Ministry of Public Health of Ukraine

Bogomolets National Medical University

Department of microbiology, virology and immunology

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STUDY GUIDE

OF THE PRACTICAL CLASSES COURSE

Part IV

Specialties:

221 "Dentistry"

222 "Medicine"

225 "Medical Psychology"

226 "Pharmacy, industrial pharmacy"

228 "Pediatrics"

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Study guide of the practical classes was approved by cycle methodical commission on hygienic disciplines of the Bogomolets National Medical University on September 23, 2020.

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Class № 25

Topic: "Staphylococci and streptococci. Microbiological diagnosis of staphylococcal and streptococcal infection"

Topic relevance:

The Gram-positive cocci include staphylococci, streptococci. They cause inflammatory processes with formation of pus. Staphylococci and streptococci are responsible for a number of local lesions in humans: hidradenitis, abscess, paronychia, blepharitis, furuncle, carbuncle, periostitis, osteomyelitis, folliculitis, sycosis, dermatitis, eczema, chronic pyodermia, peritonitis, meningitis, appendicitis, and cholecystitis. In some cases they may give rise to secondary infection in individuals suffering from smallpox, influenza, and wounds, as well as postoperative suppurations. Staphylococcal sepsis and staphylococcal pneumonia in children are particularly severe diseases. Ingestion of foodstuffs (cheese, curds, milk, rich in sugar cakes and pastry, ice cream, etc.) contaminated with pathogenic staphylococci may result in food poisoning. Acute *Streptococcus pyogenes* infections may manifest with pharyngitis, scarlet fever (rash), impetigo, cellulitis, or erysipelas. Invasive infections can result in necrotizing fasciitis, myositis and streptococcal toxic shock syndrome. Patients may also develop immune-mediated sequelae such as acute rheumatic fever and acute glomerulonephritis. *S. pneumoniae* causes pneumonia, meningitis, and sometimes occult bacteremia.

All mentioned above provide for the relevance the topic and positive motivation for the study.

Objectives:

- To study basic biological properties of staphylococci, streptococci and pneumococci;
- To master the basic methods of microbiological diagnosis, treatment and prophylaxis of the diseases caused by Gram-positive cocci.

Basic knowledge, abilities, skills necessary for the study of the theme (interdisciplinary integration).

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

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

Term	Definition
Pyogenic cocci	Large group of microbes, incorporated by similar morphology (the cells of cocci have a spherical form) and ability to cause pus formation.
Bacterial toxins	Products of metabolism, having a direct toxic influence on the microorganism cells, or causing development of intoxication symptoms with mediators' help.
Pyogenic infections	Infections accompanied with pus formation, or development of seropurulent inflammation (by suppuration). Pyogenic infections are divided by localization into general (sepsis, septicopyemia and other) and local (pyoderma, furuncle, carbuncle, abscess, phlegmon).
Staphylococcal hemotoxin (gemolysin)	Protein, causing the lysis of erythrocytes and initiator of the synthesis of antigemolytic antibodies. Gemolysin presents in the cell-free filtrates of staphylococcal cultures.
Protein A	Protein A is virulence factor of staphylococci. It is **a** surface protein of *S. aureus* which binds Ig G molecules by the Fc region. In serum, bacteria will bind IgG molecules the wrong way round by this non-immune mechanism. This will disrupt opsonization and phagocytosis.
Coagulase	Coagulase is an extracellular protein which binds to prothrombin in the host to form a complex called staphylothrombin. The protease activity characteristic of thrombin is activated in the complex, resulting in the conversion of fibrinogen to fibrin. This is the basis of the tube coagulase test. Coagulase is a traditional marker for identifying *S. aureus* in the clinical microbiological laboratory.
Streptokinase	Streptokinases are enzymes produced by most streptococci of group A. Streptokinases participate in fibrin lysis. Streptokinase has been given intravenously for treatment of pulmonary emboli, coronary artery, and venous thromboses. Mixtures of streptodornase and streptokinase are used in "enzymatic debridement." They help to liquefy exudates and facilitate removal of pus and necrotic tissue; antimicrobial drugs thus gain better access, and infected surfaces recover more quickly.

Theoretical questions to the class:

- History of discovery and study of Gram-positive cocci

- Morphology, antigen structure and cultural properties of staphylococci, streptococci and pneumococci.
- Enzymes and virulence factors of staphylococci, streptococci and pneumococci.
- Epidemiology, pathogenesis and clinical picture of diseases, caused by staphylococci, streptococci and pneumococci.
- Microbiological diagnosis of the infections caused by Gram-positive cocci.
- Treatment and prophylaxis of staphylococcal, streptococcal and pneumococcal infections.

Students' practical activities:

- To study morphological, cultural and biochemical properties of staphylococci, streptococci and pneumococci (demonstration).
- To inoculate pus onto yolk-salt agar.
- To inoculate patient's blood with suspicion on a sepsis in sugar MPB.
- To examine susceptibility of microorganisms using disk diffusion method
- To create the scheme of microbiological diagnosis of staphylococcal and streptococcal infection.
- To familiarize with preparations for diagnosis, specific prophylaxis and therapy of the diseases caused by Gram-positive cocci.

Topic content:

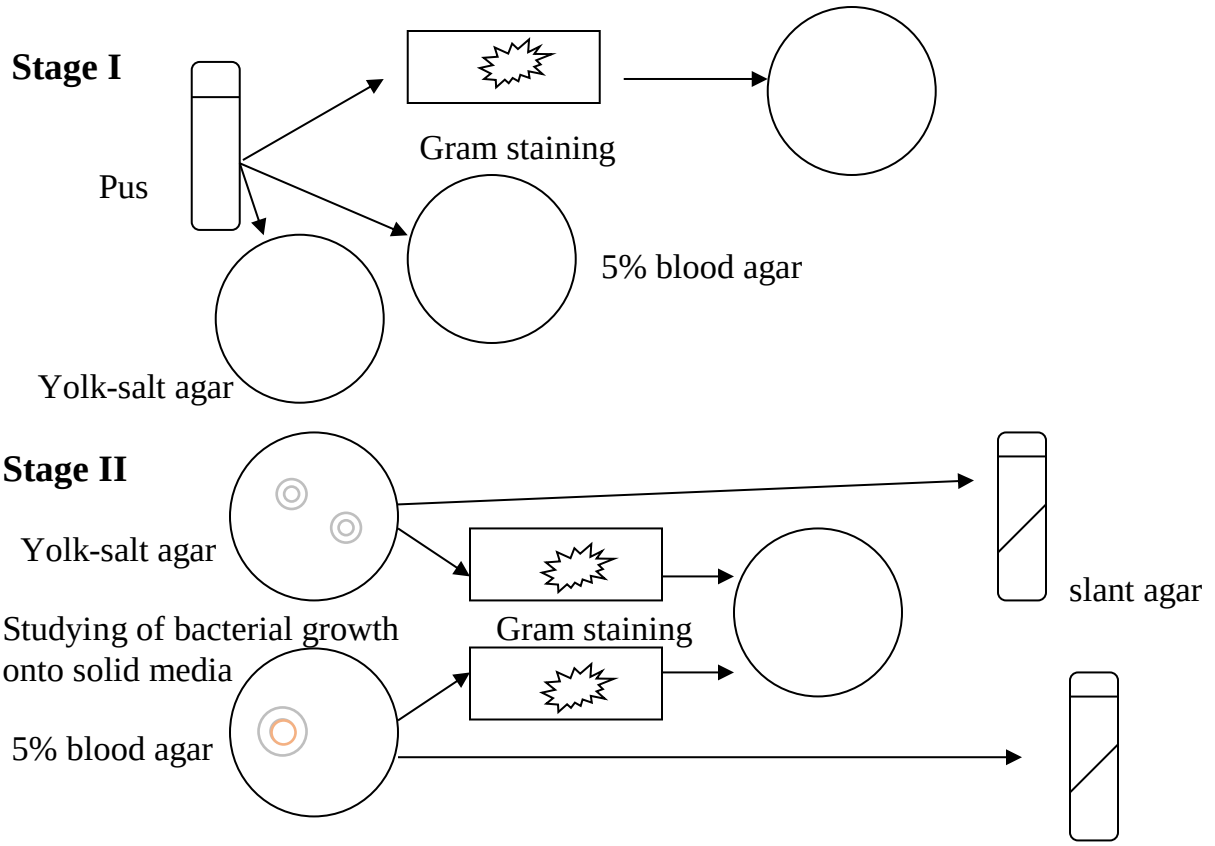
The class students prepare smears from the pure cultures of staphylococci and streptococci, stain them by Gram's technique, study morphological and tinctorial properties by microscopical examination of smears. Students study cultural, biochemical and other biological properties of Gram-positive cocci, create the scheme of microbiological diagnosis of staphylococcal and streptococcal infections. Students familiarize themselves with cultivation methods of staphylococci and streptococci and determine staphylococcal susceptibility to antibiotics using disk diffusion method. They start microbiological diagnosis of staphylococcal infection by inoculation pus onto yolk-salt agar, and microbiological diagnosis of septic process by inoculation patient's blood with suspicion on a sepsis in sugar MPB. Students describe their work and obtained results in the protocol and the teacher signs it.

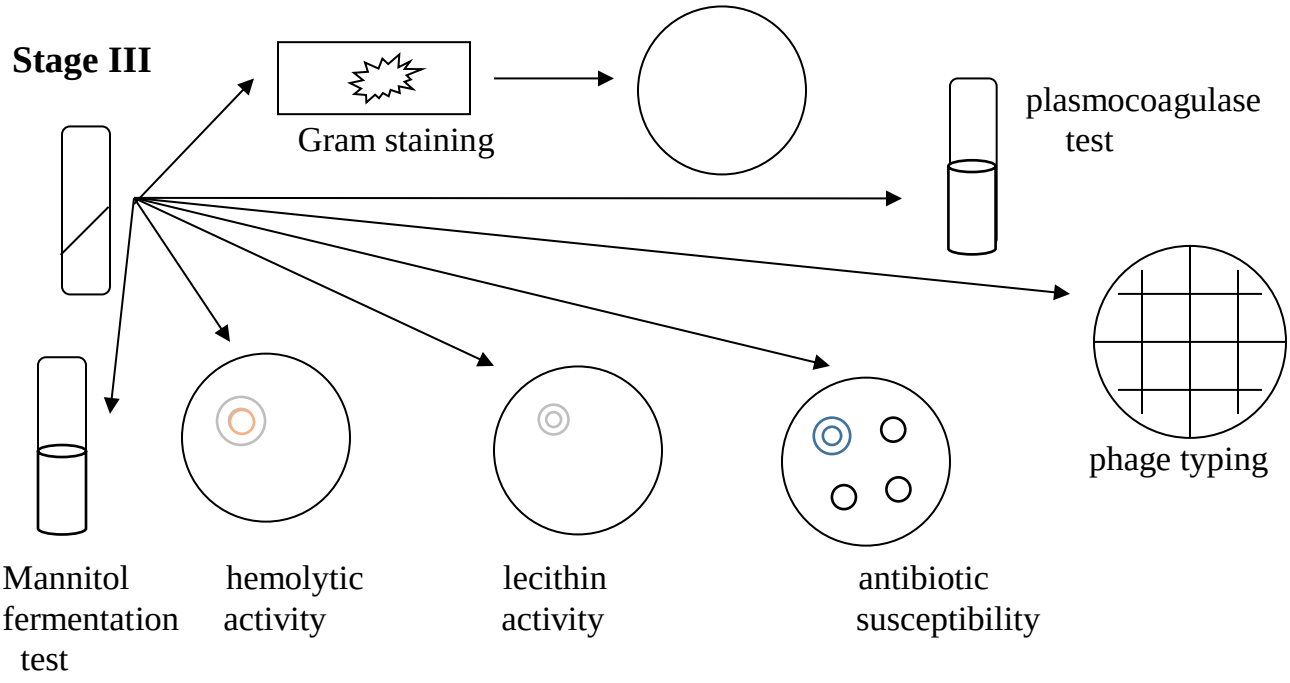
Recommendations for the protocol design

The following items should be included in the protocol.

- Differentiative signs of some species of staphylococci.
- Differentiative signs of some genes of *Micrococcaceae* family.
- Virulence factors of staphylococci and streptococci.
- The schemes of microbiological diagnosis of pyogenic process caused by staphylococci and streptococci.
- List of diagnostic, prophylactic and medical preparations.

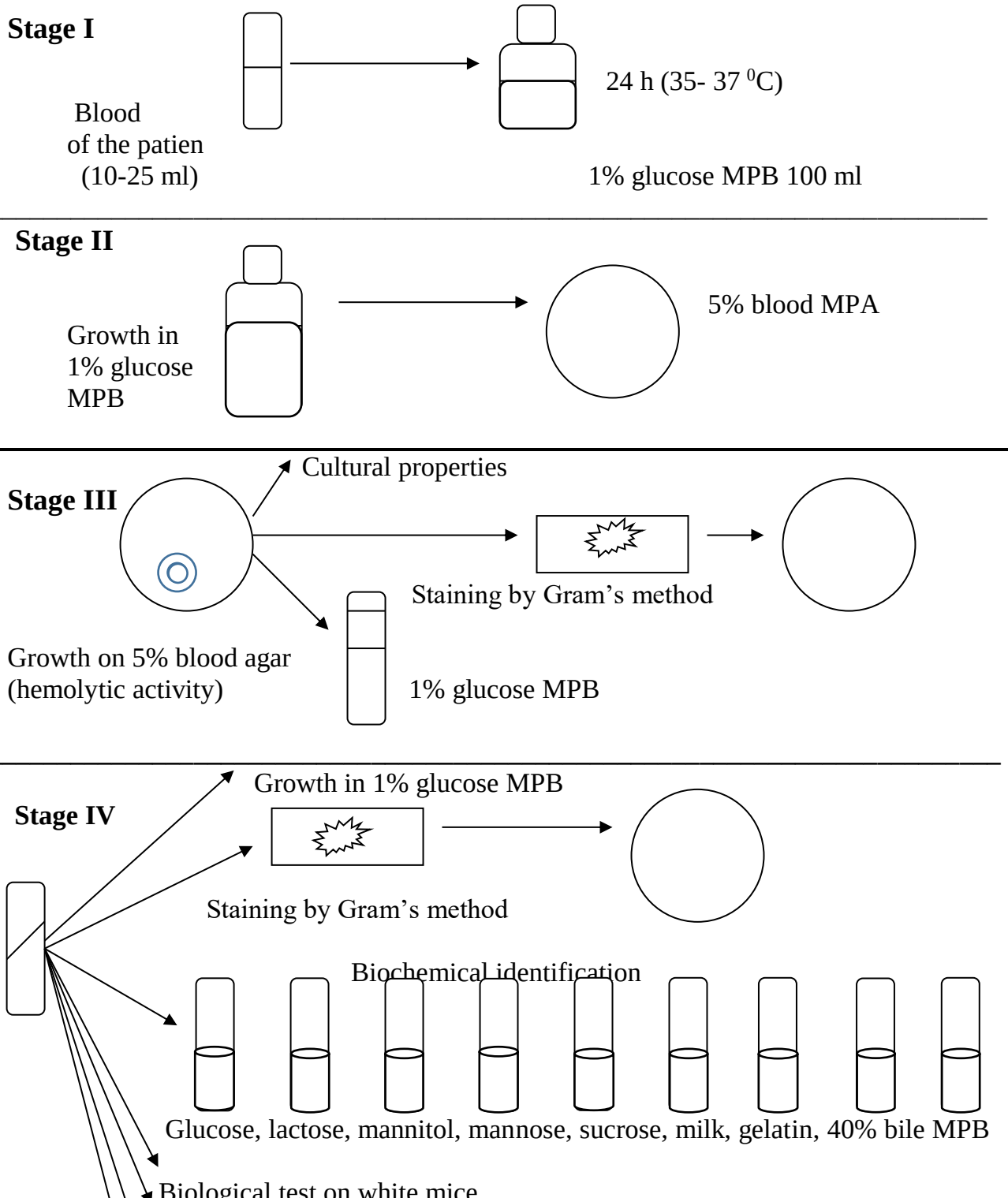
Laboratory diagnosis of staphylococcal infection





Stage IV **Conclusion**

Laboratory diagnosis of streptococcal process



Stage V **Conclusion**

Differentiation of *Staphylococci*

Main characteristics	Species		
	S. aureus	*S. epidermidis;*	*S. saprophyticus*
Plasmacoagulase	+	—	—
Phosphatase	+	+	—
Arginine dehydrolase nitrate	+	+	—
Reductase	+	+	—
Protein A in superficial antigen	+	—	—
Mannitol	+	—	+
Trehalose	+	—	+
Galactose	+	+	—
Ribose	+	—	—
Production of alpha-toxin	+	–	–
Resistance to novobiocin	S	S	R
Growth in the presence of biotin	–	+	NT

Note: *S*, *sensitive;* ***R***, *resistant,* ***NT***, *not tested.*

Virulence factors of staphylococci

I. <u>Toxins</u>

- **Membranotoxins (α-, β-, δ- and γ-hemolysins)** are characterized by lethal, haemolytic, and necrotic activity.
- **Leucocidin (δ-toxin)** destroys leucocytes, haematoblasts of the bone marrow and nerve cells.
- **Enterotoxins A-G** are toxins with superantigen activity. Enterotoxins cause diarrhea and vomiting when ingested and are responsible for staphylococcal food poisoning.
- **Epidermolytic (exfoliative) toxin (ET) –** causes the scalded skin syndrome in neonates, with widespread blistering and loss of the epidermis.
- **Toxic shock syndrome toxin (TSST-1)** are toxins with superantigen activity. TSST-1 is responsible for 75% of toxic shock syndrome, including all menstrual cases.

II. <u>Surface structure</u>

- **Protein A** is a surface protein of *S. aureus* which binds Ig G molecules by the Fc region. In serum, bacteria will bind IgG molecules the wrong way round by this non-immune mechanism. In principle this will disrupt opsonization and phagocytosis.
- **Teichoic acids** activate Co by alternative pathway, activate coagulate and kalicrein-kinine systems, facilitate adhesion to epiteliocytes.
- **Capsule** confer resistance to phagocytosis, facilitate adhesion.
- **Peptidoglycan** stimulates production of endogenic pyrogenes

III. Enzymes

- **Plasmocoagulase** binds to prothrombin; together they become enzymatically active and initiate fibrin polymerization. Coagulase may deposit fibrin on the surface of staphylococci, perhaps altering their ingestion by phagocytic cells or their destruction within such cells.
- **Clumping factor** is a surface *S. aureus* compound that is responsible for adherence of the organisms to fibrinogen and fibrin. When mixed with plasma, *S. aureus* forms clumps. Clumping factor is distinct from coagulase
- **Staphylokinase** resulting in fibrinolysis but acting much more slowly than streptokinase
- **Hyaluronidase** – spreading factor
- **DNase**
- **Lipases**
- **Proteinase**
- **β-lactamase**

Virulence factors of streptococci

I. Toxins:

- **Streptolysin O** is a protein that is hemolytically active in the reduced state (available –SH groups) but rapidly inactivated in the presence of oxygen. Streptolysin O is responsible for some of the hemolysis seen when growth is in cuts deep into the medium in blood agar plates. It combines with **antistreptolysin O,** an antibody that appears in man following infection with any streptococci that produce streptolysin O.
- **Streptolysin S** is the agent responsible for the hemolytic zones around streptococcal colonies growing on the surface of blood agar plates. It is elaborated in the presence of serum—hence the name streptolysin S. It is not antigenic, but it may be inhibited by a nonspecific inhibitor that is frequently present in the sera of humans and animals and is independent of past experience with streptococci.
- **Leukocydine**
- **Cytotoxins**
- **Pyrogenic exotoxins (eritrogenic toxin) A, B, C** have been associated with streptococcal toxic shock syndrome and scarlet fever. The pyrogenic exotoxins act as superantigens, which stimulate T cells by binding to the class II MHC in the V_{β}- region of the T cell receptor. The activated T cells release cytokines that mediate shock and tissue injury.

II. Enzymes:

- **Hyaluronidase** splits hyaluronic acid, an important component of the ground substance of connective tissue. Thus, hyaluronidase aids in spreading infecting microorganisms (spreading factor).
- **Streptokinase (Fibrinolysin)** transforms the plasminogen of human plasma into plasmin, an active proteolytic enzyme that digests fibrin and other proteins.
- **Proteinase**
- **Streptodornase A-D** (streptococcal deoxyribonuclease, DNase) possess deoxyribonuclease activity; B and D possess ribonuclease activity as well.

- **C5a-peptidase** destroys C5a-component of complement.
- **PNA-ase, ATP-ase** – inhibition of activity of phagocytes.

III. <u>Surface structure:</u>

- **Capsule** confers resistance to phagocytosis, facilitate adhesion
- **M-protein** are clearly virulence factors associated with resistance to phagocytosis. Both the M proteins and lipoteichoic acid are supported externally to the cell wall on fimbriae, and the lipoteichoic acid, in particular, appears to mediate bacterial attachment to host epithelial cells. M protein, peptidoglycan, N-acetylglucosamine, and group-specific carbohydrate portions of the cell wall have antigenic epitopes similar in size and charge to those of mammalian muscle and connective tissue.

Self-test questions:

- What diseases do staphylococci, streptococci and pneumococci cause?
- What material is explored for laboratory diagnosis of staphylococcal infection?
- What basic methods of laboratory diagnosis of staphylococcal, streptococcal and pneumococcal infection do you know?
- What virulence factors are produced by staphylococci, streptococci and pneumococci?
- What media are used for cultivation of staphylococci and streptococci?
- How is plasmocoagulation test carried out?
- What serogroup of streptococci is the most pathogenic for a man?
- What purpose is the phage typing carried out for?

Class №26

Topic: "Meningococci and gonococci. Microbiological diagnosis of the diseases caused by meningococci and gonococci"

Topic relevance:

Two species of Gram-negative cocci cause serious disease in humans – *Neisseria meningitidis,* the etiologic agent of epidemic meningitis, and *Neisseria gonorrhoeae*, the cause of the sexually transmitted disease, gonorrhea. Symptomatic or asymptomatic localized gonococcal infections include urethritis, cervicitis, proctitis, pharyngitis, and conjunctivitis. Disseminated infections occur either by extension to adjacent organs (pelvic inflammatory disease, epididymitis) or by bacteremic spread (skin lesions, tenosynovitis, septic arthritis, endocarditis, and meningitis). Infection with *N. meningitidis* has two presentations, meningococcemia, characterized by skin lesions, and acute bacterial meningitis. Fulminant disease (with or without meningitis) is characterized by multisystem involvement and high mortality.

All mentioned above provide for the relevance the topic and positive motivation for the study.

Concrete objectives:

- To study basic biological properties of meningococci and gonococci.
- To master the main methods of microbiologic diagnosis of diseases caused by meningococci and gonococci
- To analyse modern approaches to the treatment and prophylaxis of meningococcal infection and gonorrhoea.

Basic knowledge, skills, needed to study topic (interdisciplinary integration). See class №25.

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

Term	Definition
Meningococcal infection	Acute infectious disease of man, caused by *Neisseria meningitides*, transmissible by aspiration of infective particles (meningococci are spread via respiratory droplets), which attach to epithelial cells of the nasopharyngeal and oropharyngeal mucosa, cross the mucosal barrier, enter the bloodstream (and cause meningococcemia) and/or the central nervous system (and cause meningitis).
Gonococcal infection	Acute or chronic infectious disease of man, caused by *Neisseria gonorrhoeae* with sexual transmission and characterized by festering inflammation of mucous membrane of urinoexcretory

	ways (gonorrhea), conjunctiva of eye (gonococcal ophthalmia neonatorum), other organs, by intoxication.
Uncompleted phagocytosis	Microorganisms (gonococci and meningococci) are not destroyed within the phagocytic vacuole.
IgA proteases	Two distinct extracellular proteins, which cleave the heavy chain of human immunoglobulin A1 (IgA1) at different points within the hinge region and protect microorganisms from the action of antibodies.

Theoretical questions:

- History of exploration of *Neisseria*.
- Morphological, cultural properties of causative agents of meningococcal and gonococcal infections.
- Antigenic structure of *Neisseria meningitidis* and *Neisseria gonorrhoeae*.
- Biochemical properties and factors of pathogenicity of *Neisseria meningitidis* and *Neisseria gonorrhoeae*.
- Epidemiology, pathogeny and clinical picture of diseases caused by *Neisseria meningitidis* and *Neisseria gonorrhoeae*.
- Microbiological diagnosis of infections caused by *Neisseria meningitidis* and *Neisseria gonorrhoeae*.
- Treatment and prophylaxis of an acute and chronic gonorrhea.
- Treatment and prophylaxis of meningococcal infection.

Practical activities performed in class:

- To study morphological, cultural and fermentative properties of *Neisseria meningitidis* and *Neisseria gonorrhoeae* (demonstration).
- To create the schemes of microbiological diagnosis of meningococcal infection and meningococcal carriage.
- To create the schemes of microbiological diagnosis of gonorrhea.
- To learn basic principles of microbiological diagnosis of gonorrhea.
- To study preparations for diagnosis, specific prophylaxis and therapy of meningococcal infection and gonorrhea.

Topic content:

The students study morphology of meningococci and gonococci in the smear by microscopical examination of the smears prepared from the pure culture of meningococci, meningococci in a liquor, stained by Gram, and phenomenon of uncomplete phagocytosis of gonococci in material from urethra. Students familiarize with cultural, biochemical and other biological properties of meningococci and gonococci and make the scheme of microbiological diagnosis of meningococcal disease. Students describe their work and obtained results in the protocol and the teacher signs it.

Virulence factors of *N. gonorrheae*

- **Pili (Fibriae)** enhance attachment to host cells and resistance to phagocytosis.
- **Capsule** confers resistance to phagocytosis, facilitates adhesion
- **Por protein** extends through the gonococcal cell membrane and forms pores in the surface through which some nutrients enter the cell. Por proteins may impact intracellular of gonococci within neutrophils by preventing phagosome-lysosome fusion.
- **Opa proteins** function in adhesion of gonococci within colonies and in attachment of gonococci to host cells, especially cells that express carcinoembryonic antigens (CD66).
- **Rmp (Protein III)** is an outer membrane protein found in all strains of N gonorrhoeae. Antibodies to Rmp, induced either by a neisserial infection or by colonization with E coli, block bactericidal antibodies directed against Por and LOS. Rmp antibodies may facilitate infection with N gonorrhoeae.
- **Lipooligosaccharide (LOS)** produces mucosal damage in fallopian tube organ cultures and brings about the release of enzymes.
- **Ig A_1-protease** destroys Ig A_1 and protects bacteria from action of antibodies.
- **β-lactamases (plasmid encoded)** open the **β**-lactam ring of penicillins and cephalosporins and abolish their antimicrobial activity.

Virulence factors of *N. meningitides*

- **Meningococcal LPS (endotoxin)** suppresses leukotriene B4 synthesis in human polymorphonuclear leukocytes. The loss of leukotriene B4 deprives the leukocytes of a strong chemokinetic and chemotactic factor.
- **Pili (Fibriae)** enhance attachment to host cells and resistance to phagocytosis.
- **The capsule** is antiphagocytic and it is an important virulence factor.
- **Ig A_1-protease** destroys Ig A_1 and protects bacteria from action of antibodies.

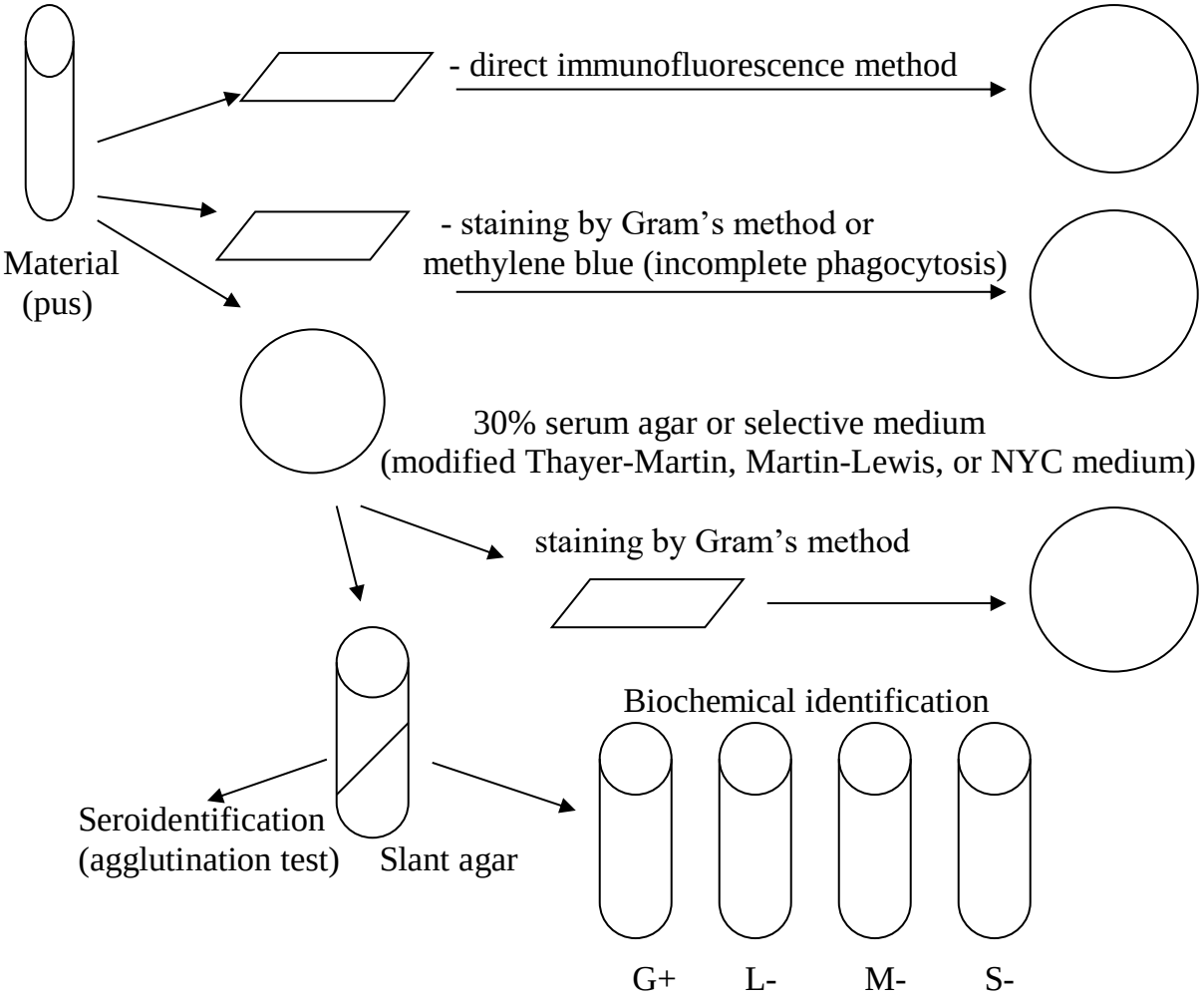
Recommendations for the protocol design

Biochemical Properties of the Neisseriae

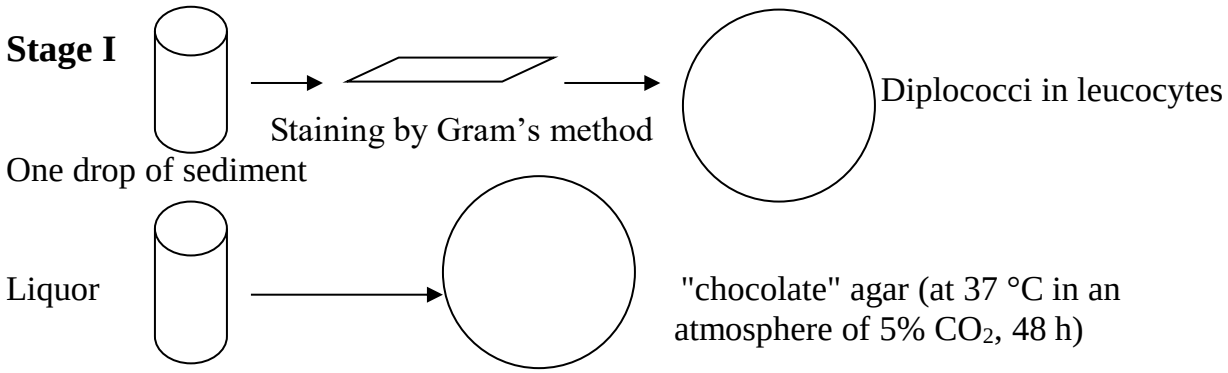
	Growth on MTM, ML, or NYC medium*	**Acid formed from**				**DNase**
		Glucose	Maltose	Lactose	Sucrose or Fructose	
N gonorrhoeae	+	+	-	-	-	-
N. meningitidis	+	+	+	-	-	-
N. sicca	-	+	+	-	+	-
N. catarrhalis	-	-	-	-	-	+

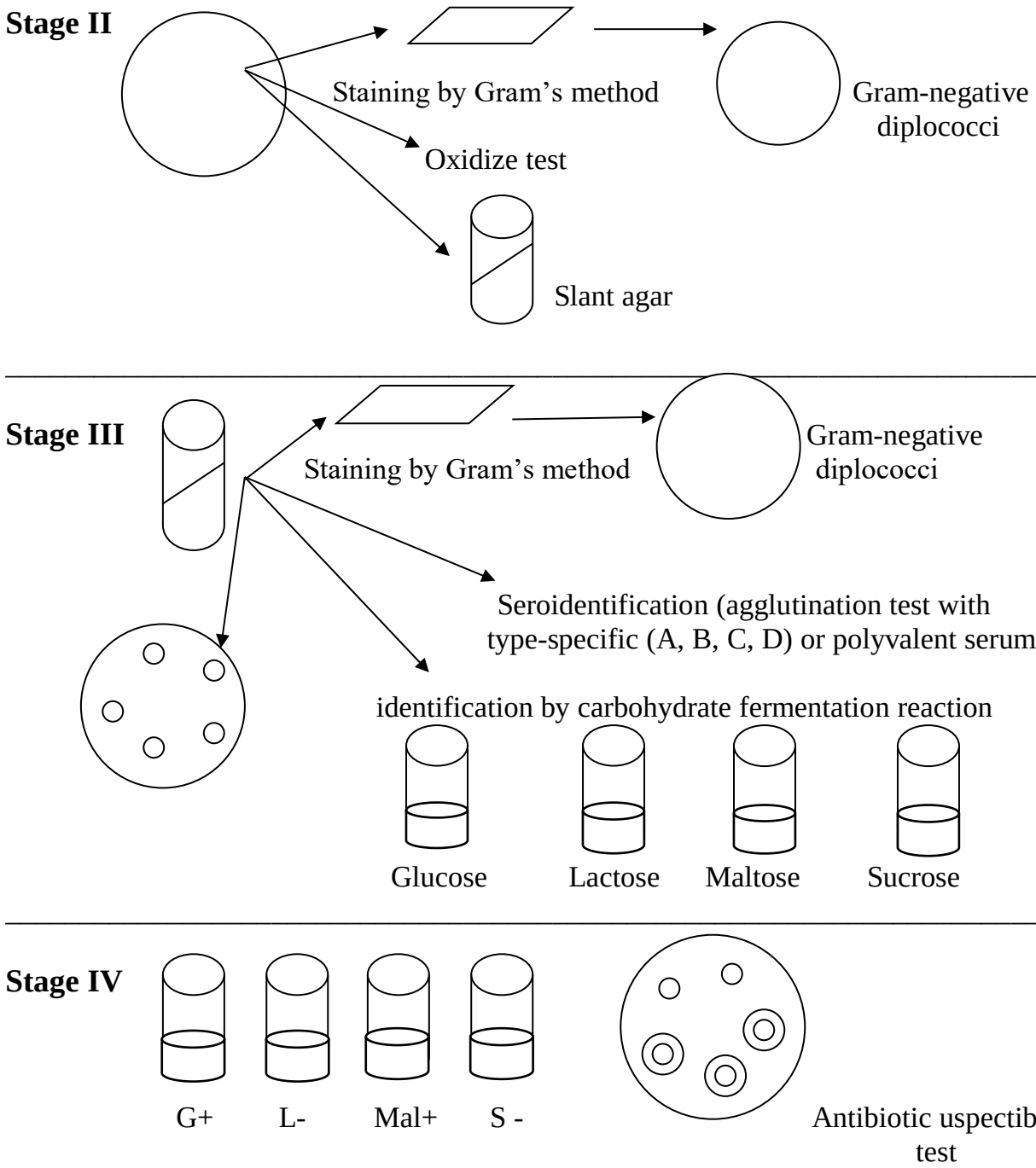
**MTM = modified Thayer-Martin medium; ML = Martin-Lewis medium; NYC = New York City medium.*

The scheme of microbiological diagnosis of gonorrhea



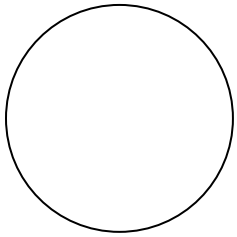
Microbiological diagnosis of meningococcal infection





Conclusion

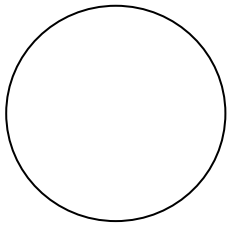
Practical activity №1. To examine the urethral smear for the presence of *N. gonorrhoeae* (demonstration).



Methylene blue staining

Conclusion. The classical appearance of acute gonorrhea is the groups of intracellular kidney-shaped diplococci with flattened, opposed margins within polymorphonuclear leucocytes. There is a high correlation (up to 98%) of these findings with a confirmed diagnosis of acute gonorrhea in the male. Due to normal flora, similar findings in the female are not diagnostic without culture confirmation from a well-collected specimen.

Practical activity №2. To examine the smear from pure culture of *N. meningitidis* (demonstration).



Gram staining

Conclusion.

Control questions:

- What diseases does meningococci cause?
- Name the portal of entry for meningococci.
- What toxin do meningococci produce?
- Describe the antigenic structure of meningococci and their classification. How many serogroups of meningococci are known?
- Name media for cultivation of meningococci and factors for their growth.
- What resistance to the physical and chemical factors do meningococci offer?
- What tested material can doctor take for isolation of gonococci?
- What method of research is basic at the acute form of gonorrhoea?
- What reaction is used for serological diagnosis of gonorrhea?

Class №27

Topic: "Escherichia. Microbiological diagnosis diseases caused by E.coli"

Topic relevance:

Intestinal infections occupy one of leading places in infectious pathology of man. The problem of acute intestinal infections is very actual owing to their wide distribution and high vulnerability of all human population. Escherichiosis are diseases caused by different strains of *E. coli* that take special place among all acute intestinal infections. Pathogenic *E. coli* cause coli-enteritis in the early age children and diseases similar to dysentery and cholera in children and adults. Escherichiosis is 15-30% among all cases of intestinal infections. Clinical symptoms of escherichiosis are very similar to other intestinal infections, such as dysentery, salmonellosis and other, this is why it is necessary to perform microbiological diagnostics of escherichiosis.

All mentioned above provide for the relevance the topic and positive motivation for the study.

Objectives:

- To speak about biological properties of *E.coli*, causative agent of coli-enteritis.
- To explain pathogenesis of diseases caused by pathogenic *E. coli*.
- To study the methods of microbiological diagnosis, etiotropic therapy and prophylaxis of coli-enteritis.

Basic knowledge, abilities, skills necessary for the study of the theme (interdisciplinary integration). See class №25.

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

Term	Definition
Escherichiosis	Infectious diseases group caused by *Escherichia*. There are two types of escherichiosis: 1) The diseases, caused by E. coli like a common member of the normal flora of the large intestine; 2) The diarrheal diseases caused by virulent Escherichia coli strains (coli-enteritis, coli-infection).
E. coli-Associated Diarrheal Diseases	*E. coli* that cause diarrhea are extremely common worldwide. These *E. coli* are classified by the characteristics of their virulence properties, and each group causes disease by a different mechanism. The principal groups of this organism responsible for enteric disease include the classical enteropathogenic serotypes (EPEC), enterotoxigenic (ETEC), enteroinvasive (EIEC), enterohemorrhagic (EHEC), and enteroaggregative (EAEC) strains.

Enteropathogenic *E. coli* (EPEC)	Enteropathogenic *E. coli* (EPEC) is an important cause of diarrhea in infants, especially in developing countries. EPEC adhere to the mucosal cells of the small bowel. The result of EPEC infection is watery diarrhea, which is usually self-limited but can be chronic. EPEC diarrhea has been associated with multiple specific serotypes of *E coli;* strains are identified by O antigen and occasionally by H antigen typing.
Enterotoxigenic *E. coli* (ETEC)	Enterotoxigenic *E. coli* (ETEC) is a common cause of "traveler's diarrhea" and a very important cause of diarrhea in infants in developing countries. ETEC colonization factors specific for humans promote adherence of ETEC to epithelial cells of the small bowel. Some strains of ETEC produce a heat-labile exotoxin (LT) that is under the genetic control of a plasmid. LT is antigenic and cross-reacts with the enterotoxin of *Vibrio cholerae.* The ETEC virulence plasmids carry the genes for enterotoxins (heat-labile enterotoxin (LT) and heat-stable enterotoxin (ST)) may also carry genes for the colonization factors that facilitate the attachment of *E coli* strains to the intestinal epithelium. The pathogenesis of ETEC diarrhea involves two steps: intestinal colonization, followed by elaboration of diarrheagenic enterotoxin(s).
Enterohemorrhagic *E. coli* (EHEC)	Enterohemorrhagic *E. coli* (EHEC) produces verotoxin, named for its cytotoxic effect on Vero cells. EHEC has been associated with hemorrhagic colitis, a severe form of diarrhea, and with hemolytic uremic syndrome, a disease resulting in acute renal failure, microangiopathic hemolytic anemia, and thrombocytopenia. Verotoxin has many properties that are similar to the Shiga toxin produced by some strains of *Shigella dysenteriae* type 1; however, the two toxins are antigenically and genetically distinct.
Enteroinvasive *E. coli* (EIEC)	Enteroinvasive *E.coli* (EIEC) produces a disease very similar to shigellosis. The disease occurs most commonly in children in developing countries and in travelers to these countries. Like shigella, EIEC strains are nonlactose or late lactose fermenters and are nonmotile. EIEC produces disease by invading intestinal mucosal epithelial cells.
Enteroaggregative *E. coli* (EAEC)	Enteroaggregative *E. coli* (EAEC) causes acute and chronic diarrhea (> 14 days in duration) in persons in developing countries. These organisms are also the cause of food-borne illnesses in industrialized countries. They are characterized by their characteristic pattern of adherence to human cells. EAEC produce ST-like toxin and a hemolysin.

Theoretical questions:

- Classification and general description of the family *Enterobacteriaceae*
- Modern views on the evolution of *Enterobacteria*.
- Morphological, cultural properties of causative agents of *E.coli*.
- Antigen structure of *E.coli*.
- The role of *E. coli* in physiology and pathology of man.
- The diseases caused by *E.coli* - Associated Diarrheal Diseases.
- The methods of microbiological diagnosis of *E.coli*.
- The treatment and prophylaxis of coli-enteritis

Practical activities:

- To study morphological, cultural and biochemical properties of *E.coli*.
- To create the scheme of microbiological diagnosis of coli-enteritis.
- To carry out presumptive agglutination test with typical sera and culture of an unknown serovar of *E. coli*.
- To study specific diagnostic agglutination sera and medicine for treatment of patient with diseases caused by *E. coli*.

Topic content.

The students study morphological, cultural, biochemical and antigenic properties of *E. coli*, carry out presumptive agglutination test with typical sera and culture of an unknown serovar of *E. coli,* make conclusions. Students describe their work and obtained results in the protocol and the teacher signs it.

Recommendations for the protocol design

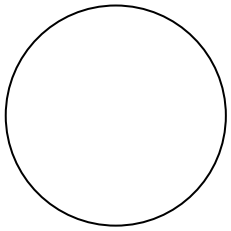
The following items should be included in the protocol.

***E. coli* Virulence Factors**

Diarrhea-producing *E. coli*	**Virulence Factors**
Enteroroxigenic *E. coli*	Heat-labile toxin (LT) Heat-stable toxin (ST) Colonization factors (fimbriae)
Enterohernorrhagic *E. coli*	Shiga-like toxin (SLT-I) Shiga-like toxin II (SLF-II) Colonization factors (fimbriae)
Enteroinvasive *E. coli*	Shiga-like toxin (SLT-I) Shiga-like toxin II (SLF-II) Ability to invade epithelial cells
Enteropathogenic *E. coli*	Adhesin factor for epithelial cells

Urinary trace infections	P- fimbriae
Meningitis	K-1 capsule

Task 1. To examine under microscope the smears of *E. coli* stained by Gram's method.



E.coli

Conclusion:

Task 2. To examine biochemical activity of *E. coli* on Hiss' media

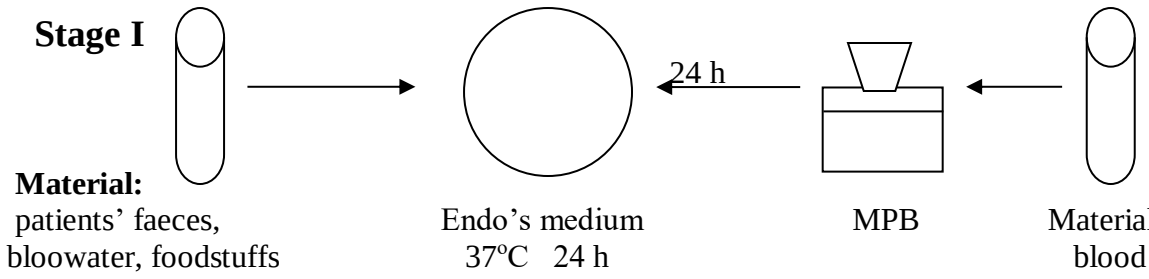
Microbe	**Fermentation of**				**Production of**	
	Glucose	Mannitol	Lactose	Sucrose	H_2S	Indole
E. coli						

Conclusion:

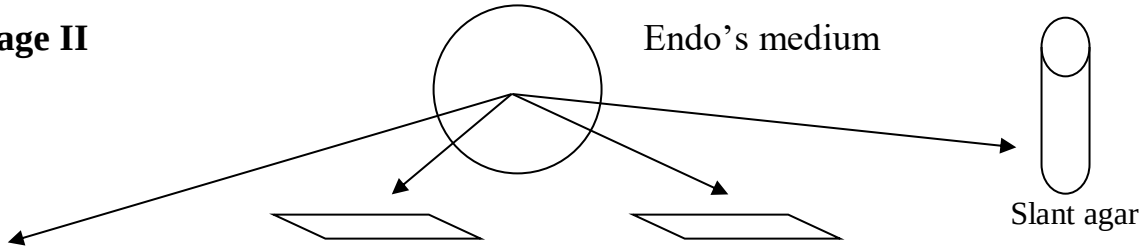
Task 3. To familiarize with specific diagnostic agglutination sera and medicine for treatment of patient with diseases caused *E. coli*.

- *Diagnostic agglutination OK serum for agglutination test.*
- *Bacteriophages for phage typing.*
- *Antibiotics and chemotherapeutic drugs: ampicillin, sulfonamides, tetracycline, trimethoprim-sulfamethoxazole, nalidixic acid, ciprofloxacin, and nitrofurantoin, gentamicin, amikacin, tobramycin, ticarcillin/clavulate, imipenem, aztreonam, third-generation cephalosporins.*
- *Probiotics: Bifidumbacterinum, colibacterinum, lactobacterinum, bificolum, coli-proteus bacteriophage.*

Microbiological diagnosis of colienteritis



Stage II



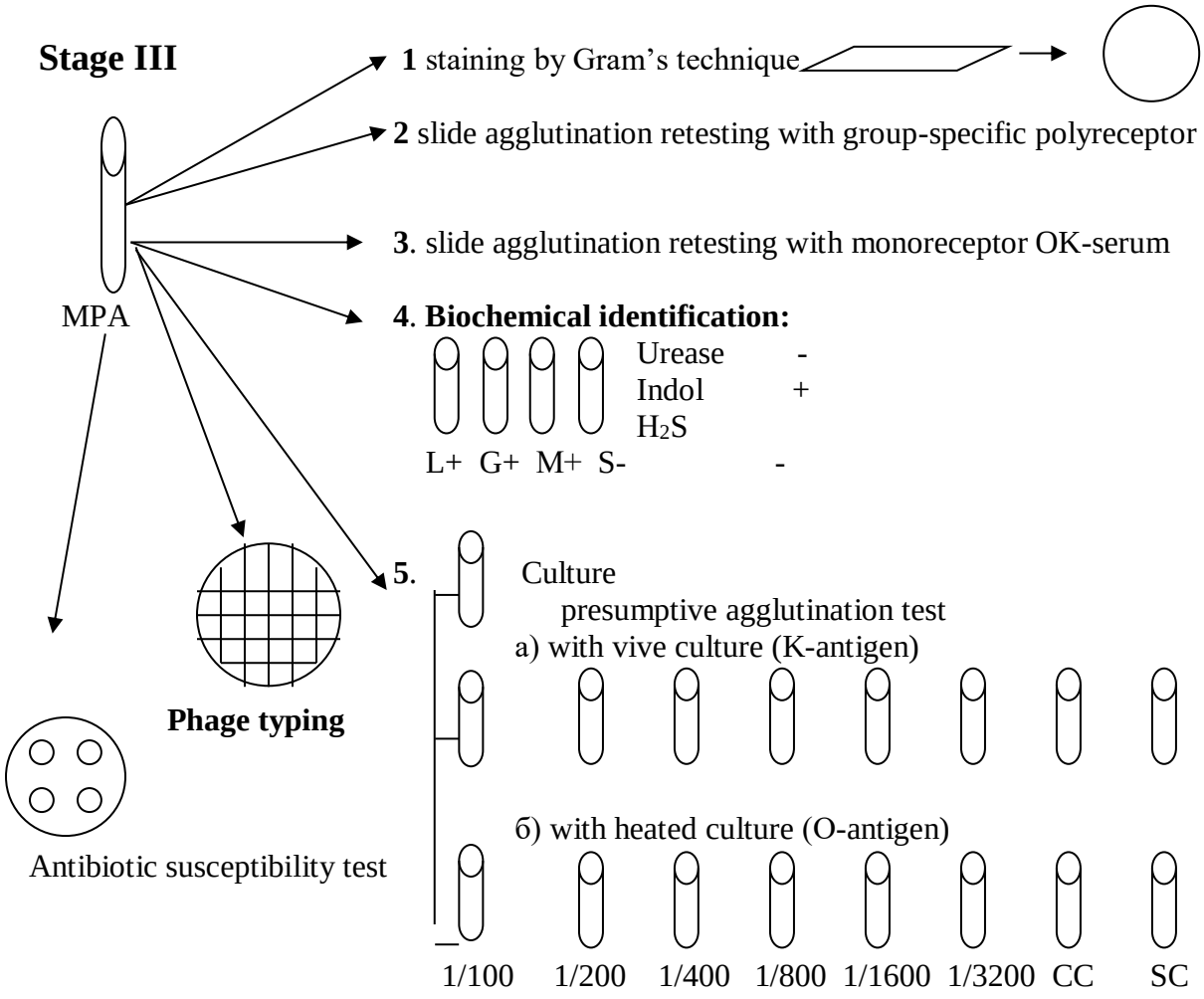
1.Cultural properties: of pink colonies

2.Gram staining morphological and tinctorial properties

3.Differentiation of pathogenic strains: slide agglutination test with group-specific polyvalent OK-serum (most speeded group EPEC)

4. Accumulation pure culture

Stage III



Stage IV

Conclusion

Self-test questions.

- Morphological, tinctorial, cultural properties of *E.coli*.
- Antigen structure of *E.coli*.
- Classification of *E. coli* by the characteristics of their virulence properties.
- Virulence factors of EPEC.
- Pathogenesis of colienteritis caused by EPEC, ETEC, EIEC, EHEC, EAEC.
- Microbiological diagnosis of escherichiosis.
- Speed-up diagnosis of colienteritis.
- Treatment of colienteritis.

Class № 28

Topic: "Salmonellas. Microbiological diagnosis of enteric fever and salmonellosis"

Topic relevance:

Salmonellae are ubiquitous human and animal pathogens, and salmonellosis, a disease that affects an estimated 2 million Americans each year. *S typhi* and *S paratyphi* infections occur worldwide but primarily in developing nations where sanitary conditions are poor. Typhoid and paratyphoid fevers are endemic in Asia, Africa, Latin America, the Caribbean, and Oceania. Typhoid fever affects 13-17 million people yearly and kills an estimated 600,000. Salmonellosis in humans usually takes the form of a self-limiting food poisoning (gastroenteritis), but occasionally manifests as a serious systemic infection (enteric fever) which requires prompt antibiotic treatment. The symptoms of enteric fevers are nonspecific and include fever, anorexia, headache, myalgias, and constipation. Enteric fevers, caused by *S. typhi*, *S. paratyphi*-A, and *S. schottmuelleri*, are severe infections and may be fatal if antibiotics are not promptly administered. Due to the wide range in the severity of typhoid fever from gravely fatal cases to mild ambulant forms it cannot be differentiated from paratyphoids and other infections by clinical symptoms. Laboratory diagnosis of these diseases is of decisive importance. Early antibiotic therapy has transformed a previously life-threatening illness of several weeks' duration with an overall mortality rate approaching 20% into a short-term febrile illness with negligible mortality. Case fatality rates of 10-50% have been reported from endemic countries when diagnosis is delayed.

All mentioned above provide for the relevance the topic and positive motivation for the study.

Objectives:

- To speak about biological properties of causative agents of typhoid fever and paratyphoids A and B.
- To explain the regularities of pathogenesis of infectious process, caused by S. *typhi, S. paratyphi* A and B.
- To study the methods of microbiological diagnosis, etiotropic therapy and prophylaxis of typhoid fever, paratyphoid A and B.

Basic knowledge, abilities, skills necessary for the study of the theme (interdisciplinary integration). See class №25.

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

Term	Definition
Enteric fever	Enteric fevers are severe systemic forms of salmonellosis. This syndrome is produced by only a few of the salmonellae (*S. typhi* produces typhoid

	fever, *S. paratyphi*-A and *S. schottmuelleri* are causative agents of paratyphoids A and B), of which *Salmonella typhi* is the most important. The ingested salmonellae reach the small intestine, from which they enter the lymphatics and then the bloodstream. They are carried by the blood to many organs, including the intestine. The organisms multiply in intestinal lymphoid tissue and are excreted in stools. Typhoid fever is a severe multisystemic illness characterized by the classic prolonged fever, sustained bacteremia without endothelial or endocardial involvement, and bacterial invasion of and multiplication within the mononuclear phagocytic cells of the liver, spleen, lymph nodes, and Peyer patches. Typhoid fever is potentially fatal if untreated.
Antigenic structure and classification of salmonella	Salmonellae are grouped based on the somatic O antigen and further divided into serotypes based on flagellar H and surface virulence (Vi) antigens. In particular, *S. typhi*, the cause of typhoid fever, has O and H antigens, an envelope (K) antigen, and a lipopolysaccharide macromolecular complex called endotoxin that forms the outer portion of the cell wall. *S. typhi, S. paratyphi C,* and *Salmonella Dublin*are the only *Salmonella* serotypes that carry Vi antigen. Based on DNA studies, all salmonellae are now considered one of two species: *Salmonella enterica* (formerly called *Salmonella choleraesuis*) and *Salmonella* bongori. *S. enterica* has 6 subspecies (I, II, IIIa, IIIb, IV, VI); *S. bongori* has one (V). *S. typhi* and *S. paratyphi* are *S.enterica* I subspecies, serotypes *typhi* and *paratyphi.*
Material for bacteriological diagnosis of enteric fever	Definitive diagnosis of typhoid fever generally requires isolation of the organism from blood, bone marrow, vomits, fresh stool, or urine. A bone marrow aspirate (BMA) culture is the most sensitive method of isolating *S. typhi*. The sensitivity is 90% at any point in the disease course for as long as 5 days after the initiation of antibiotics. If patients present within the first week of the disease, blood, intestinal secretions, and stool culture results are usually positive in approximately 85-90% of patients with typhoid fever. They decline to 20-30% later in the course of the disease. Multiple positive blood culture results are 73-97% specific for typhoid fever. Large-volume blood culture and clot culture after serum removal increase sensitivity. Stool culture alone is less than 50% sensitive, and urine even less so. Cultures of punch biopsy samples of rose spots reportedly have a sensitivity of 63% and may show positive results even after antibiotics. A single rectal swab culture at hospital admission can be expected to detect *S. typhi* in 30-40% of patients. *S. typhi* has also been isolated from the cerebrospinal fluid, peritoneal fluid, mesenteric lymph nodes, resected intestine, pharynx, tonsils, abscess, bone, and urine, among others. After disease resolution, 3 stool cultures one month apart should be taken to detect carrier state.

Widal test	Widal's reaction is employed in serological diagnosis. Antibodies to the causative agents of typhoid, paratyphoid A and paratyphoid B fevers can be recovered in the patient's blood serum starting from the 8th-10th day of the disease. The serum of patient is diluted in three parallel rows of test tubes from 1:100 to 1:1600 and 0-diagnosticums (usual or erythrocyte ones) of *Salmonella typhi* is introduced into test tubes of the first row, 0-diagnosticums of *Salmonella paratyphi A* is introduced into test tubes of the second row, and 0-diagnosticums of *Salmonella paratyphi B* is introduced into test tubes of the third row. The use of 0-diagnosticums makes it possible to reveal 0-antibodies which appear in the blood during the second week of the disease and disappear by the end of the illness. The diagnostic titre of antibodies in the Widal test in non-immunized subjects is 1:100 and higher. The Widal test is more than a century old and was the standard serologic test for typhoid fever diagnosis for decades. Now, it is mostly of historical interest.
Specific serological tests	In acute infection, O antibody appears first, rising progressively, later falling, and often disappearing within a few months. H antibody appears slightly later but persists longer and can be used to distinguish between various types of enteric fever. Indirect hemagglutination, indirect fluorescent Vi antibody, and indirect enzyme-linked immunosorbent assay for immunoglobulin M (IgM) and IgG antibodies to *S typhi* polysaccharide are available. Monoclonal antibodies against *S typhi* flagellin are promising developments.
Specific prevention of enteric fever	Routine typhoid vaccination is not recommended except endemic area. Vaccination is indicated for travelers to endemic areas, persons with intimate exposure (e.g., household contact) to a documented *S. typhi* carrier, and microbiology laboratory personnel who frequently work with *S. typhi*. Travelers should be vaccinated at least 1 week prior to departing for an endemic area. Typhoid vaccines lose effectiveness after several years. The following 3 typhoid vaccines are used: 1. Vi capsular polysaccharide (ViCPS) antigen vaccine (Typhim Vi, Pasteur Merieux) is composed of purified Vi antigen, the capsular polysaccharide elaborated by *S. typhi* isolated from blood cultures. Primary vaccination with ViCPS consists of a single parenteral dose of 0.5 mL one week before travel. Booster doses are needed every 2 years to maintain protection if continued or renewed exposure is expected. 2. Ty21a (Vivotif Berna, Swiss Serum and Vaccine Institute) is an oral vaccine that contains live attenuated *S. typhi* Ty21a strains in an enteric-coated capsule. The vaccine elicits both serum and intestinal antibodies and cell-mediated immune responses. In the United States, primary vaccination with Ty21a consists of one enteric-coated capsule taken on

	alternate days to a total of 4 capsules. The capsules must be refrigerated (not frozen), and all 4 doses must be taken to achieve maximum efficacy. The vaccine manufacturer recommends that Ty21a not be administered to children younger than 6 years. It should not be used among immunocompromised persons. 3. Acetone-inactivated parenteral vaccine is currently available only to members of the US Armed Forces. Efficacy rates for this vaccine range from 75-94%. Booster doses should be administered every 3 years if continued or renewed exposure is expected.
Classification of salmonella	All salmonellae are grouped into a single overarching species. This species, *Salmonella choleraesuis,* is divided into 7 subgroups based on DNA homology and host range. Most of the salmonellae that are pathogenic in humans belong to a single subgroup (subgroup I). Additionally, each of the salmonellae can be serotyped according to their particular complement of somatic O, surface Vi, flagellar H antigens. More than 2,300 *Salmonella* serovars exist.
Differential Medium Cultures	EMB, MacConkey's, or deoxycholate medium permits rapid detection of lactose nonfermenters (not only salmonellae and shigellae but also proteus, serratia, pseudomonas, etc). Gram-positive organisms are somewhat inhibited. Bismuth sulfite medium permits rapid detection of salmonellae which form black colonies because of H_2S production. Many salmonellae produce H_2S.
Selective Medium Cultures	The specimen is plated on salmonella-shigella (SS) agar, Hektoen enteric agar, XLD, or deoxycholate-citrate agar, which favor growth of salmonellae and shigellae over other Enterobacteriaceae.
Enrichment Cultures	The specimen (usually stool) also is put into selenite F or tetrathionate broth, both of which inhibit replication of normal intestinal bacteria and permit multiplication of salmonellae. After incubation for 1–2 days, this is plated on differential and selective media.

Theoretical questions:

- General characteristics of *Salmonella.*
- Causative agents of enteric fever.
- Structure, tinctorial properties of causative agents of enteric fever and paratyphoids. Cultivation.
- Biochemical properties of enteric fever and paratyphoids.
- Antigenic structure of salmonellae. Principles of Kauffman– White classification of Salmonellae according to their antigenic structure. Practical value of Kauffman– White classification
- Toxin production of *Salmonella*
- Epidemiology and pathogenesis of enteric fever.
- Main stages of isolation of bacteria from blood (hemoculture), feces (coproculture), and from urine.
- Serological methods of diagnosis and their practical value (Widal agglutination test, indirect haemagglutination test; indirect Vi-hemagglutination test).

- Specific prophylaxis of enteric fever and paratyphoids.

Students' practical activities:

- To study morphological, tinctorial, cultural and biochemical properties of causative agents of enteric fever.
- To create the scheme of microbiological diagnosis of enteric fever
- To identify the hemoculture isolated from the patient with enteric fever (by slide agglutination test).
- To carry out the Widal test and indirect haemagglutination test.
- To study the preparations for diagnosis and prophylaxis of typhoid fever and paratyphoids.
- To study under microscope morphological and tinctorial properties of causing agents of salmonellosis
- To study cultural and biochemical properties of causing agents of salmonellosis on Endo's, Olkenitsky's, Hiss' media, bismuth-sulphite agar, MPA.
- To carry out slide agglutination test with monoreceptor O- and H-sera and unknown culture of Salmonellae for determination of their species.
- To create the scheme of microbiological diagnosis of acute salmonella gastroenteritis.
- To study preparations for diagnosis of salmonella toxicoinfection.

Topic content

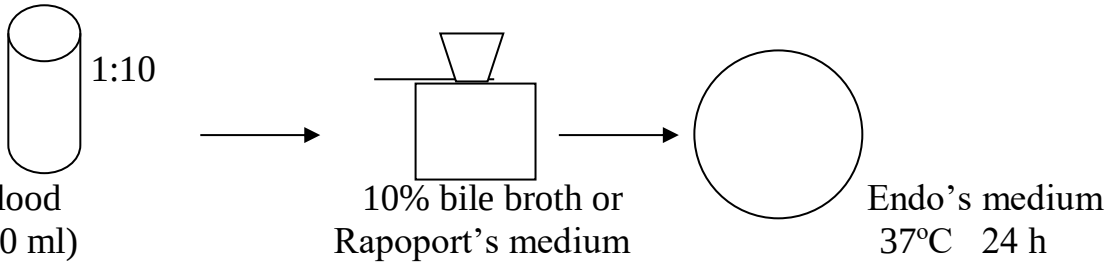
In the class the students study morphological, tinctorial, cultural and biochemical properties of causative agents of enteric fever, nutrient media for cultivation of salmonellas. They create the scheme of microbiological diagnosis of typhoid fever based on pathogenesis of this disease. The students identify the hemoculture isolated from the patient with enteric fever They familiarize with biological preparation used for laboratory diagnosis and specific prophylaxis of typhoid fever and paratyphoids A and B. Students describe their work and obtained results in the protocol and the teacher signs it.

Recommendations for the protocol design.

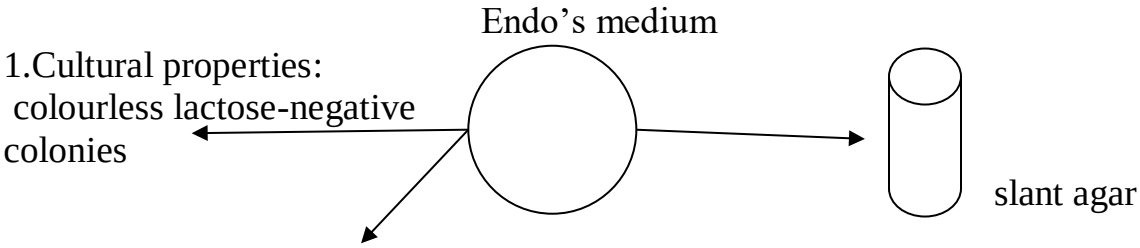
The following items should be included in the protocol.

The scheme of microbiological diagnosis of typhoid fever and paratyphoids A, B

Stage I



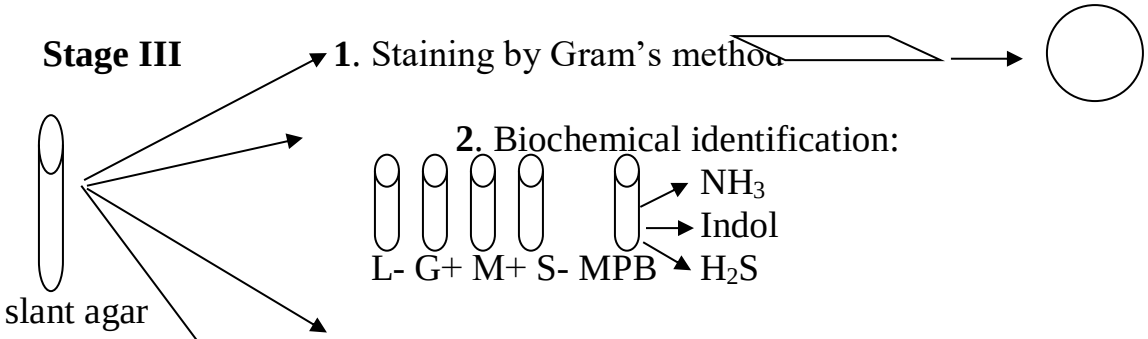
Stage II



2.Staining by Gram's method:
Morphological and tinctorial properties
(Gram-negative rods)

3. Sub-inoculation of colonies onto solid media to enrich for a pure culture.

Stage III



3. Slide agglutination test for serological identification according to Kauffman– White classification: with O-salmonellal sera (subspecies) and H- salmonellal sera (subtypes);

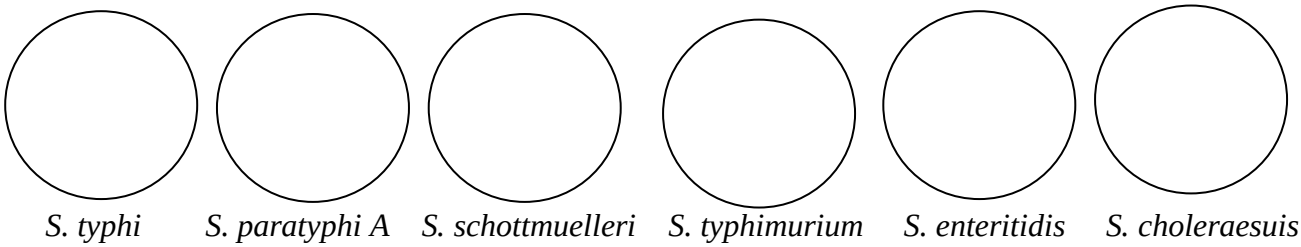
4. Phage typing

5. Antibiotic susceptibility test

Stage IV Conclusion

Task 1. To study morphological, tinctorial, cultural and biochemical properties of causative agents of enteric fever and salmonellosis (demonstration).

Morphological and tinctorial properties
(all smears are stained by Gram's method)



S. typhi *S. paratyphi A* *S. schottmuelleri* *S. typhimurium* *S. enteritidis* *S. choleraesuis*

Biochemical properties of causative agents of enteric fever

	Fermentation of				Production		
	Lactose	Glucose	Mannitol	Sucrose	Indol	NH_3	H_2S
S. typhi	-	A	A	-	-	-	+
S. paratyphi A	-	AG	AG	-	-	-	-
S. schottmuelleri	-	AG	AG	-	-	+	+

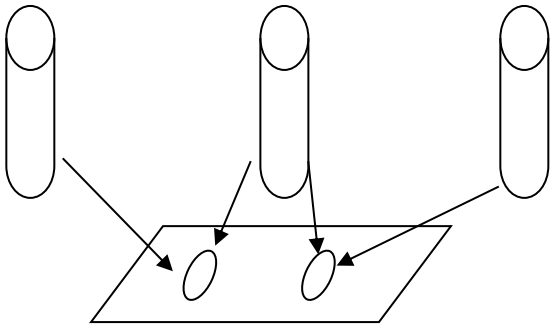
Conclusion:

Task 2. To identify the haemoculture isolated from the patient with enteric fever (by slide agglutination test).

haemoculture

O-serum

IX subspecies



H-serum

I subspecies, **d** phase

Conclusion:

Task 3. To carry out the Widal test.

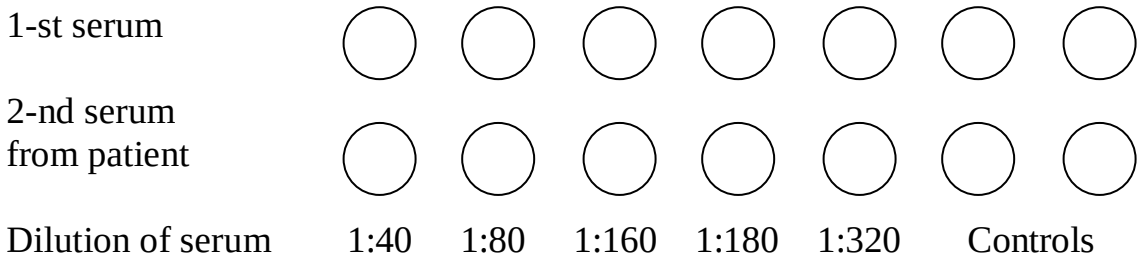
Schematic Description of the Widal Reaction

Ingredient	Number of test the tubes						
	1	2	3	4	5	**SC**	**DC**
Isotonic sodium chloride solution, ml	–	1,0	1,0	1,0	1,0	–	1,0
Patient's serum in1:100 dilution, ml	1,0	1,0	→	→	↑	1,0	–
Diagnosticum, drops	1,0	1,0	1,0	1,0	1,0	–	1,0
Serum dilution obtained	1:50	1:100	1:200	1:400	1:800	1:50	–

Results

Diagnosticum	Dilution of patient's serum					DC	SC
	1/50	1/100	1/200	1/400	1/800		
S. typhi							
S. paratyphi A							
S. schottmuelleri							

Task 4. To carry out the indirect haemagglutination test

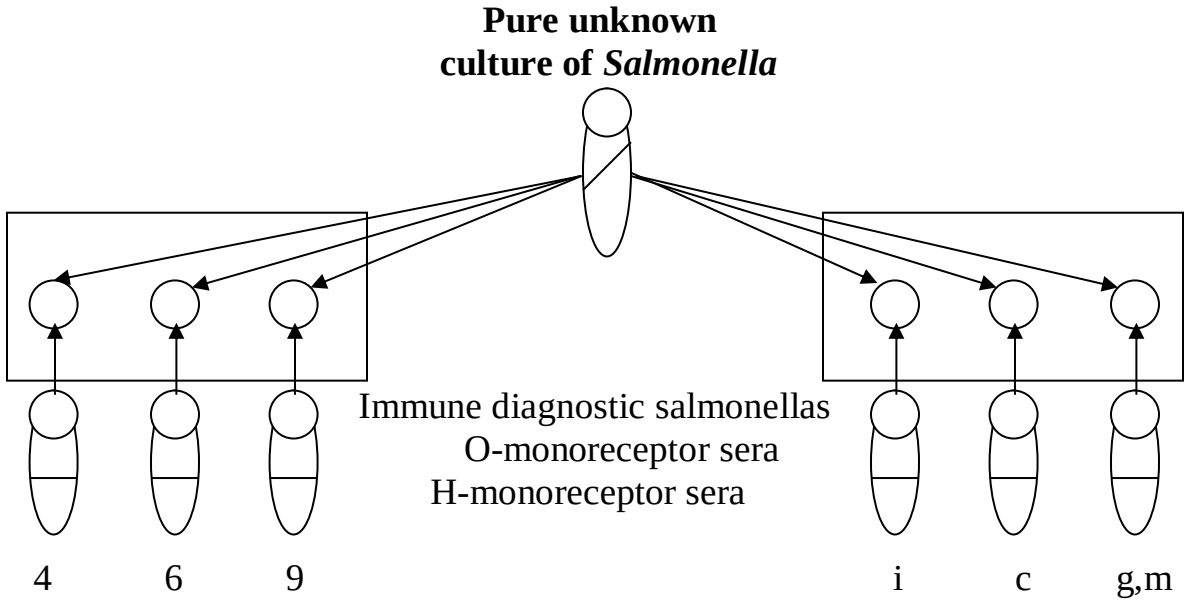


Task 5. To study cultural and biochemical properties of causing agents of salmonellosis on Endo's, Ploskirev's, Levin's, Olkenitsky's, Hiss' media, bismuth-sulphite agar, MPA.

Growth on			**Growth on Olkenitsky's medium**			**Growth on Hiss' medium**			
MPA	Endo's medium	Bismuth-sulphite agar	*Fermentation of*		Producing of H_2S	*Fermentation of*			
			Lactose	Urease		G	M	L	S
S – form of colonies	colorless colonies	black colonies	–	–	+	AG	AG	–	–

Conclusion.

Task 6. To carry out slide agglutination test with monoreceptor O- and H-sera and unknown culture of *Salmonella* for determination of their species.



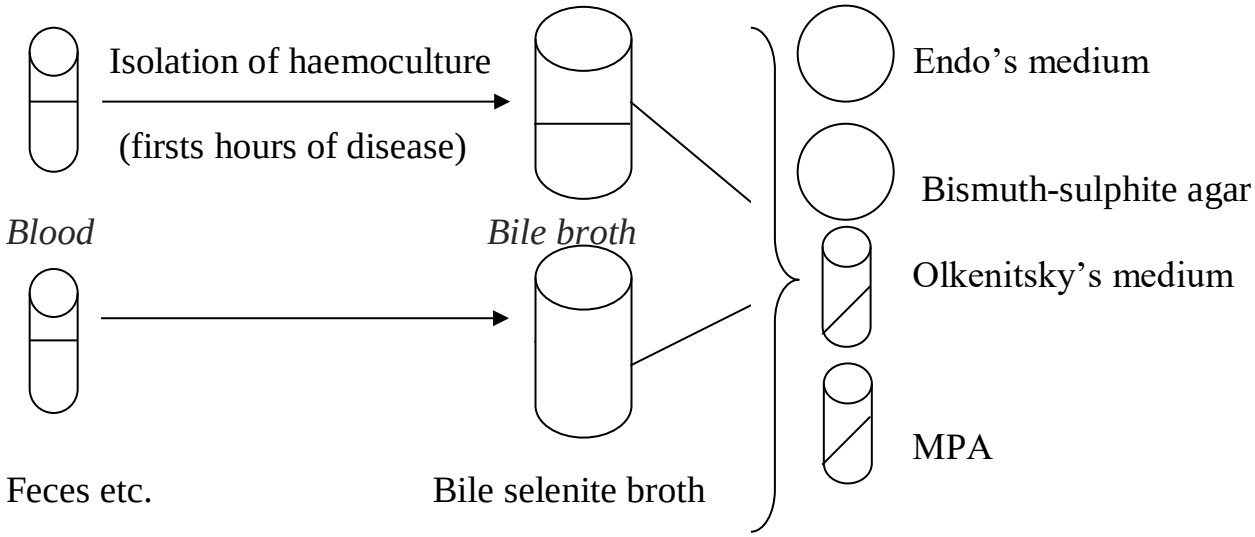
Conclusion.

Task 7. To study biological preparations, which are used for salmonellosis diagnosis.

- *Immune diagnostic salmonellal adsorbed group sera (A, B, C, D, E)*
- *Immune diagnostic salmonellal monoreceptor O-sera and H-sera*
- *Salmonellal polyvalent and group (group A, B, C, D, E) corpuscular diagnosticums*
- *Salmonellal polyvalent erythrocyte diagnosticums*

The scheme of microbiological diagnosis of acute salmonellal gastroenteritis

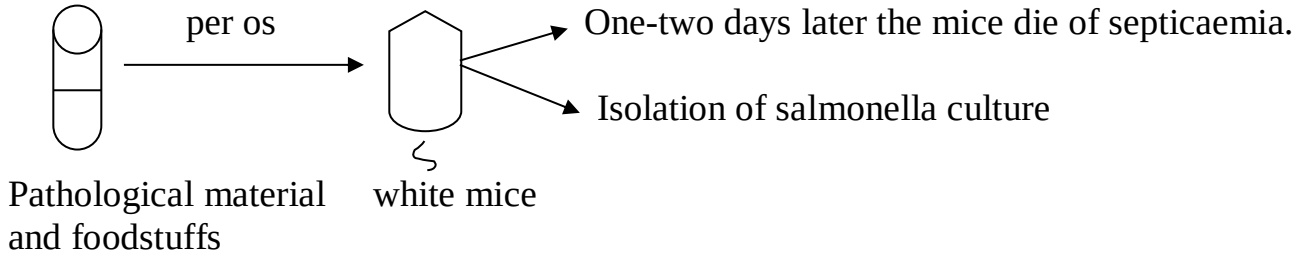
Bacteriological diagnosis



Identification of isolated culture

1. By cultural properties;
2. By morphological and tinctorial properties;
3. By biochemical properties;
   Serological identification: the agglutination test with adsorbed group O-sera (B, C, Д, E); with adsorbed O-monoreceptor sera (4, 7, 9, 10) and H-monoreceptor sera (i, r, c, g, m, e, h);
4. Phage typing

Biological examination



Serological diagnosis

The agglutination reaction and indirect haemagglutination test are employed for serological diagnosis. These may be carried out from the first days of the disease and should be repeated in 7-10 days to determine whether the titre of specific antibodies tends to increase. In conducting these tests, salmonellal polyvalent and group (group A, B, C, D, E) diagnosticums (corpuscular and erythrocyte) are utilized.

A two-four-order elevation of the antibody titre is of diagnostic importance.

Task 8. To study the preparations for diagnosis and prophylaxis of typhoid fever paratyphoids and salmonellosis.

- *S. typhi – diagnosticum.*
- *The erythrocyte monoreceptor S. typhi diagnosticum*
- *S. typhi liquid bacterriophage.*
- *The chemical associated adsorbed vaccine which contains 0- and Vi-antigens of typhoid, paratyphoid B, and a concentrated purified and sorbed tetanus anatoxin*
- *Immune diagnostic salmonellal adsorbed group sera (A, B, C, D, E)*
- *Immune diagnostic salmonellal monoreceptor O-sera and H-sera*
- *Salmonellal polyvalent and group (group A, B, C, D, E) corpuscular diagnosticums*
- *Salmonellal polyvalent erythrocyte diagnosticums*

Self-test questions.

- Morphological, tinctorial, cultural properties of *S. typhi, S. paratyphi* A and B.
- Antigenic structure of salmonellas, Kauffman– White classification.
- Salmonellas' toxins.
- Pathogenesis of typhoid fever, paratyphoids A and B.
- Microbiological diagnosis of *S. typhi, S. paratyphi* A and B.
- Preparations for diagnosis and prophylaxis of typhoid fever and paratyphoids.
- What immunity is formed in human after acute salmonella's gastroenteritis? Why are reinfection and long-term carriage possible?
- Microbiological diagnosis of salmonella's gastroenteritis

Class № 29

Topic: "Shigellae. Microbiological diagnosis of shigellosis"

Topic relevance:

Shigellosis is also known as bacterial dysentery. It is characterized by frequent watery stools that contain blood. Dysentery is an acute inflammation of the intestinal tract. The transmission of *Shigella* species occurs by the fecal-oral route with contaminated water and food often involved in outbreaks. *Shigella* species are able to penetrate the cells lining of the large intestine and multiply in those cells. Their growth leads to large numbers of *Shigella* in the feces, which are a source of spreading infection. Areas of intense inflammation develop around the multiplying of bacteria. Microabscesses form and spread within the gastrointestinal tract, leading to bleeding ulceration. Enterotoxin is generally produced by *Shigella* species but its role in the pathogenesis of this disease is unclear because strains that do not produce toxin cause the same disease. All mentioned above provide for the relevance the topic and positive motivation for the study.

Concrete objectives:

- To interpret the biological properties of shigella.
- To carry out agglutination test for identification of *Shigella*.
- To know the pathogenesis of dysentery.
- To create the scheme of microbiological diagnosis of dysentery.
- To study with preparations for diagnosis, treatment and prevention of dysentery.

Basic knowledge, skills, needed to study topic (interdisciplinary integration). See class №25.

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

Term	Definition
Dysentery	Bacillary dysentery is an acute or chronic intestinal disease caused by *Shigella*. It is characterized by a small volume of bloody, mucoid stools, and abdominal pain (cramps and tenesmus).
Classification of *Shigella*	The genus *Shigella* is differentiated into four species: *S dysenteriae* (serogroup A, consisting of 12 serotypes); *S flexn*eri (serogroup B, consisting of 6 serotypes); *S. boydii* (serogroup C, consisting of 18 serotypes); and *S sonnei* (serogroup D, consisting of a single serotype).
Shigella's toxins	Upon autolysis, all shigellae release their toxic lipopolysaccharide. This endotoxin probably contributes to the irritation of the bowel wall.

	S. dysenteriae type 1 (Shiga bacillus) produces a heat-labile exotoxin that affects both the gut and the central nervous system.
	The exotoxin is a protein that is antigenic (stimulating production of antitoxin) and lethal for experimental animals. Acting as an enterotoxin, it produces diarrhea as does the *E. coli* verotoxin, perhaps by the same mechanism. In humans, the exotoxin also inhibits sugar and amino acid absorption in the small intestine. Acting as a "neurotoxin," this material may contribute to the extreme severity and fatal nature of *S. dysenteriae* infections and to the central nervous system reactions observed in them (meningismus, coma). Patients with *S. flexneri* or *S. sonnei* infections develop antitoxin that neutralizes *S. dysenteriae* exotoxin in vitro. The toxic activity is distinct from the invasive property of shigellae in dysentery. The two may act in sequence, the toxin producing an early nonbloody, voluminous diarrhea and the invasion of the large intestine resulting in later dysentery with blood and pus in stools.
Microbiological diagnosis of dysentery	The main method of microbiological diagnosis of dysentery is bacteriological method. The materials (fresh stool, mucus flecks, rectal swabs) are streaked on differential media (MacConkey's medium or Endo's medium) and on selective media (Hektoen enteric agar or salmonella-shigella agar), which suppress other Enterobacteriaceae and gram-positive organisms. Colorless (lactose-negative) colonies are inoculated into triple sugar iron agar or Olkenitsky's medium. Organisms that fail to produce H_2S, that produce acid but not gas in the butt and an alkaline slant in triple sugar iron agar medium, and that are nonmotile should be subjected to slide agglutination by specific shigella antisera. Normal persons often have agglutinins against several *Shigella* species. However, serial determinations of antibody titers may show a rise in specific antibody. Serology is used to diagnose shigella infections rarely.
Prevention and treatment of dysentery	*Shigella* are transmitted by "food, fingers, feces, and flies" from person to person. Since humans are the main recognized host of pathogenic shigellae, control efforts must be directed at eliminating the organisms from this reservoir by (1) sanitary control of water, food, and milk; sewage disposal; and fly control; (2) isolation of patients and disinfection of excreta; (3) detection of subclinical cases and carriers, particularly food handlers; and (4) antibiotic treatment of infected individuals. Ciprofloxacin, ampicillin, doxycycline, and trimethoprim-sulfamethoxazole are most commonly inhibitory for shigella isolates and can suppress acute clinical attacks of dysentery and shorten the duration of symptoms. Multiple drug resistance can be transmitted by plasmids, and resistant infections are widespread.

Theoretical questions:

- Morphological and tinctorial properties of *Shigella*.
- Cultural and biochemical properties of *Shigella*.
- Antigenic properties and classification of *Shigella*
- Virulence factors of *Shigella*. Pathogenesis of dysentery.
- Microbiological diagnosis of dysentery.
- Prophylaxis and treatment of dysentery.

Practical activities performed in class:

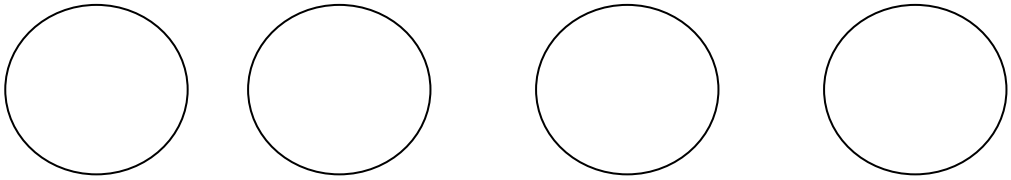
- To study under microscope morphology of various *Shigella* species.
- To study peculiarities of *Shigella* growth on MPA, Levin's and Ploskirev's media, and in MPB.
- To study biochemical properties of *Shigella* in Hiss'media. To examine species and serovar of *Shigella* in agglutination test with group and subtypes sera.
- To create the scheme of microbiological diagnosis of dysentery.

Topic content:

The students study morphological, cultural, biochemical and antigenic properties of *Shigella*. They identify the culture of *Shigella* isolated from the patient with dysentery. Students study pathogenesis of these diseases and create the scheme of diagnosis of dysentery. They study the preparations for diagnosis of salmonellas gastroenteritis. Students describe their work and obtained results in the protocol and the teacher signs it.

Recommendations for the protocol design.

Practical activity №1. To study morphological and tinctorial properties of Shigella (*Sh.dysenteriae, Sh.flexneri, Sh.boydii, Sh.sonnei*).



Sh.dysenteriae *Sh.flexneri* *Sh.boydii* *Sh.sonnei*

Microbiological diagnosis of dysentery:

1. Experess-diagnosis: IFA

2. Bacteriological diagnosis

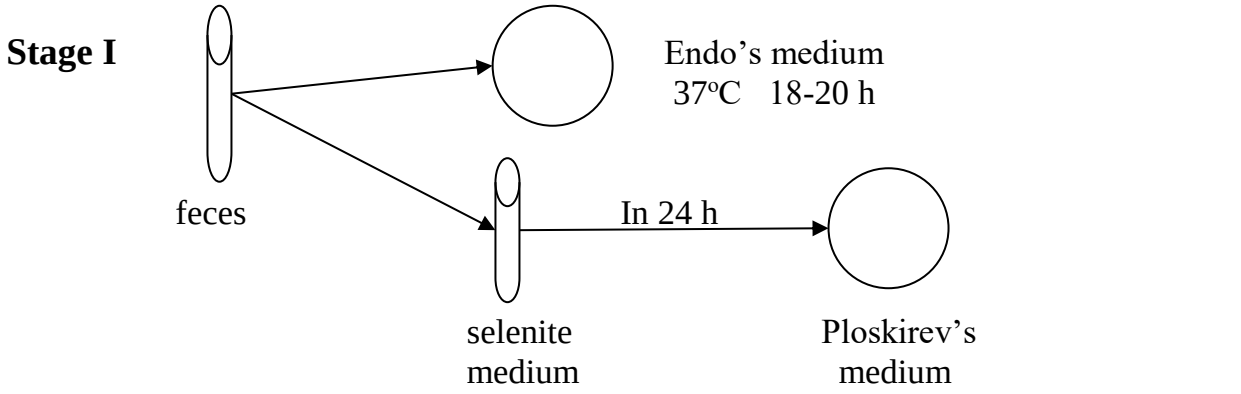
3.Serological diagnosis: indirect hemagglutination test (IHAT) with erythrocyte diagnosticums of Flexneri and Zonnei is used on the 5-th - 8-th day of diseases; titer of

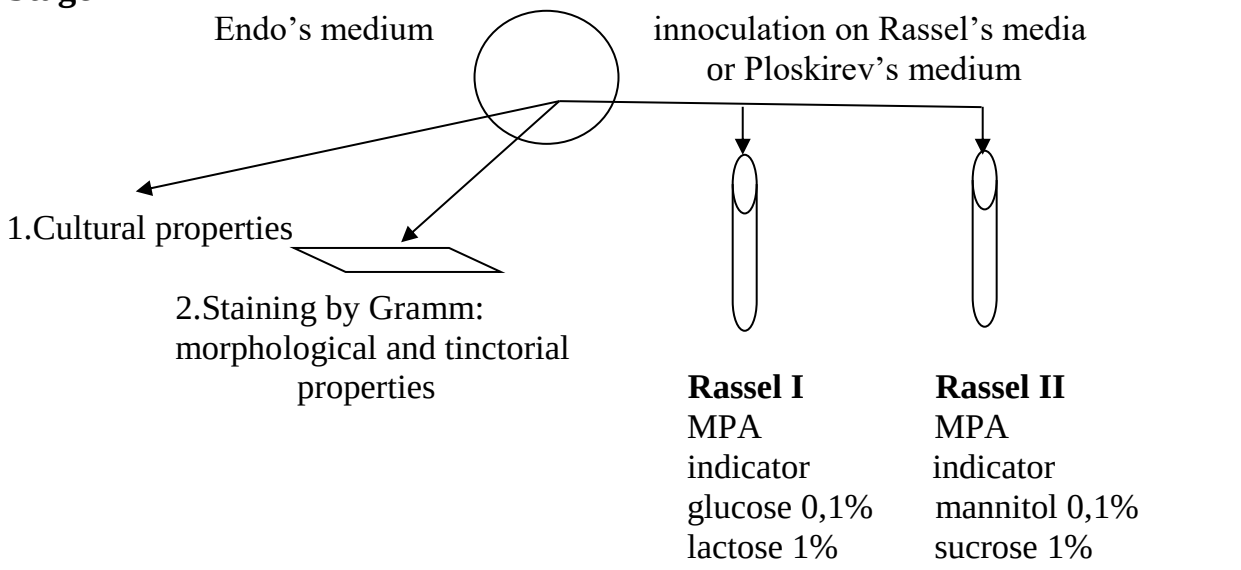
antibodies is 1:160 and higher in a positive case. If we use pair sera (taken from the patient on the 1st day of disease and in 10-14 days), we detect 4 times increasing of titer of antibodies in positive case (the patient is ill). Diagnostic titer of antibodies is 1:200 to *Sh.flexneri* and 1:100 to *Sh.sonnei*.

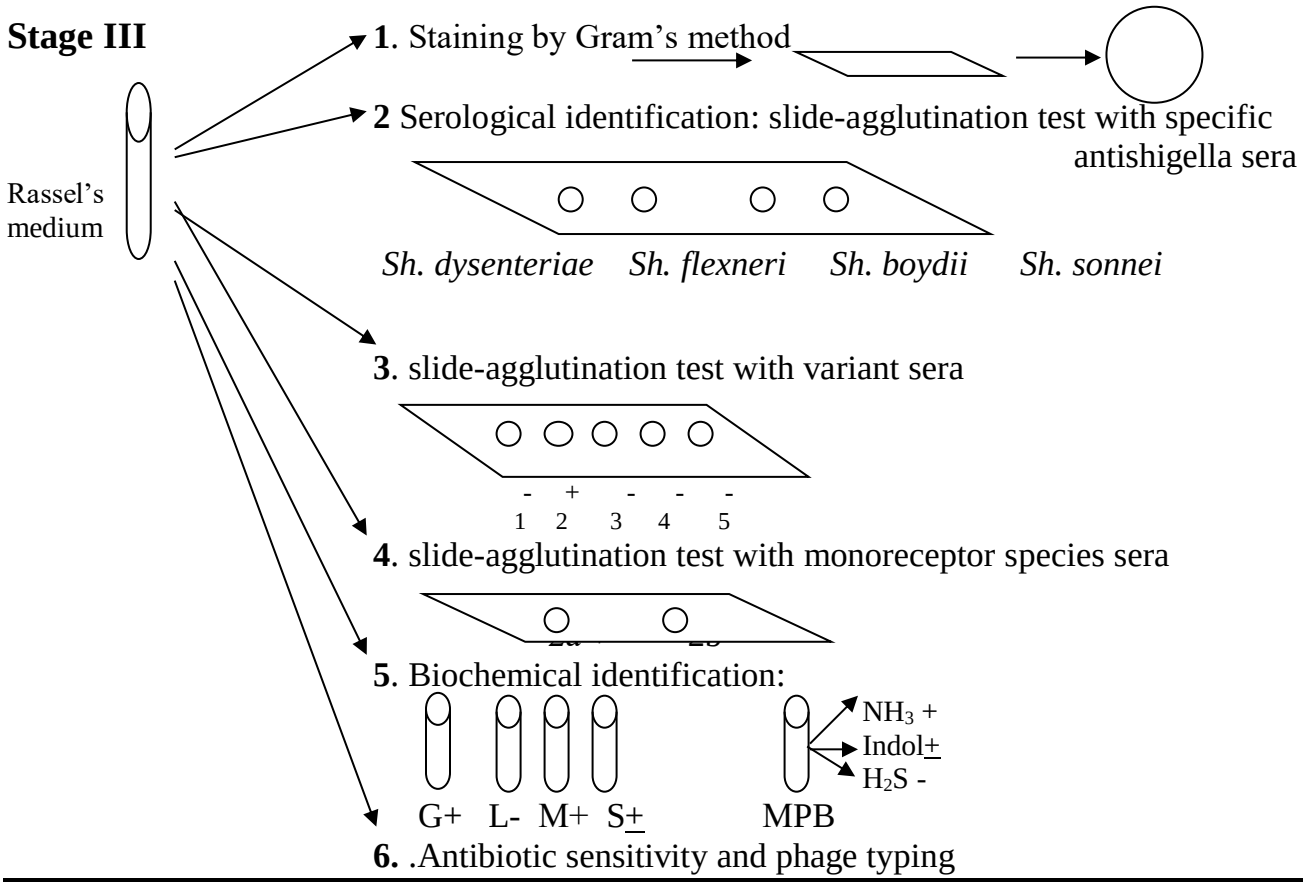
4. Allergic test consisting of intracutaneous injection of 0.1 ml of dysenterin is applied in the diagnosis of dysentery in adults and children. Hyperemia and a papule 2 to 3.5 cm in diameter develop at the site of the injection for 24 hours in a person who has dysentery. The test is strictly specific.

5. Biological method is not used, because a dysentery is an antroponosis.

Microbiological diagnosis of dysentery







Stage IV **Conclusion**

Practical activity №2. To study biochemical properties of *Shigella*.

Species	Fermentation of carbohydrates				Production of		Motility	Presence of exotoxin
	Glucose	Lactose	Mannitol	Sucrose	Indol	H_2S		
Sh.dysenteriae (12 serotypes)	A	-	-	-	-	-	-	+
Sh.flexneri (9 serotypes)	A	-	A	-	-	-	±	-
Sh.boydii (18 serotypes)	A	-	A	-	+	-	-	-
Sh.sonnei (1 serotypes)	A	A after 48 h	A	A after 72 h	-	-	-	-

Practical activity №3. Preparations for treatment of dysentery:

- *sulfonamides (norsulfazole, ftalazole, sulfamethoxazole etc)*
- *antibiotics (monomycinum, ampicillin, ciprofloxacin)*
- *dysenteric dry bacteriophage with acid resistance coverage (for prophylaxis and treatment).*
- *bificol, lactobacterin*

Control questions:

- Morphological, cultural, biochemical properties of *Shigella*.
- Antigenic structure of *Shigella*.
- Classification of *Shigella*.
- What virulence factors result in Shigella pathogenicity?
- Pathogenesis of dysentery.
- Microbiological diagnosis of dysentery.
- Specific and nonspecific prophylaxis of dysentery. Treatment.

Class №30

Topic: “Vibrio. Microbiological diagnosis of cholera”

Topic relevance:

Cholera is caused by *Vibrio cholerae*, the most feared epidemic diarrheal disease because of its severity. Dehydration and death can occur within hours of infection. Cholera is very dangerous infectious disease which causes epidemics in the world. Since 1817, 7 cholera pandemics have occurred. The first 6 occurred from 1817-1923 and were probably the result of *V. cholerae* O1 of the classic biotype. The pandemics originated in Asia, with subsequent spread to Europe and the Americas.

The seventh pandemic was caused by *V. cholerae* O1 El Tor, which was first isolated in Egypt in 1905. The pandemic originated from the Celebes Islands, Indonesia, in 1961; this pandemic affected more countries and continents than the previous 6 pandemics. The last extension of this pandemic was into Latin America. In October 1992, an epidemic of cholera emerged from Madras, India, as a result of a new serogroup, O139 (also known as Bengal). This Bengal strain has now spread throughout Bangladesh and India and into neighboring countries in Asia. Some experts regard this as an eighth pandemic. Cholera is a potentially epidemic and life-threatening secretory diarrhea characterized by numerous, voluminous watery stools, often accompanied by vomiting, and resulting in hypovolemic shock and acidosis. Rapid microbiological diagnosis is very necessary for successful treatment of patient and prevention of distribution of cholera. All mentioned above provide for the relevance the topic and positive motivation for the study.

Objectives:

- To study biological properties of *V. cholerae* and classification of *Vibrio*.
- To study epidemiology, pathogenesis, and clinical manifestation of cholera.
- To create the scheme of microbiological diagnosis of cholera.
- To study preparations for diagnosis, prophylaxis and treatment of cholera.

Basic knowledge, abilities, skills necessary for the study of the theme (interdisciplinary integration). See class №25.

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

Term	Definition
Cholera	The word cholera is derived from a Greek term that means "flow of bile." Cholera is caused by *Vibrio cholerae*, the most feared epidemic diarrheal disease because of its severity. Dehydration and death can occur within hours of infection. Cholera is very dangerous infectious disease which causes epidemics in the world.

Nutrition media for cultivation of *V. cholerae*	Alkaline meat broth, peptone water, TCBS *(thiosulphate-citrate-bromthymol sucrose)*, alkaline taurocholate-tellurite-peptone medium (Monsur's liquid medium), *Aronson's medium*
Heiberg's classification	Heiberg differentiated vibrios into biochemical types according to their property of fermenting mannose, arabinose, and saccharose. Eight groups of vibrios are known to date; the cholera vibrios of the cholerae and El Tor biovar belong to biochemical variant 1.
Cholerogen	The cholera vibrio produces an exotoxin (cholerogen) which is marked by an enterotoxic effect and plays an important role in the pathogenesis of cholera; the endotoxin also exerts a powerful toxic effect.
Antigenic structure	The cholera vibrios have thermostable O-antigens (somatic) and thermolabile H-antigens (flagellar). The O-antigen possesses species and type specificity; the H-antigen is common for the genus Vibrio. According to the O-antigen content, the vibrios are separated into subgroups of which there are more than 140. *V cholerae* O1 and *V cholerae* O139 are associated with epidemic cholera. The cholerae vibrios, El Tor biovars and biovars cholera belong to the O-1 subgroup. In the 0-1 subgroup there are three O-antigens (A, B, and C) according to the combination of which three serological variants, Ogawa (AB), Inaba (AC) and an intermediate variant Hikojima (ABC), are distinguished.
Classification of *V. cholerae*	Vibrio cholerae belongs to family *Vibrionaceae*, genus *Vibrio* consisting of 5 species. The species *Vibrio cholerae* is subdivided into four biological variants: biovar cholerae, biovar El Tor, biovar Proteus, and biovar albensis. Biovar cholerae and biovar El Tor of *Vibrio cholerae* are the causative agents of human cholera. Biovar Proteus of *Vibrio cholerae* causes diarrhoea in birds and gastroenteritis in humans; biovar albensis of *Vibrio cholerae* was revealed in fresh water and in human faeces and bile.

Theoretical questions:

- Structure and tinctorial properties of causative agents of cholera.
- Cultivation of *Vibrio cholerae*. Main nutrient media.
- Biochemical properties of *Vibrio cholerae*.
- Antigenic structure and classification of *Vibrio cholerae*.
- Toxin formation of *Vibrio cholera*e.
- Epidemiology of cholera
- Pathogenesis and clinical findings of cholera.
- Laboratory diagnosis of cholera
- Treatment and prophylaxis of cholera

Students' practical activities:

- To study morphological, tinctorial and biochemical properties of *V. cholerae*.
- To study nutrient media for cultivation of *V. cholerae*
- To create the scheme of microbiological diagnosis of cholera
- To study the tests for differentiation of *V. cholerae* biovariants
- To identify pure culture of *Vibrio* with O-I serum, Ogawa and Inaba sera
- To study biological preparation used for laboratory diagnosis and specific prophylaxis of cholera.

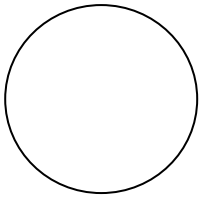
Topic content:

In the class the students study morphological properties of *V. cholerae*, nutrient media for cultivation of *V. cholerae*. They create the scheme of microbiological diagnosis of cholera based on pathogenesis of this disease. The students carry out the serological identification of *Vibrio* with O-I serum, Ogawa and Inaba sera. They study the biological preparation used for laboratory diagnosis and specific prophylaxis of cholera. Students describe their work and obtained results in the protocol and the teacher signs it.

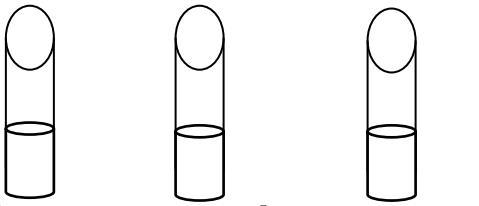
Protocol preparation.

The following items should be included in the protocol.

Task 1. To study morphological, tinctorial and biochemical properties of *V. cholerae*.



Vibrio cholerae

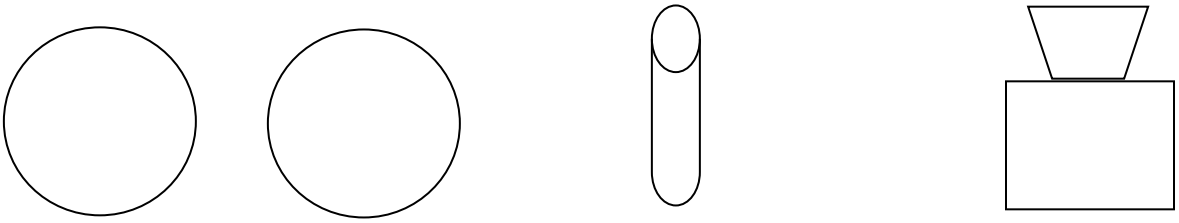


Arabinose - Mannitol + Sucrose +

Heiberg's classification of vibrios according to their biochemical property

Group	Mannose	Sucrose	Arabinose
I	+	+	-
II	-	+	-
III	+	+	+
IV	-	+	+
V	+	-	-
VI	-	-	-
VII	+	-	+
VIII	-	-	+

Task 2. To study nutrient media for cultivation of *V. cholerae*



Alkaline agar TCBS Alkaline meat broth Monsur's liquid medium

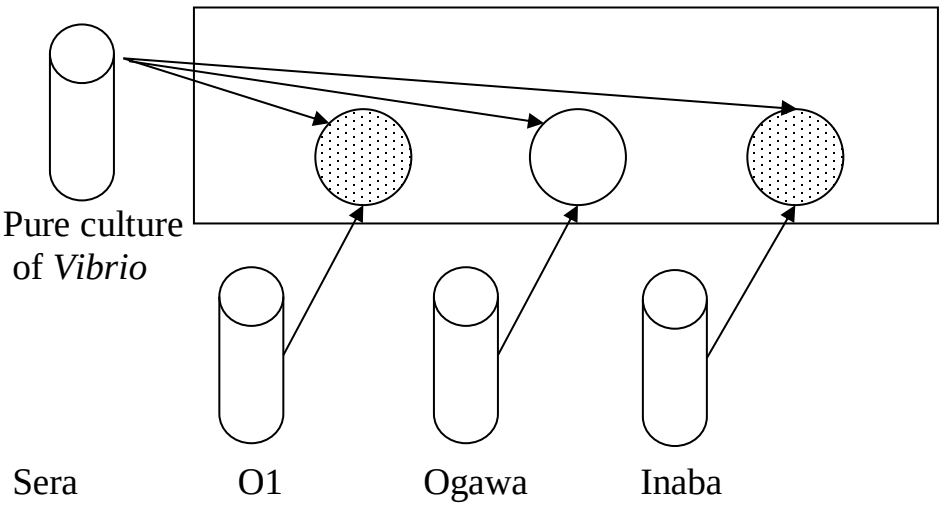
Task 3. To study the tests for differentiation of biovars ***V. cholerae***

Differentiation of Biovars of *V. cholerae*

Features	**Biovars**	
	V. cholerae, cholerae	*V.cholerae,* El-tor
1. Sensitivity to selected bacteriophages: - phage «C» - phage «El-Tor»	 + -	 - +
2. Erytrocyte agglutination	-	+
3. Sensitivity to polymixin B	+	-
4. Seep erythrocyte hemolysis	-	+

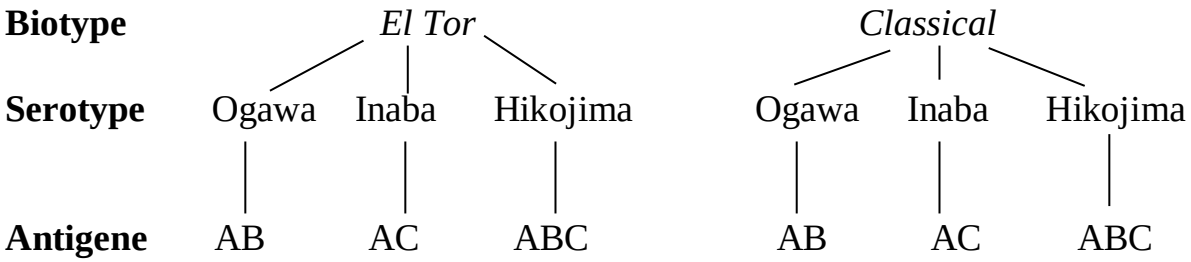
Conclusion:

Task 4. To identify pure culture of *Vibrio* with O-I serum, Ogawa and Inaba sera.



Conclusion:

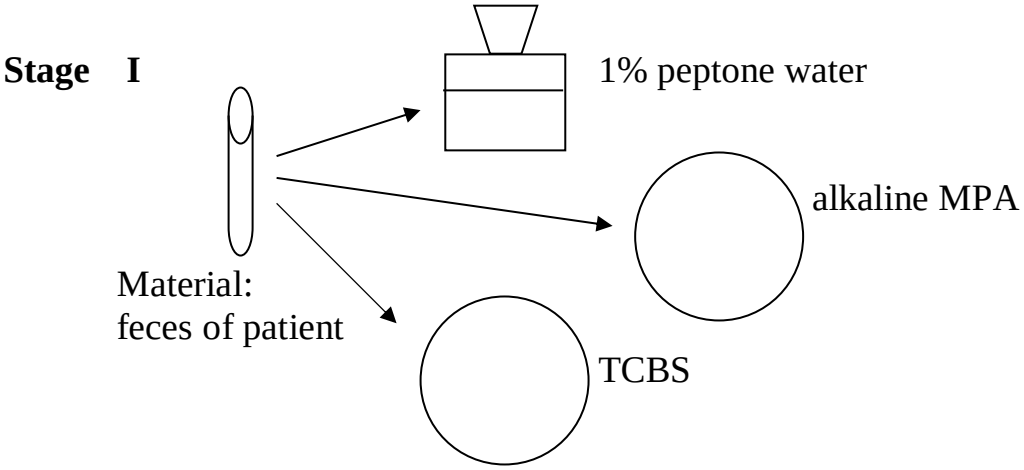
V. cholerae (O group 1 antigen) classification



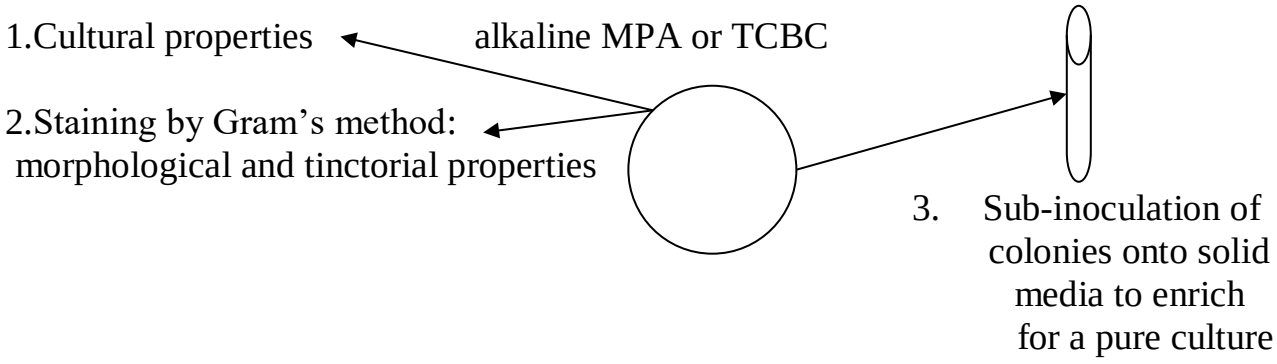
Conclusion.

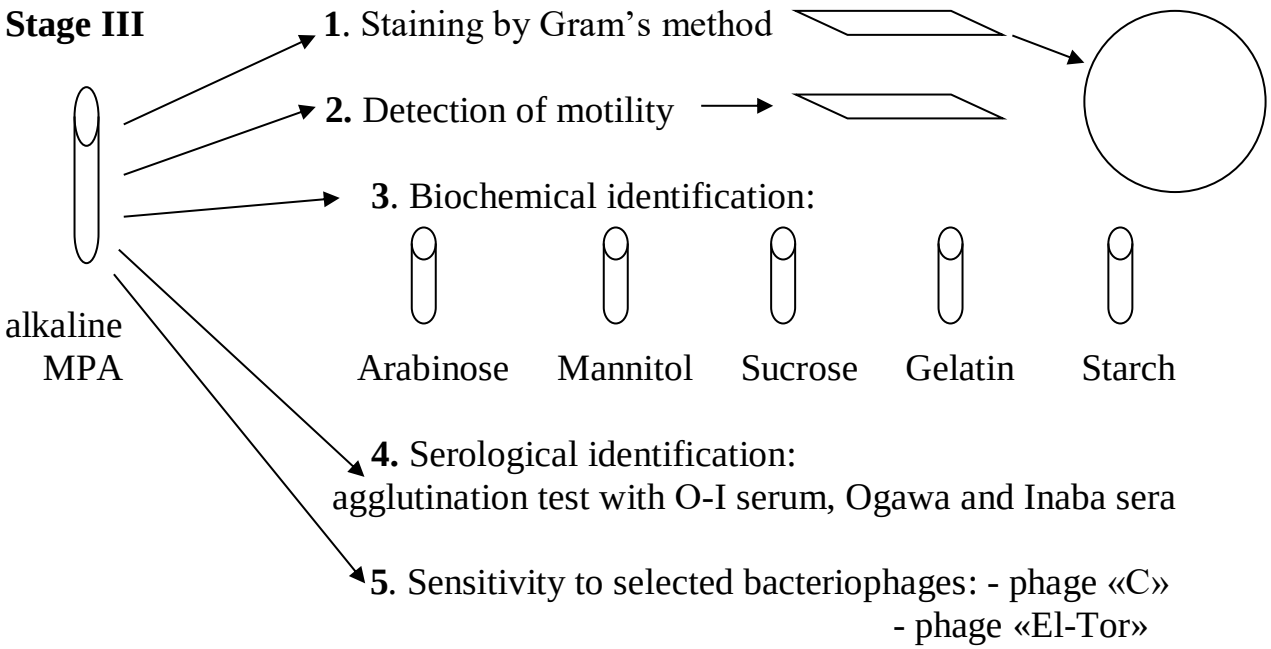
Task 5. To study biological preparation used for laboratory diagnosis and specific prophylaxis of cholera.

Microbiological diagnosis of cholera



Stage II





Stage IV **Conclusion**

Self-test questions.

- Structure, tinctorial, cultural properties of *V.cholerae*.
- Antigenic structure of *V.cholerae*.
- Classification of
- Fermentative properties and toxin production of *V. cholerae*.
- Epidemiology and pathogenesis of cholera.

Class №31

Topic: "Corynebacteria. Microbiological diagnosis of diphtheria"

Topic relevance

Diphtheria is one of the most important infectious diseases. There is a large number of *Corynebacteriae* familae, some of them are pathogenic, some are opportunistic, and others - nonpathogenic. Diphtheria is topical issue due to the increase of morbidity among children and adults.

The etiologic diagnosis of diphtheria, determining the toxigenicity, timely specific treatment and preventive measures are very important. Carriers of toxigenic and nontoxigenic strains should be detected for subsequent sanation to prevent disease spreading.

All these reasons confirm the importance of this topic in the special microbiology course.

Educational purposes:

- To learn the biological properties of causative agent of diphtheria.
- To study the pathogenetic patterns of diphtheria.
- To learn methods of microbiological diagnosis, etiotropic therapy and prevention of diphtheria

Basic knowledge, abilities, skills necessary for the study of the theme (interdisciplinary integration). See class №25.

List of terms, parameters, characteristics that a student should learn during class:

Term	Definition
Diphtheria	Diphtheria is an acute infectious disease caused by toxigenic Corynebacterium diphtheriae, transmitted by airborne droplets and characterized by a local fibrinous inflammation of the mucous oro-and nasopharynx membranes predominantly as well as intoxication, damage of the cardiovascular, nervous and excretory systems.
Biological features of diphtheria	Morphology: Gram+ rods. Complex method of staining by Neisser and simple method by Loeffler are used for staining. It is characterized by the presence of the volutin inclusions at the ends of the rods (the phenomenon of metachromasia). Bacteria are located by angle to each other. Phenomenon of polymorphism is characteristic. With cultural and biochemical properties 4 biotypes are distinguished: gravis, mitis, intermedius, belfanti.

Toxigenicity	Production of exotoxin.
The phenomenon of «phage conversion»	A change in one or more phenotypic characteristics of a host bacterium as a result of infection by a temperate bacteriophage. Phage conversion leading to the ability to produce toxin by *C. diphtheria*.
Determination of toxigenicity of *Corynebacterium diphtheriae* -«in vitro»	Precipitation tests in gel are used. Antitoxin can be able to diffuse into the gel unilaterally. If diphtheria exotoxin is present it diffuses into the gel too. The line of precipitation forms in the area of interaction between antitoxin and exotoxin.
Microbiological diagnosis of diphtheria	It is very important. The study of morphological, cultural, biochemical properties, the definition of biotypes and toxigenicity of *Corynebacterium diphtheriae* is at particular importance as the differentiation between nonpathogenic and pathogenic corynebacteria.
Specific prevention	Diphtheria toxoid is used for antitoxic immunity formation. It is specific, active, schedule prevention
Emergency prevention and treatment	Antidiphtheria serum. It is specific, passive, urgent prevention or treatment

Theoretical questions for class preparing:

- The evolution of *Corynebacteria*.
- Biological properties of *Corynebacterium diphtheriae*.
- Differentiating features between *Corynebacterium diphtheriae* and diphtheroids .
- Differentiating features between biotypes of *Corynebacterium diphtheria*.
- Toxigenicity. Genetic determinants of toxigenicity Units of toxin. Methods of measuring of toxin units.
- Pathogenicity and pathogenesis of diphtheria.
- Methods of microbiological diagnosis.
- Preparations for the prevention and treatment of diphtheria.

Tasks, performing during class:

- Learn morphological properties of *Corynebacterium diphtheriae*.
- Determination of toxigenicity of *Corynebacterium diphtheriae* in vitro.
- Write down tables "Differentiation of *Corynebacterium* with biochemical properties", "*C. diphtheriae* biotypes differences".
- Learn scheme of microbiological diagnosis of diphtheria.
- Determine the diphtheria bacteria with microbiological diagnosis.
- Study the preparations for specific prevention and therapy of diphtheria

Content of the topic:

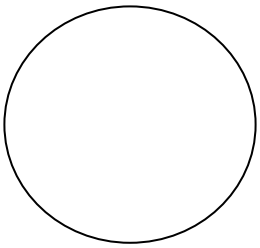
At the practical lesson the students study the morphological, cultural, biochemical and antigenic properties of *Corynebacterium diphtheriae*; pathogenicity factors, differences between the biotypes; perform inoculation of specimen from patient into nutrient media for microbiological diagnosis of carrier state. The students write down the completed tasks to protocol and teacher sign it.

Recommendations for design of the protocol:

The protocol must contain:

- *Corynebacteria diphtheriae* stained with methylene blue by Loeffler.
- Tables: "The differentiation of corynebacteria by biochemical properties", "C. diphtheriae biotypes differences".
- Demonstration the gel precipitation test during determining the toxigenicity of Corynebacterium diphtheria.
- The scheme of microbiological diagnosis of diphtheria.
- The scheme of planting material and inoculation into nutrient media for microbiological diagnosis of diphtheria bacteriacarrying.
- The list of preparations for prevention and treatment .

Practical activity № 1. Study the morphological and tinctorial properties of *Corynebacterium diphtheriae*.



Corynebacterium diphtheriae
(staining with methylene blue by Loeffler)

Conclusion: *Corynebacterium diphtheria*e is characterized by volutin granules, the phenomenon of metachromasia, the location of pathogens at an angle to each other and polymorphism.

Practical activity №2. Microbiological diagnosis of diphtheria.

Day 1. Take specimens (mucus from the throat and nose, films from the affected mucosa). Bacterioscopic study of the smears (staining by Gram, by Loeffler, Neisser) - a preliminary diagnosis.

Day 2. Study the type of colonies. Inoculation into coagulated serum for pure culture.

Day 3. Identification of pure culture: inoculation into Giss media, determing the hemolysis, the determining the toxigenicity; test for revealing cystinase and ureasis activity.

Day 4. Confirm the diagnosis.

Practical activity №3. Main properties of *Corynebacterium*.

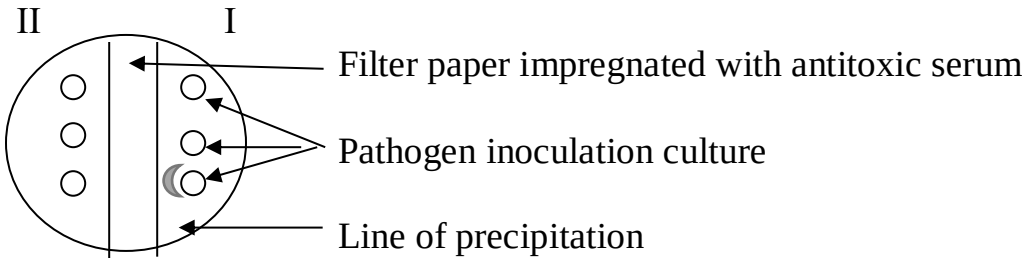
Differentiation of *Corynebacterium* with biochemical activity

Species of *Corynebacterium*	**Hemolysis**	**Fermentation of sucrose**	**Reduction of nitrates**	**Gelatinase**	**Urease**	**Cystinase**	**Toxine synthesis**
Cor. diphtheriae	+	-	+	-	-	+	+ (-)
Cor. pseudodiphthericum (hofmannii)	-	-	+	-	+	-	-
Cor. xerosis	-	+	+	-	-	-	-
Cor. ulcerans	+	-	-	+	+	+	-

Differentiating features of *Corynebacterium diphtheriae* biotypes

Biotypes	**Toxigenicity**	**CAMP-test**	**Fermentation of**						**Reduction of nitrates to nitrites**
			Cysteinase	Pyrasinamide	Glucose	Sugarose	Starch	Urea	
C. diphtheriae var. gravis	V	-	+	-	+	-	+	-	+
C. diphtheriae var. mitis	V	-	+	-	+	-	-	-	+
C. diphtheriae var. intermedius	-/+	-	+	-	+	-	-	-	+
C. diphtheriae var. belfanti	-/+	-	+	-	+	-	-	-	-

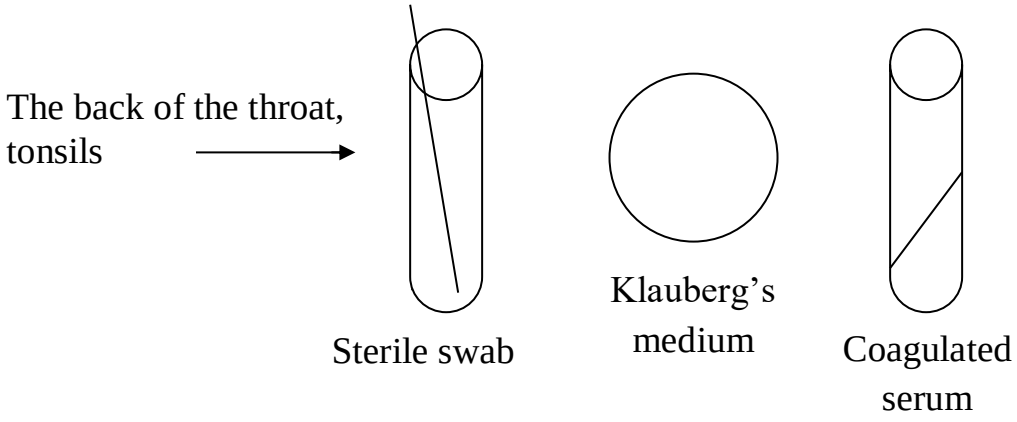
Practical activity №4. Precipitation test in gel for study the toxigenicity of *Corynebacterium diphtheriae* (demonstration).



Petri dish with a gel

Conclusion: if bacteria produce the toxin, the line of precipitation, as a result of interaction between exotoxin and antitoxin, forms in the gel (part I of the Petry dish). In control specimen the lines are absent because exotoxin is absent.

Practical activity№ 5. Microbiological diagnosis of diphtheria bacteria carrying.



Practical activity №6. List of preventive and curative preparations.

DPT - diphtheria, pertussis, tetanus vaccine;

DT-toxoid (purified adsorbed liquid diphtheria and tetanus toxoid);

DT-m-toxoid (purified, adsorbed with a reduced content of antigens, liquid diphtheria and tetanus toxoid);

Diphtheria antitoxic serum (horse purified concentrated liquid diphtheria serum).

Questions for self-control.

- Stages of diphtheria.
- Biological properties of *Corynebacteriae*.
- Evolution of *Corynebacteriae*.
- Properties of *Corinebacteria* biotypes.
- Toxigenicity. Characreristics of exotoxin. Genetic determinates of toxigenicity. Doses and their measuring.
- Pathogenic factors of *Corynebacteria*. The pathogenesis of diphtheria. Immunity.
- Methods of microbiological diagnosis of diphtheria and diphtheria carrying.
- Theoretical basis for specific prevention of diphtheria. Preparation for prevention and treatment of diphtheria.

Class №32

Topic: "Mycobacteria. Microbiological diagnosis of tuberculosis"

Actuality:

Genus *Mycobacterium* include more than 50 species and subspecies of Mycobacteria – pathogenic, opportunistic and saprophytic bacteria are widespread in environment. At least 25 species play an important role in human pathology and are the causative agents of tuberculosis, mycobacteriosis and leprosy. Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis*. It is characterized by the development of granulomas in infected tissues, the polymorphism of clinical signs, intoxication and local syndromes. Depending on the localization of tuberculosis lesions, tuberculosis of the lungs, skin, lymph nodes, meninges, bones and joints, genital and urinary system, abdominal cavity are distinguished. Nowadays the morbidity of tuberculosis increases due to severe course of the disease, higher mortality and the number of resistant strains.

Objective purposes:

- Form the scheme of microbiological diagnosis of tuberculosis.
- Study the morphological, tinctorial, cultural properties of *Mycobacterium tuberculosis* and media for their cultivation.
- Make bacterioscopic method for diagnosis of tuberculosis.
- Study preparations for diagnosis, prevention and therapy of tuberculosis.

Basic knowledge, practical skills, experiences needed for study of the topic (interdisciplinary integration). See class №25.

Enumeration of basic terms, parameters, characteristics that students have to learn during the preparation to the lesson:

Term	Significance
Tuberculosis	Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis*. It is characterized by the development of the granulomas in infected tissues, the polymorphism of clinical signs, intoxication and local syndromes. Depending on the localization of tuberculosis lesions tuberculosis of the lungs, skin, lymph nodes, meninges, bones and joints, genital and urinary system, abdominal cavity are distinguished.
Tuberculin	It was obtained by R. Koch in 1890 from *Mycobacterium tuberculosis*. Some variants of tuberculin were obtained. The first tuberculin ATK (Alttuberculin Koch) was filtrate of 5-6 weeks culture of Mycobacteria in glycerol broth condensed to 1/10 from initial volume. Present tuberculin by Koch is dried and pounded in 50% glycerol to

	homogeneous mass of *Mycobacterium tuberculosis*. Tuberculin is obtained from *Mycobacterium bovis*. It contains proteins, fatty acids, lipids, neutral fats, crystalline alcohol. In addition, free of ballast substances tuberculin – PPD (Purified Protein Derivative) and PT (Purificatum Tuberculinum) are present. PPD is produced in two forms: dry purified tuberculin with 50000 TU and PPD with standard dilution in ampoules with 3 ml. Single dose (2 TU) is contained in 0.1 ml of solution.
BCG	The vaccine was obtained by A. Calmette and C. Guerin from attenuated strain of *M. bovis* with the years of inoculation. It is used for active immunization against tuberculosis.
Mantoux test	Allergic test. It is used to determine the tuberculosis among population, screening to tuberculosis children and adolescents, the selection of individuals needed of revaccination, determining of its efficiency, diagnosis of tuberculosis and activity of the process. Tuberculin is used for this test.
Multi-drug-resistant tuberculosis (MDR-TB)	MDR-TB is a form of tuberculosis (TB) infection caused by bacteria that are resistant to treatment with at least two of the most powerful first-line anti-TB medications (drugs), isoniazid and rifampin.
Extensively drug-resistant TB (XDR TB)	XDR TB is a rare type of multidrug-resistant tuberculosis (MDR TB) that is resistant to isoniazid and rifampin, plus any fluoroquinolone and at least one of three injectable second-line drugs (i.e., amikacin, kanamycin, or capreomycin).
Totally drug-resistant tuberculosis (TDR-TB)	TDR-TB is a generic term for tuberculosis strains that are resistant to a wider range of drugs than strains classified as extensively drug-resistant tuberculosis.

Theoretical questions for the lesson:

- Mycobacteria, history of discovery and learning.
- The taxonomic position of mycobacteria.
- The morphological, tinctorial, cultural properties of the causative agent of tuberculosis.
- Factors of pathogenicity of *Mycobacterium tuberculosis*.
- Pathogenic and immunological properties of tuberculosis.
- Laboratory diagnosis of tuberculosis.
- Preparations for the treatment and specific prevention of tuberculosis.

Practical activities are performed at the class:

- Examine culture properties of Mycobacteria and the media for their cultivation

- Make the bacterioscopic diagnosis of tuberculosis with staining smears by the method of Ziehl-Nielsen and study demonstrational drugs.
- Study the methods of microbiological diagnosis of tuberculosis.
- Study the classification of mycobacteria.
- Learn the specific drugs for the prevention, diagnosis and treatment of tuberculosis.

Content of the topic:

At the practical lesson the students study the taxonomic position of Mycobacteria and their biological properties; discuss the pathogenetic and immunological features of tuberculosis; laboratory methods for diagnosis of tuberculosis; perform bacterioscopic method; study the nutrient media for cultivation of Mycobacteria; study the preparations for the specific prevention, diagnosis and treatment of tuberculosis; the classification of Mycobacteria; methods of microbiological diagnosis of tuberculosis. Completed tasks students write to protocol and sign it with a teacher. The students write down the completed tasks to protocol and teacher sign it.

Recommendations for the registration of the protocol:

Classification of Mycobacteria.

1. With pathogenicity.

а) Pathogenic: *M. tuberculosis* (90-97%), *M. bovis* (2-8%), *M. africanum*, *M. avium*

б) Opportunistic: *M. kansasii*, *M. ulcerans*, *M. marinum*, *M. fortuitum*, *M. scrofulaceum*, *M. chelonii*, *M. xenopi*.

в) Acid-resistant bacteria are not pathogenic for humans and animals.

2. With nature of the pigment formation:

а) Photochromogenic (form a pigment with light) – *M. kansasi*;

б) Scotochromogenic (form a pigment with light and darkness) – *M. scrofulaceum*;

в) Nonchromogenic (usually colorless) – *M. avium* (the cause of human tuberculosis)

3. With growth rate:

а) Fast-growing – visually large colonies appear earlier than 7 days of incubation: *M. chelonii*; *M. paratuberculosis*;

б) Slow-growing – visually large colonies appear after 7 days of incubation and later: *M. tuberculosis*; *M. africanum*; *M. scrofulaceum*;

в) Mycobacteria need of special conditions for the cultivation (do not grow in ordinary nutrient media): *M. lepraemurium*; *M. leprae*.

The method of staining by Ziehl-Neelsen (Acid-fast staining).

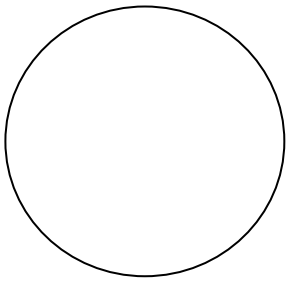
The authors proposed last version in 1884. Staining technique involves several steps:

1. Filter paper is applied to the fixed with the flame smear, pour fusion by Zielh and warm three times before steam appears (not boil); then smear with fusion is left for 1-2 minutes to cooling; we pour off the color, remove the paper, wash it with water.

2. The smear is decolorized with 5% sulfuric acid or hydrochloric acid until the yellow color (10-30 sec) and washed several times with water.
3. In addition, Leffler methylene blue is used for the next step, we color it and wash it with water, dry and examine under a microscope.

Microscopic picture: on general blue (light blue) field acid-resistant bacteria looked like the ruby-red ones.

Task 1. Study the morphological and tinctorial properties of *Mycobacterium tuberculosis* (prepare the smear with patient's material; staining by Ziehl-Neelsen, drawing the scheme)



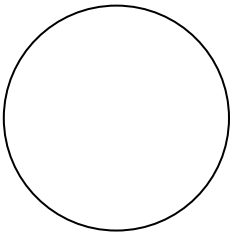
Tubercle bacilli in sputum. Staining by Ziehl-Neelsen.

Methods of laboratory diagnosis of tuberculosis.

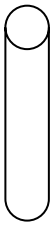
Bacterioscopic	a) *Material:* sputum, exudate ↓ *Enrichment methods* (flotation, homogenization) ↓ *Microscopy* (staining by Ziehl-Neelsen) b) *Luminescence microscopy* (stain by auramine- -rhodamine)
Bacteriological	a) *Process of material before inoculation:* Petroff's method, NALC (N-acetyl-L-cysteine) + 2% NaOH. ↓ inoculation into Petragnani; Petrova; Lowenstein-Jensen medium b) *The method of microcultures:* inoculation into citrate-blood medium to 3-7 days. ↓ *Microscopy* (staining by Ziehl-Nielsen) *c) Biochemical identification, antibiotic susceptibility test*
Biological	After processing H_2SO_4 and washing with physiological solution guinea pig is infected ↓ Macro- and microscopy of internal organs of guinea pig
Serological	*Material:* Patient's serum

	a) CFT b) Indirect hemagglutination test c) ELISA d) Western blotting
Allergy tests	a) Reaction by Moro; b) Reaction by Pirke; c) Reaction by Mantoux.
Genetic	**a)** *PCR* **b**) *Xpert MTB/RIF* is a cartridge-based nucleic acid amplification test (NAAT) for simultaneous rapid tuberculosis diagnosis and rapid antibiotic sensitivity test. It is an automated diagnostic test that can identify Mycobacterium tuberculosis (MTB) DNA and resistance to rifampicin (RIF). c) *TB-LAMP* – loop-mediated isothermal amplification d) *DNA sequencing***.**

Task № 2. Study the media for cultivation of *Mycobacterium tuberculosis* (demonstration; draw).



Medium by Lowenstein-Jensen.



Medium Finn-II.

Preparations treatment of tuberculosis.

First line of antituberculosis drugs: isoniazid (H), rifampicin (R), pyrazinamide (Z), ethambutol (E) and streptomycin (S).

Second line of antituberculosis drugs – subdivided into three groups:

1. *Fluoroquinolons* – Ofloxacin (OFX), Levofloxacin (LEV), moxifloxacin (MOX), and ciprofloxacin (CIP).
2. *Injectable antituberculosis drugs* – Kanamycin (KAN), amikacin (AMK) and capreomycin (CAP).
3. *Less-effective second-line antituberculosis drugs* – Ethionamide ETH)/Prothionamide (PTH), Cycloserine (CS)/Terizidone, P-aminosalicylic acid (PAS).

Questions for self-control.

- History of the discovery and study of the tuberculosis causative agent.
- The classification of Mycobacteria.
- The biological properties of *Mycobacterium tuberculosis*.

- Pathogenic and immunobiological features of tuberculosis.
- Factors of pathogenicity of *Mycobacterium tuberculosis*.
- Preparations for the specific prevention of tuberculosis.
- Methods for the microbiological diagnosis of tuberculosis.
- Differentiation of *Mycobacterium tuberculosis* between opportunistic and saprophytic Mycobacteria. Differentiation between causes of tuberculosis.

Class №33

Topic: "The causative agents of anaerobic infections. Microbiological diagnosis of anaerobic infections"

Actuality:

Pathogenic spore-forming anaerobes are included in the family *Clostridiaceae*, genus *Clostridium*. They are causative agents of tetanus, botulism, anaerobic infection of wounds and pseudomembranous ulcerative colitis. These are large polymorphic gram-positive bacteria; most species are motile (peritrichous); in the environment they form spherical or oval spores that are bigger than the diameter of the cell. They take its name from closter - *gr*. – spindle. Most species grow in anaerobic conditions and have different enzymatic activity. Pathogenic species of anaerobic bacteria produce a toxin; usually they inhabit the intestine of humans and animals and are excreted into the environment in feces; as spores survive in the soil, sea and fresh water for a long time (years).

Objective purposes:

- Study the morphological and tinctorial properties of the main species of pathogenic clostridia.
- Study the biological properties of the causative agents of tetanus, botulism, anaerobic infection of wounds.
- Form a scheme of microbiological diagnosis of anaerobic infections of wounds, tetanus, botulism.
- Carry out some steps of microbiological diagnosis of wound infection (microscopy of patient's material; cultivation and identification of Clostridia – causes of anaerobic infections of wounds).
- Study the preparations for the diagnosis, prevention and treatment of specific anaerobic infections.

Basic knowledge, practical skills, experience needed for study of the topic (interdisciplinary integration). See class №25.

Enumeration of basic terms, parameters, characteristics that students have to learn during the preparation to the lesson:

Term	Significance
Obligate anaerobes	Presence of molecular oxygen is harmful for these bacteria.
Facultative anaerobes	These bacteria can reproduce with molecular oxygen or without it.
Microaerophiles	Microaerophiles can multiply at low oxygen concentrations. High concentrations of oxygen do not kill these bacteria, but retard their growth.
Tetanus	Acute wound infection of humans and animals develops as a result of affect ion of neuromotor cells of spinal cord and brain

	by toxin. It manifests as the development of tonic and tetanic contraction of the muscles.
Botulism	Botulism is a severe and often fatal poisoning. It occurs as a result of ingestion of *Clostridium botulinum* toxins; it was characterized by specific lesions of the nervous system.
Anaerobic gas infection (clostridial myositis, gas gangrene)	These is acute, painful, polyetiological wound infection caused by bacteria of the genus *Clostridium* with association between each other or with opportunistic microorganisms.
Medium for the cultivation of anaerobic microorganisms	These media are Kitta-Tarotstsi's, blood sugar agar by Tseysler, medium by Wilson-Blair, medium by Vrublevsky.

Theoretical questions for the lesson:

- General characteristics of the genus *Clostridium*.
- The biological properties of the tetanus, botulism and anaerobic infection of wounds.
- Pathogenesis of tetanus, botulism and anaerobic infection of wounds.
- Principles of microbiological diagnostics of *Clostridia*; material for investigation of these diseases.
- Immunity at the anaerobic infections; the principles of specific therapy and prevention of the diseases. Preparations.
- Clostridia as a causes of food poisoning.

Practical tasks for the classroom:

- Study the morphological and tinctorial properties of the main pathogenic clostridia.
- Form the scheme of microbiological diagnostics of botulism, tetanus and anaerobic infection of wounds.
- Perform account of rapid diagnosis of anaerobic wound infection.
- Study preparation for the diagnosis, prevention and treatment of clostridiosis.

Content of the topic:

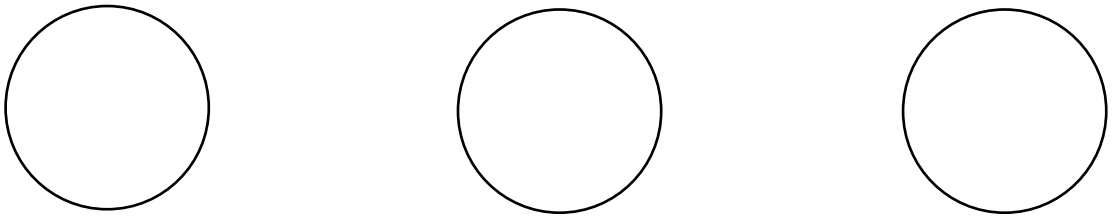
At the practical lesson the students study the general characteristics of the genus *Clostridium*, morphological and tinctorial properties of the main pathogenic clostridia; examine the biological properties of tetanus, botulism and anaerobic infections of wounds and pathogenesis of diseases caused by these bacteria; form a scheme of microbiological diagnosis of anaerobic infections; put the diagnosis of anaerobic wound infection; study preparations for the diagnosis, prevention and treatment of clostridiosis.

The students write down the completed tasks to protocol and teacher sign it.

Recommendations for the registration of the protocol:

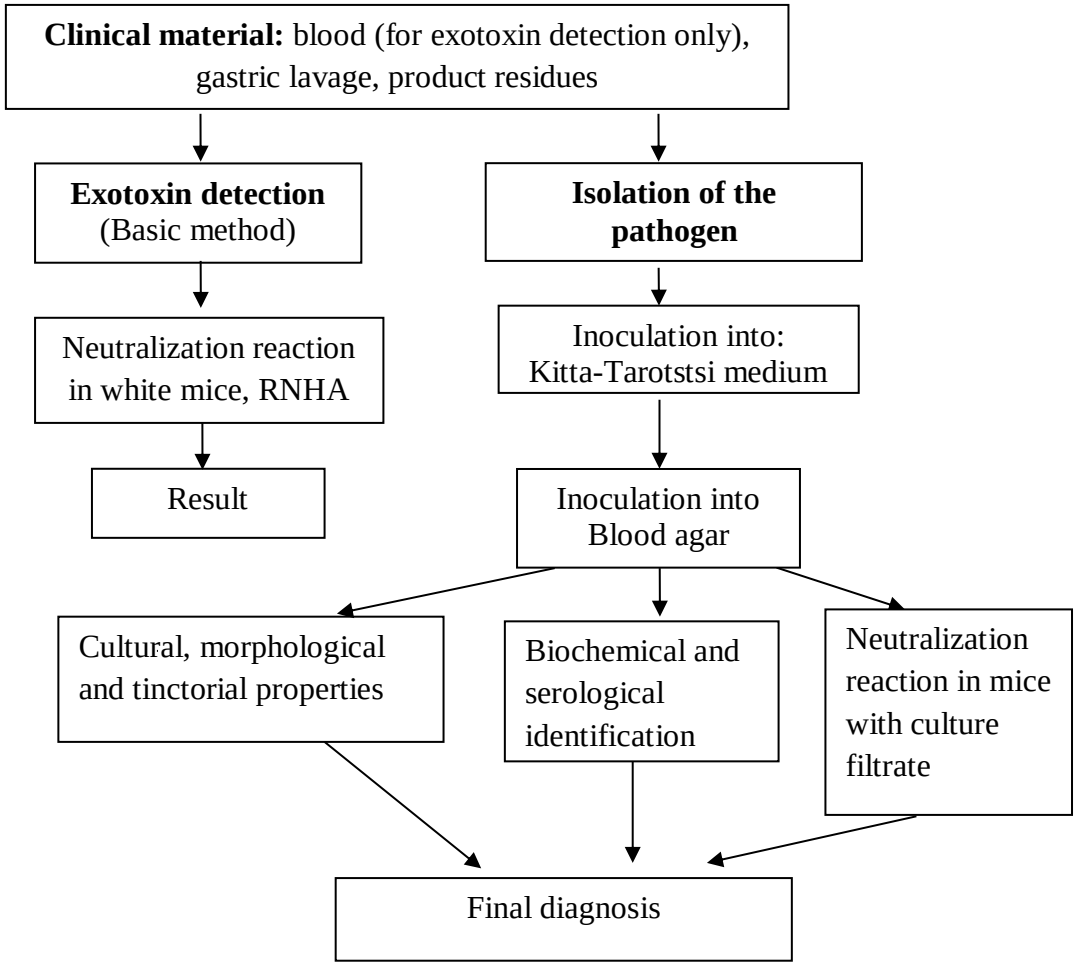
Practical activity №1. Study the morphological and tinctorial properties of Clostridia (demostration; drawing into protocol)

Microscopic preparations Gram staining

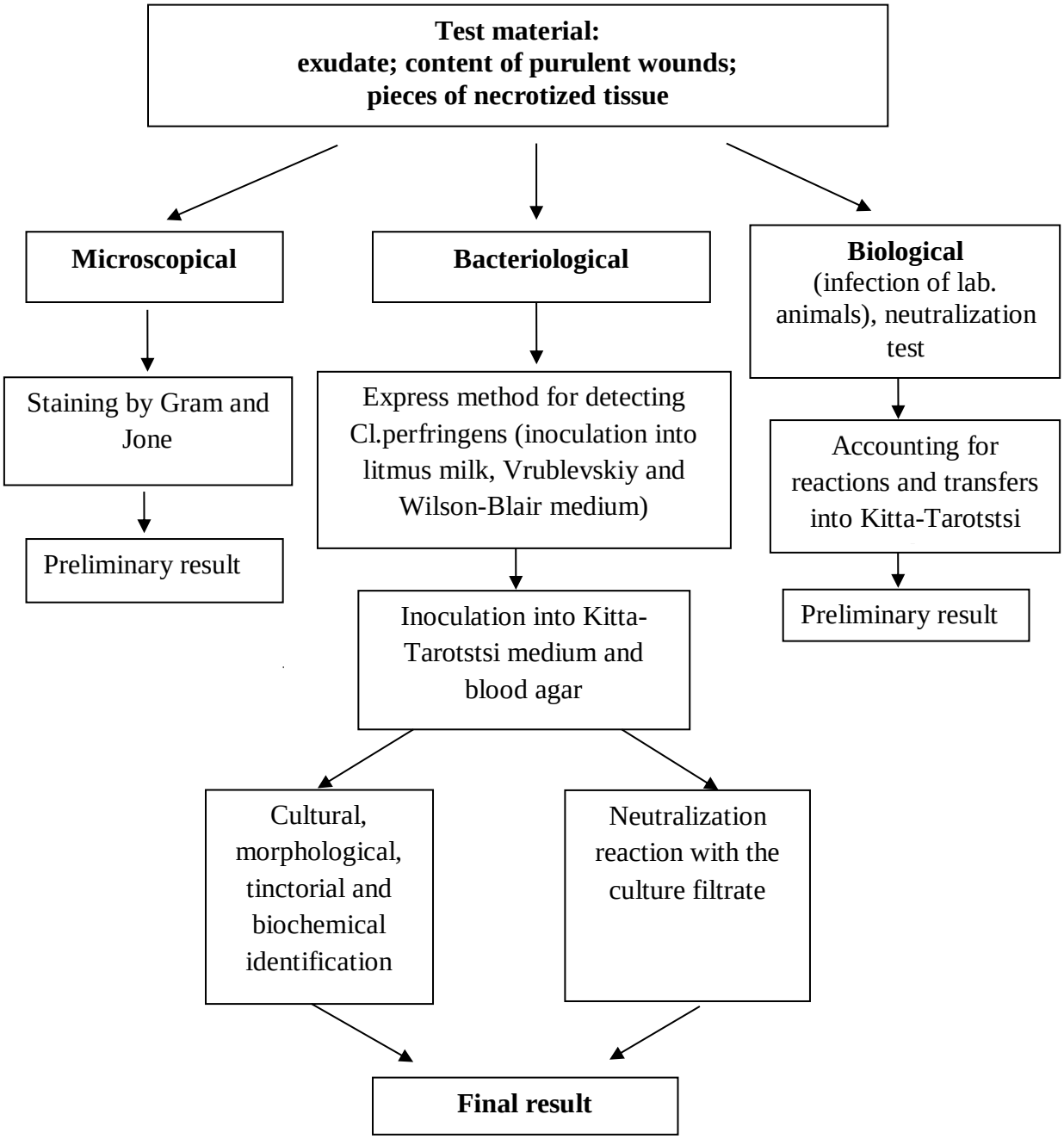


Clostridium tetani	*Clostridium botulinum*	*Clostridium perfringens*
Spores are rounded, terminal, "drumsticks"	Spores are oval, subterminal "tennis rackets"	Spores are oval, central or subterminal

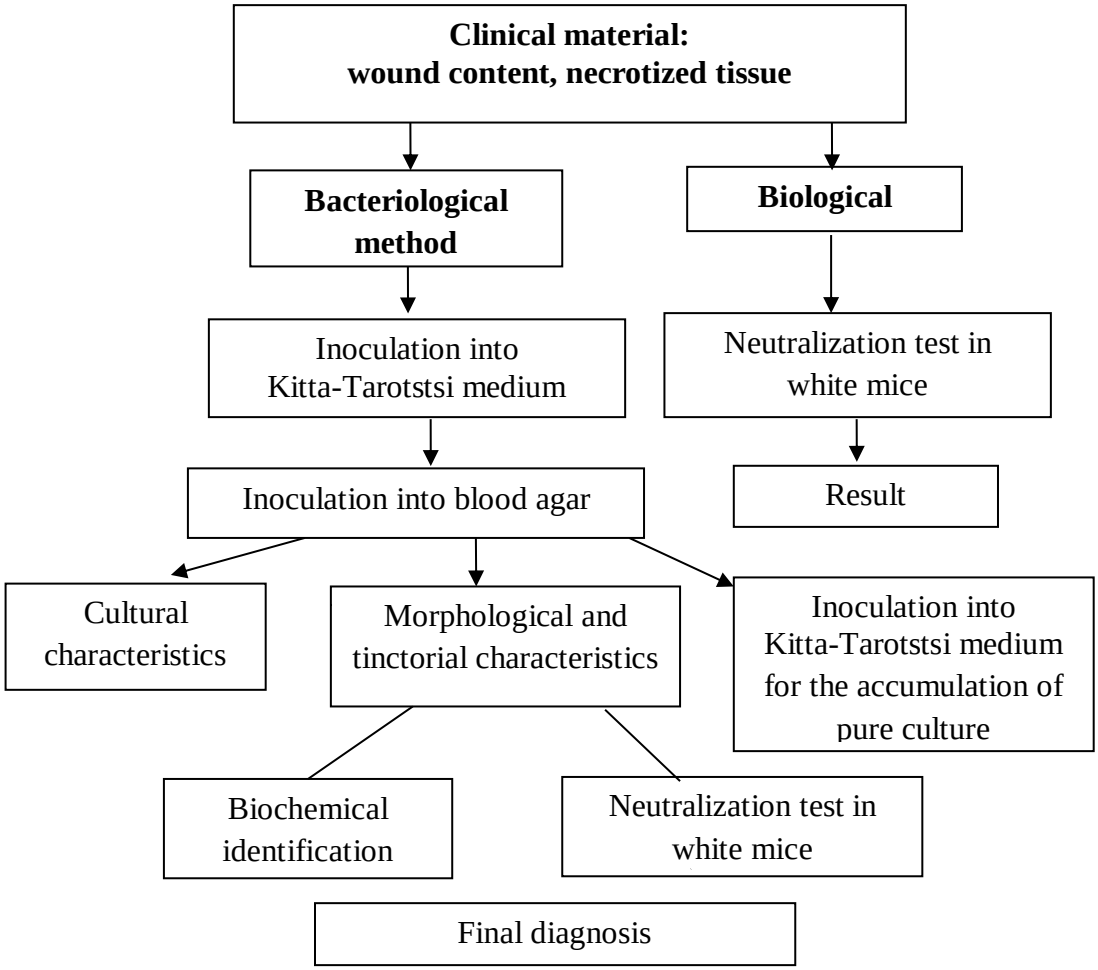
The scheme of research for identify of botulism



The scheme of research for identification of gas gangrene



The scheme of research for identification of the tetanus.



The main anaerobic microorganisms important for medical microbiology

Spore-forming:	
Gram positive bacteria	
• Clostridium (about 100 species)	
Non spore-forming:	
Gram positive bacteria	***Gram negative bacteria and spiral shapes***
• *Actinomyces* • *Bifidobacterium* • *Lactobacillus* • *Propoinobacterium*	• *Prevatella* • *Campylobacter* • *Bacteroides* • *Leptotrichia* • *Fusobacterium* • *Porhpyromonas* • *Borellia* • *Treponema*

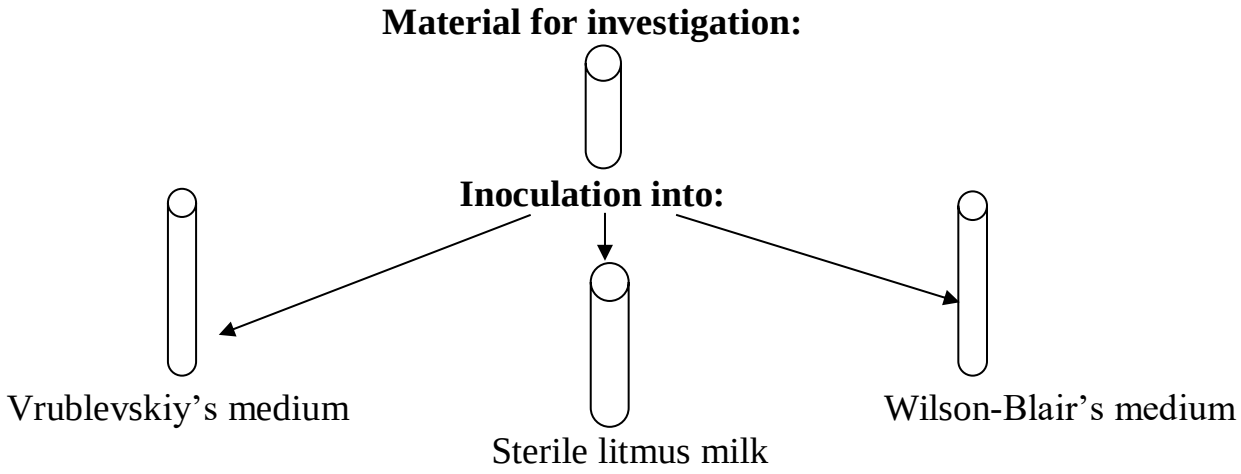
Gram positive cocci	*Gram negative cocci*
• *Peptococcus* • *Peptostreptococcus* • *Sarcina*	• *Veilonella*

Demonstration of specific preparation for the prevention, diagnosis and therapy of anaerobic infections:

- APDT;
- Tetanus toxoid;
- Antitoxic tetanus serum;
- Antotixic botulinum serum;
- Antitoxic gangrenuos serum.

Practical activity №2. Perform account of the rapid diagnosis of anaerobic wound infection.

The basis of rapid diagnostics is the high enzymatic activity of the main pathogen – *Cl. perfringens*. Changes in the medium are rapid (3-6 hours) and intense.



Questions for self-control.

- History of the discovery of anaerobic causes.
- Classification of anaerobes.
- Morphological and biological properties of the causative agents of tetanus, botulism, anaerobic infection of wounds.
- Pathogenic and immunobiological features of tetanus, botulism and anaerobic wound infection.
- Factors of pathogenecity of tetanus, botulism and anaerobic wound infection.
- Methods of the microbiological diagnosis of anaerobic infections.
- Preparations for prevention and treatment of tetanus, botulism, anaerobic infection of wounds.

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