SPECIAL AND CLINICAL MICROBIOLOGY

Laboratory workbook

Student name _____

Faculty _____

Group _____

Minsk BSMU 2016

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

ЧАСТНАЯ И КЛИНИЧЕСКАЯ МИКРОБИОЛОГИЯ

SPECIAL AND CLINICAL MICROBIOLOGY

Лабораторный практикум

2-е издание

Минск БГМУ 2016

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Содержит информацию для подготовки к практическим занятиям по разделам частной и клинической микробиологии. Приведены схемы, алгоритмы, справочные сведения, методики выполнения лабораторных работ. Первое издание вышло в 2015 году.

Предназначен для студентов 2-го курса медицинского факультета иностранных учащихся, обучающихся на английском языке.

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ЧАСТНАЯ И КЛИНИЧЕСКАЯ МИКРОБИОЛОГИЯ

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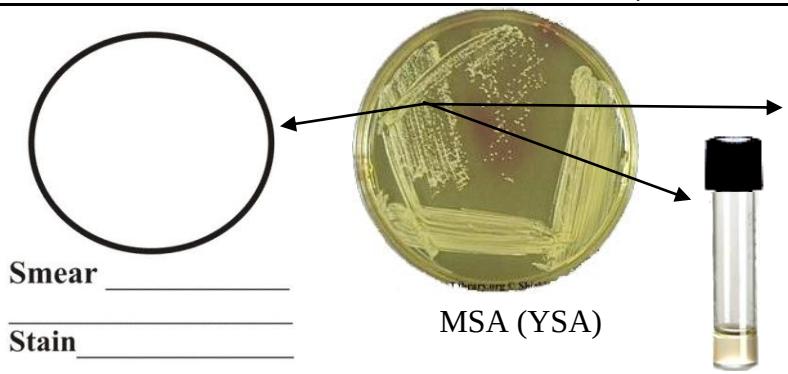
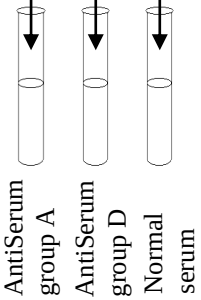
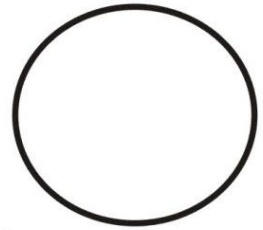
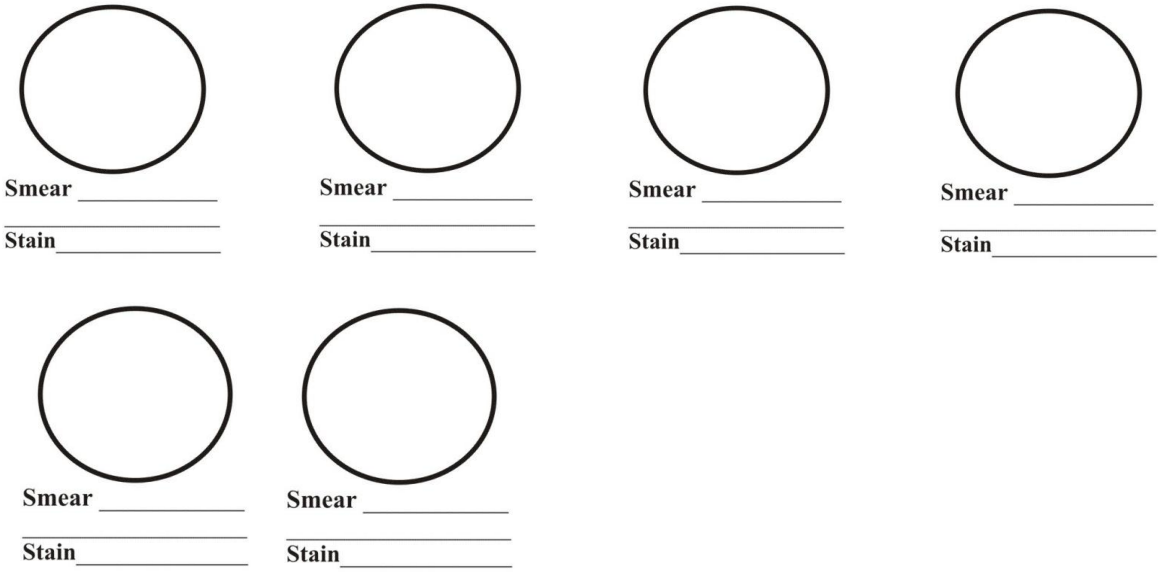
Class № 1. **Microbiological diagnostics of diseases caused by staphylococci, streptococci, neisseria** **Date_____**

The subject to study:
Staphylococci, systematics, general characteristics. Methods of microbiological diagnostics of staphylococcal infections. The material for the research depending on the form of the infection. Scheme of pure culture isolation (from pus, mucus, blood, etc.). Identification methods, phage typing. Specific prevention and treatment of staphylococcal infections.
Streptococci. Systematics. Pyogenic streptococci. Pneumococci. General characteristics. Antigenic structure. Acute and chronic diseases, pathogenesis, immunity. Specific antibodies to streptococcal antigens, diagnostic value. Methods for streptococcal infections diagnosis. Bacteriological method, study design. Material for studies depending on the form of the infection, the rules and methods for taking material. Principles of therapy and prevention pro-streptococcal infections.
Neisseria. Systematics, general characteristics.
Characteristics of the causative agent, mechanisms of pathogenesis, immunity, methods of microbiological diagnosis of acute and chronic gonorrhea.
Characteristics of the causative agent, mechanisms of pathogenesis, immunity, diagnosis and prevention of meningococcal infection-howl. sources:

Laboratory work

Tasks	Methods, results
1. Microbiological diagnostics of staphylococcal infection, Step 2 a) macro- and microscopic examination of the colonies on YSA; b) plasmacoagulase test.	 Smear _____ _____ Stain_____ MSA (YSA) Stabilized rabbit plasma, 37 °C, 2–4–24 h. **Conclusion**: according to morphological, cultural and biochemical properties unknown bacterium is identified as

Feature	Staphylococcal colonies
Shape	
Size	
Surface	
Edge	
Color	
Cionsistency	
Transparency	
Lecithinase	

2. Microbiological diagnostics of streptococcal infection, Step 3: a) a description of Streptococci growth in serum broth; b) determining the morphology of streptococci, Gram staining; c) determination of streptococcus serogroup by ring precipitation test.	 Serum broth Colonies characteristics_____ Cell wall antigens extract  AntiSerum group A AntiSerum group D Normal serum Ring precipitation test  **Smear** _____ _____ **Stain**_____ Conclusion: according to morphological, cultural and biochemical properties unknown bacterium is identified as _____
Demonstration. 1. Staphylococcus aureus in pus, Gram staining. 2. Streptococcus pneumoniae, pure culture, Gram staining. 3. Streptococcus pneumoniae, white mice, Gram staining. 4. Gonococcus in pus, Gram staining. 5. Meningococcus in cerebrospinal fluid, methylene blue. 6. The growth of staphylococci on YSA, blood agar, broth. 7. The growth of streptococci on blood agar and broth. 8. Plasmacoagulase test 9. Anaerobic mannitol fermentation. 10. Phage typing of staphylococci.	 **Smear** _____ _____ **Stain**_____ **Smear** _____ _____ **Stain**_____ **Smear** _____ _____ **Stain**_____ **Smear** _____ _____ **Stain**_____ **Smear** _____ _____ **Stain**_____ **Smear** _____ _____ **Stain**_____

Signature of the tutor_____

Complementary materials to class 5.

Staphylococcus genus characteristics

Main pathogenic species	
Morphology (size, shape, relative positions of cells)	
Spores development	
Capsule	
Flagella (motility)	
Gram staining	
Catalase activity	
Main pathogenicity factors	

Methods for staphylococcal infection diagnostics

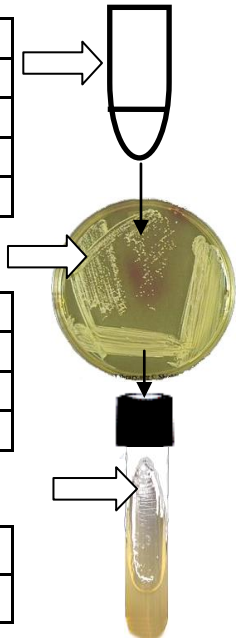
Method	Usage (+/-)
Microscopic	
Cultural	
Biological	
Serological	
Allergic	
Molecular-genetic	

Bacteriological diagnostics of staphylococcal infection

Material for investigation

Media for pure culture isolation

Medium for pure culture accumulation



Staphylococcal infections

Staphylococcus indentification

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

Streptococcus genus characteristics

Main pathogenic species	***S. pyogenes***	***S. pneumoniae***
Morphology		
Spores development		
Capsule		
Flagella (motility)		
Gram staining		
Group antigen		
Type-specific antigen (M-protein)		
Capsule polysaccharide		
Catalase activity		

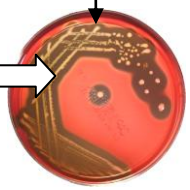
Methods for streptococcal infections diagnostics

Methods	**Usage (+/-)**	
	S. pyogenes	***S. pneumoniae***
Microscopic		
Cultural		
Biological		
Serological		
Allergic		
Molecular-genetic		

Bacteriological diagnostics of streptococcal infection

Material for investigation

Media for pure culture isolation



Medium for pure culture accumulation

***S. pyogenes* infections**
***S. pneumoniae* infection**
Other important Str. species

Streptococci identification

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

Neisseria genus characteristics

Features	*N. meningitidis*	*N. gonorrhoeae*
Morphology (size, shape, relative positions of cells)		
Spores development		
Capsule		
Flagella (motility)		
Gram staining		
Oxidase activity		
Pathogenicity factors		

Methods for neisserial infections diagnostics

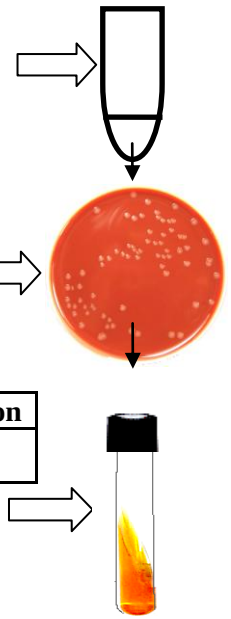
Methods	Usage (+/-)	
	N. meningitidis	*N. gonorrhoeae*
Microscopic		
Cultural		
Biological		
Serological		
Allergic		
Molecular-genetic		

Bacteriological method for the *Neisseria* infections diagnostics

Material for the investigation

Media for the pure culture isolation

Mediym for the pure culture accumulation



N. meningitidis infections

N. gonorrhoeae infections

Neisseria differentiation

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

Class № 2. **Microbiological diagnostics of acute enteric infections caused by enterobacteria** **Date**_____

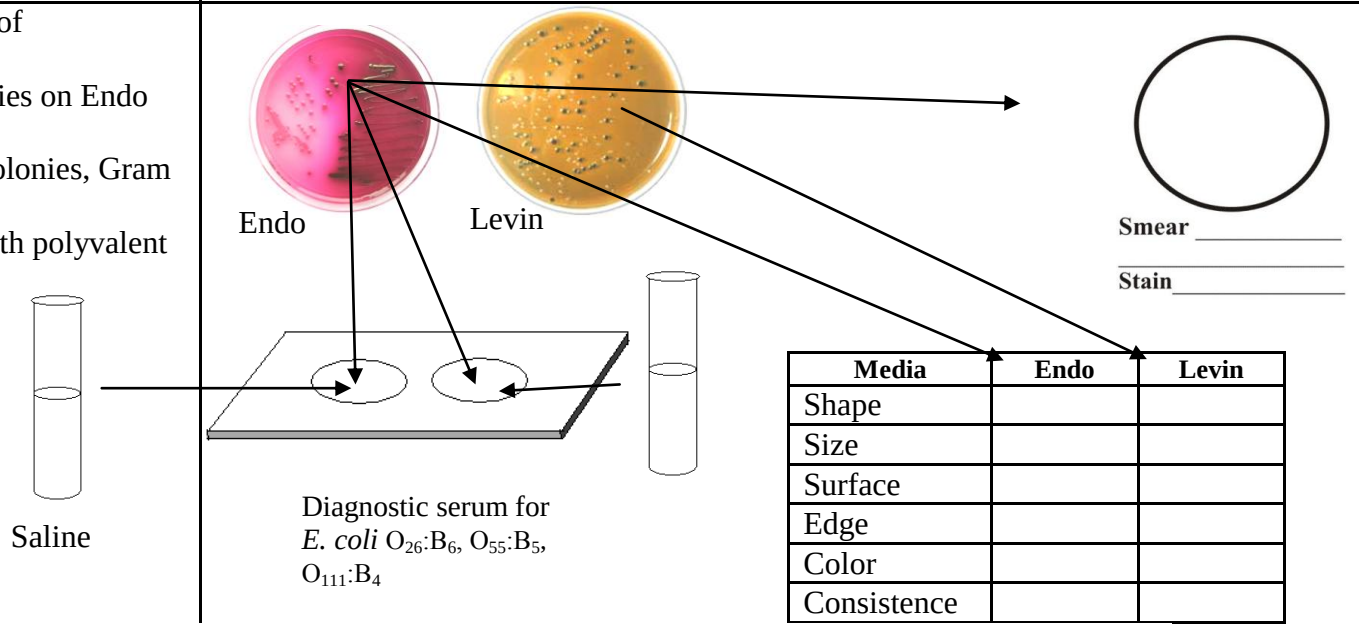
Questions to study: General characteristics of Enterobacteriaceae family. Differences between genera. General principles of diagnostics of acute enteric infections caused by pathogenic enterobacteria. Differential diagnostic media, composition, plinciple of work.

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

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

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

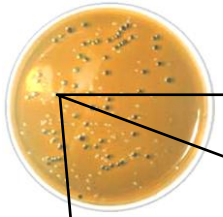
Laboratory work

Task	Methods, results
1. Bacteriological diagnostics of escherichiosis, 2-nd step: а) exploring of E. coli colonies on Endo and Levin media; б) slides preparation from colonies, Gram staining; в) slide agglutination test with polyvalent diagnostic OK-serum.	

Media	Endo	Levin
Shape		
Size		
Surface		
Edge		
Color		
Consistence		

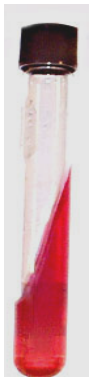
Conclusion: according to morphological, cultural and biochemical properties unknown bacterium is identified as _____

2. Bacteriological diagnostics of typhoid:
2-nd step of coproculture isolation:
a) describe colonies on Levin medium;
b) prepare slide from colonies, Gram staining;
c) inoculate Kligler medium.



Levin medium

TSI (Kligler) medium



Smear _____

_____

Stain_____

Feature	Levin medium
Shape	
Size	
Surface	
Edge	
Color	
Consistence	

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

Smear _____

_____

Stain_____

Smear _____

_____

Stain_____

Smear _____

_____

Stain_____

Smear _____

_____

Stain_____

Teacher signature_____

Complementary materials to class 2.

***Enterobacteriaceae* genera of medical importance**

General characteristics of *Enterobacteriaceae family*

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

***Escherichia coli* characteristics**

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

Methods for diagnostics of escherichiosis and salmonellosis

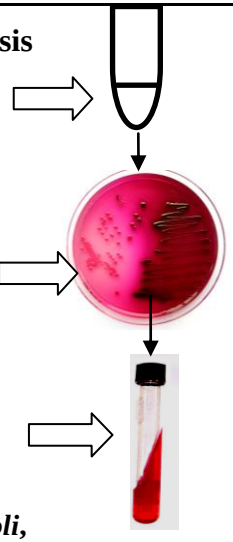
Methods	Usage (+/-)	
	Escherichiosis	***Typhoidand paratyphoid***
Microscopic		
Cultural		
Biological		
Serological		
Allergic		
Molecular-genetic		

Bacteriological diagnostics of escherichiosis

Material for the investigation

Media for pure culture isolation

Medium for the pure culture accumulation



Biological properties *E. coli*, as normal microflora representatives

Positive	Negative

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

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

Methods of microbiological typhoid diagnostics depending on the pathogenesis phase

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

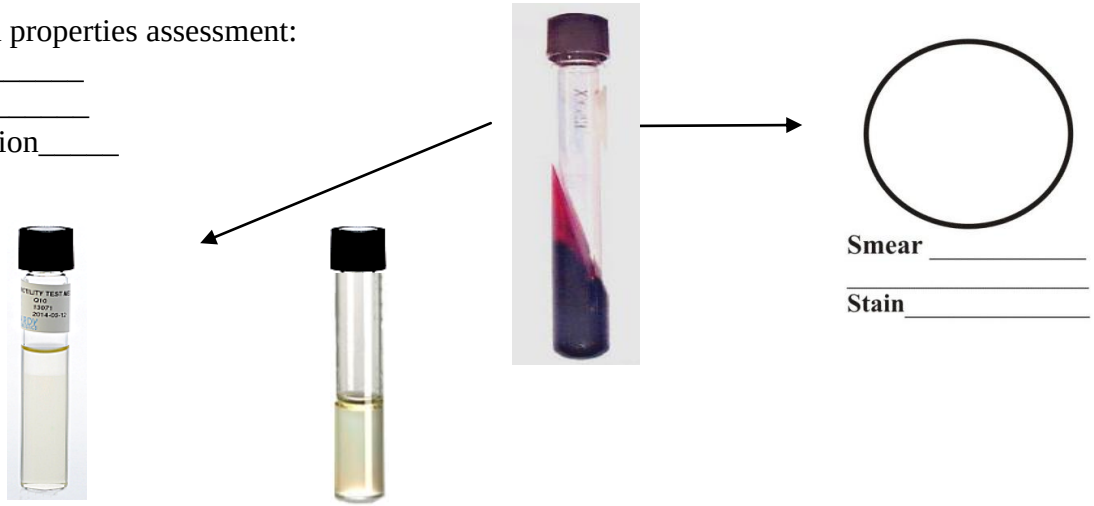
Class № 3. **Microbiological diagnostics of acute enteric diseases caused by enterobacteria** **Date_____**

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

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

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

Laboratory work

Task	Methods, results
1. Microbiological diagnostics of typhoid fever: 3-rd step. 2. a) Describe the growth on the Kligler medium; 3. b) prepare the slide from the colonies, Gram staining; 4. a) check the culture for motility and indol production; 5. d) determination of the antigenic structure of the culture isolated in slide agglutination test.	Biochemical properties assessment: Lactose_____ Glucose_____ H_2S production_____  Smear _____ _____ Stain_____ Motility test _____ _____ Indol production test _____ _____

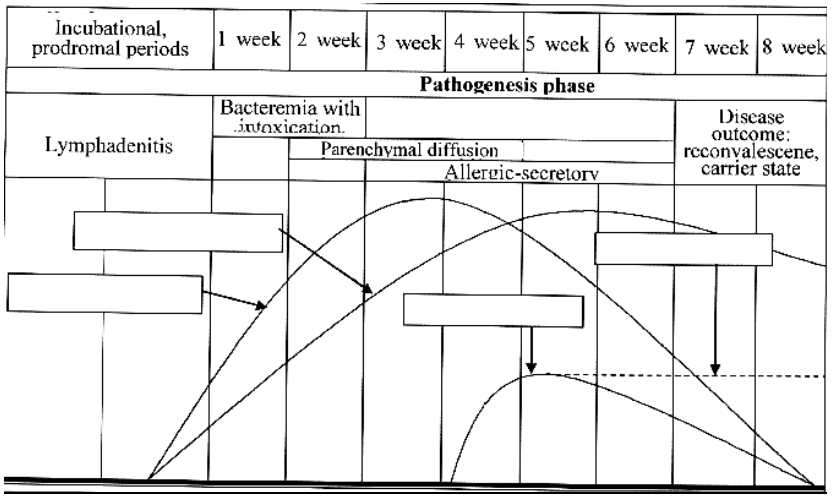
2. Assessment of Vidal test

Vidal agglutination test (AT)

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

Conclusion:_____.
(Diagnostic titer_____).

Immunoglobulines dynamics in typhoid fever



_____
_____
_____
_____
_____

Demonstration

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

Passive *Vi* – hemagglutination test

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

Conclusion:_____ .
(Diagnostic titer_____).

Signature of the tutor_____

Supporting materials to class 3.

Shigella classification

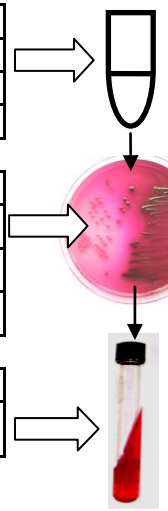
Shigella species	Serovarients number

Cultural method for the shigellosis diagnostics

Materials for the research

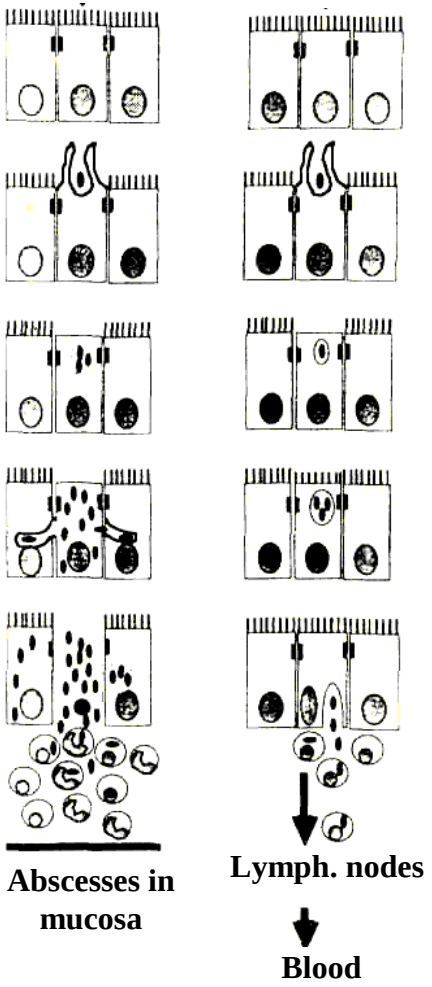
Media for the pure culture isolation

Medium for the pure culture accumulation



Shigella characteristics

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



Shigellosis and salmonellosis pathogenesis

Main salmonellosis pathogens

Bacteriological method for salmonellosis diagnostics

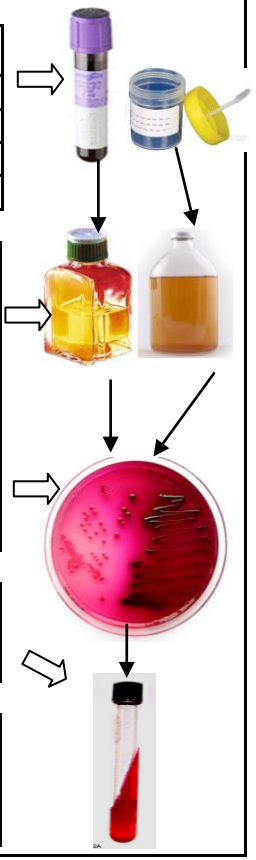
Material for the research

Medium for the material enrichment	

Medium for pure culture isolation

Medium for pure culture accumulation

Methods for salmonella identification



Class № 4. Microbiological diagnostics of diseases caused by Klebsiella, Iersinia, Campylobacter and pseudomonada. Methods for food poisoning diagnostics **Data_____.**

List of questions to study: Klebsiella, classification and general characteristics, main diseases caused. Pathogenesis, immunity, methods of microbiological diagnosis of acute and chronic klebsiellosis.

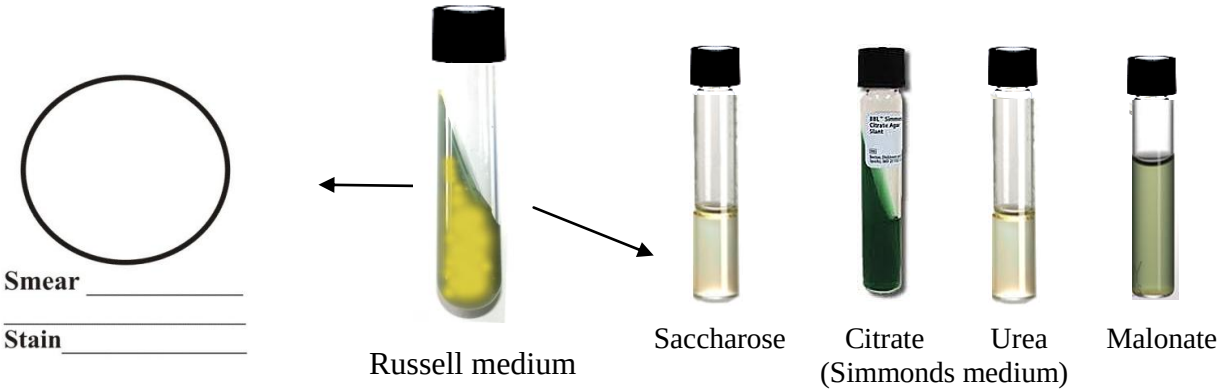
The causative agent of intestinal yersiniosis, general characteristics. Pathogenesis, immunity, methods of microbiological diagnostics.

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

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

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

Laboratory work

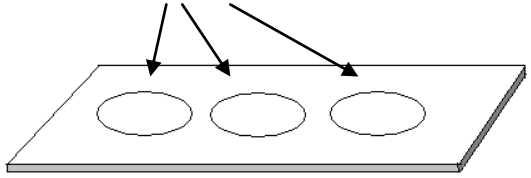
Tasks	Methods, results
1. Independent work "Microbiological klebsiellosis diagnostics": A. Examine the growth of Klebsiella on differential-diagnostic media. B. Determine the capsule presence. C. Determine the biochemical properties of Klebsiella. D. Perform slide agglutination test with anti-capsule diagnostic sera and determine the K-antigen. E. Determine the titer of CFT for serological diagnosis of scleroma.	

Klebsiella characteristics

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

Slide agglutination test with anti-capsule serum

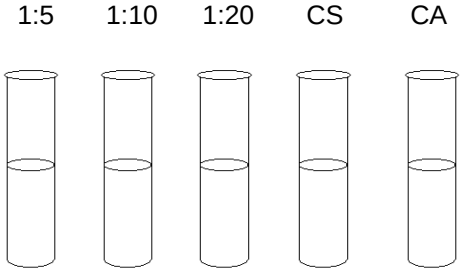
Pure culture



Conclusion:_____

Complement fixation test

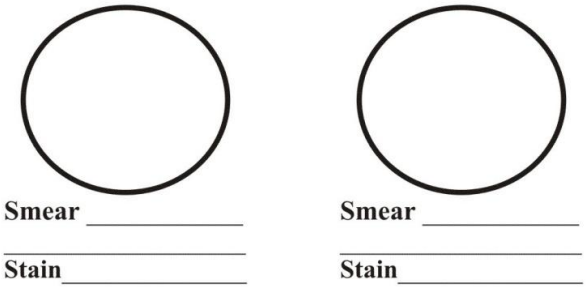
Variant	Serum dilutions			CS	CA	Result
	1:5	1:10	1:20			
1	++++	++++	++++	-	-	Very positive
2	++++	++++	-	-	-	Positive
3	+++	-	-	-	-	Weak positive
4	-	-	-	-	-	Negative



Conclusion:_____-

Demonstration.

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



Signature of the tutor_____

Additional materials for class 4

Causative agents	Disease	Materials for bacteriological diagnostics
K. pneumoniae s. rhinoscleromatis		
K. pneumoniae s. ozaenae		
K. pneumoniae s. pneumoniae		
Y. enterocolitica		
C. jejuni		
H. pylori		
P. aeruginosa		

Methods of laboratory diagnostics

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

Diagnosis of bacterial food poisoning

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

Foodborne diseases (FBD): FBDs result from ingestion of products massively colonized by certain bacteria. Pathogens: opportunistic members of the family Enterobacteriaceae - E. coli, Proteus (P. vulgaris, P. mirabilis), Morganella morganii, Citrobacter, Enterobacter, Hafnia, Klebsiella pneumoniae; Sem. Vibrionaceae - V. parahaemolyticus; Sem. Bacillaceae - B. cereus, C. perfringens serovar A; Sem. Streptococcaceae - S. faecalis; Sem. Pseudomonadaceae - P. aeruginosa, and others.	**Microbial food toxicosis (intoxication):** acute illness arising from eating food, which containes a large amount of exotoxin (as a result of massive reproduction of microbes). These include botulism, toxicosis caused by staphylococcal enterotoxin, toxins from microscopic fungi and others.
Pathogenesis. Pathogen replicates in the intestine, penetrates into lymphoid tissue, where it is killed with the release of endotoxin, which causes damage to the intramural bowel NS, CNS and blood vessels. Bacteria cause inflammation of the intestinal wall.	**Pathogenesis** is based on the microbial exotoxin, which is not destroyed by food processing, digestive enzymes and acidic stomach contents.

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

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

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

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

Class № 5. **Microbiological diagnostics of diseases caused by Corynebacteria, bordetella, haemophilus, legionella, listeria Date_____**

List of questions to study:

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

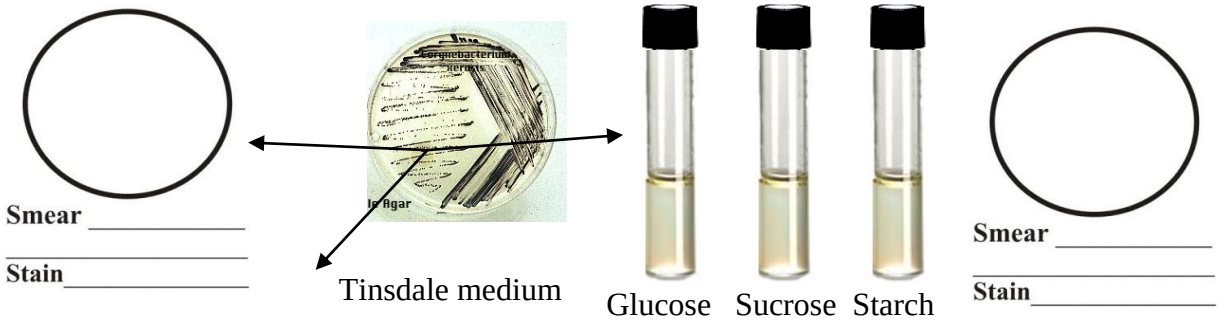
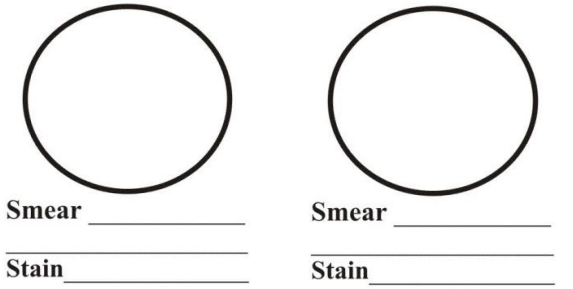
Bordetella pertussis. Characteristics of the pathogen, pathogenicity factors. Differentiation with parapertussis agent. The pathogenesis of pertussis, immunity, diagnostics. Principles of therapy and prevention of pertussis.

Haemophilus, general characteristics, role in human pathology.

Legionella, general characteristics, role in human pathology.

Listeria, general characteristics, role in human pathology.

Laboratory work

Tasks	Methods. results
1. Bacteriological diagnosis of diphtheria, 2nd step: a) Describe the colonies Corynebacterium on potassium tellurite serum agar b) Seed bacteria from typical colonies onto Hiss media (glucose, sucrose, starch).	
Demonstration. 1. Corynebacterium diphtheriastained by: a) Neisser; b) Leffler. 2. Test for Corynebacterium diphtheria toxigenicity. 3. Preparations for specific prevention and treatment of diphtheria and pertussis. 4. Growth of Bordetella pertussis and parapertussis on CCA, NA with tyrosine, urease test. 5. Bordetella pertussis, Gram staining 6. Assessment of antidiphtheria immunity intensity	

Feature	Colonies on serum tellurite agar
Shape	
Size	
Surface	
Edge	
Color	
Consistency	

Signature of the tutor_____

Additional materials and independent work for Class № 5.

Corynebacterium characteristics

Properties	*C. diphtheriae*
Morphology (size, shape, relative positions of cells)	
Spores development	
Capsule	
Flagella (motility)	
Gram staining	
Pathogenicity factors	

Medically important corynebacteria

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

C. diphtheriae pathogenicity factors

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

Laboratory diagnostics and specific prophylaxis of diphtheria

Method	Properties
Microscopic	
Cultural	
Molecular-genetic	
Specific prophylaxis	

Bordetella pertussis characteristics

Properties	*B. pertussis*
Morphology (size, shape, relative positions of cells)	
Spores development	
Capsule	
Flagella (motility)	
Gram staining	

Bordetella differentiation

Feature	*B. pertussis*	*B. parapertussis*

***B. pertussis* pathogenicity factors**

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

Laboratory diagnostics and specific prophylaxis of pertussis

Method	Properties
Bacteriological	
Serological	
Specific prophylaxis	

***Haemophilus* genus representatives and respective diseases**

Species	Diseases
H. influenzae	
H. ducreyi	
H. aphrophilus, H. parainfluenzae, H. haemolyticus, H. parahaemolyticus и др.	

***Haemophilus* genus characteristics**

Properties	*H. influenzae*
Morphology	
Spores development	
Capsule	
Flagella (motility)	
Gram staining	
Antigens	

***H. influenzae* pathogenicity factors**

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

Laboratory diagnostics and specific prophylaxis of infections caused by *Haemophilus*

Method	Properties
Microscopic	
Cultural	
Serological	
Specific prophylaxis	

Legionella characteristics

Properties	*Legionella pneumophila*
Morphology (size, shape, relative positions of cells)	
Spores development	
Capsule	
Flagella (motility)	
Gram staining	

***Legionella pneumophila* pathogenicity factors**

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

Laboratory diagnostics and specific prophylaxis of legionellosis

Method	Properties
Microscopic	
Cultural	
Serological	
Molecular-genetic	
Specific prophylaxis	

Listeria characteristics

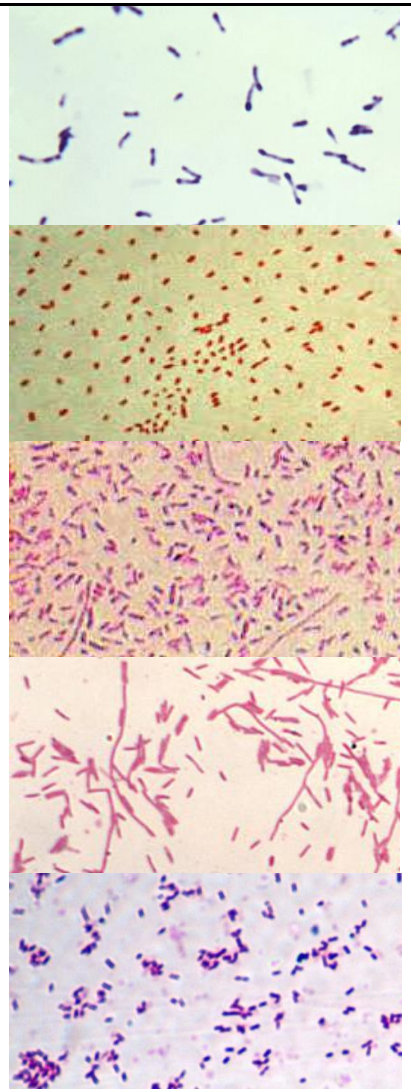
Properties	*L. monocytogenes*
Morphology (size, shape, relative positions of cells)	
Spores development	
Capsule	
Flagella (motility)	
Gram staining	

Listeria pathogenicity factors

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

Laboratory diagnostics and specific prophylaxis of listeriosis

Method	Properties
Microscopic	
Cultural	
Serological	
Molecular-genetic	
Specific prophylaxis	



C. diphtheriae

B. pertussis

H. influenzae

L. pneumophila

L. monocytogenes

Class № 6. **Methods of microbiological diagnosis of diseases caused by mycobacteria and actinomycetes. Methods of microbiological diagnostics anaerobic infections** **Data _____**

The list of questions to study:

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

Mycobacteria classification. TB germs, general characteristics. Pathogenesis, immunity, methods of microbiological diagnostics, principles of treatment and prevention of tuberculosis. Mantoux test.

The causative agent of leprosy, general characteristics, role in human pathology.

Mycobacteriosis. Nocardia.

Anaerobes, classification, general characteristics. Causative agents of gas gangrene, tetanus, botulism. Systematics and general characteristics. Exotoxins.properties Principles of therapy and prevention of anaerobic infections.

Clostridial gastroenteritis. Clostridium difitsile role in human pathology.

Nonspore anaerobes. Bacteroides. Peptococci. General characteristics, pathogenicity factors, role in human pathology.

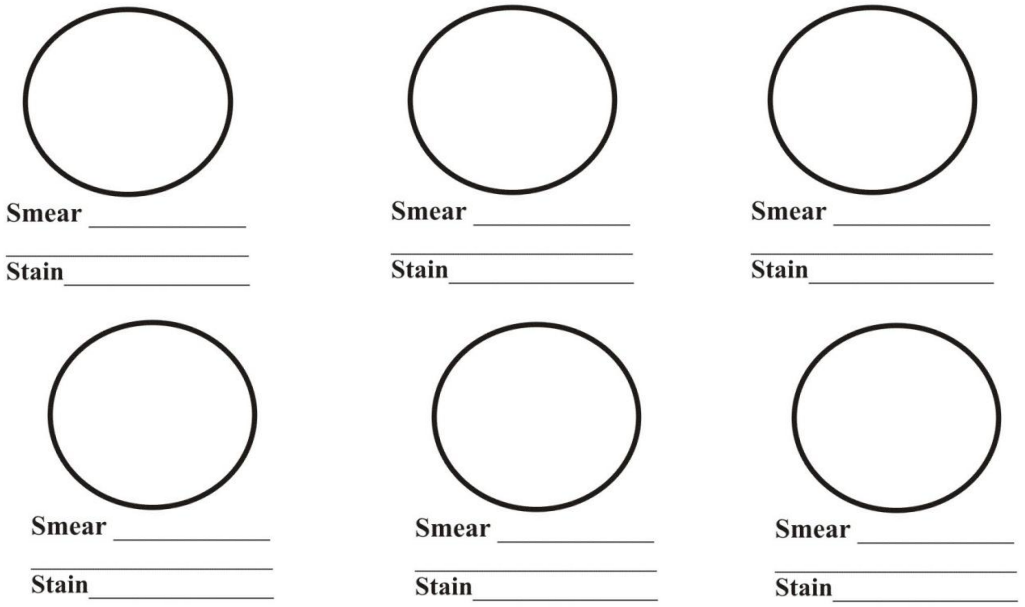
General principles and methods for anaerobic infections diagnosis. Molecular biological diagnostics - PCR.

Laboratory work

Tasks	Methods, results
1. The assessment of enzymatic activity of corynobacteria, identification	**Biochemical properties of sertain corynobacteria**

Corynobacteria spp.	Enzymatic activity					Nitrate reduction
	Glucose	Sucrose	Starch	Cysteinase	Urease	
C. diphtheriae						
gravis	+	-	+			+
mitis	+	-	-			+
C. pseudodiphtheriae (hofmani)	-	-	-	-	+	+
C. xerosis	+	+	-	-	+	+
C. ulcerans	+	-	+	+	+	-

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

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

Signature of the tutor_____

Materials for independent work for class № 6

Actinomyces characteristics

Characteristics	***Actinomyces israelii***
Morphology (size, shape, relative positions of cells)	
Spores development	
Capsule	
Flagella (motility)	
Gram staining	
Pathogenicity factors	

Microbiological diagnostics and specific prophylaxis of actinomycosis

Method	**Description**
Microscopic	
Cultural	
Specific prophylaxis	

Classification of medically important culturable mycobacteria

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

Myvobacteria characteristics

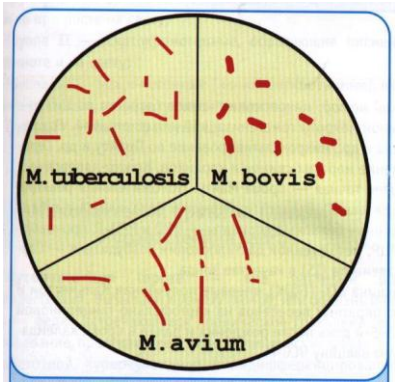
Characteristics	*M. tuberculosis*	*M. leprae*
Morphology (size, shape, relative positions of cells)		
Spores development		
Capsule		
Flagella (motility)		
Gram staining		
Pathogenicity factors		

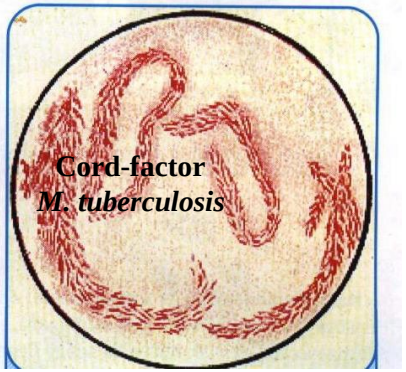
M. tuberculosis pathogenicity factors

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

Microbiological diagnostics and specific prophylaxis of tuberculosis

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





Microbiological diagnostics and specific prophylaxis of leprosy

Methods	Renarks
Microscopic	
Allergic	
Biological	
Specific prophylaxis	

Ecological group of anaerobic bacteria

Gram-negative nonsporeing rods	Diseases induced
Bacteroides species	
Fusobacterium species	
Leptotrichia bucalis	
Prevotella species	
Porphyromonas species	
Bilophila wadsworthia	

Grampositive spore forming rods		
Clostridia	*Clostridium tetani*	Tetanus (Lockjaw)
	Clostridium perfringens, C. novyi, C. ramosum, C. histolyticum, C. septicum	Gas gangrene, necrotizing enteritis
	Clostridium botulinum	Botulism
	Clostridium difficile	Pseudomembranous colitis, antibiotic-associated diarrhea

Gramnegative cocci	
Veillonella	Septic infections

Grampositive cocci	
Enterococcus species	Septic infections
Peptococcus species	
Peptostreptococcus spp.	

Bacteroides pathogenicity factors

Pathogenicity factors		Biological effect
toxins	endotoxin	general toxic effect
	leukocidin	damages leukocytes
enzymes	collagenase	destroys the collagen fibers of the connective tissue (spread of purulent process)
	DNAse, heparinase	cause intravascular blood clotting
	fibrinolysin	dissolves blood clots

Clostridia characteristics

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

Clostridium perfringens pathogenicity factors

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

	beta-lactamase	destroys the beta-lactam antibiotics
surface cell structure	pili	adhesion to the substrate
	capsule	protects the bacteria from phagocytosis
Metabolites	fatty acid	inhibit the chemotaxis and cytotoxicity of leukocyte

Microbiological diagnostics of septic infections caused by bacteroides

Method	Remarks
Microscopic	
Cultural	
Serological	
Molecular-genetic	

Microbiological diagnostics and specific prophylaxis of gas gangrene

Method	Remarks
Microscopic	
Cultural	
Biological	
Specific prophylaxis	

***Clostridium tetani* pathogenicity factors**

Pathogenicity factors	Biological effects
Tetanus toxin	

Microbiological diagnostics and specific prophylaxis of tetanus

Methods	Remarks
Microscopic	
Biological	
Cultural	
Specific prophylaxis	

***Clostridium botulinum* pathogenicity factors**

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

Microbiological diagnostics and specific prophylaxis of botulism

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

Class № 7. **Microbiological diagnostics of especially dangerous infections** **Date**_____

The list of questions to study:

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

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

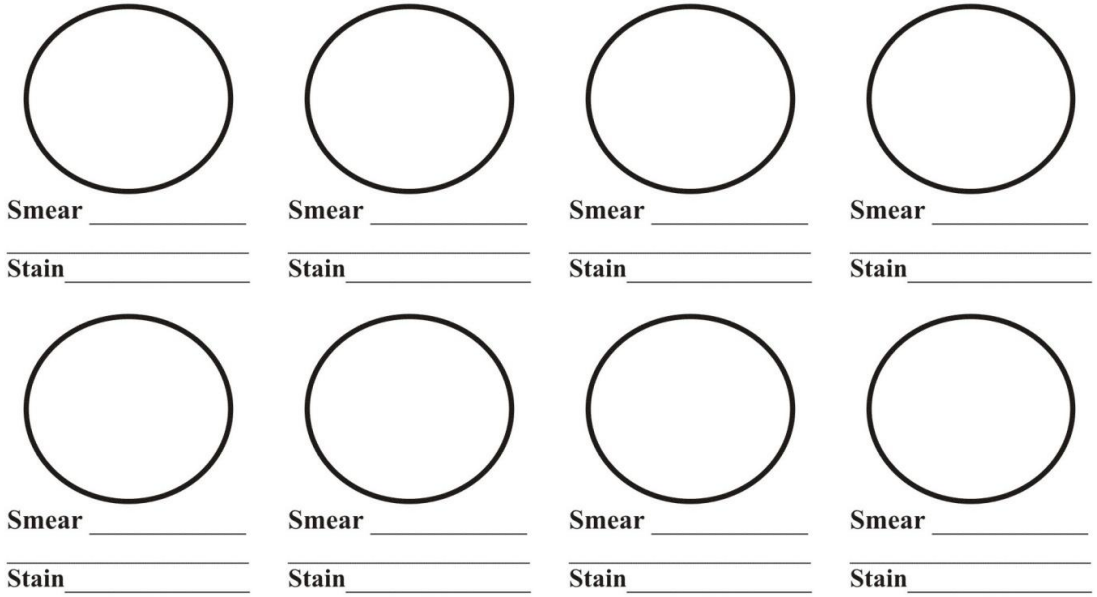
The causative agent of plague, systematic position, characteristics, pathogenicity factors. Differences from other Yersinia. Pathogenesis, principles of treatment and prevention of plague.

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

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

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

Laboratory work

Tasks	Methods, results
Demonstration. 1. Growth of vibrio cholera on alkaline agar, TCBS, peptone water. 2. Phage lysability of vibrio cholera classica and El Tor. 3. Tube agglutination test. 4. Biochemical properties of V. cholerae. 5. Mobility of Vibrio spp. 6. V.cholera, pure culture, Gram staining. 7. I.pestis in the organs, Leffler staining. 8. The causative agent of tularemia (pure culture), Gram staining. 9. Preparations for specific prophylaxis of especially dangerous infections. 10. The causative agent of brucellosis, Gram staining. 11. The growth of Bacillus spp. on nutrient media. 12. B.anthracis in organs, Gram staining. 13. B.anthracis, pure culture, Gram staining. 14. B.anthracis spores, Ozheshko staining.	 Signature of the tutor_____

Additional materials for independent study for class №7

V. cholera characteristics

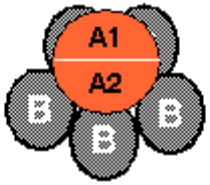
Characteristics	*Vibrio cholerae*
Morphology (size, shape, relative positions of cells)	
Spores development	
Capsule	
Flagella (motility)	
Gram staining	
Pathogenicity factors	

Vibrio cholerae pathogenicity factors

Pathogenicity factors	Biological effects
Exotoxin (choleragen)	Violation of water-salt metabolism, the cytotoxic effect on the epithelium of the small intestine
Endotoxin	Inhibition of phagocytosis, drop in blood pressure; infectious-toxic shock
Pili	Adhesion to mucosal cells
Fibrinolysin hyaluronidase	Enzymes invasion (aggression)

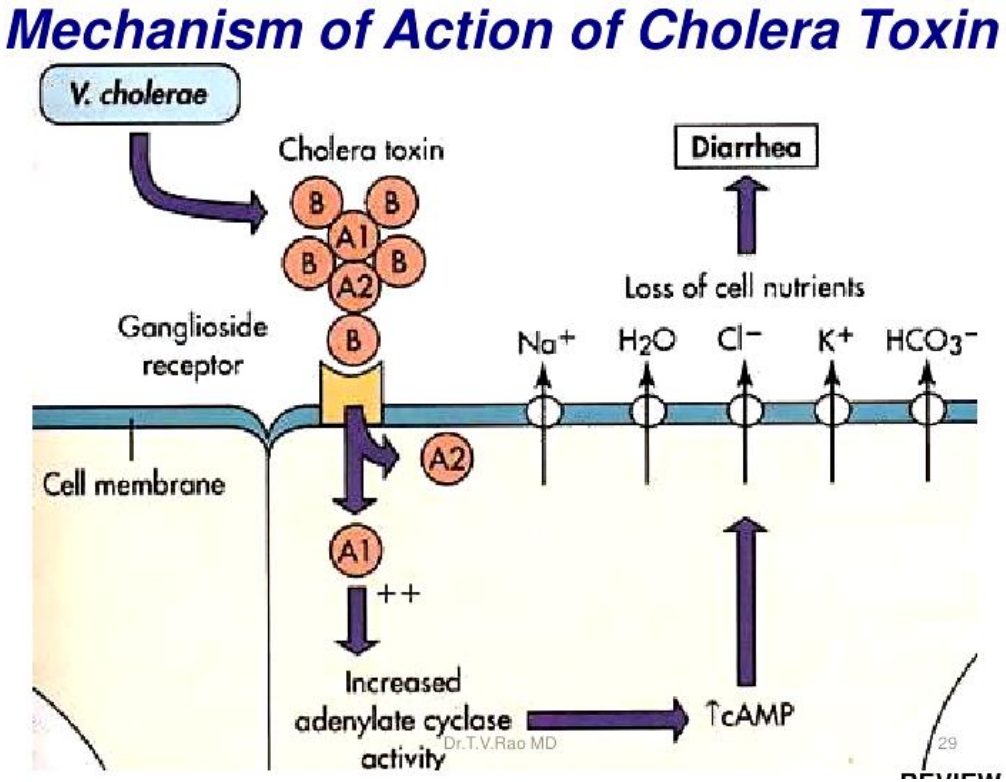
Microbiological diagnostics and specific prophylaxis of cholera

Method	Remarks
Microscopic	
Cultural	
Serological	
Molecular-genetic	
Specific prophylaxis	



CHOLERA TOXIN

5 B Subunits (Delivery)
1 A Subunit (Enzyme)



I. pestis characteristics

Characteristics	*Yersinia pestis*
Morphology (size, shape, relative positions of cells)	
Spores development	
Capsule	
Flagella (motility)	
Gram staining	
Pathogenicity factors	

Y. pestis pathogenicity factors

Pathogenicity factors	Biological effects
Capsular Ag, F1-Ag, fraction 1)	protection against the absorption of phagocytes, non-toxic, the immunogen
Plasminogen activator - protease	activates lysis of fibrin clots, and inactivates C5a
V/W(Vi)-Ag	Includes protein (V-phase) and LPS (W-phase); exhibits antiphagocytic properties, promotes intracellular bacterial growth
Murine toxin	adrenergic receptor antagonist, proteinaceous substance, localizes intracellularly
Bacteriocins (pestitsiny)	Immunogenic properties

Microbiological diagnostics and specific prophylaxis of plague

Method	Remarks
Microscopic	
Cultural	
Molecular-genetic	
Biological	
Specific prophylaxis	

F. tularensis characteristics

Characteristics	*Francisella tularensis*
Morphology (size, shape, relative positions of cells)	
Spores development	
Capsule	
Flagella (motility)	
Gram staining	
Pathogenicity factors	

F. tularensis pathogenicity factors

Pathogenicity factors	Biological effects
Intracellular parasitism	Inhibition of phagocytes lysosomal function,
Capsule	Protection from phagocytosis
Endotoxin	Less active than other Gram-negative rods endotoxin (e.g., E. coli)

Microbiological diagnostics and specific prophylaxis of tularemia

Method	Remarks
Microscopic	
Cultural	
Serological	
Molecular-genetic	
Allergic	
Biological	
Specific prophylaxis	

Brucellosis agents characteristics

Characteristics	*Brucella spp.*
Morphology (size, shape, relative positions of cells)	
Spores development	
Capsule	
Flagella (motility)	
Gram staining	
Pathogenicity factors	

***Brucella* pathogenicity factors**

Pathogenicity factors	Biological effects
Endotoxin	Systemic toxic effect
Hyaluronidase	Breaks down hyaluronic acid
Outer Membrane Proteins	Adhesion

Microbiological diagnostics and prophylaxis of brucellosis

Method	Remarks
Microscopic	
Cultural	
Serological	
Allergic	
Molecular-genetic	
Biological	
Specific prophylaxis	

Anthracs pathogen characteristics

Characteristics	*B. anthracis*
Morphology (size, shape, relative positions of cells)	
Spores development	
Capsule	
Flagella (motility)	
Gram staining	
Pathogenicity factors	

***Bacillus anthracis* pathogenicity factors**

Pathogenicity factors	Biological effects
Protein exotoxin (synthesis is controlled plasmid)	Exotoxin contains three factors: **lethal factor** - the cytotoxic effect, pulmonary edema, **protective Ag** - interacts with cell membranes mediates the activity of others. components, edematous factor - the increase in the concentration of cAMP, the development of edema
Capsule	Antiphagocytic activity

Microbiological diagnostics and specific prophylaxis of anthrax

Method	Remarks
Microscopic	
Cultural	
Serological	
Allergic	
Molecular-genetic	
Biological	
Specific prophylaxis	

Class № 8 Microbiological diagmostics0f diseases caused by spirochetes **Date_____**

List of questions to study:

Spirochetes, classification, general characteristics.

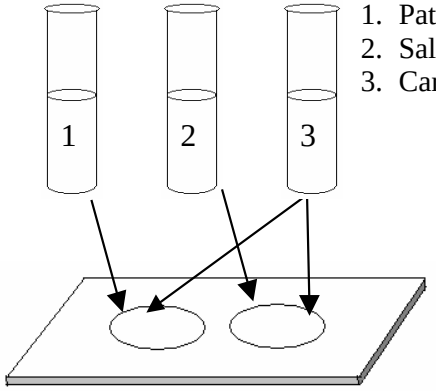
Treponema. Systematics and general characteristics. Pathogenesis and immunity in syphilis. Material for the study. Methods of microbiological diagnosis of syphilis. Principles of therapy and prevention of syphilis.

Fusospirochetosis pathogens

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

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

Laboratory work

Tasks	Methods, results
1. Perform the slide microprecipitation reaction (VDRL) for the syphilis serodiagnosis. 2. Assess ELISA (Wasserman test) for the syphilis diagnostics.	**Slide microprecipitation test**  1. Patient serum 1:20 2. Saline sol. 3. Cardiolipin Ag Conclusion:_____

Demonstration.
1. Leptospires, dark field microscopy.
2. Borrelia in blood, Romanovsky-Giemsa staining.
3. Wasserman test (ELISA).
4. Treponema in dental plaque, Gram staining.
5. Treponema pallidum, pure culture, Romanovsky-Giemsa staining.

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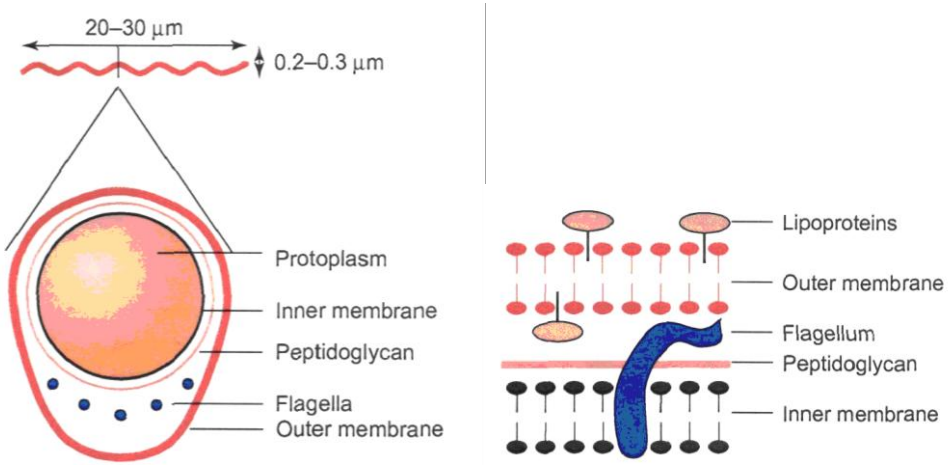
Stain_____ Stain_____ Stain_____ Stain_____

Signature of the tutor_____

Additional materials for independent study for class № 7.

Spirochetes structure



Pathogenic spirochetes characteristics

Features		Spirochetes genera		
		Treponema	*Borrelia*	*Leptospira*
Size	Length	5-20	3-20	7-14
	Width	0,09-0,5	0,2-0,5	0,1-0,15
Helixes number		8-12	2-8	12-24
Helix shape		Uniform, correct,	Uneven, different size	Uniform, correct secondary curls
Romanovsky-Giemsa staining		Pink	Blue Purple	Pink, Red

Diseases caused by treponema

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

Methods for spirochetosis diagnostics

Methods	**Method usage (+/-)**				
	Syphilis	Epidemic relapsing fever	Endemic relapsing fever	Lyme disease	Lepto-spirosis
Microscopic					
Cultural					
Serological					
Allergic					
Molecular-genetic					
Biological					

Pathogenesis of syphilis

Disease stage	**Period**	**Main pathogenetic mechanisms**
Primary		
Secondary		
Tertiary		

Serological diagnosis of syphilis:

CFT (Wasserman) with treponemal and cardiolipin antigens in primary syphilis becomes positive in the 6th week of the disease in 25-50% of patients, in 7-8 weeks - 75-90%. In secondary syphilis it is positive in 98-100% cases. In tertiary syphilis CFT is positive in only 60-70% patients. CFT for syphilis diagnostics has unsatisfactory sensitivity and specificity and is replaced now by ELISA.

ELISA is the common used technics for syphilis diagnostics.

Confirmatory tests:

– treponema immobilization test is rather specific, but time and labor consuming, subjective, requires treponema culture;

– immunofluorescence (IF) with serum from patients.

Screening tests: slide microprecipitation test, ELISA

Laboratory diagnosis of Lyme disease (Lyme borreliosis):

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

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

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

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

Class № 8. **Microbiological diagnostics of diseases caused by Rickettsia, Chlamydia and Mycoplasma** **Date**_____

List of questions to study:

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

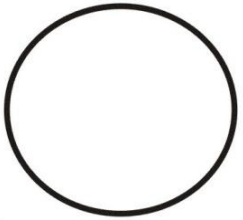
Chlamydia, general characteristics, role in human pathology. Pathogens of psittacosis, trachoma, respiratory and urogenital chlamydiosis. Methods of microbiological diagnosis of chlamydiosis. PCR in chlamydiosis diagnostics.

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

Laboratory work

Tasks

1. Perform CFT for the typhus diagnostics



Smear _____

_____

Stain_____

Methods, results

Reagents	1	2	3	4	5	6	7	SC	DC	Hemolytic system
	1:5	1:10	1:20	1:40	1:80	1:160	1:320			
Saline sol.	-	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	4 ml 3% erythrocytes suspension + 4 ml hemolytic serum
Serum of the patient	0,5	0,5	-	-	-	-	-	0,5	-	
Diagnosticum	0,5	0,5	0,5	0,5	0,5	0,5	0,5	-	0,5	
Complement	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	
Incubation 30 minut at 37° C										
Hemolytic system										
Incubation for 30′ at 37 °C										
Assessment										
Conclusion:										

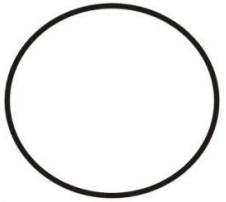
0,5

Demonstration.

1. Passive blood aggl;utination test for differential diagnostics of epidemic and residual typhus
2. Chlamydia spp. in cell culture, Romanovsky-Giemsa staining.
3. R. prowazeki, pure culture, Zdrodovski staining.

1/10 1/20 1/40 1/80 1/160 1/320 1/640 SC DC

Conclusion:_____



Smear _____

_____

Stain_____

Signature of the tutor_____

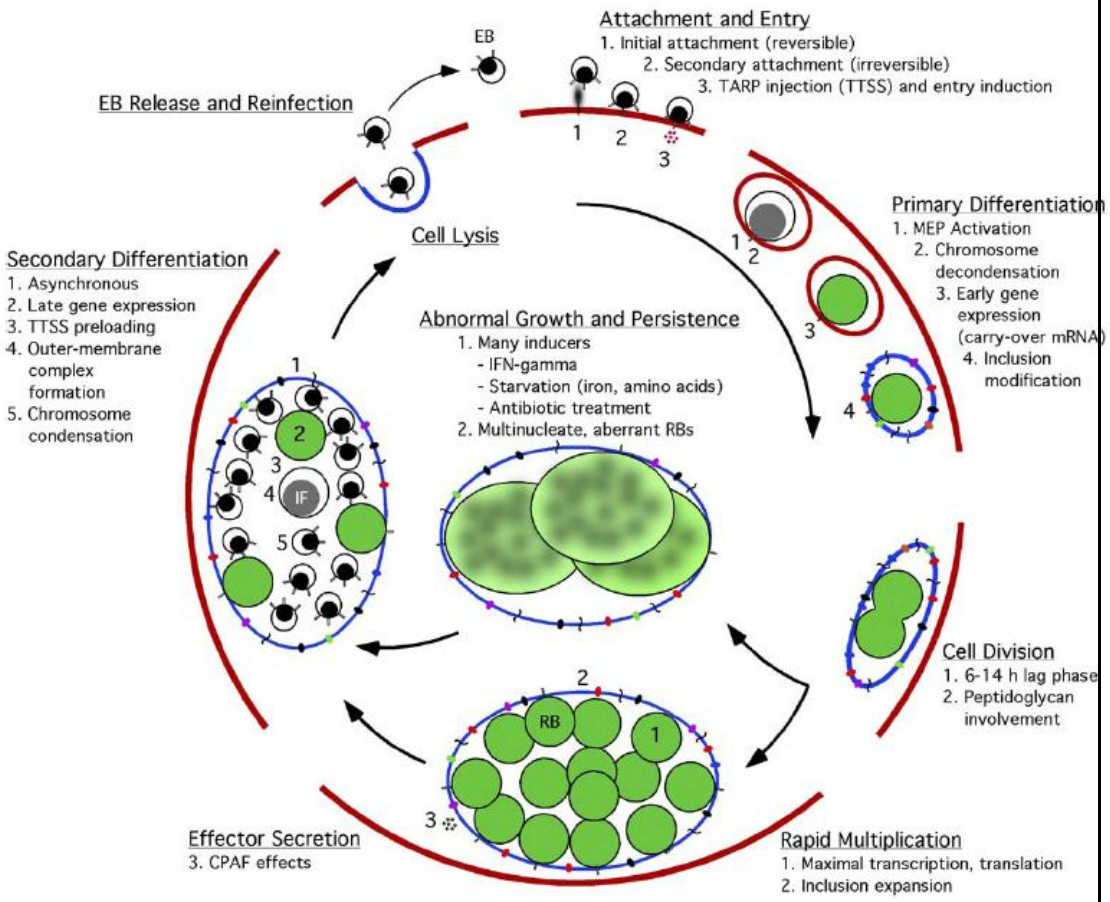
Additional materials for independent work wor class № 8

Actual classification of Rickettsia:

On the basis of a molecular genetic studies (genome sequencing, PCR) classification of microorganisms belonging to the order Rickettsiales has undergone significant changes. The genus Coxiella with C.burnetti was excluded from the family and added in the order Legionellales, family Coxiellaceae. Genus Rochalimaea was removed, and its representatives - R. quintana (Trench fever) and R. henselae (cat scratch disease) were included in the family Bartonellaceae, genus Bartonella.
The family Rickettsiaceae now include three genera: Rickettsia, Orientia, Wolbachia. The medical importance of the latter genus is still unclear.

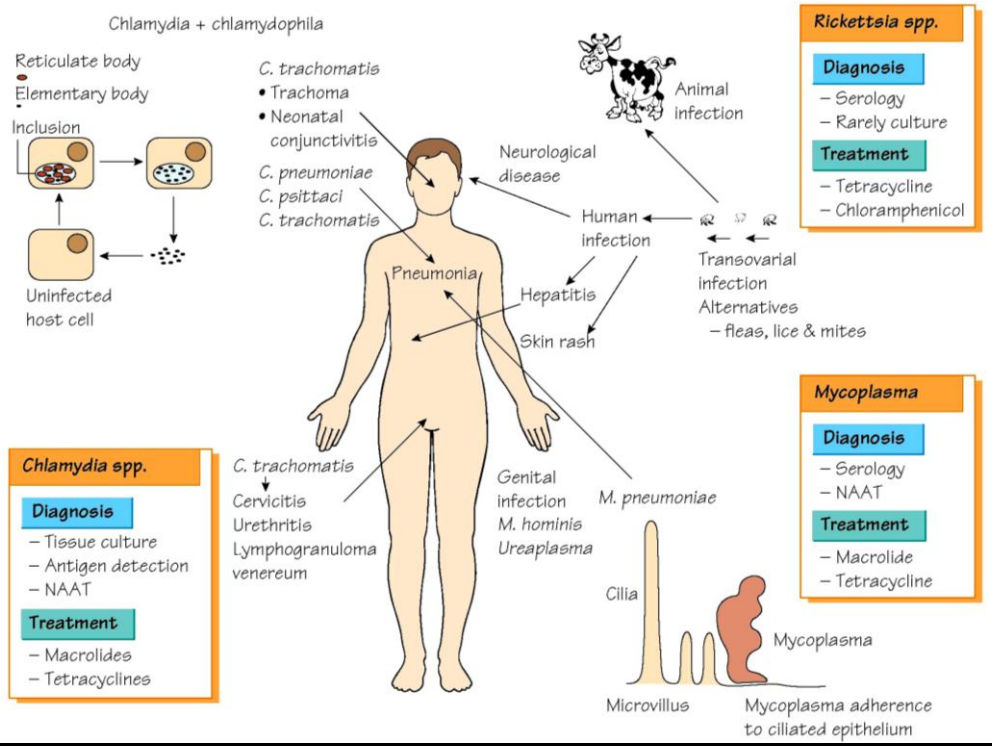
- Order ***RICKETTSIALES***
  - Family ***RICKETTSIACEAE***
    - Genus ***ORIENTIA***
      - *O. tsutsugamushi*
    - Genus ***RICKETTSIA***
      - Typhus group, spp.
        - *R. prowazekii*
        - *R. typhi*
        - *R. felis*
      - Tick rickettsiosis group, spp.
        - *R. rickettsii*
        - *R. conorii*
        - *R. sibirica*
        - *R. akarii*
        - *R. japonica*
        - *R. australis*
        - *R. honei*

The scheme of intracellular Chlamydia cycle



Laboratory diagnostics of diseases caused by Rickettsia, Chlamydia and Mycoplasma

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



Chlamydiosis characteristics

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

Mycoplasma and mycoplasmosis characteristics

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

Class № 9 **Concluding test "Special microbiology"** **Date**_____

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

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

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

7. Determine the morphology vibrio, pure culture, Gram stain.
8. Determine the morphology of Brucella, a pure culture, Gram stain.
9. Determine the morphology corynebacteria, pure culture, Leffler stain.
10. Determine the morphology of Klebsiella, pure culture, Hins-Burri stain.
11. Determine the morphology of mycobacteria in sputum, Ziehl-Neelsen stain.
12. Determine the biochemical properties of enterobacteria on Kligler iron agar medium.

Class № 10. **Clinical microbiology. Microbiological diagnostics of sepsis and purulent infections of the skin** **Date_____**

List of questions to study:

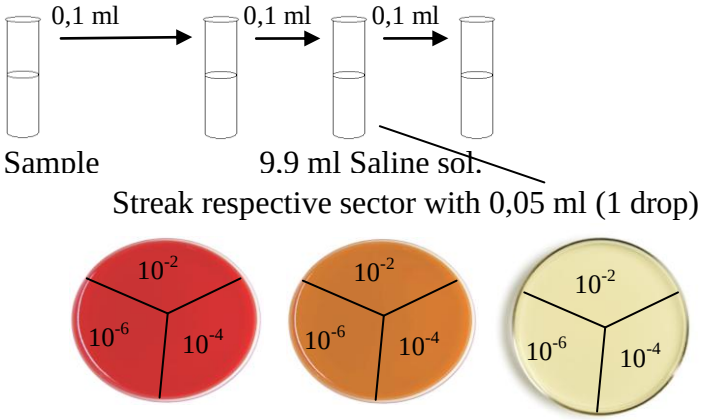
Clinical Microbiology: definition, objectives. Opportunistic microbes (OPM). Epidemiology, pathogenesis, diagnosis of diseases caused by UPM. Criteria of etiological significance.

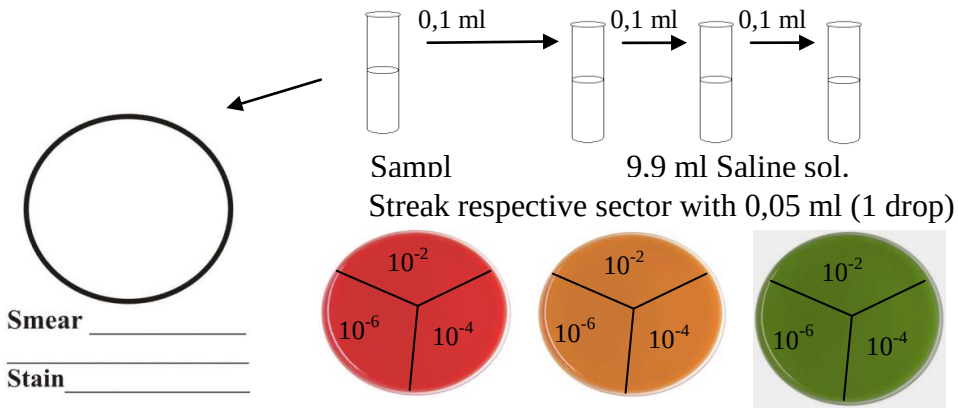
Clinical forms and the etiology of septic infections of the skin and subcutaneous tissue. Methods of microbiological diagnostics.

Bacteriological method. Material for the research (pus, exudate), rules and methods of sampling. Criteria for assessment of the etiological significance of isolated microorganisms. Susceptibility to antibiotics.

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

Laboratory work

Tasks	Methods, results
1. Independent work "Research of the sample from the burnt wound". a) Make serial dilution of the sample 1:100; 1:10000; 1:1000000) b) Streak respective sectors on nutrient media 2. Research of the blood sample from the patient with suspected sepsis. Smear _____ _____ Stain_____	**Exsudate sample research (Ist step)**  **Blood sample research (Ist step)**  Blood, 10 ml Biphasic (enrichment) media, 37 °C

3. Research of the sample from the bronchi washings: a) Prepare the slide from the material, Gram staining; b) perform quantitative seeding of the material on selective media.	**Bronchi washings sample research (Ist step)** 

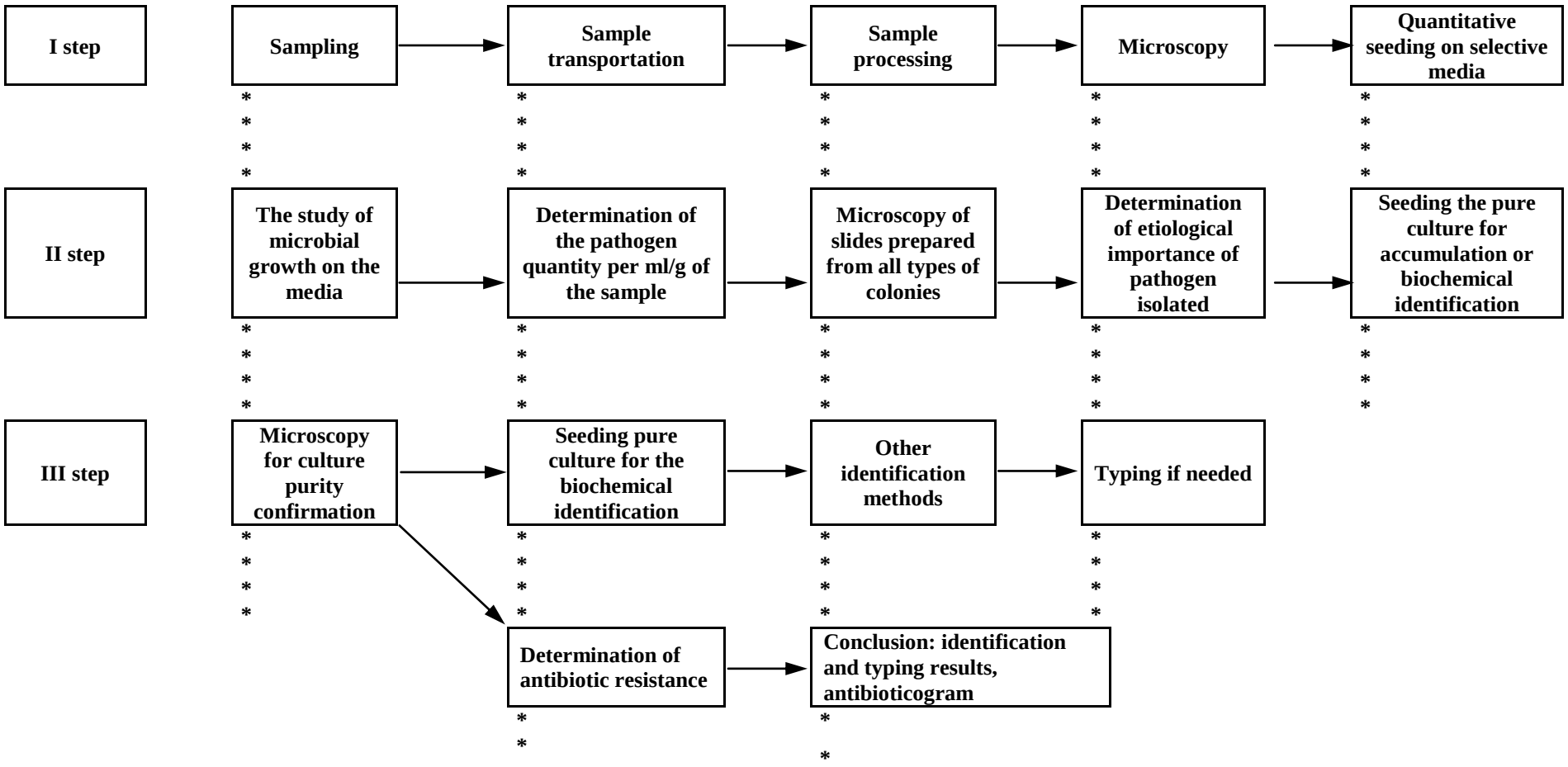
Signature of the tutor_____

Additional materials for independent work for class № 10.

Criteria of etiological importance off opportunistic pathogens
1.
2.
3.
4.
5.
6.
7.
8.
9.
10.

Etiology (main pathogens) of purulent infection of the skin
1.
2.
3.
4.
5.

The scheme of the microbiological diagnostics of purulent-septic infections



Class № 11. **Clinical microbiology. Microbiological diagnostics of purulent infections of urinary tract. Hospital-acquired infection.** **Date**_____

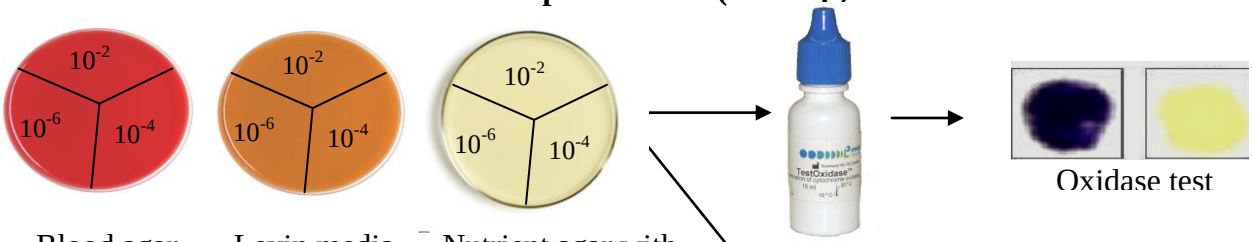
The list of questions to study:

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

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

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

Laboratory work

Tasks	Methods, results
1. Independent work: "Research of the sample from the burnt wound"	**Exsudate sample research (IIst step)**  Blood agar Levin media Nutrient agar with furaginum *p*-Phenylenediamine (PPD) Colonies characteristics: _____ _____ _____ Calculation of bacteria quality per ml/g of the sample: $N\ (CFU/ml) = n \times 20 \times 10^{x}$, n – colonies quantity in respective sector, 20 – conversion factor for 1 ml, 10^{x} – the degree of the sample dilution. **Smear** _____ _____ **Stain**_____ N = _____CFU/ml Conclusion_____

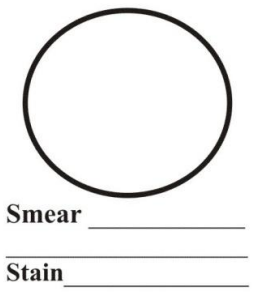
2. Research of the blood sample from the patient with suspected sepsis.

3. Research of the sample from the bronchi washings

Demonstration.

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

Research of the blood sample (II step)

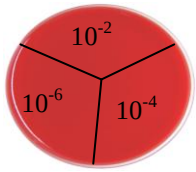


Blood culture

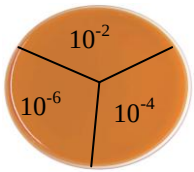
Blood agar

Yolk-salt agar

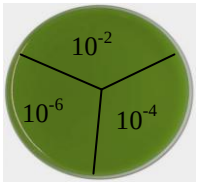
Bronchi washings sample research (II st step)



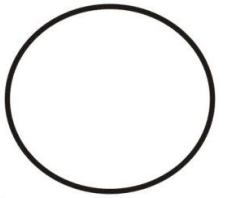
Blood agar



Levin media



Nutrient agar with lactose and bromothymol blue



Colonies characteristics:

_____

_____

_____

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

$N\ (CFU/ml) = n \times 20 \times 10^{x}$,

n – colonies quantity in respective sector,

20 – conversion factor for 1 ml,

10^{x} – the degree of the sample dilution.

N = _____CFU/ml

Russell medium

Conclusion _____

Signature of the tutor_____

Additional materials for independent work for the class № 10
Etiology (main pathogens) of respiratory septic-purulent diseases

1.
2.
3.
4.
5.

Etiology (main pathogens) of urogenital septic-purulent diseases

1.
2.
3.
4.
5.

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

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

Etiology (main pathogens) of nosocomial infections

1.
2.
3.
4.
5.

CLASSIFICATION of HAI

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

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

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

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

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

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

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

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

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

Class № 12. **Microbiological diagnostics of fungal and protozoan imfections** **Date**_____

The list of questions to study:

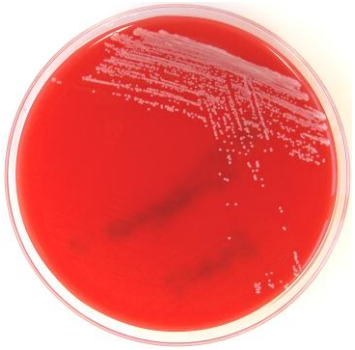
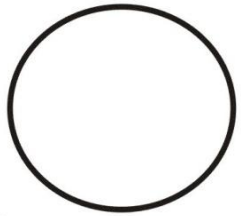
General characteristics and classification of protozoa. Pathogenic representatives. Laboratory diagnosis of malaria, toxoplasmosis, amebiasis, giardiasis, trichomoniasis.

The causative agent of cryptosporidiosis.

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

Pathogen of pneumocystosis.

Laboratory work

Tasks	Methods, results
1. Research of the blood sample from the patient with suspected sepsis. 2. Research of the sample from the bronchi washings	**Blood sample research (III step)**     Blood agar YSA **Smear** _____ _____ **Stain**_____ **Coagulase test** Stabilized rabbit plasm: 37 °C – 2, 4, 24 h Colonies characteristics: _____ _____ _____ **Conclusion:** _____

<table>
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Research of bronchial washings sample (III syep)
Russell medium →
Fermentation:
Lactose _____
Glucose _____
Smear _____
_____
Stain_____
Conclusion _____
</td>
</tr>
<tr>
<td>Demonstration.
1. Pathogenic protozoa.
2. Candida, Gram stain.
3. Candida growth on Saburo medium</td>
<td>
Smear _____ _____ Stain_____
Smear _____ _____ Stain_____
Smear _____ _____ Stain_____
Smear _____ _____ Stain_____
Smear _____ _____ Stain_____
Smear _____ _____ Stain_____
Smear _____ _____ Stain_____
Smear _____ _____ Stain_____
Smear _____ _____ Stain_____
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Signature of the tutor_____

Additional materials for independent study for the class № 12.

DIAGNOSTICS OF MYCOSIS

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

Serological method:

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

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

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

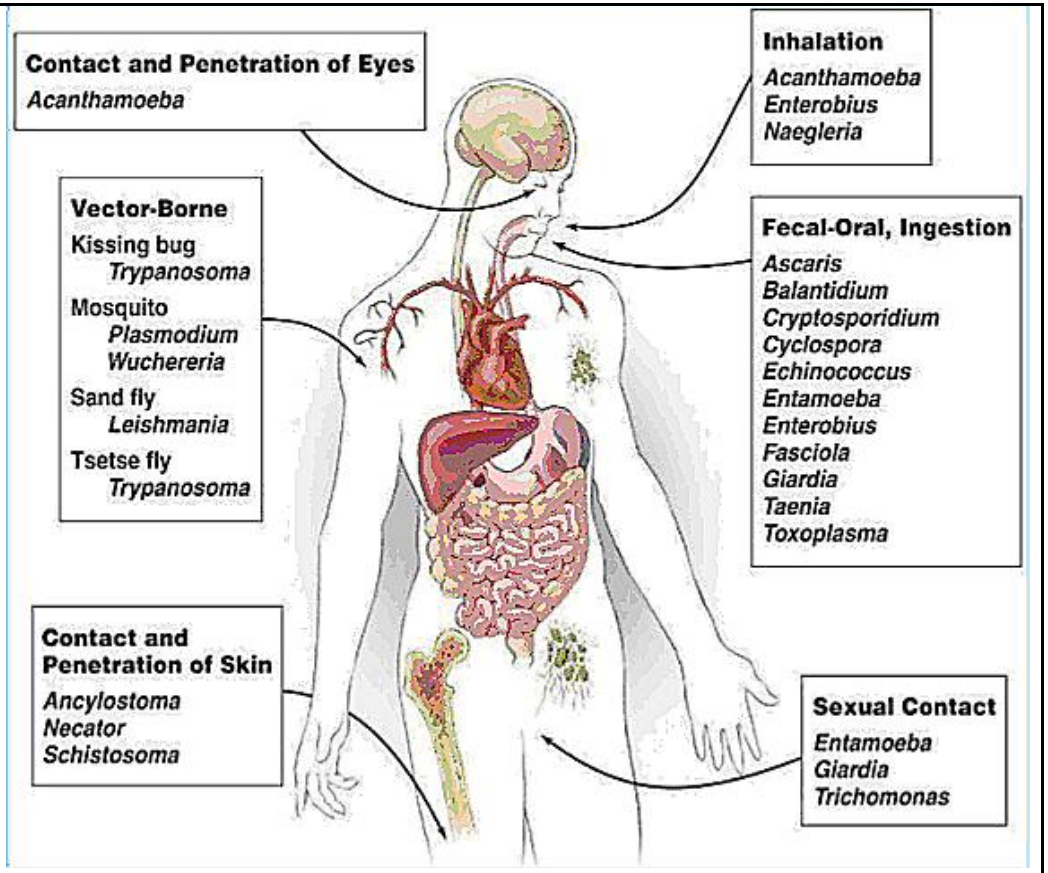
Allergic method. Skin tests are performed with fungal allergens (eg. Candide). Method is not very specific because of the group antigens presence.

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

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

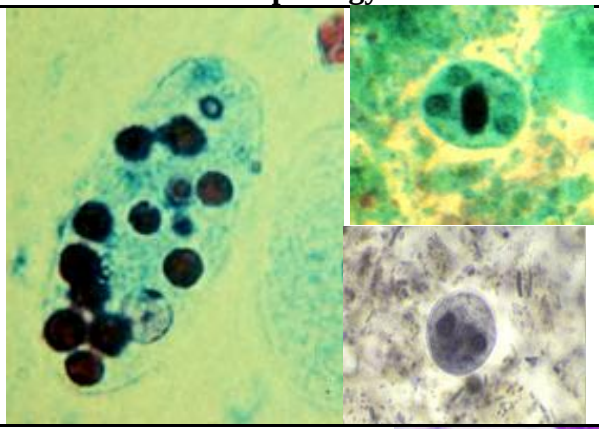
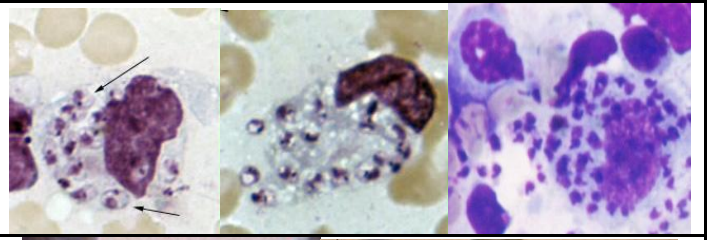
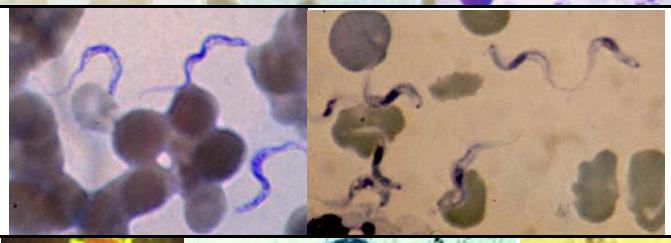
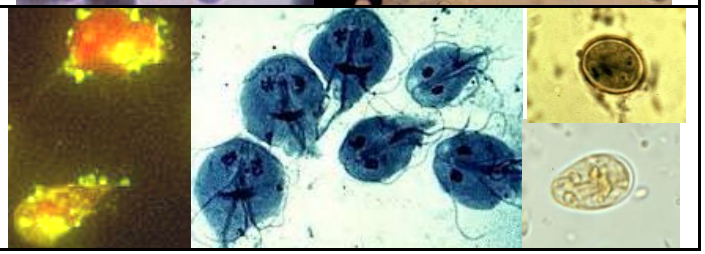
The comparison of eu- and prokaryotic cell

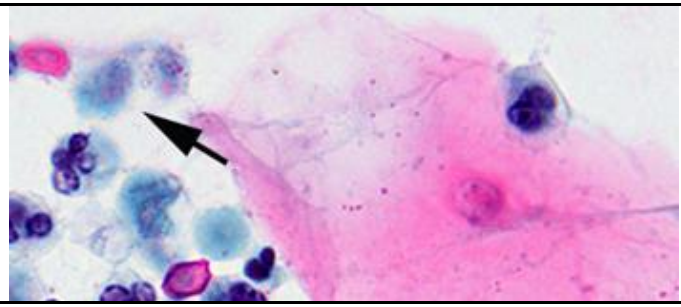
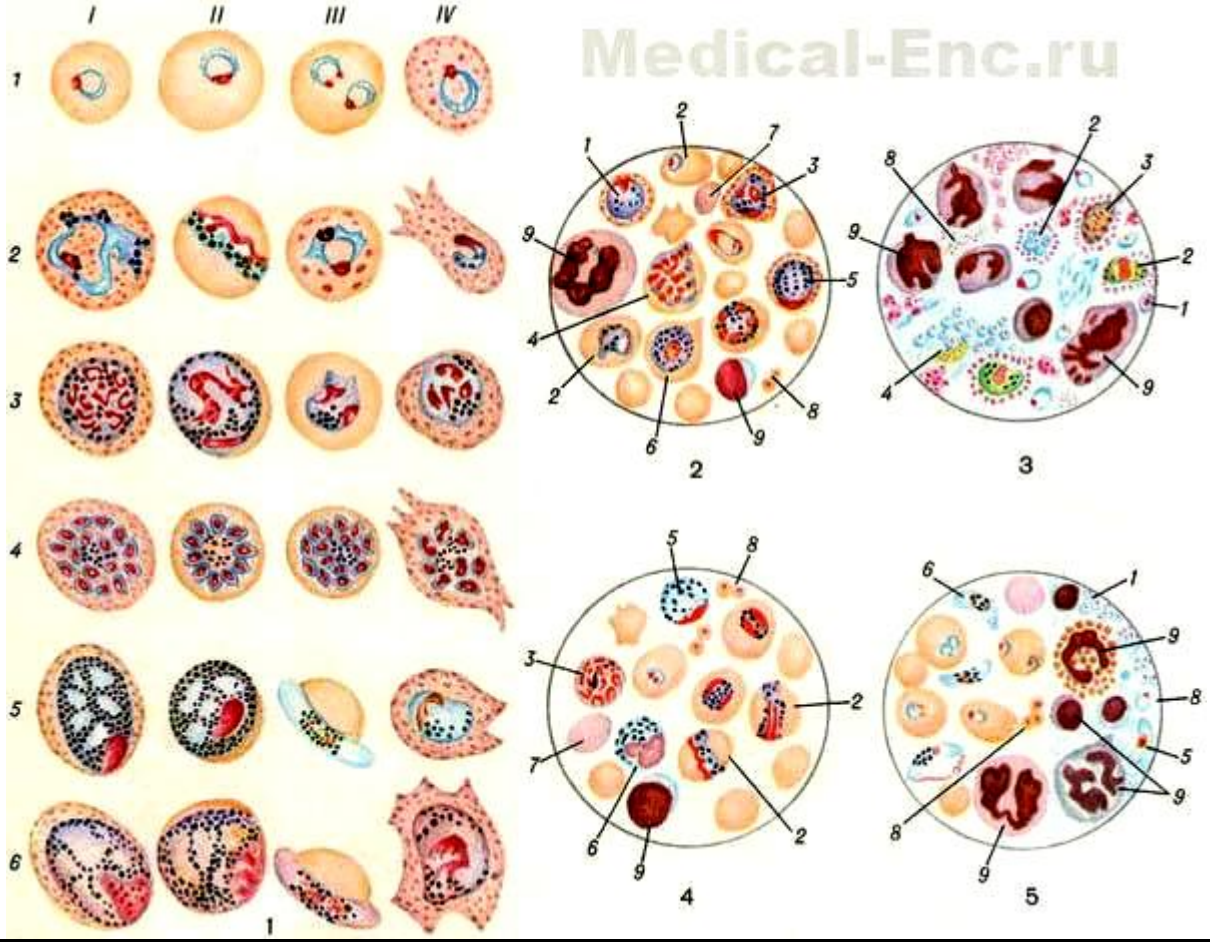
Main characteristics	Prokaryotic cell	Eukaryotic cell
Cell size	Average 0,2-2,0 mkm	
Nucleus	Does not have a true nucleus. Nucleoid, is not separated from the cytoplasm by a membrane	
Chromosomes	Ring-like	
Number of chromosomes per cell	Usually one	
Mitochondria	No	
Endoplasmatic reticulum	No	
Ribosomes location	Dispersed in cytoplasm	
Sedimentation constant	70S	
Teichoic asides in cell wall	Gram positive bacteria	
Peptidoglycane in cell wall	All bacteria with exception of mycoplasm	
Endospores	Some has	
Division of cell	Binary (mitosis)	
Gametes, zygotes	No	

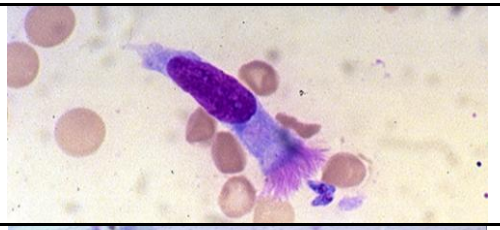
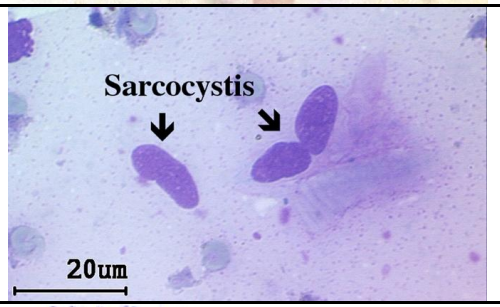
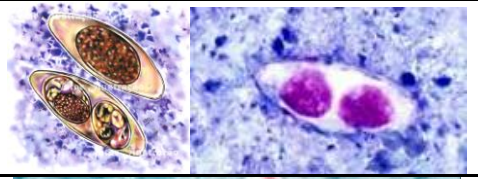
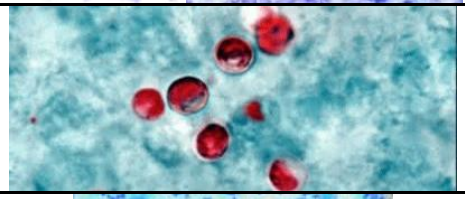
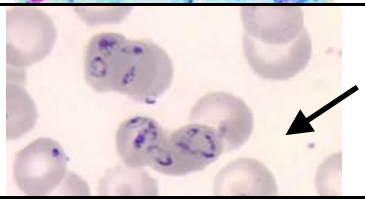


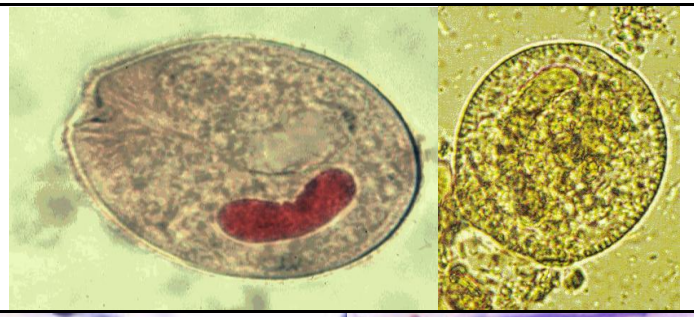
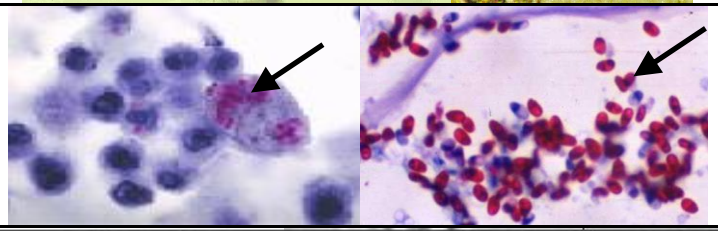
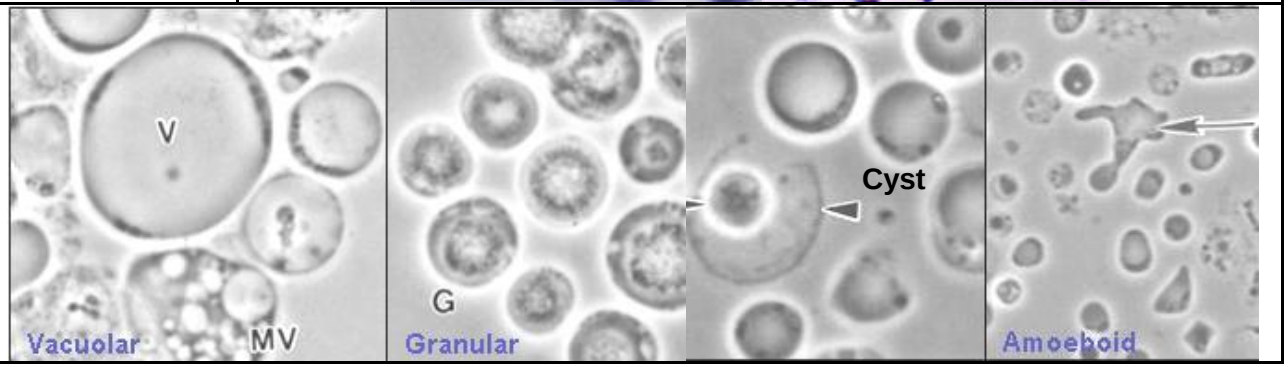
Main routs of pathogenic parasites invasion

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

Taxons	Representatives	Disease	Morphology
TYPE *SARCOMASTIGOPHORA* subtype *Sarcodina*	**AMOEBAE** *Entamoeba histolytica*	Amebiasis	
	Naegleria, acanthamoeba, hartmanella	Amoebic meningoencephalitis, keratitis	
subtype *Mastigophora*	**LEISHMANIA** *Leishmania species*	Leishmaniasis	
	TRYPANOSOMES *Tripanosoma gambiense, Tripanosoma rodesiense Tripanosoma cruzi*	African trypanosomiasis (sleeping disease) Chagas disease (American trypanosomiasis)	
	GIARDIA: *Lamblia intestinalis (Giardia lamblia)*	Diarrhea, malabsorption syndrome	

	Trichomonas *Trichomonas vaginalis*	Trichomonas vaginalis vaginitis, urethritis, prostatitis	
TYPE – *APICOMPLEXA* class – *Sporozoa*	**PLASMODIUM MALARIA:** *Plasmodium vivax* *Plasmodium ovale* *Plasmodium malariae* *Plasmodium falciparum*		

	<u>TOXOPLASMA:</u> *Toxoplasma gondii*	Toxoplasmosis	
	<u>SARCOCYST:</u> *Sarcocystis species*	Sarcocystosis	
	<u>ISOSPORA:</u> *Isospora species*	Diarrhea	
	<u>CRYPTOSPORIDIUM:</u> *Cryptospodium species*	Diarrhea	
	<u>CYCLOSPORA:</u> *Cyclospora cauetanensis*	Diarrhea	
	<u>BABESIA:</u> *Babesia species*	Babesiosis	

TYPE – CILIOPHORA class *Kinetofragminophorea*	**BALANTIDIUM:** *Balantidium coli*	Balantidiasis	
TYPE – MICROSPORA class *Microsporea*	**MICROSPORIDIA:** *Encephalitozoon species* *Enterocytozoon species*	Microsporidiasis	
	BLASTOCYST: *Blastocystis hominis*		

MICROBIOLOGICAL DIAGNOSTICS OF PROTOZOAN INVASIONS

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