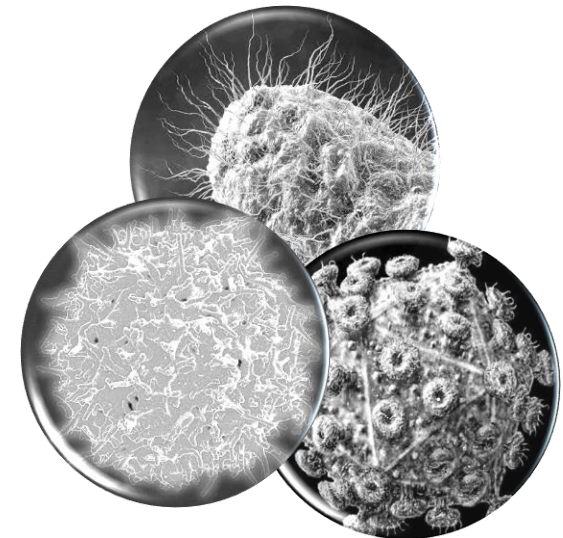


MICROBIOLOGY, VIROLOGY, IMMUNOLOGY

Laboratory workbook

Student _____ group of dental faculty



Minsk BSMU 2022

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

МИКРОБИОЛОГИЯ, ВИРУСОЛОГИЯ, ИММУНОЛОГИЯ

MICROBIOLOGY, VIROLOGY, IMMUNOLOGY

Лабораторный практикум

6-е издание



Минск БГМУ 2022

УДК 579+578+612.017.1(076.5)(075.8)-054.6

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Отражены вопросы общей и частной медицинской микробиологии, вирусологии и иммунологии. Даны алгоритмы, схемы, некоторые справочные сведения, методики выполнения лабораторных работ по дисциплине «Микробиология, вирусология и иммунология». Первое издание вышло в 2017 году.

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GLOSSARY

aerobic — Using oxygen for growth and metabolism.

agar — A gelling agent used in bacterial growth media that allows liquids to become a gel-like solid.

anaerobic — Not requiring any oxygen for growth.

antigen — Part of an organism that is foreign to our bodies and stimulates an immune response.

asexual organisms — Living creatures (usually bacteria) that are neither male nor female, and therefore do not reproduce by exchanging genetic material.

biofilm — A complex community of microorganisms living together and attached to a surface.

capsule — A structure that surrounds or encapsulates many bacteria and may serve to protect them from harsh conditions or to assist with adherence to surfaces.

cariology — The study of cavities.

collagenase — An enzyme produced by some bacteria that breaks down the connective tissue collagen.

colonies — Masses of bacteria that arise from a single cell on solid growth media.

colonization — The act of attaching to and inhabiting a surface.

conjugation — The process of DNA transfer from one bacterial cell to another.

culturing — The act of growing bacteria in a laboratory.

cytokine — Proteins that are made by cells that alter the properties and behavior of other cells.

cytosol — The interior of a cell that contains the cell's inner components, or "guts".

dissemination — The process by which a pathogen is transmitted from one host to another.

DNA fingerprint — A characteristic sequence of nucleic acid bases (A, G, C, T) that is unique to and defines a given bacterial species.

endodontic infections — Infections that occur within the pulp of the tooth.

endoplasmic reticulum — In a eukaryotic cell, the structure on which ribosomes reside.

extracellular — The environment outside of a cell.

flagella — Flexible rope-like structures that help bacteria swim and move in different environments.

genome — The complete DNA material of an organism.

genus — The designation for a group of organisms highly related to each other.

gingivitis — Gum disease.

glucan — A general term for sugar or polysaccharide.

Gram negative — Bacteria that appear pink after the Gram stain procedure due to their thin peptidoglycan cell wall.

Gram positive — Bacteria that appear purple after the Gram stain procedure due to their thick peptidoglycan cell wall.

growth media — The food and nutrients on which bacteria grow in the laboratory.

Hemagglutination — The clumping together of red blood cells.

hemolysin — A bacterial toxin that is able to destroy red blood cells.

hemolysis — The act of lysing, or killing, a red blood cell.

host — The organism, usually a human, that a pathogen lives in or on.

immuno-compromised — A state where an individual's immune system is weakened, usually by an infection or disease.

incubate — To allow microorganisms to grow in the lab under favorable growth conditions.

inflammation — The process whereby immune cells and chemicals accumulate at the site of infection and result in swelling and redness.

inner membrane — The phospholipid-containing structure around a Gram-negative cell.

invasin — A protein that a pathogen uses to enter into a host cell.

lectin — A protein that binds to a specific type of sugar.

leukotoxin — A bacterial toxin that is able to destroy white blood cells.

lipid A — The innermost portion of lipopolysaccharide (LPS) that anchors it into the outer membrane of Gram-negative bacteria; composed of lipid.

lipopolysaccharide (LPS) — The outer part of the outer membrane of Gram-negative bacteria; composed of lipid and sugars.

localized — Found only at a specific location.

macroscopic — Large enough to be seen with the naked eye.

metabolize — To utilize a nutrient source for growth and maintenance.

microbiologist — A professional who studies organisms too small to be seen with the naked eye.

migration — The act of moving throughout the body and occupying a new environment.

mucins — Large proteins in saliva that give it hydrating properties.

normal flora — The community of microorganisms that is found in an environment during good health.

nucleoid — The region of the bacterial cell cytosol that contains the chromosome.

O-antigen — The outermost portion of lipopolysaccharide; composed of sugars linked together in chains.

oligosaccharide core — The central portion of lipopolysaccharide that links the O-antigen to lipid A; composed of sugars.

organelles — Discrete structures that carry out specific functions within a cell.

outer membrane — The outermost layer of a Gram-negative cell that contains both phospholipids and lipopolysaccharide.

pathogen — An organism that can cause disease.

peptide — A short sequence of amino acids linked together in a chain.

peptidoglycan — Chemical that makes up a bacterial cell wall; composed of a mixture of amino acids and sugars.

persistent — A state where a pathogen remains in an environment for a prolonged period of time.

pH — The measure of how acidic or basic a substance is; acids have low pH values and bases have high pH values.

phagocytes — Cells of the immune system that are able to engulf pathogens and parts of them.

phospholipid bilayer — The composition of cell membranes, made up of phosphate groups attached to lipid molecules.

pili — Bacterial hair-like projections that are made of protein and aid in attachment to surfaces and other bacteria.

plaque — The bacterial biofilm that accumulates on teeth.

polymerase chain reaction (PCR) — The method by which the amount of genetic material (DNA) can be selectively increased.

polymicrobial infection — An infection caused by more than one microorganism.

resolution — The ability to distinguish two objects as separate entities.

ribosome — The structure on which amino acids are synthesized into a protein.

saliva — The liquid produced in our mouths by the salivary glands that helps to maintain good oral health.

salivary antibody — Proteins in the mouth that are directed against specific pathogens.

salivary glands — The organs in the mouth that produce saliva.

secretion systems — Components that bacteria use to export material from the inside of their cells to the outside.

sialidase — An enzyme produced by some bacteria that breaks apart specific types of sugars.

species — The designation for organisms that are biologically identical to each other.

transpeptidation — Linking together sugar chains with peptides.

vaccine — A substance that can boost the immune response and protect us from subsequent infection by a specific pathogen.

virulence — The ability to cause disease.

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 (Ceepym) 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 ___/___/_____
 - c. Exercise number Ex. 5
14. Telephone number to call in case of an emergency 101, 103.

Practical class 1. METHODS IN DIAGNOSTIC MICROBIOLOGY. MICROSCOPIC METHOD OF EXAMINATION (MME). BASIC MORPHOLOGICAL FORMS OF BACTERIA. SIMPLE METHODS OF STAINING

Suggested reading for self-study:

History of the microbiology, virology, immunology department; 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 II 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, method evaluation. 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.

Signature of the tutor _____

Oral quiz	Laboratory work	Individual work	Tests	Total results
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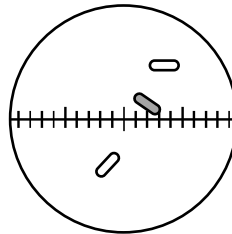
Laboratory work

Laboratory exercises

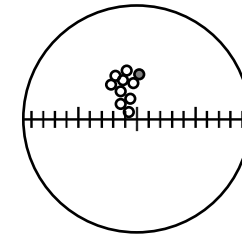
1. Prepare heat-fixed slide of *Escherichia coli*, cultured on agar medium, stain with methylene blue, examine under the oil immersion lens and complete the report.
2. Prepare heat-fixed slides of *Staphylococcus spp.*, cultured on liquid medium, stain with basic fuchsin, examine under the oil immersion lens and complete the report.
3. Complete the drawings of slides seen in demonstration room:
 - *Streptococcus spp.*, pure culture, stained with crystal violet;
 - *Vibrio spp.*, pure culture, stained with basic fuchsin;
 - *Bacillus spp.*, pure culture, stained with crystal violet.

Laboratory report

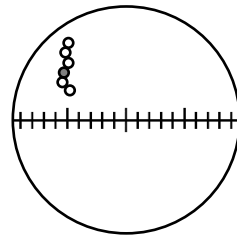
1 Smear _____
Stain _____



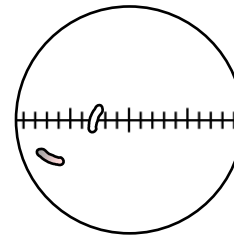
2 Smear _____
Stain _____



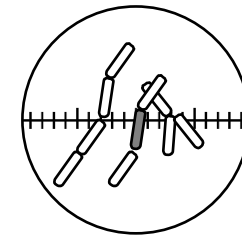
3 Smear _____
Stain _____



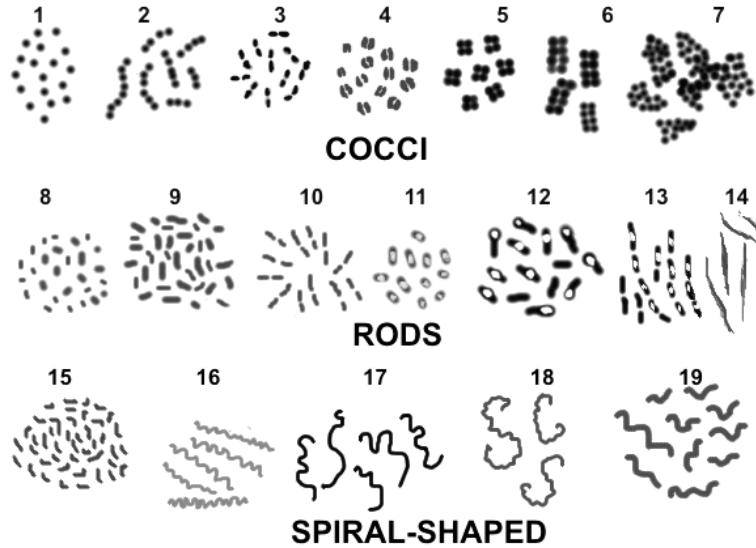
4 Smear _____
Stain _____



5 Smear _____
Stain _____



INDIVIDUAL WORK



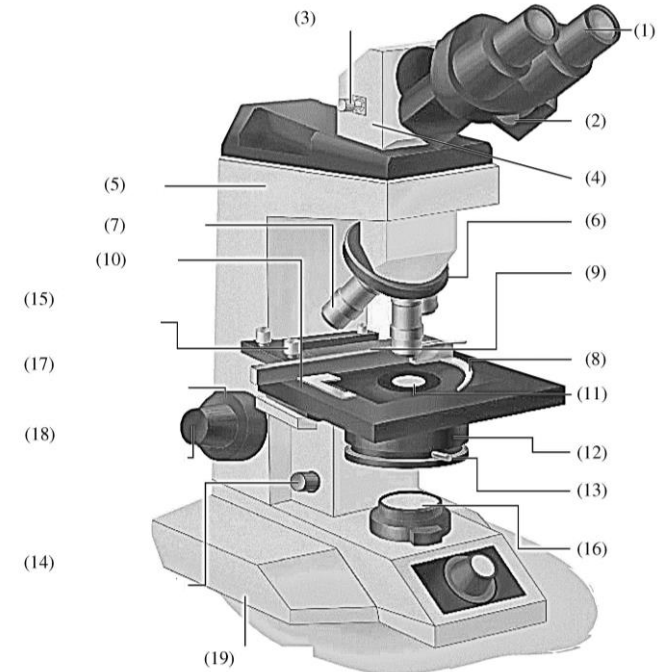
Fill the numbers in the table according to the picture above:

	bacterium
	bipolar-staining bacterium
	clostridium
	coccobacterium
	diplobacterium
	diplococcus
	fusobacterium
	micrococcus
	sarcinae
	spirillum
	spirochete (borrelia)
	spirochete (leptospira)
	spirochete (treponema)
	staphylococcus
	streptobacillus
	streptococcus
	tetrad
	vibrio

Biosafety Levels for Infectious Agents BSL
Fill in the empty cells examples of microorganisms in accordance with the level of risk

1	Agents that typically do not cause disease in healthy adults; they generally do not pose a disease risk to humans	
2	Agents that can cause disease in healthy adults; they pose moderate disease risk to humans	
3	Agents that can cause disease in healthy adults; they are airborne and pose a more serious disease risk to humans	
4	Agents that can cause disease in healthy adults; they pose lethal disease risk to humans; no vaccines or therapy available	

Write the names of the parts of a microscope



Questions for self-control and discussion:

1. What are the two purposes of heat fixation?
2. What is the purpose of simple staining?
3. Why are basic dyes more successful in staining bacteria than acidic dyes?
4. Name three basic stains.
5. Why is time an important factor in simple staining?
6. How would you define a properly prepared bacterial smear?
7. Why should you use an inoculating needle when making smears from solid media? An inoculating loop from liquid media?
8. Why is oil necessary when using the 90× to 100× objective?
9. What are three bacterial shapes that you have observed?
10. How can you increase the resolution on your microscope?
11. In microbiology, what is the most commonly used objective?

Steps of the microscopic method of examination (write in the cell)

1	
2	
3	
4	
5	

Practical class 2. MME. THE MORPHOLOGY AND FINE STRUCTURE OF BACTERIA. DIFFERENTIAL METHODS OF STAINING

Suggested reading for self-study:

Distinctive features of prokaryotic and eukaryotic cells. Basic bacterial cell structure: components of bacterial cell. The composition, function, detection methods of bacterial cell wall. Gram stain: medical application, principles, procedure for Gram stain.

The composition, function of capsule, flagella, pili (fimbriae) and methods for their detection. Detection of capsule using negative staining.

The cytoplasmic membrane: structure, function. The most important bacterial cytoplasmic membrane proteins. Bacterial core: cytoplasm, cytoplasmic structures (nucleoid, plasmids, ribosomes, and mesosomes). Inclusion bodies — storage granules (starch, fat, sulfur, polymetaphosphate (volutin)). 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: medical application, principle, procedure.

Signature of the tutor

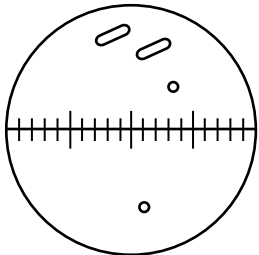
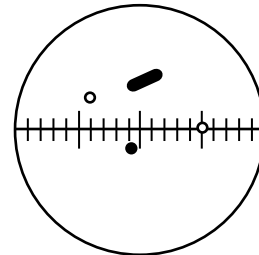
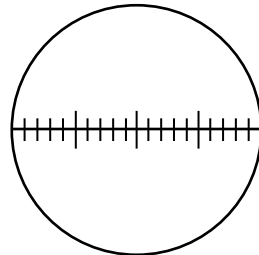
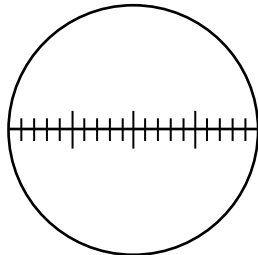
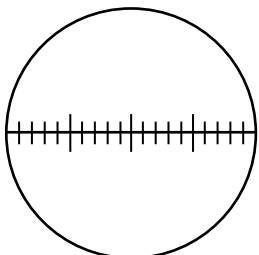
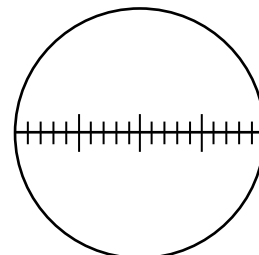
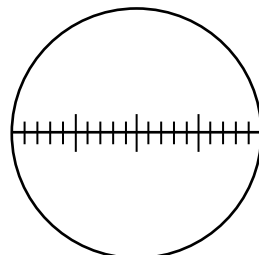
Oral quiz	Laboratory work	Individual work	Tests	Total results

Laboratory work

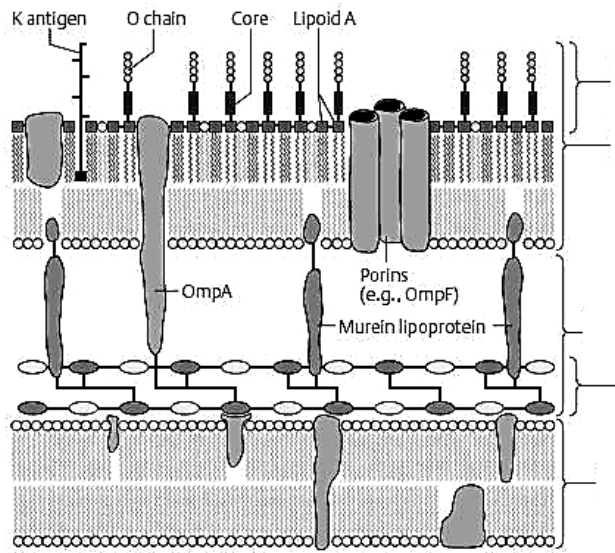
Laboratory exercises

- Prepare heat-fixed slide of the mixed culture of *Escherichia coli* (gram-negative) and *Staphylococcus aureus* (gram-positive), Gram stain, examine under oil immersion and complete the report.
- Complete the drawings of slides seen in demonstration room:
 - slide with capsule of *Klebsiella pneumoniae*, negative staining;
 - slide with mixture of *Escherichia coli* (gram-negative) and *Staphylococcus aureus* (gram-positive), Gram stain;
 - slide with volutin granules of *Corynebacterium diphtheriae*, Loeffler staining;
 - slide with volutin granules of *Corynebacterium diphtheriae*, Neisser staining;
 - slide of the mixed culture of acid-fast and acid-labile microorganisms, staining Ziehl–Neelsen.

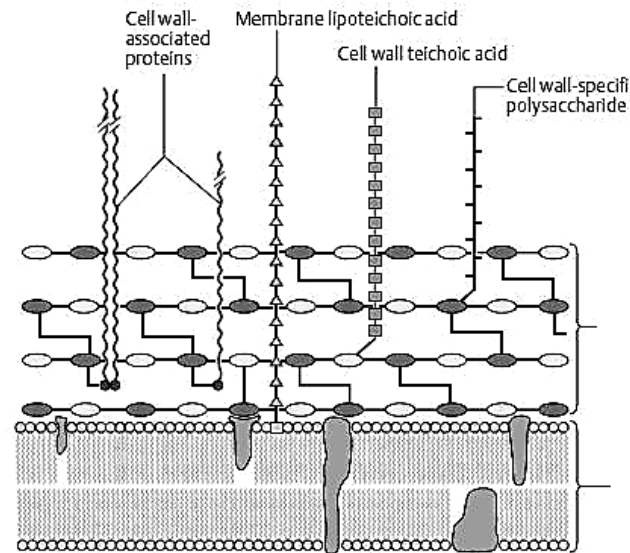
Laboratory report

<p>1 Smear _____ Stain _____</p> 	<p>2 Smear _____ Stain _____</p> 	<p>3 Smear _____ Stain _____</p> 	<p>4 Smear _____ Stain _____</p> 
<p>5 Smear _____ Stain _____</p> 	<p>6 Smear _____ Stain _____</p> 	<p>7 Smear _____ Stain _____</p> 	

INDIVIDUAL WORK (See continued on page 18)



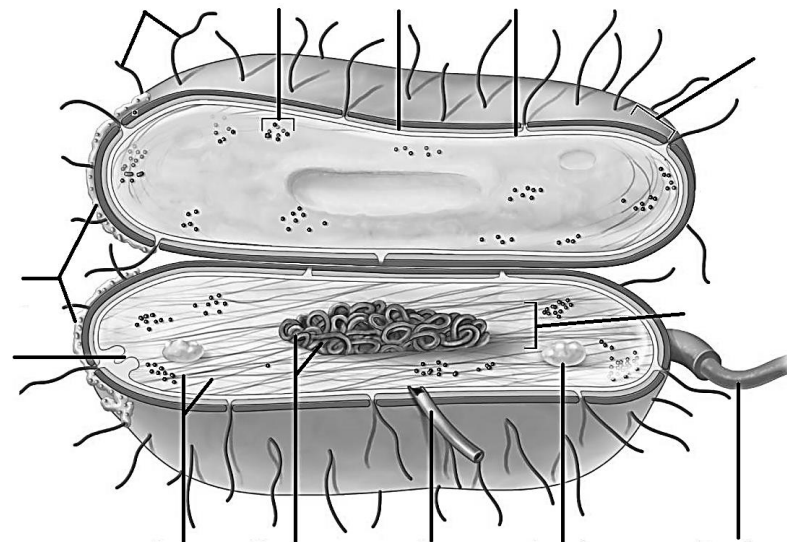
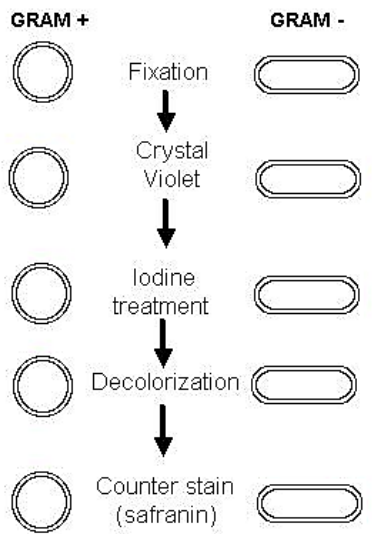
A
1
2
3
4
5



B
4
5

Write the component name of the wall	
1	
2	
3	
4	
5	
A	
B	

Paint bacteria by Gram' stage:



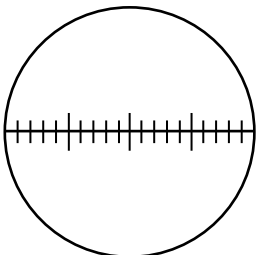
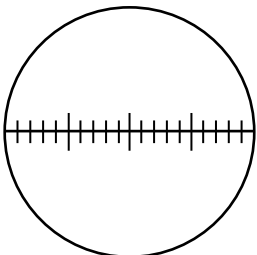
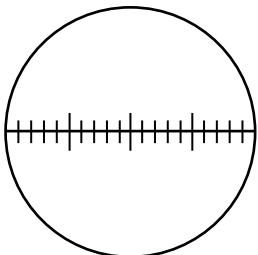
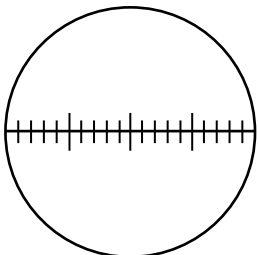
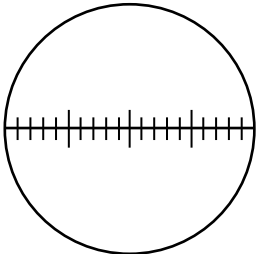
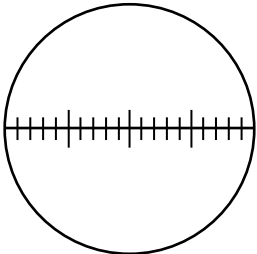
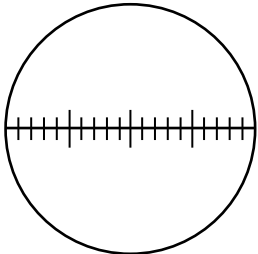
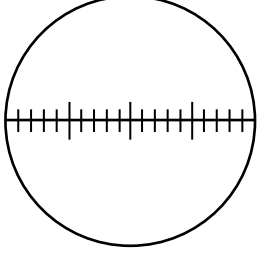
Enter the cell names of structures

1 -
2 -
3 -
4 -
5 -
6 -
7 -
8 -
9 -
10 -
11 -
12 -
13 -

Practical class 3. MME. THE MORPHOLOGY OF THE SPIROCHETES, ACTINOMYCES, RICKETTSIA, CHLAMYDIA, MYCOPLASMAS

<p>Suggested reading for self-study: Bacterial forms with defective cell wall (protoplasts, spheroplasts and L-forms): factors inducing cell wall removal, medical importance of L-forms. Resting forms of microorganisms. Bacterial endospores: medical importance, properties of endospore, the periods of endospore formation, detection methods. Spore stain using Ozheshko method: principle, procedure. Taxonomy, morphology, medical significance of the Spirochetes, Actinomyces, Rickettsiae, Chlamydiae, Mycoplasmas. Romanowsky–Giemsa stain. Dark-field light microscopy. Phase-contrast light microscopy. Fluorescence microscopy.</p>	Signature of the tutor _____				
	Oral quiz	Laboratory work	Individual work	Tests	Total results

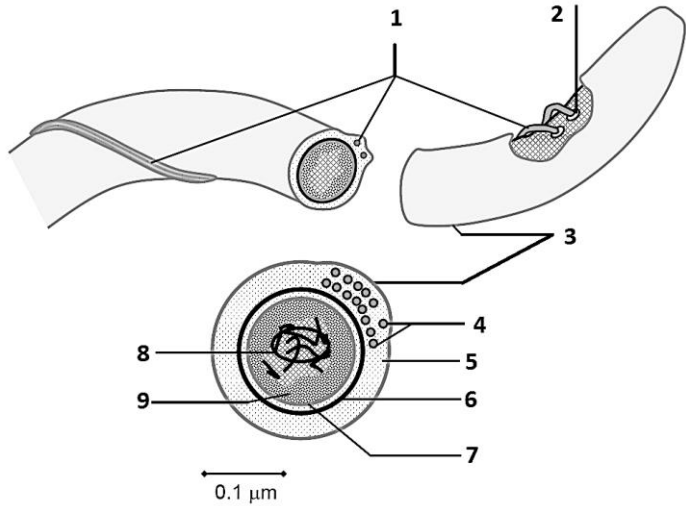
Laboratory work

Laboratory exercises	Laboratory report			
<p>1. Prepare slide of <i>Rickettsia spp.</i>, stain with fuschin, examine under the microscope, complete the report.</p> <p>2. Complete the drawings of slides seen in demonstration room:</p> <ul style="list-style-type: none"> – slide with <i>Treponema denticola</i> in dental plaque, Gram stain; – <i>Leptospira spp.</i>, dark-field microscopy; – <i>Borrelia recurrentis</i> in the blood of patient with relapsing fever, Romanowsky–Giemsa stain; – Chlamydia inclusions in cytoplasm of host-cell, Romanowsky–Giemsa stain; – slide with <i>Actinomyces spp.</i>, pure culture, Gram stain; – slide with spores of <i>Bacillus anthracis</i>, <i>Ozheshko staining</i>; – slide with <i>E. coli</i>, pure culture, acridine orange stain. 	<p>1 Smear _____ Stain _____</p> 	<p>2 Smear _____ Stain _____</p> 	<p>3 Smear _____ Stain _____</p> 	<p>4 Smear _____ Stain _____</p> 
	<p>5 Smear _____ Stain _____</p> 	<p>6 Smear _____ Stain _____</p> 	<p>7 Smear _____ Stain _____</p> 	<p>8 Smear _____ Stain _____</p> 

INDIVIDUAL WORK

Morphology of Spirochetes (write in cells names of structures)

Endoflagella (axial filaments) beneath outer membrane, Basal body, Outer membrane, Endoflagella, Periplasm, Cell wall (peptidoglycan), Inner (cell/plasma) membrane, DNA in nucleoid, cytoplasm



Confront Gram-positive and Gram-negative bacteria

1	Characteristic	Gram-Positive	Gram-Negative
2	Number of peptidoglycan layers		
3	Overall thickness in nm		
4	Specific compounds		
5	Interbridges between tetra peptides of neighbor glycan chains		
6	Outer membrane		
7	Periplasmic space		
8	Porin proteins		
9	Permeability		

The technique of Gram stain (write the component and exposure time)

Component: crystal violet, tag water, basic fuchsine or safranin, ethanol, iodine

	Component	Exposure time, sec			
1			Main mechanisms of genetic exchange		
2			Cell wall deficient forms in vitro		
3			Ability to produce spores		
4			Ability to produce long filamentous		
5			Susceptibility to Lysozyme		
6			Adhesion by pili		
7	Tag water (wash slide thoroughly)	5	Pathogenicity islands		
			Gram stain (fill)		

INDIVIDUAL WORK

Questions for self-control and discussion (Practical class 2)		Questions for self-control and discussion (Practical class 3)	
What is the function of the iodine solution in the Gram stain? If it were omitted, how would staining results be affected?		result	For what diseases would you use an acid-fast stain?
What is the purpose of the alcohol solution in the Gram stain?			What chemical is responsible for the acid-fast property of mycobacteria?
What counterstain is used? Why is it necessary? Could colors other than red be used? What is the advantage of the Gram stain over the simple stain?		result	How should the acid-fast stain of a sputum specimen from a patient with suspected pulmonary Nocardia infection be performed?
Describe at least two conditions in which an organism might stain gram variable.			Is a Gram stain an adequate substitute for an acid-fast stain? Why?
Which step is the most crucial or most likely to cause poor results in the Gram stain? Why?			Are acid-fast bacteria gram positive or gram negative? Explain your answer.
Why must young cultures be used when doing a Gram stain? What is meant by gram variable?			Why is it important to know whether bacterial cells possess flagella, or endospores?
What part of the bacterial cell is most involved with Gram staining, and why?			What do endospore stains have in common with the Ziehl–Neelsen acid-fast stain? Is bacterial sporulation a reproductive process? Explain.
What is an advantage of negative staining?			What is the purpose of the heat during the acid-fast staining procedure?
Why is negative staining also called either indirect or background staining?			Why are endospores so difficult to stain?

Practical class 4. ECOLOGY OF MICROORGANISMS. ASEPSIS. METHODS OF STERILIZATION, DISINFECTION AND ANTISEPSIS

Suggested reading for self-study:

Ecology of microorganisms. Interspecific and intraspecific relations. Symbiosis, its variants. Antagonistic microbial relationships, its background and medical importance. Bacteriocins.

Definition of terms asepsis, sterilization, disinfection, antiseptics. Methods of sterilization: physical, chemical, mechanical. Differences between sterilization and disinfection. Types and methods of disinfection. Types and methods of antiseptics. Practical antiseptics. Classification of antiseptics, origin and characteristics of groups. Mechanisms of action on microorganisms. Antimicrobial management in dentistry.

Signature of the tutor _____

Oral quiz	Laboratory work	Individual work	Tests	Total results

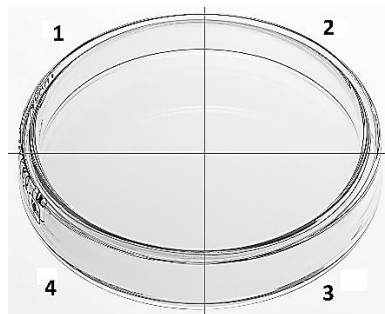
Laboratory work

Laboratory exercises

1. Test the effectiveness of hygienic and surgical hand antiseptics. **The result is taken into account in the next practical class.**

Laboratory report

1. Divide a nutrient agar plate into 4 sections with a marking pen or pencil. Mark each section of the plate with numbers 1, 2, 3, 4.
2. Mark each plate with your group number and your name.
3. On the surface of agar medium at section N 1 make a fingerprint of skin untreated with any antiseptic (control).
4. Wash your hands with soap as you do it usually at home and make a fingerprint on the surface of the agar medium at section N2.
5. Wash your hands with soap twice and then your 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 N 3.
6. Do not wash your hands and fingers with antiseptic (1 % of iodopyron) — 2 minutes, neutralize iodopyron with neutralizer (1 % of sodium thiosulfate) for 2 minutes and make a fingerprint on the surface of agar medium at section N 4.
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 antiseptics.



Section	Experiment description	Quantity of CFU
1	Control	
2	Hygienic hand antiseptics (washing with soap)	
3	Surgical hand antiseptics	
4	Antiseptics with iodopyron	

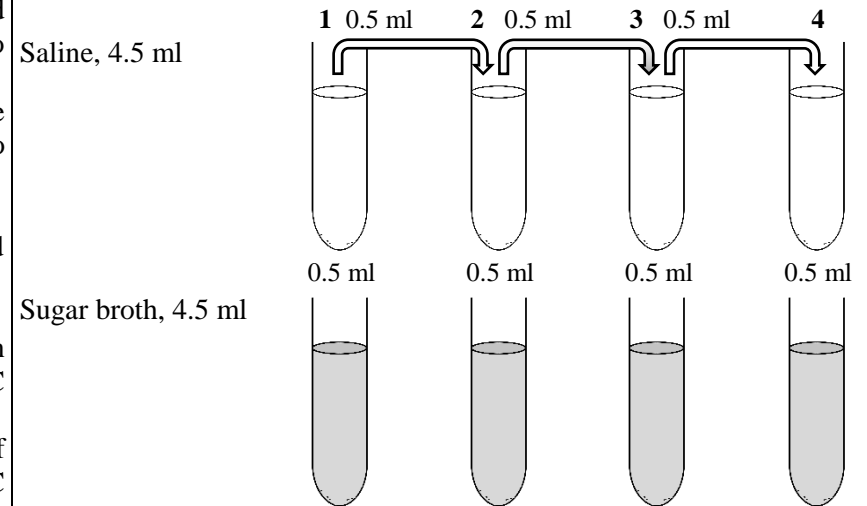
Conclusion:

2. Test the effectiveness of hygienic oral antiseptics. **The result is taken into account in the next practical class.**



1. Mark the Petri plate "Experience" and "Control".
2. Rinse mouth with sterile saline 45 seconds, and spit in the plate "Control".
3. Rinse the mouth with 1% solution of boric acid 45 seconds and spit into the sink.
4. Rinse mouth with sterile saline, and spit in the plate of "Experience".
5. Using a sterile pipette and spray bulb make breeding materials:
 - a) prepare 4 test tubes with 4.5 ml of sterile saline, label 1C, 2C, 3C, 4C;
 - dial 0.5 ml of material from the plate "Control" and release into the tube 1C. Reset the pipette into a porcelain cup;
 - other pipette to mix the contents of the tube 1C, type 0.5 ml tube and release in 2C. Reset the pipette into a porcelain cup. Do this with the other tubes.
 - b) analogous prepare "Experience" material.
6. Use a glass pipette and spray bulb produce seed dilutions on sugar broth:
 - prepare 4 tubes with Sugar broth sign 1C, 2C, 3C, 4C;
 - sterile pipette to stir the contents of the tube 4C gain of diluted material 0.5 ml in a test tube and release 4C broth;
 - without changing the pipette, transfer 0.5 ml of the diluted material from the tube into the tube 3C broth; do this with the other tubes.
7. Analogous prepare "Experience" material.
8. Incubate all tubes at 37 °C for 24 hours. After incubation observe each tube for growth (+) or absence of growth (-). Complete the table by recording your own results and formulate the conclusion regarding effectiveness of oral antiseptics.

"Experience" / "Control"



Result Experience				
Control				

Conclusion:

INDIVIDUAL WORK

Enter in cells possible methods of sterilization		Give the definition of the following terms:	
Bacteriological loops		Antisepsis -	
Gauze, cotton, bandage		Asepsis -	
Rubber, plastic products		Disinfection -	
Glass products		Sterilization -	
Air in operating room		Modes of action of disinfectants and antiseptics (write in cells)	
General-purpose media		Mode	Disinfectants or antiseptics
Enriched media with serum or blood			
Solution which is inactivated at above 60 °C			
Borer			
Dental mirror			
Tooth brush			

Practical class 5. BACTERIOLOGICAL METHOD OF LABORATORY DIAGNOSIS OF INFECTIOUS DISEASES. TECHNIQUES FOR PURE CULTURE ISOLATION AND MAINTENANCE

Suggested reading for self-study:

Metabolism and energy exchange in microbes. Constructive and energy metabolism. Types and methods of feeding, nutrient transport through the membrane. Breathing microbes, breathing apparatus, ways of biological oxidation. Aerobic, anaerobic, facultative anaerobes.

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 aerobic and anaerobic microorganisms isolation in pure culture. Bacterial colony characteristics.

Signature of the tutor _____				
Or al qu iz	Laboratory work	Individual work	Tests	Total results

Laboratory work

Laboratory exercises

1. Register the results of experiment on antisepsis (see class N 4).
2. Perform the 2nd period of bacteriological diagnosis (inspection and accumulation of aerobic microorganisms pure cultures isolation):
 - characterize morphology of colonies two different types present on agar medium;
 - determine morphology and purity of colonies two different types using Gram stain;
 - use aseptic technique and transfer the colony of Gram-negative microorganisms for subculturing on a surface of agar slant for microbial biomass accumulation.

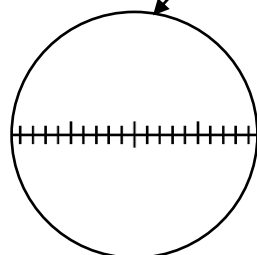
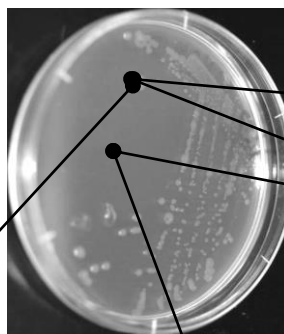
Laboratory report

The 2ND PERIOD OF BACTERIOLOGICAL DIAGNOSIS

Incubation 24 hours, 37 °C

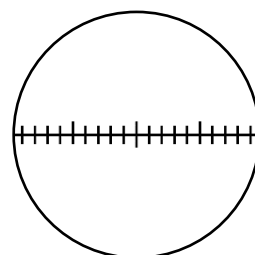
Inoculation of slant media with isolated colony of gram-negative bacteria

Nutrient agar with isolated colonies



Morphology of culture 1

Stain _____



Morphology of culture 2

Stain _____

Morphology of colony	Colony of culture 1	Colony of culture 2
Shape		
Size		
Surface		
Edge		
Color		
Consistency		
Transparency		
Gram stain		


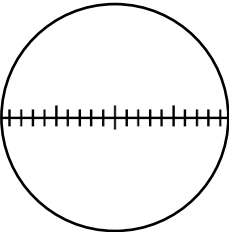
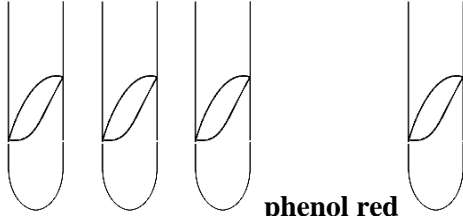

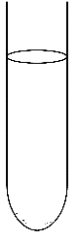

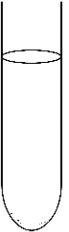
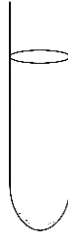
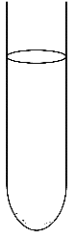
INDIVIDUAL WORK

Questions for self-control and discussion:

Define a pure culture, a mixed culture.	
Define a bacterial colony. List four characteristics by which bacterial colonies may be distinguished.	
Why should a Petri dish not be left open for any extended period?	
Why does the streaking method of plates inoculation result in isolated colonies?	
Why are culture media sterilized before use?	
Discuss the relative value of broth and agar media in isolating bacteria from mixed cultures.	
At what temperature does agar solidify? At what temperature does agar melt?	
Define a culture medium.	
Discuss some of the physical and chemical factors involved in the composition, and in the preparation, of a culture medium.	
Why is it necessary to isolate individual colonies from a mixed growth?	
Are the large numbers of microorganisms found in the mouth cause for concern? Explain.	
Why are plate cultures incubated in the inverted position?	
How do you decide which colonies should be picked from a plate culture of a mixed flora?	
Why is it necessary to make pure subcultures of organisms grown from clinical specimens?	
How can you determine whether a culture or subculture is pure?	
What kinds of clinical specimens may yield a mixed flora in bacterial cultures?	
When more than one colony type appears in a pure culture, what are the most likely sources of the extraneous organisms?	

Practical class 6. BACTERIOLOGICAL METHOD OF INFECTIOUS DISEASES LABORATORY DIAGNOSIS.
TECHNIQUES FOR PURE CULTURE IDENTIFICATION

<p>Suggested reading for self-study:</p> <p>Identification of microorganisms: approaches and methods. Bacterial species: term definition, species criteria and methods for discovering bacterial species.</p> <p>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 (carbohydralyses, amylase); c) lipolytic (lipases, lecithinase); d) oxidative-reductive (dehydrogenase, oxidase, catalase); e) hemolysins; α-, β-, γ-, -hemolysis.</p> <p>Rapid multitest systems for microorganisms identification. Automatic bacteriological analyzers: structure and principle of bacterial identification.</p>	Signature of the tutor				
	Oral quiz	Laboratory work	Individual work	Tests	Total results

Laboratory work	
Laboratory exercises	Laboratory report
<p>1. Perform the 3rd period of bacteriological diagnosis (identification of aerobic microorganisms pure cultures):</p> <ul style="list-style-type: none"> - determine morphology and confirm purity of agar slant culture; - using stab technique inoculate Hiss media with sucrose, maltose, mannitol for the determination of bacterial carbohydrate hydrolyses; - using stab and streaking technique inoculate Kligler Iron agar for the determination of bacterial carbohydrate hydrolyses and H₂S production; - using stab technique inoculate semisolid tube medium to detect motility; - inoculate nutrient broth and test the culture for the indole production. <p>2. Demonstration:</p> <ul style="list-style-type: none"> - semisolid and liquid Hiss media with different pH indicators; - hemolysis on blood agar medium, lecithinase activity, indol detection; - differentiate among members of the family <i>Enterobacteriaceae</i> using Kligler Iron agar; - rapid multitest systems for identification of microorganisms. 	<div style="text-align: right; margin-bottom: 10px;"> <p>Key YELLOWY 6.8 < RED < 8.2 CRIMSON</p> </div> <div style="display: flex; justify-content: space-around;"> <div style="text-align: center;"> <p>Smear _____</p> <p>Stain _____</p>  </div> <div style="text-align: center;">  </div> <div style="text-align: center;">  <p>phenol red</p> </div> </div> <div style="display: flex; justify-content: space-around; margin-top: 20px;"> <div style="text-align: center;"> <p>Triple sugar iron agar</p>  <p>glucose, lactose H₂S production</p> <p>Carbohydrases cysteinedesulfurase</p> </div> <div style="text-align: center;"> <p>Semiliquid nutrient medium</p>  <p>motility detection</p> </div> <div style="text-align: center;"> <p>Hiss medium sucrose</p>  <p>Carbohydrase</p> </div> <div style="text-align: center;"> <p>Hiss medium maltose</p>  <p>Carbohydrase</p> </div> <div style="text-align: center;"> <p>Hiss medium mannitol</p>  <p>Carbohydrase</p> </div> <div style="text-align: center;"> <p>Nutrient bullion</p>  <p>indole detection tryptophanase</p> </div> </div>

INDIVIDUAL WORK

BACTERIOLOGICAL METHOD OF LABORATORY DIAGNOSIS – 5 P's

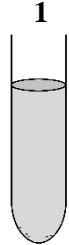
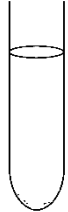
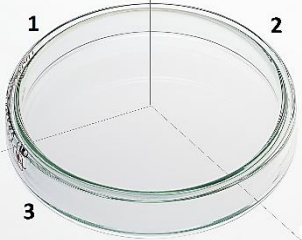
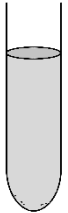
1	2	3	4

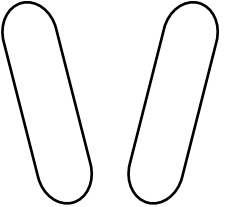
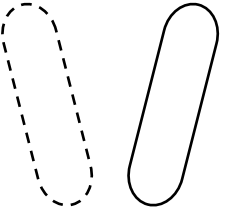


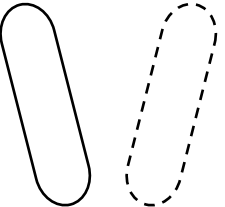
Practical class 7. MOLECULAR BASIS OF BACTERIAL GENETICS. MOLECULAR METHODS OF INFECTIOUS DISEASES DIAGNOSIS AND BACTERIAL GENETIC INVESTIGATIONS

<p>Suggested reading for self-study: The structure of bacterial genetic apparatus. Regulation of gene expression. General properties and varieties of plasmids. Detection of plasmids. Bacterial variability: phenotypic and genetic. Practical significance of bacterial variability. Mechanisms of genetic variability: Mutation and recombination. Classification of mutations. Methods of mutant bacteria selection. Molecular methods: tasks, specimens for investigation, advantages of the 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 thermal cycle, detection of amplicons, interpretation of results. Equipment for PCR. Practical application of PCR.</p>	Signature of the tutor _____				
	Oral quiz	Laboratory work	Individual work	Tests	Total results

Laboratory work

Laboratory exercises	Laboratory report										
<p>1. Identify isolated pure culture and complete the final report: – register the biochemical properties of tested pure culture in the table; – analyze the results and determine the species of tested pure culture.</p>	Species	Morphology	Biochemical characteristics							<p>Conclusion: According to morphological, cultural, biochemical properties X-microbe is attributed to _____</p> <p>* “A” — acid, “G” — gas</p>	
			Glucose	Lactose	Maltose	Mannitol	Sucrose	H₂S	Indole		Motility
	E. coli	Gram- rods	AG	AG	AG	AG	–	–	+		+
	S. Typhi	Gram- rods	A*	–	A	A	–	+	–		+
	S. Paratyphi A	Gram- rods	AG	–	AG	AG	–	–	–		+
	S. Schottmuelleri	Gram- rods	AG	–	AG	AG	–	+	–	+	
	X-microbe										
<p>2. Perform PCR for the detection of M.tuberculosis in the sputum of the patient with tuberculosis suspected.</p> <p>Identification of M. tuberculosis in sputum is based on the detection of gen MPB64 unique for M. tuberculosis and M. bovis. PCR amplifies the fragment with the size 357 bp. of this gene.</p>	<p>Procedure of PCR DNA extraction: Mark the tubes with the volume 1.5 ml with letters S (sputum) and NC (control). Add 100 µl of the sputum to the tube with letter S and 100 µl of negative control to the tube marked with letter NC. Shake the tubes thoroughly and boil in the water bath for 10 minutes (in room 507). PCR cocktail preparation: Mark the tubes with the volume 0.5 ml with letters S (sputum) and NC (control). These tubes contain primers, dNTPs, MgCl₂. Add 10 µl of prepared DNA and 10 µl of liquid into PCR' tube. Amplification in special equipment — thermocycler — for approximately 1 hour. Detection of PCR products: Electrophoresis of PCR products in agarose gel. UV detection of specific PCR-products in gel with ethidium bromide. Report: Specific products sized 357 bp were / not detected. Sputum is positive / negative for Mycobacterium tuberculosis.</p>										

Laboratory exercises	Laboratory report				
<p>3. Perform the bacterial conjugation experiment:</p> <ul style="list-style-type: none"> – prepare the mating mixture by aseptically transferring 0.5 ml of an overnight meat-peptone both culture of donor and recipient <i>E. coli</i> into the separate tube; – mix and incubate at 37 °C for 1 hours; – confirm the resistance status and leucine and threonine production by the culturing donor, recipient and recombinant <i>E. coli</i> on minimal medium supplemented with streptomycin. 	<p>In bacterial conjugation experiment donor <i>E. coli</i> is susceptible to streptomycin and synthesize threonine and leucine. Recipient <i>E. coli</i> displays complementary properties: resistant to streptomycin and unable to synthesis threonine 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.</p>	<p><i>E. coli</i> D (donor)</p> <p>F⁺ tre⁺ leu⁺ str^S</p> 	<p>3</p>   <p>Minimal medium without threonine and leucine, with streptomycin 100 µg/ml</p>	<p>Recombinant <i>E. coli</i></p> <p>F⁻ tre⁻ leu⁻ str</p> <p>1 – donor 2 – Recipient 3 – recombinant</p> <p>Registration of THE results after 24 hours incubation at 37 °C</p>	<p><i>E. coli</i> R (recipient)</p> <p>F⁻ tre⁻ leu⁻ str^R</p> 

INDIVIDUAL WORK				
Bacterial conjugation — Draw a process diagram				
0 min	2 min	10 min	15 min	20 min
<p>0 min</p> 	<p>2 min Pilus formation</p> 	<p>10 min DNA replication with continued pilus formation</p> 	<p>15 min DNA transfer</p> 	<p>20 min Conjugates separate</p> 

INDIVIDUAL WORK

The polymerase chain reaction (PCR), complete cells

Stages

Amplification

Evaluation of method

Practical application

Practical class 8. INFECTIONS. APPLICATION OF LABORATORY ANIMALS IN MICROBIOLOGY. ANTIBIOTIC SUSCEPTIBILITY TESTING OF MICROORGANISMS

Suggested reading for self-study:

Defenition of infection. Classification of infections. Bacterial pathogenicity and virulence. Measurements of virulence: ID50, LD50, DLM. The genetics of bacterial pathogenicity. Pathogenicity islands. Pathogenicity factors: adhesins, invasins, impedins, agressins, modulins. The role of bacterial biofilms. Methods of adhesins, capsule, invasins, toxigenicity detection.

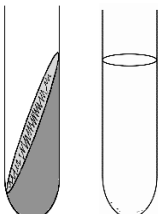
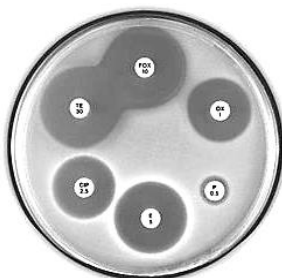
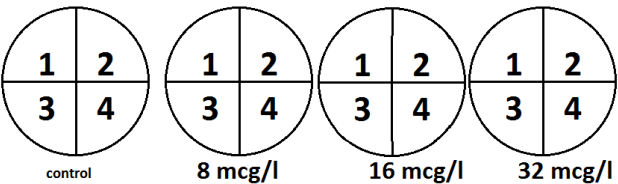
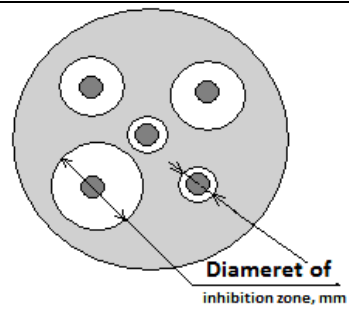
Biological method (application of laboratory animals in microbiology): tasks, phases, evaluation of the method. Animal models for infectious diseases. Routs for animal infection. Ethical, humane and legal considerations involved in the use of laboratory animals.

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. Antibiotic susceptibility testing of microorganisms: methods and principles.

Signature of the tutor

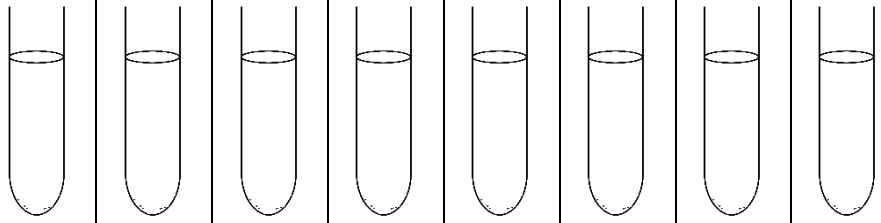
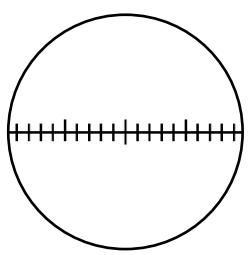
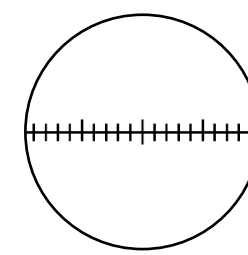
Oral quiz	Laboratory work	Individual work	Tests	Total results

Laboratory work

Laboratory exercises	Laboratory report				
<p>1. Perform the disk diffusion method (Kirby–Bauer) for determination of antibiotic susceptibility of four different microorganisms which often infect humans — <i>Staphylococcus aureus</i>, <i>Escherichia coli</i>, <i>Pseudomonas aeruginosa</i>, and <i>Klebsiella pneumoniae</i>.</p>	<p>Pure culture</p>  <p>1.0 ml of inoculum of microorganisms</p>	<p>Inoculation on Mueller–Hinton agar</p> <p>Mueller–Hinton agar (composition): <i>meat extract</i> — 2.0 g; <i>casein hydrolysate</i> — 17.5 g; <i>corn starch</i> — 1.5 g; <i>agar</i> — 17.0 g; <i>aqua distillate</i> — 1 l; pH 7.4±0.2</p>	<p>Incubation at 35 °C 24 h</p>  <p>Mueller–Hinton agar application of antimicrobial discs to the surface of the inoculated agar plate</p>		
	<p>2. Determine antibiotic susceptibility of microorganisms by agar dilution test. Complete the report.</p>	 <p>control 8 mcg/l 16 mcg/l 32 mcg/l</p> <p>Conclusion:</p>	<p>Registration of results</p> 		
<p>Petri dishes with serial doubled dilutions of Ampicillin in agar media</p>					
<p>Interpretation of results, MIC, mcg/l</p>					
antibiotic		resistant		susceptible	
Ampicillin		≥ 32		≤ 8	
Microbial culture		MIC, mcg/ml		Interpretation of results	
Culture 1					
Culture 2					
Culture 3					
Culture 4					

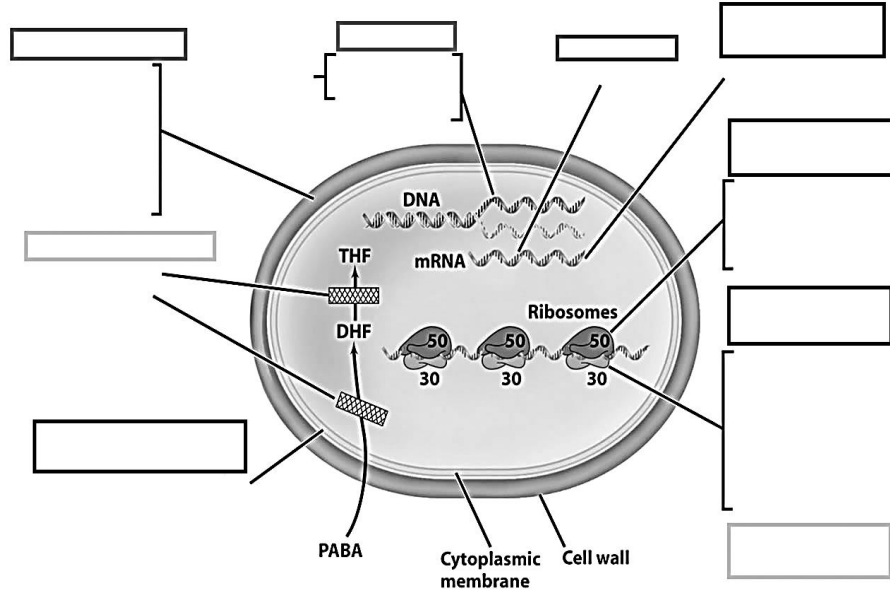
3. Determine antibiotic susceptibility of microorganisms by disk diffusion method, complete the report (perform it at classes N 9).

4. Demonstration:
 - agar disk diffusion test for antibiotic susceptibility testing of microorganisms;
 - rapid test for antibiotic susceptibility testing of microorganisms;
 - slide of *Bacillus anthracis* in tissues of white mouse, Gram stain;
 - slide of *Y. pestis* in tissues of white mouse, Gram stain;
 - slide of *Klebsiella pneumoniae rhinoscleromatis* in tissues of white mouse, Gram stain.

Results of pure culture testing by disc diffusion method								Antibiotic	Diameter of inhibition zones (mm)			
Antibiotic		Diameter of inhibition zone, mm		Interpretation of results					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	
0.5 μg/ml	1.0 μg/ml	2.0 μg/ml	4.0 μg/ml	8.0 μg/ml	16.0 μg/ml	32.0 μg/ml	Control	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	
DDM report (formulate what antibiotics can be recommended for the therapy):								4-1 Smear _____ Stain _____	4-2 Smear _____ Stain _____			
BDT report: minimal inhibitory concentration of antibiotic is _____ μg/ml.												

INDIVIDUAL WORK

Define the target action of antibiotics



DNA-directed RNA polymerase, Cell wall synthesis, RNA elongation, protein synthesis (50S inhibitors), protein synthesis (30S inhibitors), Folic acid metabolism, Cytoplasmic membrane structure, protein synthesis (tRNA)

Mechanisms of action of antimicrobial drugs (write in cells)

**Side effects of antimicrobial drugs
(write in cells)**

**Pathogenicity factors' groups
(write in cells)**

**Mechanisms of resistance of bacteria to an antimicrobial agents
(write in cells)**

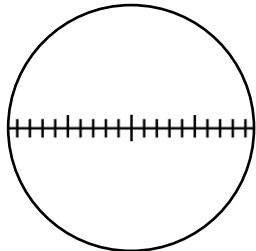
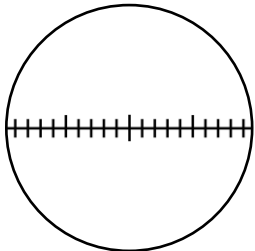
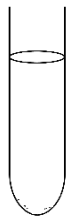
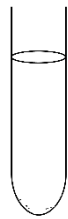

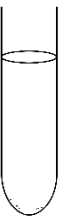

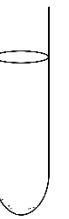
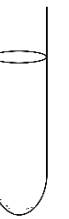
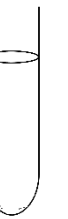
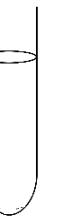
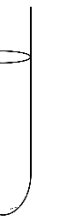
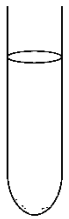
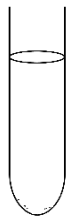
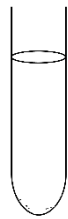

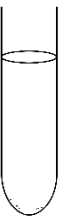

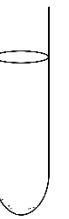
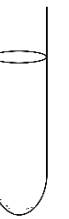
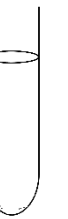
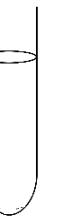
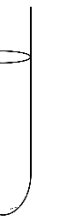
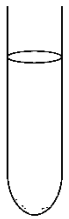
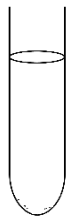
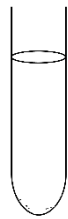

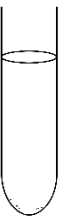

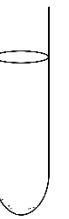
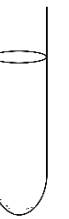
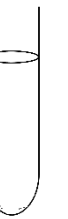
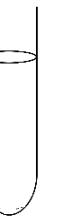
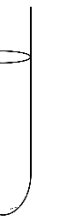
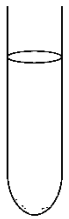
Practical class 9. CREDIT “MORPHOLOGY AND PHYSIOLOGY OF MICROORGANISMS”

List of questions	Oral quiz	Script	Tests	Total results
<p>1. History of microbiology as a science. Periods. The founders of microbiology main routs.</p> <p>2. Microscopic method of examination: tasks, procedure, evaluation of the method.</p> <p>3. Bright-field light microscope: components and proper use of the microscope. Dark-field 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.</p> <p>4. Type of microscopic preparations. Smear preparation and fixation. Simple methods of staining.</p> <p>5. Differential stains of microorganisms. Gram stain: medical application, principles, procedure for Gram stain.</p> <p>6. Morphology of bacteria. Distinctive features of prokaryotic and eukaryotic cells. Basic morphological forms of bacteria. Morphological characteristics of cocci, rods and spiral-shaped bacteria. Motility of bacteria, methods of detection.</p> <p>7. Structure and function of cell envelope and appendages. Capsule. Detection methods of the capsule.</p> <p>8. The composition, function, detection methods of bacterial cell wall. 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.</p> <p>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.</p> <p>10. Resting forms of microorganisms. Bacterial endospores: medical importance, properties of endospore, the periods of endospore formation, detection methods (principles, procedures).</p> <p>11. Taxonomy of microorganisms: classification and nomenclature. Modern approaches to taxonomy of microorganisms. Taxonomic ranks. Vars (types), strains, clones, pure cultures.</p> <p>12. Taxonomy, morphology, medical significance of the spirochetes. Methods for spirochetes detection.</p> <p>13. Taxonomy, morphology, medical significance of Actinomyces.</p> <p>14. Taxonomy, morphology, medical significance of Mycoplasmas. Methods for Mycoplasmas investigations.</p> <p>15. Taxonomy, morphology, medical significance of Chlamydiae and Rickettsiaceae.</p> <p>16. Nutrition of microorganisms. Source of macro- and micronutrients, growth factors. Nutritional types. Transport mechanisms for nutrient absorption.</p> <p>17. Energy strategies in microorganisms. Aerobic and anaerobic respiration. Structures involved in respiration in microorganisms.</p> <p>18. Reproduction of microorganisms. Mechanisms and phases of bacterial division.</p> <p>19. Bacteriological method of laboratory diagnosis: tasks, procedure, evaluation of the method.</p> <p>20. 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.</p> <p>21. Methods of aerobic microorganisms isolation in pure culture.</p> <p>22. Methods of anaerobic microorganisms isolation in pure culture. Cultivation of anaerobic bacteria: culture media, techniques, equipment.</p> <p>23. Identification of microorganisms: morphological, cultural, serologic, biological, genetic.</p>	<p>24. 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.</p> <p>25. The structure of bacterial genetic apparatus. Phenotype, genotype, genome, genes. Regulation of gene expression. General properties and varieties of plasmids. Detection of plasmids.</p> <p>26. Bacterial variability: phenotypic and genetic. Practical significance of bacterial variability. Population variability.</p> <p>27. Molecular methods in diagnosis of infection diseases: aims, methods, advantages. Molecular hybridization and polymerase chain reaction: principles of the methods.</p> <p>28. Doctrine regarding infections. Terms for emergence of infectious disease. Basic terminology of infectology. Classification of infections.</p> <p>29. Role of microorganisms in infection emergence. Bacterial pathogenicity and virulence. The genetics of bacterial pathogenicity. Pathogenicity islands. Pathogenicity factors: adhesins, invasins, impedins, agressins, modulins.</p> <p>30. Role of microorganisms, social and physical factors in infection emergence.</p> <p>31. Biological method (application of laboratory animals in microbiology): tasks, phases, evaluation of the method.</p> <p>32. Chemoprophylaxis and chemotherapy; antimicrobial chemotherapeutic agents and antibiotics. Sources of antibiotics. Especially the use of antibiotics in dentistry.</p> <p>33. Mechanisms of antibiotics action. Side effects of antibiotics. Principles for rational antimicrobial therapy.</p> <p>34. The problem of resistance to antimicrobials: definitions (intrinsic, acquired resistance), incidence, significance. Resistance mechanisms.</p> <p>35. Antibiotic susceptibility testing of microorganisms: methods and principles.</p> <p>36. Ecology of microorganisms. Basic terminology of ecology.</p> <p>37. Asepsis: definition, surgical, medical asepsis, asepsis in microbiological laboratory.</p> <p>38. Sterilization: definition, methods of sterilization (physical, chemical, mechanical), quality control.</p> <p>39. Disinfection: definition, methods of disinfection.</p> <p>40. Antisepsis: definition, methods of antisepsis. Disinfectant and antiseptics: classification and modes of action.</p> <p>List of practice.</p> <p>1. Prepare heat-fixed slide of bacteria, cultured on agar medium, stain with methylene blue.</p> <p>2. Prepare heat-fixed slides of bacteria, cultured on liquid medium, stain with basic fuchsin.</p> <p>3. Prepare heat-fixed slides of bacteria, cultured on liquid medium, stain by Gram.</p> <p>4. Technology immersion microscopy.</p> <p>5. Determine the morphology of Staphylococcus, pure culture, Gram stain.</p> <p>6. Determine the morphology of E. coli, pure culture, Gram stain.</p> <p>7. Determine the morphology of Gram+ and Gram- bacteria into the mix, Gram stain.</p> <p>8. Determine the morphology of the culture in smear colored by Ginsu-Burri.</p> <p>9. Define streptobacill pure culture morphology, Gram stain coloring.</p> <p>10. Determine antibiotic susceptibility of microorganisms by disk diffusion method.</p> <p>11. Characterize morphology of two different types of colonies present on agar medium.</p>			

Practical class 10. IMMUNE SYSTEM. INNATE IMMUNITY. METHODS FOR INNATE IMMUNITY FACTORS EVALUATION

<p>Suggested reading for self-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. Methods for estimation of complement system activity. Lysozyme, b-lysins. Polynuclear and mononuclear phagocytes systems. Phagocytosis: phases, intracellular killing mechanisms, outcomes. Dendritic cells. Methods for estimation of phagocytosis. Natural killer cells. Antigen-presenting cells. TOLL-like receptors.</p>	Signature of the tutor _____				
	Oral quiz	Laboratory work	Individual work	Tests	Total results

Laboratory work

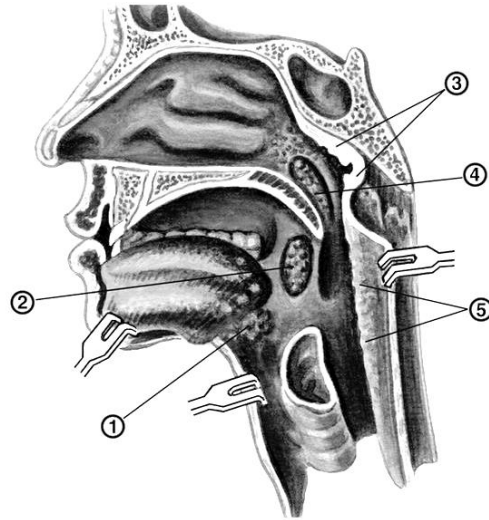
Laboratory exercises	Laboratory report																																	
<p>1. Determine phagocytosis parameters in prepared slides stained by Gimza method.</p> <p>2. Complete the drawings of slides seen in demonstration room:</p> <ul style="list-style-type: none"> – incomplete phagocytosis of <i>N. gonorrhoea</i>. – incomplete phagocytosis of <i>K. rhinoscleromatis</i>. <p>3. Register the complement system activity by 50 % hemolysis method.</p> <p>Serum is diluted and added in wells 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 well. Reaction is incubated at 37 °C for 45 min, cooled at 4 °C and centrifuged at 1500 rpm for 5 min. The well in which 50 % hemolysis occurred is determined visually. This means the volume of patient's serum that contains one unit of CH₅₀. Then the CH₅₀ for the whole serum is calculated.</p>	<p>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 phagocytosing leucocytes and phagocytosed staphylococci are counted and phagocytosis parameters calculated.</p> <p>PI (Phagocytosis index) = Number of phagocytosing leucocytes / All leucocytes counted Norma* — 40–60 %.</p> <p>PN (Phagocytosis number) = Number of phagocytosed staphylococci / Number of phagocytosing leucocytes Norma* — 4–7.</p>	<p>Smear _____ Stain _____</p> <div style="text-align: center;">  </div>	<p>Smear _____ Stain _____</p> <div style="text-align: center;">  </div>																															
	<p style="text-align: center;">Volume of diluted (1 : 10) serum, ml</p> <table style="width: 100%; text-align: center;"> <tr> <td>0.05</td><td>0.1</td><td>0.15</td><td>0.2</td><td>0.25</td><td>0.3</td><td>0.35</td><td>0.4</td><td>0.45</td><td>0.5</td><td>50 % hemolysis</td> </tr> <tr> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> </tr> </table> <p>Results:</p> <table border="1" style="width: 100%; height: 30px;"> <tr> <td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td> </tr> </table>	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5	50 % hemolysis																						
0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5	50 % hemolysis																								
																																		

INDIVIDUAL WORK

<p>Fill cells with types of immunity</p> <p>immunity, adoptive, passive, natural, artificial, immune factors, humoral, cellular, non-immune factors, active</p> <div style="background-color: #cccccc; width: 100%; height: 40px; margin: 10px 0;"></div> <div style="display: flex; justify-content: space-between;"> <div style="background-color: #cccccc; width: 15%; height: 100px; margin: 10px 0;"></div> <div style="background-color: #cccccc; width: 10%; height: 100px; margin: 10px 0; text-align: center; font-size: 24px; color: white;">INNATE</div> </div> <div style="display: flex; justify-content: space-between; margin: 10px 0;"> <div style="background-color: #cccccc; width: 7%; height: 40px; margin: 10px 0;"></div> <div style="background-color: #cccccc; width: 7%; height: 40px; margin: 10px 0;"></div> <div style="background-color: #cccccc; width: 7%; height: 40px; margin: 10px 0;"></div> <div style="background-color: #cccccc; width: 7%; height: 40px; margin: 10px 0;"></div> <div style="background-color: #cccccc; width: 7%; height: 40px; margin: 10px 0;"></div> <div style="background-color: #cccccc; width: 7%; height: 40px; margin: 10px 0;"></div> </div> <div style="display: flex; justify-content: space-between; margin: 10px 0;"> <div style="background-color: #cccccc; width: 4%; height: 40px; margin: 10px 0;"></div> <div style="background-color: #cccccc; width: 4%; height: 40px; margin: 10px 0;"></div> <div style="background-color: #cccccc; width: 4%; height: 40px; margin: 10px 0; text-align: center;">active</div> <div style="background-color: #cccccc; width: 4%; height: 40px; margin: 10px 0;"></div> <div style="background-color: #cccccc; width: 4%; height: 40px; margin: 10px 0;"></div> <div style="background-color: #cccccc; width: 4%; height: 40px; margin: 10px 0;"></div> </div>	Fill with sample of					
	Organs of immune system		Cells of immune system		Molecules of immune system	
	Write in cells ligand of receptors			Associate the scientist and his discovery		
	Pattern Recognition Receptors	Ligand pathogen-associated molecular patterns		Edward Anthony Jenner		Phagocytosis, Cell-mediated immunity
	TLR1			Élie Metchnikoff		Chemical structure of antibodies
	TLR2			Polly Celine Eveline Matzinger		Smallpox vaccine, vaccination
	TLR3			Charles Alderson Janeway		side chains, humoral immune response
	TLR4			Rodney Robert Porter Gerald M. Edelman		Diphtheria antitoxin
	TLR5			Karl Landsteiner		Danger model, danger theory
	TLR6			Paul Ehrlich		Immune tolerance
TLR7			Jules Jean-Baptiste Vincent Bordet		pattern recognition theory	
TLR8			Emil Adolf von Behring		complement	
TLR9			Frank Macfarlane Burnet		blood group system, Rh factor, poliovirus	

INDIVIDUAL WORK

Compare	
INNATE IMMUNITY	ADOPTIVE/ACQUIRED IMMUNITY

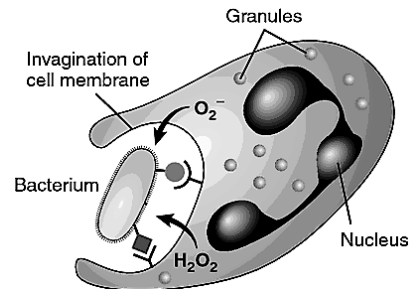


Nose-Associated Lymphoid Tissue

- 1 –
- 2 –
- 3 –
- 4 –
- 5 –

Complement system			Phases of phagocytosis (write in cells)
Activation pathway			
activators			
C3-convertase			
C5-convertase			
MAC development			

The illustration shows the process of phagocytosis. Draw a picture of the possible outcomes of the process in adjacent cells and named them.



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Practical class 11. ANTIGENS. ANTIBODIES. IMMUNE RESPONSE

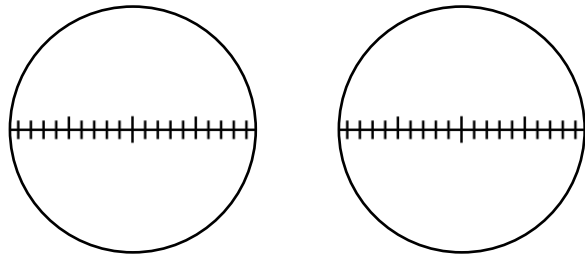
Suggested reading for self-study:

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.
 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.
 Cellular immune response and its phenomena. Interaction and control of the immune system.
 Methods for evaluation of T- and B-lymphocytes system: quantitative and functional tests.

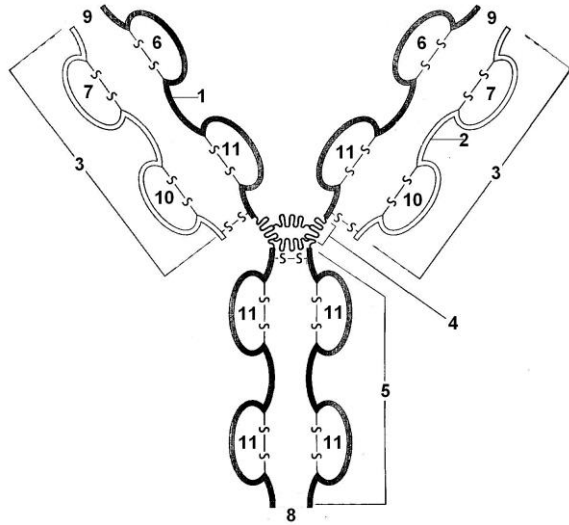
Signature of the tutor _____

Oral quiz	Laboratory work	Individual work	Tests	Total results

Laboratory work

Laboratory exercises		Laboratory report						
1. Determine the quantity of B-cells by immune rosettes methods in ready-made slides. 2. Complete the drawings of slides seen in demonstration room: – immune rosettes method for T-cell quantity determination (Romanowsky–Giemsa stain); – blast transformation of lymphocytes (Romanowsky–Giemsa stain); – determine an IgG, A, M concentration in serum by Manchini method (simple radial gel immunodiffusion).	N	Count	N	Count	N	Count	The method reveals CD20 antigen on B-cell surface; Normal B-cells count by CD20 = 8–20 % total blood lymphocytes. $B_{CD20} = \text{rosette's Cell}/30 =$ Conclusion:	Smear _____ Stain _____ 
	1		11		21			
	2		12		22			
	3		13		23			
	4		14		24			
	5		15		25			
	6		16		26			
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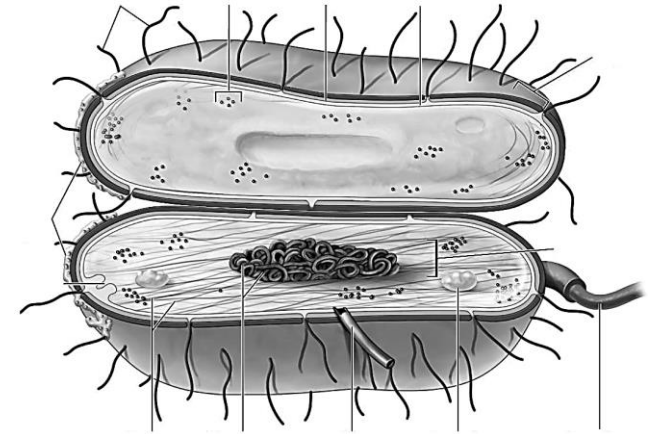
INDIVIDUAL WORK



Write figures for elements of an immunoglobulin molecule indicated on scheme

	Light chain (L)
	Variable domen of the light chain
	Constant domen of the light chain
	Heavy chain (H)
	Variable domen of the heavy chain
	Constant domen of the heavy chain
	Hinge fragment
	Fc- fragment
	Fab- fragment
	Active center
	Fc-receptor ligand

Enter the names of structures of bacteria, which are antigens



Write the main cells and molecules that are involved in the humoral immune response

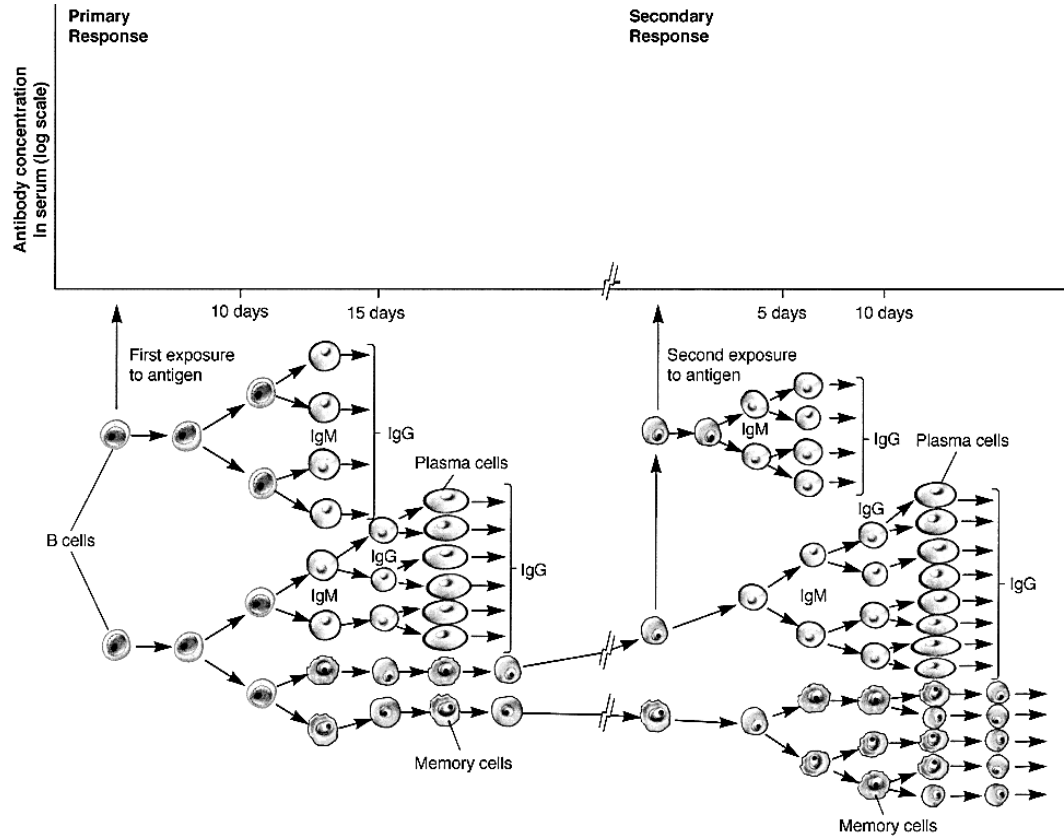
Cells

Molecules

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

Structure	Characteristics	Class
		Ig A
		Ig D
		Ig E
		Ig G
		Ig M

INDIVIDUAL WORK

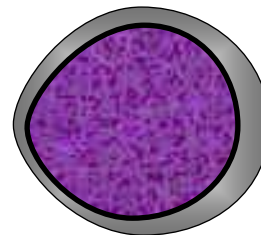


According to the following diagram, draw a graph of dynamics of immunoglobulins G and M classes for primary and secondary immune responses.

Write methods of the study of cellular immunity

Source: Ryan KJ, Ray CG: *Sherris Medical Microbiology, 5th Edition*: www.accessmedicine.com
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Draw the B-lymphocyte



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

Practical class 12. SEROLOGICAL METHOD


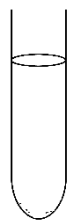


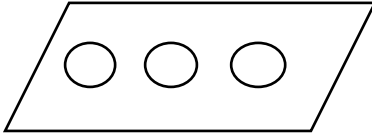
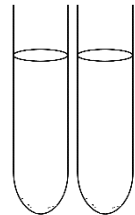
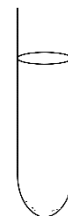




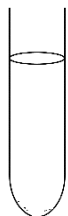
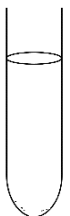

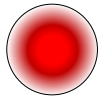
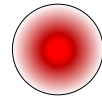
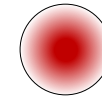
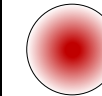
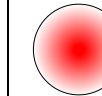
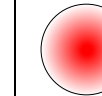
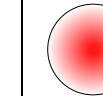
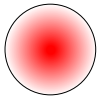
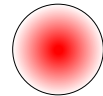
Suggested reading for self-study:

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.
 Immune lysis reactions. Complement fixation test: ingredients, implementation, characteristics.
 Immunofluorescence test: direct and indirect variants. Immunoenzyme test. ELISA. Radioimmune test.





Signature of the tutor _____

Oral quiz	Laboratory work	Individual work	Tests	Total results

Laboratory work

Laboratory exercises	Laboratory report											
1. Perform slide agglutination test to identify an X-bacteria.		1. antiserum S. Typhi		2. antiserum E. coli		3. Saline		X-bacteria		Conclusion: X-microbe is _____		
2. Determine the result of the complement fixation test.	CFT		1:20	1:40	1:80	1:160	1:320			SC	AC	
												
	Key “+” “-“		Assess:									
Conclusion:												
3. Determine the result of passive hemagglutination reaction.	PASSIVE BLOOD AGGLUTINATION TEST											
	Key “+” “-“		1/10	1/20	1/40	1/80	1/160	1/320	1/640		SC	AC
												
Assess:												
Conclusion:												

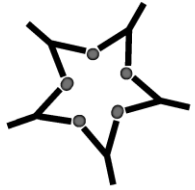
Laboratory exercises	Laboratory report																																																									
<p>4. Perform ELISA for HBs antigen detection in donor serum:</p> <p>a) put 100 mcl of control serum and samples according to test scheme;</p> <p>b) put 50 mcl of conjugate in each well;</p> <p>c) incubate for 1 hour at 37 °C;</p> <p>d) wash the strip 5 times;</p> <p>e) put 100 mcl of chromogen in each well;</p> <p>f) incubate for 30 min at 37 °C;</p> <p>g) put 50 mcl of stop-reagent in each well;</p> <p>h) measure the strip on ELISA reader and print out the results;</p> <p>i) fill in the report: check the test validity and make the final conclusion about results.</p>	<p>ELISA test for HBs-Ag detection in the serum</p>	<table border="1"> <tr> <td></td> <td>1</td> <td>2</td> <td>3</td> <td>4</td> <td>5</td> </tr> <tr> <td>A</td> <td>○</td> <td>○</td> <td>○</td> <td>○</td> <td>○</td> </tr> <tr> <td>B</td> <td>○</td> <td>○</td> <td>○</td> <td>○</td> <td>○</td> </tr> <tr> <td>C</td> <td>○</td> <td>○</td> <td>○</td> <td>○</td> <td>○</td> </tr> <tr> <td>D</td> <td>○</td> <td>○</td> <td>○</td> <td>○</td> <td>○</td> </tr> <tr> <td>E</td> <td>○</td> <td>○</td> <td>○</td> <td>○</td> <td>○</td> </tr> <tr> <td>F</td> <td>○</td> <td>○</td> <td>○</td> <td>○</td> <td>○</td> </tr> <tr> <td>G</td> <td>○</td> <td>○</td> <td>○</td> <td>○</td> <td>○</td> </tr> <tr> <td>H</td> <td>○</td> <td>○</td> <td>○</td> <td>○</td> <td>○</td> </tr> </table>		1	2	3	4	5	A	○	○	○	○	○	B	○	○	○	○	○	C	○	○	○	○	○	D	○	○	○	○	○	E	○	○	○	○	○	F	○	○	○	○	○	G	○	○	○	○	○	H	○	○	○	○	○	<p>Negative control</p> <p>Negative control</p> <p>Low positive control</p> <p>High positive control</p> <p>Sample 1</p> <p>Sample 2</p> <p>Sample 3</p> <p>Sample 4</p>	<p>Test validity:</p> <ul style="list-style-type: none"> – average OD of negative controls must be < 0.15 OD(NC) (negative controls) = – OD negative controls must range from 0.6 to 1.4 of average OD(NC) 0.6 OD(NC) = 1.4 OD(NC) = – average positive controls OD must be more than four times as much as OD(NC): average OD(PC)/ OD(NC) = – Low positive control OD must be higher than cut-off level <p>Cut-off calculation:</p> <p>Cut-off = OD(NC) + 0.04</p>
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INDIVIDUAL WORK		
Write down the following definitions:		
Titer -		
Diagnostic titer -		
Diagnosticum -		
Diagnostic serum -		
<p>Direct variant</p> <p>_____</p>	<p>Draw the scheme of ELISA</p> <p>Antigen — </p> <p>Antibody — </p> <p>Anti-Ig antibody — </p> <p>Enzyme — </p>	<p>Indirect variant</p> <p>_____</p>

INDIVIDUAL WORK

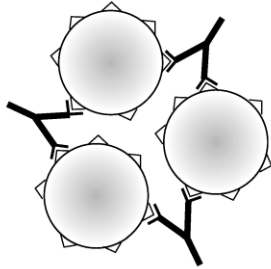
Write name of reaction' type in the first and in the second case:

1



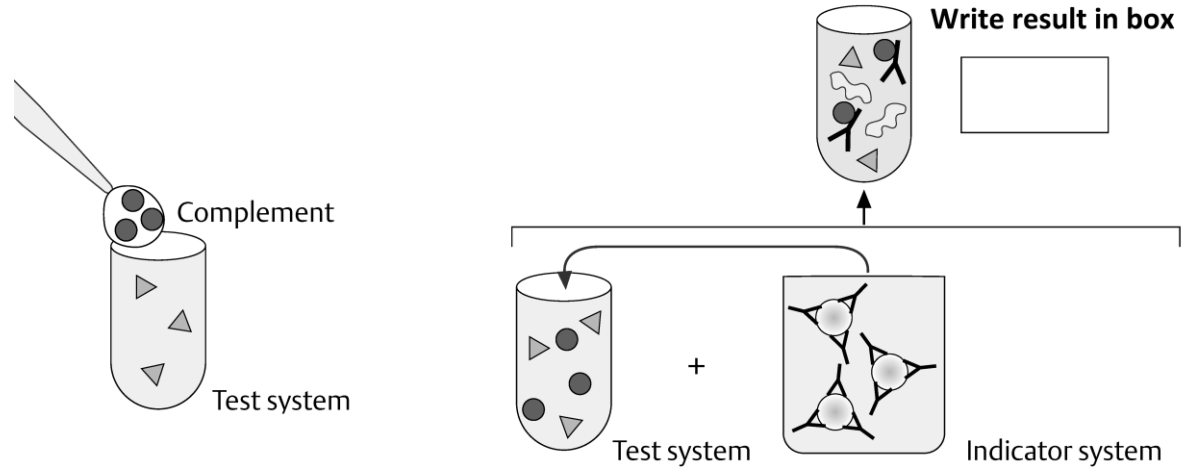
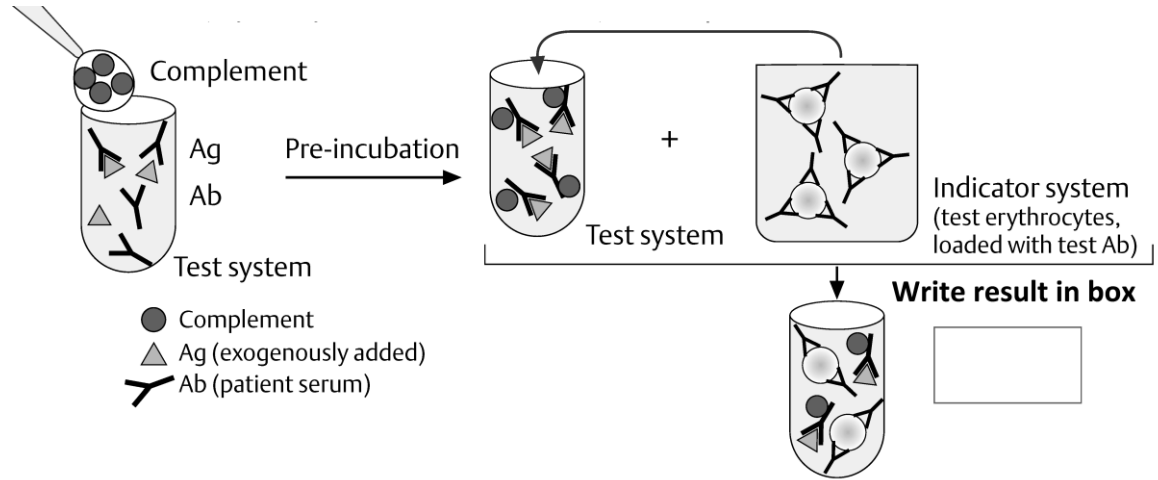
Immune complex formation with molecular antigens

2



Immune complex formation with antigenic particles (e.g. erythrocytes, latex particles)

What type of serologic test is depicted? Give explanations and result for both variant:



Write labels for next types of assays:

Immunofluorescence

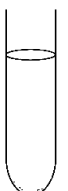
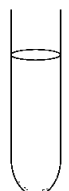

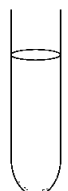


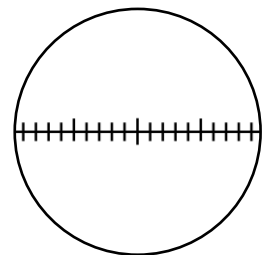
ELISA

Radioimmune test

Practical class 13. IMMUNOPROPHYLAXIS AND IMMUNOTHERAPY. IMMUNOPATHOLOGY AND CLINICAL IMMUNOLOGY

<p>Suggested reading for self-study: Immunoprophylaxis and immunotherapy. Vaccines, classification, essential characteristics. Vaccinal immunity, factors affecting its development. Methods of vaccinal immunity evaluation. Passive immunoprophylaxis. Immune sera and serum preparations; methods of its production and application. Allergy, periods, types. Immediate type of hypersensitivity mechanisms: mediator type (I), cytotoxic type (II), immune complex type (III). Delayed type of hypersensitivity mechanism (IV). Drug allergy. Allergens in dentistry. Methods for allergic conditions diagnostics. Clinic immunology: definition. Immune status. Immunogram. Primary and secondary immunodeficiency. Autoimmune disease. Causes, manifestation. Autoantibodies, diagnostic value, methods of determination. Antitumor immunity. Methods of immune status correction. Immunosuppression. Immunostimulation. Immunomodulators. Thymus, spleen, bone marrow substances. Interleukins, interferons.</p>	<p>Signature of the tutor _____</p>				
	Oral quiz	Laboratory work	Individual work	Tests	Total results

Laboratory work

Laboratory exercises	Laboratory report						
<p>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 antibody (IgM) to IgG. It is found in certain autoimmune diseases (SLE, RA etc.) and is useful for diagnostics.</p> <p>2. Perform the LA test to detect autoantibodies to thyroglobulin Latex diagnosticum = latex microsphaera coated with thyroglobulin molecules</p> <p>3. Demonstration: – degranulation of mast cells, Romanowsky–Giemsa stain; – Allergens; – Medicine for correction.</p>	<p>1. Saline</p> 	<p>2. Patient's serum</p> 	<p>3. ER Diagnosticum</p> 	<p>1. Saline</p> 	<p>2. Patient's serum</p> 	<p>3. Latex diagnosticum</p> 	<p>Smear _____ Stain _____</p> 
	<p>Conclusion:</p>			<p>Conclusion:</p>			

INDIVIDUAL WORK

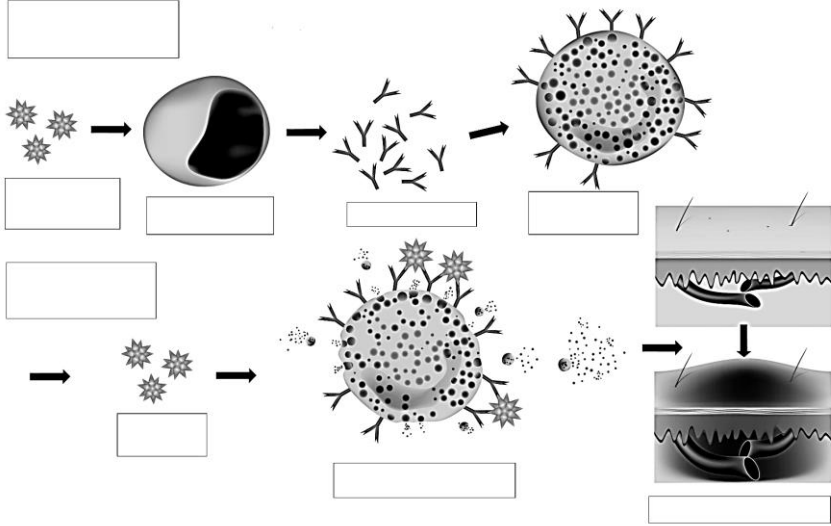
Write down the types of allergy by P.G.H. Gell and P.R.A. Coombs (1964):

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INDIVIDUAL WORK

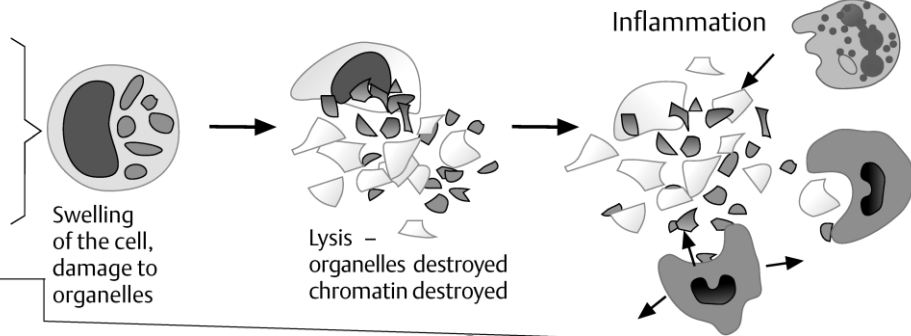
What type of allergy phenomena is depicted? Give explanations.

The vaccines for active immunization can be divided into four groups:



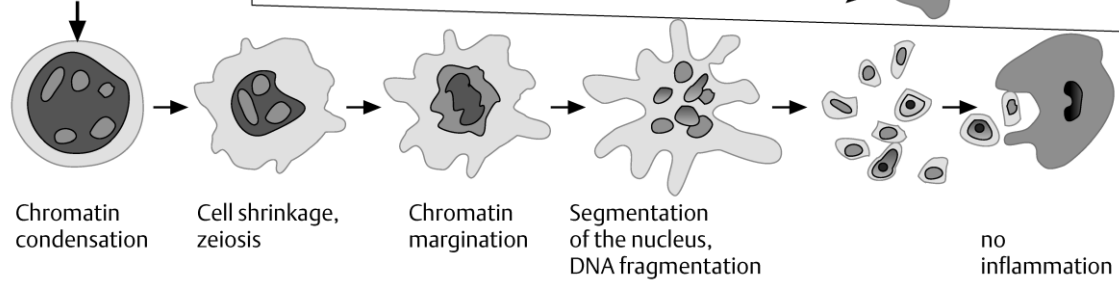
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- 1** Ischemia
 - Hyperthermia
 - Hypothermia
 - Physical or chemical damage
 - Trauma



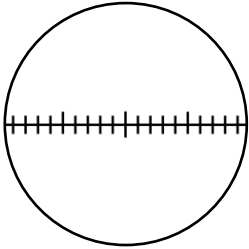
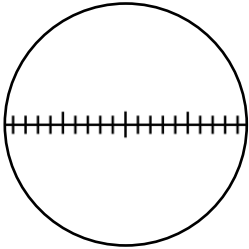
What are the two phenomena are depicted in the diagram. Give explanations.

2 signal



Write major allergens of drug allergy:

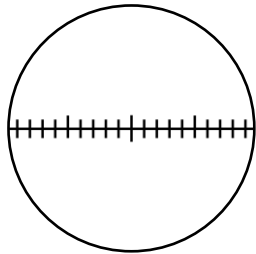
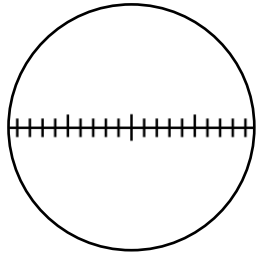
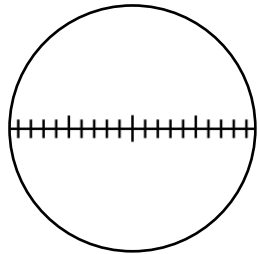

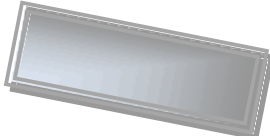
Practical class 15. MICROBIOLOGICAL DIAGNOSTICS OF DISEASES CAUSED BY STAPHYLOCOCCI, STREPTOCOCCI, NEISSERIA

<p>Suggested reading for self-study: Staphylococci, general characteristics. Pathogenicity factors. Staphylococcal infection, including dentistry. Staphylococci as causative agents of nosocomial infections. Methods of staphylococcal infections microbiological diagnostics. The material for the research depending on the infection form. Scheme of pure culture isolation (from pus, mucus, blood, etc.). Identification methods, phagotyping of Staphylococci. Specific prevention and treatment of staphylococcal infections. Streptococci, systematics, general characteristics. Antigenic structure. <i>S. pyogenes</i>, <i>S. pneumoniae</i>, <i>S. mutans</i> and other spp. of the oral cavity. The role in the health and pathology of the oral cavity. Acute and chronic diseases, pathogenesis, immunity. 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 streptococcal infections. Neisseria. Systematics, general characteristics. The role in the health and pathology of the oral cavity. Meningococcus, gonococcus. Pathogenicity factors. Pathogenesis and immunity. Microbiological diagnostics, material for studies. Specific prevention and treatment.</p>		<p>Signature of the tutor _____</p>				
	Or al qui z	Laboratory work	Individual work	Tests	Total results	
<p align="center">Laboratory work — practical class' duration in second semester is 2 academic hours 15 minutes</p>						
<p align="center">Laboratory exercises</p>		<p align="center">Laboratory report</p>				
<p>1. Microbiological diagnostics of staphylococcal infection, 2nd period: – macro- and microscopic examination of the colonies on YSA; – plasmacoagulase test (stabilized rabbit plasma, 37 °C, 2-4-24 h).</p>	Smear _____		<p>Staphylococcal colonies</p>			
	Stain _____		shape (form)			
			size/elevation			
			surface (appearance)			
			edge (margin)			
			pigmentation			
			consistency			
			transparency			
	lecithinase					
<p>Conclusion: according to morphological, cultural and biochemical properties unknown bacterium is identified as _____</p>						
<p>2. Microbiological diagnostics of streptococcal infection, 3rd period: – the description of Streptococci growth in serum broth; – determining the morphology of streptococci, Gram staining; – determination of streptococcus serogroups by ring precipitation test.</p>	Smear _____					
	Stain _____					
<p>Conclusion: according to morphological, cultural and biochemical properties unknown bacterium is identified as _____</p>						

Laboratory exercises	Laboratory report			
<p>3. Demonstration:</p> <ul style="list-style-type: none"> - <i>Staphylococcus aureus</i> in pus, Gram staining; - <i>Streptococcus pneumoniae</i>, pure culture, Gram staining; - <i>S. pneumoniae</i>, white mice, Gram staining; - <i>Neisseria gonorrhoeae</i> in pus, Gram staining; - <i>Neisseria meningitidis</i> in cerebrospinal fluid, methylene blue; - the growth of staphylococci on YSA, blood agar, broth; - the growth of streptococci on blood agar and broth; - coagulase test (plasma); - anaerobic mannitol fermentation; - phage typing of staphylococci. 	Smear _____	Smear _____	Smear _____	Smear _____
	Stain _____	Stain _____	Stain _____	Stain _____
	Smear _____			
	Stain _____			

INDIVIDUAL WORK																																											

Practical class 16. MICROBIOLOGICAL DIAGNOSTICS OF ACUTE ENTERIC INFECTIONS CAUSED BY ENTEROBACTERIA. METHODS FOR FOOD POISONING DIAGNOSTICS

<p>Suggested reading for self-study: General characteristics of Enterobacteriaceae family. Escherichia, general characteristics. The biological role of Escherichia coli in health and pathology. Salmonella, classification and general characteristics. The role in the pathology, the pathogenesis of typhoid, manifestations in the oral cavity. Shigella, classification, general characteristics. The role in pathology. Common principle of microbiological diagnosis of acute intestinal infection. Etiology of food poisoning. Principles of microbiological diagnostics.</p>		<p>Signature of the tutor _____</p>				
		Oral quiz	Laboratory work	Individual work	Tests	Total results
Laboratory work						
Laboratory exercises	Laboratory report					
<p>1. Demonstration: – <i>E. coli</i>, pure culture, Gram staining; – <i>Salmonella typhi</i> pure culture, Gram staining; – <i>Shigella flexneri</i> pure culture, Gram staining; – clean media: Endo, Levin, Ploskirev, bismuth sulfite agar, Rapoport, magnesium, Kliglera; – the same media with the growth of <i>E. coli</i>, <i>Salmonella</i>, <i>Shigella</i>; – biochemical activity of <i>E. coli</i> and <i>Salmonella</i>;</p> <p>2. Slide agglutination test with diagnostic O and H-serum for identification of <i>Salmonella</i>.</p>	<p>Smear _____ Stain _____</p> <div style="text-align: center; margin: 20px 0;">  </div> <p>Smear _____ Stain _____</p> <div style="text-align: center; margin: 20px 0;">  </div>	<p>Smear _____ Stain _____</p> <div style="text-align: center; margin: 20px 0;">  </div> <p>Slide agglutination test</p> <div style="display: flex; align-items: center; justify-content: space-around;">   <div style="text-align: right;"> <p>Conclusion:</p> </div> </div>				

Practical class 17. FINAL TEST “GENERAL MICROBIOLOGY. IMMUNOLOGY”

List of questions	Oral quiz	Script	Tests	Total results
<ol style="list-style-type: none"> 1. Microbiology: definition, area and fields of microbiology, methods of investigation. Dental microbiology: goals, objectives, role in the dentist’s practice. 2. Milestones (periods) in microbiology. Work of Louis Pasteur, Robert Koch, Ilya Mechnikov. Evolution of microorganisms and infectious diseases. 3. Common with other organisms and the unique features of microorganisms. Principles of microorganisms systematics . Classification and nomenclature of microorganisms. The term of “species” in bacteria: group of traits for species identification (criteria for speciation). 4. Morphology of bacteria. Basic morphological forms of bacteria. The bacterial cell structure. Functions of the surface and cytoplasmic structures of the bacterial cell. Mechanism of Gram staining. Forms of bacteria with the cell wall defects. 5. Unique features of metabolism in prokaryotes. Nutrition of bacteria: types, requirements of bacteria, nutrients and pathways of nutrients penetration into the bacterial cell. Nutrient media: specification (what they should be to provide the best growth of bacteria), classification. 6. Respiration of microorganisms: types, pathways of energy production. Enzymes and cell structures involved into the process of respiration. Classification of bacteria regarding their oxygen requirements. 7. Growth and reproduction of bacteria. The mechanism of simple division and its phases. Dormant forms of microorganisms: general characteristics, factors inducing their formation, medical importance. 8. Sampling for microbiological studies: types of samples, the rules of sampling, storage, transportation. Principles of organization, equipment and levels of biosafety in microbiological laboratories. 9. Microscopic (bacterioscopic) method of diagnosing the infectious diseases: definition, aim and tasks, steps and evaluation of specificity, sensitivity, disadvantages of the method. Types of microscopic preparations. Staining of microorganisms: methods. Types of microscopes. 10. The bacteriological method of the infectious diseases diagnosing: aim, tasks, phases, and evaluation of specificity, sensitivity, disadvantages of the method. 11. Methods for isolation identification of aerobic and anaerobic bacteria pure cultures. Identification of microorganisms without pure culture isolation. 	<ol style="list-style-type: none"> 12. Genetic apparatus of bacteria (nucleoid, plasmids, transposons, IS-elements) characteristics, functions, effect and importance. The concept of genetic engineering and biotechnology. 13. Inheritance and variability of microorganisms. Types of variability. Mutations. The genetic recombination of bacteria. Phenotypic variability. The practical significance of the variability of microorganisms in the diagnosis, treatment and prevention of infectious diseases. 14. Molecular biological method of diagnosing the infectious diseases (molecular hybridization, polymerase chain reaction): definition, the principle of the methods, application in dentistry. 15. Effect of physical and chemical factors on microorganisms. Disinfection: definition of the term, aim and tasks, types, disinfectants, methods of disinfection quality control. 16. Sterilization: the term definition, methods, quality control. Sterilization of instruments and medical devices. Consequences of sterilization errors. 17. Infection (infection process): the term definition, causes and conditions of infectious diseases emergence. Differences in communicable and non-communicable diseases. Periods of infectious diseases. Infectious disease classification and outcomes. 18. The role of microorganisms in the infectious process. Pathogenicity and virulence. Factors of pathogenicity of microorganisms. Pathogenicity Island. Microbial toxins. Types of exotoxins and their biological properties. Mechanisms of microbial persistence and latency in host’s organisms. 19. The role of host, social, environmental factors in the infectious process. 20. The biological (experimental) method of diagnosing the infectious diseases: definition of the term, aim, tasks, phases, evaluation. Disbiosis: causes, consequences, prevention. Gnotobiology. 21. The ecology of microorganisms. Types of ecological relationships in microorganisms. The role of microorganisms in the genesis and development of the Biosphere (the concept of the microbial dominance). Spread of microorganisms in the nature. 22. The chemotherapy and chemoprophylaxis of infectious diseases. Groups of antimicrobial chemotherapeutic agents, mechanisms and spectrum of action on microbial cells. Chemotherapeutic index. 			

- | | |
|--|---|
| <p>23. The antiseptics: definition of the term, types, categories, methods of application. Antiseptic agents: classification, mechanism of action, side effects. Principles of rational antiseptics in dental practice.</p> <p>24. The antibiotics: characteristics, classification, mechanisms of action. The rational antibiotic therapy principles. Antibiotics for bacterial complications prophylaxis. Side effects of antibiotics.</p> <p>25. Natural and acquired resistance of microorganisms to antibiotics. The genetic and biochemical mechanisms of microorganisms resistance. Genotypic and phenotypic methods for determining the microorganisms susceptibility to antibiotics.</p> <p>26. Immunology: definition of the term, aim and task, methods, history of development, branches. Immunity: definition, types of immunity.</p> <p>27. The immune system. Central and peripheral organs of the immune system. Immunocompetent cells: classification, function, molecules.</p> <p>28. Innate immunity. Innate immunity versus acquired immunity. Immune and non-immune factors of innate immunity. Mechanisms of recognition in the innate immune system.</p> <p>29. The complement system: definition, main components, activators and activation pathways, functions of components and their fragments. Methods of the complement system activity evaluation.</p> <p>30. Natural killer cells and mechanisms of cytotoxicity. Phagocytes, classification. Phagocytosis reaction: phases, mechanisms of intracellular microorganisms killing, outcomes. Methods of phagocytosis evaluation. Phagocytic reaction indexes, definition and importance in clinical practice.</p> <p>31. Antigens: structure, properties, classification. T-dependent and T-independent antigens. Superantigens.</p> <p>32. Antigens of microorganisms. Antigenic structure of bacteria. Type, species, group antigens. Protective antigens. Cross-reactive antigens, medical importance.</p> <p>33. Immune response: definition, conditions for development. Humoral immune response: definition, development. Activation, proliferation, differentiation and interactions of cells involved. T-dependent and T-independent response. Primary and secondary humoral immune response characteristics.</p> <p>34. B cells: development, markers, antigen-specific B-cell receptor. Methods for B-lymphocytes quantity and functional activity as saying.</p> <p>35. Antibodies (immunoglobulins): structure, properties, classification, immunoglobulins biosynthesis. The mechanism of interaction of antibodies with antigens: specificity, phases, manifestations. Affinity and avidity.</p> | <p>36. Methods for the immunoglobulins concentration detection: simple radial immunodiffusion, ELISA, nephelometry. Monoclonal antibody: principles of production, application.</p> <p>37. Serological method of investigation: general definition of the term, objectives, basic concepts (diagnosticum, diagnostic serum, titer, diagnostic titer, paired sera). Samples for serological examination. General characteristics of the method. Use of serological method for infectious and non-infectious diseases diagnostics.</p> <p>38. Agglutination: ingredients, main variants of performance, registration, evaluation, application. Indirect (passive) and reverse passive agglutination: ingredients, mechanism, methodology, registration of results, practical use.</p> <p>39. Immunoprecipitation reaction: ingredients, mechanism, main methods of performance, application. Reaction of the immune lysis. Complement fixation test: ingredients, mechanism, registration of results.</p> <p>40. Solid phase immunoassay reactions. Immunofluorescence (fluorescent antibodies test, FAT), main variants, ingredients, mechanisms, registration of results, practical use. ELISA: ingredients, mechanisms, registration of results, practical use. Immunoblotting (IB). Radioimmunoassay (RIA).</p> <p>41. T-cells: development, markers, subpopulations. Helper T-cells, main types (Th1, Th2, Th3, Th17), spectrum of cytokines produced. Control of the immune response of T lymphocytes (Th3, T-regulators, CD4+CD25+Tcells). Methods for assaying of the amount and functional activity of T-lymphocytes.</p> <p>42. T-cell receptor: structure, types, genetic control, variety. T-dependent antigens. T-cell epitopes. T-cell restriction.</p> <p>43. Cellular immune response: definition, development, main periods, manifestation. The model of two (three) signals: the response, anergy, apoptosis. Manifestation of cellular immune response. Immunological memory.</p> <p>44. Anti-infection immunity and its types depending on pathogen nature. Innate and acquired defines mechanisms. Protective immunity. Mechanisms of antitoxic, antibacterial, antifungal, antiparasite immunity. Maternal immunity: mechanisms, significance.</p> <p>45. Immunoprophylaxis and immunotherapy for infectious diseases. Active immunoprophylaxis. Vaccines: requirements, characteristics of main vaccines types (live, inactivated (corpuscular, chemical, conjugated, split, subunit), toxoids, genetic engineered). The concept of "ideal vaccine". Adjuvants mechanisms of action. New approaches for the vaccine development. Side effects of vaccination: sever vaccinal reaction, post-vaccination complications.</p> |
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<p>46. Post-vaccination immunity: mechanisms and factors influencing its development. Indications and contraindications to vaccination. Immunization schedule. Expanded Programme on immunization. Collective immunity to infectious diseases, importance.</p> <p>47. Passive immunoprophylaxis and immunotherapy of infectious diseases: indications, principles, complications. Classification of serum preparations (specificity, the manufacturing method, object of the antibodies action, purpose).</p> <p>48. Allergology: the definition, objectives. Allergens. Allergy: the periods, types of reactions.</p> <p>49. Allergic reaction in the oral cavity. Allergic method of investigation: definition, objectives, general characteristics, periods, evaluation.</p> <p>50. Immediate type hypersensitivity (ITH). Mediator type (I) ITH: allergens, mechanism, development, manifestation, prevention of anaphylaxis. Cytotoxic (II) type ITH: allergens, development, mechanisms, manifestations. Immunocomplex (III) type ITH: allergens, development, mechanisms, manifestations.</p> <p>51. Delayed type of hypersensitivity (IV): allergens, development, mechanism, manifestation (infection and contact allergy), importance in oral cavity.</p>	<p>52. Drug allergy: major allergens, the mechanisms and types of allergic reactions, methods for diagnostics and prevention.</p> <p>53. Food allergy. Main allergens. Prevention of food allergy. Paraallergy. Idiosyncrasy.</p> <p>54. Autoantibodies: origin, role in the pathology. Autoimmune diseases: definition, classification, etiology, mechanisms of tissue damage, manifestations. Principles of treatment. Prophylaxis.</p> <p>55. Transplantation immunity. Histocompatibility antigens. Graft reaction types, mechanisms of development, prevention. Immunological tolerance: mechanisms, significance.</p> <p>56. Clinical Immunology: definition, objectives, main concepts. Immune status: principle and methods of examination. Immunogram. Immunodeficiency conditions: classification, causes of development, methods for detection, principles for correction. Antitumor immunity. The concept of immune surveillance. Mechanisms of tumour escape from immune surveillance.</p>
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	MICROBIOLOGY	IMMUNOLOGY
INDIVIDUAL WORK		
TEST		
PRACTICAL SKILLS		
AVERAGE GRADE		
ABSENCE FROM PRACTICAL CLASS		
ABSENCE FROM LECTURE		
RATING		
Credit (CROSS)	«PASSED»	«NOT PASSED»

Practical class 1(18). MICROBIOLOGICAL DIAGNOSTICS OF DISEASES CAUSED BY KLEBSIELLA, CAMPYLOBACTER, HELICOBACTER AND PSEUDOMONADA

Suggested reading for self-study:

Klebsiella, classification and general characteristics, main diseases caused.
 Campylobacter, general characteristics, role in human pathology. Mechanisms of pathogenesis. Diagnosis of campylobacteriosis. Helicobacter.
 Pseudomonas aeruginosa, general characteristics, role in human pathology.

Signature of the tutor _____

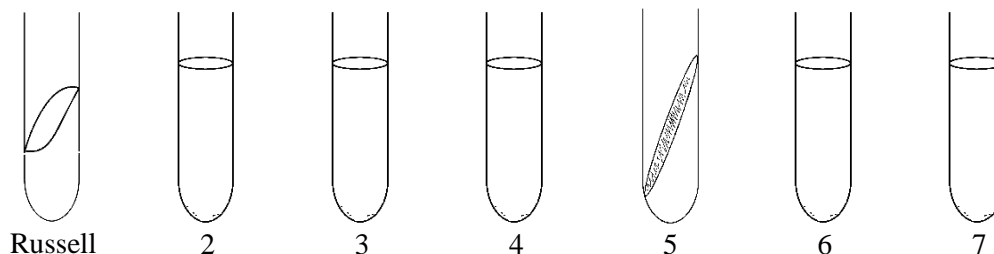
Oral quiz	Laboratory work	Individual work	Tests	Total results

Laboratory work

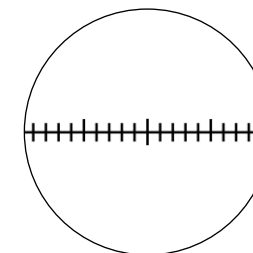
Laboratory exercises

1. Microbiological diagnostics of Klebsiellosis, 3rd period:
- determine the biochemical properties of Klebsiella;
 - perform slide agglutination test with anti-capsule diagnostic sera and determine the K-antigen;
 - determine the titer of CFT for serological diagnosis of Scleroma.

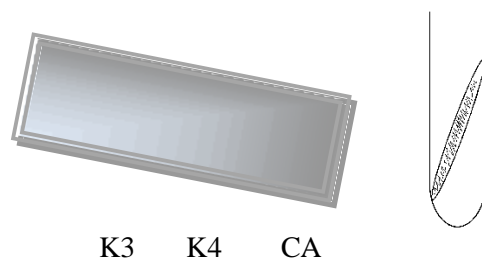
Laboratory report



Smear _____
 Stain _____


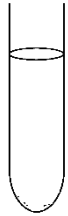


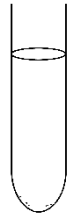
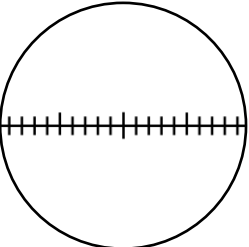
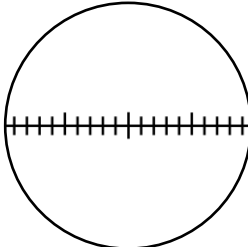
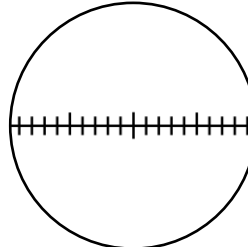
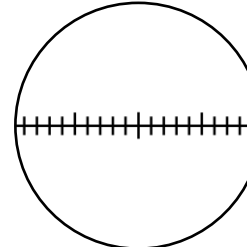


Slide agglutination test with anti-capsule serum



Conclusion: _____

Biochemical properties	K. pneumoniae		
	s. rhinoscleromatis	s. ozaenae	s. pneumoniae
1, 2 Glucose (A+G)	-	+/-	+
1, 3 Lactose	-	+/-	+
4 Saccharose (4 th day)	-	+/-	+
5 Citrate	-	+/-	+
6 Urea	-	-/+	+
7 Malonate	+	-	+
8 Antigens	O2a:K3	O2b:K4	O1,3-5:K1-3

Laboratory exercises	Laboratory report											
2. Demonstration: – <i>K. pneumonia s. rhinoscleromatis</i> capsule (Hins–Burri staining); – <i>K. pneumonia s. rhinoscleromatis</i> , pure culture, Gram staining; – <i>Pseudomonas aeruginosa</i> , pure culture, Gram staining; – <i>C. jejuni</i> , pure culture, Gram staining; – Klebsiella growth on differential diagnostic media; – oxidase test.	1 : 5	1 : 10	1 : 20	SA	AC	COMPLEMENT FIXATION TEST						
						Var	Serum dilutions			SC	AC	Result
				1 : 5	1 : 10		1 : 20					
				1	++++	++++	++++	–	–	Very positive		
				2	++++	++++	–	–	–	Positive		
			3	+++	–	–	–	–	Slight positive			
			4	–	–	–	–	–	Negative			
Smear _____ Smear _____ Smear _____ Smear _____												
Stain _____ Stain _____ Stain _____ Stain _____												
<div style="display: flex; justify-content: space-around; align-items: center;">     </div>												

Practical class 2 (19). MICROBIOLOGICAL DIAGNOSIS METHODS OF DISEASES CAUSED BY CORYNEBACTERIA, BORDETELLA

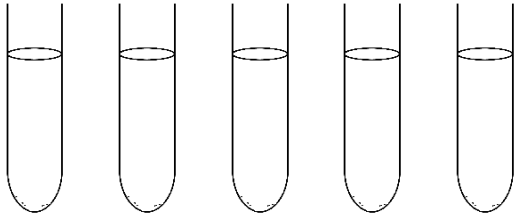
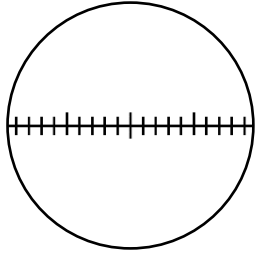
Suggested reading for self-study:

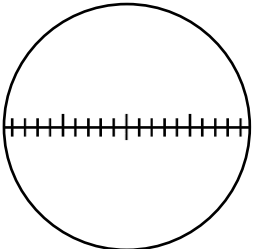
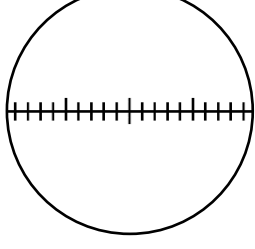
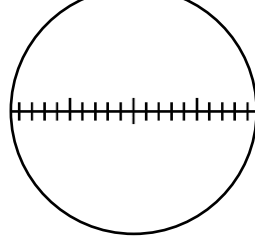
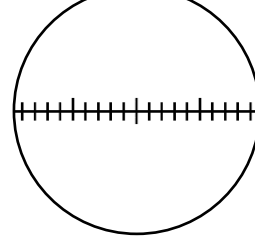
Corynebacterium diphtheria, general characteristics of the pathogen. Types of Corynebacterium diphtheria, their distinctive features. Diphtheria toxin and antitoxic serum. The pathogenesis of diphtheria. Diphtheria in the oral cavity. Methods of diphtheria microbiological and molecular biological diagnosis. Principles of diphtheria therapy and prevention.

Bordetella pertussis and parapertussis. Characteristics of the pathogen, pathogenicity factors. The pathogenesis of pertussis, manifestation in the oral cavity, immunity, diagnostics. Principles of pertussis therapy and prevention.

Oral quiz	Laboratory work	Individual work	Tests	Total results
Signature of the tutor _____				

Laboratory work

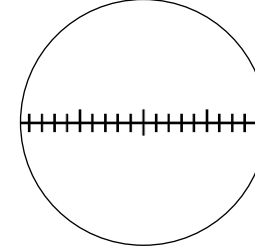
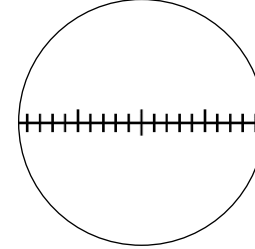
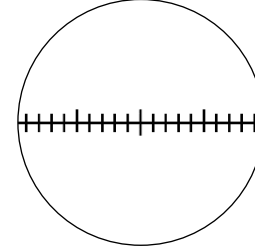
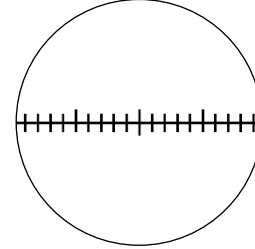
Laboratory exercises	Laboratory report					
1. Bacteriological diagnosis of diphtheria, the 2 nd period: – describe the colonies Corynebacterium on potassium tellurite serum agar; – seed bacteria from typical colonies into Hiss media (glucose, sucrose, starch).	Smear _____	Feature	Colonies on serum tellurite agar			
	Stain _____	Shape				
		Size				
		Surface				
		Edge				
		Color				
		Consistency				
	Biochemical properties of certain corynobacteria					
			Enzymatic activity			
			with Acid production			Cysteinase
Corynobacteria spp.	Glucose	Sucrose	Starch			
	+	–	+	+	–	
	+	–	–	+	–	
	–	–	–	–	+	
	+	+	–	–	+	
	+	–	+	+	+	
	<i>X-microbe</i>					
Conclusion: according to morphological, cultural and biochemical properties unknown bacterium is identified as _____						

2. Demonstration: – <i>Corynebacterium diphtheria</i> stained by Neisser; – <i>C. diphtheria</i> stained by Leffler; – <i>Bordetella pertussis</i> , Gram staining; – test for <i>Corynebacterium diphtheria</i> toxigenicity; – preparations for specific prevention and treatment of diphtheria and pertussis; – Growth of <i>Bordetella pertussis</i> and parapertussis on CCA, NA with tyrosine, urease test; – assessment of antidiphtheria immunity intensity.	Smear _____	Smear _____	Smear _____	Smear _____
	Stain _____	Stain _____	Stain _____	Stain _____
				

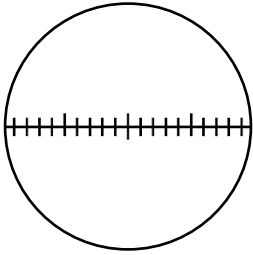
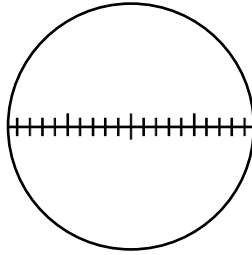
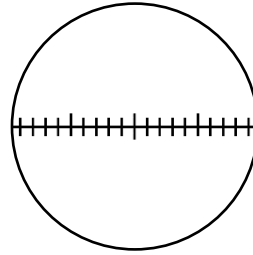
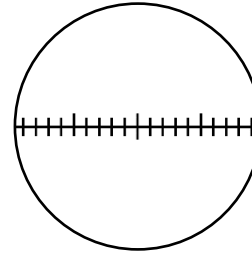
Practical class 3 (20). MICROBIOLOGICAL DIAGNOSIS METHODS OF DISEASES CAUSED BY MYCOBACTERIA AND ACTINOMYCETES

Suggested reading for self-study: Actinomycetes, systematic position, general characteristics, prevalence, role in the oral cavity pathology. Etiology, pathogenesis, microbiological diagnostics principles of the head and neck tissues actinomycosis. Mycobacteria, general characteristics, resistance to acids. The causative agents of tuberculosis, species composition, morphology, nutritional needs, pathogenicity factors, differences from non-tuberculosis mycobacteria. The pathogenesis of tuberculosis, infectious granuloma, immunity, allergy, anergy. Principles of microbiological diagnostics of tuberculosis, immunoprophylaxis. TB chemotherapeutic drugs. TB symptoms in the oral cavity.	Oral quiz	Laboratory work	Individual work	Tests	Total results
Signature of the tutor _____					

Laboratory work

Laboratory exercises	Laboratory report			
1. Bacteriological diagnosis of diphtheria, the 3 rd period: – the assessment of Corynobacteria enzymatic activity, identification, conclusion. 2. Demonstration: – Cord factor of <i>M. tuberculosis</i> , Ziehl–Neelsen staining; – <i>Actinomycetes spp.</i> , pure culture, Gram staining; – <i>M. leprae</i> , Ziehl–Neelsen staining; – <i>M. tuberculosis</i> in sputum, Ziehl–Neelsen staining; – Mycobacteria growth on nutrient media; – Flotation method; – determination of <i>M. tuberculosis</i> drug resistance.	Smear _____	Smear _____	Smear _____	Smear _____
	Stain _____	Stain _____	Stain _____	Stain _____
				

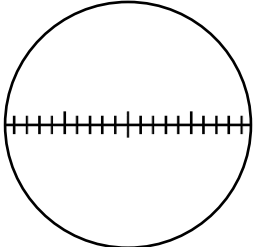
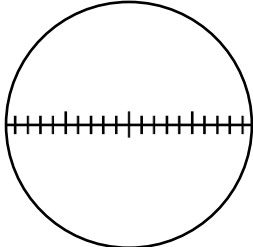
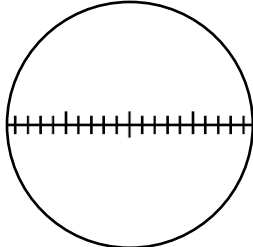
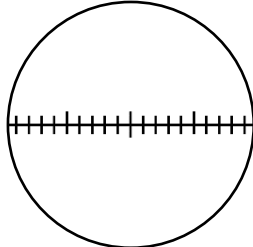
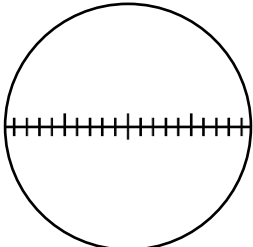
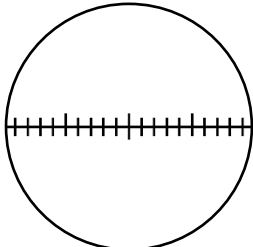
Practical class 4 (21). METHODS OF ANAEROBIC INFECTIONS MICROBIOLOGICAL DIAGNOSTICS

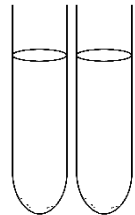


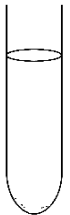
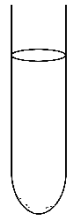
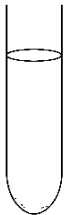

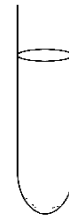
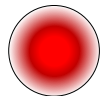
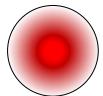
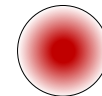
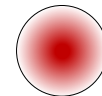
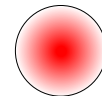
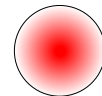
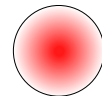
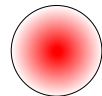
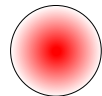
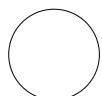
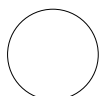
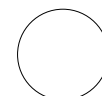
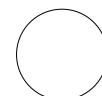
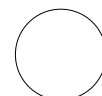
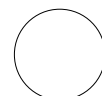
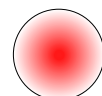
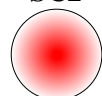
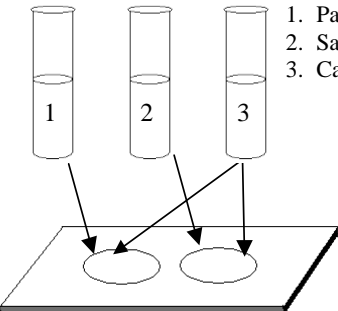
<p>Suggested reading for self-study: Anaerobes, classification, general characteristics. Non-spore anaerobes of the oral cavity (streptococci, bacteroides, fusobacteria, peptococci, peptostreptococci, veillonella, fusobacterial, leptotrichi, prevotella, bilophila), role in pathology. Causative agents of gas gangrene, tetanus, botulism, general characteristics. Pathogenicity factors, exotoxins. Clostridium role in dentistry. General principles and methods for anaerobic infections diagnosis. Molecular biological diagnostics — PCR. Principles of anaerobic infections therapy and prevention.</p>				Oral quiz	Laboratory work	Individual work	Tests	Total results	
				<p>Signature of the tutor _____</p>					
Laboratory work									
Laboratory exercises			Laboratory report						
<p>1. Bacteriological diagnosis of diphtheria, the 3rd period: – the assessment of Corynebacteria enzymatic activity, identification, conclusion. 2. Demonstration: – Clostridium, Gram staining; – Bacteroides, Gram staining; – veillonella spp., Gram staining; – fusobacterial spp., Gram staining; – anaerobes growth on nutrient media.</p>			Smear _____	Smear _____	Smear _____	Smear _____	Stain _____	Stain _____	Stain _____
			Stain _____	Stain _____	Stain _____	Stain _____	Stain _____	Stain _____	Stain _____
									

Practical class 5 (22). MICROBIOLOGICAL DIAGNOSTICS OF DISEASES CAUSED BY SPIROCHETES, RICKETTSIA, CHLAMYDIA, MYCOPLASMA

<p>Suggested reading for self-study: Spirochetes, classification, general characteristics. Treponema. Systematics and general characteristics. Pathogenesis and immunity in syphilis, manifestations in the oral cavity. Methods of syphilis microbiological diagnosis. Principles of syphilis therapy and prevention. Fusospirochetosis pathogens. Leptospira, Borrelia. Role in human pathology. The causative agent of Lyme borreliosis. Rickettsiae, systematic position, classification, general characteristics, role in human pathology. Rickettsia typhi, pathogenesis, immunity and methods of microbiological diagnostics. Other pathogenic rickettsia. Chlamydia, systematics and general characteristics, role in human pathology. Mycoplasma, systematics and general characteristics, role in human pathology.</p>	Oral quiz	Laboratory work	Individual work	Tests	Total results
	<p>Signature of the tutor _____</p>				

Laboratory work

Laboratory exercises	Laboratory report			
<p>1. Demonstration: – <i>Leptospira</i> spp., dark field microscopy; – <i>Borrelia recurrentis</i> in blood, Romanovsky–Giemsa staining; – <i>Treponema</i> spp. in dental plaque, Gram staining; – <i>Treponema pallidum</i>, pure culture; Romanovsky–Giemsa staining; – <i>Chlamydia</i> spp. in cell culture, Romanovsky–Giemsa staining; – <i>R. prowazeki</i>, pure culture, Zdrodovski staining; – Wasserman test (ELISA).</p>	Smear _____ Stain _____	Smear _____ Stain _____	Smear _____ Stain _____	Smear _____ Stain _____
				
	Smear _____ Stain _____	Smear _____ Stain _____		
				

Laboratory exercises	Laboratory report											
2. Assess CFT for the epidemic typhus diagnostics.	4. CFT		1 : 20	1 : 40	1 : 80	1 : 160	1 : 320			SC	AC	
												
	Key “+” “-“		Assess:									
	Conclusion:											
3. Passive blood agglutination test for differential diagnostics of epidemic and residual typhus.	PASSIVE BLOOD AGGLUTINATION TEST											
	1/10	1/20	1/40	1/80	1/160	1/320	1/640		SC1	AC		
												
												
Conclusion:												
4. Perform the slide microprecipitation reaction (VDRL) for the syphilis serodiagnosis. 5. Assess ELISA (Wasserman test) for the syphilis diagnostics.				1. Patient serum 1:20 2. Saline sol. 3. Cardiolipin Ag Slide microprecipitation reaction (VDRL) for the syphilis serodiagnosis Conclusion:	Assess ELISA (Wasserman test) for the syphilis diagnostics. Conclusion:							

Practical class 6 (23). TEST “SPECIAL BACTERIOLOGY”

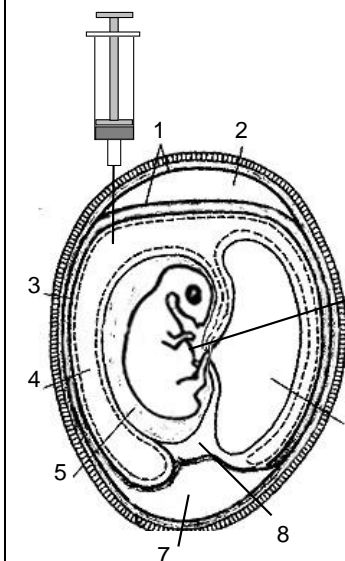
List of questions	Oral quiz	Script	Tests	Total results
<p>1. Staphylococci, classification, general characteristics. Staphylococcal infections, pathogenesis and immunity. Role in oral cavity pathology. Microbiological diagnosis. Principles of staphylococcal infections treatment and prevention.</p> <p>2. Streptococci, classification, general characteristics, antigenic structure. Acute and chronic streptococcal infections. Oral streptococci. The role of streptococci in oral pathology. Methods of streptococcal infections diagnostics. Principles of therapy and prophylaxis.</p> <p>3. Classification of Neisseria. Meningococcus, general characteristics. Meningococcal infections, mechanisms of pathogenesis, immunity, methods of diagnosis, prevention.</p> <p>4. Gonococci, general characteristics. Mechanisms of pathogenesis and immunity. Microbiological diagnosis of acute and chronic gonorrhoea. Principles of therapy and prophylaxis. Gonorrhoeal stomatitis.</p> <p>5. General characteristics of the family. Enterobacteriaceae.</p> <p>6. General Principles of acute intestinal infections (AII) bacteriological diagnosis. E. coli, common characteristic. The biological role of Escherichia coli. Diseases caused by Escherichia.</p> <p>7. Salmonella. General characteristics. Members of the genus. Diseases caused by Salmonella.</p> <p>8. Pathogens of typhoid, paratyphoid A and B, general characteristic. Pathogenesis, immunity, prophylaxis and methods of microbiological diagnosis of typhoid and paratyphoid.</p> <p>9. The etiology of bacterial origin food poisoning and intoxication. Materials and methods of diagnosis.</p> <p>10. Shigella. Classification. Characteristics. Pathogenesis, immunity of dysentery.</p> <p>11. Klebsiella, general characteristics. Role in human pathology. Methods of klebsiellosis microbiological diagnostics.</p> <p>12. Pseudomonas aeruginosa, general characteristics, pathogenicity factors. Role in human pathology.</p> <p>13. C.diphtheria, general characteristics. Pathogenesis of diphtheria. Manifestation of diphtheria in oral cavity. Immunity in diphtheria. Methods of microbiological diagnostics, principles of diphtheria therapy and prevention.</p> <p>14. The causative agent of whooping cough, general characteristics. Differentiation with paraptussis agent. Pathogenesis, immunity. Microbiological diagnosis, principles of pertussis treatment and prevention.</p> <p>15. Actinomycetes, general characteristics. Role in the oral cavity pathology. Actinomycosis, characteristic of pathogen diagnostic techniques.</p> <p>16. Classification of Mycobacteria. General characteristics of the tuberculosis causative agents. Pathogenesis, immunity, diagnostic, principles of tuberculosis therapy and prophylaxis. Manifestations of tuberculosis in the oral cavity.</p>	<p>17. Quarantine infection. Classification mode. Basic rules of infectious material sampling, sending and transportation. General principles of diagnosis.</p> <p>18. V.cholera, general characteristics. Pathogenesis, immunity, principles of treatment and prevention.</p> <p>19. Classification and general characteristics of anaerobes. Clostridia. Nonspore anaerobes. Role in the oral cavity pathology. 20. The causative agent of tetanus, general characteristics. Pathogenesis, immunity, principles of tetanus treatment and prevention. Gas gangrene pathogens, general characteristics. Pathogenesis, principles of gas gangrene treatment and prevention.</p> <p>21. The causative agent of botulism, general characteristic. Pathogenesis, principles of botulism prevention and therapy.</p> <p>22. Methods of anaerobic infections diagnosis.</p> <p>23. Classification and general characteristics of spirochetes. Borreliosis and leptospirosis agents.</p> <p>24. Classification of treponemes and treponemal diseases. Characteristics of syphilis causative agent. Pathogenesis, immunity, principles of syphilis therapy and prophylaxis, manifestations in the oral cavity. Methods of syphilis diagnosis.</p> <p>25. Oral spirochetes. Fusospirochaetosis.</p> <p>26. Rickettsia. Role in human pathology. Pathogenesis, immunity, methods of typhus diagnosis.</p> <p>27. Chlamydia. Role in human pathology. Pathogenesis, immunity, methods of diagnosis.</p> <p>28. Mycoplasma. Role in human pathology. Pathogenesis, immunity, methods of diagnosis.</p>			
	<p>Practical skills</p> <p>1. Determine the morphology of Staphylococcus, pure culture, Gram stain.</p> <p>2. Determine the morphology of Streptococcus, pure culture, Gram stain.</p> <p>3. Determine the morphology of Gonococci in pus, Gram stain.</p> <p>4. Determine the morphology of Enterobacteria, pure culture, Gram stain.</p> <p>5. Determine the morphology of the mixture of S.aureus and Escherichia coli, Gram stain.</p> <p>6. Determine the morphology of B.anthraxis, pure culture, Gram stain.</p> <p>7. Determine the morphology Vibrio, pure culture, Gram stain.</p> <p>8. Determine the morphology of Brucella, a pure culture, Gram stain.</p> <p>9. Determine the morphology Corynebacteria, pure culture, Leffler stain.</p> <p>10. Determine the morphology of Klebsiella, pure culture, Hins-Burri stain.</p> <p>11. Determine the morphology of Mycobacteria in sputum, Ziehl-Neelsen stain.</p> <p>12. Determine the biochemical properties of enterobacteria on Kligler iron agar medium.</p>			

Practical class 7 (24). METHODS OF INVESTIGATIONS IN VIROLOGY. BACTERIOPHAGES

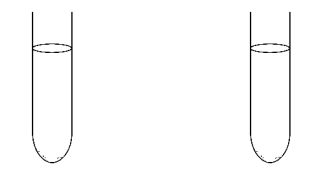



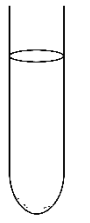
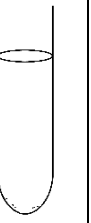

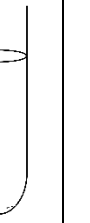
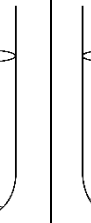

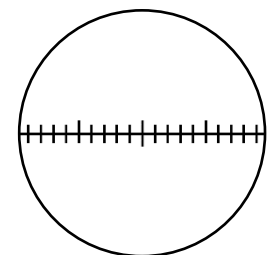
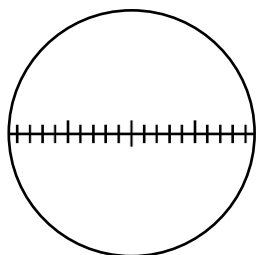
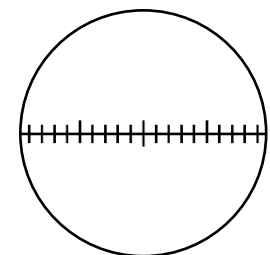
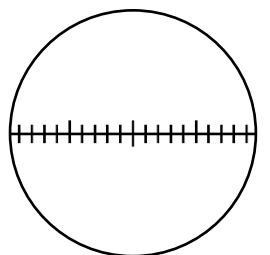
Suggested reading for self-study: Viruses. Taxonomy and morphology of viruses. Mechanisms of reproduction. Strict parasitism and cytotropism of viruses. The types of viral infection. The mechanisms of antiviral immunity. Principles for the prevention of viral infections in the dental practice. Methods of viral infections diagnostics. Culturing of viruses. Viruses of bacteria (bacteriophages), characteristics of bacteriophages. Use of bacteriophages in medical practice.	Oral quiz	Laboratory work	Individual work	Tests	Total results
	Signature of the tutor				

Laboratory work

Laboratory exercises	Laboratory report
<p>1. Chicken embryo inoculation with influenza virus in allantois cavity.</p>	<p>1. Study the structure of hen embryo (8–11 days)</p> <p>2. Examine hen embryo in ovoscope and determine the vitality signs:</p> <ol style="list-style-type: none"> the dimensions of the embryo shape presence of the developed blood vessels pattern active mobility of the embryo mark the air cavity border <p>3. Set embryo on the egg rack and work with the shell as follows:</p> <ol style="list-style-type: none"> 70 % alcohol 5 % iodine <p>4. Inoculate embryo as follows:</p> <ol style="list-style-type: none"> flame scissors carefully pierce the shell for 3–5 mm above the air cavity border introduce 0.2 ml of viral material (live influenza vaccine) into the syringe put the needle into the embryo (25 mm) vertically and introduce the material. <p>5. Repeat the shell manipulations according to p.3.</p> <p>6. Seal the shell with tape or melted wax. Mark the embryo (group number).</p> <p>Inoculation of the Allantois cavity:</p> <ol style="list-style-type: none"> Use cotton wool and 70 percent alcohol to swab the eggs end to be inoculated. Allow the alcohol to evaporate. Swab the eggshell punch with 70 percent of alcohol solution. Place used cotton wool in discard tray. Pierce a hole in the end of the egg at the marked inoculation site. Attach needle to 1 mL syringe. Draw inoculum into 1 mL syringe. Keeping the needle and syringe vertically, run through the eggshell hole approximately for 16 mm into the egg to reach the allantois cavity. Inject 0.1 mL of inoculum into the egg. Take the needle out from the egg. Seal the hole in the shell with stationery tape or melted wax. Discard the used needles and syringes. Put the inoculated eggs into an incubator.



- Shell membrane
- Air sac
- Chorioallantoic membrane
- Allantois cavity
- Amnion cavity
- Yolk sac
- Albumin
- Extraembryonic cavity
- Embryo

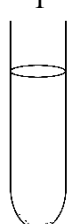


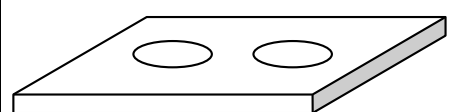
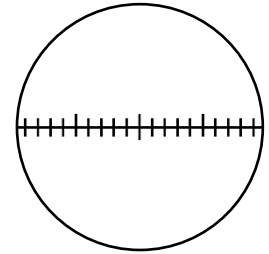
2. Virus titration by color test.		10⁻¹	10⁻²	10⁻³	10⁻⁴	10⁻⁵	10⁻⁶	10⁻⁷	CC	VC
	KEY									
										
	pH>=7,2 pH<7,2									
	Conclusion:									
3. Demonstration: – chicken fibroblasts, eosin stain; – Hep2 cell line, normal, eosin stain; – cytopathic effect of adenoviruses, eosin stain; – hemadsorption test.	Smear _____	Smear _____	Smear _____	Smear _____						
	Stain _____	Stain _____	Stain _____	Stain _____						
										

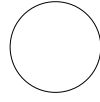
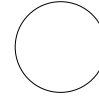
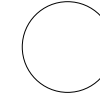
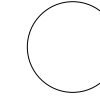
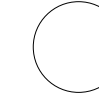
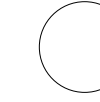
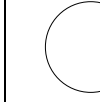
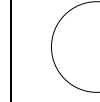
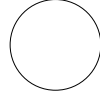
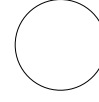
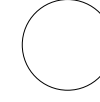
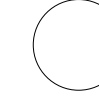
INDIVIDUAL WORK							
According to Baltimore classification, viruses are divided into the following seven classes (fill table)							
Class	I	II	III	IV	V	VI	VII
Description of genome and replication strategy							
tip	T-C-A-G A-G-T-C	T-C-A-G	U-C-A-G A-G-U-C	U-C-A-G	U-C-A-G-	U-C-A-G↑	T-C-A-G ↓ A-G-T-C

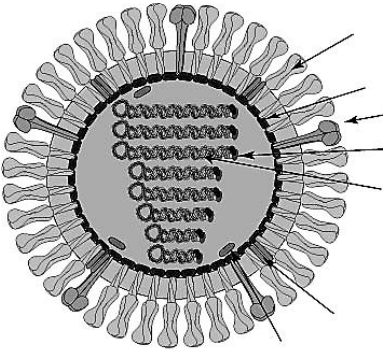
Practical class 8 (25). VIROLOGY DIAGNOSTICS OF DISEASES CAUSED BY ORTHOMYXOVIRUSES, PARAMYXOVIRUSES. TOGAVIRUSES

<p>Suggested reading for self-study: Orthomyxoviruses. Taxonomy and characteristics of the family. Influenza viruses, morphology, antigenic structure and antigenic diversity (shift and drift) and its consequences. Methods for influenza diagnostics. Principles of therapy and prophylaxis. Paramyxoviruses. Taxonomy and characteristics of the family. Differentiation with Orthomyxoviruses, Parainfluenza viruses, Mumps virus, Morbillivirus, HRSV. Pathogenesis, immunity, specific prophylaxis. Rubella virus. General characteristics. Role in pathology. Manifestations of rubella in the maxillofacial region. Prevention of rubella.</p>	Oral quiz	Laboratory work	Individual work	Tests	Total results
	<p>Signature of the tutor _____</p>				

Laboratory work







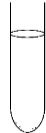





Laboratory exercises	Laboratory report		
<ol style="list-style-type: none"> 1. Chicken embryo autopsy. 2. Virus indication by slide HT. 3. Evaluation of HIT for influenzavirus identification. 	<ol style="list-style-type: none"> 1. Before autopsy embryo should be cooled for 2–3 hours at 4–6 °C for blood vessels constriction. 2. Treat the eggshell with 70 % alcohol 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 eyes. Usually influenza viruses produce no CPE. 6. Perform slide HT for virus indication 		
	<div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;"> <p>1</p>  </div> <div style="text-align: center;"> <p>2</p>  </div> <div style="text-align: center;"> <p>3</p>  </div> </div> <div style="text-align: center; margin-top: 20px;">  </div>	<p style="text-align: center;">SLIDE HT</p> <p>Put two drops of 5 % chicken erythrocytes suspension onto glass slide. 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.</p> <ol style="list-style-type: none"> 1. Allantois liquid. 2. Saline. 3. 5 % chicken erythrocytes. 	<p>Smear _____</p> <p>Stain _____</p> <div style="text-align: center; margin-top: 20px;">  </div>

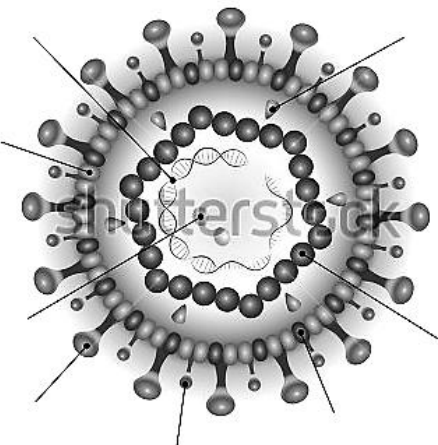
Laboratory work									
Laboratory exercises	Laboratory report								
4. Evaluation of HIT for influenza virus identification	L patient's virus	Anti H ₁ N ₁	Anti H ₃ N ₂	Anti H ₅ N ₁	EC	VC	K _{анти} C1	K _{анти} C2	K _{анти} C3
									
	D patient's virus								
Conclusion:									

INDIVIDUAL WORK									
 <p>1. Hemagglutinin 2. Neuraminidase 3. Lipid bilayer membrane 4. Matrix protein M1 5. Ion channel protein M2 6. Nucleoprotein 7. Nuclear export protein 8. Polymerase complex</p> <p>Virion of _____ virus (identify numerals virion structure)</p> <p>Baltimore Group _____</p>	Fill the table								
	Host	Tropism	Diseases	Transmission	Vaccine	Antiviral drugs	Samples	Laboratory diagnostics	
	Influenza A virus								
Measles virus									

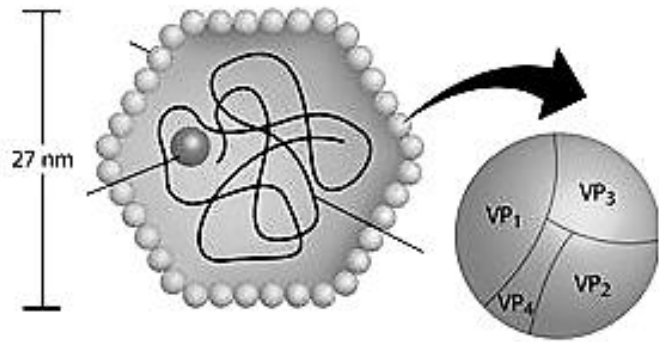
Practical class 9 (26). VIROLOGIC DIAGNOSTICS OF DISEASES CAUSED BY PICORNAVIRUSES AND HEPATITIS VIRUSES

<p>Suggested reading for self-study: Picornaviruses. Characteristics of the family, importance for human pathology. Etiology, pathogenesis, immunity, diagnostics and immunoprophylaxis of poliomyelitis. Coxsackieviruses and ECHOviruses. Stomatitis in diseases caused by RNA-viruses. Hepatitis viruses A, B, C, D, E. Taxonomy and characteristics, role in human pathology. Pathogenesis and immunity in hepatitis B. Laboratory diagnostics. Specific and non-specific prophylaxis in dentistry.</p>					Oral quiz	Laboratory work	Individual work	Tests	Total results																																										
					<p>Signature of the tutor _____</p>																																														
Laboratory work																																																			
Laboratory exercises					Laboratory report																																														
<p>1. Performance of ELISA for VHC diagnostics.</p> <p>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.</p>					<p>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.</p> <p>Reaction scheme: a) HCV antigens are adsorbed on the strip wells as follows: rows A, E — core rows B,F — NS3 rows C,G — NS4 rows D, H — NS5</p>					<p>b) put 100 µl of control sera and samples according to the plate layout's) close strip with adhesive tape and incubate for 1 hour at 37 °C; d) wash wells 5 times; e) put 100 µl of conjugate in each well; f) seal strip with tape and incubate for 30 min at 37 °C; g) wash 5 times; h) put 100 µl of substrate in each well; i) incubate for 30 min at 37 °C; j) put 50 µl of stop solution in each well; k) measure the plate by ELISA reader; l) evaluate results.</p>					<p>C- — negative control; C+ — positive control; X₁ — serum patient 1; X₂ — serum patient 2; «1», «2» — plate vertical rows; A-H — plate horizontal rows;</p> <table border="1"> <tr><td>Core</td><td>A</td><td>C-</td><td>X₁</td></tr> <tr><td>NS₃</td><td>B</td><td>C-</td><td>X₁</td></tr> <tr><td>NS₄</td><td>C</td><td>C-</td><td>X₁</td></tr> <tr><td>NS₅</td><td>D</td><td>C-</td><td>X₁</td></tr> <tr><td>Core</td><td>E</td><td>C+</td><td>X₂</td></tr> <tr><td>NS₃</td><td>F</td><td>C+</td><td>X₂</td></tr> <tr><td>NS₄</td><td>G</td><td>C+</td><td>X₂</td></tr> <tr><td>NS₅</td><td>H</td><td>C+</td><td>X₂</td></tr> </table> <p>Card STATEMENT</p>					Core	A	C-	X ₁	NS ₃	B	C-	X ₁	NS ₄	C	C-	X ₁	NS ₅	D	C-	X ₁	Core	E	C+	X ₂	NS ₃	F	C+	X ₂	NS ₄	G	C+	X ₂	NS ₅	H	C+	X ₂
Core	A	C-	X ₁																																																
NS ₃	B	C-	X ₁																																																
NS ₄	C	C-	X ₁																																																
NS ₅	D	C-	X ₁																																																
Core	E	C+	X ₂																																																
NS ₃	F	C+	X ₂																																																
NS ₄	G	C+	X ₂																																																
NS ₅	H	C+	X ₂																																																
Antigens	Row	OD control	OD probe	Cut-off	Results	<p>1. Test results validation: Negative control OD < 0,2 Mean negative control OD = Mean positive control OD > 0,8 Mean positive control OD =</p> <p>2. Cut-off level for each antigen: Cut-off (core-Ag) = NC ODO(core) + 0,2 = Cut-off (NS3-Ag) = NC OD (NS3) + 0,2 = Cut-off (NS4-Ag) = NC OD (NS4) + 0,2 = Cut-off (NS5-Ag) = NC OD (NS5) + 0,2 =</p> <p>3. Positivity index determination for each antigen:</p>				<p>PI(core-Ag) = OD sample(core)/ Cut-off(core-Ag) = PI(NS3-Ag) = OD sample (NS3)/Cut-off(NS3-Ag) = PI(NS4-Ag) = OD sample (NS3)/Cut-off(NS4-Ag) = PI(NS5-Ag) = OD sample (NS3)/Cut-off(NS5-Ag) =</p> <p>4. Results evaluation: a) If PI less than 1, sample is considered negative; б) the results are considered positive if IP exceeds 1 for: core-Ag any two antigens в) result is considered uncertain if IP exceeds 1 for one nonstructural protein only.</p>																																									

2. Neutralization test on cell culture in paired sera for poliomyelitis serodiagnostics – accounting of reaction.	NT IN PAIRED SERA FOR POLIOMYELITIS SERODIAGNOSTICS									Signature of the tutor _____
		1/10	1/20	1/40	1/80	1/160	SC ₁	VC	CC	
	Patient Z' serum									
Patient X' serum							SC ₂			
Conclusion:										

INDIVIDUAL WORK									
	Fill the table								
		Host	Tropism	Diseases	Trans- mission	Vaccine	Antiviral drugs	Samples	Laboratory diagnostics
	1. DNA 2. DNA Polymerase 3. Lipid bilayer membrane 4. Large HBsAg 5. Medium HBsAg 6. Small HBsAg 7. Core HBcAg 8. HBeAg	Hepatitis B virus							
Virion of _____ virus (identify numerals virion structure) Baltimore Group _____	Hepatitis C virus								

INDIVIDUAL WORK



1. RNA
2. Capsid polypeptides
3. VPg

**Virion of _____ virus
(identify numerals virion structure)**

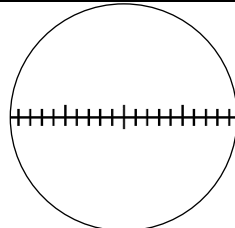
Baltimore Group _____

Fill the table

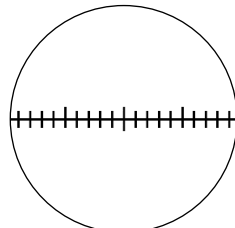
	Host	Tropism	Diseases	Trans- mission	Vaccine	Antivira l drugs	Samples	Laboratory diagnostics
Hepatitis E virus								
Hepato- virus A								

Virus	Family-Genus-Species	Genome	The structure, size of the virion, nm	High-risk group
HAV	<i>Picornaviridae – Hepatovirus – Hepatitis A virus</i>			
HBV	<i>Hepadnaviridae – Orthohepadnavirus – Hepatitis B virus</i>			
HCV	<i>Flaviviridae – Hecacivirus – Hepatitis C virus</i>			
HDV	Unassigned – <i>Deltavirus – Hepatitis delta virus</i>			
HEV	<i>Hepeviridae – Hepevirus – Hepatitis E virus</i>			

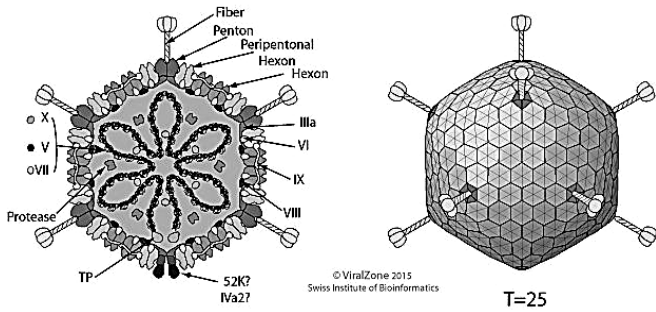
Practical class 10 (27). METHODS OF DIAGNOSTICS FOR DISEASES CAUSED BY RETROVIRUSES AND RABDOVIRUSES

Suggested reading for self-study: Retroviruses. Taxonomy and characteristics of the family. Human immunodeficiency virus (HIV-1, HIV-2). Pathogenesis. AIDS-associated diseases. Manifestations in the oral cavity. HIV diagnostics, prophylaxis, treatment. HIV in Belarus. Rabdoviruses. Taxonomy and characteristics of rabdoviruses. Pathogenesis, immunity and specific prophylaxis of rabies.		Oral quiz	Laboratory work	Individual work	Tests	Total results
		Signature of the tutor _____				
Laboratory work						
Laboratory exercises		Laboratory report				
1. Demonstration: – Negry bodies in mouse brain homogenate, Muromtcev stain.		Smear _____ Stain _____				

Practical class 11 (28). METHODS OF DIAGNOSTICS FOR DISEASES CAUSED BY HERPES- AND ADENOVIRUSES DISEASES IN ORAL CAVITY

Suggested reading for self-study: Herpes viruses. Taxonomy and family characteristics. HSV-1, HSV-2, properties, role in human pathology, pathogenesis, immunity, diagnostics, chemo and immunotherapy. Herpetic stomatitis, keratoconjunctivitis, facial skin lesions and red lip rims. A virus of chicken pox and herpes zoster. Cytomegalovirus, properties, forms of infection. Cytomegalovirus parotitis. Epstein-Barr virus, properties, role in human pathology. Infectious mononucleosis. Herpesviruses of human 6, 7, 8 types, role in human pathology. Immunity, diagnosis, chemotherapy and immunotherapy of herpetic infections. Adenoviruses. Characteristics. Human adenoviruses. Virions structures, pathogenesis, immunity, laboratory diagnostics.		Oral quiz	Laboratory work	Individual work	Tests	Total results
		Signature of the tutor _____				
Laboratory work						
Laboratory exercises		Laboratory report				
1. Demonstration: – CPE of adenoviruses.		Smear _____ Stain _____				

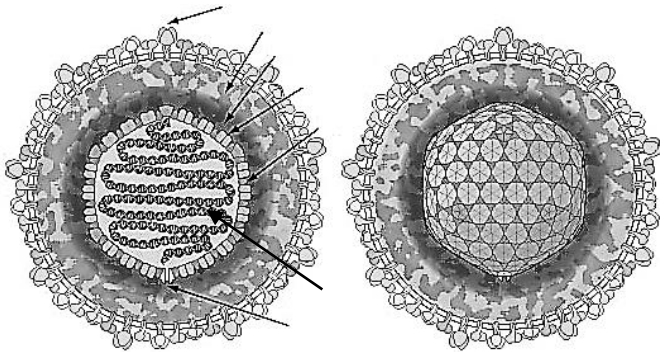
INDIVIDUAL WORK



Virion of _____ virus
(identify numerals virion structure)

Baltimore Group _____

Fill the table								
	Host	Tropism	Diseases	Transmission	Vaccine	Antiviral drugs	Samples	Laboratory diagnostics
Human adenovirus								
Human herpesvirus 1, 2								
Human herpesvirus 4								
Human herpesvirus 5								
Human herpesvirus 6, 7								
Human herpesvirus 8								



1. Envelope proteins
2. Outer tegument
3. Inner tegument
4. Major capsid protein
5. Triplex
6. Portal vertex
7. DNA

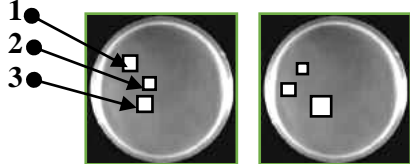
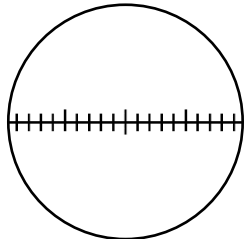
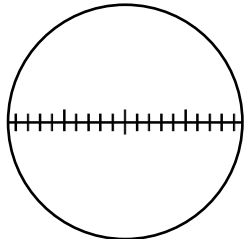
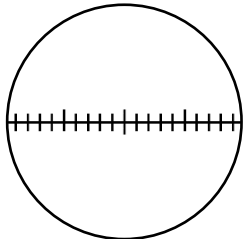
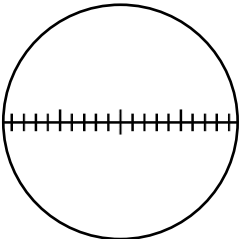
Virion of _____ virus
(identify numerals virion structure)

Baltimore Group _____

Practical class 12 (29). DENTAL MICROBIOLOGY. METHODS OF ORAL CAVITY NORMAL FLORA INVESTIGATION. ETIOLOGY AND PATHOGENESIS OF CARIES

<p>Suggested reading for self-study: Dental microbiology, goals and objectives. Normal microflora of the oral cavity, characteristic. Ontogeny of normal microflora. Influence of genetic and non-genetic factors on the composition of the oral cavity microflora (which regulates the role of saliva, teeth, soft tissue, contact with alien microorganisms, diet and oral hygiene). The value of normal microflora. Methods of study. Dysbacteriosis of the mouth, causes, diagnostic methods. The etiology of caries. Causal importance of microorganisms. S. mutans, properties. Subsidiary germs. Pathogenesis. Conditions conducive to the caries development. Prophylaxis and therapy of caries. Rules and methods of sampling for the study of cariesogenic microflora. Criteria for assessment of the isolated microorganisms etiological significance.</p>	Oral quiz	Laboratory work	Individual work	Tests	Total results
	<p>Signature of the tutor</p> <p>_____</p>				

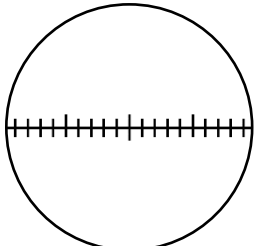




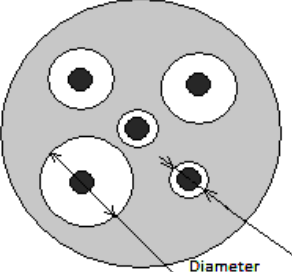
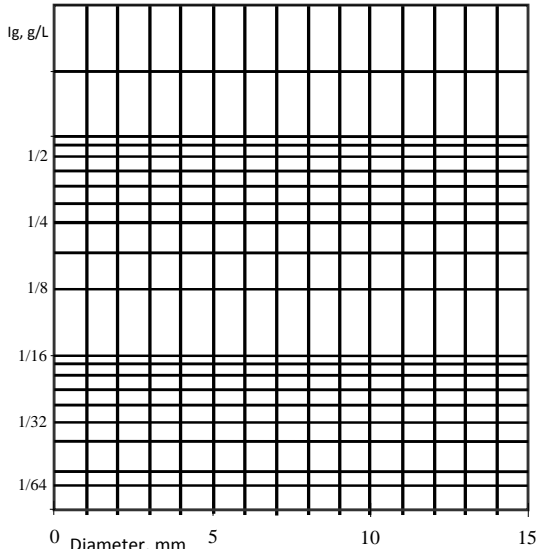
Laboratory work

Laboratory exercises	Laboratory report				
<p>1. Perform isolation of normal flora from mucus of oral cavity membrane surfaces to gain the microorganisms diversity understanding at these body locations and exclude/confirm dysbacteriosis.</p>	<p>- Divide agar plates into four sections with a marking pen or pencil. Mark each section with 1, 2, 3, 4. - Mark each plate with group number and your name. - Add sterile isotonic solution to the Petri dish with sterile filter paper squares (1×1 cm); - Use flamed forceps to cover the squares of the various body sites in which normal flora is to be investigated (saliva, lips, gum, mucus membranes of tongue, cheeks) with filter paper for 30 sec. - Put the squares of filter paper for 60 sec on the surface of blood and MacConkey agar. - Fill in the table with the sites in which the microbial flora is under study. Incubate the plates at 37 °C for 24–48 hours.</p>			<p style="text-align: center;">Blood agar MacConkey agar</p> 	
<p>2. Register the results of experiment on normal flora isolation from mucus membrane surfaces, Gram stain different types of colonies, explore under microscope, complete the report. <i>(The task will be given at the next lesson).</i></p>	<p>Results of registration of dysbacteriosis:</p> <p>Conclusion: _____</p>	<p>Body site</p> <p>1 – _____</p> <p>Amount of colonies and their description</p>	<p>2 – _____</p>	<p>3 – _____</p>	
<p>3. Prepare heat-fixed smear from dental plaque, Gram stain, explore under microscope, complete the report.</p>	<p>3 Smear _____ 1 – Stain _____ 2 – _____ 3 – _____ 4 – _____ 5 – _____ 6 – _____ 7 – _____ 8 – _____ 9 – _____ 10 –</p> 	<p>Gram stain</p>	<p>Smear _____ Stain _____</p> 	<p>Smear _____ Stain _____</p> 	<p>Smear _____ Stain _____</p> 
<p>4. Demonstration: – slide with dental plaque, Gram stain; – methods for detection of pathogenicity factors (capsule, hemolysins, lecithinase, coagulase).</p>					

Practical class 13 (30). DENTAL MICROBIOLOGY. METHODS OF ORAL CAVITY IMMUNITY FACTORS INVESTIGATION

Suggested reading for self-study: Immune and non-immune mechanisms in the oral cavity (natural and acquired). Protective mechanisms of saliva, mucous membranes of the oral cavity, enamel, dentin and pulp of the teeth. Importance of phagocytosis. Immunoglobulins of the oral cavity. Secretory immunoglobulin A. Cell-mediated immunity. Mechanisms of antibacterial and antiviral immunity in the oral cavity.	Oral quiz	Laboratory work	Individual work	Tests	Total results
	Signature of the tutor _____				

Laboratory work

Laboratory exercises	Laboratory report												
1. Determine the content of lysozyme in saliva. – collect 1–1.5 ml saliva in a tube; – mark the Petri dish with the ready-hole seeded Micrococcus lysodeikticus , according to the scheme; – pipette in the wells of the lysozyme appropriate dilutions 50 µl (from low to high concentration); – in the central well of the test add 50 µl of saliva; – incubate the plate for 24 hours; – construct a calibration curve and determine the concentration of lysozyme in your sample; – compare with the standard and make a conclusion.	Smear _____ Stain _____ 	1  6.25 mcg/ml	2  12.50 mcg/ml	3  25.00 mcg/ml	4  50.00 mcg/ml	Saliva, 1–1.5 ml 							
		<table border="1"> <thead> <tr> <th>Standard of Lysozyme, mcg/ml</th> <th>Zone of inhibition, diameter in mm</th> </tr> </thead> <tbody> <tr> <td>6.25 (1/8)</td> <td></td> </tr> <tr> <td>12.50 (1/4)</td> <td></td> </tr> <tr> <td>25.00 (1/2)</td> <td></td> </tr> <tr> <td>50.00 (1)</td> <td></td> </tr> <tr> <td>X sample</td> <td></td> </tr> </tbody> </table> <p>Conclusion:</p>	Standard of Lysozyme, mcg/ml	Zone of inhibition, diameter in mm	6.25 (1/8)		12.50 (1/4)		25.00 (1/2)		50.00 (1)		X sample
Standard of Lysozyme, mcg/ml	Zone of inhibition, diameter in mm												
6.25 (1/8)													
12.50 (1/4)													
25.00 (1/2)													
50.00 (1)													
X sample													

Laboratory exercises	Laboratory report																													
<p>2. Determine the IgA concentration in saliva by Mancini method (simple radial gel immunodiffusion). sIgA standard — 2.0 g per liter.</p> <p>3. Register the experiment results on normal flora isolation from mucus membrane surfaces, Gram stain different types of colonies, explore under the microscope, complete the report.</p>		Standart curve Standard sIgA = 2 g/l																												
		<table border="1"> <thead> <tr> <th></th> <th style="text-align: center;">Titer</th> <th style="text-align: center;">Concentration, g/l</th> <th style="text-align: center;">Diameter, mm</th> </tr> </thead> <tbody> <tr> <td>Point 1</td> <td style="text-align: center;">1</td> <td style="text-align: center;">2,000</td> <td></td> </tr> <tr> <td>Point 2</td> <td style="text-align: center;">1/2</td> <td style="text-align: center;">1,000</td> <td></td> </tr> <tr> <td>Point 3</td> <td style="text-align: center;">1/4</td> <td style="text-align: center;">0,500</td> <td></td> </tr> <tr> <td>Point 4</td> <td style="text-align: center;">1/8</td> <td style="text-align: center;">0,250</td> <td></td> </tr> <tr> <td>Point 5</td> <td style="text-align: center;">1/16</td> <td style="text-align: center;">0,125</td> <td></td> </tr> <tr> <td>X-sample</td> <td></td> <td></td> <td></td> </tr> </tbody> </table>		Titer	Concentration, g/l	Diameter, mm	Point 1	1	2,000		Point 2	1/2	1,000		Point 3	1/4	0,500		Point 4	1/8	0,250		Point 5	1/16	0,125		X-sample			
	Titer	Concentration, g/l	Diameter, mm																											
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Point 5	1/16	0,125																												
X-sample																														
Conclusion:																														

Practical class 14 (31). DENTAL MICROBIOLOGY. MICROBIOLOGY OF PERIODONTAL AND PERI-IMPLANTITIS DISEASES

Suggested reading for self-study:

Plaque: stages of formation, microorganisms-colonizers. Plaque as a biofilm. Periodontal diseases: classification, etiology, risk factors. Theories of the pathogenesis of periodontitis. Properties of periodontopathogenic microorganisms, mechanisms of invasion and persistence. Microbial complexes (Socransky, 1998). Immune mechanisms in diseases of the tissues of the periodioth. Principles of prevention and treatment of periodontitis. Dynamics of microflora with successful and complicated dental implantation.

Oral quiz	Laboratory work	Individual work	Tests	Total results
Signature of the tutor _____				

Laboratory work

Laboratory exercises	Laboratory report
1. Determine the content of lysozyme in saliva — ending (see practical class 12).	

INDIVIDUAL WORK

SUBLINGVAL MICROBIAL COMPLEXES by SOCRANSKY

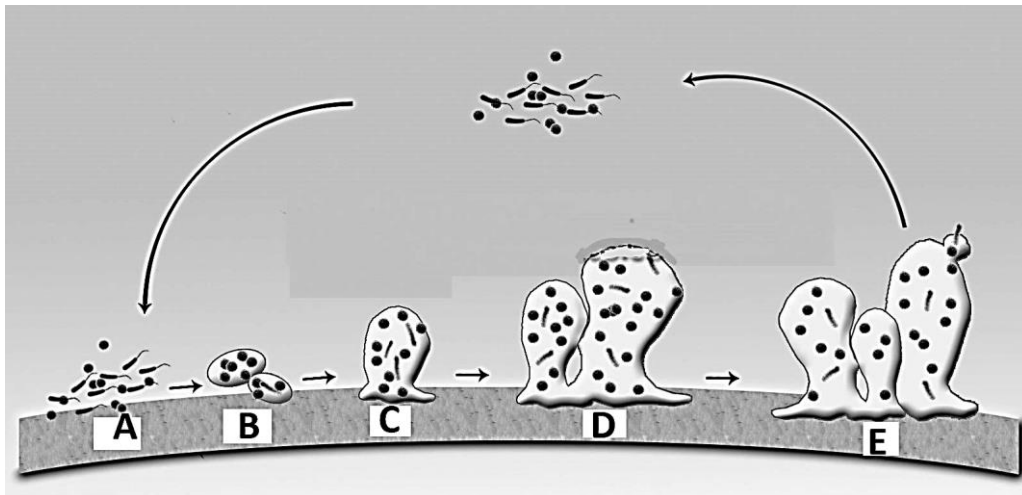
(enter the names of the species of bacteria in each complex according to color)

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INDIVIDUAL WORK

BIOFILM FORMATION
(write stage of formation of biofilm)



- A –
- B –
- C –
- D –
- E –

detachment and recolonization – multiplication – co-adhesion – colonization – reversible adhesion

Practical class 15 (32). DENTAL MICROBIOLOGY. METHODS OF MICROBIOLOGICAL DIAGNOSTICS OF STOMATITIS. MICROBIOLOGICAL DIAGNOSTICS OF FUNGAL INFECTIONS

Suggested reading for self-study:

Inflammatory diseases of the oral mucosa. Bacterial stomatitis: specific (gonococcal, typhoid fever, antrax stomatitis, manifestations in the oral cavity of syphilis, tuberculosis, actinomycosis, scarlet fever) and nonspecific. Viral stomatitis.

Classification and general characteristics of fungi. Classification of mycosis. Candida, general characteristics. Role in human pathology. Soor. General principles of fungal infections diagnostics.

Oral quiz	Laboratory work	Individual work	Tests	Total results
Signature of the tutor _____				

Laboratory work


Laboratory exercises

- Research of the sample of the patient's pus with an abscess subcutaneous tissue of maxillofacial area, the 1st period:
 - microscopy of pus (smear, Gram stain);
 - preparation of inverse hundredfold dilutions material in sterile saline (1 : 100; 1 : 10000; 1 : 1000000);
 - quantitative (50 mcl) streak respective sectors dilutions of pus on solid nutrient media (MSA, Endo, blood agar, NA with furagin) depending on the results of microscopy.
- Research of the blood sample from the patient with stomatogenic sepsis, the 1st period:
 - microscopy of blood, smear "thick drop", methylene blue stain or Romanovsky;
 - Crop material in the liquid medium of the primary crop (enrichment) in a ratio of 1 : 10–60;
 - Incubation of cultivation in an incubator at 37 °C — 18–24 hours and up to 14 days.

All inoculations are placed in a incubator for 24 hours, then transferred to a refrigerator for 14 days.

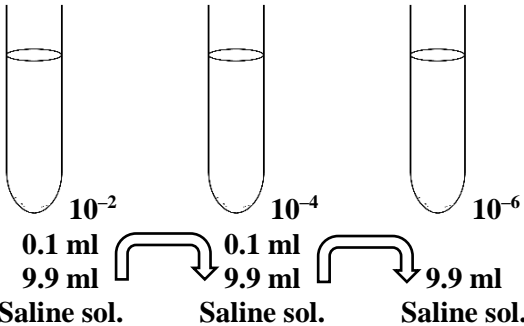
Laboratory report

Sample of pus from



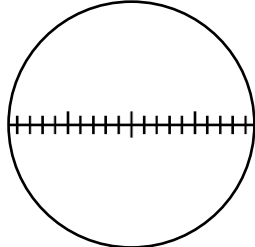
Burnt wound

Serial dilution of the sample




Steak respective sector with 0.05 ml (1 drop)

Smear 2.1 _____
Stain _____

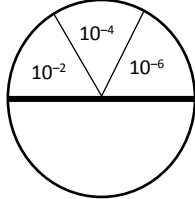


Blood sample examination, 1st period

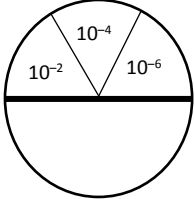


10 ml:60 ml

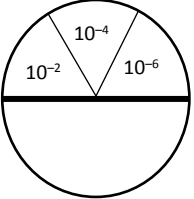
Medium



Blood agar

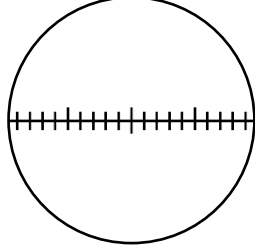



Levin



Nutrient agar with

Smear 3.1 _____
Stain _____





Laboratory exercises	Laboratory report																		
<p>3. Snyder's caries susceptibility test</p> <p>The degradation of enamel and dentin in the formation of tooth decay (dental caries) occurs as a result of the production of lactic acid by bacteria (<i>Streptococcus mutans</i> and others) in the presence of sucrose high levels. Of the various methods that have been devised to determine one's susceptibility to tooth decay, M. L. Snyder's caries susceptibility test is a relatively simple test that has been shown to have a high reliability correlation.</p> <p>This method relies on the rapidity of organisms in saliva to lower the pH in the medium that contains 2 % dextrose (Snyder test agar). Since decalcification of enamel begins at pH of 5.5, and progresses rapidly as the pH is lowered to 4.4 and less, the demonstration of pH lowering becomes evidence of susceptibility to caries.</p> <p>To indicate the presence of acid production in the medium, the indicator bromocresol green is incorporated in it. This indicator is green at pH 4.8 and becomes yellow at pH 4.4, remaining yellow below 4.4.</p> <p>Figure illustrates the procedure that is used in the Snyder caries susceptibility test. Note that 0.2 ml of saliva is added to the tube of liquefied Snyder test agar (50 °C) and mixed well by rotating the tube between the palms of both hands. After the medium has solidified, the tube is incubated at 37 °C for a period of 24–72 hours. If the medium turns yellow in 24–48 hours, the individual is said to be susceptible to caries.</p> <p>Although we will be performing this test only once, it should be noted that test reliability is enhanced by performing the test on three consecutive days at the same time each day. If the test is performed correctly after tooth brushing, it is not as reliable as if 2 or 3 hours have elapsed after brushing.</p>	<ol style="list-style-type: none"> Liquefy a tube of Snyder test agar and cool it to 50 °C. After allowing a piece of paraffin to soften under the tongue for a few minutes, start chewing it. Chew it for 3 minutes, moving it from one side of the mouth to the other. <i>Do not swallow the saliva.</i> As it accumulates, deposit it in the small sterile beaker. Vigorously shake the sample in the beaker from side to side for 30 seconds to disperse the organisms. With a 1 ml pipette transfer 0.2 ml of saliva to the tube of agar. Do not allow the pipette to touch the side of the tube or agar. 	<ol style="list-style-type: none"> Before the medium solidifies, mix the contents of the tube by rotating the tube vigorously between the palms of the hands. Write your name on a gummed label and attach it to the tube. Incubate the tube at 37 °C. Examine the tube every 24 hours to see if the bromocresol green indicator has changed to yellow. If it has, the test is positive. The degree of caries susceptibility is determined from the table below. Record your results on the Laboratory Report. 																	
<p>Materials:</p> <p>1 tube of Snyder test agar (5 ml in 15 mm dia tube)</p> <p>1 30 ml sterile beaker</p> <p>1 piece of paraffin (1/4" 1/4" 1/8")</p> <p>1 ml pipette</p> <p>1 gummed label</p>	<p style="text-align: center;">CARIES SUSCEPTIBILITY</p> <p style="text-align: center;">Marked</p> <p style="text-align: center;">Moderate</p> <p style="text-align: center;">Slight</p> <p style="text-align: center;">Negative</p>	<p style="text-align: center;">MEDIUM TURNS YELLOW IN:</p> <table border="1" style="width: 100%; text-align: center;"> <thead> <tr> <th style="width: 25%;">24 HOURS</th> <th style="width: 25%;">48 HOURS</th> <th style="width: 25%;">72 HOURS</th> </tr> </thead> <tbody> <tr> <td>Positive</td> <td></td> <td></td> </tr> <tr> <td>Negative</td> <td>Positive</td> <td></td> </tr> <tr> <td>Negative</td> <td>Negative</td> <td>Positive</td> </tr> <tr> <td>Negative</td> <td>Negative</td> <td>Negative</td> </tr> </tbody> </table>			24 HOURS	48 HOURS	72 HOURS	Positive			Negative	Positive		Negative	Negative	Positive	Negative	Negative	Negative
24 HOURS	48 HOURS	72 HOURS																	
Positive																			
Negative	Positive																		
Negative	Negative	Positive																	
Negative	Negative	Negative																	

Practical class 16 (33). TEST “GENERAL AND SPECIAL VIROLOGY. DENTAL MICROBIOLOGY”

List of questions	Oral quiz	Script	Tests	Total results
<p>1. Virology, tasks and methodologies. The systematic position and classification of viruses.</p> <p>2. Forms of viruses existence. The morphology of virions. The interaction of viruses with susceptible cells.</p> <p>3. Features of infection and immunity in viral infections.</p> <p>4. Methods of virus cultivation (cell culture, chicken embryo, laboratory animals).</p> <p>5. General principles of viral infections diagnostics.</p> <p>6. Influenza viruses. General characteristics. Pathogenesis, specific and non-specific treatment and prevention, influenza laboratory diagnosis. Manifestations in the oral cavity.</p> <p>7. Paramyxoviruses, general characteristics. Mumps virus, respiratory-syncytial virus, measles virus, parainfluenza viruses. Manifestations in the oral cavity.</p> <p>8. Enteroviruses, general characteristics, role in human pathology. Poliovirus, pathogenesis and laboratory diagnostics, specific prevention. Manifestations of enteroviruses infection in oral cavity.</p> <p>9. Classification of hepatitis viruses. Characterization of hepatitis A, B, C virus. Pathogenesis, immunity, laboratory diagnosis, prevention.</p> <p>10. Retroviruses. Human immunodeficiency virus (HIV-1, HIV-2). Pathogenesis. AIDS-associated diseases in dentistry. HIV diagnostics, prophylaxis.</p> <p>11. Adenoviruses, general characteristics. Pathogenesis, laboratory diagnostics of adenoviral infections. Manifestations in oral cavity.</p> <p>12. Herpes viruses. Classification. General characteristics, disease. Herpetic stomatitis.</p> <p>13. Bacterial viruses (bacteriophages), properties, classification. The practical use of bacteriophages.</p> <p>14. The microflora of the oral cavity (indigenous, transient). Ontogeny of normal oral flora.</p> <p>15. Representatives of the normal oral flora: Gram-positive and Gram-negative cocci (streptococci, peptostreptococci, staphylococci, veillonella, Neisseria), their role.</p> <p>16. Representatives of the normal oral flora: Gram-positive (propionibacterium, lactobacillus, actinomyces, corynebacterium) and Gram-negative rods (bacteroides, prevotella, porphyromonas, fusobacterium, leptotrichia), their role.</p>	<p>17. Representatives of the normal oral flora spiralshaped bacteria (vibrio, wolinella, centipedia, selenomonas, campylobacter, spirochetes), mycoplasma, protozoa, fungi, and their role.</p> <p>18. Microflora of specific areas of the mouth: saliva, dorsum of the tongue, dental pocket, mucous membranes. Methods of study of oral microflora.</p> <p>19. Influence of environmental factors and physiological features on oral flora. The role of the oral cavity normal microflora (positive and negative). Disbacteriosis of the oral cavity: causes, outcome, prevention, principles of correction.</p> <p>20. Antigens and the immune system of the oral cavity. Citrullinated antigens. Immune mechanisms in the oral cavity. Antimicrobial factors of saliva: defensins, cathelicidin, mucins, histatin, statherin, cystatins. Proinflammatory cytokines.</p> <p>21. Nonspecific mechanisms of defense of the mucous membranes, saliva, gingival fluid, tooth enamel, normal microflora's.</p> <p>22. Factors and mechanisms of acquired immunity of oral cavity. Local Immunity of the oral cavity. Immunological aspects of relationship of inflammatory periodontal diseases, cardiovascular and rheumatic diseases.</p> <p>23. Types of inflammatory processes of the oral cavity, their characteristics. Cytokines of early and late phase of inflammation: cell producers, properties. Methods of cytokines detection: obtaining of specimens, storage, methods of determination (ELISA, genetic).</p> <p>24. The etiology of dental caries. Features of cariogenic microorganisms. Cariogenic streptococci. Characteristics of <i>S.mutans</i>. Characteristics of lactobacilli. Associative (additional) microorganisms. The role of the microorganism in the development of caries.</p> <p>25. Cariogenesis: mechanisms of streptococci adhesion to teeth and their role in dental plaque formation. Role of glucans and their characteristics. Factors responsible for caries development. Resistance to caries. Prevention of dental caries.</p> <p>26. Odontogenic infections: etiology, types. The role of microorganisms in the etiology and pathogenesis of gingivitis. Dynamics of the microflora of implants in case of successful implantation and complicated.</p> <p>27. The role of microorganisms in the pathogenesis of pulpitis, acute and chronic periodontitis ray, periostitis, osteomyelitis, abscesses and soft tissue abscesses.</p>			

28. Periodontal diseases: classification, risk factors. General properties of periodontopathogenic microorganisms. Red complex microorganisms: *Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola*. Characterization, pathogenicity factors and their role in the pathogenesis of periodontitis. Characteristics of *Aggregatibacter actinomycetemcomitans* and role in the development of aggressive periodontitis.

29. Dental Plaque: microflora, formation stages. The role of dental plaque in the development of periodontitis. Microorganisms of orange and yellow complexes, their role in the development of periodontal disease. Plaque as a biofilm. The role of quorum sensing factors in the formation of plaque. New approaches to reduce the bioburden of plaque.

30. Immune mechanisms in the development of periodontal diseases. Factors contributing invasion of microorganisms. Mechanisms to protect tissues from microbial invasion. Principles of prevention and treatment of periodontitis

31. The role of microorganisms in the formation of dental calculus. Pathogenesis of the carie dental calculus formation.

32. Inflammatory diseases of the oral mucosa: classification, the role of microorganisms in their development. Specific and nonspecific stomatitis.

33. Stomatitis caused by obligate pathogens and opportunistic bacteria.

34. Fusospirochetal diseases: etiology, characteristics of pathogens, pathogenesis, clinical forms.

35. Actinomyces spp.: systematics, classification, characteristics, antigenic structure, factors of pathogeneity. Cervico-maxillo-facial actinomycosis: pathogenesis, immunity, microbiological diagnosis, prevention.

36. Viral stomatitis.

37. Candida: systematics, properties, pathogenicity factors. Candidosis: factors responsible for the development, methods of diagnosis and prevention.

38. Methods of studying the normal oral flora. Methods of sampling for dental diseases diagnosis.

39. Manifestations of allergic and immunodeficiency conditions in the oral cavity. Recurrent viral aphthous stomatitis.

40. Types and etiology of stomatogenic infections.

Dental Clinical Microbiology. Opportunistic pathogens. Specific features opportunistic pathogens and infections caused by them. Specific features of pathogenesis and diagnosis of opportunistic diseases. Criteria of Etiological significance of isolated bacteria from a specimen.

Practical class 17 (34). DENTAL MICROBIOLOGY. METHOD OF MICROFLORA INVESTIGATION IN DISEASES OF THE TEETH AND ORAL CAVITY SOFT TISSUES

Suggested reading for self-study:

Odontogenic inflammation. Microflora, pathogenesis, microbiological diagnosis of pulpitis, periodontitis, periostitis, osteomyelitis, odontogenic abscesses and phlegmon.

Purulent-inflammatory dental diseases of soft tissues and bones of the maxillofacial area. Pathogens, pathogenesis, methods of microbiological diagnostics (material for research, rules and methods of sampling, a scheme for bacteriological examination of pus, criteria for the etiological role of isolated microorganisms). Determination of sensitivity to antibiotics. Dental sepsis. Pathogens, methods of microbiological diagnosis.

Oral quiz	Laboratory work	Individual work	Tests	Total results

Signature of the tutor _____

Laboratory work

Laboratory exercises	Laboratory report				
<p>1. Research of the sample of the patient's pus with an abscess subcutaneous tissue of maxillofacial area, 2nd period:</p> <ul style="list-style-type: none"> – microscopy of slides prepared from all types of colonies; – the study of microbial growth on the media; – determination of the pathogen quantity per ml/g (CFU) of the sample with formula; – oxidase test; – coagulase test; – seeding the pure culture for accumulation and biochemical identification, incubation in an incubator at 37 °C — 18–24 hours. 	Colonies characteristics	Medium ____	Medium ____	Smear _____ Stain _____	
	Shape				
Size					
Surface					
Edge					
Color					
Consistency					
Transparency					
	Determination of CFU				
	Calculation of bacteria quality per ml/g of the sample:				
	$N_{(CFU/ml)} = n \times 20 \times 10^x$,				
	n – colonies quantity in respective sector,				
	20 – conversion factor for 1 ml,				
	10 ^x – the degree of the sample dilution.				
	$N_{(CFU/ml)} =$				
<p>2. Research of the blood sample from the patient with stomatogenic sepsis, the 2nd period:</p> <ul style="list-style-type: none"> – the study of microbial growth on the media; – microscopy of slides prepared from the media; – seeding on the blood and Yolk-salt agar for the pure culture. 					

Practical class 18 (35). CLINICAL MICROBIOLOGY. MICROBIOLOGICAL DIAGNOSTICS OF PURULENT INFECTIONS OF BRONCHI AND LUNGS. HOSPITAL-ACQUIRED INFECTION

Suggested reading for self-study:

Dental bronchopulmonary diseases. Pathogens. Pathogenesis. Conditions of occurrence. Methods of microbiological diagnosis (materials for research, rules and methods of sampling, a scheme for bacteriological sputum examination, bronchial washings, criteria for the etiological role of isolated microorganisms).

Determination of sensitivity to antibiotics.

Nosocomial infections. Pathogens, features in the practice of a dentist, principles of diagnosis. Anti-epidemic regime in dental practice. Principles of microbiological diagnosis. Prevention.

Oral quiz	Laboratory work	Individual work	Tests	Total results

Signature of the tutor _____

Laboratory work

Laboratory exercises

- Research of the blood sample from the patient with stomatogenic sepsis, the 3rd period:
 - the study of microbial growth on the medium;
 - microscopy of slides prepared from all types of colonies;
 - oxidase test;
 - coagulase test;
 - seeding the pure culture for accumulation and biochemical identification, incubation in an incubator at 37 °C — 18–24 hours.
 - incubation at 37 °C — 18–24 hours.

- Research of the blood sample from the patient with stomatogenic sepsis, the 4th period:
 - the study of tests used for identification of cultures and antimicrobial sensitivity level in DDM.

Laboratory report

Blood agar YSA MH agar Coagulase test Glucose and mannitol fermentation (anaerobic)

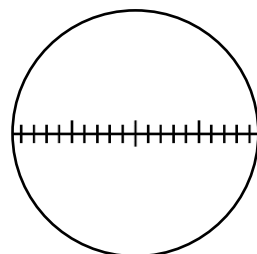
Exp Control

Hemolyses _____ Lecithinase _____

Kirby–Bauer method

Stabilized rabbit plasm: 37 °C — 2, 4, 24 h

Smear _____
Stain _____



Colonies characteristics	Medium _____	Medium _____
Shape		
Size		
Surface		
Edge		
Color		
Consistency		
Transparency		

Conclusion:

Exam' questions for the dental faculty students

List of questions

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| <ol style="list-style-type: none"> 1. Microbiology: definition, area and fields of microbiology. Objects and methods of research. Dental microbiology: goals, objectives, role in the dentist's practice. 2. Milestones (periods) in microbiology. Work of L. Pasteur, R. Koh, I.I. Mechnikov. Evolution of microorganisms and infectious diseases. 3. Common with other organisms and the unique features of microorganisms. Principles of systematics of microorganisms. Classification and nomenclature of microorganisms. The term of "species" in bacteria: group of traits for species identification (criteria for speciation). 4. Morphology of bacteria. Basic morphological forms of bacteria. The structure of a bacterial cell. Functions of the surface and cytoplasmic structures of a bacterial cell. Mechanism of Gram staining. Forms of bacteria with the cell wall defects. 5. Unique features of metabolism in prokaryotes. Nutrition of bacteria: types, requirements of bacteria, nutrients and pathways of nutrients penetration into the bacterial cell. 6. Respiration of microorganisms: types, pathways of energy production. Enzymes and cell structures involved in the process of respiration. Classification of bacteria regarding their oxygen requirements. 7. Growth and reproduction of bacteria. The mechanism of simple division and its phases. Dormant forms of microorganisms: general characteristics, factors inducing their formation, medical importance. 8. Sampling for microbiological studies: types of samples, the rules of sampling, storage, transportation. Principles of organization, equipment and levels of biosafety in microbiological laboratories. 9. Microscopic (bacterioscopic) method of diagnosing the infectious diseases: definition, aim and tasks, steps and evaluation of specificity, sensitivity, disadvantages of the method. Types of microscopic preparations. Staining of microorganisms: methods. Types of microscopes. 10. The bacteriological method of diagnosing the infectious diseases: aim, tasks, phases, and evaluation of specificity, sensitivity, disadvantages of the method. 11. Cultivation of bacteria, nutrient media: requirements, classification. Methods for the isolation of pure cultures of aerobic and anaerobic bacteria. 12. Methods of identification of aerobic and anaerobic bacteria pure cultures. Identification of microorganisms without isolation of a pure culture. 13. Genetic apparatus of bacteria (nucleoid, plasmids, transposons, IS-elements) characteristics, functions, effect and importance. The concept of genetic engineering and biotechnology. | <ol style="list-style-type: none"> 14. Inheritance and variability of microorganisms. Types of variability. Mutations. The genetic recombination of bacteria. Phenotypic variability. The practical significance of the variability of microorganisms in the diagnosis, treatment and prevention of infectious diseases. 15. Molecular biological method of diagnosing the infectious diseases (molecular hybridization, polymerase chain reaction): definition, the principle of the methods, application in dentistry. 16. Infection (infection process): definition of the term causes and conditions of infectious diseases emergence. Differences in communicable and non-communicable diseases. Periods of infectious diseases. Infectious disease classification and outcomes. 17. Classification of infectious processes: the nature of the pathogen, the source of infection, the mechanisms and routes of infection, prevalence, the multiplicity of infection, duration. 18. The role of microorganisms in the infectious process. Pathogenicity and virulence. Factors of pathogenicity of microorganisms. Pathogenicity island. Microbial toxins. Types of exotoxins and their biological properties. Mechanisms of microbial persistence and latency in host's organisms. 19. The role of host, social, environmental factors in the infectious process. 20. The biological (experimental) method of diagnosing the infectious diseases: definition of the term, aim, tasks, phases, evaluation. 21. The ecology of microorganisms. Types of ecological relationships in microorganisms. The role of microorganisms in the genesis and development of the Biosphere (the concept of the microbial dominance). Spread of microorganisms in the nature. 22. The characteristic of normal human microflora and its biological role. Methods of study. Disbiosis: causes, consequences, prevention. Gnotobiology. 23. Sterilization: definition of the term, methods, quality control. Sterilization of instruments and medical devices. Consequences of sterilization errors. 24. Disinfection: definition of the concept, types, methods of conducting. Groups of disinfectants used in dentistry. 25. The antisepsis: definition of the term, types, categories, methods of application. Antiseptic agents: classification, mechanism of action, side effects. Principles of rational antisepsis in dental practice. |
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| <p>26. The chemotherapy and chemoprophylaxis of infectious diseases. Groups of antimicrobial chemotherapeutic agents, mechanisms and spectrum of action on microbial cells. Chemotherapeutic index.</p> <p>27. Antibiotics: characteristic, classification. Requirements for antibiotics. Mechanisms of action of antibiotics.</p> <p>28. Principles of a rational antibiotic therapy in stomatology. Antibiotics for prophylaxis of bacterial complications. Side effects of antibiotics. New approaches to the development of antibiotics.</p> <p>29. Natural and acquired resistance of microorganisms to antibiotics. The genetic and biochemical mechanisms of resistance of microorganisms.</p> <p>30. Genotypic and phenotypic methods for determining the susceptibility of microorganisms to antibiotics. Instruments and test systems for the automated detection of antibiotic susceptibility of microorganisms</p> <p>31. Immunology: definition of the term, aim and task, methods, history of development, branches. Immunity: definition, types of immunity.</p> <p>32. Immune system of the body: organs, cells, molecules of the main histocompatibility complex (structure, distribution on cells, biological role), cytokines (classification, functions).</p> <p>33. Innate immunity. Immune and non-immune factors of innate immunity. Mechanisms of recognition in the innate immune system.</p> <p>34. Phagocytes, classification. Phagocytosis reaction: phases, mechanisms of intracellular microorganisms killing, outcomes. Methods of phagocytosis evaluation. Phagocytic reaction indexes, definition and importance in clinical practice.</p> <p>35. The complement system: definition, main components, activators and activation pathways, functions of components and their fragments. Methods of evaluation of the complement system activity.</p> <p>36. Antigens: structure, properties, classification. T-dependent and T-independent antigens. Superantigens.</p> <p>37. Antigens of microorganisms. Antigenic structure of bacteria. Type, species, group antigens. Protective antigens. Cross-reactive antigens, medical importance.</p> <p>38. Antigen presenting cells: types, characteristics. B-lymphocytes: development, markers, antigen-specific B-cell receptor.</p> <p>39. Humoral immune response: definition, development. Activation, proliferation, differentiation and interactions of cells involved. T-dependent and T-independent response. Primary and secondary humoral immune response characteristics.</p> | <p>40. Antibodies (immunoglobulins): structure, properties, classification, Immunoglobulins biosynthesis. The mechanism of interaction of antibodies with antigens: specificity, phases, manifestations. Affinity and avidity. Monoclonal antibody: principles of production, application.</p> <p>41. Serological method of investigation: general definition of the term, objectives, basic concepts (diagnosticum, diagnostic serum, titer, diagnostic titer, paired sera). Samples for serological examination. General characteristics of the method. Use of serological method for infectious and noninfectious diseases diagnostics.</p> <p>42. Agglutination: ingredients, main variants of performance, registration, evaluation, application. Indirect (passive) and reverse passive agglutination: ingredients, mechanism, methodology, registration of results, practical use.</p> <p>43. Immunoprecipitation reaction: ingredients, mechanism, main methods of performance, application. Reaction of the immune lysis. Complement fixation test: ingredients, mechanism, registration of results.</p> <p>44. Immunofluorescence (fluorescent antibodies test, FAT), main variants, ingredients, mechanisms, registration of results, practical use. ELISA: ingredients, mechanisms, registration of results, practical use. Immunoblotting (IB). Radioimmunoassay (RIA).</p> <p>45. T-cells: development, markers, subpopulations. Helper T-cells, main types (Th1, Th2, Th3, Th17), spectrum of cytokines produced. T-cell receptor: structure, types, genetic control, variety.</p> <p>46. Cellular immune response: definition, development, main stages, manifestation. The model of two (three) signals: the response, anergy, apoptosis. Manifestation of cellular immune response. Immunological memory.</p> <p>47. Anti-infection immunity and its types depending on pathogen nature. Mechanisms of antitoxic, antibacterial, antifungal, antiparasite immunity.</p> <p>48. Immunoprophylaxis and immunotherapy for infectious diseases. Active immunoprophylaxis. Vaccines: requirements, characteristics of main types of vaccines. Adjuvants mechanisms of action. Side effects of vaccination: severe vaccinal reaction, post-vaccination complications.</p> <p>49. Post-vaccination immunity: mechanisms and factors influencing its development. Indications and contraindications to vaccination. Immunization schedule. Expanded Programme on immunization. Collective immunity to infectious diseases, importance.</p> <p>50. Passive immunoprophylaxis and immunotherapy of infectious diseases: indications, principles, complications.</p> |
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<p>51. Allergology: the definition, objectives. Allergens. Allergy: the stages, types of reactions. Classification of allergens. Allergens in dentistry.</p> <p>52. Immediate type hypersensitivity (ITH). Mediator type (I) ITH: allergens, mechanism, development, Manifestations in the oral cavity, ways to prevent anaphylaxis.</p> <p>53. Cytotoxic (II) type ITH: allergens, development, mechanisms, manifestations. Immunocomplex (III) type ITH: allergens, development, mechanisms. Manifestations of allergic reactions II and III types in the oral cavity.</p> <p>54. Delayed type of hypersensitivity (IV): allergens, development, mechanism, manifestation (infection and contact allergy), importance in oral cavity.</p> <p>55. Drug allergy: major allergens, the mechanisms and types of allergic reactions, methods for diagnostics and prevention. Food allergy. Main allergens. Prevention of food allergy. Idiosyncrasy.</p> <p>56. Methods of diagnosing allergic diseases. Prevention of allergy.</p> <p>57. Antitumor immunity. The concept of immune surveillance. Mechanisms of tumor escape from immune surveillance.</p> <p>58. Clinical Immunology: definition, objectives, main concepts. Immune status: principle and methods of examination. Methods for determining the amount and functional activity of T-and B-lymphocytes.</p> <p>59. Autoantibodies: origin, role in pathology. Autoimmune diseases: definition, classification, aetiology, mechanisms of tissue damage, manifestations.</p> <p>60. Immunodeficiency conditions: classification, causes of development, methods for detection, principles for correction.</p> <p>61. Staphylococci: classification, characterization, antigenic structure, pathogenicity factors. Staphylococcal infections: pathogenesis, immunity, microbiological diagnosis and principles of prevention, immunotherapy. Staphylococcal carriage: diagnosis, significance. Staphylococcus aureus: MRSA, antibiotics of choice for their therapy.</p> <p>62. Streptococci: classification, characterization, antigenic structure, pathogenicity factors. Streptococcal disease: pathogenesis, immunity, microbiological diagnosis, and prevention. Pneumococci: classification, characterization, antigenic structure, pathogenicity factors. Pneumococcal infections.</p> <p>63. Neisseria meningitidis: systematics, characterization, antigenic structure, pathogenicity factors. Meningococcal infections: pathogenesis, immunity, microbiological diagnosis, prophylaxis.</p> <p>64. Neisseria gonorrhoeae: systematics, characterization, antigenic structure, pathogenicity factors. Pathogenesis, immunity, microbiological diagnosis of acute and chronic gonorrhoea, prophylaxis. Prevention of gonorrhoea and gonorrhoeal conjunctivitis, stomatitis.</p>	<p>65. Family of Enterobacteria: classification, characterization, pathogenicity factors. Principle of microbiological diagnosis of GIT diseases caused by Enterobacteria. Principles of identification of enterobacteria.</p> <p>66. Escherichia: systematics, characterization, antigenic structure, pathogenicity factors. Pathogenic and opportunistic Escherichia coli. The biological role of Escherichia coli. Escherichiosis: pathogenesis, immunity, microbiological diagnosis and prevention.</p> <p>67. Salmonella: systematics and classification, characterization, antigenic structure, pathogenicity factors, role Salmonella in pathology. Salmonellosis and Typhoid fever: pathogenesis, immunity, prevention.</p> <p>68. Shigella: classification, characteristics, antigenic structure, pathogenicity factors. Bacterial dysentery: pathogenesis, immunity, microbiological diagnosis, prophylaxis.</p> <p>69. Food poisoning of microbial aetiology: classification, etiology, pathogenesis, principles of microbiological diagnosis, prophylaxis.</p> <p>70. Klebsiella: classification, characteristics, antigenic structure, pathogenicity factors, Klebsiella diseases. Pseudomonas: characteristics, antigenic structure, pathogenicity factors, role in the pathology.</p> <p>71. Campylobacter, Helicobacter: characteristics, role in pathology.</p> <p>72. Corynebacterium: classification, characteristics, antigenic structure, pathogenicity factors. Diphtheria: pathogenesis, immunity, microbiological diagnostics, immunotherapy and aetiological therapy of diphtheria, prophylaxis. Manifestation of diphtheria in oral cavity.</p> <p>73. Bordetella: classification, characteristics, antigenic structure, pathogenicity factors. Whooping cough: pathogenesis, immunity, microbiological diagnosis, prophylaxis. Haemophilus spp.: characteristics, role in pathology, prophylaxis Hib-infections.</p> <p>74. Actinomyces: classification, characterization, antigenic structure, pathogenicity factors. Cervico-maxillofacial actinomycosis: pathogenesis, immunity, microbiological diagnosis, prevention.</p> <p>75. Mycobacteria: classification, characteristics, antigenic structure, pathogenicity factors. Tuberculosis: pathogenesis, immunity, methods of diagnosis, principle of prevention and treatment. Mycobacterioses. Manifestation of tuberculosis in oral cavity.</p> <p>76. Obligate anaerobes. Classification and characteristics. Clinical signs of anaerobic infection. Features of taking the material in case of suspected anaerobic infection.</p> <p>77. Gas gangrene Clostridia spp.: classification, characteristics, antigenic structure, pathogenicity factors. Anaerobic myonecrosis: pathogenesis, immunity, microbiological diagnostics and prophylaxis, aetiological treatment.</p>
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<p>78. Clostridium tetani: systematics, characterization, antigenic structure, pathogenicity factors. Tetanus: pathogenesis, immunity, microbiological diagnosis, prevention, aetiological treatment.</p> <p>79. Nonsporforming anaerobes: classification, characteristics, role in pathology of oral cavity. Principles of sampling in anaerobic bacteriology. Principle of bacteriological diagnosis of infections caused by nonsporforming anaerobes.</p> <p>80. Quarantine diseases: characteristics, classification. Principles of collection, transportation and investigation of specimens with pathogens of 3d and 4th biosafety levels.</p> <p>81. Vibrio: classification, characteristics, antigenic structure, pathogenicity factors. Cholera: pathogenesis, immunity, microbiological diagnosis, prophylaxis.</p> <p>82. Classification and characteristics of causative agents of plague, tularemia, pathogenicity factors, microbiological diagnosis, prophylaxis.</p> <p>83. Classification and characteristics of causative agents of brucellosis, anthrax, pathogenicity factors, microbiological diagnosis, prophylaxis.</p> <p>84. Spirochetes: classification, characteristics, antigenic structure, pathogenicity factors. Role of Borrelia spp. in human pathology. Lyme borreliosis: aetiology, pathogenesis, immunity, microbiological diagnosis, prophylaxis. Role of Leptospira in human pathology, prophylaxis of leptospirosis.</p> <p>85. Treponema: classification, characteristics, antigenic structure, pathogenicity factors. Syphilis: pathogenesis, immunity, microbiological diagnosis, prophylaxis. Manifestation of Syphilis in oral cavity.</p> <p>86. Treponema of oral cavity and their role in pathology. Fusospirochetozes: etiology, characteristics of pathogens, pathogenesis, clinical forms.</p> <p>87. Chlamydia: classification, characterization, development cycle, antigenic structure, pathogenicity factors, role in pathology. Microbiological diagnostics and prevention.</p> <p>88. Mycoplasma spp.; classification, characteristics, role in pathology.</p> <p>89. Rickettsia: classification, characteristics, role in pathology.</p> <p>90. Pathogenic fungi: classification, characteristics. Fungal infections promoting factors and conditions. Role microfungi in human pathology. Prophylaxis of mycoses.</p> <p>91. Virology: definition, objectives, methods. Systematic position and classification of viruses. History. D.Ivanovski works importance. Forms of existence of viruses. Morphology and biochemical structure of virions. Structure, function and properties of virion nucleic acid, proteins, lipids and carbohydrates. Prions, role in human pathology.</p> <p>92. Interaction of the virus and susceptible cell. Strict parasitism and cytotropism of viruses. Cell receptors for viruses. Viral genome organization. Reproduction strategy of DNA and RNA viruses.</p>	<p>93. Types of viral infection of cell. Changes in the host cells in the process of a viral infection. Peculiarities of viral infections of an organism. Acute, chronic and slow infection. Local and systemic mechanisms of antiviral immunity. Factors of innate and adaptive antiviral immunity. Interferons: classes, properties, mechanisms of antiviral activity.</p> <p>94. Principles of etiologic diagnostics of viral infections. Rapid methods. Serological diagnostics: principles, criteria for diagnosis. Principles of viral infections chemotherapy. Groups of antiviral drugs.</p> <p>95. Cultivation of viruses. Indication and identification of viruses.</p> <p>96. The aetiology of acute respiratory viral infections. Influenza viruses: classification, characteristics, antigenic properties. Influenza: pathogenesis, immunity, prevention, etiologic diagnostics of influenza, chemotherapy and chemoprophylaxis of influenza.</p> <p>97. Paramyxoviruses: classification, characteristics, role in pathology. Prevention of infection caused by paramyxoviruses.</p> <p>98. Rabies virus: classification, characteristics, specific inclusion. Rabies: pathogenesis, etiologic diagnosis, prevention.</p> <p>99. Rubella virus. General characteristics. Role in pathology. Prevention of rubella.</p> <p>100. Enteroviruses: classification, characteristics. Enterovirus infections: pathogenesis, prevention. Role in pathology of oral cavity.</p> <p>101. Viral hepatitis A: pathogenesis, immunity, etiologic diagnosis, prevention.</p> <p>102. Parenteral hepatitis viruses: classification, characteristics. Parenteral hepatitis: pathogenesis, immunity, etiologic diagnostics, prevention.</p> <p>103. Retroviruses. Human immunodeficiency virus (HIV). HIV infection: pathogenesis, immunity, etiologic diagnostics, principles of therapy, prophylaxis. AIDS-related illnesses. HIV-associated diseases in oral cavity.</p> <p>104. Herpesviruses: classification, characterization, role in pathology. Herpetic stomatitis. Chickenpox. Herpes viruses of 4II8 types, their role in human pathology.</p> <p>105. Adenoviruses: classification, characteristic. Adenoviral infections: pathogenesis, immunity, etiological diagnosis. Papillomaviruses: characteristics, role in pathology, disease prevention.</p> <p>106. Dental microbiology: definition, goals, objectives. General principles of microbiological diagnosis of dental diseases.</p> <p>107. The microflora of the oral cavity (indigenous, transient). Ontogeny of normal oral flora.</p> <p>108. The role of normal microflora of the oral cavity (positive and negative). Dysmicrobiosis of the oral cavity: causes, effects, prevention, principles of correction. Influence of environmental factors, physiological features of the oral cavity and other factors of the microorganism on the microflora of the oral cavity.</p>
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<p>109. Representatives of the normal microflora of the oral cavity: aerobes and facultative anaerobes (streptococci, corynebacteria, staphylococci, Neisseria), their role. General characteristics of streptococci of the oral cavity.</p> <p>110. Representatives of the normal oral flora: anaerobes (velonella, propionibacterium, lactobacillus, actinomyces, bacteroides, prevotella, porphyromonas, fusobacterium, leptotrichia), their role.</p> <p>111. Representatives of the normal oral flora spiralshaped bacteria (vibrio, wolinella, centipedia, selenomonas, campylobacter, spirochetes), mycoplasma, protozoa, fungi, and their role.</p> <p>112. Microflora of specific areas of the mouth: saliva, dorsum of the tongue, dental pocket, mucous membranes. Features of these biotopes, affecting microorganisms.</p> <p>113. Methods of study of oral microflora. Methods of sampling material for dental diseases. Environments for the isolation of cariogenic streptococci, lactobacilli.</p> <p>114. Nonspecific mechanisms of defense of the mucous membranes, saliva, gingival fluid, tooth enamel, normal microflora's, system of polymorphonuclear leukocytes.</p> <p>115. Functions of saliva. Antimicrobial factors of saliva: defensins, cathelicidin, mucins, histatin, statherin, cystatins, peroxidase.</p> <p>116. The role of factors and mechanisms of acquired immunity of the oral cavity. Local immunity of the oral cavity. Functions of secretory immunoglobulins A.</p> <p>117. Dental plaque: the stages of formation, microorganisms-colonizers. Plaque as a biofilm. The role of factors in the quorum of sensing in the formation of plaque. New approaches to reducing the bioburden of plaque.</p> <p>118. Etiology of caries. Criteria of cariogenicity. Cariesogenic streptococci. Characteristic of <i>S. mutans</i> et <i>sobrinus</i>. Characteristics of lactobacilli. Associative (auxiliary) microorganisms. The role of the macroorganism in the development of caries.</p> <p>119. Pathogenesis of caries: mechanisms of adhesion (carbohydrate-dependent and carbohydrate-independent) streptococci and mechanisms of destruction of tooth tissues. The role of streptococci in coaggregation. Glukans. Conditions for the development of caries. Caries resistance. Prophylaxis of caries. Fluorides and their influence are microorganisms.</p> <p>120. Odontogenic inflammation: etiology, types and phases of inflammation. Significance in pathology of foci of chronic odontogenic infection. Immunological aspects of the relationship between inflammatory periodontal diseases, cardiovascular and rheumatic diseases.</p> <p>121. Types of microorganisms and their role in the origin and pathogenesis of pulpitis, acute and chronic apical periodontitis, periostitis, osteomyelitis, abscesses and phlegmon soft tissues.</p>	<p>122. Periodontal disease: classification, risk factors for development. The role of microorganisms in the etiology and pathogenesis of gingivitis. Dynamics of microflora of implants in case of successful and complicated implantation.</p> <p>123. The role of dental plaque in the development of periodontitis. The role of microorganisms in the formation of dental plaque. Pathogenetic importance of dental plaque.</p> <p>124. General properties of periodontopathogenic microorganisms. Microorganisms of the red complex: Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola. Characteristics, pathogenicity factors, their role in the pathogenesis of periodontitis.</p> <p>125. Microorganisms of orange, green and yellow complexes, their role in the development of periodontal diseases. Characteristics Aggregatibacter actinomycetemcomitans, pathogenicity factors, the mechanism of invasion and persistence, a role in the development of periodontitis.</p> <p>126. Immune mechanisms in diseases of periodontal tissues. Factors contributing to the invasion of microorganisms. Mechanisms of tissue protection from microbial invasion. Principles of prevention and treatment of periodontitis.</p> <p>127. Inflammatory diseases of the oral mucosa: specific and nonspecific bacterial stomatitis.</p> <p>128. Viral stomatitis.</p> <p>129. Candida: systematics, properties, pathogenicity factors. Candidosis: factors responsible for the development, methods of diagnosis and prevention.</p> <p>130. Manifestations of allergic and immunodeficiency conditions in the oral cavity. Recurrent viral aphthous stomatitis.</p> <p>131. Dental Clinical Microbiology. Opportunistic pathogens. Specific features opportunistic pathogens and infections caused by them. Specific features of pathogenesis and diagnosis of opportunistic diseases. Criteria of Etiological significance of isolated bacteria from a specimen.</p> <p>132. Etiology and principles of microbiological diagnosis of opportunistic diseases of skin and subcutaneous tissue of stomatogenic origin.</p> <p>133. Etiology and principles of microbiological diagnosis of opportunistic diseases of bronchopulmonary tract of stomatogenic origin.</p> <p>134. Etiology and principles of microbiological diagnosis of bacteremia, sepsis of stomatogenic origin.</p> <p>135. Nosocomial infections: definition of the term, etiology, incidence and spread, principles of microbiological diagnosis, prevention. Antiepidemic control in stomatology.</p>
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PRACTICAL SKILLS FOR DEMONSTRATION (PRE-EXAM)

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|---|---|
| 1. Prepare a smear from bullion culture of bacteria and stain by Gram method. | 13. Identify capsule of <i>Klebsiella spp.</i> (negative contrasting) |
| 2. Prepare a smear from agar medium culture of bacteria and stain by Gram method. | 14. Identify <i>Mycobacterium</i> in sputum (Ziehl–Neelsen stain stain) |
| 3. Identify <i>Staphylococcus spp.</i> | 15. Demonstrate inoculation technique on plated agar medium from slant media. |
| 4. Identify <i>Streptococcus spp.</i> | 16. Demonstrate inoculation technique on slant agar medium from plated medium. |
| 5. Identify <i>Neisseria gonorrhoeae</i> . | 17. Demonstrate inoculation technique on slant medium from slant medium. |
| 6. Identify <i>Escherichia coli</i> . | 18. Register and assess the results antibiotic susceptibility testing by disc diffusion method. |
| 7. Identify a mixture of <i>Staphylococcus spp.</i> and <i>Escherichia coli</i> . | 19. Assess the results of agglutination reaction in tubes. |
| 8. Identify a causative agent of anthrax – <i>Bacillus anthracis</i> . | 20. Assess the results of Complement fixation test. |
| 9. Identify <i>Vibrio spp.</i> | 21. Assess the results of Indirect (passive) agglutination test. |
| 10. Identify <i>Brucella spp.</i> | 22. Assess the results of haemagglutination inhibition test. |
| 11. Identify <i>Candida spp.</i> | 23. Demonstrate the technique of slide agglutination testing. |
| 12. Identify <i>Corynebacterium diphtheria</i> (Löffler stain). | |

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CLASSIFICATION OF BACTERIA. PROCARIOTE by Bergy, 2001 DOMAIN BACTERIA

PHYLUM	CLASS	ORDER	FAMILY	GENUS	SPECIES	
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rickettsiales</i>	<i>Rickettsiaceae</i>	<i>Rickettsia</i>	<i>R. prowazekii</i> , <i>R. typhi</i> , <i>R. felis</i> , <i>R. rickettsii</i> , <i>R. conorii</i> , <i>R. australis</i> , <i>R. akari</i> , <i>R. sibirica</i> , <i>R. japonica</i> , <i>R. honei</i>	
				<i>Orientia</i>	<i>O.tsutsugamushi</i>	
			<i>Ehrlichiaeceae</i>	<i>Ehrlichia</i>	<i>E. chaffeensis</i> , <i>E. sennetsu</i> , <i>E. equilike</i> (<i>E. phagocytophila</i>)	
		<i>Rhizobiales</i>	<i>Bartonellaceae</i>	<i>Bartonella</i>	<i>B. quintana</i> , <i>B. henselae</i> , <i>B. bacilliformis</i> , <i>B. chlaridgeae</i> , <i>B. elizabethae</i>	
			<i>Brucellaceae</i>	<i>Brucella</i>	<i>B. melitensis</i> , <i>B. abortus</i> , <i>B. suis</i> u òp.	
		<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Burkholderiaceae</i>	<i>Burkholderia</i>	<i>B. mallei</i> , <i>B. pseudomallei</i> , <i>B. cepacia</i> u òp.
	<i>Alcaligenaceae</i>			<i>Alcaligenes</i>	<i>A. faecales</i> u òp.	
	<i>Bordetella</i>			<i>B. pertussis</i> , <i>B. parapertussis</i> , <i>B. bronchiseptica</i> u òp.		
	<i>Neisseriales</i>		<i>Neisseriaceae</i>	<i>Neisseria</i>	<i>N. gonorrhoeae</i> , <i>N. meningitidis</i> , <i>N. sicca</i> , <i>N. subflava</i> u òp.	
				<i>Eikenella</i>	<i>E. corrodens</i>	
				<i>Kingella</i>	<i>K. kingae</i> u òp.	
	<i>Nitrozomonadales</i>	<i>Spirillaceae</i>	<i>Spirillum</i>	<i>S. minus</i> u òp.		
	<i>Gammaproteobacteria</i>	<i>Thiotrichales</i>	<i>Francisellaceae</i>	<i>Francisella</i>	<i>F. tularensis</i>	
		<i>Legionellales</i>	<i>Legionellaceae</i>	<i>Legionella</i>	<i>L. pneumophila</i> u òp.	
			<i>Coxiellaceae</i>	<i>Coxiella</i>	<i>C. burnetii</i>	
		<i>Pseudomonadales</i>	<i>Pseudomonadaceae</i>	<i>Pseudomonas</i>	<i>P. aeruginosa</i> u òp.	
			<i>Moraxellaceae</i>	<i>Moraxella</i>	Ποδποδ <i>Moraxella</i> (<i>M. lacunata</i> u òp.); Ποδποδ <i>Branhamella</i> (<i>B. catarrhalis</i> u òp.)	
				<i>Acinetobacter</i>	<i>A. calcoaceticus</i> u òp.	
		<i>Vibrionales</i>	<i>Vibrionaceae</i>	<i>Vibrio</i>	<i>V. cholerae</i> (βιοβαρυ: <i>cholerae</i> , <i>eltor</i>), <i>V. parahaemolyticus</i> , <i>V. vulnificus</i> , <i>V. sputorum</i> u òp.	
		<i>Aeromonadales</i>	<i>Aeromonadaceae</i>	<i>Aeromonas</i>	<i>A. hydrophilia</i>	
		<i>Enterobacteriales</i>	<i>Enterobacteriaceae</i>	<i>Citrobacter</i>	<i>C. freundii</i> , <i>C. amalonaticus</i> , <i>C. diversus</i> u òp.	
				<i>Enterobacter</i>	<i>E. cloacae</i> , <i>E. sakazakii</i> , <i>E. agglomerans</i> , <i>E. gergoviae</i> u òp.	
				<i>Escherichia</i>	<i>E. coli</i> , <i>E. fergusonii</i> , <i>E. germannii</i> , <i>E. vulneris</i> , <i>E. blattae</i>	
				<i>Klebsiella</i>	<i>K. pneumoniae</i> (ποδβυδυ: <i>ozaenae</i> , <i>rhinoscleromae</i> , <i>pneumoniae</i>), <i>K. oxytoca</i> , <i>K. planticola</i> , <i>K. terrigena</i>	
				<i>Salmonella</i>	<i>S. enterica</i> , <i>S. bongori</i> . Βυδ <i>S. enterica</i> <i>cocmoum</i> υδ 6 ποδβυδοδ (<i>subsp.</i> : <i>arizonae</i> , <i>diarizonae</i> , <i>enterica</i> , <i>houtenae</i> , <i>indica</i> , <i>salamae</i>). Σεροβαρυ: <i>S. Typhi</i> , <i>S. Paratyphi</i> A, <i>S. Schottmuelleri</i> , <i>S. Enteritidis</i> , <i>S. Typhimurium</i> , <i>S. Choleraesuis</i> u òp.	
				<i>Shigella</i>	<i>S. dysenteriae</i> , <i>S. flexneri</i> , <i>S. boydii</i> , <i>S. sonnei</i>	
				<i>Erwiniaceae</i>	<i>Erwinia</i>	<i>E. amylovora</i> u òp.
				<i>Hafniaceae</i>	<i>Hafnia</i>	<i>H. alvei</i>
		<i>Edwardstiella</i>	<i>E. tarda</i> u òp.			
		<i>Morganellaceae</i>	<i>Morganella</i>	<i>M. morganii</i>		
			<i>Proteus</i>	<i>P. vulgaris</i> , <i>P. mirabilis</i> u òp.		
			<i>Providencia</i>	<i>P. alcalifaciens</i> u òp.		
<i>Yersiniaceae</i>	<i>Yersinia</i>	<i>Y. pestis</i> , <i>Y. enterocolitica</i> , <i>Y. pseudotuberculosis</i> u òp.				
	<i>Serratia</i>	<i>S. marcescens</i> u òp.				
<i>Pasteurellales</i>	<i>Pasteurellaceae</i>	<i>Haemophilus</i>	<i>H. influenzae</i> , <i>H. ducreyi</i> u òp.			

PHYLUM	CLASS	ORDER	FAMILY	GENUS	SPECIES
	<i>Epsilonproteobacteria</i>	<i>Campylobacteriales</i>	<i>Campylobacteriaceae</i>	<i>Campylobacter</i>	<i>C. jejuni</i> , <i>C. fetus</i> , <i>C. coli</i> u òp.
			<i>Helicobacteriaceae</i>	<i>Helicobacter</i>	<i>H. pylori</i> , <i>H. heilmanii</i> u òp.
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Clostridiaceae</i>	<i>Clostridium</i>	<i>C. botulinum</i> , <i>C. perfringens</i> , <i>C. novyi</i> , <i>C. histolyticum</i> , <i>C. septicum</i> , <i>C. tetani</i> , <i>C. defficile</i> u òp.
			<i>Peptostreptococcaceae</i>	<i>Peptostreptococcus</i>	<i>P. anaerobius</i> u òp.
			<i>Peptococcaceae</i>	<i>Peptococcus</i>	<i>P. niger</i>
				<i>Centipeda</i>	<i>C. periodontii</i>
				<i>Mitsuokella</i>	<i>M. dentalis</i>
			<i>Acidaminococcaceae</i>	<i>Selenomonas</i>	<i>S. sputigena</i>
		<i>Veillonella</i>	<i>V. parvula</i> u òp.		
	<i>Mollicutes</i>	<i>Mycoplasmatales</i>	<i>Mycoplasmataceae</i>	<i>Mycoplasma</i>	<i>M. pneumoniae</i> , <i>M. hominis</i> , <i>M. fermentans</i> , <i>M. salivarum</i> , <i>M. orale</i> , <i>M. arthritis</i> u òp.
				<i>Ureaplasma</i>	<i>U. urealiticum</i> u òp.
	<i>Bacilli</i>	<i>Bacillales</i>	<i>Bacillaceae</i>	<i>Bacillus</i>	<i>B. anthracis</i> , <i>B. cereus</i> u òp.
			<i>Listeriaceae</i>	<i>Listeria</i>	<i>L. monocytogenes</i> u òp.
			<i>Staphylococcaceae</i>	<i>Staphylococcus</i>	<i>S. aureus</i> , <i>S. epidermidis</i> , <i>S. saprophyticus</i> u òp.
		<i>Lactobacillales</i>	<i>Lactobacillaceae</i>	<i>Lactobacillus</i>	<i>L. casei</i> , <i>L. fermentum</i> , u òp.
			<i>Enterococcaceae</i>	<i>Enterococcus</i>	<i>E. faecalis</i> , <i>E. faecium</i> u òp.
			<i>Leuconostocaceae</i>	<i>Leuconostoc</i>	<i>L. mesenteroides</i>
<i>Streptococcaceae</i>			<i>Streptococcus</i>	<i>S. pyogenes</i> , <i>S. pneumoniae</i> , <i>S. agalactiae</i> , <i>S. anginosus</i> , <i>S. bovis</i> , <i>S. mutans</i> , <i>S. mitis</i> , <i>S. salivarius</i> , <i>S. sanguis</i> , <i>S. milleri</i> u òp.	
	<i>Lactococcus</i>	<i>L. lactis</i> u òp.			
<i>Actinobacteria</i>	<i>Actinobacteria</i>	<i>Actinomycetales</i>	<i>Actinomycetaceae</i>	<i>Actinomyces</i>	<i>A. israelii</i> , <i>A. naeslundii</i> , <i>A. viscosus</i> , <i>A. odontolyticus</i> , <i>A. pyogenes</i> ,
			<i>Micrococcaceae</i>	<i>Micrococcus</i>	<i>M. lysodeicticum</i> , <i>M. luteus</i> u òp.
			<i>Corynebacteriaceae</i>	<i>Corynebacterium</i>	<i>C. diphtheriae</i> , <i>C. ulcerans</i> , <i>C. urealyticum</i> , <i>C. xerosis</i> u òp.
			<i>Mycobacteriaceae</i>	<i>Mycobacterium</i>	<i>M. tuberculosis</i> , <i>M. bovis</i> , <i>M. africanum</i> , <i>M. leprae</i> , <i>M. kasasii</i> , <i>M. avium</i> , <i>M. ulcerans</i> , <i>M. fortuitum</i> u òp.
			<i>Nocardiaceae</i>	<i>Nocardia</i>	<i>N. asteroides</i> , <i>N. farcinica</i> u òp.
			<i>Propionibacteriaceae</i>	<i>Propionibacterium</i>	<i>P. acnes</i> , <i>P. propionicus</i> u òp.
	<i>Bifidobacteriales</i>	<i>Bifidobacteriaceae</i>	<i>Bifidobacterium</i>	<i>B. bifidum</i> u òp.	
		<i>Gardnerella</i>	<i>G. vaginalis</i>		
<i>Chlamydiae</i>	<i>Chlamydiae</i>	<i>Chlamydiales</i>	<i>Chlamydiaceae</i>	<i>Chlamydia</i>	<i>C. trachomatis</i>
				<i>Chlamydophila</i>	<i>C. psittaci</i> , <i>C. pneumoniae</i>
<i>Spirochaetes</i>	<i>Spirochaetes</i>	<i>Spirochaetales</i>	<i>Spirochaetaceae</i>	<i>Borrelia</i>	<i>B. recurrentis</i> , <i>B. burgdorferi</i> , <i>B. duttoni</i> , <i>B. persica</i> u òp.
				<i>Treponema</i>	<i>T. pallidum</i> (нодсуды — <i>pallidum</i> , <i>endemicum</i> , <i>pertenue</i>), <i>T. carateum</i> , <i>T. denticola</i> , <i>T. minutum</i> , <i>T. refringens</i> , <i>T. scoliodontum</i> , <i>T. vincentii</i> u òp.
			<i>Leptospiraceae</i>	<i>Leptospira</i>	<i>L. interrogans</i> , <i>L. biflexa</i>
<i>Bacteroidetes</i>	<i>Bacteroidetes</i>	<i>Bacteroidales</i>	<i>Bacteroidaceae</i>	<i>Bacteroides</i>	<i>B. fragilis</i> , <i>B. gingivalis</i> u òp.
			<i>Porphyromonadaceae</i>	<i>Porphyromonas</i>	<i>P. gingivalis</i> , <i>P. endodontales</i> u òp.
			<i>Prevotellaceae</i>	<i>Prevotella</i>	<i>P. melaninogenica</i> , <i>P. denticola</i> u òp.
	<i>Flavobacteria</i>	<i>Flavobacteriales</i>	<i>Flavobacteriaceae</i>	<i>Flavobacterium</i>	<i>F. meningosepticum</i> , <i>F. breve</i> u òp.
<i>Fusobacteria</i>	<i>Fusobacteria</i>	<i>Fusobacteriales</i>	<i>Fusobacteriaceae</i>	<i>Fusobacterium</i>	<i>F. nucleatum</i> , <i>F. necroforum</i> , <i>F. vincentii</i> u òp.
				<i>Leptotrichia</i>	<i>L. buccalis</i> u òp.
				<i>Streptobacillus</i>	<i>S. moniliformis</i>

CLASSIFICATION OF VIRUSES (updates approved during EC 48, Budapest, Hungary, August 2016; Email ratification 2017)

GENOME	ORDER	FAMILY	SUBFAMILY	GENUS	SPECIES
dsDNA	<i>Herpesvirales</i>	<i>Herpesviridae</i>	<i>Alphaherpesvirinae</i>	<i>Simplexvirus</i>	<i>Human alphaherpesvirus 1, 2</i>
dsDNA	<i>Herpesvirales</i>	<i>Herpesviridae</i>	<i>Alphaherpesvirinae</i>	<i>Varicellovirus</i>	<i>Human alphaherpesvirus 3</i>
dsDNA	<i>Herpesvirales</i>	<i>Herpesviridae</i>	<i>Betaherpesvirinae</i>	<i>Cytomegalovirus</i>	<i>Human betaherpesvirus 5</i>
dsDNA	<i>Herpesvirales</i>	<i>Herpesviridae</i>	<i>Betaherpesvirinae</i>	<i>Roseolovirus</i>	<i>Human betaherpesvirus 6A, 6B, 7</i>
dsDNA	<i>Herpesvirales</i>	<i>Herpesviridae</i>	<i>Gammaherpesvirinae</i>	<i>Lymphocryptovirus</i>	<i>Human gammaherpesvirus 4</i>
dsDNA	<i>Herpesvirales</i>	<i>Herpesviridae</i>	<i>Gammaherpesvirinae</i>	<i>Rhadinovirus</i>	<i>Human gammaherpesvirus 8</i>
dsDNA	<i>Unassigned</i>	<i>Adenoviridae</i>		<i>Mastadenovirus</i>	<i>Human mastadenovirus A-F</i>
dsDNA	<i>Unassigned</i>	<i>Iridoviridae</i>	<i>Alphairidovirinae</i>	<i>Lymphocystivirus</i>	<i>Lymphocystis disease virus 1</i>
dsDNA	<i>Unassigned</i>	<i>Papillomaviridae</i>		<i>Alphapapillomavirus</i>	<i>Alphapapillomavirus 1-72</i>
dsDNA	<i>Unassigned</i>	<i>Papillomaviridae</i>		<i>Betapapillomavirus</i>	<i>Betapapillomavirus 1</i>
dsDNA	<i>Unassigned</i>	<i>Papillomaviridae</i>		<i>Deltapapillomavirus</i>	<i>Deltapapillomavirus 1</i>
dsDNA	<i>Unassigned</i>	<i>Papillomaviridae</i>		<i>Gammapapillomavirus</i>	<i>Gammapapillomavirus 1</i>
dsDNA	<i>Unassigned</i>	<i>Polyomaviridae</i>		<i>Alphapolyomavirus</i>	<i>Human polyomavirus 12</i>
dsDNA	<i>Unassigned</i>	<i>Polyomaviridae</i>		<i>Betapolyomavirus</i>	<i>Human polyomavirus 1</i>
dsDNA	<i>Unassigned</i>	<i>Polyomaviridae</i>		<i>Deltapolyomavirus</i>	<i>Human polyomavirus 6</i>
dsDNA	<i>Unassigned</i>	<i>Poxviridae</i>	<i>Chordopoxvirinae</i>	<i>Molluscipoxvirus</i>	<i>Molluscum contagiosum virus</i>
dsDNA	<i>Unassigned</i>	<i>Poxviridae</i>	<i>Chordopoxvirinae</i>	<i>Orthopoxvirus</i>	<i>Vaccinia virus</i>
dsDNA	<i>Unassigned</i>	<i>Poxviridae</i>	<i>Chordopoxvirinae</i>	<i>Orthopoxvirus</i>	<i>Variola virus</i>
dsDNA	<i>Unassigned</i>	<i>Poxviridae</i>	<i>Chordopoxvirinae</i>	<i>Orthopoxvirus</i>	<i>Monkeypox virus</i>
ssDNA(-)	<i>Unassigned</i>	<i>Anelloviridae</i>		<i>Alphatorquevirus</i>	<i>Torque teno virus 1</i>
ssDNA(-)	<i>Unassigned</i>	<i>Anelloviridae</i>		<i>Betatorquevirus</i>	<i>Torque teno mini virus 1</i>
ssDNA(-)	<i>Unassigned</i>	<i>Anelloviridae</i>		<i>Gammatorquevirus</i>	<i>Torque teno midi virus 1</i>
ssDNA(+/-)	<i>Unassigned</i>	<i>Circoviridae</i>		<i>Circovirus</i>	<i>Human associated circovirus 1</i>
ssDNA(+/-)	<i>Unassigned</i>	<i>Genomoviridae</i>		<i>Gemykibivirus</i>	<i>Human associated gemykibivirus 1</i>
ssDNA(+/-)	<i>Unassigned</i>	<i>Genomoviridae</i>		<i>Gemyvongvirus</i>	<i>Human associated gemyvongvirus 1</i>
ssDNA(+/-)	<i>Unassigned</i>	<i>Parvoviridae</i>	<i>Parvovirinae</i>	<i>Bocaparvovirus</i>	<i>Ungulate bocaparvovirus 1</i>
dsDNA-RT	<i>Unassigned</i>	<i>Hepadnaviridae</i>		<i>Orthohepadnavirus</i>	<i>Hepatitis B virus</i>
ssRNA(-)	<i>Bunyavirales</i>	<i>Nairoviridae</i>		<i>Orthonairovirus</i>	<i>Crimean-Congo hemorrhagic fever orthonairovirus</i>
ssRNA(-)	<i>Bunyavirales</i>	<i>Peribunyaviridae</i>		<i>Orthobunyavirus</i>	<i>Bunyamwera orthobunyavirus</i>
ssRNA(-)	<i>Bunyavirales</i>	<i>Peribunyaviridae</i>		<i>Orthobunyavirus</i>	<i>California encephalitis orthobunyavirus</i>
ssRNA(-)	<i>Mononegavirales</i>	<i>Bornaviridae</i>		<i>Bornavirus</i>	<i>Mammalian 1 bornavirus</i>
ssRNA(-)	<i>Mononegavirales</i>	<i>Filoviridae</i>		<i>Ebolavirus</i>	<i>Bundibugyo/Reston/Sudan/Tai Forest/Zaire ebolavirus</i>
ssRNA(-)	<i>Mononegavirales</i>	<i>Filoviridae</i>		<i>Marburgvirus</i>	<i>Marburg marburgvirus</i>
ssRNA(-)	<i>Mononegavirales</i>	<i>Paramyxoviridae</i>		<i>Henipavirus</i>	<i>Hendra henipavirus</i>
ssRNA(-)	<i>Mononegavirales</i>	<i>Paramyxoviridae</i>		<i>Morbillivirus</i>	<i>Measles morbillivirus</i>
ssRNA(-)	<i>Mononegavirales</i>	<i>Paramyxoviridae</i>		<i>Respirovirus</i>	<i>Human respirovirus 1, 3</i>
ssRNA(-)	<i>Mononegavirales</i>	<i>Paramyxoviridae</i>		<i>Rubulavirus</i>	<i>Human rubulavirus 2, 4</i>
ssRNA(-)	<i>Mononegavirales</i>	<i>Paramyxoviridae</i>		<i>Rubulavirus</i>	<i>Mumps rubulavirus</i>
ssRNA(-)	<i>Mononegavirales</i>	<i>Pneumoviridae</i>		<i>Metapneumovirus</i>	<i>Human metapneumovirus</i>
ssRNA(-)	<i>Mononegavirales</i>	<i>Pneumoviridae</i>		<i>Orthopneumovirus</i>	<i>Human orthopneumovirus</i>
ssRNA(-)	<i>Mononegavirales</i>	<i>Rhabdoviridae</i>		<i>Lyssavirus</i>	<i>Rabies lyssavirus</i>
ssRNA(-)	<i>Mononegavirales</i>	<i>Rhabdoviridae</i>		<i>Vesiculovirus</i>	<i>Indiana vesiculovirus</i>
ssRNA(-)	<i>Unassigned</i>	<i>Orthomyxoviridae</i>		<i>Influenzavirus A</i>	<i>Influenza A virus</i>
ssRNA(-)	<i>Unassigned</i>	<i>Orthomyxoviridae</i>		<i>Influenzavirus B</i>	<i>Influenza B virus</i>
ssRNA(-)	<i>Unassigned</i>	<i>Orthomyxoviridae</i>		<i>Influenzavirus C</i>	<i>Influenza C virus</i>

GENOME	ORDER	FAMILY	SUBFAMILY	GENUS	SPECIES
ssRNA(-)	Unassigned	Orthomyxoviridae		Influenzavirus D	Influenza D virus
ssRNA(-)	Unassigned	Orthomyxoviridae		Quarantavirus	Quarantavirus
ssRNA(-)	Unassigned	Orthomyxoviridae		Thogotovirus	Thogoto virus
ssRNA(-)	Unassigned	Unassigned		Deltavirus	Hepatitis delta virus
ssRNA(+/-)	Bunyvirales	Phenuiviridae		Phlebovirus	Rift Valley fever phlebovirus
ssRNA(+/-)	Bunyvirales	Phenuiviridae		Phlebovirus	Uukuniemi phlebovirus
ssRNA(+/-)	Unassigned	Arenaviridae		Mammarenavirus	Junin mammarenavirus
ssRNA(+/-)	Unassigned	Arenaviridae		Mammarenavirus	Lassa mammarenavirus
ssRNA(+/-)	Unassigned	Arenaviridae		Mammarenavirus	Lymphocytic choriomeningitis mammarenavirus
ssRNA(+/-)	Unassigned	Arenaviridae		Mammarenavirus	Machupo mammarenavirus
ssRNA(+)	Nidovirales	Coronaviridae	Coronavirinae	Alphacoronavirus	Human coronavirus 229E, NL63
ssRNA(+)	Nidovirales	Coronaviridae	Coronavirinae	Betacoronavirus	Human coronavirus HKU1
ssRNA(+)	Nidovirales	Coronaviridae	Torovirinae	Torovirus	Human torovirus
ssRNA(+)	Picornavirales	Picornaviridae		Aphthovirus	Foot-and-mouth disease virus
ssRNA(+)	Picornavirales	Picornaviridae		Cardiovirus	Cardiovirus A
ssRNA(+)	Picornavirales	Picornaviridae		Cosavirus	Cosavirus A
ssRNA(+)	Picornavirales	Picornaviridae		Enterovirus	Enterovirus C
ssRNA(+)	Picornavirales	Picornaviridae		Enterovirus	Rhinovirus A
ssRNA(+)	Picornavirales	Picornaviridae		Hepatovirus	Hepatovirus A
ssRNA(+)	Picornavirales	Picornaviridae		Kobuvirus	Aichivirus A
ssRNA(+)	Picornavirales	Picornaviridae		Parechovirus	Parechovirus A, B, C
ssRNA(+)	Picornavirales	Picornaviridae		Rosavirus	Rosavirus A
ssRNA(+)	Picornavirales	Picornaviridae		Salivirus	Salivirus A
ssRNA(+)	Unassigned	Astroviridae		Mamastrovirus	Mamastrovirus 1
ssRNA(+)	Unassigned	Caliciviridae		Norovirus	Norwalk virus
ssRNA(+)	Unassigned	Caliciviridae		Sapovirus	Sapporo virus
ssRNA(+)	Unassigned	Flaviviridae		Flavivirus	Dengue virus
ssRNA(+)	Unassigned	Flaviviridae		Flavivirus	Japanese encephalitis virus
ssRNA(+)	Unassigned	Flaviviridae		Flavivirus	Murray Valley encephalitis virus
ssRNA(+)	Unassigned	Flaviviridae		Flavivirus	Omsk hemorrhagic fever virus
ssRNA(+)	Unassigned	Flaviviridae		Flavivirus	Tick-borne encephalitis virus
ssRNA(+)	Unassigned	Flaviviridae		Flavivirus	West Nile virus
ssRNA(+)	Unassigned	Flaviviridae		Flavivirus	Yellow fever virus
ssRNA(+)	Unassigned	Flaviviridae		Flavivirus	Zika virus
ssRNA(+)	Unassigned	Flaviviridae		Hepacivirus	Hepacivirus C
ssRNA(+)	Unassigned	Flaviviridae		Pegivirus	Pegivirus H
ssRNA(+)	Unassigned	Hepeviridae		Orthohepevirus	Orthohepevirus A
ssRNA(+)	Unassigned	Togaviridae		Alphavirus	Chikungunya virus
ssRNA(+)	Unassigned	Togaviridae		Alphavirus	O'nyong-nyong virus
ssRNA(+)	Unassigned	Togaviridae		Alphavirus	Semliki Forest virus
ssRNA(+)	Unassigned	Togaviridae		Alphavirus	Sindbis virus
ssRNA(+)	Unassigned	Togaviridae		Alphavirus	Venezuelan equine encephalitis virus
ssRNA(+)	Unassigned	Togaviridae		Rubivirus	Rubella virus
dsRNA	Unassigned	Picobirnaviridae		Picobirnavirus	Human picobirnavirus
dsRNA	Unassigned	Reoviridae	Sedoreovirinae	Rotavirus	Rotavirus A-G
dsRNA	Unassigned	Reoviridae	Spinareovirinae	Coltivirus	Colorado tick fever virus
ssRNA-RT	Unassigned	Retroviridae	Orthoretrovirinae	Deltaretrovirus	Primate T-lymphotropic virus 1
ssRNA-RT	Unassigned	Retroviridae	Orthoretrovirinae	Lentivirus	Human immunodeficiency virus 1, 2
ssRNA-RT	Unassigned	Retroviridae	Spumaretrovirinae	Spumavirus	Simian foamy virus

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