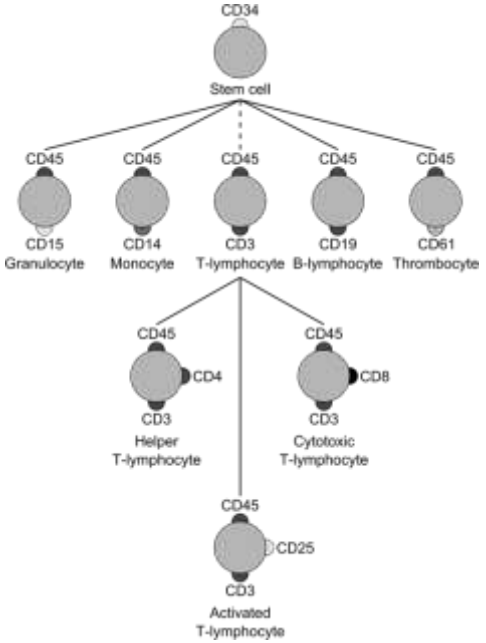
Acute phase proteins are referred as humoral factors of an innate immunity and permanently present in blood. But in systemic inflammation condition under proinflammatory cytokines influence (IL1, TNF alpha, IL6) their production by reticular-endothelial cells and hepatocytes increases dramatically. They include: fibrinogen, C-reactive protein (CRP), plasma amyloidal protein, mannose binding protein, alpha-1-antitrypsin etc. Acute phase proteins determination (CRP) is used in clinic for the inflammation intensity evaluation.

Protein	Characteristics	Function
CRP, Plasma amyloidal protein	Belong to pentraxin family (are composed of 5 subunits); Normal concentration ~ 1 mg/L; in systemic inflammation — up to 2 g/L	After binding pentraxin can activate complement by classic and alternative pathways. Bond CRP is a chemoattractant for neutrophils and can stimulate phagocytosis
Mannose-binding protein	Belong to collectin family. Normal concentration is 0.1–1 mg/L; in systemic inflammation — 10 times as much	After binding it turns to serine protease and can activate complement by lectin pathway. Activated MBP can also cleave C2 and C4 (activation complement by classic pathway)

Differences between innate and acquired immunity:		
Feature	**Innate immunity**	**Acquired immunity**
Specificity		
Diversity		
Memory		
Components:		
Anatomic and physiologic barriers		
Blood proteins		
Cells		

Cluster of differentiation (CD):

The cluster of differentiation (CD) is a nomenclature system that identifies and classifies antigens found on the cell surface of leukocytes. Under the CD system, antigens that are well characterized are assigned an arbitrary number (eg CD1, CD2, etc.). CD molecules are commonly used as cell markers, allowing the identification and isolation of leukocyte populations, subsets, and differentiation stages.

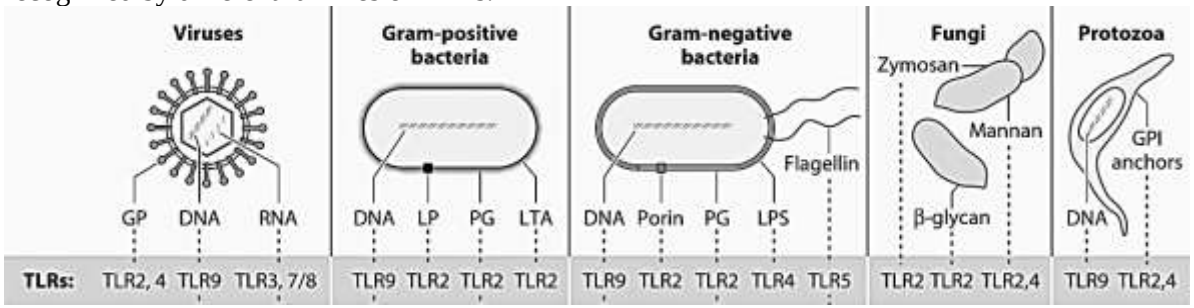


Write in cells CD markers of cells:

CD marker	Type of cell
CD34	
CD3	
CD4	
CD8	
CD19, CD20, CD72, CD79 et al.	
CD16/CD56	
CD14,CD64	

Recognition mechanisms in the innate immune system:

Viruses, bacteria, fungi, and protozoa display several different PAMPs, some of which are shared between different classes of pathogens. Major PAMPs are nucleic acids, including DNA, dsRNA, ssRNA, as well as surface glycoproteins (GP), lipoproteins (LP), and membrane components (peptidoglycans (PG), lipoteichoic acid (LTA), LPS, and GPI anchors). These PAMPs are recognized by different families of PRRs.



Write in cells ligand of receptors:

PRRs	Ligand
TLR2	
TLR3	
TLR4	
TLR5	
TLR7	
TLR8	
TLR9	

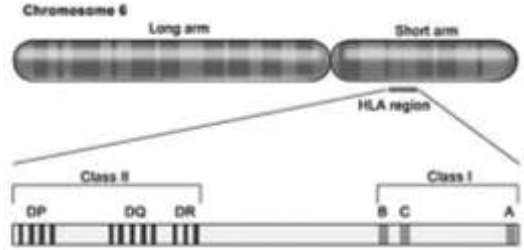
3. Define the following terms with examples:

Pattern recognition receptors (PRRs) —

Pathogen-associated molecular patterns (PAMPs) —

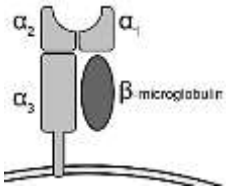
MHC GENES AND ANTIGENS

Human Leucocyte Antigens (HLA) are glicoproteins encoded by genes of Major Histocompatibility Complex (MHC). In immune system the glycoproteins perform a very important function: they determine biologic individuality of each human being and take part in peptide antigen presentation to T-lymphocytes by antigen-presenting cells (APC). HLA-molecules encoded by MHC-genes are subdivided into glycoprotein of **class I** MHC (HLA-A, HLA-B, and HLA-C; these glycoproteins presented on the surface of all somatic cells excluding the extravillous trophoblast cells and erythrocytes) and **class II** MHC (HLA-DP, HLA-DQ, and HLA-DR; they are predominantly expressed on membranes of the APC. The MHC complex includes 2000 allelic genes. The map of the human MHC is shown in the picture:



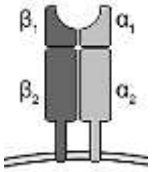
MHC-I. Includes loci HLA-A, HLA-B and HLA-C and encodes class I MHC molecules.
MHC-II. Includes loci HLA-DP, HLA-DQ and HLA-DR and encodes class II MHC molecules.
MHC-III. The term «MHC-III» designates the region MHC-I and MHC-II genes. It includes genes of complement components (C4a and C4b, C2, factor B), cytokines (TNF-a and lymphotoxin), etc.

MHC-I molecules:



– presented on the surface of all nucleated cells
– consist of transmembrane α-chain + short β2-microglobulin.
– genes are located at loci A, B, C.
– present intracellular antigens to CD8+ T-cells.

MHC-II molecules:



– expressed on membranes of the APC
– consist of two polypeptide chains (α- and β)
– genes are located at loci DP, DQ, DR
– present extracellular antigens to CD4+ T-cells

Regulatory cytokines of the inflammatory cascade:

Cytokine	Principal cell source	Biologic effects
Interleukin-1 (IL-1)	Macrophages, endothelial cells, some epithelial cells	Endothelial cells: activation (inflammation, coagulation); hypothalamus (fever); liver (synthesis of acute-phase proteins)
Interleukin-6 (IL-6)	Macrophages, endothelial cells, T cells	Liver (synthesis of acute-phase proteins); B cells (proliferation of antibody-producing cells)
Interleukin-12 (IL-12)	Macrophages, dendritic cells	T cells (T_H1 differentiation); NK cells and T cells (IFN-γ synthesis, increased cytolytic activity)
Interleukin-10 (IL-10)	Macrophages, T cells (mainly T_H2)	Macrophages, dendritic cells: inhibition of IL-12 production and expression of costimulators and class II MHC molecules
Chemokines	Macrophages, endothelial cells, T cells, fibroblasts, platelets	Leukocytes: chemotaxis, activation; migration into tissues
Type I IFNs (IFN-α, IFN-β)	IFN-α: macrophages IFN-β: fibroblasts	All cells: antiviral state, increased class I MHC expression. NK cells: activation
Tumor necrosis factor (TNF)	Macrophages, T cells	Endothelial cells: activation (inflammation, coagulation); neutrophils (activation); hypothalamus (fever); muscle (fat: catabolism (cachexia)); liver (synthesis of acute-phase proteins); many cell types (apoptosis)
Transforming growth factor-β (TGF-β)	T cells, macrophages, other cell types	T cells: inhibition of proliferation and effector functions B cells: inhibition of proliferation; IgA production Macrophages: inhibition

METHODS FOR INNATE IMMUNE SYSTEM DIAGNOSTICS

Quantification of neutrophils

Neutrophils are routinely quantified in the five-part white cell differential count obtained from hematology analyzers.

An apparently low neutrophil count should always be confirmed by examining a blood film. Alternatively white blood cells can be quantified by counting cell suspension in special camera under microscope. Exact quantity of blood cells can be calculated after their percentage evaluation in blood film.

Prior to considering neutrophil function testing, a blood film should be performed to assess neutrophil morphology, as neutrophils that appear abnormal rarely function normally. Rare genetic abnormalities of neutrophil granulation as well as myelodysplasia (a premalignant condition in which neutrophil development and function is abnormal) can readily be recognized on a blood film. In these conditions formal neutrophil testing rarely adds to the management of the patient.

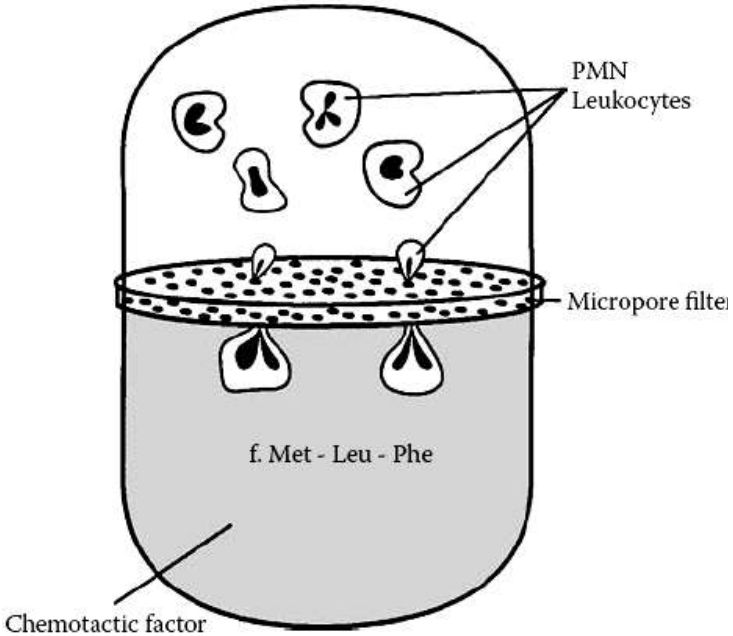
Phagocytosis

Phagocytosis of organisms is measured using latex particles or stained organisms incubated with patient cells. The percentage of cells that have ingested particles (phagocytosis index) and the number of particles ingested (phagocytosis number) is compared to controls. If fluorescent particles are used, phagocytosis can be measured by flow cytometry. In both assays distinguishing adherent particles on the cell surface from those that have been phagocytosed is difficult.

Adhesion

Measurement of key neutrophil adhesion molecules using standard flow cytometry techniques is commonly used. The adhesion molecules which may be measured in this way include CD15 — sialyl Lewis x, CD11a, CD11b, CD11c and CD18.

Neutrophil adhesion assays involve allowing neutrophils to adhere to plastic, fibronectin coated glass or cultured endothelium. Unbound neutrophils are washed away and the adherent neutrophils quantified either by microscopy or measurement of a neutrophil specific protein such as myeloperoxidase.



Chemotaxis.

Respiratory burst

The nitroblue tetrazolium (NBT) test relies on reduction of NBT to formazan by oxygen radicals produced by stimulated neutrophils. Neutrophils are incubated with colorless NBT, appropriately stimulated and the presence of formazan is assessed visually using a microscope or by spectrophotometry. Intracellular dyes, which become fluorescent after reduction by reactive oxygen intermediates, have led to flow cytometric equivalents of the NBT test.

Chemotaxis

Neutrophil chemotaxis (migration in response to chemotactic stimuli) may be measured under agarose. Wells cut into agarose are filled with neutrophils, chemoattractant or control saline, and the numbers of cells migrating after a defined period are counted. A control is always included and the results are compared with age-matched controls. Alternatively, specialized Boyden chambers may be used.

Class № 10. METHODS OF CLINICAL AND INFECTIOUS IMMUNOLOGY. ANTIGENS. HUMORAL IMMUNE RESPONSE. ANTIBODIES

The subject to study:

Immune response, definition, main factors. Antigens: definition, main features, classification.

B-lymphocytes system. B cells genesis. B cell receptor (BCR). B-cell activation, proliferation, differentiation to plasmocyte, immunoglobulin production. Humoral immune response. Primary and secondary humoral response. Immunoglobulins: structure, functions. Classes and subclasses of immunoglobulins. Monoclonal immunoglobulins.

Methods of B-lymphocytes evaluation: quantitative and functional tests.

Laboratory work

Laboratory exerscises

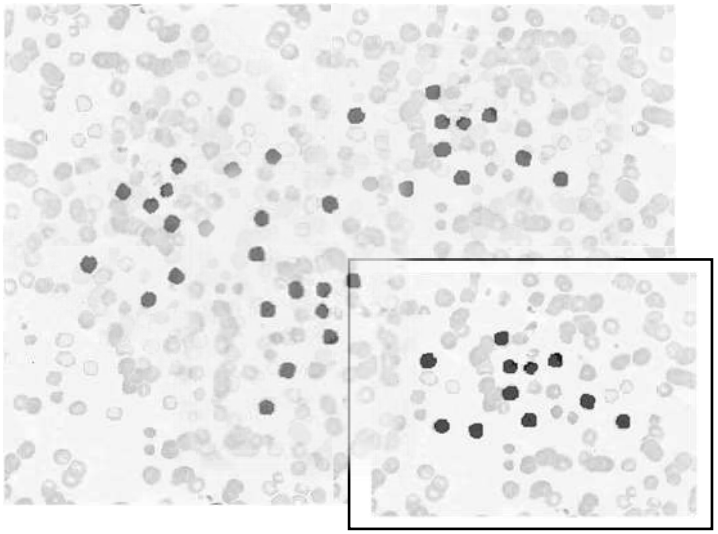
1. Determine the quantity of B-cells by immunocytochemistry methods in ready made slides.

Immunocytochemistry Protocol:

1. Permeabilize Membrane: Add one drop of PBS/0.1 % Triton X-100 to each well to permeabilize lymphocytes previously separated from the blood. Incubate slides for 1 min. Wash the slides twice in PBS and place the slides onto a tray.
2. Blocking: Soak slides in 1.5 % H_2O_2 / PBS solution for 15 min. Wash twice in PBS on the shaker. Incubate with 5 % BSA into each well to block for overnight at 4 °C in a humid chamber.
3. Primary Antibody (PA): Dilute the PA to the recommended concentration in 1 % BSA diluent. Add 35 µL of PA to each well. Incubate for one hour at room temperature. Wash slides three times in PBS, 5 minutes each on the shaker.
4. Secondary Antibody (SA) and Detection: Dilute the biotinylated SA to 1 : 200 in a solution of 1 % BSA diluent. Add one drop SA solution into each well. Incubate for one hour.Wash in PBS three times. Add one drop streptavidin-HRP to each well. Incubate for 30 minutes. Wash three times 5 minutes in PBS. Add DAB solution to each cell well. Once the cells start turning brown wash twice in PBS for 5 minutes each time on the shaker.
5. Optional Counterstain: Dip the slide rack with the slides into a staining dish of hematoxylin for 30 seconds. Remove and place into an acid bath. Rinse with DI H_2O.
6. Count the cells: both brown-stained and blue-stained cells are counted per 100 cells.

Laboratory report

1. Determine the number and percentage of B lymphocytes in the figure below (*count the cells in the highlighted element, where the total number of lymphocytes is 100 cells*):

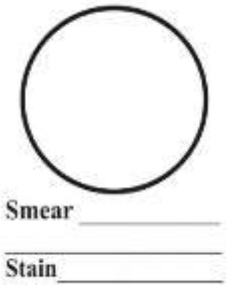


All cell count — _____ cells.

Result: Number of B-cells — _____.

Percentage of B-lymphocytes — ____ %.

2. Determine the quantity of B-cells in ready made slides.

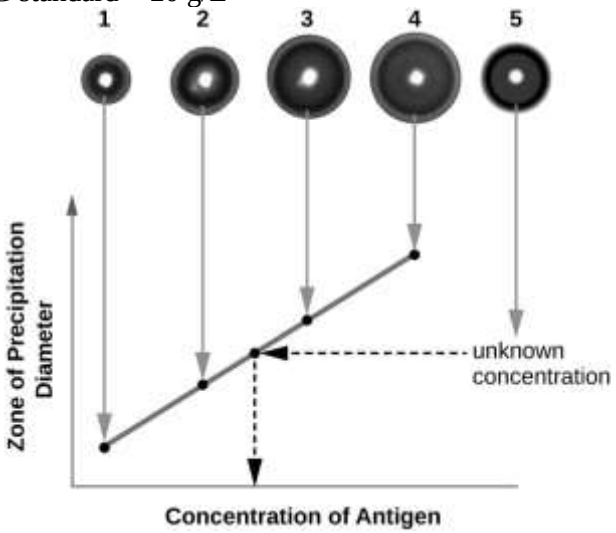


Normal B-cells count by CD20 = 8–20 % total blood lymphocytes.

Result: _____

2. Determine an IgG concentration in serum by Manchini method (simple radial immunodiffusion (SRID)).

IgG standard = 20 g/L

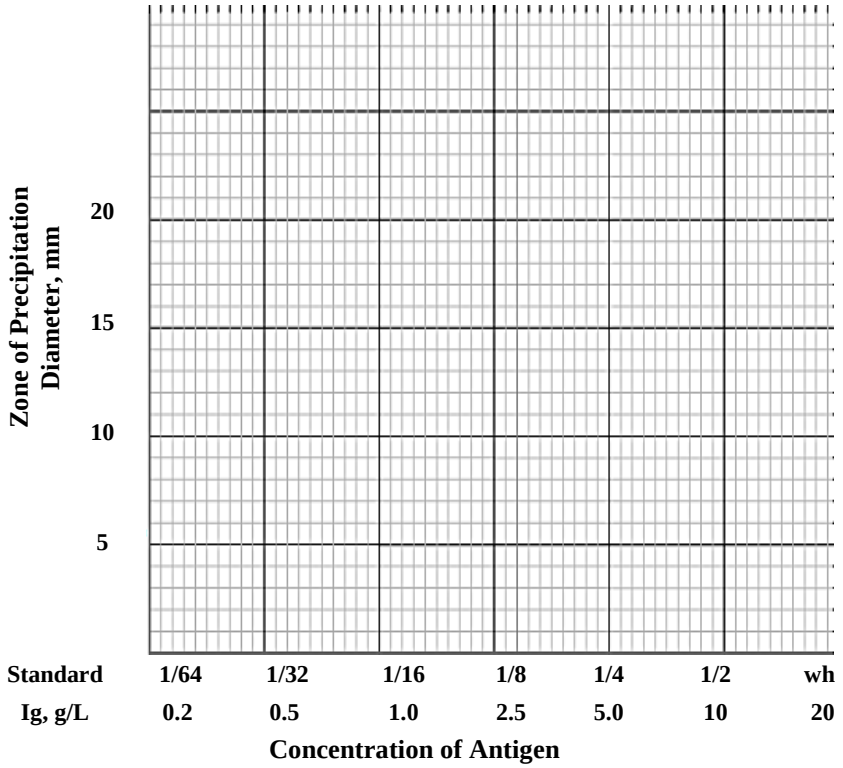


SRID is a technique to quantify antigens. Known also as the Mancini technique.

Antibody is incorporated into agar in plates, wells are cut, and precise quantities of antigen are placed in the wells. The antigen is permitted to diffuse into the agar containing antibody and produce a ring of precipitation upon interaction with the antibody. As diffusion proceeds, an excess of antigen develops in the area of the precipitate which causes it to dissolve, only to form again at a greater distance from the site of origin. At the point where antigen and antibody reach equivalence in the agar, a precipitation ring is produced. The ring encloses an area proportional to the concentration of antigen measured 48 to 72 hours following diffusion.

Standard curves are employed using known antigen standards. The antigen concentration is determined from the diameter of the precipitation ring. This method can detect as little as 1 to 3 μg/mL of antigen.

Standart curve



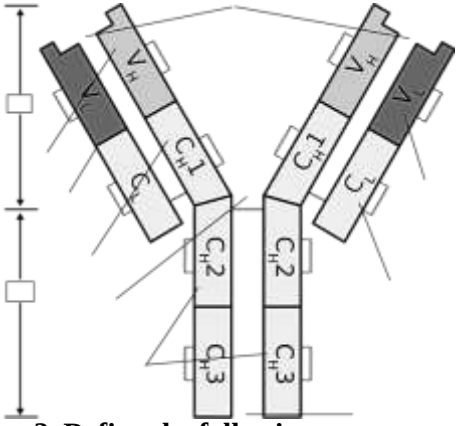
	Titer	Concentration, g/L	Diameter, mm
1 point			
2 point			
3 point			
4 point			
5 point			
Experiment			

Normal IgG ranged 9,5–14,5 g/L

Conclusion _____

Signature of the tutor _____ Date ____/____/2024

Indicate in the figure the elements of the immunoglobulin molecule:



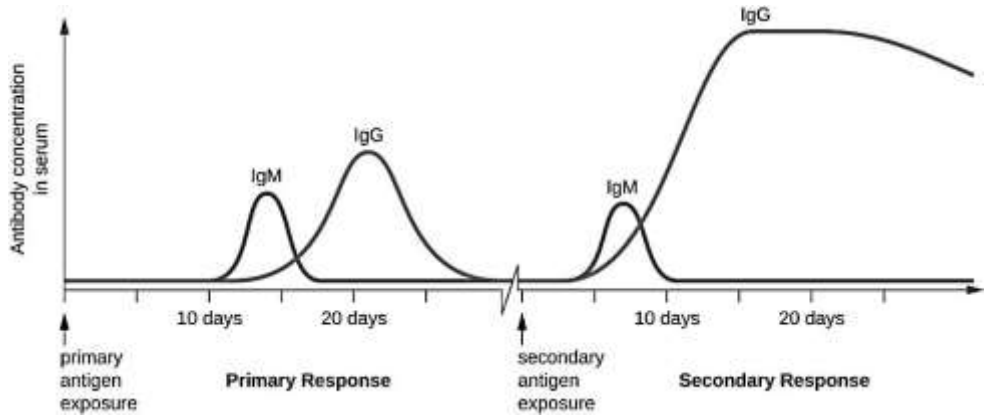
1	Antigen binding site
2	Light chain (L)
3	Heavy chain (H)
4	Variable region of a light chain
5	Constant region of a light chain
6	Hinge region
7	Variable region of a heavy chain
8	Constant region of a heavy chain
9	Fc-fragment
10	Fab-fragment
11	Fc-receptor ligand

3. Define the following terms:

Antibody affinity — _____
_____

Antibody avidity — _____
_____

Antibody valence — _____
_____



Write down the characteristics of immunoglobulin according to class and molecule structure:

Class	Structure	Characteristics
Ig__		
Ig__		
Ig__		
Ig__		
Ig__		

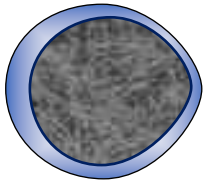
Primary Immune Response & Secondary Immune Response:

Characteristics	Primary Immune Response	Secondary Immune Response
Definition		
Antibody Peak		
Affinity of Antibody		
Responding Cells		
Lag Phase		
Types of Antibody		

Primary humoral immune response development:

Localization	Stages
I. Induction of T-effectors (helpers and other subpopulations)	
Tissue	1. APCs capture antigen (protein, microbes), process it and transport to regional lymphatic nodes
Secondary lymphoid organs	2. APCs present antigens by endosome pathway to CD4+ naïve T-cells
	3. T-cells activate, proliferate and differentiate into effector cells (Th1, Th2, Th3, Tr1, Tr2, CD4+CD25+ etc.)
Blood, tissues	4. T-effectors recirculate through the organism
II. Induction of B-effectors (plasma cells)	
Tissues	1. APCs for B cells (follicular dendritic cells) capture antigen and transported it to secondary lymphoid organs (lymphatic nodes, Peyer patches, etc.). Antigen is not processed and conserved on DCs surface for a long time (up to year or longer)
	B-lymphocyte captures antigen and present it in complex with type II MHC molecule; due to activation the expression of CD86 increases on its surface
	T-effector receives activating signals
	Activated T-effector expresses CD40 L and secrets cytokines (IL-4,5,6)
	B-lymphocyte proliferates and differentiates to plasma cells
Secondary lymphoid organs, BM, blood	2. B-lymphocyte captures antigen, processes it and presents to T-effector. Specific T-effector is activated and activates B-cell with contact (CD40L) and distant (cytokines) interactions
	3. B-lymphocyte proliferates, enters bloodstream and reaches secondary lymphoid organs and BM
	4. B-lymphocytes turn to plasmacytes and produce immunoglobulins for some time (up to 3 months)
	5. Some B-lymphocytes return to the quiescent state and become the memory B-cells
III. Immunoglobulins realize their functions	

Draw the B-lymphocyte:



CD4	**CD8**	**CD40b**	**BCR**
sIgM	**CD3**	**TCRα,β**	**TCR**
sIgD	**CD19**	**IL4r**	**ACR**
CD52	**CD20**	**ILR**	**HLA**
CD45	**CD23**	**CD37**	**CD11C**
	CD79a	**CD79b**	**CD38**

Antibody functions:

B-lymphocytes subpopulations:

Feature	B-1 lymphocytes	B-2 lymphocytes
Genesis	Separate stem cell; leaves BM early in ontogenesis	BM, common stem cell
Area	Body cavities (pleural, abdominal)	BM, peripheral organs of an immune system
Specificity		
Function		

Selected methods for immune cells isolation and evaluation:

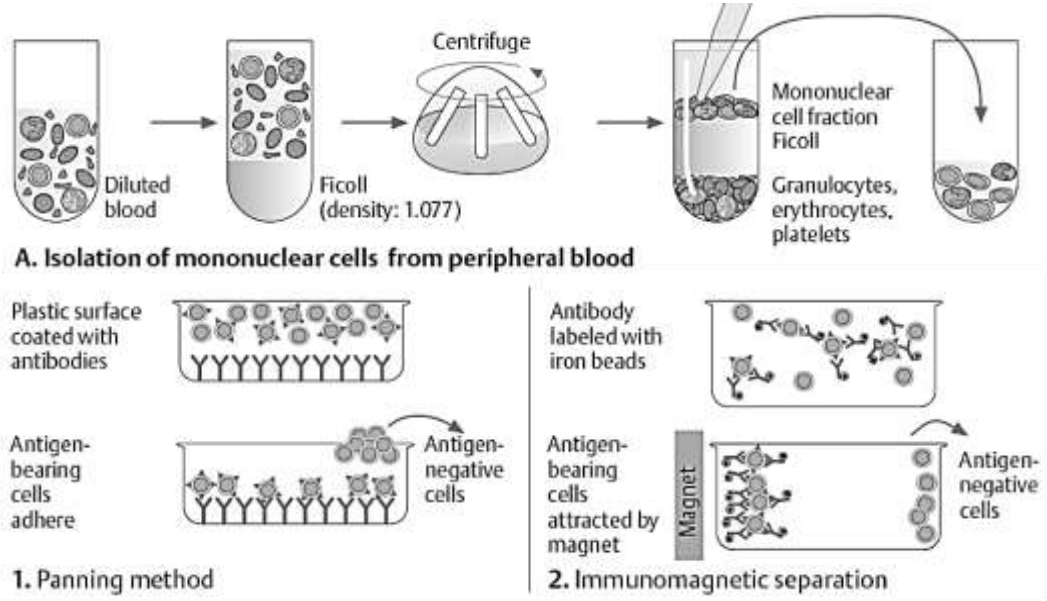
The first step in studying lymphocytes is *to isolate* them so that their behavior can be analyzed in vitro.

Human lymphocytes can be isolated most readily from peripheral blood *by density centrifugation over a step gradient* consisting of a mixture of the carbohydrate polymer FicollTM and the dense iodine-containing compound metrizamide (1.077). This yields a population of mononuclear cells at the interface that has been depleted of red blood cells and most polymorphonuclear leukocytes or granulocytes (granulocytes readily can be isolated as well by proper gradient = 1.09–12). The resulting population, called peripheral blood mononuclear cells, consists mainly of lymphocytes and monocytes.

A particular cell population can be isolated from a sample or culture by binding to antibody-coated plastic surfaces, a technique known as *panning*, or *by removing unwanted cells by reatment with specific antibody and complement* to kill them.

Cells can also be *passed over columns of antibody-coated, nylon-coated steel wool* and different populations differentially eluted. This technique extends affinity chromatography to cells, and is now a very popular way to separate cells.

All these techniques can also be used as a pre-purification step prior to sorting out highly purified populations by FACS. Isolation of lymphocytes from tissues other than blood.

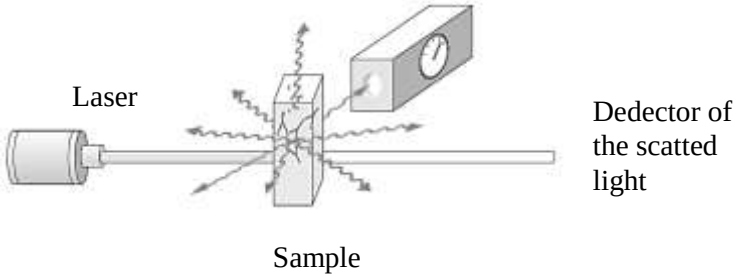


Morphological and functional methods for B-cells system evaluation:

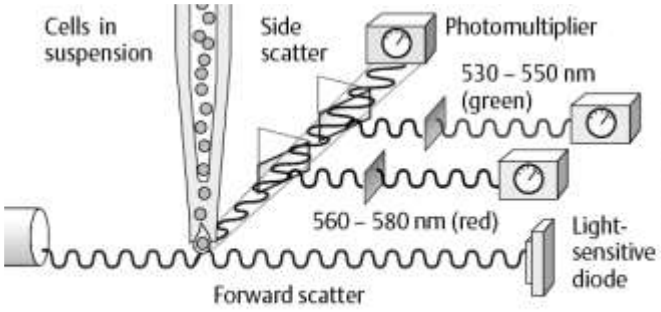
1. B-cell quantity in the blood can be determined by flow cytometry.

2. B-cell function may be assayed by immunoglobulins concentration measurement in the serum:

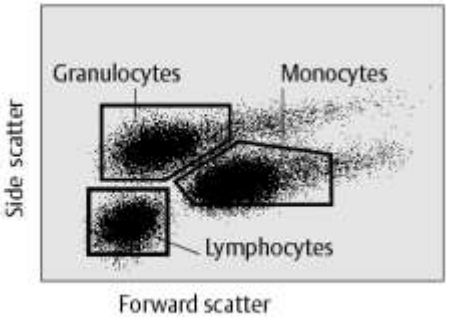
a) *Immunonephelometry*. A technique to assay proteins and other biological materials through the formation of a precipitate of antigen and homologous antibody. The assay depends on the turbidity or cloudiness of a suspension and is based on determination of the degree to which light is scattered when a helium-neon laser beam is directed through the suspension. Measurement is made at 340 to 360 nm using a spectrophotometer. The antigen concentration is ascertained using a standard curve devised from the light scatter produced by solutions of known antigen concentration. This method is used by many clinical immunology laboratories for the quantification of complement components and immunoglobulins in patients' sera or other body fluids.



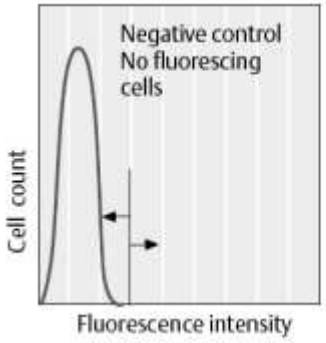
b) *Simple radial immunodiffusion (SRID).*

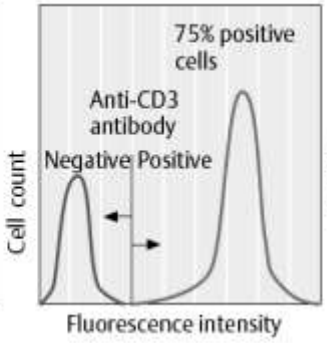


Flow cytometry

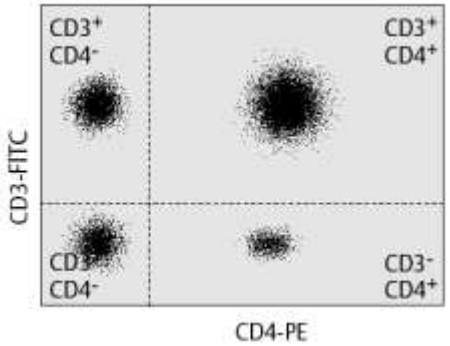


Cell separation by granularity and size





Fluorescence intensity histograms
Cell separation by fluorescence



Dot-plot, two color analysis

Flow cytometry:

An analytical technique to phenotype cell populations. It requires a special apparatus, termed a flow cytometer, that can detect fluorescence on individual cells in suspension and thereby ascertain the number of cells that express the molecule binding a fluorescent probe.

Cell suspensions are incubated with fluorescent-labeled monoclonal antibodies or other probes, and the quantity of probe bound by each cell in the population is assayed by passing the cells one at a time through a spectrofluorometer with a laser-generated incident beam. Sample cells flow single file past a narrowly focused excitation light beam that is used to probe the cell properties of interest. As the cells pass the focused excitation light beam, each cell scatters light and may emit fluorescent light, depending on whether or not it is labeled with a fluorochrome or is autofluorescent.

Scattered light is measured in both the forward and perpendicular directions relative to the incident beam. The fluorescent emissions of the cell are measured in the perpendicular directions by a photosensitive detector.

Measurements of light scatter and fluorescent emission intensities are used to characterize each cell as it is processed.

Flow cytometry is a fast, accurate way to measure multiple characteristics of a single cell simultaneously.

Three-color flow cytometry is used to analyze blood cells by size, cytoplasmic granularity, and surface markers labeled with different fluorochromes.

Class № 11. METHODS OF CLINICAL AND INFECTIOUS IMMUNOLOGY. CELLULAR IMMUNE RESPONSE. ALLERGY AND ECOLOGICAL IMMUNOLOGY

The subject to study:

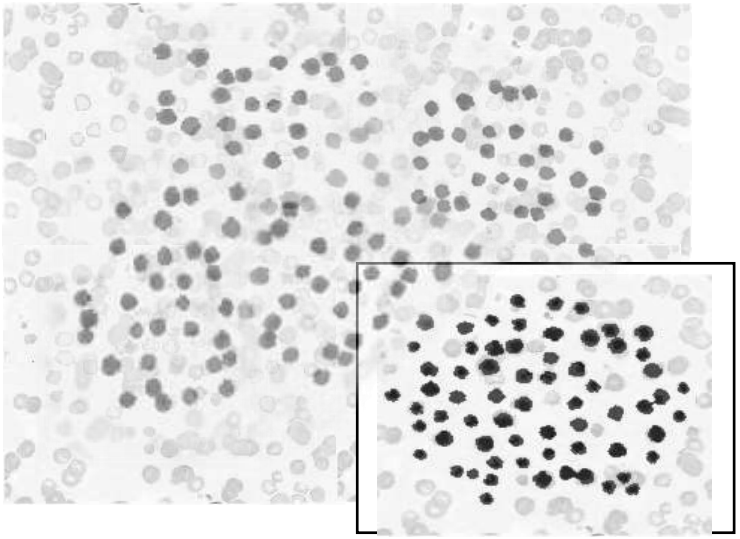
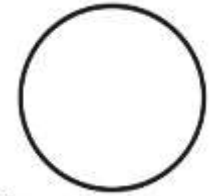
Cellular immune response and its phenomena.

T-lymphocyte system. T-cell markers. TCR. Genetic control of TCR diversity. T-lymphocytes subpopulations: helpers, killers, DTH-effectors, regulators. T-helpers of 1, 2, 3 and 17 types. Methods for evaluation of T-lymphocytes system: quantitative and functional tests.

Allergy, stages, types. Immediate type of hypersensitivity mechanisms: mediator type (I), cytotoxic type (II), immune complex type (III). Delayed type of hypersensitivity mechanism (IV). Drug allergy. Methods for allergic conditions diagnostics.

Ecological immunology: definition, objects of research. Immunotropic environmental factors, mechanisms and conditions of their action on the human immune system.

Laboratory work

Laboratory exerscises	Laboratory report
1. Determine the quantity of T-cells by immunocytochemistry methods in ready made slides. Immunocytochemistry Protocol: see class № 10.	1. Determine the number and percentage of T-lymphocytes in the figure below (*count the cells in the highlighted element, where the total number of lymphocytes is 100 cells*):  All cell count — _____ cells. **Result: Number of T-cells — _____.** **Percentage of T-lymphocytes — ____%.** Normal T-cells count by CD3 = 62–70 % total blood lymphocytes. 2. Determine the quantity of T-cells in ready made slides.  Smear _____ _____ Stain_____ **Result: _____**

Demonstration:

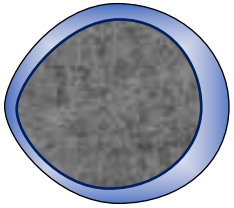
1. Immune rosettes method for T-cell quantity determination (Romanowsky–Giemsa stain).
2. Blast transformation of lymphocytes (Romanowsky–Giemsa stain).
3. Degranulation of mast-cells (Romanowsky–Giemsa stain).

Smear _____ Smear _____ Smear _____

Stain_____ Stain_____ Stain_____

Signature of the tutor_____ Date ____/____/2024

Draw the cytotoxic T-lymphocyte (CTL):



CD4	**CD8**	**CD40b**	**BCR**
sIgM	**CD3**	**TCRα,β**	**TCR**
HLAI	**Perforin**	**IL4r**	**ACR**
CD52	**CD20**	**ILR**	**HLA II**
CD45	**CD23**	**CD37**	**CD11C**
	FasL	**CD79b**	**CD28**

Define the following terms:

Anergy — _____

_____

Hypersensitivity — _____

_____

Allergen — _____

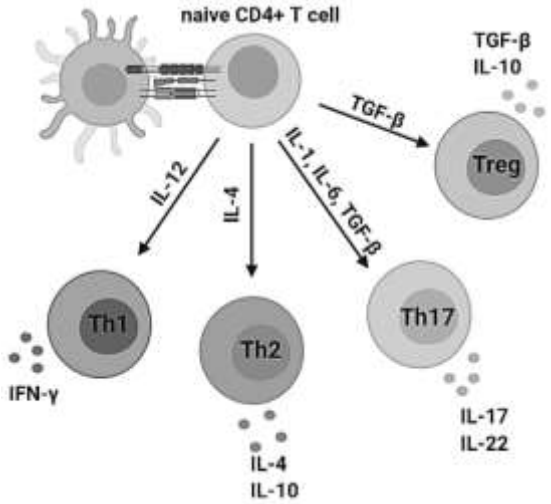
_____

Sensitization — _____

_____

Markers and receptors of T-lymphocytes:

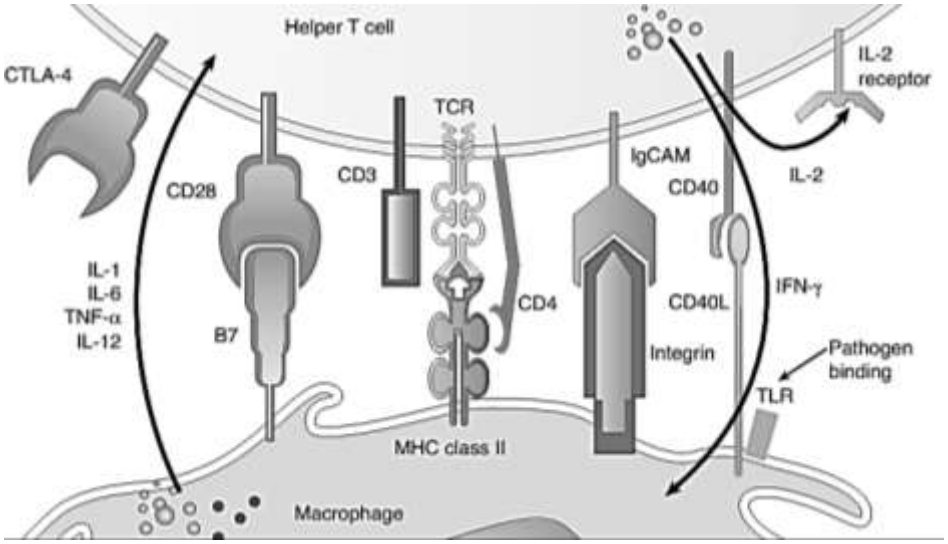
Main T-cells markers:	CD2 — the receptor to sheep erythrocytes (human ligand to CD2 is CD58 adhesion molecule); CD3 — TCR co-receptor	**Molecules for distant interactions:**	CD25/122/132 — alpha, beta and gamma chains of IL2 receptor CD121 — IL1 receptor CD117 — stem cells growth factor receptor CD124/132 — IL4 receptor CD127/132 — IL7 receptor CD129/132 — IL9 receptor
T-cell receptor (TCR):	Heterodimer composed of two chains. They both are transmembrane proteins of immunoglobulin superfamily. Extracellular part includes variable and constant domens. Together with another chain they form an agretope (antigen specific structure) which is responsible for antigen binding and recognition. Membrane parts stabilize the TCR structure and both membrane and intracellular parts transduce the activational signal to the nucleus		
Co-receptors:		**Activation markers:**	CD69 — early activation marker (function unknown) CD25 — alpha chain of IL2 receptor CD71 — transferrin receptor CD95 — receptor for activation-induced apoptosis HLA-II — type II MHC molecules
Co-stimulatory molecules:			
Adhesion molecules:			



Simplified diagram of the Th1, Th2, Th17, and Treg subsets, showing key cytokines produced by these cells.

	T-helper subsets			
	T-helper 1	**T-helper 2**	**T-helper 3**	**T-helper 17**
Inducing agents				
Function				
Principal cytokine				
Host responses				

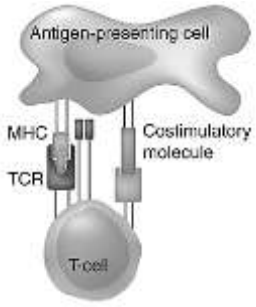
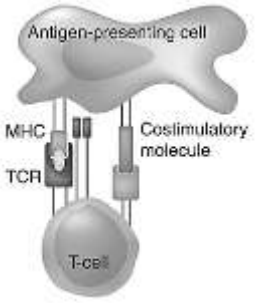
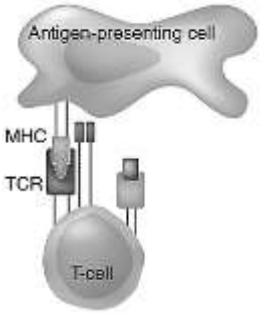
Signals involved in the activation of naïve T cells by APC:



Two-signal model of T-cell activation:

Signal 1	
Signal 2	

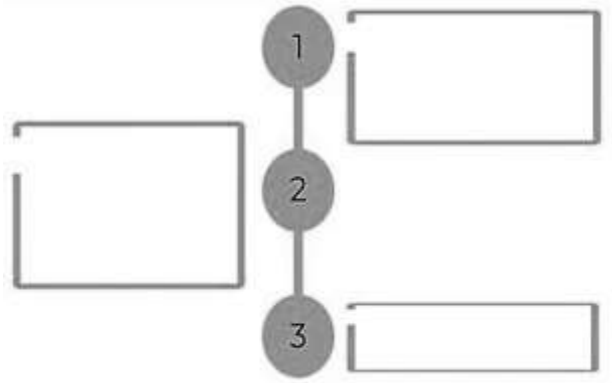
Describe the result of T-lymphocyte activation:

Signal 1 and 2	**No signal 1**	**No signal 2**
		

The scheme of the cellular immune response development (primary):

Location	Stages
I. Induction of CD+ T-effectors	
Tissues	1. Antigen (proteins or protein conjugates) is captured by APC, processed and transported to regional lymphatic nodes.
Secondary lymphoid organs	2. APCs process antigen through endosome pathway and present it to CD4+ naïve T-cells.
	3. T cells become activated, proliferate and differentiate into CD4+ effectors (Th1, Th2, Th3, Tr1, Tr2, CD4+CD25+ etc.): a) T-cell and APC adhere each other (LFA1+ICAM1 etc.); b) Antigen recognition (TCR+ MHC II-Ag) and costimulation occurs (CD28 + CD80, 86); c) T cells begin to express CD25 and thus form complete IL2 receptor, produce IL2, accept it and proliferation starts; d) Th0 cells differentiate into Th1 under the influence of IL12 produced by APC; differentiation into Th2 occurs spontaneously (IL4 stimulate this process); Th3 appear under the influence of large doses of IL10 and/or TGF beta; e) mature T-effectors enter recirculation. Usually they die from apoptosis within several weeks; some of them become memory cells.
Blood, tissues, secondary lymphoid organs	4. **Mature T-effectors:** a) can be activated by interaction with unprofessional APC; b) are able to produce cytokines of different profile; c) are able to recirculate in certain tissues in normal conditions; d) are able to enter any tissues under inflammation; e) die within weeks from apoptosis without activation; f) can postpone apoptosis for some time when activated. **CD4+ T-effectors** function as: **a) T-helpers**: 1. Help B-cells to produced immunoglobulins: activate and make growth factors for B-cells (IL6, IL2); cause the immunoglobulin isotype changing, differentiation in plasmacytes. 2. Help naïve CD8+ T-killer precursors: activate and make growth factors (IL2), control differentiation. **b) T-effectors of DTH**: they produce cytokines (proinflammatory cytokines, chemokines, anti-inflammatory cytokines, growth factors for broad types of cells (fibroblasts, nerve cells, endothelium etc.)); **c) T-regulators**: they can produce inhibitory cytokines (IL10, TGF beta) or express surface inhibitory factors (CTLA4); **d) T-killers** (insignificant part of CD4+ cells): CD4+ cells induce apoptosis of target cells in herpes infection.
II. Induction of CD8+ T-cell (T-killers)	
Tissues, secondary lymphoid organs, blood	1. Induction of CD4+ T-effectors (see above).
	2. APCs capture the antigen and transport it into secondary lymphoid organs:one should take into account that for T-killers the antigen must be processed and presented by cytoplasmic pathway. Therefore: a) APC capture antigen by endosome pathway and somehow transfer it into cytoplasmic one (so-called cross presentation); b) other considerations are even more doubtful. 3. APCs present antigen to CD8+ naïve T-cells by cytoplasmic pathway: a) naïve T-killer precursors are considered not able to kill APC during primary activation.
	4. CD8+ cells proliferate, differentiate, enter bloodstream and recirculate (see p. 4 above): a) CD8+ cells need IL2 from CD4+ T-effectors; b) the requirement for simultaneous activation of CD4+ and CD8+ lymphocytes testifies for triple component model (APC+Th1+Tk) and cross presentation.
	5. **CD8+ T-effectors** perform the next functions: a) killing. Activated mature T-killers have no need in additional signals and immediately lyse target cell after recognition of the antigen on its surface. Activated T-killer is able to lyse several target cells. Within few weeks T-killer dies from apoptosis. Some cells can return to the quiescent state and become the memory cells; b) cytokine production (less potent than CD4+ T-effectors). CD8+ cytokine producers can be distinguished in type I and II (like Th1 and Th2); c) immune response regulation (killing of APCs, production of pro or anti-inflammatory cytokines).

Stages of allergic reaction:



Hypersensitivity reactions

	Type 1	Type 2	Type 3	Type 4
Onset				
Antigen				
Mediators				
Effector mechanism				
Examples				

Cytokines of Adaptive Immunity:

Cytokine	Principal cell source	Biologic effects
Interleukin-2 (IL-2)	T cells	T cells: proliferation, increased cytokine synthesis; potentiates Fas-mediated apoptosis NK cells: proliferation, activation B cells: proliferation, antibody synthesis (*in vitro*)
Interleukin-4 (IL-4)	$CD4^+$ T cells (T_H2), mast cells	B cells: isotype switching to IgE T cells: T_H2 differentiation, proliferation Macrophages: inhibition of IFN-γ-mediated activation Mast cells: proliferation (*in vitro*)
Interleukin-5 (IL-5)	$CD4^+$ T cells (T_H2)	Eosinophils: activation, increased production B cells: proliferation, IgA production
Interferon-γ(IFN-γ)	T cells (T_H1, $CD8^+$T cells), NK cells	Macrophages: activation (increased microbicidal functions) B cells: isotype switching to opsonizing and complement-fixing IgG subclassesT cells: T_H1 differentiation Various cells: increased expression of class I and class II MHC molecules, increased antigen processing and presentation to T cells
Transforming growth factor-β (TGF-β)	T cells, macrophages, other cell types	T cells: inhibition of proliferation and effector functions B cells: inhibition of proliferation; IgA production Macrophages: inhibition
Lymphotoxin (LT)	T cells	Recruitment and activation of neutrophils Lymphoid organogenesis
Interleukin-13 (IL-13)	$CD4^+$ T cells (T_H2)	B cells: isotype switching to IgE Epithelial cells: increased mucus production Macrophages: inhibition

Selected methods for allergic diseases diagnostics:

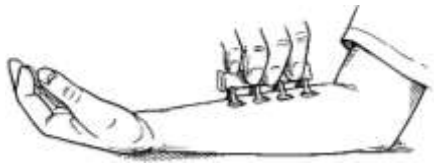
1. **Common considerations**:

1. Skin testing should be performed only in remission.

2. Any kind of skin test may cause severe systemic (anaphylaxis) or local reaction.

3. Skin test should be performed specially trained medical nurse; medical doctor should be present at procedure and register the results.

4. Before skin testing patient should stop antihistamine treatment (usually 48 hours before the procedure).



Prick testing

2. **Prick-test:** Allergens, positive and negative controls are introduced into epidermis by special disposable lancets. Testing usually performed on the palmary surface of the forearm. The skin is washed by ethanol, lancet or special multilancet device are dipped into allergen solutions and press against skin tightly. The registration of the results is performed in 20 min by measuring of hyperemia area.

Advantages of prick tests:

1. Easy to perform
2. Arbitrary safe
3. Painless
4. Not expensive

Disadvantages: low sensitivity (10–100 times less sensitive than traditional skin test).

Criteria for prick test interpretation

Interpretation		Reaction description
Negative	–	Like negative control
Low positive	+	Urtica 3–5 mm with hyperemia up to 10 mm
Positive	++	Urtica 5–10 mm, with hyperemia up to 10 mm
Highly positive	+++	Urtica 10–15 mm, with hyperemia above 10 mm
	++++	Urtica above 15 mm with pseudopodia, hyperemia above 20 mm
Doubtful	±	Only hyperemia

Mistakes in allergy diagnostics by skin tests:

A. False negative results:

1) absence of the target allergen in panel; 2) improper allergen storing and testing technique; 3) diminished skin reactivity because of age, individuality etc.; 4) temporary desensibilization after systemic allergic reactions (reflects internalization of IgE by mast cells and decrease of its expression on the surface. Usually skin tests should not be performed up to 3–4 weeks after anaphylaxis; 5) influence of anti-allergic drugs.

B. False positive results: 1) improper testing technique and/or allergen storing; 2) using drugs or food which can liberate histamine; 3) pronounced dermagraphism.

C. Testing results should be correlated with clinic examination data.

Selected laboratory tests for allergy diagnostics:

1. Methods for the detection of total IgE immunoglobulins in serum.

ELISA — the solid phase method in which surface-bound antibody traps a protein (IgE) by binding to one of its epitopes. An enzyme linked antibody specific for a different epitope on the protein surface is employed to detect the trapped protein. The concentration of the target protein can be calculated by standard curve.

IgE concentration in serum

Age group	KU/ml
New-born	0–2
3–6 months	3–10
1 year	8–20
5 years	10–50
10 years	15–60
Adults	20–100

2. Methods for the detection of specific IgE immunoglobulins in serum.

RAST (radioallergosorbent test) — the solid phase method involves binding of the allergen–antigen complex to an insoluble support such as dextran particles or Sepharose®. The serum is then passed over the allergen support complex that permits specific

IgE antibodies in the serum to bind with the allergen. After washing to remove nonreactive protein, radiolabeled anti-human IgE antibody is then placed in contact with the insoluble support, where it reacts with the bound IgE antibody. Both the allergen and anti-IgE antibody must be present in excess for the test to be accurate. The amount of radioactivity on the beads is proportional to the quantity of serum antibody that is allergen-specific.

3. At present an immuno chemiluminescent analysis is also used.

Class № 12. IMMUNODIAGNOSTICS OF INFECTIOUS DISEASES. SEROLOGICAL RESEARCH METHOD

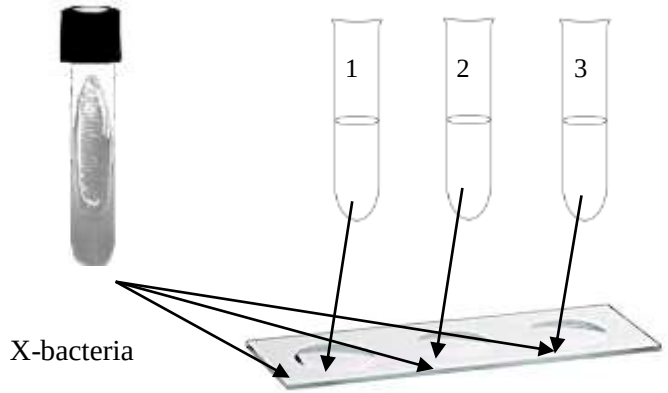
The subject to study:

Serological method, characteristics. Antibody titre. Diagnostic titre. Diagnosticum. Diagnostic serum.

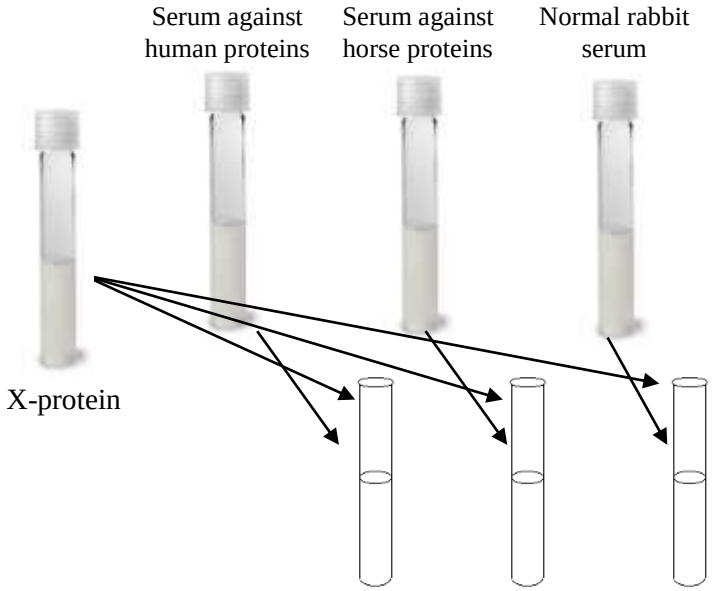
Agglutination, passive agglutination, reversed passive agglutination, latex agglutination.

Precipitation. Ring precipitation test, double immunodiffusion in a gel (by Ouchterlony), simple radial immunodiffusion in a gel (by Mancini), immunoelectrophoresis, electroimmunodiffusion.

Laboratory work

Laboratory exerscises	Laboratory report
1. Perform slide agglutination test to identify an X-bacteria	 1. Serum against *S. typhi* 2. Serum against *E. coli* 3. Saline solution **Conclusion:** _____
2. Determine the result of tube agglutination reaction	 **Conclusion:** _____

3. Perform the ring precipitation reaction to identify the X-antigen

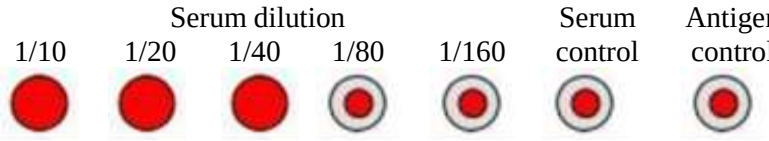


Positive result Negative result

Conclusion: _____

4. Determine the result of passive hemagglutination reaction

Passive hemagglutination reaction:

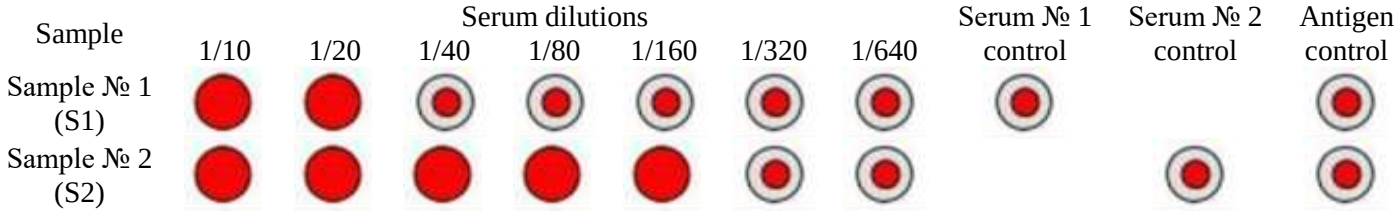


Conclusion: _____

5. Determine the result of passive hemagglutination reaction with pared sera

Ingredients:
– sera of the patient:
– S1 — taken at admission;
– S2 — taken in two weeks;
– erythrocytes suspension;
– saline solution.

Passive hemagglutination reaction with pared sera:

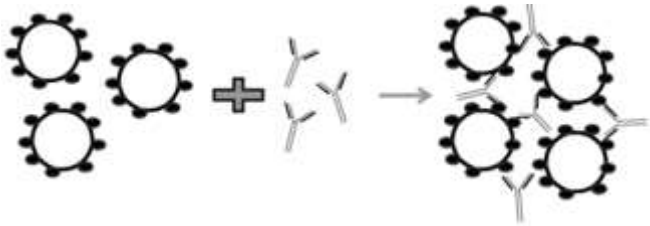


Conclusion: _____

Signature of the tutor _____ Date ____/____/2024

AGGLUTINATION

The combination of soluble antibody with particulate antigens in an aqueous medium containing electrolyte, such as erythrocytes, latex particles bearing antigen, or bacterial cells, to form an aggregate that may be viewed microscopically or macroscopically. Agglutination is the basis for multiple serological reactions, including blood grouping, diagnosis of infectious diseases, rheumatoid arthritis (RA) testing, etc. Red blood cells may serve as carriers for adsorbed antigen. Like precipitation, agglutination is a secondary manifestation of antigen–antibody interaction. As specific antibody crosslinks particulate antigens, aggregates that form become macroscopically visible and settle out of suspension. Thus, the agglutination reaction has a sensitivity 10 to 500 times greater than that of the precipitin test with respect to antibody detection.



Agglutinin. An antibody that interacts with antigen on the surfaces of particles such as erythrocytes, bacteria, or latex cubes to cause their aggregation or agglutination in an aqueous environment containing electrolyte.

Agglutinogen. Antigens on the surfaces of particles such as red blood cells that react with the antibody known as agglutinin to produce aggregation or agglutination. The most widely known agglutinogens are those of the ABO and related blood group systems.

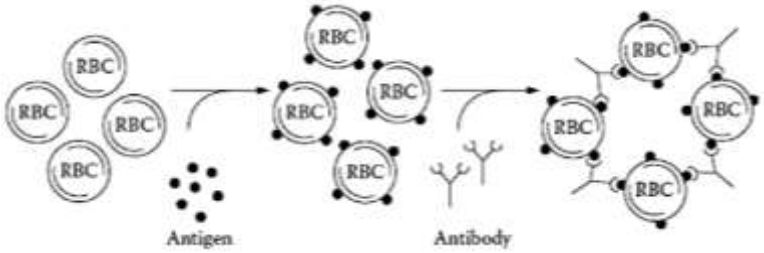
Antibody titer. The amount or level of circulating antibody in a patient with an infectious disease. For example, the reciprocal of the highest dilution of serum (containing antibodies) that reacts with antigen (e.g. produces an agglutination) is the titer.

Agglutination titer. The highest dilution of a serum that causes clumping of particles such as bacteria. It is determined by preparing serial dilutions of antibody to which a constant amount of antigen is added. The end point is the highest dilution of antiserum in which a visible reaction with antigen can be detected.

Slide agglutination test. The aggregation of particulate antigen using red blood cells, microorganisms, or latex particles coated with antigen within 30 seconds following contact with specific antibody. The reactants are usually mixed by rocking the slide back and forth, and agglutination is observed macroscopically and microscopically. The test has been widely used for screening but is unable to distinguish reactions produced by cross reacting antibodies that can be ruled out in a tube test that allows dilution of the antiserum.

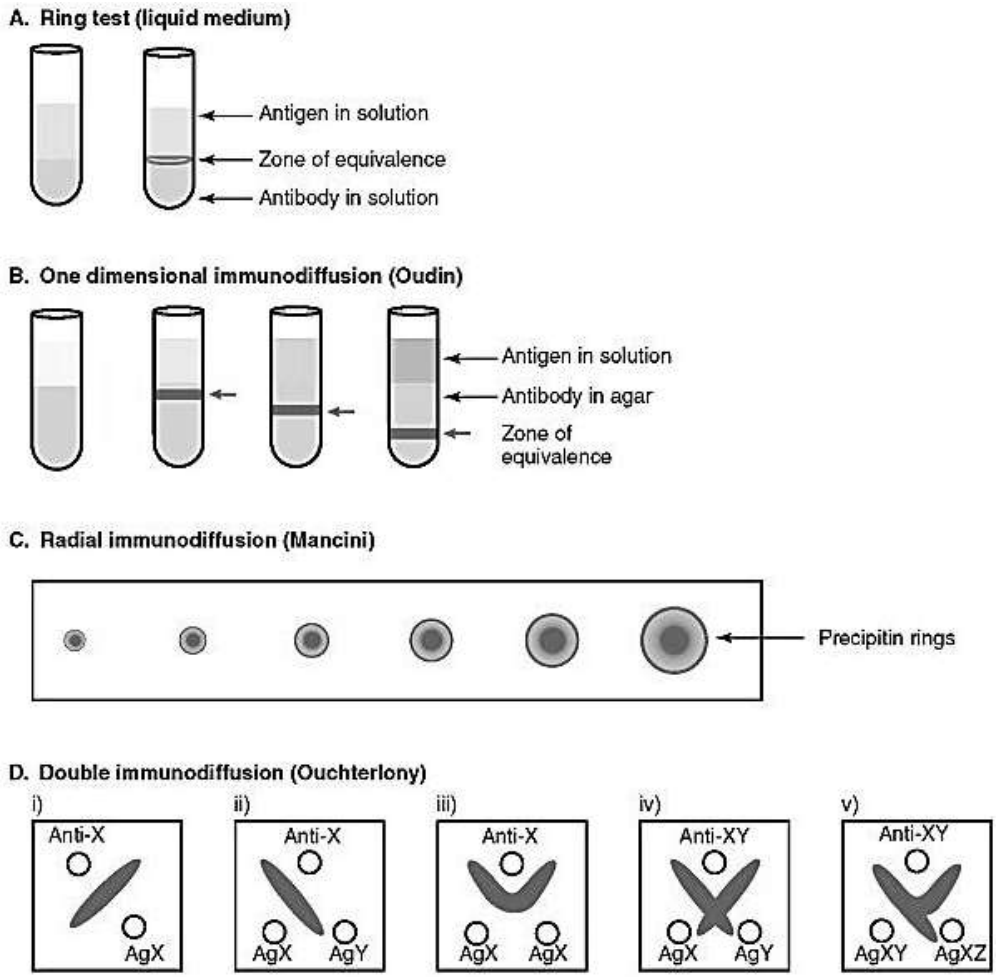
Tube agglutination test. An agglutination assay that consists of serial dilutions of antiserum in serological tubes to which a particulate antigen such as a microorganism is added.

Passive agglutination. The aggregation of particles with soluble antigens adsorbed to their surfaces by a homologous antibody. The soluble antigen may be linked to the particle surface through covalent bonds rather than by mere adsorption. Red blood cells, latex, bentonite, or collodion particles may be used as carriers for antigen molecules. When a red blood cell is used as a carrier particle, its surface has to be altered to facilitate maximal adsorption of the antigen to its surface. Several techniques are employed to accomplish this. One is the tanned red blood cell technique (treating the cells with a tannic acid solution), a second method is the treatment of red cell preparations with chemicals such as bis-diazotized benzidine. Because red blood cells are the most commonly employed particles, the technique is referred to as *passive hemagglutination*. Latex particles are used in the rheumatoid arthritis test, in which pooled IgG molecules are adsorbed to latex particles and reacted with the sera of patients with rheumatoid arthritis that contain rheumatoid factor to produce agglutination.



PRECIPITATION

Following the union of soluble macromolecular antigen with a homologous antibody in the presence of electrolytes *in vitro* and *in vivo* that occurs within seconds after contact, complexes of increasing density form in a lattice arrangement and settle out of solution, as in the precipitation or precipitin reaction. The materials needed for a precipitin reaction include antigen, antibody, and electrolyte. The reaction of soluble antigen and antibody in the precipitin test may be observed in liquid or gel media. The reaction in liquid media may be qualitative or quantitative. Following discovery of the precipitin reaction by Kraus, quantitative and semiquantitative measurements of antibody could be made. The term *precipitinogen* is sometimes employed to designate the antigen, and *precipitin* is the antibody in a precipitation reaction.



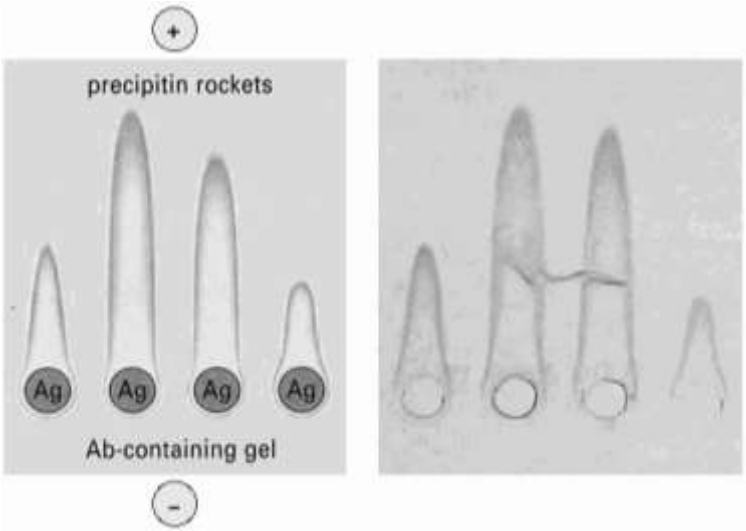
Simple immunodiffusion test:

1. A technique in which antibody is incorporated into agar gel and antigen is placed in a well cut into the surface of the antibody-containing agar. Following diffusion of the antigen into the agar, a ring of precipitation forms at the point where antigen and antibody reach equivalence. The diameter of the ring is used to quantify the antigen concentration by comparison with antigen standards.

2. The addition of antigen to a tube containing gel into which specific antibody has been incorporated. Lines of precipitation form at the site of interaction between equivalent quantities of antigen and antibody.

Ring precipitation test. A ring precipitation test developed by Rebecca Lancefield to classify streptococci according to their group-specific polysaccharides. The polysaccharide antigen is derived by treatment of cultures of the microorganisms with HCl, formimide, or a *Streptomyces albus* enzyme. Antiserum is first placed into a serological tube, followed by layering the polysaccharide antigen over it. A positive reaction is indicated by precipitation at the interface.

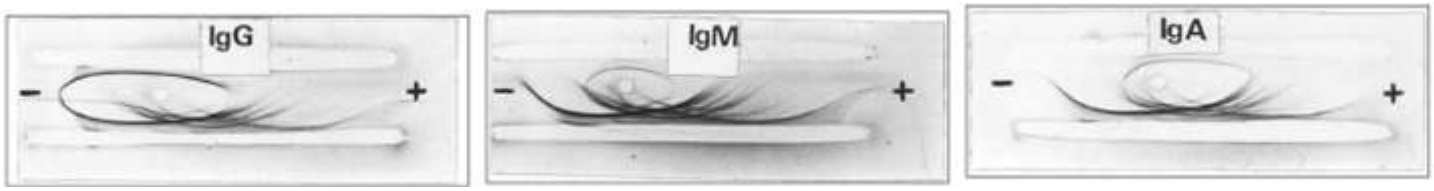
Double immunodiffusion test (Ouchterlony test). A double diffusion in a gel type precipitation test. Antigen and antibody solutions are placed in separate wells cut into an agar plate prepared with electrolyte. As the antigen and antibody diffuse through the gel medium, a line of precipitation forms at the point of contact between antigen and antibody. Results are expressed as a reaction of identity, reaction of partial identity, or reaction of nonidentity (refer to those entries for further details).



Electroimmunodiffusion. A double-diffusion in-gel method in which antigen and antibody are forced toward one another in an electrical field. Precipitation occurs at the site of their interaction. Also called counter immunoelectrophoresis.

Rocket electrophoresis. The electrophoresis of antigen into an agar-containing specific antibody. Through electroimmunodiffusion, lines of precipitation formed in the agar by the antigen–antibody interaction assume the shape of a rocket. The antigen concentration can be quantified because the size of the rocket-like area is proportional to the antigen concentration. This can be deduced by comparing with antigen standards. This technique has the advantage of speed. It can be completed within hours instead of longer periods required for single radial immunodiffusion. Also called Laurell rocket electrophoresis.

Immunoelectrophoresis (IEP or IE). A method to identify antigens on the basis of their electrophoretic mobility, diffusion in gel, and formation of precipitation arcs with specific antibody. Electrophoresis in gel is combined with diffusion of a specific antibody in a gel medium containing electrolyte to identify separated antigenic substances. This allows determination of the presence or absence of immunoglobulin molecules of various classes in a serum sample. One percent agar containing electrolyte is layered onto microscope slides and allowed to gel, and patterns of appropriate troughs and wells are cut in the solidified medium. Antigen to be identified is placed in the circular wells cut into the agar medium. This is followed by electrophoresis that permits separation of the antigenic components according to their electrophoretic mobility. Antiserum is placed in a long trough in the center of the slide. After antibody has diffused through the agar toward each separated antigen, precipitin arcs form where the antigen and antibody interact. Abnormal amounts of immunoglobulins result in changes in the shape and position of precipitin arcs when compared with the arcs formed by antibody against normal human serum components. With monoclonal gammopathies, the arcs become broad, bulged, and displaced. The absence of immunoglobulin classes such as those found in certain immunodeficiencies can also be detected with IEP.



Write down the following definitions:

Titer — _____

Diagnostic titer — _____

Diagnosticum — _____

Diagnostic serum — _____

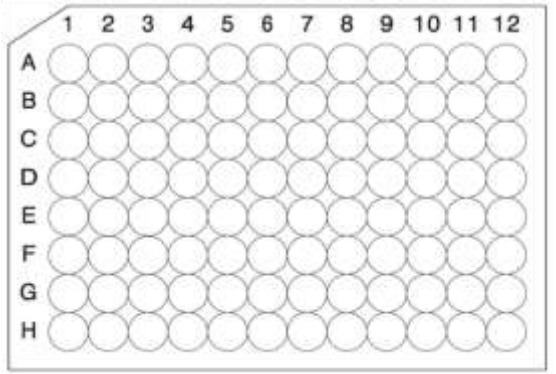
Class № 13. METHODS OF CLINICAL AND INFECTIOUS IMMUNOLOGY. SOLID PHASE IMMUNOLOGICAL ASSAY

The subject to study:

Immune lysis reactions.

Immunofluorescence test: direct and indirect variants. Immunoenzyme test. ELISA. Radioimmune test. Immunochromatographic analysis.

Laboratory work

Laboratory exerscises	Laboratory report
1. Perform ELISA for HBs antigen detection in donor serum. **Enzyme-linked immunosorbent assay (ELISA).** A binder-ligand immunoassay that employs an enzyme linked to either anti-immunoglobulin or antibody specific for antigen and detects either antibody or antigen. This method is based on the sandwich technique in which an enzyme is used as the label. Antibody is attached to the plastic tube, well, or bead surface to which the antigen-containing test sample is added. If antibody is sought in the test sample, the antigen should be attached to the plastic surface. Following antigen–antibody interaction, the enzyme–anti-immunoglobulin conjugate is added. The ELISA test is read by incubating the reactants with an appropriate substrate to yield a colored product that is measured in a spectrophotometer. Alkaline phosphatase and horseradish peroxidase enzymes are often employed.	**REPORT** **ELISA test for HBs-Ag detection in the serum** 1. Put 100 µl of control sera and samples according to to test scheme: rows A, B — negative control rows C — low positive control rows D — high positive control rows E–H — sample (1–4) 2. Place 50 µl of conjugate in each well; 3. Incubate for 1 hour at 37 °C; 4. Wash the strip 5 times; 5. Place 100 µl of chromogen in each well; 6. Incubate for 30 min at 37 °C; 7. Place 100 µl of stop-reagent in each well; 8. Measure the strip on ELISA reader and print out the results; 9. Fill in the report: check the test validity and make the final conclusion about results.  Test validity: a) average OD of negative controls must be < 0,15: OD(NC) (negative controls) = b) OD negative controls must range from 0,6 to 1,4 of average OD(NC): 0,6 OD(NC) = 1,4 OD(NC) = c) average positive controls OD must be more than four times as much as OD(NC): average OD(PC)/ OD(NC) = d) Low positive control OD must be higher than cut-off level: **Cut-off = OD(NC) + 0,04**

Antigen	OD	Result
Sample 1		
Sample 2		
Sample 3		
Sample 4		

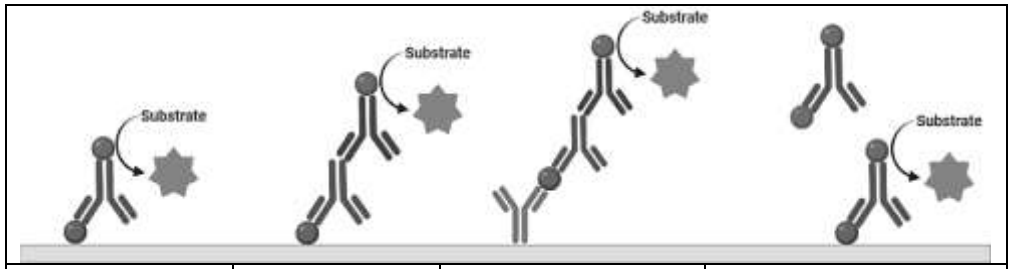
Conclusion

_____

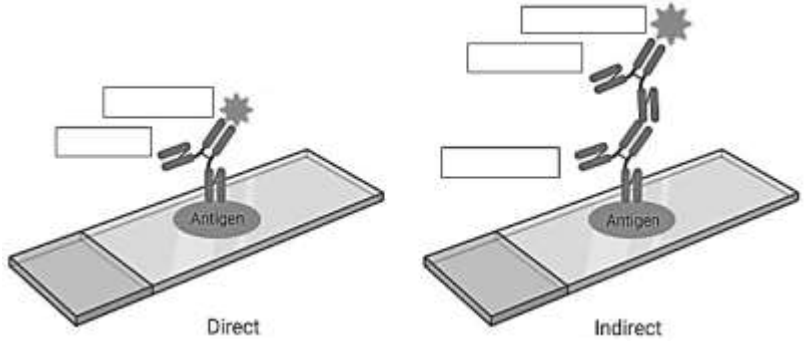
Signature of the tutor _____

Date ____/____/2024

Specify the type of ELISA:

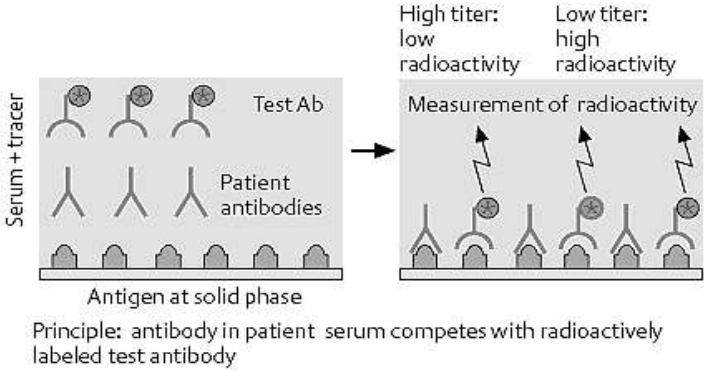


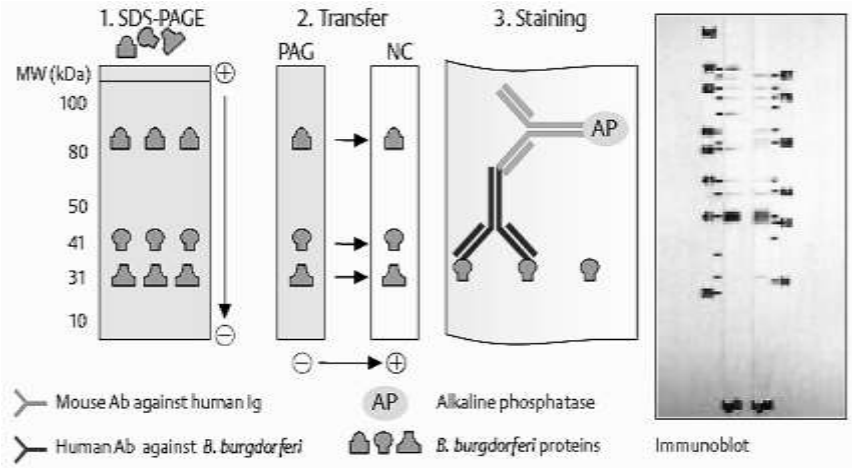
Describe the scheme of immunofluorescence reaction:



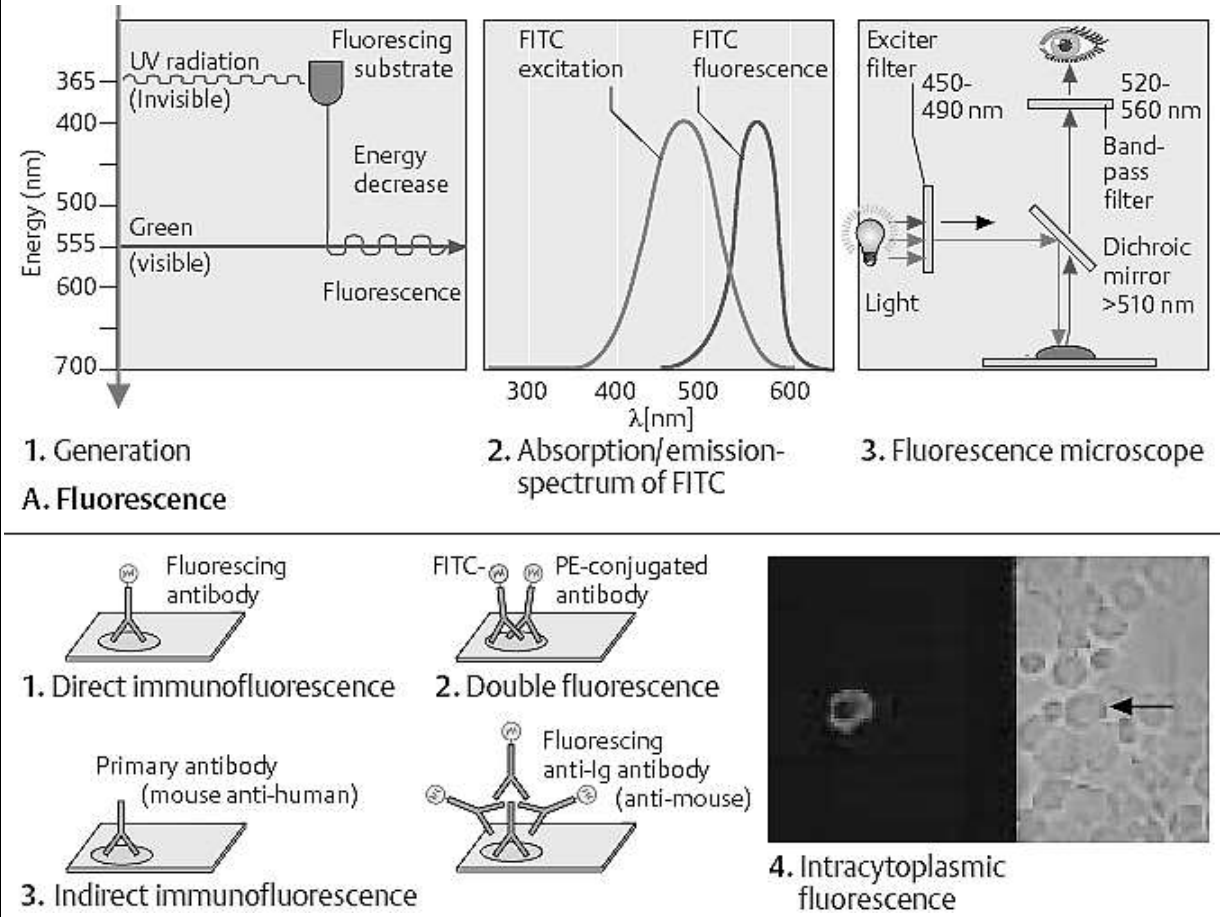
Radioimmunoassay (RIA). A binder ligand technique used to assay antigen or antibody; it is based on competitive inhibition by a radiolabeled antigen of the binding of an unlabeled antigen to specific antibodies. Minute quantities of enzymes, hormones, and other immunogenic substances can be assayed by RIA. Enzyme immunoassays have largely replaced RIAs because of the problems associated with radioisotope regulation and disposal.

Western blot (immunoblot). A method to identify antibodies against proteins of precise molecular weight. It is widely used as a confirmatory test for HIV-1 antibody following screening via the ELISA assay. Following separation of proteins by one- or twodimensional electrophoresis, they are blotted or transferred to a nitrocellulose or nylon membrane followed by exposure to biotinylated or radioisotope-labeled antibody; the antigen under investigation is revealed by either a color reaction or autoradiography, respectively.





RAST (radioallergosorbent test). A technique to detect specific IgE antibodies in serum. This solid phase method involves binding of the allergen–antigen complex to an insoluble support such as dextran particles or Sepharose®. The serum is then passed over the allergen support complex that permits specific IgE antibodies in the serum to bind with the allergen. After washing to remove nonreactive protein, radiolabeled anti-human IgE antibody is then placed in contact with the insoluble support, where it reacts with the bound IgE antibody. Both the allergen and anti-IgE antibody must be present in excess for the test to be accurate. The amount of radioactivity on the beads is proportional to the quantity of serum antibody that is allergen-specific.



Immunofluorescence. A method for the detection of antigen or antibody in cells or tissue sections through the use of fluorescent labels (fluorochromes) by fluorescent light microscopic examination. The most commonly used fluorochromes are fluorescein isothiocyanate, which imparts an apple-green fluorescence, and rhodamine B isothiocyanate, which imparts a reddish-orange tint. This method, developed by Albert Coons in the 1940s, has a wide application in diagnostic medicine and research. In addition to antigens and antibodies, complements and other immune mediators may also be detected by this method. It is based on the principle that, following adsorption of light by molecules, cells or tissues dispose of their increased energy by an emission of light of longer wavelength. Several immunofluorescence techniques are available. In the direct test, smears of the substance to be examined are fixed with heat or methanol and followed by flooding with a fluorochrome–antibody conjugate. This is followed by incubating in a moist chamber for 30 to 60 minutes at 37 °C, after which the smear is washed first in buffered saline for 5 to 10 minutes and then in tap water for another 5 to 10 minutes. The washing procedures remove uncombined conjugated globulin. After adding a small drop of buffered glycerol and a cover slip, the smear may be examined with a fluorescence light microscope. In the indirect test, which is more sensitive, a smear or tissue section is first flooded with unlabeled antibody specific for the antigen sought. After washing, fluorescein-labeled antiimmunoglobulin of the species of the primary antibody is layered over the section. After appropriate incubation and washing, the section is cover slipped and examined as in the direct method. Variations such as complement staining are also available. The indirect method is more sensitive and considerably less expensive; one fluorochrome-labeled antiimmunoglobulin may be used with multiple primary antibodies specific for a battery of antigens.

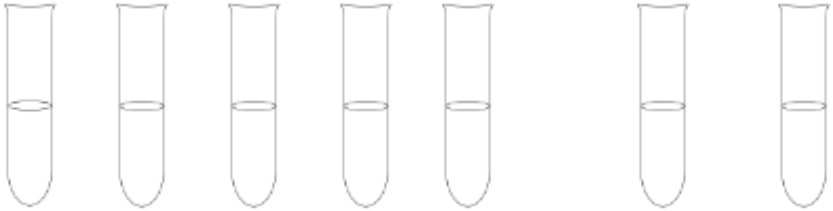
Class № 14. ANTI-INFECTIVE IMMUNITY. IMMUNOPROPHYLAXIS AND IMMUNOTHERAPY OF INFECTIOUS DISEASES. METHODS OF VACCINAL IMMUNITY EVALUATION

The subject to study:

Anti-infective immunity. Mechanisms of antitoxic, antibacterial, antifungal, antiparasitic immunity. Protective immunity. Maternal immunity: mechanisms, significance.

Immunoprophylaxis and immunotherapy. Vaccines, classification, essential characteristics. Vaccinal immunity, factors affecting its development. Primary and secondary immune response. Booster reaction. Methods of vaccinal immunity evaluation. Passive immunoprophylaxis. Immune sera and serum preparations; methods of its production and application.

Laboratory work

Laboratory exerscises	**Laboratory report**
1. Register the agglutination reaction for the evaluation of immunity to whooping cough.	1/50 1/100 1/200 1/400 1/800 Serum control Antigen control  **Conclusion: _____**
2. Register the passive hemagglutination test for the evaluation of immunity to diphtheria. ***Protective titer*** *— surrogate marker of the organism immunity (due to antibodies formation) to particular infection.*	**Passive hemagglutination reaction:** Serum dilution 1/10 1/20 1/40 1/80 1/160 1/320 1/640 Serum control Antigen control  Protective titer = 1 : 40 **Conclusion:_____** **Signature of the tutor _____ Date ____/____/2024**

Type of vaccine			Examples: (write in cells)
Live Attenuated Vaccine		Living pathogen that has been weakened in the laboratory	
Inactivated Vaccine		Whole pathogen killed by heat, chemicals or radiation	
Subunit Vaccine	Protein Subunit	Purified viral antigens	
	Polysaccharide/Conjugate	Surface polysaccharide antigens, primarily from bacterial pathogens	
	Toxoid	Chemically inactivated toxins from pathogen	
	Virus-Like Particles	Particles that contain virus surface proteins that can elicit an immune response, but lack viral genetic material (so cannot replicate)	*Human papillomavirus vaccine*
Viral Vaccine	Replicating	A carrier virus that is able to infect human cells (such as an adenovirus) is introduced carrying genetic material that codes for the specific viral antigen in order to elicit the immune response	*SARS-CoV-2 vaccine in development*
	Non-Replicating	A carrier virus (such as an adenovirus) that is able to infect human cells **but cannot replicate** is introduced carrying genetic material that codes for the specific viral antigen in order to elicit the immune response	*SARS-CoV-2 vaccine*
Nucleic Acids	DNA Vaccine	DNA plasmid containing DNA sequences encoding for viral/pathogenic antigen	*Ebola vaccine*
	RNA Vaccine	mRNA sequences that encode for viral antigenic proteins. Often carried by lipid nanoparticles	*SARS-CoV-2 vaccine*

Define the following terms:

Vaccine — _____

Vaccination — _____

Immunization — _____

Passive immunoprophylaxis — _____

Active immunoprophylaxis — _____

Adjuvant — _____

Booster shots — _____

Immune sera and serum preparations:

Antisera and immunoglobulin preparations are routinely used for:

1. Immunodeficiency therapy;
2. Immune cells depletion (treatment for autoimmune diseases);
3. Cancer therapy (immunotoxins);
4. Prevention of the alloimmunization of Rhesus-negative women;
5. Prevention and treatment of infection:

The ability of antibody to neutralize toxins and organisms is exploited to prevent several infectious diseases. Antibodies used include human hyperimmune globulin, equine serum and humanized MAbs.

Indications include exposure to:

– *Tetanus* — the causative toxin can be neutralized by antibodies.

Human hyperimmune serum is given following a high-risk injury in non-immune patients.

– *Hepatitis B* — the risk of infection is reduced by neutralizing antibodies, which inhibit viral entry into cells.

Human hyperimmune serum is administered after high-risk exposure in non-immune individuals.

– *Varicella zoster (VZV)* — in non-immune, immunocompromised or pregnant patients, primary infection can be fatal. Neutralizing antibodies can reduce the risk of infection as well as the severity.

Specific VZV immunoglobulin, or batches of intravenous immunoglobulin known to have high anti-VZV titers may be used post-exposure in high-risk groups.

– *Cytomegalovirus* — infection in immunosuppressed patients can cause severe disease. Immunoglobulin treatment was widely used as prophylaxis; however antiviral agents like ganciclovir are now used more commonly.

– *Hepatitis A* — immunoglobulin can be used in immunocompromised individuals, following exposure or prior to travel. Vaccination is preferred in immunocompetent individuals.

– *Rabies and botulism* — equine antibodies given post exposure may be of value in these life-threatening infections.

– *Respiratory syncytial virus* — *RSV* causes bronchiolitis in young children, and severe illness in children born prematurely, particularly if they have had bronchopulmonary dysplasia.

Palivizumab, a humanized neutralizing MAb, can be administered monthly during the RSV season to reduce the risk of infection.

6. Immunomodulation:

Immunoglobulin preparations can be used for immunomodulation:

– Infliximab is a humanized MAb, which inhibits TNF, and etanercept is a TNF receptor grafted onto an IgG molecule. Anti-TNF therapy is used in rheumatoid disease, Crohn’s disease and juvenile arthritis.

– Monoclonal antibodies are also used for IgE elimination and allergy treatment.

– Neutralization of envenomation.

Class № 15. BASICS OF CLINICAL IMMUNOLOGY. METHODS OF DETERMINATION AND ESTIMATION OF THE IMMUNE STATUS. IMMUNOPATHOLOGY. TRANSPLANTATION IMMUNITY. ANTITUMOR IMMUNITY

The subject to study:

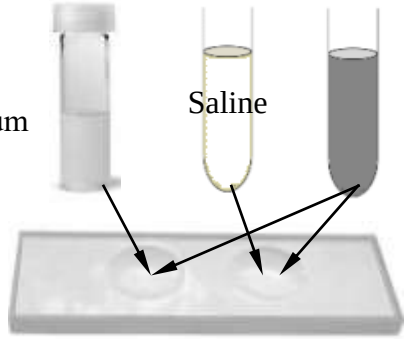
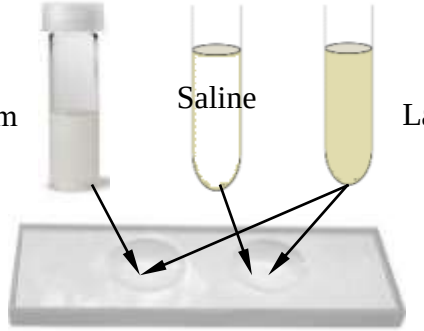
Clinical immunology: definition, tasks. The immune status of the organism. 1st and 2nd levels of assessment of the immune status. Immunogram and its types.

Primary and secondary immunodeficiencies. Autoimmune diseases. Causes, manifestations. Autoantibodies, diagnostic value, methods of determination. Methods for correcting immune status disorders. Immunosuppression. Immunostimulation. Immunomodulators. Means thymus, spleen, bone marrow. Interleukins, interferons.

Transplantation immunity. Histocompatibility antigens. Transplant reactions: types, mechanisms of development, prevention. Immunological tolerance: mechanisms, significance.

Tumor antigens. Mechanisms of antitumor immunity. Escape mechanisms of tumors from immune surveillance.

Laboratory work

Laboratory exerscises	Laboratory report
1. Perform the passive hemagglutination test for the detection of rheumatoid factor. ***Diagnosticum*** = armed bull erythrocytes coated with human IgG. Rheumatoid factor is an autological antibodies (IgM) to IgG. It is found in certain autoimmune diseases (SLE, RA etc.) and is useful for diagnostics.	 **Conclusion: _____**
2. Perform the latex agglutination test to detect autoantibodies to thyreoglobulin. ***Latex diagnosticum*** = latex microsphera coated with thyreoglobulin molecules.	 **Conclusion: _____**

3. Perform semi-quantitative ELISA test for the determination of human autoantibodies of the IgG class against cyclic citrullinated peptides (CCP) in patient serum.

The most commonly performed serological test in suspected RA cases was until now the determination of rheumatoid factors (RF) in addition to general inflammatory parameters.

40–60 % of RA patients also exhibit autoantibodies against epidermal filaggrin (RA keratin, anti-perinuclear factor) in their serum. Filaggrin is a protein of the epidermis, which links keratin filaments to one another.

Amino acid citrulline, which is present in filaggrin, is a substantial component of the antigenic epitope. Autoantibodies against cyclic citrullinated peptides (CCP) are highly specific marker for rheumatoid arthritis.

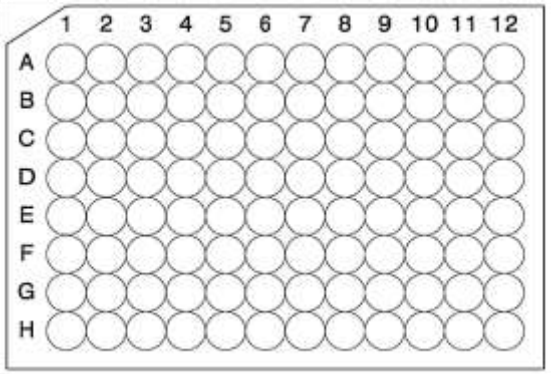
Antibodies against CCP are predominantly of class IgG. They are a predictive marker since they can be found in the serum and the synovial liquid of 70–80 % of patients very early during the development of the disease, often even many years before the onset of the first symptoms.

The importance of antibodies against CCP as a serological marker becomes apparent in comparison with rheumatoid factors (RF) which have a significantly lower specificity (anti-CCP: 96–100 %, RF: 63 %) at almost the same sensitivity (anti-CCP: 80 %, RF: 79 %). Antibodies against CCP can also be used as a marker in differential diagnostics, e.g. in the differentiation of hepatitis-associated arthropathies from rheumatoid arthritis.

REPORT

semi-quantitative ELISA test for CCP detection in the serum

1. Put 100 µl of of the calibrators, controls and samples according to to test scheme:
rows A — calibrator 2
rows B — positive control
rows C — negative control
rows D–H — sample (1–5).
2. Incubate for 60 minutes at room temperature.
3. Wash the strip 3 times.
4. Place 100 µl of enzyme conjugate (peroxidase-labelled anti-human IgG) in each well.
5. Incubate for 30 minutes at room temperature.
6. Wash the strip 3 times.
7. Place 100 µl of chromogen in each well.
8. Incubate for 30 min at 37 °C.
9. Place 100 µl of stop-reagent in each well.
10. Measure the strip on ELISA reader and print out the results.



Antigen	OD	Ratio	Result
Calibrator 2			
Sample 1			
Sample 2			
Sample 3			
Sample 4			
Sample 5			

Calculation of results:

Results can be evaluated semiquantitatively by calculating a ratio of the extinction value of the control or patient sample over the extinction value of calibrator 2. Calculate the ratio according to the following formula:

Ratio = Extinction of the control or patient sample / Extinction of calibrator 2
Result: Ratio ≤ 1.0: negative
Ratio > 1.0: positive

Conclusion _____

Signature of the tutor _____ **Date** ____/____/**2024**

The names of therapeutic monoclonal antibodies:

Prefix + Target or disease (*li (m) — for lymphocyte or immune; tu (m) — for tumor*) + Sourse + Stem (- mab)

Sourse	Mouse -o- 	Chimeric -xi- 	Humanized -zu- 	Fully Human -u- 
Examples				

Clinical Tumor Markers:

Cancer	Marker	Marker Description
Breast Cancer	CA125	
	CEA	
	HER2	
Pancreas Cancer	CA125	
	CEA	
Liver Cancer	AFP	
Prostate Cancer	PSA	
Testicular Cancer	AFP	
	HCG	

Evasion of immune responses by tumors:

1. Immune Checkpoints. Tumors evade antitumor T cell responses by engaging inhibitory molecules (CTLA-4 (cytotoxic T-lymphocyte – associated protein 4) and PD-1 (programmed cell death protein-1), two of the best-defined inhibitory pathways in T cells. LAG-3, TIM-3, and TIGIT also may contribute to inhibition of antitumor immune responses.
2. Secreted products of tumor cells may suppress antitumor immune responses. An example of an immunosuppressive tumor product is TGF-β, which is secreted by many tumors and inhibits the proliferation and effector functions of lymphocytes and macrophages.
3. Regulatory T cells may suppress T cell responses to tumors.
4. Myeloid-derived suppressor cells (MDSCs) are immature myeloid precursors that accumulate in bone marrow, lymphoid tissues, blood, and suppress innate and T cell–mediated antitumor immune responses. MDSCs suppress innate and adaptive immune responses by many different mechanisms, including secretion of immunosuppressive cytokines, such as IL-10 and TGF-β, and of prostaglandins, and to promote Treg differentiation.
5. M2 macrophages activated by tumors may also inhibit antitumor immunity and promote tumor growth.
6. Loss of Tumor Antigen Expression. Immune responses to tumor cells impart selective pressures that result in the survival and outgrowth of variant tumor cells with reduced immunogenicity. This phenomenon has been called immune editing, implying that the immune response directs changes in tumors that help them evade the response.
7. Class I MHC expression may be downregulated on tumor cells so they cannot be recognized by CTLs.

Class № 16. CONCLUDING SESSION: «THEORETICAL AND APPLIED MEDICAL IMMUNOLOGY»

List of questions:

1. Immunology. Definition, tasks, methods. History of immunology.
2. Immune system. Characteristics. Organs, cells.
3. Molecules of an immune system: receptors, MHC molecules of I, II and III types, adhesins, immunoglobulins superfamily.
4. Cytokines. Definition, classification. Biological importance, clinical application. Chemokines and its receptors.
5. Immunity: definition, classification. Characteristics of innate and acquired immunity. Anti-infection immunity.
6. Innate immunity: definition, immune and non immune factors, characteristics.
7. Complement system: definition, ways of activation, functions. Medical importance. Methods of complement activity evaluation.
8. Phagocytosis. Phagocytes. Phagocytosis phases. Intracellular killing mechanisms. Phagocytosis outcome (complete, incomplete). Chemotaxins, opsonins: origin and medical importance.
9. Phagocytosis evaluation methods.
10. Immune response and factors influencing its strength. Genetic control of humoral and cellular immune response.
11. Humoral immune response. Primary and secondary immune response.
12. B-lymphocytes, characteristics, main markers. Methods for B-lymphocytes quantity and functional activity evaluation.
13. Antigens: structure, classification, characteristics.
14. Bacteria antigenic structure. Group, species and type antigens. Cross-reacting antigens. Antigenic formula.
15. Antibodies, structure-functional organization of immunoglobulin molecule, characteristics. Antiidiotypic antibodies.
16. Classes of immunoglobulins, characteristics. Immunoglobulins Subclasses, allotypes, isotypes, idiotypes. Methods of immunoglobulins concentration determination.
17. Mechanisms of antigens and antibodies interactions. Specificity. Phases. Affinity. Avidity.
18. Serology reactions, characteristics. Serum titer, diagnosticum, diagnostic serum, clinical importance.
19. Agglutination reaction. Methods of conduction and result registration. Medical importance.
20. Passive hemagglutination, ingredients. Methods of conduction and result registration. Medical importance. Reversed passive agglutination test. Latex agglutination.
21. Precipitation reaction. Methods of conduction and result registration. Medical importance.
22. Immunofluorescence test. Medical importance.
23. Immunoenzyme analysis. ELISA. Ingredients, methods of conduction, results registration, characteristics. Medical importance.
24. Immune lysis reactions.
25. Cellular immune response, main phenomena. Immunological memory.
26. Subpopulations of T-lymphocytes (T-helpers, killers, regulators), characteristics. Main markers, TCR. Genetic control of TCR diversity.
27. T-lymphocyte activation. Costimulation. Two signals model. Anergy. Apoptosis.
28. Methods for T-lymphocytes quantity and functional activity evaluation.

29. Local immunity, main components. Medical importance.
30. Allergy: definition, classification. Allergy phases.
31. Allergens: definition, classification, characteristics.
32. Allergic reaction of immediate type, clinical phenomena.
33. Mediator type of ITH: definition, mechanisms, clinical phenomena, approaches for prophylaxis.
34. Cytotoxic (II) and immunocomplex (III) ITH types: definitions, mechanisms, clinical phenomena.
35. Hypersensitivity of delayed type (IY): definition, classification, clinical phenomena.
36. Methods for ITH diagnostics (in vivo and in vitro).
37. Methods for DTH diagnostics (in vivo and in vitro).
38. Immune tolerance: definition, mechanisms, medical importance.
39. Transplantation immunity. MHC antigens of I, II, III types, role for an immune response development. Transplantological reactions. Mechanisms of transplant rejection. Prophylaxis.
40. Clinical immunology: definition, aims. Ecological immunology, main immunotropic ecological factors.
41. Primary and secondary immunodeficiencies: definitions, classification, medical importance.
42. Immune status: definition, methods for evaluation. Immunogram. Influence of way of life on the immune system function.
43. Autoimmune diseases, classification. Autoantigens. Mechanisms of autoimmunity. Tumor associated immunity.
44. Immunoprophylaxis and immunotherapy of infections. Achievements and problems.
45. Vaccines, main demands. Classification, characteristics, approaches to development. New vaccines.
46. Vaccinal immunity. Factors influencing vaccinal immunity. Methods of evaluation. Collective immunity, methods of evaluation.
47. Passive immunoprophylaxis. Antisera for therapy and prophylaxis, medical importance.
48. Immunocorrection. Methods for suppression and stimulation of an immune response, drugs for immunocorrection.

List of practice:
1. Register the result of agglutination test.
2. Register the result of gel immunoprecipitation test.
3. Register the result of complement fixation test.
4. Register the result of passive hemagglutination test.
5. Perform the slide agglutination test
6. Determine the immunoglobulins concentration.
7. Determine T-lymphocytes quantity in ready slide by immune rosettes method.
8. Determine phagocytosis indices in ready slides.

Signature of the tutor _____ Date ____/____/2024

Class № 17. MICROBIOLOGICAL DIAGNOSTICS OF DISEASES CAUSED BY STAPHYLOCOCCI, STREPTOCOCCI, NEISSERIA

The subject to study:

Staphylococci, systematics, general characteristics. Methods of microbiological diagnostics of staphylococcal infections. The material for the research depending on the form of the infection. Scheme of pure culture isolation (from pus, mucus, blood, etc.). Identification methods, phage typing. Specific prevention and treatment of staphylococcal infections.

Streptococci. Systematics. Pyogenic streptococci. Pneumococci. General characteristics. Antigenic structure. Acute and chronic diseases, pathogenesis, immunity. Specific antibodies to streptococcal antigens, diagnostic value. Methods for streptococcal infections diagnosis. Bacteriological method, study design. Material for studies depending on the form of the infection, the rules and methods for taking material. Principles of therapy and prevention pro-streptococcal infections.

Neisseria. Systematics, general characteristics. Characteristics of the causative agent, mechanisms of pathogenesis, immunity, methods of microbiological diagnosis of acute and chronic gonorrhea. Characteristics of the causative agent, mechanisms of pathogenesis, immunity, diagnosis and prevention of meningococcal infection.

Laboratory work

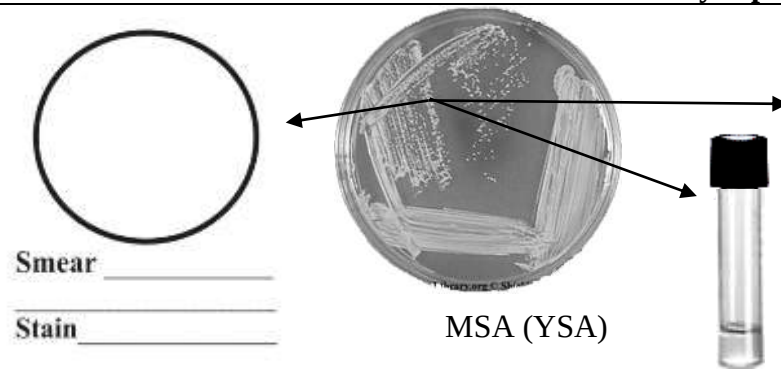
Laboratory exerscises

1. Microbiological diagnostics of staphylococcal infection, Step 2:

a) macro- and microscopic examination of the colonies on YSA;

b) plasmacoagulase test.

Laboratory report



Feature	Staphylococcal colonies
Shape	
Size	
Surface	
Edge	
Color	
Cionsistency	
Transparency	
Lecithinase	

Stabilized rabbit plasma, 37 °C, 2–4–24 h.

Conclusion: according to morphological, cultural and biochemical properties unknown bacterium is identified as _____.

2. Microbiological diagnostics of streptococcal infection, 3[d] step:

a) a description of Streptococci growth in serum broth;

b) determining the morphology of streptococci, Gram staining;

c) determination of streptococcus serogroup by ring precipitation test.

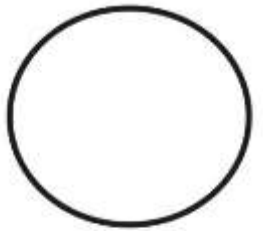
Serum broth

Colonies characteristics _____

Cell wall antigens extract

AntiSerum group A | AntiSerum group D | Normal serum

Ring precipitation test



Smear _____

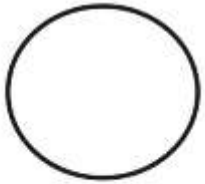
_____

Stain_____

Conclusion: according to morphological, cultural and biochemical properties unknown bacterium is identified as _____

Demonstration:

1. *Staphylococcus aureus* in pus, Gram staining.
2. *Streptococcus pneumoniae*, pure culture, Gram staining.
3. *Streptococcus pneumoniae*, white mice, Gram staining.
4. *Gonococcus* in pus, Gram staining.
5. *Meningococcus* in cerebrospinal fluid, methylene blue.
6. The growth of staphylococci on YSA, blood agar, broth.
7. The growth of streptococci on blood agar and broth.
8. Plasmacoagulase test.
9. Anaerobic mannitol fermentation.
10. Phage typing of staphylococci.



Smear _____

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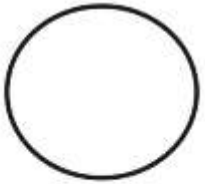
Stain_____



Smear _____

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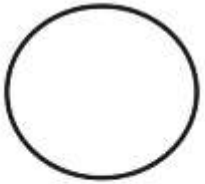
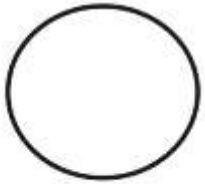
Stain_____



Smear _____

_____

Stain_____



Smear _____

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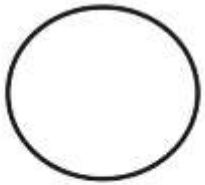
Stain_____



Smear _____

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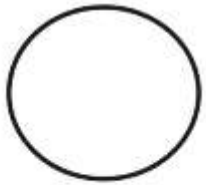

Smear _____

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Characteristics of staphylococci, streptococci, neisseria						
Genus	***Staphylococcus***	***Streptococcus***		***Enterococcus***	***Neisseria***	
Species	*S. aureus* *S. epidermidis,* *S. saprophyticus*	*S. pyogenes*	*S. pneumoniae*	*E. faecalis*	*N. meningitidis*	*N. gonorrhoeae*
Morphology						
Spores						
Capsule						
Flagella (motility)						
Gram staining						
Catalase activity						
Main pathogenicity factors						

Methods for diagnostics:

Method/ Genus	***Staphylococcus***	***Streptococcus***		***Enterococcus***	***Neisseria***	
	S. aureus, *S. epidermidis,* *S. saprophyticus*	*S. pyogenes*	*S. pneumoniae*	*E. faecalis*	*N. meningitidis*	*N. gonorrhoeae*
Microscopic						
Cultural						
Biological						
Serological						
Allergic						
Molecular-genetic						

***Staphylococci identification*:**

Species	Plasma-coagulation test	Anaerobic mannitol fermentation	DNA-se	Lecithinase	Protein-A
S. aureus					
S. epidermidis					
S. saprophyticus					

***Staphylococci identification*:**

Species	Growth in nutrition broth	Hemolysis (α, β, γ)	Precipitation test	Capsule swelling test	Inulin fermentation	Optochin test	Bile test
S. pyogenes							
S. pneumoniae							
E. faecalis							

Neisseria differentiation:

Species	Growth on nutrition agar	Growth at 20ºC	Colonies color	Fermentation	
				Glucose	Maltose
N. meningitidis					
N. gonorrhoeae					
Opportunistic species					

Bacteriological diagnostics of staphylococci, streptococci, neisseria

Staphylococcus:	***Streptococcus:***	***Neisseria:***
Media for pure culture isolation	**Media for pure culture isolation**	**Media for pure culture isolation**
Medium for pure culture accumulation	**Medium for pure culture accumulation**	**Medium for pure culture accumulation**
Material for investigation	**Material for investigation**	**Material for investigation**

CREDIT «GENERAL MICROBIOLOGY. IMMUNOLOGY»

Questions for the credit were uploaded to the e-test (section «Final knowledge control»).

Class № 1 (18). MICROBIOLOGICAL DIAGNOSTICS OF ACUTE ENTERIC INFECTIONS CAUSED BY ENTEROBACTERIA

The subject to study:

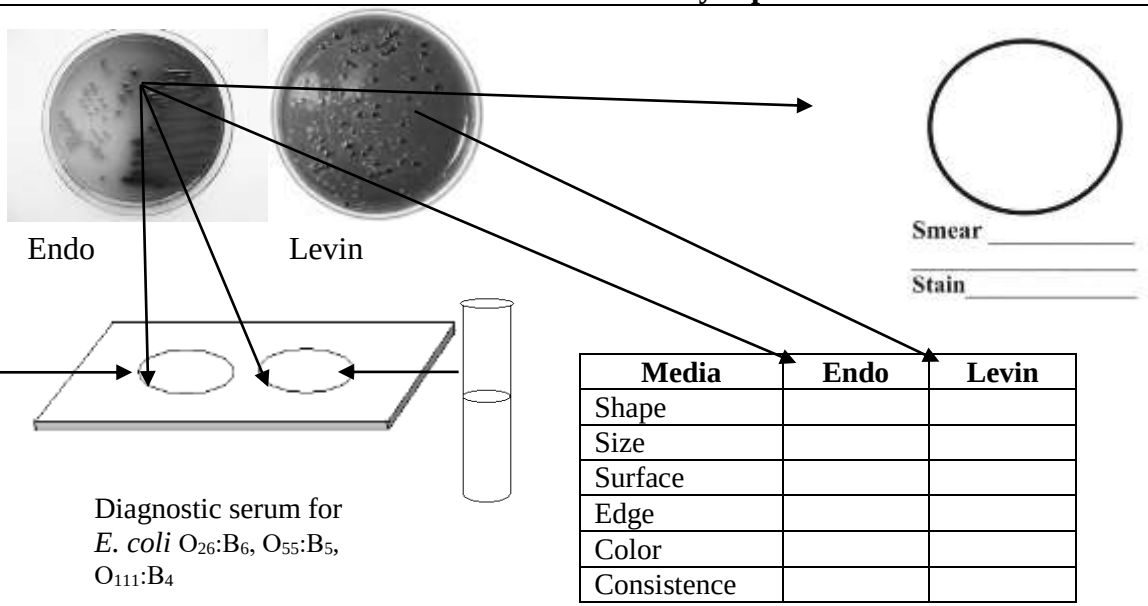
General characteristics of Enterobacteriaceae family. Differences between genera. General principles of diagnostics of acute enteric infections caused by pathogenic enterobacteria. Differential diagnostic media, composition, plinciple of work.

Escherichia, systematic position, general characteristics. The biological role of Escherichia coli. Molecular mechanisms of escherihiosis pathogenesis. Enteropathogenic, enterotoxigenic, enteroinvasive and enterohaemorrhagic Escherichia coli. Escherihiosis diagnostics. Antibiotic treatment.

Salmonella, classification and general characteristics. Serological classification of Salmonella. Identification of Salmonella. Molecular biological typing.

Causative agents of typhoid and paratyphoid. The pathogenesis of typhoid. Microbiological diagnostics of typhoid fever, depending on the stage of pathogenesis.

Laboratory work

Laboratory exerscises	**Laboratory report**
1. Bacteriological diagnostics of escherichiosis, 2nd step: a) exploring of E. coli colonies on Endo and Levin media; b) slides preparation from colonies, Gram staining; c) slide agglutination test with polyvalent diagnostic OK-serum.	 **Media** / **Endo** / **Levin** Shape Size Surface Edge Color Consistence **Conclusion:** according to morphological, cultural and biochemical properties unknown bacterium is identified as _____.

2. Bacteriological diagnostics of typhoid: 2nd step of coproculture isolation:

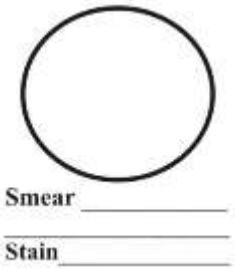
a) describe colonies on Levin medium;

b) prepare slide from colonies, Gram staining;

c) inoculate Kligler medium.

Levin medium

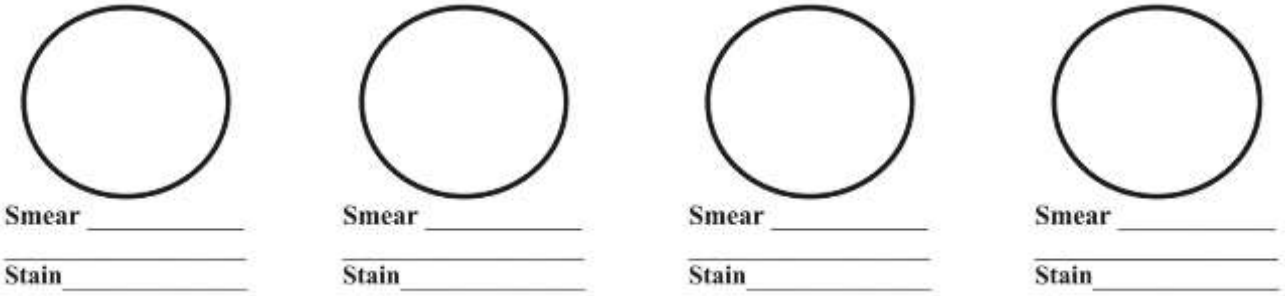
Feature	Levin medium
Shape	
Size	
Surface	
Edge	
Color	
Consistence	



TSI (Kligler) medium

Demonstration:

1. Clean media: Endo, Levin, Ploskirev, bismuth sulfite agar, Rapoport, magnesium, Kligler agar.
2. The same media with the growth of E. coli, Salmonella, Shigella.
3. Biochemical activity of E. coli and Salmonella.
4. Dendrograms of Salmonella molecular typing.
5. Tube agglutination test with killed E. coli culture.
6. The morphology of E. coli, Salmonella, Shigella (Gram staining).



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Enterobacteriaceae genera of medical importance:

General characteristics of Enterobacteriaceae family:

Characteristics	***Enterobacteriaceae***
Morphology	
Spores development	
Capsule	
Flagella (motility)	
Gram staining	
Antigens	
Exotoxins	
Endotoxins	

Escherichia coli characteristics:

Characteristics	***Escherichia coli***
Morphology	
Spores development	
Capsule	
Flagella (motility)	
Gram staining	
Antigens	
Number of serovars	
E. coli classification according to pathogenicity factors	1. 2. 3. 4.
Diseases caused by *E. coli*	

Methods for diagnostics of escherichiosis and salmonellosis

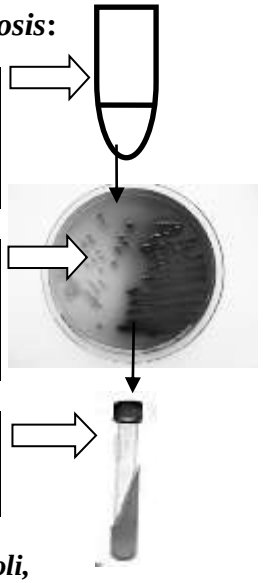
Methods	**Usage (+/–)**	
	Escherichiosis	***Typhoid and paratyphoid***
Microscopic		
Cultural		
Biological		
Serological		
Allergic		
Molecular-genetic		

***Bacteriological diagnostics of escherichiosis*:**

Material for the investigation

Media for pure culture isolation

Medium for the pure culture accumulation



Biological properties E. coli, as normal microflora representatives:

Positive	**Negative**

Characteristics of certain species from *Escherichia* and *Salmonella* genera:

Species	Fermentation					Indol production	H_2S production	Catalase activity	Antigenic formula (O, H, K)	
	Glucose	Lactose	Mannitol	Maltose	Saccharose					
E. coli										
S. typhi										
S. paratyphi A										
S. schottmuelleri										
S. typhimurium										

Methods of microbiological typhoid diagnostics depending on the pathogenesis phase:

Pathogenesis phase		Bacteriological method				Serological method	
		Hemoculture	Urinoculture	Coproculture	Bileculture	Vidal test	BPAT with Vi-Ag
Incubation period							
Prodromal period							
Midst of illness	Bacteremia and intoxication						
	Parenchymal diffusion						
	Allergic-secretory						
Reconvalescence							
Bacteria carrier state							

Class № 2 (19). MICROBIOLOGICAL DIAGNOSTICS OF ACUTE ENTERIC DISEASES CAUSED BY ENTEROBACTERIA

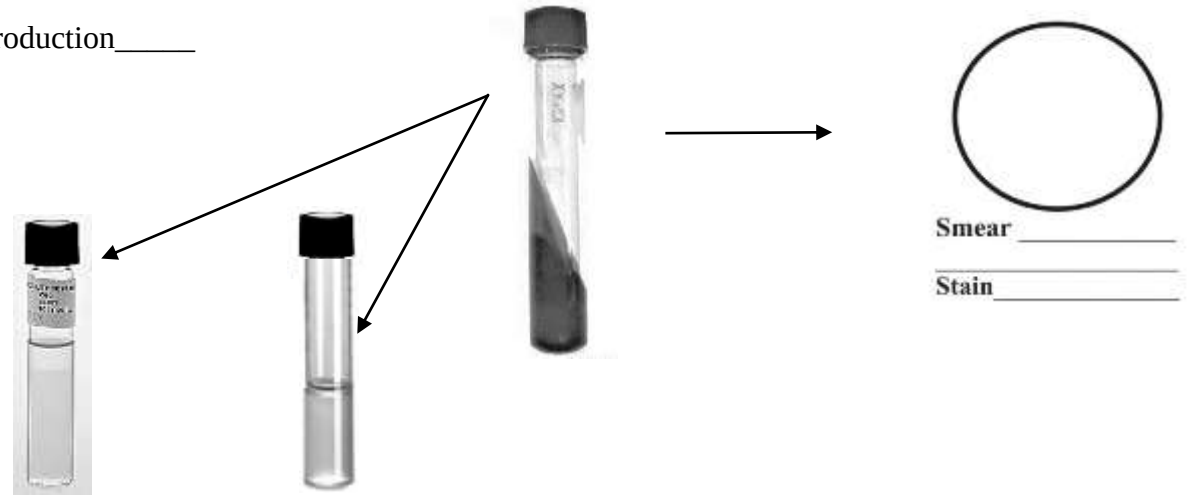
The list of questions to study:

Characteristics of immunity in typhoid and paratyphoid fever. Serological diagnosis of typhoid and paratyphoid fever. Formulation and analysis of Vidal reaction. Methods for distinguishing infection, anamnestic and postvaccinal titer. Diagnosis of bacteria carrier state in typhoid fever.

Salmonella — causative agents of acute gastroenteritis. Salmonella phage typing and indication.

Shigella. Causative agents of dysentery, classification, general characteristics. Molecular mechanisms of pathogenesis, immunity, methods of laboratory diagnosis of acute and chronic dysentery. Approaches to the prevention of dysentery. Antibiotic treatment.

Laboratory work

Laboratory exerscises	Laboratory report
1. Microbiological diagnostics of typhoid fever (3^{d} step): a) Describe the growth on the Kligler medium; b) prepare the slide from the colonies, Gram staining; c) check the culture for motility and indol production; d) determination of the antigenic structure of the culture isolated in slide agglutination test.	Biochemical properties assessment: Lactose_____ Glucose_____ H_2S production_____  Motility test _____ _____ Indol production test _____ _____

2. Assessment of Vidal test.

Widal reaction

Bacterial agglutination test used to diagnose enteric infections caused by *Salmonella*. Doubling dilutions of patient serum are combined with a suspension of microorganisms known to cause enteric fever such as *S. typhi*, *S. paratyphi* B, and *S. paratyphi* A and C. The test microorganisms should be motile and smooth and in the specific phase. Formalin-treated suspensions are used to assay H agglutinins, and alcohol-treated suspensions assay O agglutinin. The Widal test is positive after the 10th day of the disease. Results may be false-positive if an individual previously received a TAB vaccine.

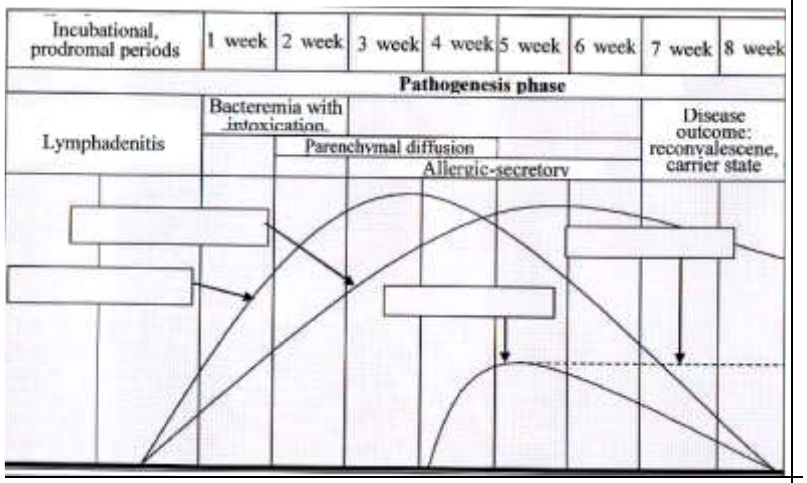
Vidal agglutination test (AT):

Diagnosticum	1:50	1:100	1:200	1:400	1:800	AC	SC
O9							
Hd							
A (OH)							
B (OH)							

Conclusion: _____.

(Diagnostic titer _____).

Immunoglobulines dynamics in typhoid fever:



Demonstration:

1. Shigella growth on differential-diagnostic media.
2. Shigella and Salmonella growth on Kligler medium.
3. Biochemical activity of enterobacteria.
4. Salmonella phage-typing.
5. Vi-passive hemagglutination test.
6. Preparations for the specific prophylaxis of typhoid and paratyphoid fever.

Passive *Vi* — hemagglutination test

1/10	1/20	1/40	1/80	1/160	1/320	1/640	SC	AC
◯	◯	◯	◯	◯	◯	◯	◯	◯

Conclusion: _____

(Diagnostic titer _____).

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Shigella classification:

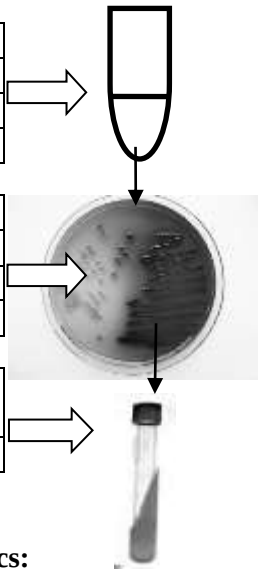
Shigella species	Serovarients number

Bacteriological method for the shigellosis diagnostics:

Materials for the research

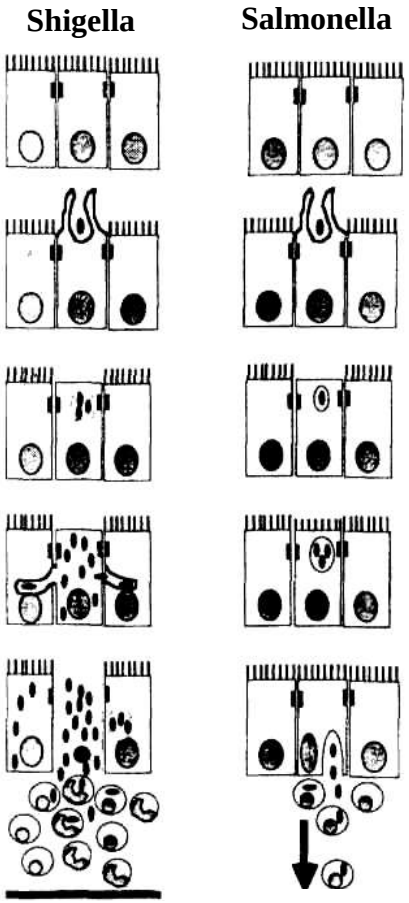
Media for the pure culture isolation

Medium for the pure culture accumulation



Shigella characteristics:

Feature	*S. sonnei*	*S. flexneri*	*S. dysenteriae*
Glucose (A+G)			
Lactose			
Mannitol			
Serogroup			



Shigellosis and salmonellosis pathogenesis

Main salmonellosis pathogens:

Bacteriological method for salmonellosis diagnostics:

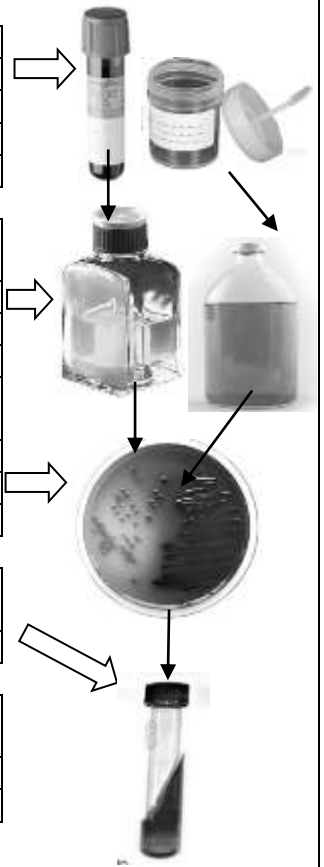
Material for the research

Medium for the material enrichment	

Medium for pure culture isolation

Medium for pure culture accumulation

Methods for salmonella identification



Class № 3 (20). MICROBIOLOGICAL DIAGNOSTICS OF DISEASES CAUSED BY KLEBSIELLA, IERSINIA, CAMPYLOBACTER AND PSEUDOMONADA. METHODS FOR FOOD POISONING DIAGNOSTICS

List of questions to study:

Klebsiella, classification and general characteristics, main diseases caused. Pathogenesis, immunity, methods of microbiological diagnosis of acute and chronic klebsiellosis.

The causative agent of intestinal yersiniosis, general characteristics. Pathogenesis, immunity, methods of microbiological diagnostics.

Campylobacter, general characteristics, role in human pathology. Mechanisms of pathogenesis. Diagnosis of campylobacteriosis. Helicobacter.

Pseudomonas aeruginosa, general characteristics, pathogenicity factors, role in human pathology. Methods of microbiological diagnostics Pseudomonas infection.

Classification, etiology of food poisoning. Principles of microbiological diagnostics.

Laboratory work

Laboratory exerscises

1. Independent work «Microbiological klebsiellosis diagnostics»:

a) Examine the growth of Klebsiella on differential-diagnostic media.

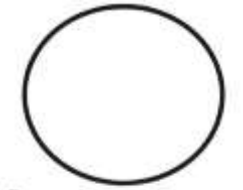
b) Determine the capsule presence.

c) Determine the biochemical properties of Klebsiella.

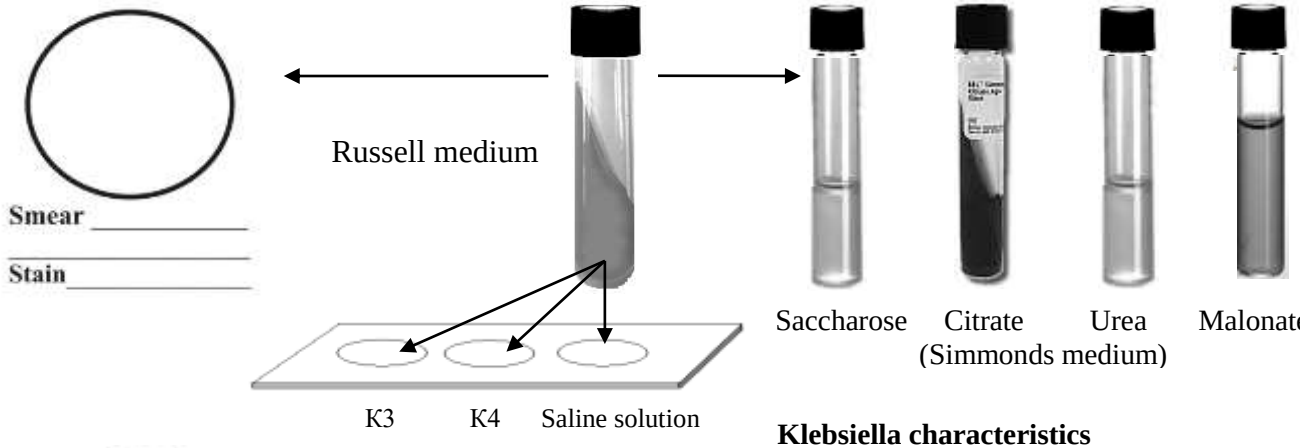
d) Perform slide agglutination test with anti-capsule diagnostic sera and determine the K-antigen.

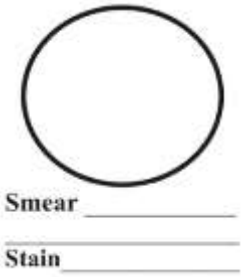
Demonstration:

1. Klebsiella growth on differential diagnostic media.
2. *Klebsiella scleroma* capsule (Hins-Burri staining).
3. Pseudomonas aeruginosa, pure culture, Gram staining.
4. Oxidase test.



Laboratory report





Klebsiella characteristics

Biochemical properties	*K. pneumoniae s. rhinoscleromatis*	*K. pneumoniae s. ozaenae*	*K. pneumoniae s. pneumoniae*
Glucose (A+G)	–	+/–	+
Lactose	–	+/–	+
Saccharose (4th day)	–	+/–	+
Citrate	–	+/–	+
Urea	–	–/+	+
Malonate	+	–	+

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Возбудители	Вызываемые заболевания	Материалы для культуральной диагностики
K. pneumoniae s. rhinoscleromatis		
K. pneumoniae s. ozaenae		
K. pneumoniae s. pneumoniae		
Y. enterocolitica		
C. jejuni		
H. pylori		
P. aeruginosa		

Methods of laboratory diagnostics:

Method	**Usage (+/–)**			
	Klebsiella	Campylo-bacter	Yersinia	Pseudomonas aeruginosa
Microscopic				
Cultural				
Biological				
Serological				
Allergic				
Molecular-genetic				

Diagnosis of bacterial food poisoning

Food poisoning — acute systemic diseases resulting from ingestion of food, massively contaminated with microorganisms or microbial exotoxins. Food poisoning is divided into bacterial foodborne diseases and food poisoning (toxicosis), as well as poisoning of mixed etiology.

Foodborne diseases (FBD): FBDs result from ingestion of products massively colonized by certain bacteria. Pathogens: opportunistic members of the family Enterobacteriaceae — E. coli, Proteus (P. vulgaris, P. mirabilis), Morganella morganii, Citrobacter, Enterobacter, Hafnia, Klebsiella pneumoniae; Sem. Vibrionaceae — V. parahaemolyticus; Sem. Bacillaceae — B. cereus, C. perfringens serovar A; Sem. Streptococcaceae — S. faecalis; Sem. Pseudomonadaceae — P. aeruginosa, and others.

Microbial food toxicosis (intoxication): acute illness arising from eating food, which containes a large amount of exotoxin.(as a result of massive reproduction of microbes). These include botulism, toxicosis caused by staphylococcal enterotoxin, toxins from microscopic fungi and others.

Pathogenesis. Pathogen replicates in the intestine, penetrates into lymphoid tissue, where it is killed with the release of endotoxin, which causes damage to the intramural bowel NS, CNS and blood vessels. Bacteria cause inflammation of the intestinal wall.

Pathogenesis is based on the microbial exotoxin, which is not destroyed by food processing, digestive enzymes and acidic stomach contents.

Materials for the research: vomit, stomach washings, feces, urine, blood, sectional material (in the case of death), the remains of the suspected food, raw and semi-finished products used, daily samples of food, swabs and scrapings from kitchen utensils.

Lab. Diagnosis: isolation of obligate pathogenic or opportunistic enterobacteria and Vibrio, staphylococci and their toxins, streptococci, bacillus, as well as (if indicated) — botulism pathogens and toxins.

To evaluate the etiologic role of opportunistic bacteria (OB) certain criteria are used.

Main criterion is quantitative: Etiologically significant number of OB is 10^5–10^6 or more CFU per 1g of material. The diagnosis is more reliable while simultaneous detecting same germs or toxins in suspected food. Other criteria are: repeated isolation of same germs from the material of the patient, the identity of the pathogen strains (serovars and phage-vars) in a large number of patients in group food poisoning, as well as the increase in antibody titer in the dynamics of the disease.

Class № 4 (21). MICROBIOLOGICAL DIAGNOSTICS OF DISEASES CAUSED BY CORYNEBACTERIA, BORDETELLA, HAEMOPHILUS, LEGIONELLA, LISTERIA

List of questions to study: Corynebacterium diphtheria. Systematics, general characteristics of the pathogen. Types of Corynebacterium diphtheria, their distinctive features. Diphtheria toxin and antitoxic serum. The pathogenesis of diphtheria. Methods of microbiological and molecular biological diagnosis of diphtheria. Principles of therapy and prevention of diphtheria. Determination of the effectiveness of post-vaccinal immunity.

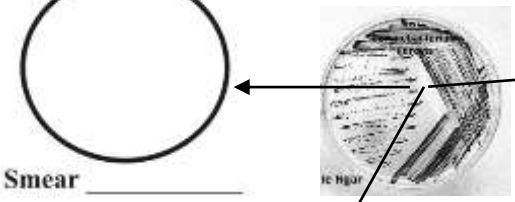
Bordetella pertussis. Characteristics of the pathogen, pathogenicity factors. Differentiation with parapertussis agent. The pathogenesis of pertussis, immunity, diagnostics. Principles of therapy and prevention of pertussis.

Haemophilus, general characteristics, role in human pathology.

Legionella, general characteristics, role in human pathology.

Listeria, general characteristics, role in human pathology.

Laboratory work

Laboratory exerscises	**Laboratory report**
1. Bacteriological diagnosis of diphtheria, 2nd step: a) Describe the colonies Corynebacterium on potassium tellurite serum agar. b) Seed bacteria from typical colonies onto Hiss media (glucose, sucrose, starch).	Tinsdale medium  Smear _____ _____ Stain_____  Glucose Sucrose Starch Smear _____ _____ Stain_____ Smear _____ _____ Stain_____
Demonstration: 1. *Corynebacterium diphtheria* stained by: a) Neisser; b) Leffler. 2. Test for *Corynebacterium diphtheria* toxigenicity. 3. Preparations for specific prevention and treatment of diphtheria and pertussis. 4. Growth of *Bordetella pertussis and parapertussis* on CCA, NA with tyrosine, urease test. 5. *Bordetella pertussis*, Gram staining. 6. Assessment of antidiphtheria immunity intensity.	**Feature** / **Colonies on serum tellurite agar** Shape Size Surface Edge Color Consistency **Signature of the tutor _____ Date ____/____/2024**

Characteristics of Corynebacteria, bordetella, haemophilus, legionella, listeria:

Species	***C. diphtheriae***	***B. pertussis***	***Legionella pneumophila***	***L. monocytogenes***	***H. influenzae***
Morphology (size, shape, relative positions of cells)					
Spores development					
Capsule					
Flagella (motility)					
Gram staining					
Pathogenicity factors					

Laboratory diagnostics and specific prophylaxis:

Method / Species	***C. diphtheriae***	***B. pertussis***	***Legionella pneumophila***	***L. monocytogenes***	***H. influenzae***
Microscopic					
Cultural					
Biological					
Serological					
Molecular-genetic					
Specific prophylaxis					

Medically important corynebacteria:

Species	**Diseases**
C. diphtheriae	Diphtheria
C. ulcerans, *C. minutissimum,C. xerosis, C. pseudodiphtheriticum*	Opportunistic infections

C. diphtheriae pathogenicity factors:

Pathogenicity factors	**Biological effect**
Protein exotoxin (includes A and B subunits)	Protein synthesis arrest, specific damage of the myocardium, adrenal glands and nerve ganglia
Glycolipid (6-6'-diester-trehalose)	Phagocytosis impairment
Hyaluronidase	permeability of tissues violation
Neuraminidase	

Legionella pneumophila pathogenicity factors:

Pathogenicity factors	Biological effect
1. Optional intracellular parasitism	
Toxin (peptide)	inhibiting the «oxidative burst» during phagocytosis
Catalase	inactivation of toxic metabolites during macrophage activation
Factors of unknown nature	inhibit fusion of phagosomes and lysosomes, electron transport
2. Production of toxins, enzymes	
Labile exotoxin (Cytotoxin and hemolysin)	dysfunction or cell lysis
Endotoxin	dysfunction or cell lysis
Proteolytic enzymes: phosphatase, lipase, nuclease	degradation of host cells
3. Suppression of the expression of MHC class II molecules on macrophages, violation of Ag-presenting functions — the suppression of cellular immune response	

Haemophilus genus representatives and respective diseases:

Species	Diseases
H. influenzae	
H. ducreyi	
H. aphrophilus, *H. parainfluenzae,* *H. haemolyticus,* *H. parahaemolyticus et al.*	

H. influenzae pathogenicity factors:

Pathogenicity factors	Biological effect
Polysaccharide capsule	Inhibition of phagocytosis
Pili and other adhesins	Attaching to epithelial cells
Lipopolysaccharide and glycopeptide	Epithelium surface and cilia damage
Ig A protease	Suppression of local immunity

Bordetella differentiation:

Feature	B. pertussis	B. parapertussis

B. pertussis pathogenicity factors:

Pathogenicity factors	Biological effect
Filamentous hemagglutinin	Binds cell membrane glycolipid of ciliated airway epithelium, binds surface R3-glycoprotein receptor and initiates phagocytosis
Pertussis toxin (Pertussin)	S1 — Pertussin subunit ribosylates membrane protein Gi; toxin inhibits the activity of phagocytes and monocyte migration. S2 — subunit binds to the respiratory tract cell surface glycolipid; S3 — subunit binds to phagocytes surface gangliosides
Pili	Adhesion to the ciliated epithelium of the respiratory tract
Pertactin	Adhesion to the ciliated epithelium of the respiratory tract
Adenylate cyclase	Suppresses killing- activity of phagocytes and monocytes migration
Dermatonekrotoksin	Damages the skin and is lethal to laboratory animals
Tracheal toxin	Peptidoglycan fragment — destroys ciliated cells of the respiratory tract; stimulates interleukin-1 secretion (fever)
Endotoxin (LPS)	Activates complement and stimulate the production of cytokines

Listeria pathogenicity factors:

Pathogenicity factors	Biological effect
Endotoxin	Toxic effects
Internalin — membrane protein	Listeria entry into macrophages and endothelial cells (from phagosome into the cytoplasm)
Listeriolysin O	Hemolysin, cause phagolysosomes membrane disruption
Phospholipase	Membrane damage and penetration into the cell

Class № 5 (22). METHODS OF MICROBIOLOGICAL DIAGNOSIS OF DISEASES CAUSED BY MYCOBACTERIA AND ACTINOMYCETES. METHODS OF MICROBIOLOGICAL DIAGNOSTICS ANAEROBIC INFECTIONS

List of questions to study:

Actinomycetes, systematic position, general characteristics, role in human pathology.

Mycobacteria classification. TB germs, general characteristics. Pathogenesis, immunity, methods of microbiological diagnostics, principles of treatment and prevention of tuberculosis. Mantoux test. The causative agent of leprosy, general characteristics, role in human pathology. Mycobacteriosis. Nocardia.

Anaerobes, classification, general characteristics. Causative agents of gas gangrene, tetanus, botulism. Systematics and general characteristics. Exotoxins, properties Principles of therapy and prevention of anaerobic infections. Clostridial gastroenteritis. Clostridium difitsile role in human pathology.Nonspore anaerobes. Bacteroides. Peptococci. General characteristics, pathogenicity factors, role in human pathology.

General principles and methods for anaerobic infections diagnosis. Molecular biological diagnostics — PCR.

Laboratory work

Laboratory exerscises

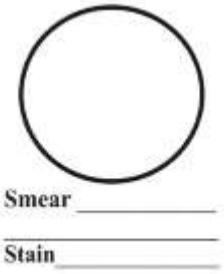
1. The assessment of enzymatic activity of corynebacteria, identification.
2. Microscopy of ready smear of tuberculosis patient sputum, Ziehl–Neelsen staining.

Demonstration:

1. Mycobacteria growth on nutrient media.
2. Flotation method.
3. Determination of *M. tuberculosis* drug resistance.
4. Cord factor of *M.tuberculosis*, Ziehl–Neelsen staining.
5. *Actinomycetes*, pure culture, Gram staining.
6. *M. leprae*, Ziehl-Neelsen staining.
7. *M. tuberculosis* in sputum, Ziehl–Neelsen staining.
8. Anaerobes growth on nutrient media.
9. *Clostridium spp.*, Gram staining.
10. *Bacteroides spp.*, Gram staining.

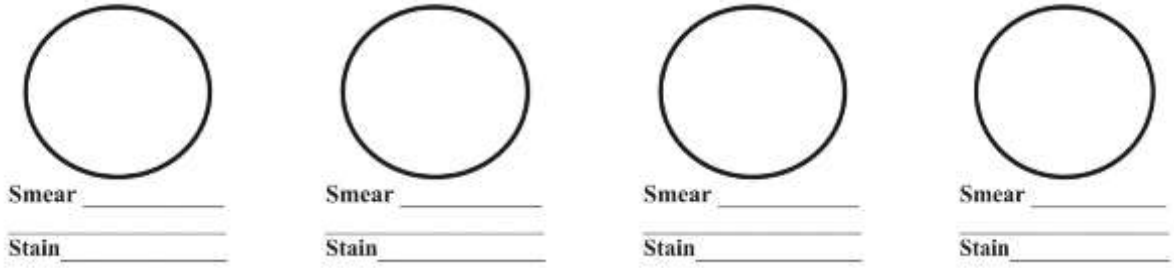
Laboratory report

Biochemical properties of sertain corynebacteria:



Corynebacteria spp.	Enzymatic activity					Nitrate reduction
	Acid producrion			Cysteinase	Urease	
	Glucose	Sucrose	Starch			
C.diphtheriae						
gravis	+	–	+	+	–	+
mitis	+	–	–	+	–	+
C.pseudodiphtheriae (hofmani)	–	–	–	–	+	+
C. xerosis	+	+	–	–	+	+
C. ulcerans	+	–	+	+	+	–

Conclusion: according to morphological, cultural and biochemical properties unknown bacterium is identified as _____.



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Actinomyces characteristics:

Characteristics	***Actinomyces israelii***
Morphology	
Spores development	
Capsule	
Flagella (motility)	
Gram staining	
Pathogenicity factors	

Microbiological diagnostics and specific prophylaxis of actinomycosis:

Method	**Remarks**
Microscopic	
Cultural	
Specific prophylaxis	

Classification of medically important culturable mycobacteria:

Slowly growing			***Fast growing***	
Tuberculosis agents	**Non chromogenic**	**Chromogenic**	**Non chromogenic**	**Chromogenic**
M. tuberculosis *M. bovis* *M. africanum*	*M. avium complex* *M. xenopi* *M. haemophilum* *et al.*	*M. kansasii* *M. marinum* *M. simae* *et al.*	*M. fortuitum* *M. chelonae* *M. smegmatis* *et al.*	*M. phlei* *M. vaccae*

Mycobacteria characteristics:

Characteristics	***M. tuberculosis***	***M. leprae***
Morphology		
Spores development		
Capsule		
Flagella (motility)		
Gram staining		
Pathogenicity factors		

M. tuberculosis pathogenicity factors:

Pathogenicity factors	**Biological effects**
Cord-factor (trehalose-6,6-dimycolate)	
Sulphatides (sulfur-containing glycolipids)	
Antigens	

Microbiological diagnostics and specific prophylaxis of tuberculosis:

Methods	**Remarks**
Microscopic	
Cultural	
Serological	
Biological	
Molecular-genetic	
Allergic	
Specific prophylaxis	

Microbiological diagnostics and specific prophylaxis of leprosy:

Methods	**Remarks**
Microscopic	
Allergic	
Biological	
Specific prophylaxis	

Ecological group of anaerobic bacteria:

Gram-negative nonsporeing rods	**Diseases induced**	
Bacteroides species		
Fusobacterium species		
Leptotrichia bucalis		
Prevotella species		
Porphyromonas species		
Bilophila wadsworthia		
Grampositive spore forming rods		
Clostridia	*Clostridium tetani*	Tetanus (Lockjaw)
	Clostridium perfringens, C. novyi, C. ramosum, C. histolyticum, C. septicum	Gas gangrene, necrotizing enteritis
	Clostridium botulinum	Botulism
	Clostridium difficile	Pseudomembranous colitis, antibiotic-associated diarrhea
Gramnegative cocci		
Veillonella	Septic infections	
Grampositive cocci		
Enterococcus species	Septic infections	
Peptococcus species		
Peptostreptococcus spp.		

Bacteroides pathogenicity factors:

Pathogenicity factors		**Biological effect**
Toxins	endotoxin	general toxic effect
	leukocidin	damages leukocytes
Enzymes	collagenase	destroys the collagen fibers of the connective tissue (spread of purulent process)
	DNAse, heparinase	cause intravascular blood clotting
	fibrinolysin	dissolves blood clots
	beta-lactamase	destroys the beta-lactam antibiotics
Surface cell structure	pili	adhesion to the substrate
	capsule	protects the bacteria from phagocytosis
Metabolites	fatty acid	inhibit the chemotaxis and cytotoxicity of leukocyte

Clostridia characteristics:

Characteristics	***C. perfringens***	***C. tetani***	***C. botulinum***
Morphology (size, shape, relative positions of cells)			
Spores development			
Capsule			
Flagella (motility)			
Gram staining			
Pathogenicity factors			

Clostridium perfringens pathogenicity factors:

Pathogenicity factors		**Biological effects**
Main toxins	alpha-toxin (Lecithinase)	cleaves lecithin in cell membranes; increases vascular permeability destroying erythrocytes; necrotizing activity
	beta-toxin	necrotizing activity; induction of hypertension as a result of formation of catecholamines
	epsilon-toxin	increases vascular permeability of the gastrointestinal tract
	iota-toxin	necrotizing activity and increased vascular permeability
	Enterotoxin	violates the permeability of the mucosa of the small intestine
Minor toxin	delta-toxin	hemolysis
	theta-toxin	hemolysis, cytolysis
	kappa-toxin	collagenase, gelatinase, necrotizing activity
	lambda-toxin	protease
	mu-toxin	hyaluronidase: increases the permeability of tissues
	nu-toxin	DNAse; hemolytic, necrotizing activity
	Neuraminidase	damages gangliosides cell receptor, promotes thrombosis in capillaries

Microbiological diagnostics of septic infections caused by bacteroides:

Method	Remarks
Microscopic	
Cultural	
Serological	
Molecular-genetic	

Microbiological diagnostics and specific prophylaxis of gas gangrene:

Method	Remarks
Microscopic	
Cultural	
Biological	
Specific prophylaxis	

Clostridium tetani pathogenicity factors:

Pathogenicity factors	Biological effects
Tetanus toxin	

Microbiological diagnostics and specific prophylaxis of tetanus:

Methods	Remarks
Microscopic	
Biological	
Cultural	
Specific prophylaxis	

Clostridium botulinum pathogenicity factors:

Pathogenicity factors	Biological effects
Botulinum exotoxin	Blocks the transmission of nerve impulses in the peripheral cholinergic synapses, providing neurotoxic effects (lethal dose for humans is about 0.3 g)

Microbiological diagnostics and specific prophylaxis of botulism

Methods	Remarks
Serological	
Biological	
Cultural	
Specific prophylaxis	Botulinum toxoids A, B, E, are used according to indications. For urgent passive prophylaxis specific antitoxic serum is used.

Class № 6 (23). MICROBIOLOGICAL DIAGNOSTICS OF ESPECIALLY DANGEROUS INFECTIONS

List of questions to study:

Classification and general characteristics of the especially dangerous infections. Demands to collection and transportation of biological material. Principles of diagnostics.

Vibrio cholerae, the systematic position. Classification and general characteristics, pathogenicity factors. Biovars. Differentiation from non-cholera vibrio. Pathogenesis of cholera. Methods of microbiological diagnostics. Rapid methods. Principles of treatment and prevention.

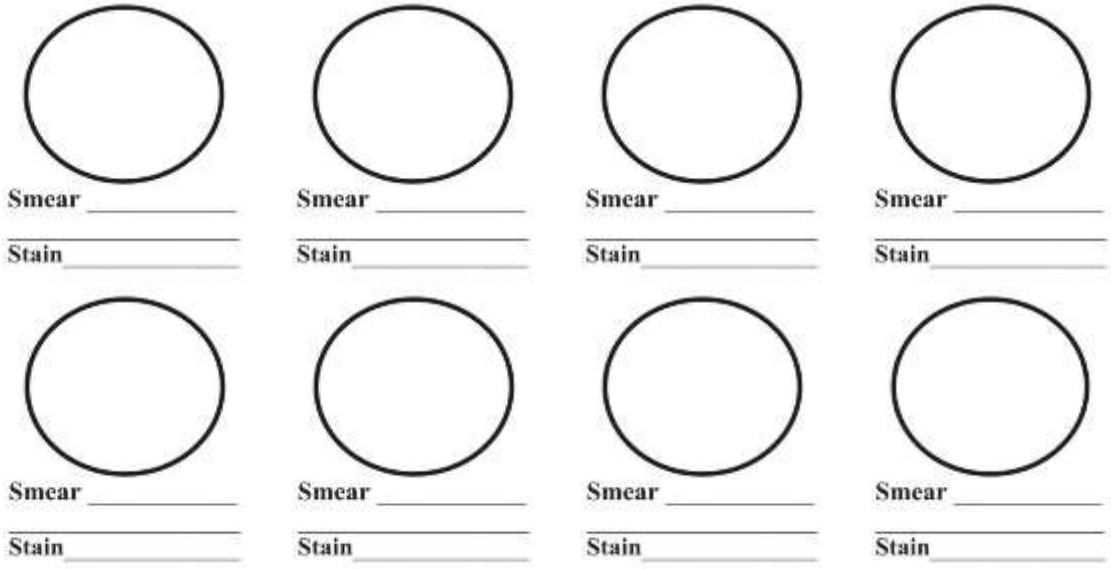
The causative agent of plague, systematic position, characteristics, pathogenicity factors. Differences from other Yersinia. Pathogenesis, principles of treatment and prevention of plague.

The causative agent of tularemia, systematics, general characteristics. Pathogenesis, principles of treatment and prevention.

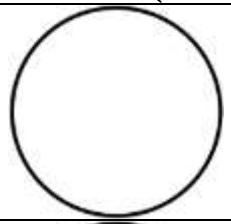
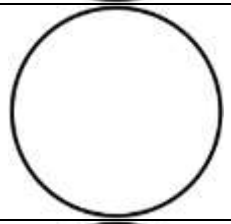
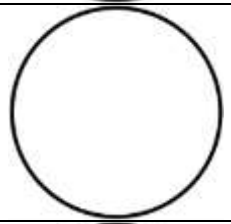
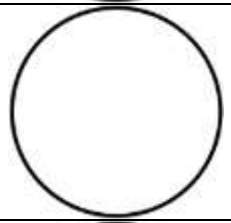
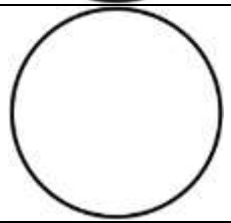
Causative agents of brucellosis. Systematics and general characteristics, pathogenicity factors, pathogenesis. Microbiological diagnosis of brucellosis. Principles of treatment and prevention.

Anthrax. Systematics and general characteristics, pathogenicity factors. Differences from non-pathogenic bacilli. Pathogenesis. Microbiological diagnosis of anthrax. Principles of treatment and prevention.

Laboratory work

Laboratory exerscises	Laboratory report
Demonstration: 1. Growth of *Vibrio cholera* on alkaline agar, TCBS, peptone water. 2. Phage lysability of *Vibrio cholera Classica and El Tor*. 3. Tube agglutination test. 4. Biochemical properties of *V. cholerae*. 5. Mobility of *Vibrio spp*. 6. *V.cholera*, pure culture, Gram staining. 7. *Y.pestis* in the organs, Leffler staining. 8. The causative agent of tularemia (pure culture), Gram staining. 9. Preparations for specific prophylaxis of especially dangerous infections. 10. The causative agent of brucellosis, Gram staining. 11. The growth of *Bacillus spp*. on nutrient media. 12. *B. anthracis* in organs, Gram staining. 13. *B. anthracis*, pure culture, Gram staining. 14. *B. anthracis* spores, Ozheshko staining.	 **Signature of the tutor _____ Date ____/____/2024**

Methods for microbiological diagnosis of cholera, plague, tularemia, brucellosis, anthrax.
Microorganisms of pathogenicity groups 3–4 (*fill in the table*)

Disease	Pathogen	Morphology	Gram stain (draw)	Cultural properties
Plague				
Tularemia				
Anthrax				
Brucellosis				
Cholera				

Pathogen	Pathogenicity factor	Biological effect	Disease Source of infection, mechanisms of infection, clinical manifestations
Vibrio cholerae	Exotoxin (cholera toxin)	violation of water-salt metabolism, the cytotoxic effect on the epithelium of the small intestine	
	Endotoxin	inhibition of phagocytosis, drop in blood pressure; infectious-toxic shock	
	Pili	adhesion to mucosal cells	
	Fibrinolysin Hyaluronidase	enzymes invasion (aggression)	
Yersinia pestis	Capsular Ag, F1-Ag, fraction 1)	protection against the absorption of phagocytes, non-toxic, the immunogen	
	Plasminogen activator — protease	activates lysis of fibrin clots, and inactivates C5a	
	V/W(Vi)-Ag	includes protein (V-phase) and LPS (W-phase); exhibits antiphagocytic properties, promotes intracellular bacterial growth	
	Murine toxin	adrenergic receptor antagonist, proteinaceous substance, localizes intracellularly	
	Bacteriocins (pestitsiny)	immunogenic properties	
Francisella tularensis	Intracellular parasitism	inhibition of phagocytes lysosomal function	
	Capsule	protection from phagocytosis	
	Endotoxin	less active than other Gram-negative rods endotoxin (e.g., E. coli)	
Brucella spp. B. melitensis, B. abortus, B. suis	Endotoxin	systemic toxic effect	
	Hyaluronidase	breaks down hyaluronic acid	
	Outer Membrane Proteins	adhesion	
	Secretion of low molecular weight proteins → survival inside phagocytes	inhibition of phagosome-lysosome fusion and oxidative burst in phagocytes	
Bacillus anthracis	Protein exotoxin (synthesis is controlled plasmid)	exotoxin contains three factors: lethal factor — the cytotoxic effect, pulmonary edema, protective Ag — interacts with cell membranes mediates the activity of others. components, edematous factor — the increase in the concentration of cAMP, the development of edema.	
	Capsule	antiphagocytic activity	

Laboratory diagnostics, specific prevention and therapy of especially dangerous infections

Methods/Diseases	***Cholera***	***Plague***	***Brucellosis***	***Tularemia***	***Anthrax***
Material for research					
Microscopic					
Bacteriological					
Serological					
Biological					
Molecular-genetic					
Allergic					
Specific prophylaxis					
Specific therapy					

Class № 7 (24). MICROBIOLOGICAL DIAGNOSTICS OF DISEASES CAUSED BY SPIROCHETES

List of questions to study:

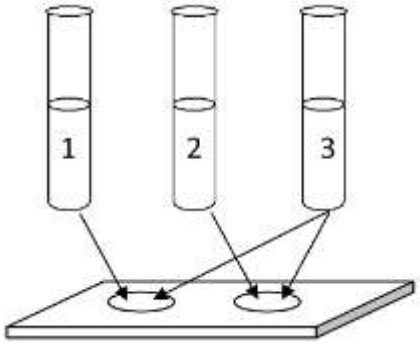
Spirochetes, classification, general characteristics.

Treponema. Systematics and general characteristics. Pathogenesis and immunity in syphilis. Material for the study. Methods of microbiological diagnosis of syphilis. Principles of therapy and prevention of syphilis. Fusospirochetosis pathogens.

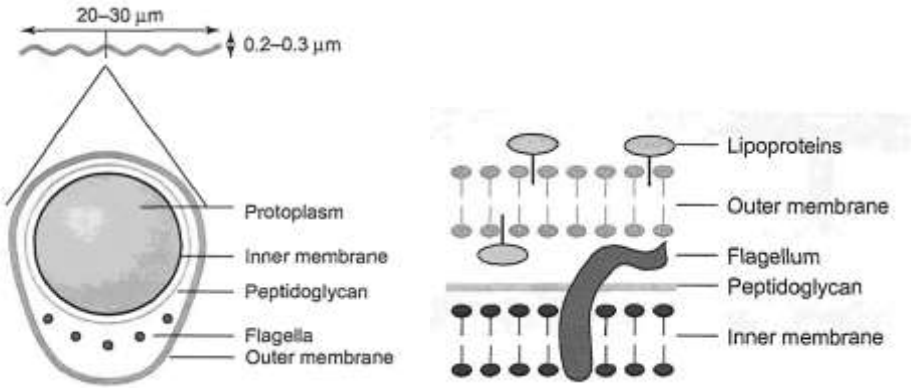
Leptospira. Systematics and general characteristics. Pathogenesis, methods of microbiological diagnostics, principles of treatment and prevention of leptospirosis.

Borrelia. Systematics and general characteristics. Pathogenesis and methods of microbiological diagnosis of relapsing fever. The causative agent of Lyme borreliosis, principles of treatment and prevention.

Laboratory work

Laboratory exerscises	Laboratory report
1. Perform the slide microprecipitation reaction (VDRL) for the syphilis serodiagnosis. 2. Assess ELISA (Wasserman test) for the syphilis diagnostics. **Demonstration:** 1. *Borrelia* in blood, Romanovsky–Giemsa staining. 2. Wasserman test (ELISA). 3. *Treponema* in dental plaque, Gram staining. 4. *Treponema pallidum*, pure culture, Romanovsky–Giemsa staining.	**Slide microprecipitation test:**  1. Patienr serum 1 : 20 2. Saline solution 3. Cardiolipin antigen **Conclusion:** _____ Smear _____ Smear _____ Smear _____ Stain_____ Stain_____ Stain_____ **Signature of the tutor _____ Date ____/____/2024**

Spirochetes structure:



Pathogenic spirochetes characteristics:

Features		Spirochetes genera		
		Treponema	***Borrelia***	***Leptospira***
Size	Length	5–20	3–20	714
	Width	0.09–0.5	0.2–0.5	0.1–0.15
Coils number		8–12	2–8	12–24
Shape		Uniform, correct,	Uneven, different size	Uniform, correct secondary curls
Romanovsky–Giemsa staining		Pink	Blue Purple	Pink, Red

Diseases caused by treponema:

Treponema spp.	Disease	Morbidity (countries, continents)
T. pallidum, subspecies *pallidum*		
T. pallidum, subspecies *bedjel* (endemicum)		
T. pallidum, subspecies *pertenue*		
T. carateum		
Opportunistic or saprophytic: *T. vincentii, T. refringens T. denticola, T. minutum T. scoliodontum*		

Pathogenesis of syphilis:

Disease stage	Period	Main pathogenetic mechanisms
Primary		
Secondary		
Tertiary		

Methods for spirochetosis diagnostics:

Methods	Using the (+/–) method				
	Syphilis	Epidemic relapsing fever	Endemic relapsing fever	Lyme disease	Leptospirosis
Microscopic					
Cultural					
Serological					
Allergic					
Molecular-genetic					
Biological					

Laboratory diagnosis of Lyme disease (Lyme borreliosis):

Microscopic method: dark-field microscopy (scrapings of skin lesions, plasma pellet, CSF, urine), microscopy of smears, impregnated with silver, IFT, and electron microscopy.

Cultural method: *B. burgdorferi* isolation is possible in 80 % cases from skin lesions (1st stage) on special nutrient media.

Molecular genetic methods: PCR allows the identification of the pathogen's DNA in the samples of the skin, blood, cerebrospinal fluid.

Serological: ELISA, indirect IFT, Western blot. Sometimes there are false-positive results due to cross-reactions among patients with syphilis, mononucleosis, rheumatoid arthritis and others.

Class № 8 (25). MICROBIOLOGICAL DIAGNOSTICS OF DISEASES CAUSED BY RICKETTSIA, CHLAMYDIA AND MYCOPLASMA

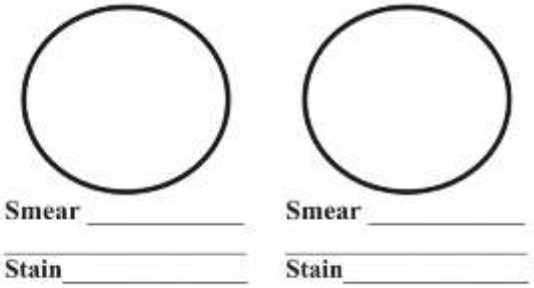
List of questions to study:

Rickettsiae, systematic position, classification, general characteristics, role in human pathology. Rickettsia typhi, pathogenesis, immunity and methods of microbiological diagnostics. Other pathogenic rickettsia.

Chlamydia, general characteristics, role in human pathology. Pathogens of psittacosis, trachoma, respiratory and urogenital chlamydiosis. Methods of microbiological diagnosis of chlamydiosis. PCR in chlamydiosis diagnostics.

Mycoplasma, general characteristics, role in human pathology. Methods of microbiological diagnostics of mycoplasmoses.

Laboratory work

Laboratory exerscises	Laboratory report
1. Passive blood agglutination test for differential diagnostics of epidemic and residual typhus. 2. Prepare slide of *Rickettsia spp*., stain with fuschin, complete the report.	**1/10 1/20 1/40 1/80 1/160 1/320 1/640 SC AC** **I** _____ ○ ○ ○ ○ ○ ○ ○ ○ ○ **II** _____ ○ ○ ○ ○ ○ ○ ○ ○ **Conclusion** _____
Demonstration: 1. *Chlamydia spp.* in cell culture, Romanovsky–Giemsa staining. 2. *R. prowazeki*, pure culture, Zdrodovski staining.	 **Smear** _____ **Smear** _____ **Stain**_____ **Stain**_____ **Signature of the tutor** _____ **Date** ____/____/2024

Actual classification of Rickettsia:

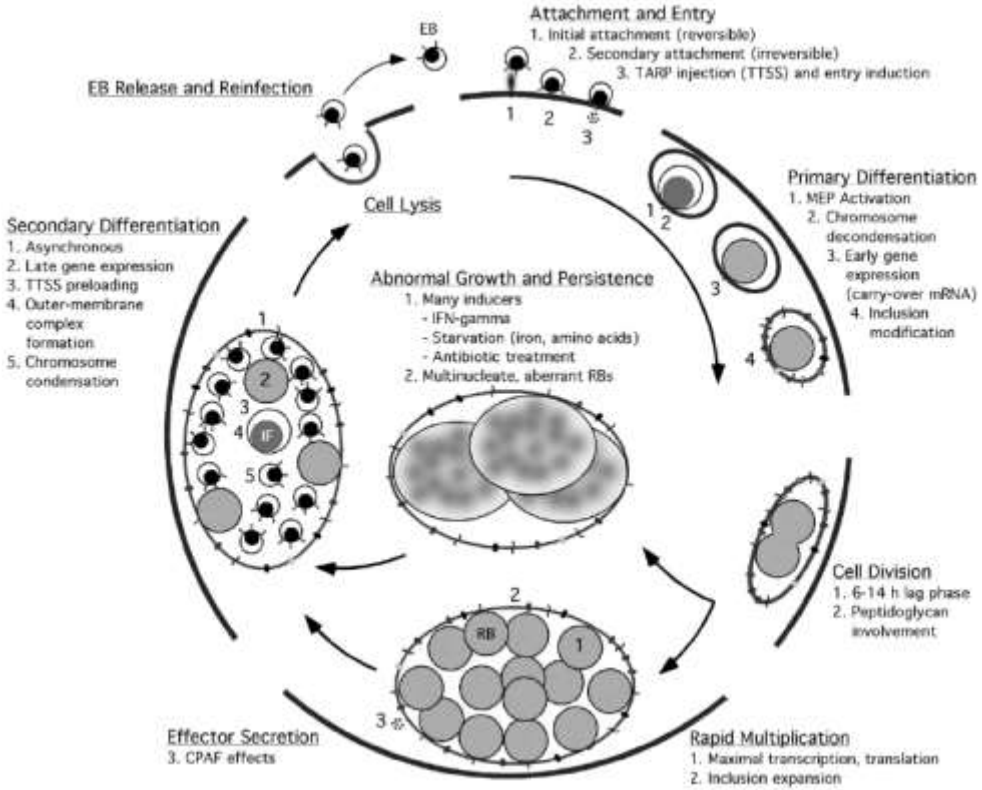
On the basis of a molecular genetic studies (genome sequencing, PCR) classification of microorganisms belonging to the order Rickettsiales has undergone significant changes.

The genus Coxiella with C.burnetti was excluded from the family and added in the order Legionellales, family Coxiellaceae. Genus Rochalimaea was removed, and its representatives — R. quintana (Trench fever) and R. henselae (cat scratch disease) were included in the family Bartonellaceae, genus Bartonella.

Rickettsial infections are caused by various bacteria within 6 genera of the order Rickettsiales: ***Rickettsia, Orientia, Anaplasma, Ehrlichia, Neoehrlichia, and Neorickettsia***. Rickettsia spp. are classically divided into the spotted fever group (SFG) and the typhus group, although more recently these have been classified into as many as 4 groups. Orientia spp. comprise the scrub typhus group, which has only recently expanded from the single species O. tsutsugamushi.

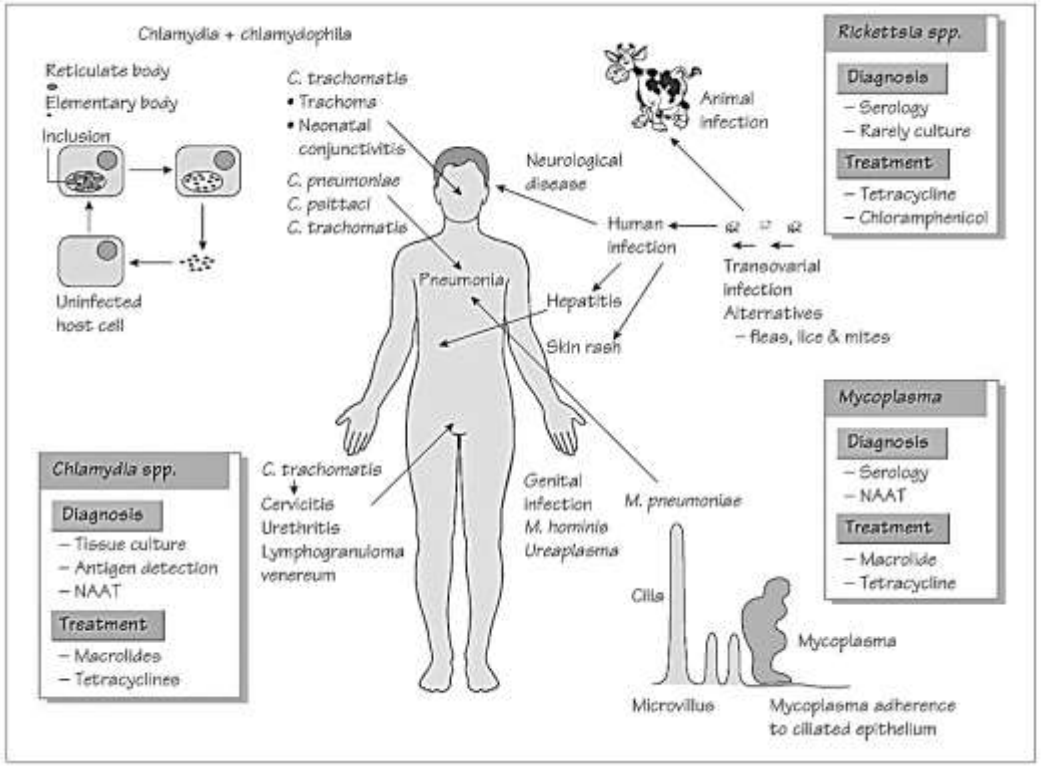
SPOTTED FEVER GROUP	*Rickettsia africae*	*R. conorii, subsp. indica*
	R. helvetica	*R. japonica*
	R. conorii, subsp. caspiae (proposed)	*R. sibirica mongolotimonae*
	R. felis	*R. parkeri*
	R. heilongjiangensis	*R. conorii, subsp. conorii(proposed)*
	R. honei	*R. massiliae*
TYPHUS GROUP	*Rickettsia prowazekii*	
	R. typhi	
SCRUB TYPHUS GROUP	*Orientia tsutsugamushi*	*O. chiloensis*
	O. chuto	
ANAPLASMA GROUP	*Anaplasma bovis*	*A. phagocytophilum*
	A. capra	*A. platys*
		A. ovis
EHRLICHIA GROUP	*Ehrlichia chaffeensis*	*E. muris muris*
	E. ewingii	*E. canis*
	E. muris eauclairensis	*E. ruminantium*
NEOEHRLICHIA GROUP	*Neoehrlichia mikurensis*	
NEORICKETTSIA GROUP	*Neorickettsia sennetsu*	

The scheme of intracellular Chlamydia cycle:



Laboratory diagnostics of diseases caused by *Rickettsia, Chlamydia and Mycoplasma:*

Method		Method usage		
		rickettsiosis	**chlamydiosis**	**mycoplasmosis**
Microscopical				
Cultural	Nutrition media			
	Chicken embryo			
	Cell culture			
	Lab animals			
Biological				
Serological				
Allergic				
Molecular-genetic				



Chlamydiosis characteristics:

Disease	Pathogen	Source	Transmission
Trachoma			
Urogenital chlamidiosis			
Veneral lymphogranulomas			
Psittacosis			
Pharyngitis, sinusitis, bronchitis, pneumonia			

Mycoplasma and mycoplasmosis characteristics:

Properties	*Mycoplasma spp.*
Size	
Cell wall, peptidoglican	
Gram staining	
Capsule	
Flagella	
Spore	
Resistance in environment	
Cultural properties	
Reproduction	
Parasitism peculiarities	
Source of infection	
Transmission mechanisms	
Immunity	

Class № 9 (26). CONCLUDING SESSION: «SPECIAL MICROBIOLOGY»

List of questions:

1. Staphylococci, general characteristics. Role in human pathology. Pathogenicity factors and mechanisms of pathogenesis of staphylococcal infections. Microbiological diagnosis. Principles of treatment and prevention of staphylococcal infections.
2. Streptococci, classification. General characteristics. Pathogenicity factors. Antigenic structure. Pathogenesis, immunity, microbiological diagnosis, principles of treatment and prevention of streptococcal infections.
3. Classification of Neisseria. Meningococcus, general characteristics. Meningococcal infections, mechanisms of pathogenesis, immunity, methods of diagnosis, prevention.
4. Gonococci, general characteristics. Mechanisms of pathogenesis and immunity. Microbiological diagnosis of acute and chronic gonorrhea.
5. General characteristics of the family Enterobacteriaceae.
6. General principles of bacteriological diagnosis of acute intestinal infections (AII). The nutrient medium for enterobacteria. Classification principles of application. Materials for researches in AII diagnostics.
7. E. coli, common characteristic. The biological role of Escherichia coli. Diseases caused by Escherichia.
8. Salmonella. General characteristics. Members of the genus. Serological classification by Kaufmann–White. Molecular biological typing.
9. Pathogens of typhoid, paratyphoid A and B, general characteristic. Phage typing. Vi-antigen and its value.
10. Pathogenesis and methods of microbiological diagnosis of typhoid and paratyphoid.
11. Immunity in typhoid fever. Serological diagnosis of typhoid and paratyphoid. Specific prophylaxis.
12. The etiology of food poisoning and intoxication of bacterial origin. Materials and methods of diagnosis.
13. Salmonellosis. Characteristics of pathogens and diagnostic methods. Nosocomial salmonellosis.
14. Shigella. Classification. Characteristics. Pathogenesis, immunity. Methods of microbiological diagnostics of acute and chronic dysentery.
15. Klebsiella. Classification, general characteristics. Pathogenesis, immunity, methods of microbiological diagnostics of klebsiellosis.
16. Pseudomonas aeruginosa, general characteristics, pathogenicity factors. Role in human pathology.
17. Pathogens of intestinal yersiniosis, general characteristics. Pathogenesis. Methods of diagnosis of yersiniosis.
18. C.diphtheria, general characteristics. Differences from non-pathogenic corynebacteria. Mechanisms of pathogenesis and microbiological diagnosis of diphtheria.
19. Diphtheria toxin and its properties. Toxoid. Immunity in diphtheria and its character. Determination of antitoxic immunity. Principles of therapy and prevention of diphtheria.
20. The causative agent of whooping cough, general characteristics. Differentiation with parapertussis agent. Pathogenesis, immunity. Microbiological diagnosis, principles of treatment and prevention of pertussis.
21. General characteristics of the causative agents of tuberculosis. Pathogenesis, immunity, diagnosis and specific prevention of tuberculosis. Mycobacteriosis.
22. The causative agent of leprosy. Characteristic, pathogenesis, immunity.
23. Especially dangerous infections. classification, Basic rules of sampling, sending and transportation of infectious material. General principles of diagnosis.

24. V. cholera. Systematics. General characteristics. Differentiation of biovars. Pathogenesis, immunity, principles of treatment and prevention. Methods of microbiological diagnostics.
25. The causative agent of plague, a general characteristic. The pathogenesis of plague. Immunity, the principles of therapy and prevention of plague.
26. B. anthracis characteristic. Pathogenesis, immunity, principles of treatment and prophylaxis of anthrax.
27. The causative agent of tularemia, general characteristic. Pathogenesis, immunity, principles of treatment and prophylaxis of tularemia.
28. Pathogens of brucellosis, a general characteristic. Differentiation of Brucella species. Pathogenesis, immunity, principles of treatment and prevention of brucellosis.
29. Spirillae family. Campylobacter, characteristics, role in human pathology. Helicobacter.
30. Classification and general characteristics of anaerobes. Clostridia. Bacteroides, Peptococci and other nonspore anaerobes. Pathogenicity factors. Role in human pathology.
31. The causative agent of tetanus, general characteristics. Pathogenesis, immunity, principles of treatment and prevention of tetanus.
32. Gas gangrene pathogens, general characteristics. Pathogenesis, principles of treatment and prevention of gas gangrene.
33. The causative agent of botulism, general characteristic. Pathogenesis, principles of botulism prevention and therapy. Clostridial gastroenteritis.
34. Methods of diagnosis of anaerobic infections.
35. Classification and general characteristics of spirochetes.
36. Classification of treponemes and treponemal diseases. Characteristics of syphilis causative agent. Pathogenesis, immunity, diagnostic tests for syphilis.
37. Leptospires. General characteristics. The pathogenesis of leptospirosis, immunity, specific prevention. Microbiological diagnosis of leptospirosis.
38. Borrelia, general characteristics. Recurrent fever pathogenesis, immunity. Microbiological diagnosis. The causative agent of Lyme borreliosis.
39. Systematic position and characterization of Rickettsia. Pathogenesis, immunity, methods of diagnosis of typhus.
40. Characteristics of chlamydia. Causative agents of trachoma, psittacosis, respiratory and urogenital chlamydiosis. Pathogenesis and methods of diagnosis of chlamydia.
41. General characteristics of mycoplasma, pathogenicity factors, role in human pathology. Methods of mycoplasmosis diagnosis.

Practical skills:

1. Determine the morphology of Staphylococcus, pure culture, Gram stain.
2. Determine the morphology of streptococcus, pure culture, Gram stain.
3. Determine the morphology of gonococci in pus, Gram stain.
4. Determine the morphology of enterobacteria, pure culture, Gram stain.
5. Determine the morphology of the mixture of S.aureus and Escherichia coli, Gram stain.
6. Determine the morphology of B.anthracis, pure culture, Gram stain.

Signature of the tutor _____ Date ____/____/2024

Class № 10 (27). METHODS OF INVESTIGATIONS IN VIROLOGY. BACTERIOPHAGES

List of questions to study:

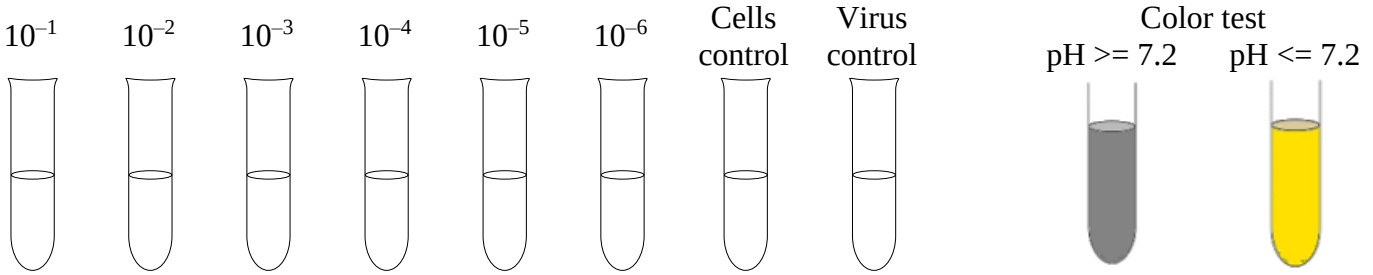
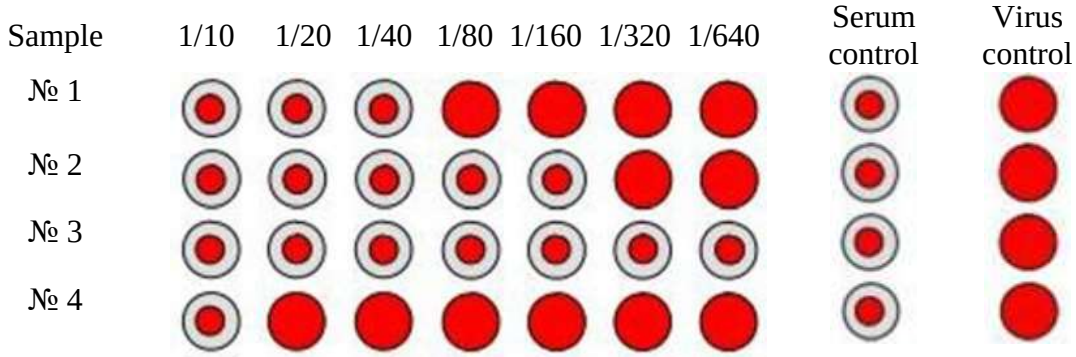
Viruses. Taxonomy and morphology of viruses. Mechanisms of reproduction. Strict parasitism and cytotropism of viruses.

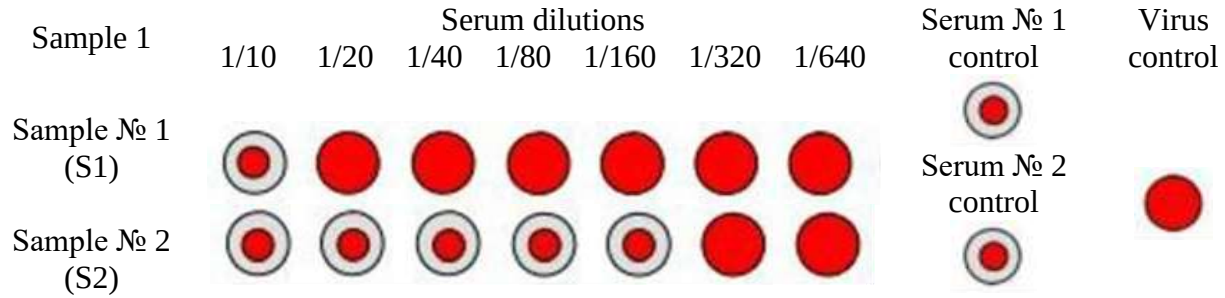
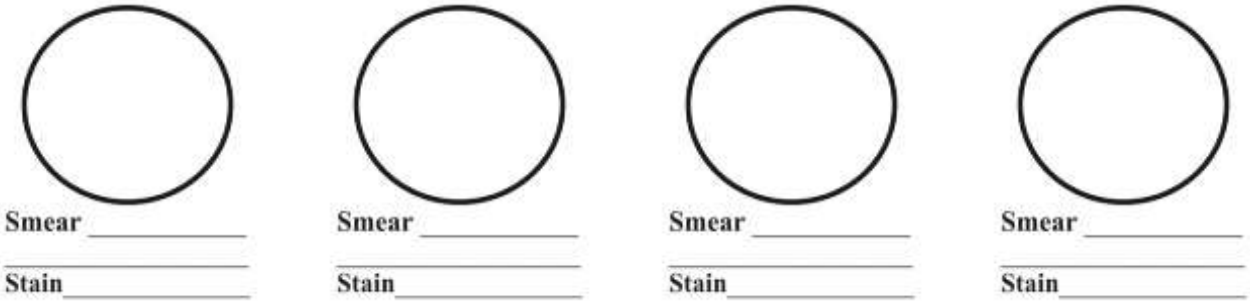
The types of viral infection. The mechanisms of antiviral immunity.

Methods of viral infections diagnostics. Culturing of viruses in hen embryos and in laboratory animals. Methods of infection, indication and identification of viruses. Serological methods in virology. Hemagglutination inhibition test (HIT), hemadsorption inhibition test, neutralization test, immunoenzyme analysis (ELISA). Molecular-genetic methods.

Viruses of bacteria (bacteriophages). Virulent and moderate bacteriophages. Methods for bacteriophages titration. Use of bacteriophages in medical practice. Phagodiagnostics and phagotyping.

Laboratory work

Laboratory exerscises	Laboratory report
1. Virus titration by color test. 2. Hemagglutination inhibition test for viral disease diagnostics. *Ingredients:* – *sera of the patient;* – *erythrocytes suspension;* – *standard virus dilution;* – *saline solution.*	**Virus titration by color test:**  **Conclusion:** _____ **Hemagglutination inhibition test for viral disease diagnostics:**  **Conclusion:** **Sample** / **Titer** № 1 № 2 № 3 № 4

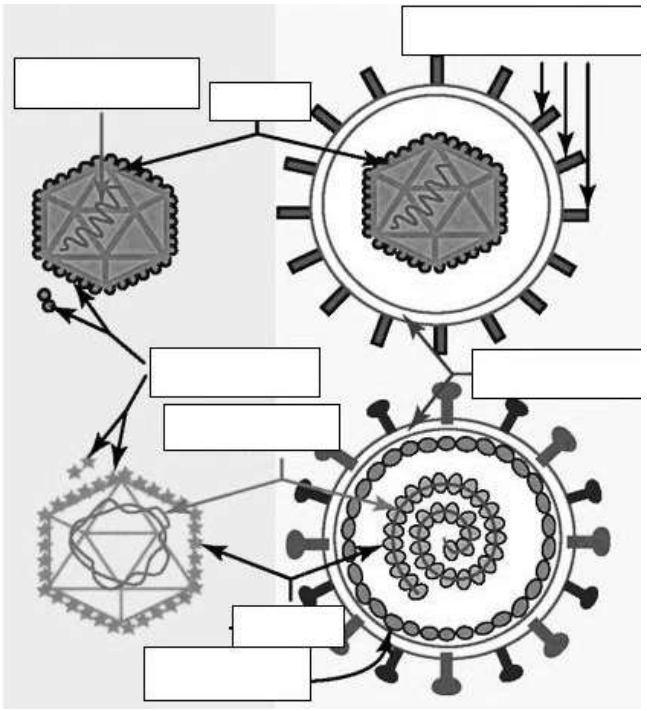
3. Hemagglutination inhibition test with pared sera for influenza serodiagnostics. *Ingredients:* *– sera of the patient: S1 — taken at admission; S2 — taken in two weeks;* *– erythrocytes suspension;* *– standard virus dilution;* *– saline solution.*	**Hemagglutination inhibition test with pared sera for viral disease diagnostics:**  **Conclusion:** _____
Demonstration: 1. Chicken fibroblasts, eosin. 2. Hep2 cell line, normal. 3. Cytopathic effect of adenoviruses. 4. Hemadsorption test.	 **Signature of the tutor _____ Date ____/____/2024**

Viral life cycle:

Stages	Naked viruses	Enveloped viruses
Viral attachment		
Penetration (virus entry)		
Uncoating		
Biosynthesis of viral components	**DNA viruses** are reproduced usually in the nucleus of the infected cells. They use cell DNA and RNA polymerases for nucleic acid replication. The majority of DNA viruses contains <u>double-stranded DNA</u>, which is transcribed into sense mRNA. The latter is used as a pattern for protein synthesis: dsDNA $\xrightarrow{\text{transcription}}$ m-RNA $\xrightarrow{\text{translation}}$ protein 2. Replication patterns of **RNA viruses** are more entangled: since eukaryotic cells possess no enzymes for RNA replication, the virus must supply the RNA-dependent RNA polymerase(s) ("replicase"). These enzymes are thus in any case virus-coded proteins, and in some cases are actually components of the virus particle. **2.1. Positive single-stranded RNA** is used as a template for direct protein synthesis: +RNA $\xrightarrow{\text{translation}}$ protein 2.2. Viruses, containing **negative genomic RNA**, synthesize mRNA by viral RNA polymerase: -RNA $\xrightarrow{\text{transcription}}$ m-RNA $\xrightarrow{\text{translation}}$ protein Viral genomic RNA is usually multiplied in the cytoplasm of infected cells with some exceptions (e.g. retroviruses). 2.3. **Retroviruses** have a unique way of transmitting genetic information: RNA $\xrightarrow{\text{reverse transcriptase}}$ DNA $\xrightarrow{\text{transcription}}$ m-RNA $\xrightarrow{\text{translation}}$ protein	
Morphogenesis with assembly of viral particles		
Virion release (or egress)		

Write down the names of the virion components:

Naked Virus **Enveloped Virus**



1. Capsid
2. Glycoprotein spikes
3. Nucleic acid
4. Envelope (phospholipid)
5. Matrix protein
6. Capsomeres

Virology Laboratory is used for laboratory diagnostic tests for viral infections, monitoring and intensity evaluation of specific post-infection and post-vaccination immunity, and participates in the prevention of viral diseases. The structure of the virology laboratory depends on the objectives and features of its activities.

Virological investigation: general principles

Virological investigation means isolation of the virus from pathological material, its serological identification and detailed study of various properties (pathogenicity, antigenicity, cultivation in the laboratory, morphological features). In every case of the disease with a suspected viral etiology it is necessary to isolate the agent from pathological material first. In this regard, the correct selection, packaging, transportation and processing of the material is of great importance for the successful diagnosics of the viral disease.

The material for the study.

From diseased, dead or slaughtered animals the material should be taken as soon as possible after the onset of clear signs of illness or no later than 2–3 hours after clinical death or slaughter. The material should be taken with regard to suspected disease pathogeneses (the entrance gate, the spread in the body, the place of reproduction and the ways of excretion).

The material for virus isolation may serve a variety of excreta and secrets, pieces of organs, blood, lymph, etc. At autopsy of animals material is collected under strict aseptic and antiseptic rules in order not to contaminate the material and do not allow the infection to spread.

Transport and storage of samples.

Samples taken should be put as quickly as possible in conditions that ensure the retardation of the virus inactivation. Such conditions mean low temperature. For this tubes with material, closed with rubber stoppers are placed in cooling mixture.

Material delivered in a laboratory should be immediately used for virus isolation. If for some reason investigation is delayed, the material is stored at minus (40–70) °C. Most viruses in the blood, cerebrospinal fluid, urine, nasal swabs and scrapings are quickly destroyed, so the success of their isolation depends on the speed of the investigation.

Preparation of virus-containing material.

In the laboratory the pathological material obtained should be freed of preservatives, thawed and washed from glycerol, weighed or measured.

Materials for infection of contamination-sensitive objects is carried out in two ways: with the antibiotics treatment or by sterile filtration.

Preparation of organs and tissues.

The virus should be freed from the cells of organs and tissues to Hank's solution. To do this, the material is thoroughly cut with scissors and grated in a mortar with sterile quartz sand. From the ground material is usually prepared 10 % suspension in Hanks solution. The resulting suspension is centrifuged at 1500–3000 rpm for 15–30 minutes, the supernatant is transferred into sterile vials and freed from bacteria by broad-spectrum antibiotics (Penicillin, Streptomycin, Nystatin, Tetracycline, etc.) treatment. Doses of antibiotics used for this purpose can vary within a wide range (from 100 to 1–2 million IU or more per 1 ml) depending on the nature of the material used. Exposure to antibiotics should be no less than 30–60 min at room temperature, then the material is subjected to a bacteriological control.

After a negative result of bacteriological control the virus-containing material can be used to infect laboratory animals, chick embryos and cell cultures. In case of a positive bacteriological control virus suspension is subjected to further processing.

Viral inclusions:

Viral inclusions (VI) are usually revealed by microscopy of infected cells and are the specific signs of viral infection of the cell. Certain viral inclusion has diagnostic importance.

VI were discovered by D. Ivanovsky (abnormal crystal intracellular inclusions in affected leaves of tobacco — Ivanovsky crystals).

VI can be revealed in nucleus and/or cytoplasm of the infected cell.

VI may be basophilic or eosinophilic and can vary in shape, quantity and location in the cell.

Characteristic nuclear VI can be observed in cells infected by herpesviruses, polyomaviruses, foot and mouth disease virus, adenoviruses, flaviviruses etc.

Cytoplasmic VI are usually noted in smallpox, influenza, rabies etc.

Cells infected by smallpox virus (Guarnieri bodies)

Cells infected by herpes virus (Cowdry bodies)

Cells infected by reovirus

Cells infected by rabies virus (Negry bodies)

Cells, infected with adenovirus

Cells, infected with influenza virus

Class № 11 (28). VIROLOGIC DIAGNOSTICS OF DISEASES CAUSED BY ORTHO-, PARAMYXOVIRUSES AND CORONAVIRUSES

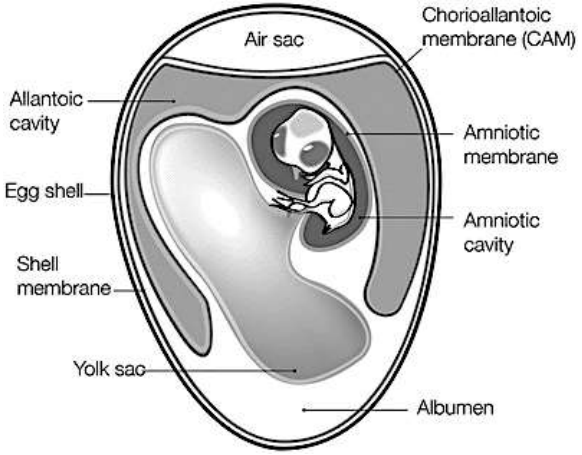
Suggested reading for self-study:

Orthomyxoviruses. Taxonomy and characteristics of the family. Influenza viruses A, B, C. Morphology. Antigenic structure and serotypes. Antigenic diversity (shift and drift) and its consequences. Influenza, prevalence, pathogenesis, immunity. Methods for influenza diagnostics. Principles of therapy and prophylaxis, preparations for specific immunoprophylaxis and chemopherapy of influenza. Avian and swine influenza.

Paramyxoviruses. Taxonomy and characteristics of the family. Differentiation with orthomyxoviruses. Parainfluenzaviruses, properties, importance for human pathology. Pathogenesis, immunity, diagnostics. Mumps virus, properties, pathogenesis, immunity, specific prophylaxis. Morbilivirus, morphology, properties, pathogenesis, immunity, specific prophylaxis. HRSV, properties, importance for human pathology.

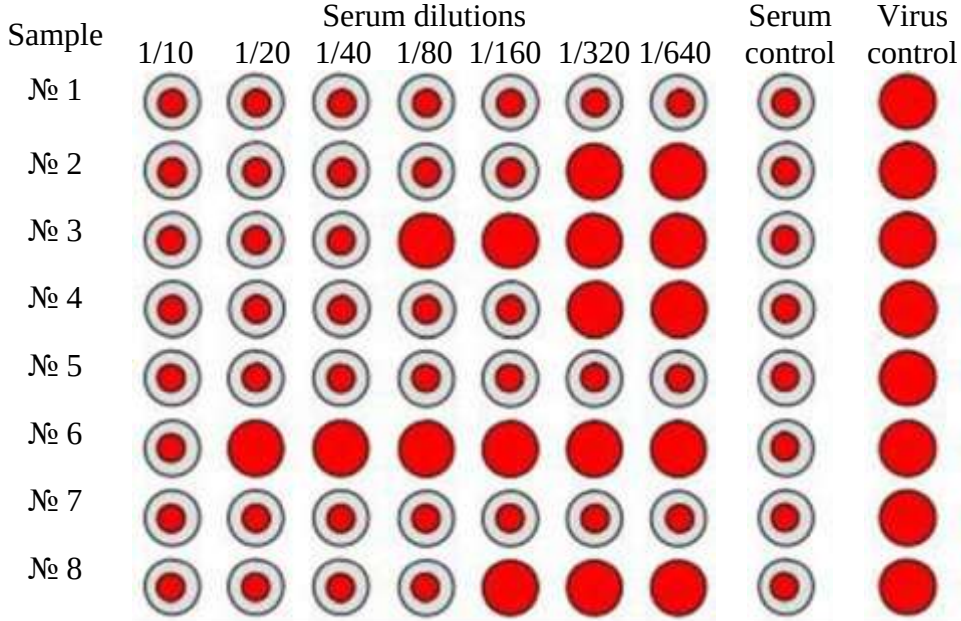
Coronaviruses: classification, characteristics, sensitivity to the physical and chemical factors. SARS-CoV coronavirus, severe acute respiratory syndrome (SARS). MERS-CoV coronavirus, Middle East respiratory syndrome (MERS). SARS-CoV-2 coronavirus. Coronavirus infection COVID-19: pathogenesis, immunity, etiological diagnosis, prevention, vaccine development, approaches for treatment, epidemiological situation in Europe and in the World.

Laboratory work

Laboratory exerscises	Laboratory report
1. Chicken embryo inoculation with influenza virus in allantois cavity.  	**Inoculation of the Allantoic cavity:** 1. Study the structure of hen embryo (8–11 days). 2. Examine hen embryo in ovoscope and determine the vitality signs: a) the dimensions of the embryo shape; b) presence of the developed blood vessels pattern; c) active mobility of the embryo; d) mark the air cavity border. 3. Set embryo on the egg rack and treat shell as follow: a) 70 % alcohol b) 5 % iodine. 4. Inoculate embryo as follow: a) flame scissors; b) carefully pierce the shell 3–5 mm above an air cavity border; c) take 0.2 ml of viral material (live influenza vaccine) in the syringe; d) keeping the needle and syringe vertical, place the needle through the hole in the eggshell approximately 16 mm into the egg to reach the allantoic cavity. Inject 0.1 mL of inoculum into the egg. 5. Repeat shell treatment according to p.3. 6. Seal the shell with tape or melted wax. Mark the embryo (group number). 7. Place the inoculated eggs into an incubator. 

2. Hemagglutination inhibition test for antibodies detection against influenzavirus type A.

Hemagglutination inhibition test for antibodies detection against influenzavirus type A:



Results:

Sample	Titer
№ 1	
№ 2	
№ 3	
№ 4	
№ 5	
№ 6	
№ 7	
№ 8	

3. Hemagglutination inhibition test with pared sera for influenza serodiagnostics.

Ingredients:

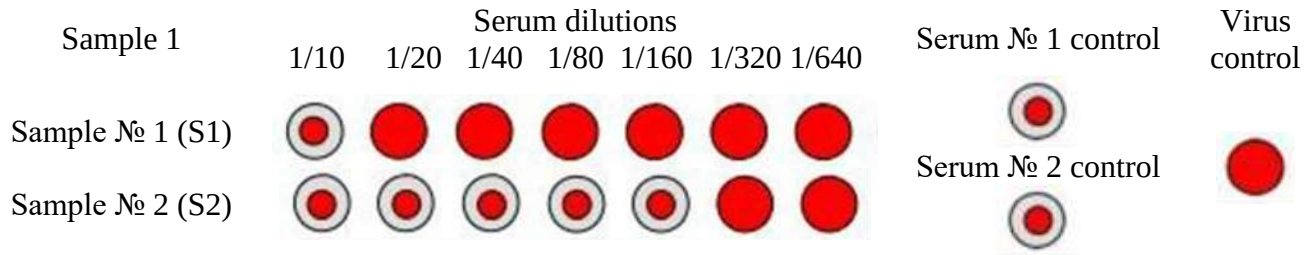
– sera of the patient: S1 — taken at admission; S2 — taken in two weeks;

– erythrocytes suspension;

– standard virus dilution;

– saline solution.

Hemagglutination inhibition test with pared sera for influenza serodiagnostics:

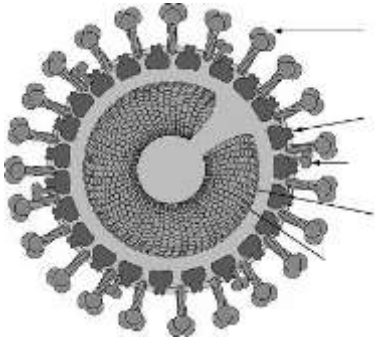


Conclusion: _____

Signature of the tutor _____ Date ____/____/2024

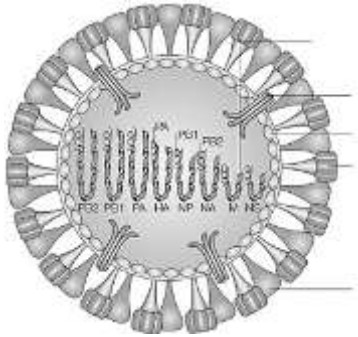
Indicate respective structural elements of virion:

The structure of _____ virus.



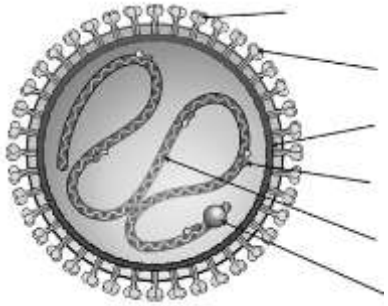
1. Spike glycoprotein (S)
2. Hemagglutinin-esteraseHE
3. Membrane protein M
4. RNA
5. Nucleoprotein

The structure of _____ virus.



1. Haemagglutinine
2. Neuraminidaze
3. Matrix protein M1
4. Protein M2
5. RNA

The structure of _____ virus.



1. Fusion protein F
2. Glicoprotein HN, G
3. Phosphopronein
4. Matrix protein
5. Nucleocapsid
6. Polymerase

Receptors of ortho- and paramyxoviruses

	H	N	F	G
influenza viruses	+	+	–	–
parainfluenza viruses	+	+	+	–
mumps virus	+	+	+	–
measles virus	+	–	+	–
respiratory syncytial virus	–	–	+	+

H — hemagglutinin, N — neuraminidase, F — fusion protein (formation of symplasts, syncytium), G — connection with cell receptors.

Laboratory diagnostics of measles:

1. Usually is not needed because the symptoms are very characteristic.
2. Used for diagnostics when:
The disease is atypical; epidemic outbreak investigation; lethal cases investigation.
3. Examination of nasopharyngeal smears by FAT, rash elements scraping, detection of characteristic multinuclear cells.
4. Virus can be isolated from blood or nasopharyngeal wash from prodromal stage till first day after rash appearance. Human embryo kidney cells, Vero or other cells are inoculated. After 3–4 days of incubation characteristic CPE can be registered: giant vacuolated multinucleated cells and cyncytium with cytoplasmic inclusions.
Also round-cell or spindle-cell degeneration with cytop;asmic or nuclear inclusions can be observed. Identification of isolated viruses is performed by FAT, HIT or NA.
5. Serologically antibodies titer increment in paired sera by ELISA (CFT, HIT) can be determined.
6. Molecular-genetic methods (PCR).

Laboratory diagnostics of mumps:

1. Usually is not needed because the symptoms are very characteristic.
2. Used for diagnostics when the disease is atypical with damage of internal organs and glands (pancreatitis, thyreoiditis, orhytis)
3. Serologically antibodies titer increment in paired sera by ELISA (CFT, HIT)can be determined.
4. Virus isolation: virus can be isolated from saliva (first 3 days of the disease), liquor (6 days) and urine (9 days):
a) mumps virus grows in chicken embryos (7–8 days old). Inoculation is performed in amnion cavity. Embryos then are incubated for 6–7 days at 35 °C. For virus indication HA is used;
b) virus also can be isolated on cell culture (human embryo kidney cells, HELA). Indication is performed by CPE registeration after 48–72 hours of incubation (giant multinucleated cells and symplasts with cytoplasmic inclusions).
c) for identification of isolated viruses — FAT, HIT, NA or CFT are used.
5. Molecular-genetic methods (PCR).

Class № 12 (29). METHODS OF DIAGNOSTICS FOR DISEASES CAUSED BY PICORNAVIRUSES, ROTAVIRUSES AND RETROVIRUSES

Suggested reading for self-study:

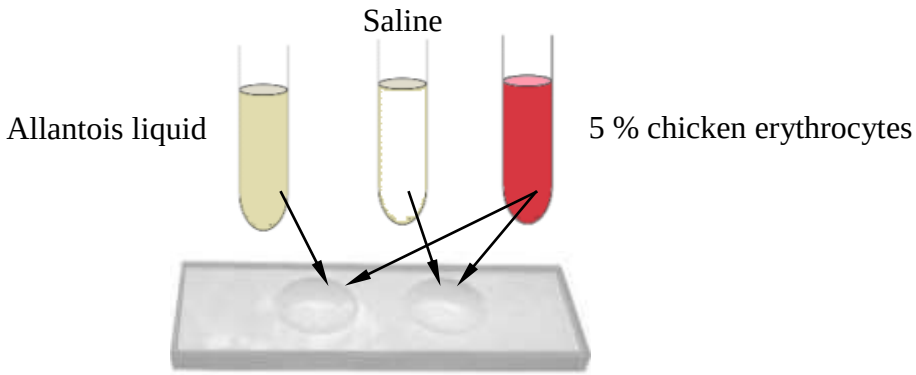
Picornaviruses. Taxonomy and characteristics of the family, importance for human pathology. Etiology, pathogenesis, immunity, diagnostics and immunoprophylaxis of poliomyelitis. Problem of poliomyelitis eradication.

Coxsackieviruses and ECHO-viruses, importance for human pathology. Methods for discrimination. Rhinoviruses. Taxonomy. Structure and characteristics. Prevalence, pathogenesis, immunity.

Rotaviruses, characteristics, role in pathology.

Retroviruses. Taxonomy and characteristics of the family. Human immunodeficiency virus (HIV-1, HIV-2). Morphology. Pathogenesis, role of CD4+ and CD8+ T-cells. AIDS-associated diseases. HIV diagnostics, prophylaxis, treatment. HIV in Belarus.

Laboratory work

Laboratory exerscises	Laboratory report
1. Chicken embryo autopsy.	1. Before autopsy embryo should be cooled for 2–3 hours at 4–6 °C for blood vessels constriction. 2. Treat the egg shell with 70 % alcohole and flamed. Repeat it once more. 3. Open the shell by sterile scissors 2–3 mm above air sack border. Remove shell membrane and aspirate 1 ml of allantois cavity liquid. 4. Amnion cavity liquid can also be taken (0.5–1.5 ml). 5. Remove an embryo on the Petri plate. Allantois membrane should be carefully examined by yes. Usually influenza viruses produce no CPE. 6. Perform slide HT for virus indication
2. Virus indication by slide hemagglutination test. 1. Put two drops of 5 % chicken erythrocytes suspension onto glass slide. 2. Add and mix one drop of allantois liquid (experiment) and saline (negative control) with each drop. The test is positive if flakes of erythrocytes are developed. The test is negative if erythrocytes remain in suspension after 5–7 min.	**Slide hemagglutination test:**  **Conclusion:** _____

3. Evaluation of HIT for influenzavirus identification.

Evaluation of HIT results for influenzavirus identification:

Serum	H1N1	H3N2	H5N1	Serum control			Virus control	**Result (serotype of influenza virus):**
				H1N1	H3N2	H5N1		
Patient A.								
Patient M.								
Patient N.								
Patient F.								
Patient K.								

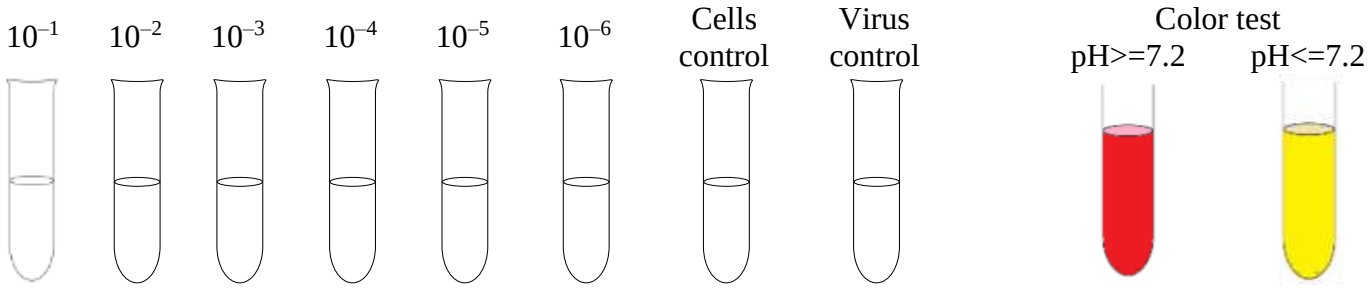
Demonstration:

1. Poliomyelitis virus titration by color test.

Color test. The test is based on the phenomenon of virus induced inhibition of cell culture metabolic activity. Healthy, uninfected culture gradually reduces the pH of the medium, and if phenol red has been added as an indicator, the color changes from red to yellow. If the cells are killed by poliomyelitis virus, metabolism ceases and the medium remains red. Conversely, if antibody-containing serum is added to virus and susceptible cells, the virus is neutralized and does not kill those cells with the result that metabolism proceeds in a normal manner and the medium becomes yellow.

Usually color test is applicable only for highly cytopathogenic viruses like enteroviruses or adenoviruses.

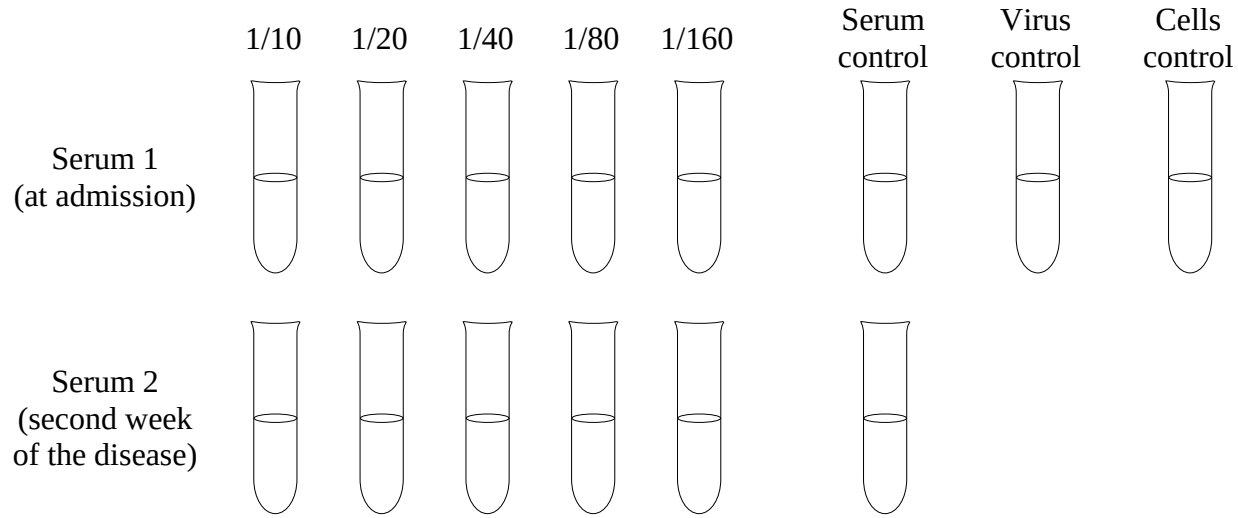
Determination of poliomyelitis virus titer by color test:



Conclusion _____

2. Neutralization test on cell culture in paired sera for poliomyelitis serodiagnostics.

Neutralization test in paired sera for poliomyelitis serodiagnostics:

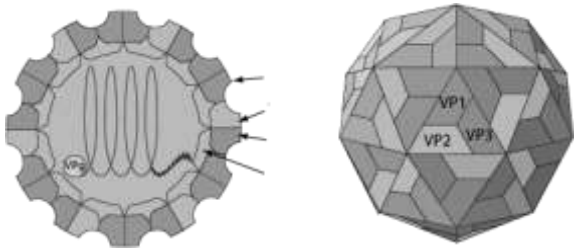


Conclusion _____

Signature of the tutor _____ Date ____/____/2024

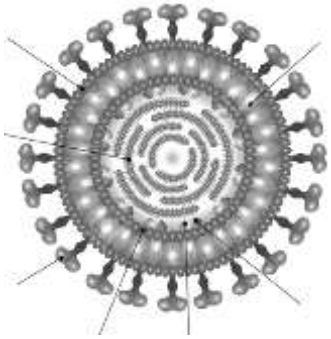
3. Write in virus family name, indicate respective structural elements of virion.

The structure of _____ virus



1. VP1
2. VP2
3. VP3
4. RNA
5. VP4

The structure of _____ virus.



1. Outer capsid VP7
2. Intermediate capsid VP6
3. Surface protein VP4
4. Inner capside protein VP2
5. Core protein VP1 (RNA polymerase)
6. RNA
7. Core protein VP3 guanylyl transferase

The structure of _____ virus.

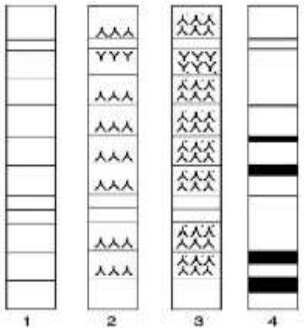
1. Capsid (p24)
2. Matrix protein (p17)
3. Revertase
4. gp120
5. gp41
6. Lipide bilayer
7. RNA

ELISA for HIV infection screening:

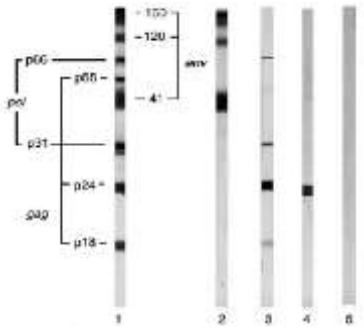
At present the fourth generation ELISA kits are used. Its main advantages include recombinant antigens, monoclonal antibodies, simultaneous detection HIV antigens (usually p24) and antibodies against surface HIV antigens

Biotin and avidin represent a pair receptor-ligand with very high affinity and specificity. Their properties allow to use this for antigens and antibodies tagging. One avidin molecule can bind four biotin molecules. That is the signal about binding would be four times higher.

Immunoblotting for HIV infection diagnostics:



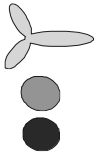
1. Blot preparing: electrophoretic separation of HIV proteins by their mass and charge. Transfer to the membrane, fixation and cutting into strips.
2. Incubation with serum examined. Washing.
3. Incubation with antibodies, tagged with peroxidase, against human antibodies.
4. After substrate is added color bands appear where patient`s antibodies bind to HIV antigens.



1. Positive result in person, infected with HIV-1.
2. Healthy person, vaccinated with surface HIV-1 glycoproteins.
3. Uncertain result in person, infected with HIV-2.
4. Uncertain result because of some unrelated antibodies presence cross-reacting with p24 antigen.
5. Negative result.

The scheme of ELISA for HIV infection screening:

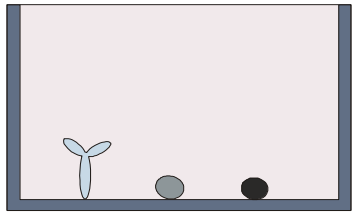
1. In wells of the plate for immunoanalysis certain ingredients are adsorbed:



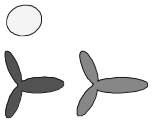
Monoclonal antibodies to p24

Recombinant epitop from gp120

Recombinant epitop from gp41

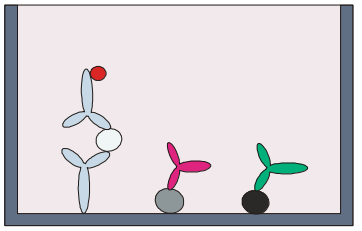


2. When patients serum is added p24 and antibodies against surface HIV glicoproteins bind their adsorbed ligands.

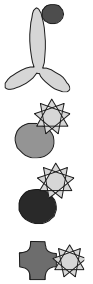


p24

Antibodies to HIV glicoproteins from patient' serum



3. Then conjugates are added:

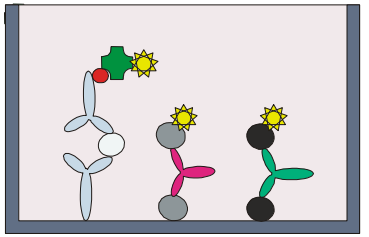


Antibodies to p24 + biotin

gp41 + peroxidase

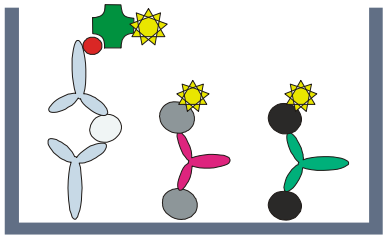
gp120 + peroxidase

Avidin + peroxidase



Conjugate molecules fix to immune complexes according to the quantity of molecules to reveal.

4. After substrate is added the dose-dependent fermentation occurs and colored product is developed.



Class № 13 (30). VIROLOGICAL DIAGNOSTICS OF DISEASES CAUSED BY ARBOVIRUSES AND ROBOVIRUSES. ONCOGENIC VIRUSES. SLOW INFECTION

Suggested reading for self-study:

General features of arboviruses and arboviral infections.

Toga-, flavi-, bunja-, arenaviruses, taxonomy, virion structure, role in human pathology. Etiology, pathogenesis, immunity, methods for tick encephalitis (Russian spring summer encephalitis) diagnostics. Hemorrhagic fever with kidney insufficiency syndrome (HFKS or HFRS).

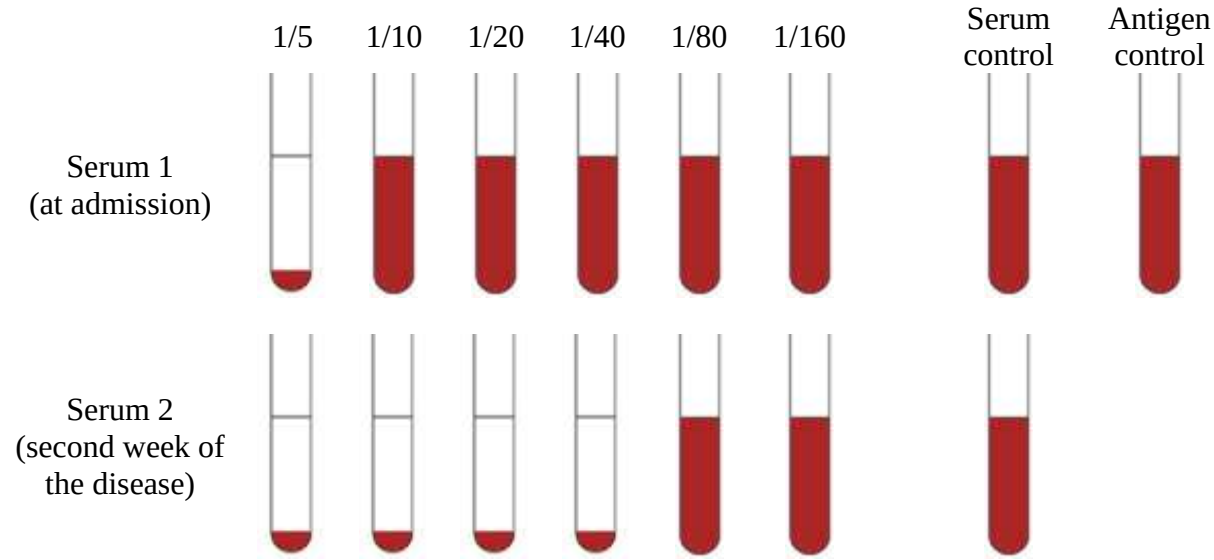
Rubella virus. General characteristics. Role in human pathology. Prophylaxis.

Rabdoviruses. Taxonomy and characteristics of rabdoviruses. Pathogenesis, immunity and specific prophylaxis of rabies.

Filoviruses. Marburg and Ebola viruses.

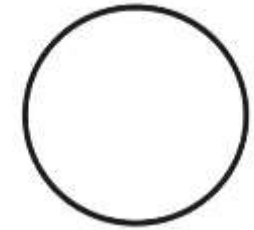
Oncogenic viruses (DNA an and RNA). Viral cancerogenesis mechanisms. Slow infections etiology.

Laboratory work

Laboratory exerscises	Laboratory report
1. Determination of titer increment in paired sera for tick encephalitis diagnostics. **Complement fixation assay:** A serologic test based on the fixation of complement by antigen–antibody complexes. The test is characterized by high sensitivity and specificity. The test are of value for virology, when high quality antigen (viral particles glicoproteins) can be easily generated in cell culture and used for the diagnostics of infection (even in the case of unknown viral infection for which modern diagnostic means are not developed) or for virus identification with the help of diagnostic antisera.	**CFT results evaluation for tick encephalitis diagnostics:**  **Conclusion** _____

Demonstration:

1. Negry bodies in mouse brain homogenate (Muromtcev stain).



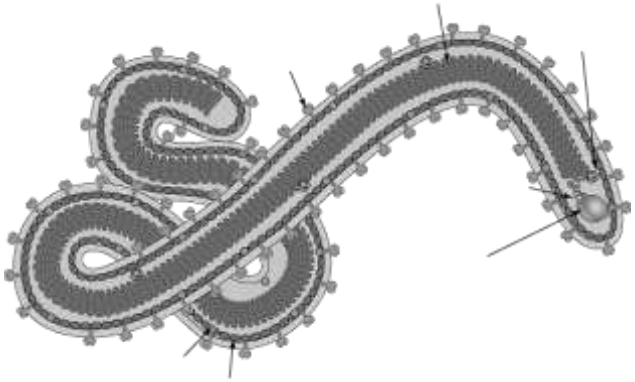
Smear _____

_____

Stain_____

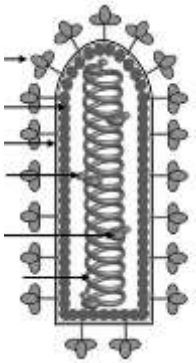
2. Write in virus family name, indicate respective structural elements of virion.

The structure of _____ virus



1. Glycoprotein (gp1+gp2)
2. Nucleoprotein (NP)
3. Transcription factor VP30
3. Polymerase cofactor VP35
4. Polymerase (L)
4. M-protein VP40
5. VP24

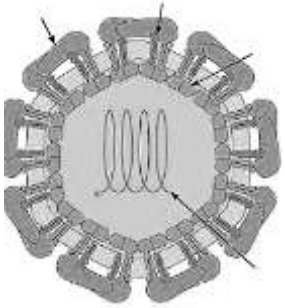
The structure of _____ virus



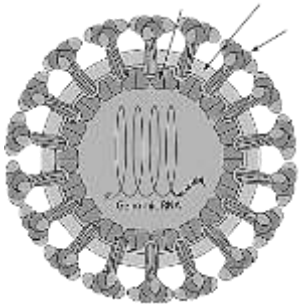
1. Glycoprotein
2.Matrix protein
3. Lipid bilayer
4. Phosphoprotein
5. Large Protein
6. Nucleocapsid-RNA

The structure of _____ virus.

1. M protein
2. Capsid
3. RNA
4. E-dimer



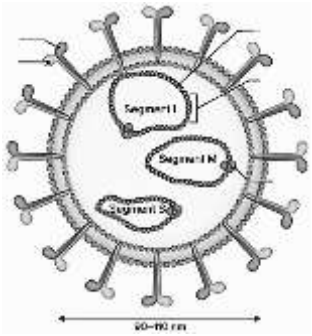
The structure of _____ virus.



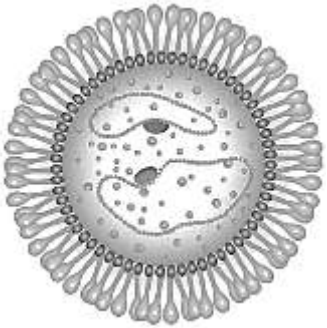
1. Capsid protein
2. Membrane
3. Trimer of E1-E2

The structure of _____ virus.

1. Glycoprotein Gc
2. Glycoprotein Gn
2. L-polymerase
3. Nucleocapsid



The structure of _____ virus.



1. Supercapsid
2. Nucleocapsid (beads on RNA)
3. Ribosomes
4. L-RNA
5. S-RNA
6. Glicoproteins

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ONCOGENIC VIRUSES

Oncogenic viruses are able to transform (make cell immortal and cause genome instability and tumor progression) normal cells in vitro and in vivo.

Transformation signs: adhesion loss; increasing mobility; invasive activity; resistance to proliferation and differentiation control mechanisms; ability to form tumors; increased rate of chromosome aberration.

RNA oncogenic viruses:

Oncogenic RNA viruses belong to 5 genera of Retroviridae family, Oncornavirinae subfamily — Alpharetrovirus avium myeloblastosis virus (AMV), Rous sarcoma virus — RSV), Betaretrovirus (Mouse mammary tumor virus — MMTV), Gammaretrovirus (Murine leukemia virus — MuLV), Deltaretrovirus (Human T-lymphotrophic virus, Bovine leukemia virus — BLV, HTLV), Epsilonretrovirus (Walleye dermal sarcoma virus — WDSV).

Mechanisms of oncogenic transformation include introduction in a cell of highly active oncogenes (normal cell genes homolog which are able to transform cells in culture. Viral oncogenes are designated v-onc, and respective cellular oncogenes — c-onc. Some RNA oncogenic viruses can transform cells without oncogenes by specific integration:

– by enforcement of normal cellular genes activity (IL2, IL2R, c-fos) with viral promotor;

– by damaging of antitumor genes activity (RBp, p53 etc.).

DNA oncogenic viruses:

DNA oncogenic viruses belong to 5 families: polyomaviruses, papillomaviruses, adenoviruses, herpesviruses, hepadnaviruses. Of course not all viruses from particular family are oncogenic and not all oncogenic viruses can induce advanced tumors.

DNA oncogenic viruses use similar transformation mechanisms:

– increase of cellular genes activity (translocation or specific integration). Cellular oncogenes are activated by strong viral or cellular promoters (e.g. TCR or immunoglobulines).

– introduction of highly active oncogenes in the cell: DNA oncogenic viruses have their own oncogenes.

The transformation mechanism of DNA oncogenic viruses includes violation of cellular apoptosis and leads to cell immortalization and tumor progression. Many viruses express mechanisms damaging the function of antitumor cellular factors: adenoviruses bind and neutralized retinoblastoma gene protein; HCV binds antioncogene p53, and papillomaviruses can target it and destroy on proteosomes.

Tick encephalitis diagnostics:

1. Materials for investigation: blood, urine, liquor, brain (autopsy). For serological diagnostics paired sera are used (first is taken at the beginning and second — on 5^{th}–7^{th} week of the disease.

2. Virus can be isolated on white mice: prepared material is injected in brain directly. After 8–12 days symptoms can be registered (irritability, unsteadiness, convulsions, paralysis, death). If no disease appear two more passages can be done.

3. Virus can be isolated on cell culture (chicken embryo fibroblasts and others). TEV usually do not produce CPE.

4. Indication/identification is performed by FAT, NT on mice, HIT or CFT with standard typospecific sera.

5. Serological tests include CFT, PHAT, HIT and most often ELISA.

Rabies diagnostics:

1. Materials for investigation: brain, salivary gland tissues, skin biopsy, hair follicles. 2. Diagnostics is based on the detection of Negry bodies or viral antigens in tissues sections or smears. Inoculation of white mice is also used:

а) Negri bodies are eosinophilic, sharply outlined, pathognomonic inclusion bodies (2–10 μm in diameter) found in the cytoplasm of certain nerve cells containing the virus of rabies, especially in Ammon's horn of the hippocampus. Often also found in the cerebellar cortex of postmortem brain samples of rabies victims. They consist of ribonuclear proteins produced by the virus. The sections are usually stained by Mann's, Giemsa, or Sellers methods which permit differentiation of rabies inclusions from other intracellular inclusions. With these stains, Negri bodies appear magenta in color and have small (0.2 μm to 0.5 μm), dark-blue interior basophilic granules. In former SU countries brain sections are stained by Muromtcev method for rabies diagnostics

б) FAT allow to reveal viral antigens in cytoplasm of the infected neurons (in brain, salivary glands, skin, hair follicles etc). When fluorescein is used for antiserum targeting viral antigens appear as greenish granules of different size (0.2–25 mkm).

Class № 14 (31). VIROLOGIC DIAGNOSTICS OF DISEASES CAUSED BY HEPATITIS VIRUSES, HERPES- AND ADENOVIRUSES

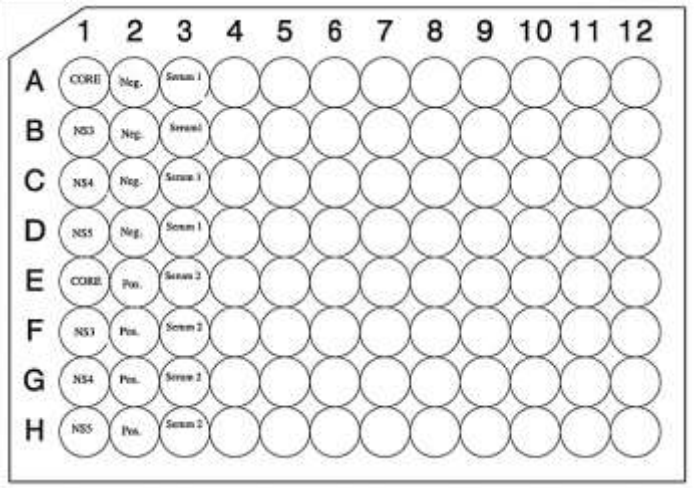
Suggested reading for self-study:

Hepatitis viruses A, B, C, D, E, F, G, TTV and SEN. Taxonomy and characteristics, role in human pathology. Pathogenesis and immunity in hepatitis A, B, C. Laboratory diagnostics. Specific and non specific prophylaxis.

Herpesviruses. Taxonomy and family characteristics. HSV-1, HSV-2, properties, role in human pathology, pathogenesis, immunity, diagnostics, chemo and immunotherapy. HZV, properties, pathogenesis, immunity, diagnostics, prophylaxis. CMV: properties, pathogenesis. EBV features, role in human pathology. Pathogenesis, immunity, diagnostics. HHV6, HHV-7, HHV-8, role in human pathology.

Adenoviruses. Taxonomy and family characteristics. Human adenoviruses. Virions structures, pathogenesis, immunity, laboratory diagnostics.

Laboratory work

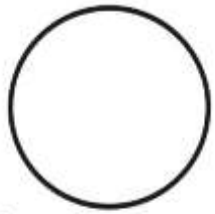
<table>
<tr><th>Laboratory exerscises</th><th colspan="2">Laboratory report</th></tr>
<tr><td rowspan="2">1. Perform of ELISA for HCV diagnostics.
The protocol is based on the commercial ELISA kit for VHC diagnostics «RecombiBest anti-HCV» by VectorBest, RF.
The method reveals antibodies (IgG and IgM) to HCV antigens.
Antibodies from patients serum bind to recombinant antigens adsorbed on the well of a plate.
Specific immune complexes then detected by conjugate antibody-enzyme and respective enzymatic reaction.
Colored product developed is measured by ELISA reader.</td><td colspan="2">HCV antigens are adsorbed on the strip wells as follows:
rows A, E — core
rows B, F — NS_3
rows C, G — NS_4
rows D, H — NS_5
1. Put 100 mkl of control sera and samples according to the plate layout.
2. Close strip with adhesive tape and incubate for 1 hour at 37 °C.
3.Wash wells 5 times.
4.Put 100 mkl of conjugate in each well.
5. Seal strip with tape and incubate for 30 min at 37 °C.
6. Wash 5 times.
7. Put 100 mkl of substrate in each well.
8. Incubate for 30 min at 37 °C.
9. Put 50 mkl of stop solution in each well.
10. Measure the plate by ELISA reader.
11. Evaluate results:
  </td></tr>
<tr><td>1. Test results validation:
Negative control OD < 0.2
Mean negative control OD =
Mean positive control OD >0.8
Mean positive control OD =
2. Cut-off level for each antigen:
Cut-off (core-Ag) = NC ODO(core) + 0.2 =
Cut-off (NS_3-Ag) = NC OD (NS3) + 0.2 =
Cut-off (NS_4-Ag) = NC OD (NS4) + 0.2 =
Cut-off (NS_5-Ag) = NC OD (NS5) + 0.2 =</td><td>3. Positivity index determination for each antigen:
PI(core-Ag) = OD sample(core)/ Cut-off(core-Ag) =
PI(NS_3-Ag) = OD sample (NS_3)/Cut-off(NS_3-Ag) =
PI(NS_4-Ag) = OD sample (NS_3)/Cut-off(NS_4-Ag) =
PI(NS_5-Ag) = OD sample (NS_3)/Cut-off(NS_5-Ag) =
4. Results evaluation:
a) If PI less than 1, sample is considered negative;
b) the results sre considered positive if IP exceeds 1 for: core-Ag any two antigens;
c) result is considered uncertain if IP exceeds 1 for one nonstructural protein only.</td></tr>
</table>

Demonstration:

1. Methods for HBs-Ag detection.
2. CPE of adenoviruses.

ELISA protocol for VHC diagnostics:

Antigen	Row	OD	Cut-off	Result
Core	A			
NS3	B			
NS4	C			
NS5	D			
Core	E			
NS3	F			
NS4	G			
NS5	H			



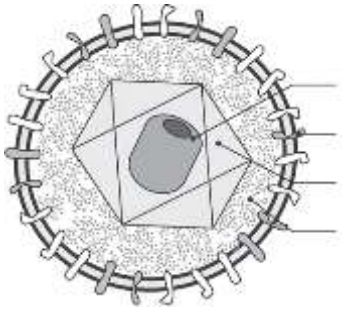
Smear _____

Stain _____

Conclusion _____

Signature of the tutor _____ Date ____/____/2024

The structure of _____ virus.



1. Envelope proteins
2. Icosahedral capsid
3. Tegument
4. DNA

The structure of _____ virus.

1. Hexon
2. Penton base
3. Penton fibre
4. Knob
5. Capsid
6. ds-DNA
7. Core

Virus	Family, genus		Genome	Virion morphology	Antigens	Mechanism of infection	Carriage, complications
HAV	*Picornaviridae*, genus *Hepatovirus*						
HBV	*Hepadnaviridae*, genus *Orthohepadnavirus*						
HCV	*Flaviviridae*, genus *Hepacivirus*						

Virus	Family, genus		Genome	Virion morphology	Antigens	Mechanism of infection	Carriage, complications
HDV	Unclassified satellite virus						
HEV	*Hepeviridae*, genus *Orthohepevirus*						

Clinical and epidemiological meaning of hepatitis A, B, C, D, E markers:

Marker	Clinical and epidemiological meaning
HAV antigen (HAV-Ag)	Detection of HAV-Ag in children feces is an indication of infection danger for persons in the seat of infection (but is not diagnostic)
Total antibodies to HAV antigen (abHAV)	Indicate the current disease or past disease and is useful for vaccination need evaluation
IgM to HAV antigen (abHAV-IgM)	Acute hepatitis A marker
RNA of HAV (RNA-HAV)	Indicate HAV presence in material
HBs-Ag (Surface HBV-antigen)	Hepatitis B marker (both acute and chronic), require confirmation by total abHBc or abHBc-IgM. One of safety criteria in transfusiology. Screening in risk groups.
Total antibodies to HBs-Ag (abHBs)	Hepatitis B staging, prognosis, specific immunity control. Vaccination efficacy control. Epidemiological research. Favorable outcome marker
Core HBV antigen (HBcAg)	Marker of HBV presence in hepatocyte (in acute or chronic hepatitis B)
IgG to HBV core antigen (abHBc)	Acute and chronic viral hepatitis B marker, carrier state marker, present or past HBV infection marker. Safety criterion in transfusiology
IgM to HBV cor antigen (abHBc-IgM)	Acute hepatitis B or chronic hepatitis B exacerbation marker
E-antigen of HBV (HBeAg)	The marker of HBV replication intencity and epidemiological danger of the patient. It is of importance for viral hepatitis differential diagnostics, prognosis of the disease outcome, evaluation of the vertical transmission risk
Total antibodies to Hbe-antigen (abHBe)	Staging of the disease, differential diagnostics. Marker of favorable disease outcome
HBV DNA	Marker of blood infectivity and active virus replication. Differential diagnostics between HBV or HBs-Ag carrier state
Total antibodies to HCV antigen (abHCV)	Hepatitis C marker. It is not suitable for disease staging
IgM to HCV cor antigen (abHCc-IgM)	Acute hepatitis C marker (but sometimes can be found in chronic hepatitis C reactivation)
HCV RNA	Marker of blood infectivity
Total antibodies to HDV (abHD)	Hepatitis D marker. It is not suitable for disease staging
IgM to HDV (abHD-IgM)	Acute hepatitis D marker
HDV RNA	Virus presence in blood
Total antibodies to HEV (abHEV)	Hepatitis E marker

Virological diagnostics for herpes infection:

A) Early diagnostics: morphological examination of damaged tissues and isolation of virus. Scrapings and smears from rash elements are used as a material.

Smears are usually stained by Gimsa method or by hematoxylin-eosin. Giant cells formation and nuclear inclusion development are characteristic for herpes infection.

Smears can be stained with fluorescent antibodies (FAT). Herpes antigens may be found in multinucleated, giant and unchanged cells. The method allows to detect herpes infection in brain, spinal cord and other tissues (liver) in lethal cases.

Virus can be isolated by 12-days chicken embryo inoculation. Material is applied on allantois membrane. Embryo is incubated for 48 hours at 35 °C. Allantois membrane damages are observed. Giant and multinucleated cells with nuclear inclusions are revealed by microscopy.

Cell culture inoculation. Typical CPE includes multinucleated cells formation with nuclear inclusions and round cell degeneration;

Suckling mice inoculation. Mice are infected in brain or in abdominal cavity. The disease appears in 3–4 days and kills animals;

Rabbits inoculation. Rabbits are infected on scarified cornea or in brain: specific keratitis or lethal encephalitis develops respectively.

Identification of isolated viruses is performed by FAT or NT.

B) Retrospective diagnostics: for serological diagnostics CFT or ELISA in paired sera are used.

Virological diagnostics for chicken pox:

A) Early diagnostics: microscopy of material from lesions, viral antigens, DNA detection or virus isolation in cell culture.

The best results are achieved by microscopy of material from fresh vesicular: multinucleated giant cells with nuclear inclusions are characteristic.

For rapid identigication FAT method is usually used. Specific antigen can be revealed extracellularly as bright grains or intracellular.

Virus can be isolated in cell culture. Characteristic CPE — the development of giant multinuclear cells or round-cell degeneration. Eosinofilic nuclear inclusions are often observed. Identification of isolated viruses is performed by FAT or NT.

B) Retrospective diagnostics: specific antibodies are revealed in ELISA, CFT or NT in paired sera.

Virological diagnostics for adenovirus infection:

1. Nasopharyngeal and conjunctival washes and scrapings, feces, urine, biopsy and autopsy are used as a material.

2. Fast methods include viral antigens and DNA detection in the material: usually FAT or ELISA in situ are used.

3. Virus isolation: different epithelial cell lines (HEK, HELA, A-549) are used. Characteristic CPE includes: small cell degeneration with cell agglomeration (grape like); cell rounding; cytoplasmic and nuclear inclusions; cells death.

Virus identification is performed by NT, FAT, CFT; PCR; EM and IEM.

4. Retrospective diagnostics (for epidemiological purposes) includes ELISA, HIT, CFT in paired sera.

Virological diagnostics for EBV infection:

1. **Heterophilic antibodies detection** — natural antibodies (IgM), which agglutinate erythrocytes of unrelated species (sheep, bull, horse etc). This phenomenon is found in approximately 90 % EBV patients. Heterophilic antibodies sometimes present in blood of healthy persons in low titer.

a) **Paul–Bunnell test** (Hanganutziu–Deicher reaction) — standard method for infection mononucleosis diagnostics. It is based on sheep erythrocytes hemagglutination by patient's serum. Diagnostic titer is 1:128–1:256. Heterophilic antibodies are found 3–4 week of the disease. Paul-Bunnell test are positive in leucosis, viral hepatitis, CMV infection, Burkitte lymphoma, rheumatoid arthritis, serum sickness.

b) **The monospot test** is a rapid test for infectious mononucleosis due to Epstein–Barr virus (EBV). The test is sensitive for heterophile antibodies which agglutinate horse erythrocytes. Commercially-available test kits are 70–92 % sensitive and 96–100 % specific. It will generally not be positive during the 4–6 week incubation period before the onset of symptoms. It will also not generally be positive after active infection has subsided, even though the virus persists in the same cells in the body for the rest of the carrier's life.

2. **Serological diagnostics**. Tests for heterophilic antibodies are relatively not sensitive and if negative can not exclude EBV infection. In this case other serological tests are useful:

a) ELISA for IgM and IgG to EBV capsid antigen. Its concentration reaches maximum in 2 weeks and diminishes during 2–3 months. IgM to EBV capsid antigen testifies for recent infection, IgG — infection in the past.

b) ELISA for antibodies to early EBV antigens. Its concentration reaches maximum in 2 weeks of the disease.

c) ELISA for antibodies to nuclear EBV antigen. They appear approximately in 4 weeks of the disease and persist lifelong.

Class № 15 (32). CONCLUDING SESSION «GENERAL AND SPECIAL MEDICAL VIROLOGY»

List of questions:

42. Virology: definition, objectives, methods. Systematic position and classification of viruses.
43. History. D.Ivanovsky works importance.
44. Forms of existence of viruses. Morphology and biochemical structure of virions. Viral genome organization. Structure, function and properties of virion nucleic acid, proteins, lipids and carbohydrates. Prions, role in human pathology.
45. Interaction of the virus and susceptible cell. Strict parasitism and cytotropism of viruses. Cell receptors for viruses. Reproduction strategy of DNA and RNA viruses.
46. Types of viral infection of cell. Changes in the host cells in the process of a viral infection.
47. Peculiarities of viral infections of an organism. Acute, chronic and slow infection.
48. Local and systemic mechanisms of antiviral immunity. Factors of innate and adaptive antiviral immunity. Control of viral reproduction by the immune system. Interferons: classes, properties, mechanisms of antiviral activity.
49. Principles of etiological diagnostics of viral infections. Rapid methods. Serological diagnostics: principles. Hemagglutination/hemadsorbtion inhibition test: mechanisms, methods of performance, registration, application. Neutralization test: mechanisms, performance, registration, application.
50. Cultivation of viruses. Cell culture: types, methods of infection. Reveling and identification of viruses in cell culture. Types of cytopathic effects (CPE) . Viral inclusion: the nature, location, diagnostic value.
51. Cultivation of viruses in the chick embryo: methods of infection, indication and identification of viruses. Cultivation of viruses in laboratory animals: methods of infection, indication and identification of viruses.
52. Principles of viral infections chemotherapy. Group of antiviral drugs. Viral load , methods of its determination.
53. The etiology of acute respiratory viral infections. Influenza viruses: classification, characteristics. Dissimilarities between influenza viruses. Influenza: pathogenesis, immunity and prevention. Rhino-viruses: classification, characteristics, role in human pathology.
54. Influenza A viruses: genome, properties and functions of proteins of the virion, the antigenic structure and its variability. Chemotherapy and chemoprophylaxis of influenza. Viruses of “bird” and “swine” flu.
55. Etiologic diagnostics of influenza: the material for the study, methods, evaluation of results.
56. Differentiation of influenza and parainfluenza viruses.
57. Paramyxoviruses: classification, characteristics, role in pathology. Prevention of mumps.
58. Coronaviruses: classification, characteristics, sensitivity to the physical and chemical factors. SARS-CoV coronavirus, severe acute respiratory syndrome (SARS). MERS-CoV coronavirus, Middle East respiratory syndrome (MERS).
59. SARS-CoV-2 coronavirus. Coronavirus infection COVID-19: pathogenesis, immunity, etiological diagnosis, prevention, vaccine development, approaches for treatment, epidemiological situation in Europe and in the World.
60. Measles virus: classification, characteristics. Measles: pathogenesis, immunity and prevention. Subacute sclerosing panencephalitis. Epidemiological situation in Europe and in the World regarding measles. Mitigated measles.

61. Rubivirus: systematics, characteristics. Rubella: pathogenesis, etiologic diagnosis, prevention. Congenital rubella syndrome.
62. Ecological group of arboviruses: definition, classification, characteristics. Arbovirus infection: features, pathogenesis.
63. Tick-borne encephalitis: pathogenesis, etiologic diagnosis, prevention. Ecological roboviruses subgroup.
64. Bunyaviruses, hemorrhagic fever with renal syndrome.
65. Zika virus and pathogenesis of diseases caused by it. Nipah virus infection. Hendra virus.
66. Ecological sub-group of roboviruses. Hemorrhagic fever with renal syndrome: characteristics, the role in pathology. Arenaviruses: classification, characteristics, role in pathology. Filoviruses: classification, characteristics, role in pathology. Ebola haemorrhagic fever: pathogenesis, diagnostics, prevention, history of its epidemics. Murburg haemorrhagic fever.
67. Rabies virus: classification, characteristics, specific inclusion. Rabies: pathogenesis, etiologic diagnosis, prevention. L. Pasteur's research and it's importance.
68. Viruses as etiological agents of GIT-infections. Enteroviruses: classification, characteristics. Enterovirus infections: pathogenesis, prevention.
69. Rotaviruses: classification, characteristics. Rotavirus infections: pathogenesis, prevention.
70. Polio viruses: classification, characteristics. Poliomyelitis: pathogenesis, immunity, etiologic diagnosis, prevention. Vaccine-associated polio.
71. Enteric hepatitis viruses. Hepatitis A virus: classification, characteristics. Viral hepatitis A: pathogenesis, immunity, etiologic diagnosis, prevention.
72. Hepatitis E virus: classification, characteristics, role in pathology, etiological diagnostics, prevention. Norovirus (Norwalk virus).
73. Parenteral hepatitis viruses: classification, characteristics. Hepatitis B virus: systematics, characteristics, antigens. Parenteral hepatitis B: pathogenesis, immunity, etiologic diagnostics, therapy, prevention. Diagnostic significance of detection of HBV DNA, IgG and IgM against antigens, and viral antigens. Hepatitis D virus: systematics, characteristics, etiologic diagnostics, prevention.
74. Hepatitis C virus: systematics, characteristics, antigens. Parenteral hepatitis C: pathogenesis, immunity, etiologic diagnostics and therapy, prevention. Diagnostic significance of detection of HCV RNA, antibodies IgG and IgM against core-Ag, NS-proteins. Hepatitis G virus: characteristics, etiologic diagnostics, prevention.
75. Retroviruses. Human immunodeficiency virus (HIV). HIV infection: pathogenesis, immunity, etiologic diagnostics, principles of therapy, prophylaxis. AIDS-related illnesses. HIV infection in Belarus and in the World. Prophylaxis of professional HIV infection in medical personal.
76. DNA viruses: classification. Smallpox: specific prevention, eradication. Adenoviruses: classification, characteristics. Adenoviral infections: pathogenesis, immunity, etiologic diagnostics. Human bocavirus: characteristics, role in pathology.
77. Herpesviruses: classification, characteristics. Human disease caused by the herpes simplex viruses: pathogenesis, immunity.
78. Varicella and herpes zoster: etiology, pathogenesis, prevention. The role of herpesvirus types 4–8 in human pathology.
79. Oncogenic DNA and RNA viruses. Viral and cellular oncogenes. Mechanisms of viral oncogenesis. Changes in the cells in the process of transformation. Papillomaviruses: characteristics, role in human pathology. Prevention of human papillomavirus infections.
80. Bacterial viruses (phages): properties, classification. Interaction of virulent and temperate phages with susceptible bacteria. Temperate phages. Lysogeny. Practical use of bacteriophages: phage typing, phage therapy and prophylaxis.

Signature of the tutor _____ Date ____/____/2024

Class № 16 (33). CLINICAL MICROBIOLOGY. MICROBIOLOGICAL DIAGNOSTICS OF SEPSIS AND PURULENT INFECTIONS OF THE SKIN

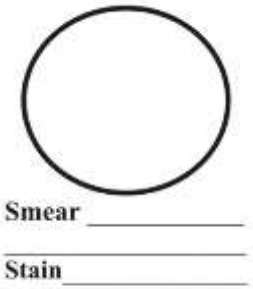
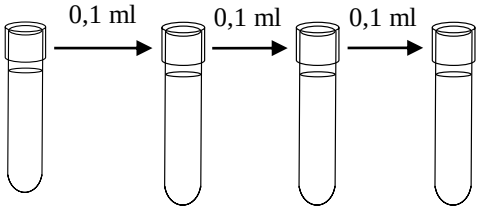
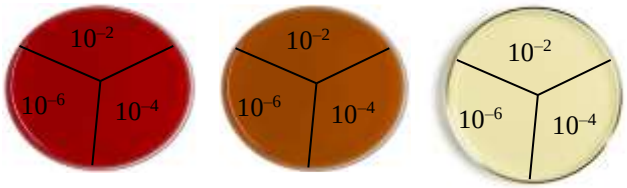
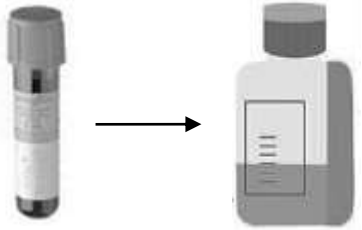
List of questions to study:

Clinical Microbiology: definition, objectives. Opportunistic microbes (OPM). Epidemiology, pathogenesis, diagnosis of diseases caused by UPM.

Clinical forms and the etiology of septic infections of the skin and subcutaneous tissue. Methods of microbiological diagnostics. Bacteriological method. Material for the research (pus, exudate), rules and methods of sampling. Criteria for assessment of the etiological significance of isolated microorganisms. Susceptibility to antibiotics.

Bacteremia. Sepsis. Pyosepticemia. Etiology, definitions. Methods of microbiological diagnosis of sepsis. Bacteriological method. Rules and methods of blood collection for the research, peculiarities of pathogen isolation and results interpretation Susceptibility to antibiotics testing.

Laboratory work

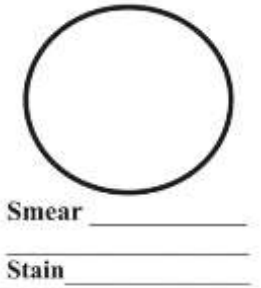
Laboratory exerscises	**Laboratory report**
1. Independent work "Research of the sample from the burnt wound": a) Make serial dilution of the sample 1:100; 1:10000; 1:1000000). b) Streak respective sectors on nutrient media. 2. Research of the blood sample from the patient with suspected sepsis.  	**Exsudate sample research (Ist step):**  Sample — 9,9 ml Saline solution Streak respective sector with 0.05 ml (1 drop):  Blood agar — Levin media — Nutrient agar with furaginum **Blood sample research (Ist step):**  Blood, 10 ml — Biphasic (enrichment) media, 37 °C

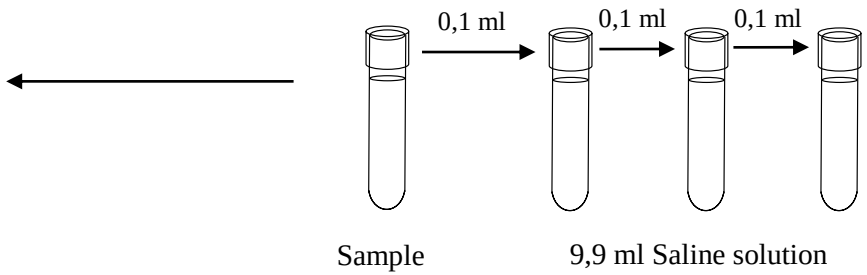
3. Research of the sample from the bronchi washings:

a) Prepare the slide from the material, Gram staining.

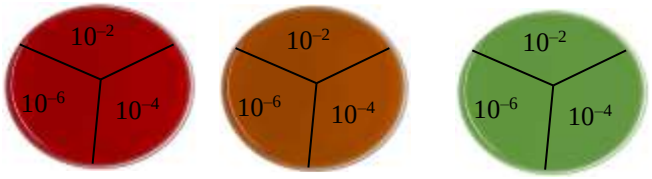
b) Perform quantitative seeding of the material on selective media.

Bronchi washings sample research (Ist step):





Streak respective sector with 0.05 ml (1 drop)



Blood agar | Levin media | Nutrient agar with lactose and bromothymol blue

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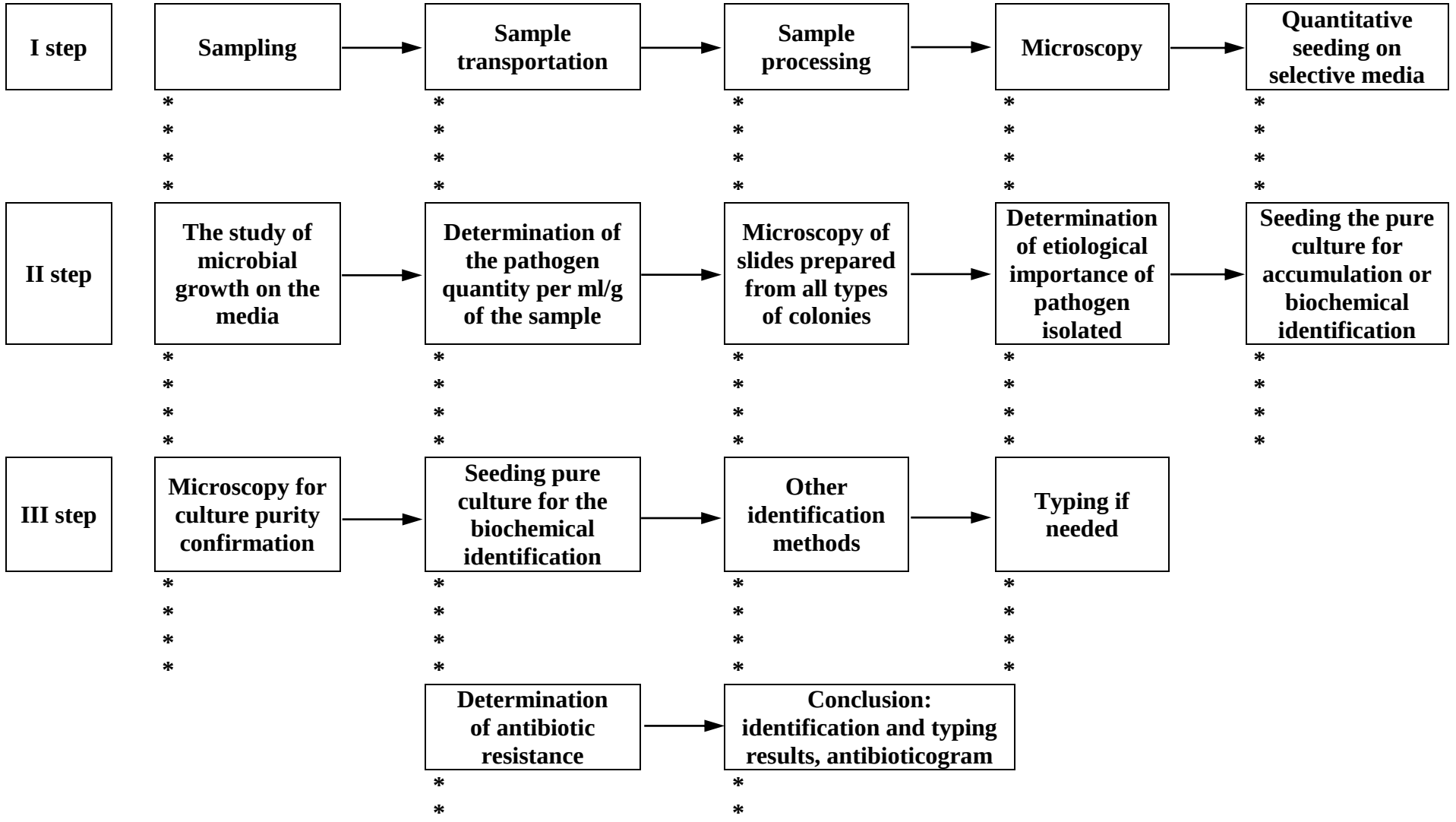
Criteria of etiological importance off opportunistic pathogens:

1.
2.
3.
4.
5.
6.
7.
8.
9.
10.

Etiology (main pathogens) of purulent infection of the skin:

1.
2.
3.
4.
5.

The scheme of the microbiological diagnostics of purulent-septic infections:



Class № 17 (34). CLINICAL MICROBIOLOGY. MICROBIOLOGICAL DIAGNOSTICS OF PURULENT INFECTIONS OF URINARY TRACT. HOSPITAL-ACQUIRED INFECTION

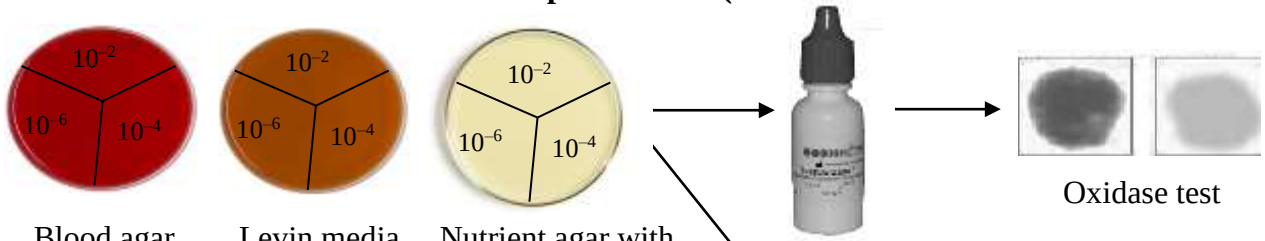
The list of questions to study:

Clinical forms and etiology of septic-purulent (opportunistic) infections of the bronchi and lungs. Methods of microbiological diagnostics. Material for the research, rules and methods of sampling. Bacteriological method. Criteria for assessing the etiological role of isolated bacteria. Susceptibility to antibiotics.

Etiology and clinical forms of septic-purulent (opportunistic) infections of the urogenital tract. Methods of microbiological diagnostics. Material for the study, rules and methods of sampling. Urine culture. Criteria for assessing the etiological role of isolated microbes. Susceptibility to antibiotics. Antibioticogramm.

Nosocomial infections. Pathogens. Principles of microbiological diagnosis. Prevention.

Laboratory work

Laboratory exerscises	**Laboratory report**
1. Independent work «Research of the sample from the burnt wound».	**Exsudate sample research (2nd step):**  Blood agar Levin media Nutrient agar with Furaginum *p*-Phenylenediamine (PPD) Oxidase test Colonies characteristics: _____ _____ _____ _____ Calculation of bacteria quality per ml/g of the sample: **N (CFU/ml) = n × 20 × 10^x,** n — colonies quantity in respective sector; 20 — conversion factor for 1 ml; 10^x — the degree of the sample dilution. N = _____ CFU/ml **Smear** _____ _____ **Stain**_____ **Conclusion** _____

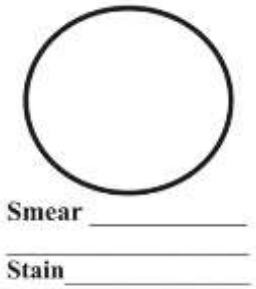
2. Research of the blood sample from the patient with suspected sepsis.

3. Research of the sample from the bronchi washings.

Demonstration.

1. *P. aeruginosa* growth on nutrient medium with furaginum (quantitative inoculation).
2. Klebsiella growth on medium with lactose and bromothimol blue (quantitative inoculation).

Research of the blood sample (2nd step):

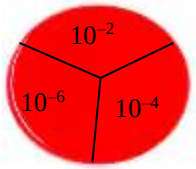


Blood culture

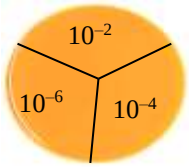
Blood agar

Yolk-salt agar

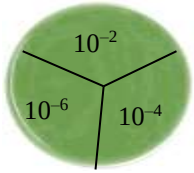
Bronchi washings sample research (2nd step):



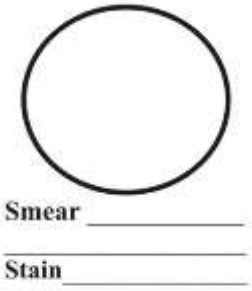
Blood agar



Levin media



Nutrient agar with lactose and bromothymol blue



Colonies characteristics:

_____
_____
_____
_____

Russell medium

Calculation of bacteria quality per ml/g of the sample:

N (CFU/ml) = n × 20 × 10^x,

n — colonies quantity in respective sector;

20 — conversion factor for 1 ml;

10^x — the degree of the sample dilution.

N = _____ CFU/ml **Conclusion** _____

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Etiology (main pathogens) of respiratory septic-purulent diseases:

1.
2.
3.
4.
5.

Etiology (main pathogens) of urogenital septic-purulent diseases:

1.
2.
3.
4.
5.

Hospital acquired infections (HAI, nosocomial infections) — any clinically recognizable infection contracted by patient due to residence or receiving various types of inpatient and outpatient medical care, the delivery of emergency medical services both in health care organizations and at home, as well as infectious disease contracted by medical staff as a result of professional activity, regardless of time of symptoms onset.

Nosocomial infections should be distinguished (introduced) from cases of infectious diseases registered in the delivery of health care in inpatient, outpatient medical institutions, or at home. Their main features are: the absence of a causal connection with the performance of therapeutic and diagnostic procedures and manipulations; acquisition of infection within the minimum incubation period before seeking medical help.

Etiology (main pathogens) of nosocomial infections:

1.
2.
3.
4.
5.

CLASSIFICATION of HAI

HAI etiology includes bacteria; viruses; fungi; protozoa and metazoa.

By source of infection HAI can be exogenous; endogenous and auto-infection.

Depending on the profile of medical care nosocomial infections are divided into: surgical infection, obstetric infections; neonatal infections; other infections.

Depending on the entrance gate and localization of infection nosocomial infections are divided into: surgical wound infections; burn wound infection; infections of skin and soft tissue; primary bloodstream infections; sepsis; cardiovascular system infection; bone and joint infections; eye infection; ear infections; infection of the nose, throat, mouth and upper respiratory tract; lower respiratory tract infections; pneumonia; infections of the central nervous system; urinary tract infections; infections of the reproductive system; infections of the gastrointestinal tract.

Depending on the type of pathogen nosocomial infections are divided into: caused by obligate pathogens and opportunistic pathogens.

Depending on the spread in the organism HAI can be divided into: localized; generalized and systemic infections.

Depending on the course character nosocomial infections are divided into: acute; subacute and chronic.

By severity nosocomial infections are divided into: pathogen caring; mild; moderate and severe form.

Depending on the mechanisms, ways and factors of transmission of nosocomial infections are divided into: aerosol; contact (direct and indirect); parenteral; fecal-oral (food and water).

Class № 18 (35). MICROBIOLOGICAL DIAGNOSTICS OF FUNGAL AND PROTOZOAN INFECTIONS

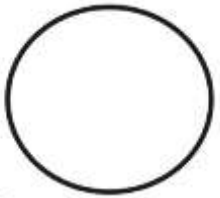
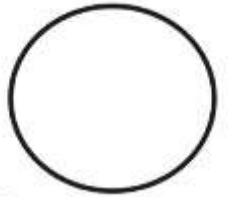
The list of questions to study:

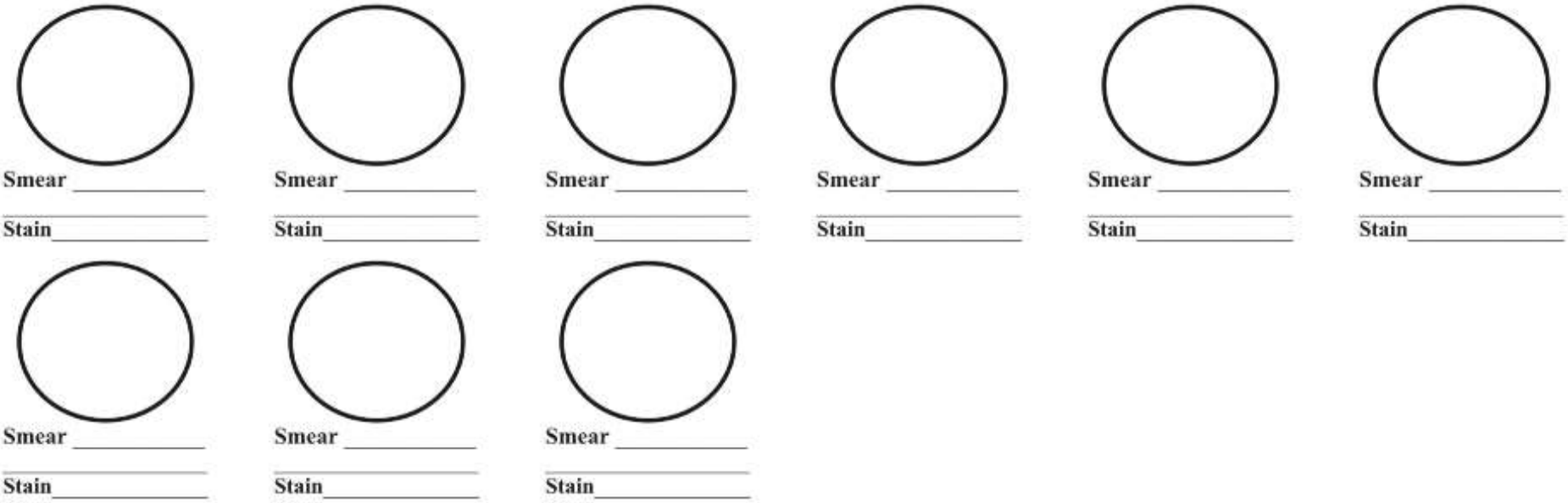
General characteristics and classification of protozoa. Pathogenic representatives. Laboratory diagnosis of malaria, toxoplasmosis, amebiasis, giardiasis, trichomoniasis.

The causative agent of cryptosporidiosis.

Classification and general characteristics of fungi. Pathogens of ringworm, keratomycosis, deep mycoses. Candidiasis and conditions which promote its development. General principles of fungal infections diagnostics. Pathogen of pneumocystosis.

Laboratory work

Laboratory exerscises	Laboratory report
1. Research of the blood sample from the patient with suspected sepsis.	**Blood sample research (3rd step):**  Blood agar  YSA   Smear _____ _____ Stain_____ **Coag** Stabilized rabbit plasm: 37 °C — 2, 4, 24 h Colonies characteristics: _____ _____ _____ **Conclusion:** _____
2. Research of the sample from the bronchi washings. **Demonstration:** 1. Pathogenic protozoa. 2. *Candida*, Gram stain. 3. Candida growth on Saburo medium.	**Research of bronchial washings sample (3rd step):** Russell medium  Fermentation: Lactose_____ Glucose_____  Smear _____ _____ Stain_____ **Conclusion** _____



Signature of the tutor _____ Date ____/____/2024

DIAGNOSTICS OF MYCOSIS:

Microscopic method. High diagnostic value of the method caused by significant differences in fungal morphology, simplicity and speed of the research. The result can be obtained within 1–2 hours. Microscopy can be conducted in native preparations without staining. For visualization of the pathogen in the biological material which is poorly transparent (hair, skin, nails, etc.) it should be processed with 10–20 % alkaline (KOH), which dissolves keratin and has no effect on the morphology of the fungal cells. Fixed smears may be stained by Gram (fungi are Gram-positive), Romanovsky–Giemsa, special techniques. Dimorphic fungi in biological material are in the form of yeast. Microscopy of histological preparations is also possible.

Culture (mycological) method. Most pathogenic fungi are mesophiles (20–45 °C) and not demanding for the nutrient medium. Optimal pH ranges from 4.0 to 6.5. Growing time depends on the kind of fungus and can be from several weeks to 2–3 days. The most frequently used medium is Saburo agar (peptone agar with glucose or maltose). The acidity of the medium and high carbohydrate content inhibits the growth of bacteria. Dimorphic fungi (pathogens caused subcutaneous and deep mycoses) grow in the mycelial form at 20–25 °C. The identification of a pure culture is carried out by morphological and biochemical characteristics.

Serological method. Immunofluorescence is sensitive, specific and rapid method based on the identification of fungal Ag in biological materials.

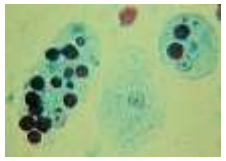
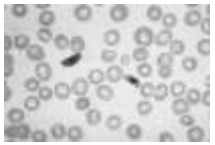
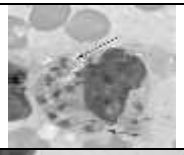
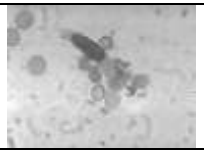
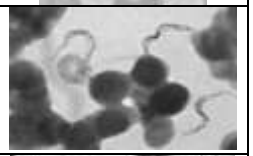
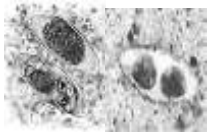
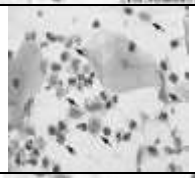
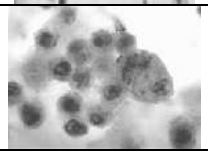
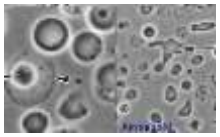
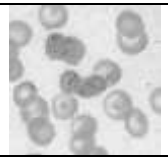
PHAT, latex agglutination, PT, CFT, ELISA are used to detect fungal antigens and antibodies in blood, CSF, urine. Serological reactions not always highly specific, but produce results earlier than culture method.

Allergic method. Skin tests are performed with fungal allergens (eg.- Candide). Method is not very specific because of the group antigens presence.

Biological method. Bioassays in laboratory animals allow us to estimate the virulence of the pathogen, get in tissue culture of the fungus (usually in a form of yeast).

Molecular genetic methods. PCR and molecular hybridization are used. Among advantages — very high sensitivity and specificity, relative safety and short time needed for results.

Protozoa belong to the domen — *EUKARYA*, kingdom — *ANIMALIA*, subkingdom — *PROTOZOA*, which includes 7 types
Four types of medical importance are showed in the table:

Taxons	Representatives	Disease	Morphology		Representatives	Disease	Morphology		Representatives	Disease
TYPE *SARCOMASTIGOPHORA* subtype *Sarcodina*	**AMOEBAE** *Entamoeba histolytica Naegleria, acanthamoeba, hartmanella*	Amebiasis Amoebic meningoencephalitis, keratitis			**PLASMODIUM MALARIA:** *Plasmodium vivax Plasmodium ovale Plasmodium malariae Plasmodium falciparum*	Malaria		**TYPE – CILIOPHORA** class *Kinetofragminophorea*	**BALANTIDIUM** *Balantidium coli*	Balantidiasis
subtype *Mastigophora*	**LEISHMANIA** *Leishmania species*	Leishmaniasis			**TOXOPLASMA:** *Toxoplasma gondii*	Toxoplasmosis				
	TRYPANOSOMES *Tripanosoma gambiense, Tripanosoma rodesiense Tripanosoma cruzi*	African trypanosomiasis (sleeping disease) Chagas disease (American trypanosomiasis)		**TYPE – *APICOMPLEXA*** class – *Sporozoa*	**SARCOCYST:** *Sarcocystis species*	Sarcocystosis				
	GIARDIA: *Lamblia intestinalis (Giardia lamblia)*	Diarrhea, malabsorption syndrome			**ISOSPORA:** *Isospora species*	Diarrhea				
	TRICHOMONAS *Trichomonas vaginalis*	Trichomonas vaginalis vaginitis, urethritis, prostatitis			**CRYPTOSPORIDIUM:** *Cryptospodium species*	Diarrhea				
TYPE – MICROSPORA class *Microsporea*	**MICROSPORIDIA** *Encephalitozoon species Enterocytozoon species*	Microsporidiasis			**CYCLOSPORA:** *Cyclospora cauetanensis*	Diarrhea				
	BLASTOCYST: *Blastocystis hominis*	Blastocystosis			**BABESIA:** *Babesia species*	Babesiosis				

MICROBIOLOGICAL DIAGNOSTICS OF PROTOZOAN INVASIONS

AMEBIASIS **Microscopic method**. Materials: samples of faeces or exudates from abscesses. Smears are stained with iodine solution or hematoxylin. Tissue forms with phagocytized erythrocytes or quad cysts. can be identified. In native specimens characteristic motile vegetative forms can be noted. IF may be used for the identification of pathogen. **Serological method**: PHA test, ELISA, CFT, and other tests may be used. The highest antibody titer can be detected in extraintestinal amebiasis. Some non-pathogenic amoeba are morphologically identical to Entamoeba histolytica. The differentiation is based on the enzymatic, immunological and molecular genetic analysis.	**LEISHMANIASIS** **Microscopic method**. Materials: skin lesions (bumps, ulcers), bone marrow. Smears are stained by Romanovsky–Giemsa method. The detection of amastigote (nucleus and kinetoplasts are of red-purple color and cytoplasm is bluish) is of importance. IFT is also used. **Cultural method**. Leishmania can be cultured on blood agar. **Biological method**. Infection of mice or hamsters is possible. **Serological method**. Specific antibodies may be detected by CFT, passive hemagglutination or ELISA. **Allergic method**. Skin test with leishmania Ags may be used.
TRYPANOSOMES **Microscopic method**. Materials: samples of blood, punctate from cervical lymphatic nodes, cerebrospinal fluid. Smears are stained by Romanovsky–Giemsa method. **Cultural method**. Trypanosomes can be cultured on a nutrient medium with blood as well as in white mice or rats. **Serological method**. The determination of specific IgM by ELISA is used.	**GIARDIASIS** **Microscopic method**. Materials: feces, duodenal secretion. In smears cysts or vegetative forms, can be detected. Iodine staining is usually used. IFT is also applicable. **Cultural method**. Giardia can be cultured nutrient media. **Serological method**. Specific antibody titers are higher in symptomatic giardiasis.
TRICHOMONIASIS **Microscopic method**. Materials: samples from urethral discharge, prostatic secretions or urine sediment are studied. Smears are stained by Romanovsky–Giemsa (trophozoite nucleus is violet-ruby, cytoplasm — blue and blefaroplast, flagella and aksostil — pink-red), methylene blue. IF is also used. **Cultural method**. In chronic trichomoniasis pathogen can be cultured on nutrient media with protein. The method gives good results when confirmation of convalescence is needed.	**BALANTIDIASIS** **Microscopic method**. Microscopy of smears from feces under low magnification allows to reveal large motile balantidiums. **Cultural method**. Possible, but rarely use.
TOXOPLASMOSIS **Microscopic method**. Materials: biopsy, samples of body fluids (blood, cerebrospinal fluid, lymph node puncture, etc.). Smears are stained by Romanovsky–Giemsa method. Toxoplasma Ags may be detected by IF test. **Cultural method**. Cultivation of Toxoplasma is possible in cell cultures and chicken embryo. **Serological method**. Detection of specific IgM indicates the early stages of the disease. IgG peaks at 4–8 week of disease. ELISA is widely used. **Biological method**. Mice are infected in the abdominal cavity or intracranially. They usually succumb 7–10 days after infection. The pathogen is identified microscopically or by serological method.	**MALARIA** **Microscopic method**. Smears of blood are stained by Romanovsky–Giemsa method. Various forms of pathogen can be identified (red nucleus, blue cytoplasm). Differentiation of species is carried out by morphological features of parasites and parasitized erythrocytes. **Serological method**. Specific antibodies are detected by ELISA. IFT is applicable for diagnostics. **Molecular genetic method**. PCR.

Appendix 1

Classification of microorganisms according to Bergey (abbreviated) — PROKARYOTES, DOMAIN (Domain) — BACTERIA

PHYLUM	CLASS	ORDER	FAMILY	GENUS	SPECIES
Proteo-bacteria	*Alphaproteo-bacteria*	*Rickettsiales*	*Rickettsiaceae*	*Rickettsia*	*R. prowazekii, R. typhi, R. felis, R. rickettsii, R. conorii, R. australis, R. akari, R. sibirica, R. japonica, R. honei*
				Orientia	*O. tsutsugamushi, O. chuto, O. chiloensis*
			Anaplasmataceae	*Ehrlichia*	*E. chaffeensis, E. canis, E. ewingii et al.*
				Anaplasma	*A. phagocytophilum, A. bovis, A. capra, A. platys, A. ovis*
				Neoehrlichia	*Neoehrlichia mikurensis*
				Neorickettsia	*N. sennetsu*
		Rhizobiales	*Bartonellaceae*	*Bartonella*	*B. quintana, B. henselae, B. bacilliformis, B. chlaridgeae, B. elizabethae*
			Brucellaceae	*Brucella*	*B. melitensis, B. abortus, B. suis et al.*
	Betaproteo-bacteria	*Burkholderiales*	*Burkholderiaceae*	*Burkholderia*	*B. mallei, B. pseudomallei, B. cepacia et al.*
			Alcaligenaceae	*Alcaligenes*	*A. faecales et al.*
				Bordetella	*B. pertussis, B. parapertussis, B. bronchiseptica et al.*
		Neisseriales	*Neisseriaceae*	*Neisseria*	*N. gonorrhoeae, N. meningitidis, N. sicca, N. subflava et al.*
				Eikenella	*E. corrodens*
				Kingella	*K. kingae et al.*
				Simonsiella	*Simonsiella muelleri*
		Nitrozomonadales	*Spirillaceae*	*Spirillum*	*S. winogradskyi et al.*
	Gamma-proteobacteria	*Thiotrichales*	*Francisellaceae*	*Francisella*	*F. tularensis*
		Legionellales	*Legionellaceae*	*Legionella*	*L. pneumophila et al.*
			Coxiellaceae	*Coxiella*	*C. burnetii*
		Pseudomonadales	*Pseudomonadaceae*	*Pseudomonas*	*P. aeruginosa et al.*
			Moraxellaceae	*Moraxella)*	*M. lacunata, M. catarralis*
				Acinetobacter	*A. calcoaceticus, A. baumannii et al.*
		Vibrionales	*Vibrionaceae*	*Vibrio*	*V. cholerae (biovars: cholerae, eltor), V. parahaemolyticus, V. vulnificus et al.*
		Aeromonadales	*Aeromonadaceae*	*Aeromonas*	*A. hydrophilia*
		Enterobacteriales		*Plesiomonas*	*P. shigelloides*
			Erwiniaceae	*Erwinia*	*E. amylovora et al.*
			Hafniaceae	*Hafnia*	*H. alvei*
				Edwardsiella	*E. tarda et al.*
			Morganellaceae	*Morganella*	*M. morganii*
				Proteus	*P. vulgaris, P. mirabilis, et al.*
				Providencia	*P. alcallifaciens et al.*
			Yersiniaceae	*Yersinia*	*Y. pestis, Y. enterocolitica, Y. pseudotuberculosis et al.*
				Serratia	*S. marcescens et al.*

PHYLUM	CLASS	ORDER	FAMILY	GENUS	SPECIES
			Enterobacteriaceae	*Enterobacter*	*E. cloacae*
				Citrobacter	*C. freundii, C. amalonaticus, C. koseri et al.*
				Escherichia	*E. coli, E. fergusonii, E. germannii, E. albertii*
				Klebsiella	*K. pneumoniae (subsp: ozaenae, rhinoscleromae, pneumoniae), K. oxytoca, K. planticola, K. terrigena, K. granulomatis*
				Salmonella	*S. enterica, S. bongori. Species S. enterica consict from 6 subsp.: arizonae, diarizonae, enterica, houtenae, indica, salamae). Serotypes: S. Typhi, S. paratyphi A, S. schottmuelleri, S. enteritidis, S. typhimurium, S. choleraesuis et al.*
				Shigella	*S. dysenteriae, S. flexneri, S. boydii, S. sonnei*
		Pasteurellales	*Pasteurellaceae*	*Haemophilus*	*H. influenzae, H. ducreyi et al.*
				Pasteurella	*P. stomatis*
	Epsilon-proteobacteria	*Campylobacteriales*	*Campylobacteriaceae*	*Campylobacter*	*C. jejuni, C. fetus, C. coli et al. C. sputorum*
			Helicobacteriaceae	*Helicobacter*	*H. pylori, H. heilmanii et al.*
				Wolinella	*W. succinogenes*
Firmicutes	*Negativicutes*	*Selenomonadales*	*Selenomonadaceae*	*Selenomonas*	*S. sputigena*
				Centipeda	*C. periodontii*
				Mitsuokella	*M. multacida*
		Veillonellales	*Veillonellaceae*	*Veillonella*	*V. parvula et al.*
	Clostridia	*Eubacteriales*	*Clostridiaceae*	*Clostridium*	*C. botulinum, C. perfringens, C. novyi, C. histolyticum, C. septicum, C. tetani et al.*
				Hathewaya	*H. histolytica*
				Sarcina	*S. ventriculi*
			Peptostreptococcaceae	*Peptostreptococcus*	*P. anaerobius et a.l*
				Clostridioides	*C. difficile*
			Peptococcaceae	*Peptococcus*	*P. niger, P. simiae*
			Mogibacteriaceae	*Mogibacterium*	*Mogibacterium timidum*
			Lachnospiraceae	*Lachnoanaerobaculum*	*Lachnoanaerobaculum saburreum*
	Bacilli	*Caryophanales*	*Bacillaceae*	*Bacillus*	*B. subtilis, B. anthracis, B. cereus et al.*
			Listeriaceae	*Listeria*	*L. monocytogenes et al.*
			Staphylococcaceae	*Staphylococcus*	*S. aureus, S. epidermidis, S. saprophyticus et al.*
		Lactobacillales	*Lactobacillaceae*	*Lactobacillus*	*L. fermentum et al.*
				Lacticaseibacillus	*L. caseii*
			Enterococcaceae	*Enterococcus*	*E. faecalis, E. faecium et al.*
			Leuconostoccaceae	*Leuconostoc*	*L. mesenteroides*
			Streptococcaceae	*Streptococcus*	*S. pyogenes, S. pneumoniae, S. agalactiae, S. anginosus, S. bovis, S. mutans, S. mitis, S. salivarius, S. sanguis, S. milleri et al.*
				Lactococcus	*L. lactis et al.*

PHYLUM	CLASS	ORDER	FAMILY	GENUS	SPECIES
Actinobacteria	*Actinobacteria*	*Actinomycetales*	*Actinomycetaceae*	*Actinomyces*	*A. israelii, A. naeslundii, A. viscosus, A. odontolyticus, A. pyogenes*
				Mobiluncus	*M. curtisii*
		Bifidobacteriales	*Bifidobacteriaceae*	*Bifidobacterium*	*B. bifidum et al.*
				Gardnerella	*G. vaginalis*
		Micrococcales	*Micrococcaceae*	*Micrococcus*	*M. lysodeicticum, M. luteus et al.*
				Rothia	*Rothia dentocariosa*
		Mycobacteriales	*Mycobacteriaceae*	*Mycobacterium*	*M. tuberculosis, M. bovis, M. africanum, M. leprae, M. kasasii, M. avium, M. ulcerans, M. fortuitum, M. smegmatis et al.*
			Corynebacteriaceae	*Corynebacterium*	*C. diphtheriae, C. ulcerans, C. urealyticum, C. xerosis et al.*
			Nocardiaceae	*Nocardia*	*N. asteroides, N. farcinica et al.*
			Nocardiaceae	*Rhodococcus*	*R. rhodochrous*
		Propionibacteriales	*Propionibacteriaceae*	*Propionibacterium*	*P. acnes, P. propionicus et al.*
		Streptomycetales	*Streptomycetaceae*	*Streptomyces*	*Streptomyces albus*
Bacteroidetes	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	*B. fragilis, B. gingivalis et al.*
			Porphyromonadaceae	*Porphyromonas*	*P. gingivalis, P. endodontales et al.*
			Prevotellaceae	*Prevotella*	*P. melaninogenica, P. dentalis et al.*
	Flavobacteriia	*Flavobacteriales*	*Flavobacteriaceae*	*Flavobacterium*	*F. brevivitae et al.*
				Capnocytophaga	*Capnocytophaga gingivalis*
		Flavobacteriales	*Weeksellaceae*	*Elizabethkingia*	*Elizabethkingia meningoseptica*
Fusobacteria	*Fusobacteria*	*Fusobacteriales*	*Fusobacteriaceae*	*Fusobacterium*	*F. nucleatum, F. necroforum, F. ulcerans*
			Leptotrichiaceae	*Leptotrichia*	*L. buccalis et al.*
			Leptotrichiaceae	*Streptobacillus*	*S. moniliformis*
Chlamydiae	*Chlamydiae*	*Chlamydiales*	*Chlamydiaceae*	*Chlamydia*	*C. trachomatis, C. psittaci, C. pneumoniae*
Spirochaetes	*Spirochaetes*	*Spirochaetales*	*Treponemataceae*	*Treponema*	*T. pallidum, T. pertenue, T. denticola, T. minutum, T. refringens,T. medium*
			Borreliaceae	*Borrelia*	*B. recurrentis, B. burgdorferi, B. duttoni, B. persica et al.*
		Leptospirales	*Leptospiraceae*	*Leptospira*	*L. interrogans, L. biflexa*
Tenericutes	*Mollicutes*	*Mycoplasmatales*	*Mycoplasmataceae*	*Mycoplasma*	*M. mycoides*
				Ureaplasma	*U. urealiticum et al.*
		Mycoplasmoidales	*Metamycoplasmataceae*	*Metamycoplasma*	*M. hominis, M. orale, M. salivarum, M. artritidis*
				Mycoplasmopsis	*M. fermentans*
		Mycoplasmoidales	*Mycoplasmoidaceae*	*Mycoplasmoides*	*M. pneumoniae*
		Acholeplasmatales	*Acholeplasmataceae*	*Acholeplasma*	*A. laidlawii*

CLASSIFICATION OF VIRUSES
(Updates approved during EC 51, Berlin, Germany, July 2019; Email ratification March 2020)

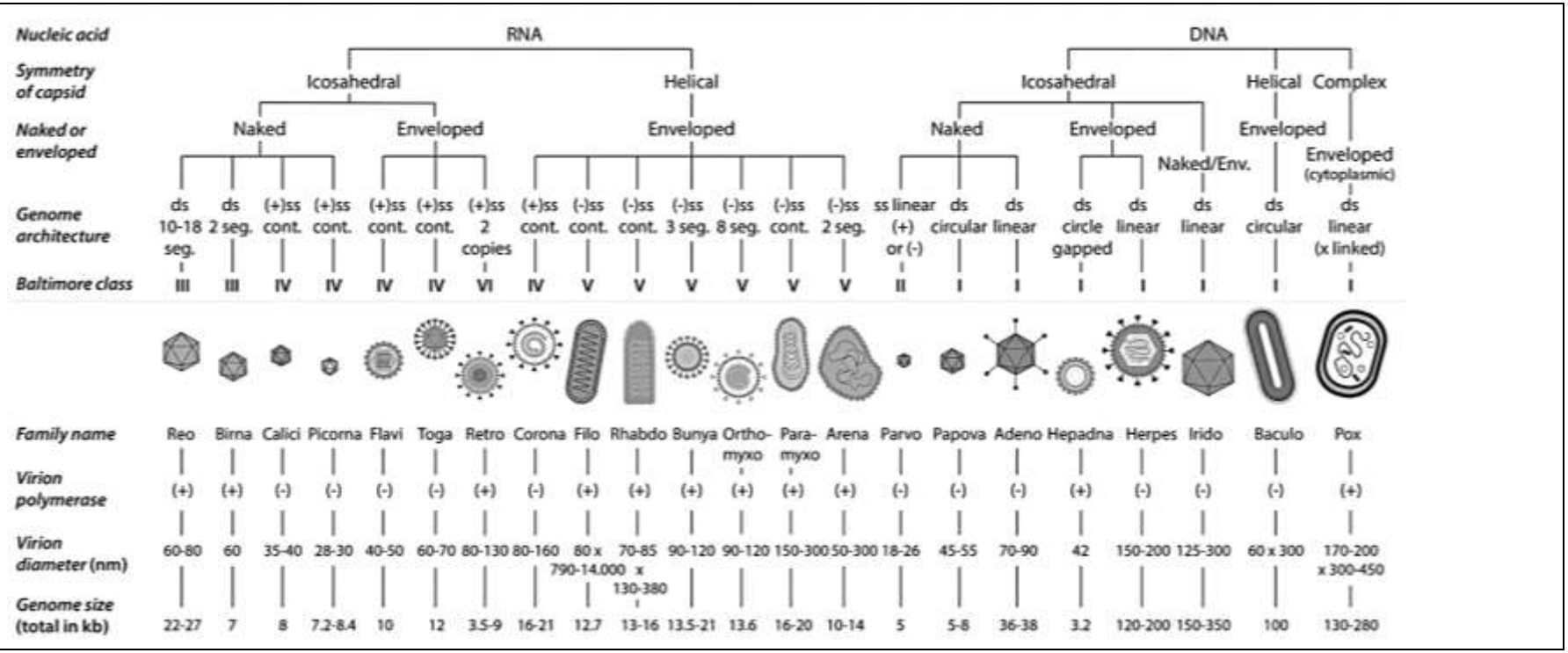
REALM	KINGDOM	PHYLUM	CLASS	ORDER	FAMILY	SUBFAMILY	GENUS	SPECIES	GENOME
Duplodnaviria	*Heunggongvirae*	*Peploviricota*	*Herviviricetes*	*Herpesvirales*	*Herpesviridae*	*Alphaherpesvirinae*	*Simplexvirus*	*Human alphaherpesvirus 1, 2*	dsDNA
						Alphaherpesvirinae	*Varicellovirus*	*Human alphaherpesvirus 3*	dsDNA
						Betaherpesvirinae	*Cytomegalovirus*	*Human betaherpesvirus 5*	dsDNA
						Betaherpesvirinae	*Roseolovirus*	*Human betaherpesvirus 6A, 6B, 7*	dsDNA
						Gammaherpesvirinae	*Lymphocryptovirus*	*Human gammaherpesvirus 4*	dsDNA
						Gammaherpesvirinae	*Rhadinovirus*	*Human gammaherpesvirus 8*	dsDNA
Monodnaviria	*Shotokuvirae*	*Cossaviricota*	*Papovaviricetes*	*Sepolyvirales*	*Polyomaviridae*		*Alphapolyomavirus*	*Human polyomavirus 5, 8, 9, 13, 14*	dsDNA
							Betapolyomavirus	*Human polyomavirus 1–4*	dsDNA
							Deltapolyomavirus	*Human polyomavirus 6, 7, 10, 11*	dsDNA
				Zurhausenvirales	*Papillomaviridae*	*Firstpapillomavirinae*	*Alphapapillomavirus*	*Alphapapillomavirus 1*	dsDNA
							Betapapillomavirus	*Betapapillomavirus 1*	dsDNA
							Gammapapillomavirus	*Gammapapillomavirus 1*	dsDNA
							Mupapillomavirus	*Mupapillomavirus 1*	dsDNA
							Nupapillomavirus	*Nupapillomavirus 1*	dsDNA
			Quintoviricetes	*Piccovirales*	*Parvoviridae*	*Parvovirinae*	*Bocaparvovirus*	*Pinniped bocaparvovirus 1*	ssDNA
							Dependoparvovirus	*Adeno-associated dependoparvovirus A, B*	ssDNA
							Erythroparvovirus	*Primate erythroparvovirus 1*	ssDNA
		Cressdnaviricota	*Arfiviricetes*	*Cirlivirales*	*Circoviridae*		*Cyclovirus*	*Human associated cyclovirus 8 (1–12)*	ssDNA
				Cremevirales	*Smacoviridae*		*Huchismacovirus*	*Human associated huchismacovirus 1, 2, 3*	ssDNA
							Porprismacovirus	*Human associated porprismacovirus 1, 2*	ssDNA
			Repensiviricetes	*Geplafuvirales*	*Genomoviridae*		*Gemykibivirus*	*Human associated gemykibivirus 1–5*	ssDNA
							Gemyvongvirus	*Human associated gemyvongvirus 1*	ssDNA
Riboviria	*Orthornavirae*	*Duplornaviricota*	*Resentoviricetes*	*Reovirales*	*Reoviridae*	*Sedoreovirinae*	*Rotavirus*	*Rotavirus A (A–J)*	dsRNA
						Spinareovirinae	*Coltivirus*	*Colorado tick fever coltivirus*	dsRNA
		Kitrinoviricota	*Alsuviricetes*	*Hepelivirales*	*Hepeviridae*		*Orthohepevirus*	*Orthohepevirus A*	ssRNA(+)
					Matonaviridae		*Rubivirus*	*Rubella virus*	ssRNA(+)
				Martellivirales	*Togaviridae*		*Alphavirus*	*Chikungunya virus*	ssRNA(+)
								Eastern equine encephalitis virus	ssRNA(+)
								Onyong-nyong virus	ssRNA(+)

REALM	KINGDOM	PHYLUM	CLASS	ORDER	FAMILY	SUBFAMILY	GENUS	SPECIES	GENOME
								Rio Negro virus	ssRNA(+)
								Ross River virus	ssRNA(+)
								Semliki Forest virus	ssRNA(+)
								Sindbis virus	ssRNA(+)
Riboviria	*Orthornavirae*	*Kitrinoviricota*	*Alsuviricetes*	*Martellivirales*	*Togaviridae*		*Alphavirus*	*Venezuelan equine encephalitis virus*	ssRNA(+)
								Western equine encephalitis virus	ssRNA(+)
			Flasuviricetes	*Amarillovirales*	*Flaviviridae*		*Flavivirus*	*Dengue virus*	ssRNA(+)
								Edge Hill virus	ssRNA(+)
								Japanese encephalitis virus	ssRNA(+)
								Murray Valley encephalitis virus	ssRNA(+)
								Omsk hemorrhagic fever virus	ssRNA(+)
								Rio Bravo virus	ssRNA(+)
								Saint Louis encephalitis virus	ssRNA(+)
								Tick-borne encephalitis virus	ssRNA(+)
								West Nile virus	ssRNA(+)
								Yellow fever virus	ssRNA(+)
								Zika virus	ssRNA(+)
							Hepacivirus	*Hepacivirus C*	ssRNA(+)
							Pegivirus	*Pegivirus A*	ssRNA(+)
		Negarnaviricota	*Monjiviricetes*	*Mononegavirales*	*Filoviridae*		*Ebolavirus*	*Zaire, Bombali, Bundibugyo, Reston, Sudan, Tai Forst ebolavirus*	ssRNA(-)
							Marburgvirus	*Marburg marburgvirus*	ssRNA(-)
					Paramyxoviridae	*Orthoparamyxovirinae*	*Henipavirus*	*Hendra henipavirus*	ssRNA(-)
							Henipavirus	*Nipah henipavirus*	ssRNA(-)
							Morbillivirus	*Measles morbillivirus*	ssRNA(-)
							Respirovirus	*Human respirovirus 1, 3*	ssRNA(-)
						Rubulavirinae	*Orthorubulavirus*	*Human orthorubulavirus 2, 4*	ssRNA(-)
								Mumps orthorubulavirus	ssRNA(-)
					Pneumoviridae		*Metapneumovirus*	*Human metapneumovirus*	ssRNA(-)
							Orthopneumovirus	*Human orthopneumovirus*	ssRNA(-)
					Rhabdoviridae		*Ledantevirus*	*Le Dantec ledantevirus*	ssRNA(-)
							Lyssavirus	*Rabies lyssavirus*	ssRNA(-)
							Vesiculovirus	*Indiana vesiculovirus*	ssRNA(-)
			Ellioviricetes	*Bunyavirales*	*Arenaviridae*		*Mammarenavirus*	*Lymphocytic choriomeningitis mammarenavirus*	ssRNA(+/-)
					Hantaviridae	*Mammantavirinae*	*Orthohantavirus*	*Hantaan orthohantavirus*	ssRNA(-)
								Khabarovsk orthohantavirus	ssRNA(-)
					Nairoviridae		*Orthonairovirus*	*Crimean-Congo hemorrhagic fever orthonairovirus*	ssRNA(-)

REALM	KINGDOM	PHYLUM	CLASS	ORDER	FAMILY	SUBFAMILY	GENUS	SPECIES	GENOME
					Peribunyaviridae		*Orthobunyavirus*	*Bunyamwera orthobunyavirus*	ssRNA(-)
								California encephalitis orthobunyavirus	ssRNA(-)
					Phenuiviridae		*Phlebovirus*	*Rift Valley fever phlebovirus*	ssRNA(+/-)
Riboviria	*Orthornavirae*	*Negarnaviricota*	*Ellioviricetes*	*Bunyavirales*	*Phenuiviridae*		*Uukuvirus*	*Uukuniemi uukuvirus*	ssRNA(+/-)
			Insthoviricetes	*Articulavirales*	*Orthomyxoviridae*		*Alphainfluenzavirus*	*Influenza A virus*	ssRNA(-)
							Betainfluenzavirus	*Influenza B virus*	ssRNA(-)
							Gammainfluenzavirus	*Influenza C virus*	ssRNA(-)
							Quaranjavirus	*Quaranfil quaranjavirus*	ssRNA(-)
							Thogotovirus	*Dhori thogotovirus*	ssRNA(-)
		Pisuviricota	*Duplopiviricetes*	*Durnavirales*	*Picobirnaviridae*		*Picobirnavirus*	*Human picobirnavirus*	dsRNA
			Pisoniviricetes	*Nidovirales*	*Coronaviridae*	*Orthocoronavirinae*	*Alphacoronavirus*	*Human coronavirus 229E*	ssRNA(+)
								Human coronavirus NL63	ssRNA(+)
							Betacoronavirus	*Human coronavirus HKU1*	ssRNA(+)
								Severe acute respiratory syndrome-related coronavirus	ssRNA(+)
				Picornavirales	*Picornaviridae*		*Cardiovirus*	*Cardiovirus A*	ssRNA(+)
							Cosavirus	*Cosavirus A*	ssRNA(+)
							Enterovirus	*Enterovirus C*	ssRNA(+)
							Enterovirus	*Rhinovirus A*	ssRNA(+)
							Hepatovirus	*Hepatovirus A*	ssRNA(+)
							Kobuvirus	*Aichivirus A*	ssRNA(+)
							Parechovirus	*Parechovirus A*	ssRNA(+)
			Stelpaviricetes	*Stellavirales*	*Astroviridae*		*Mamastrovirus*	*Mamastrovirus 1*	ssRNA(+)
	Pararnavirae	*Artverviricota*	*Revtraviricetes*	*Blubervirales*	*Hepadnaviridae*		*Orthohepadnavirus*	*Hepatitis B virus*	dsDNA-RT
				Ortervirales	*Retroviridae*	*Orthoretrovirinae*	*Deltaretrovirus*	*Primate T-lymphotropic virus 1, 2, 3*	ssRNA-RT
							Lentivirus	*Human immunodeficiency virus 1, 2*	ssRNA-RT
						Spumaretrovirinae	*Bovispumavirus*	*Bovine foamy virus*	ssRNA-RT
Varidnaviria	*Bamfordvirae*	*Nucleocytoviricota*	*Pokkesviricetes*	*Chitovirales*	*Poxviridae*	*Chordopoxvirinae*	*Molluscipoxvirus*	*Molluscum contagiosum virus*	dsDNA
							Orthopoxvirus	*Vaccinia virus*	dsDNA
								Variola virus	dsDNA
							Parapoxvirus	*Orf virus*	dsDNA
		Preplasmiviricota	*Tectiliviricetes*	*Rowavirales*	*Adenoviridae*		*Mastadenovirus*	*Human mastadenovirus C (A–G)*	dsDNA
					Anelloviridae		*Alphatorquevirus*	*Torque teno virus 1*	ssDNA(-)
							Betatorquevirus	*Torque teno mini virus 1*	ssDNA(-)
							Gammatorquevirus	*Torque teno midi virus 1*	ssDNA(-)
							Deltavirus	*Hepatitis delta virus*	ssRNA(-)

Appendix 3

INFOGRAPHICS «SYSTEMATICS OF VIRUSES»



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ISBN 978-985-21-1693-0