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 V

Specialties:

221 "Dentistry"

222 "Medicine"

225 "Medical Psychology"

226 "Pharmacy, industrial pharmacy"

228 "Pediatrics"

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Class №34

Topic: «Causative agents of brucellosis, plague, tularemia and anthrax. Microbiological diagnosis of zoonose infections»

Relevance of the topic:

Zoonose infection – is a disease in which pathogens are transmitted from animals (sik animals or carriers) to man. In zoonose pathogens organ tropism missing. Epidemiology of zoonoses is characterized by a variety of mechanisms, ways and factors of transmission. Causative agents of zoonoses - is polipathogenic microorganisms, they can infected many different species of animals. For example, the causative agent of plague - about 250, tularemia - about 50 species. It provides high stability of natural sources and makes them not demolished. Most zoonoses are diseases with natural sources.

For the diagnosis of zoonoses widely used methods for rapid diagnosis (IF test, ELISA, PCR, phago-diagnosis et al.), skin-allergic tests with appropriate diagnostic allergens. At present time the treatment of most zoonoses is quite effective (in timely diagnosis), as the causative agents of bacterial zoonoses are sensitive to antibiotics. Specific prophylaxis held on epidemic parameters through immunization by vaccines. Specific prevention is directed to the sanitary protection area, to prevent spread into or beyond their natural sources, and to conduct health and veterinary measures

Causative agents of zoonose infections belong to different families and genera: the causative agent of plague – is *Yersinia pestis*, *Francisella tularensis* – is the causative agents of tularemia, brucellas (*Brucella melitensis, Brucella abortus, Brucella suis*) – are causative agents of brucellosis in humans, *Bacillus anthracis* – is causative agents of anthrax, and others. These types of bacteria cause, as usually, infectious disease, which are characterised by sudden onset, rapid spread, massive coverage, heavy flow, high mortality. Infections diseases with these characteristics belong to especially dangerous infections (EDI), plague, besides, belong to quarantine infections. According the high biological threat causative agents of zoonoses, especially plague, anthrax, tularemia, and others., they are regarded as potential agents for use as biological weapons or use for bioterrorism.

In this regard, microbiological studies of these infections is carried out in special regime laboratories, with trained personnel in accordance with strict observance of anti-epidemic regime.

Specific objectives:

- To study the morphological and cultural properties of causative agents of plague and tularemia.
- To analyse the schemes of microbiological diagnostics of plague and tularemia.
- Examine the medicine that are used for diagnosis, specific prophylaxis and therapy of plague and tularemia.

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

The names of the previous disciplines	Skills
Human anatomy	Analyze information about the structure of the human body, systems, of which it consists, organs and tissues.
Histology, cytology, embryology	Interpret the microscopic and submicroscopic structure of cells.
Medical and biological physics	Treat common physical and biophysical principles that based biological processes.
Medical biology	Explain at the molecular and cellular level patterns of biological processes.
Medical chemistry	Treat common physical and chemical principles that based the processes of cells develoupment.

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

Terms	Definitions
Antroponosis infection	Antroponosis infection - group infectious parasitic diseases in humans in which the source and reservoir of infection is only human.
Zoonosis infection	Zoonosis infection - group infectious parasitic diseases in humans in which the source and reservoir of infection is infected animals (ills or carriers).
Sapronosis infection	Sapronosis infection - group infectious parasitic diseases in humans in which the source of infection are the objects of the environment. Sapronosis - a disease, which pathogens are not only vertebral host, but also a place of development, and reservoir inanimate origin (organic matter, including food, soil, plants).
Quarantine infection	Quarantine infection infection (convention) - an infection, system information and prevention measures which are conditioned by international agreements (convention). If cases of apparense quarantine infection in any location of world come into force, according the International Health Regulations, the following system: 1) country sends information about the occurrence in the WHO, 2) WHO processes data and sends them to all the countries of the world, 3) countries receiving information, make decisions according specific anti epidemice measures and inform the WHO about its 4) WHO processes data and sends them all over the world. The main channel of communication is a weekly epidemiological bulletin «Weekly epidemiological Record» (WER) and automatic telex accumulation and transfer of information.

Epidemic process	The epidemic process is the process of emergence and spread of specific infectious states among the human population - from carriage (without symptome) to manifest diseases that are caused by pathogens circulating among the human population.
Sporadic sick rate	Sporadic sick rate – is ordinary rate of sick with definite nosology form, into definite region, in the definite historycal term of time.
Epidemy	This sick rate with certain nosology forms in a particular area at a particular time, which greatly exceeds the level of sporadic disease.
Pandemy	This sick rate with certain nosology forms in a particular area at a particular time, which greatly exceeds the level of ordinary epidemy.

Theoretical questions to studies.

- The concept of zoonosis infections.
- Epidemiology and pathogenesis of these diseases.
- Factors of virulence of causative agents of zoonosis infections.
- Peculiarities of laboratory diagnosis of plague, tularemia, brucellosis, anthrax.
- Morphological, tynktorial and cultural peculiarities causative agents of plague, tularemia, brucellosis, anthrax.
- Medicines which are used for diagnosis, specific prophylaxis and treatment of zoonosis infections.
- Preventive measures to prevent zoonosis infections.

Practical tasks are performed in class.

- Define the terms antroponosis infectio, zoonosis infection, sapronosis infection, epidemy, pandemy, epidemic process.
- To study methods of microbiological diagnosis of plague, tularemia, brucellosis, anthrax and familiarised with morphological, tynktorial and cultural peculiarities of causative agents of these diseases.
- To read the resalts of Wright's reaction, and to carry out agglutination test on glass (Haddlson test).
- Familiarised with medicine that are used for diagnosis, specific prophylaxis and treatment of zoonosis infections.

Contents subject.

In practice, students learn the demonstration preparations with pure cultures of causative agents of plague, tularemia, brucellosis, anthrax, and drug punctate with tambourine from a patient with plague; studying nutrient medium which are used for cultivation of these pathogens and analyze the particular nature of the growth of microorganisms on solid and liquid nutrient media, that take into account for bacteriological methods of diagnosis. Students analyze methods of microbiological diagnosis of plague, tularemia, brucellosis and anthrax. Students read the results of Wright test, and carry out the agglutination test on glass (Haddlson test).

They study drugs that are used for diagnosis, specific prevention and treatment of antropozoonosis infections. Completed tasks students write in the protocol and sign it with a teacher.

Recommendations for design of the protocol.

Methods of plague diagnosis

- ***Express-diagnostics*** (RIF, indirect Haemagglutination test).
- ***Bacterioscopic method*** (smear microscopy, stained with methylene blue and Gram stain).
- ***Bacteriological method*** (inoculation on MPA and MPB media with growth stimulants, incubation at 28ºC, the isolation of pure culture and its identification). The first cases of diseases must be confirmed bacteriologically. This method is the most demonstrative.
- ***Biological method*** (inject material in the peritoneum, or rubbing the skin in guinea pigs, the isolation of pure culture and its identification). This method uses for isolation of pure culture from material contaminated by extraneous microflora.
- ***Serological tests*** are appropriate to use for retrospective diagnosis of plague, and to evaluate the effectiveness of vaccination.

Methods of tularemia diagnosis

- ***Serological diagnosis*** (agglutination test, indirect Haemagglutination test).
- ***Allergological diagnosis*** (allergic skin tests with allergen tulyaryn).
  The first two methods are the main methods diagnosis of tularemia.
- ***Bacterioscopic method*** (microscopy of smers stained by Gram method) is used to detect the causative agent in the organs of laboratory animals.
- ***Bacteriological methods*** (inoculation into special nutriant media, isolation of pure culture and its identification). The method is ineffective because it is difficult to isolate culture. The method is ineffective because of low concentration causative agent in the patient's body.
- ***Biological method*** (infection under the skin of white mice and guinea pigs). Used for diagnostics "bubonic form of" tularemia.

Methods of diagnosis of brucellosis

- ***Bacteriological methods*** (inoculation into special media, isolation of pure culture and its identification). This is most informative method compared with others, but long-term (5-6 weeks). Allows you to determine the type of brucella.
- ***Serological diagnosis*** (Wright's reaction in test tubes, and agglutination test on glass (Haddlson test)).
- ***Allergological method*** (allergic internally skin tests with allergen brutselin - Byurne test).
- ***Biological method*** (infection under the skin of guinea pigs or white mice, isolation of pure culture and its identification). Use to isolate pure culture of bacteria from spesimencel contaminated by extraneous microflora, or contains a low concentration brucella.

Methods of anthrax diagnosis

- ***Bacterioscopic method*** (smear microscopy, stained by Gram and by Orzeszkow).
- ***Bacteriological methods*** (inoculation into MPA, blood agar and MPB, isolation of pure culture and its identification).
- ***Allergological method*** (internally allergic skin tests with allergen antraksyn).
- ***Express-diagnostics*** (reaction termopretcipitation by Ascoli, RIF).
- ***Biological method*** (infection under the skin of white mice, guinea pigs or rabbits, for detection causative agent in the organs of animals).

Schematical representation agglutination test on tubes (Wright test).

Components, ml	**Tubes**						
	Test tubes					**Serum control**	**Diagnosticum control**
	1	2	3	4	5	6	7
Isotonic solution of NaCl	-	0,5	0,5	0,5	0,5	0,5	0,5
Serum in titer 1:25	0,5	0,5→	0,5→	0,5→	0,5↑	0,5	-
Diagnosticum	0,5	0,5	0,5	0,5	0,5	-	0,5
Dilution of serum	1:50	1:100	1:200	1:400	1:800		

- **Termostate 37ºC 18-24 hour**

- **Note:** *in the first and second tube add 0.5 ml of serum, mixed. 0.5 ml of the mixture is transferred from the second test tube into the next (third) test tubes, continuing dilution to the fifth tube, from which 0.5 ml of the mixture is removed.*

Differential characteristics of brucella.

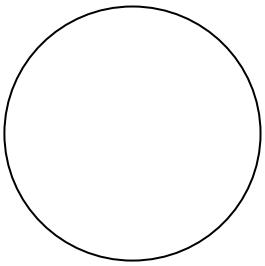
Spesies	**Necessity in CO_2**	**H_2S production**	**Growth intomedia with**		**Sencitive to T6 phage**
			basic fuchsin (1:25000)	tionin (1:50000)	
B.melitensis	-	-	+	+	-
B.abartus	+	+	+	-	+
B.suis	-	+	-	+	-

Schematical representation agglutination test on glass (Haddlson test).

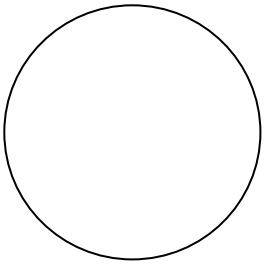
Components, ml	**Square**					
	Test				**Serum control**	**Diagnosticum control**
	1	2	3	4	5	6
Serum (not dilution)	0,08	0,04	0,02	0,01	0,02	-
Diagnosticum	0,03	0,03	0,03	0,03	-	0,03
Isotonic solution of NaCl	-	-	-	-	0,03	0,03
Dilution of serum according Wright test	1:50	1:100	1:200	1:400		

1	2	3
4	5	6

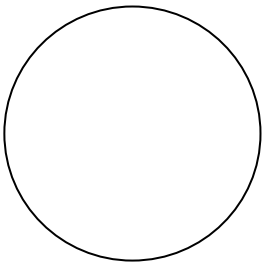
Practical activity №1. To study morphological and tinctorial properties of *Y. pestis, F. tularensis, Brucella melitensis* and *Bacillus anthracis.*



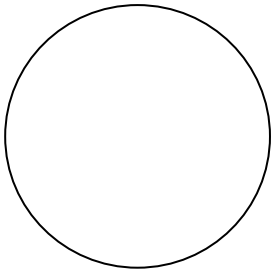
Yersinia pestis
(staining by Gram)



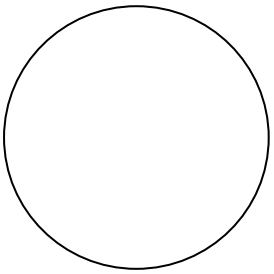
Francisella tularensis
(staining by Gram)



Punctate of bubones
(***staining by Gram)***



Brucella melitensis
(staining by Gram)



Bacillus anthracis
(staining by Gram)

Questions for self-control.

- What is quarantine the infection?
- What are the features of pathogenesis, clinical manifestations of plague, tularemia, brucellosis, anthrax?
- Describe the morphological, tynktorial and cultural properties causative agents of plague, tularemia, brucellosis, anthrax.
- Describe the growth characteristics causative agents of plague, tularemia, brucellosis, anthrax in the solid and liquid nutrient media that are considered when bacteriological methods are used.
- What are the microbiological methods used for retrospective diagnosis of plague, and for evaluate the effectiveness of the vaccination?
- Can we use plague bacteriophage for the prevention and treatment?
- What are the main methods for diagnosis of tularemia?
- What disease can be diagnosing with using Wright test in tubes and Haddlson test on glass, what is the essence of these methods?

- What allergen for Byurne test is used?
- What disease can be diagnos with allergic test with using antraksyn?
- • In which cases are used termoprecipitation test Ascoli?
- What methods of staining can be used for bacterioscopic diagnosing of anthrax?
- What medicine are used for diagnosis, specific prophylaxis and treatment of zoonosis infections?

Class №35

Topic: "Rickettsia, Mycoplasma, Chlamydia. Microbiological diagnosis of rickettsiosis, mycoplasmosis, chlamydiosis"

Relevance of the topic.

Chlamydiosis, rickettsiosis, mycoplasmosis - a different infectious disease, where causative agents have certain biological properties (fierst of all - intracellular parasitism), may persiste long time in humans body, causing acute and chronic processes, clinical and asymptomatic infections and relapses.

Chlamydia, rickettsiae and mycoplasma bealong to prokaryotes, but have certain features that distinguish them from ordinary bacteria. For example, rickettsia and chlamydia in the evolutionary and biological aspects occupy an intermediate position between the bacteria and viruses. They are obligate intracellular parasites, like viruses, mycoplasmas have no cell wall. These microorganisms are difficult to grow in the laboratory. All this complicates the diagnosis, prevention and treatment of diseases that they cause.

In class students are given the possibility to familiarize with the classification, biological properties of rickettsiae, chlamydia, mycoplasma, to study the pathogenesis, diagnosis, prevention and treatment of diseases that they cause.

All this makes the relevance of topics and aimed at a positive motivation to learn.

Specific objectives:

- To study the classification and basic biological properties of rickettsiae, chlamydia and mycoplasma, familiarized with pathogenesis and treatment of diseases that they cause.
- To familiarized the scheme of microbiological diagnostics rickettsiosis, chlamydiosis and mycoplasmosis.
- To familiarized with morphological and tynktorial properties of rickettsia and chlamydia in the demonstration smearses.
- To learn methods of serological diagnosis of typhus fever and Q-fever.
- To familiarized with medicicne that are used for diagnosis, specific prophylaxis and treatment of rickettsiosis.

Basic knowledge, skills, needed to study topics (interdisciplinary integration). See a class №34.

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

Terms	Definitions
Rickettsia	*Rickettsia* - genus of parasitic bacteria of the family Rickettsiaceae, usually coccus shape with size 0,3-0,6 x 0,8-2 mm, but there are bacillary and filamentous cells. Cell wall built by the type of gram-negative bacteria. They able to reproduce only in the cytoplasm of

	sensitive cells. In laboratory conditions, *R.* cultivated in the yolk sac of chicken embryo, cell cultures (HeLa, HEp-2, Deytroyt-6), as well as in laboratory animals (mice). They are parasites of insects (louses, fleas, ticks). Some species are pathogenic for mammals and cause them acute infectious diseases - rickettsiosis.
Rickettsiosis	Rickettsiosis - a group of acute infectious vector-borne diseases where causative agents are bacteria of Rikcettsiaceae family. Depending on the biological properties of the causative agents, clinical symtoms and epidemiology the following groups of diseases are: 1. - Epidemic typhus (louse-borne; causative agent is *R.prowazekii*), - sporadic typhus (relapse of epidemic typhus, causative agen is *R.prowazekii*), - endemic typhus (flea-borne, causative agent is - *R.typhi*). 2. Fever tsutsugamushi (Scrab typhus), causative agent is *R.tsutsugamuchi*. 3. Spotted fever group: Rocky mountain spotted fever (causative agent is *R.rickettsii*), Mediterian spotted fever (causative agent *is R.conorii*), Siberian tick typhus (North Asian tick typhus), causative agent is *R.sibirica*, Queensland tick typhus (causative agent is *R.australis*), Rickettsial pox (causative agent is *R.akari*). 4. Q fever (causative agent is *Coxiella burnetti*). 5. Trench fever (causative agent is *R.guintana*). For epidemic typhus reservoir of infection is the man, for all other rickettsiosis - reservoir of infection are animals (rats, other rodents, dogs, cattle). Infection occurs through human bites or rubbing feces of infected insects. Only Q fever can be transmitted through objects in the environment.
Epidemic Typhus	Epidemic Typhus (Weyl reaction, louse-borne), - acute, especially dangerous infection, causative agent is *R.prowazekii*. The causative agent belongs to the genus *Rickettsia*, the order *Rickettsiales* and characterized by their inherent properties. They multiply in the cytoplasm of endothelium and mononuclear cells of human, and in gastric epithelium of human lice. In artificial conditions they multiply well in yolk sac and in culture chicken embryo fibroblasts, they also causes fever in guinea pigs with intraabdominal infection. In patients who were sick of typhus, there are relapses, called Brill's disease. Relapses can occur due to many years after the underlying disease. In practice for microbiological diagnosis using serological tests: Weil-Felix (diagnostic titre 1:200 and higher), agglutination of rickettsiae (diagnostic titer is 1:100 and above), indirect agglutination (diagnostic titer is 1:160 and above), RIF, CFT (diagnostic titer is 1:80 or higher). The most specific results are obtained with CFT. In addition to diagnostic titers also need to focus on increasing antibody titer.
Q Fever	Q Fever - an acute infectious disease of humans and animals caused by Coxiella burnetti, which belongs to the family *Rickettsiaceae*. A

	person infected by large and small cattle, rats and other animals with airborne and alimentary (usually via milk) ways of transmission. Among animal infection transmitted by ixod ticks. For human infection this way is not typical.
Weil-Felix reaction	Weil-Felix reaction – is agglutination test of patients serums with typhus. In this reaction diagnosticum is OX-19 *Proteus vulgaris*, which have common antigens with causative agent of typhus. Diagnostic titer of this reaction is 1:200 and higher. Weil-Felix reaction use for serological diagnosis of typhus, but its the sensitivity and specificity is lower then agglutination test with rickettsiae.
Mycoplasma	*Mycoplasma* – is a genus of small gram-negative aerobic or anaerobic microorganisms from the family *Mycoplasmataceae*, which have no rigid cell wall and do not forme the spores. They are polymorphic: spherical, elipsovydnoyi shape, size 150-350 nm and filamentous forms, up to several mkm. Tey multiply in complex nutrient media, the temperature of cultivation - 37^0C. For reproduction they need sterols and other growth factors. On solid media they form a small (10 to 500 mkm in diameter) round with elongated center colony. In the cell cultures proliferate on the surface of cells. They are resistant to penicillin, cephalosporins, but sensitive to tetracycline and erythromycin. They are parasitic on the mucous membranes of the urinary and respiratory tract. Mycoplasma infection - are human disease that caused by *M.pneumoniae*, *M.hominis* and others. *M.pneumoniae* causes acute and not bacterial interstitial and perybronhial pneumonia and bronchiolitis. Diagnosis consists from detection iof ncreasing antibody titer in CFT, RIF, direct haemagglutination test and the test of immune inhibition of mycoplasma. Causative agent can be isolate from the throat and bronchial washing water in the early stages of the disease. Growth on media can be for 5-6 days after material inoculation. Culture identified by morphology, the nature of growth on nutrient media and with serological tests. *M.hominis* – is causative agent of acute angina and respiratory infections (usually without fever), prostatitis, urethritis. *M. (Ureaplasma) urealyticum* (former name - *T-strains*) can be isolated from patients with prostate and nehonoreynyh urethritis. It forms very small colonies (about 40 mkm), needs to grow urine, can hydrolyzed urea
Chlamydia	*Chlamydia* -is a small likeness bakteria from the class of *Rickettsiae*. *Chlamydia* has such properties: nonmotile, asporogenic, without capsul, gram-negative, obligate intracellular parasites of mammals and birds. They are one family and one genus, which include 2 types: *Ch.trachomatis* – causative agent of trachoma, conjunctivitis with inclusions, venereal granuloma and *Ch.psittaci* – causative agent of psittacosis and ornithosis. Develoupment cycle of chlamydia can be

	subdivided into not less than 2 stages of the existence, which differ from one to another morphologicaly, properties, functions: Stage of infectious particle (elementary body; EB) –is a small cell, about 0.3 x0, 5 mkm, with rigid thick cell wall, compact nukleoid, cytoplasm contains many ribosomes.This stage is a stage of rest (metabolism sharply reduced), and it designed to move from one host to another; elementary bodies have infectious properties. Theyvpenetrate host cells by phagocytosis. In phagosom infected cells elementary bodies reorganized into vegetative stage (reproductive particle), called a "reticular body"(RB). It characterized by spherical shape, about 1,5-1mkm in diameter, thin membrane, has nukleoid fibrils and rybosomal elements. At the begenning reticular body grows, then divided into daughter cells, which are again divided. Particles of final separation reorganized into elementary bodies, which are located in the cytoplasm as mikrokoloniy surrounded by a common membrane (hlamyda). Infected cells at this stage are killed, destroyed, elementary bodies go outside and phagocyted by new cells. Both forms are Gram-negative. Elementary bodies stained in red, reticular bodies - in blue (according to Romanovsky-Himza method). *Chlamydia* metabolism looks like bacteria metabolism, except for the fact that chlamydia is not capable to forme macroergic compounds (ATP, NADP), it makes them energetically dependent on the host. All *Chlamydia* grow well in chicken embryos, especially in yolk sac, some strains - in cell culture. *Chlamydia* sensitive to disinfectants, especially to formalin, phenol, and also to tetracyclines, erythromycin, levomitcetin. *Ch.trachomatis* is sensitive to sulfamid, but *Ch.psittaci* is resistans to it. *Chlamydia* caused acute and chronic, clinical and asymptomatic infection.
Trachoma	Trachoma – is acute or chronic human keratoconjunctivitis, caused by *Chlamydia*, which ends up scarring and sometimes blindness. Causative agent of Trachoma is *Ch.trachomatis*, it has the same morphology and ontogeny, as other *Chlamydia*

Theoretical questions to studies.

- Taxonomic position and basic biological properties of rickettsiae, mycoplasma and chlamydia, which are drew together them to bacteria and viruses.
- Methods of cultivation rickettsia, chlamydia, mycoplasma.
- The role of rickettsia in human pathology. Pathogenesis and immunogenesis with typhus. Brill's disease. Peculiaryties of Q-fever pathogenesis. Prevention and treatment.
- Methods of laboratory diagnosis of Tyhus fever and Q-fever. Their assessment. Serological diagnosis of rickettsiosis.
- Classification of chlamydia, diseases that they cause. Pathogenesis, immunity and peculiaryties of chlamydiosis diagnosis.

• Mycoplasma and Ureaplasma infections. Causative agents characteristics. Pathogenesis, diagnosis and treatment of these infections.

Practical tasks are performed in class:

- To determine the taxonomic position and basic biological properties of rickettsiae, chlamydia and mycoplasma. Familiarised with the epidemiology and pathogenesis of diseases that they cause.
- To study the methods of laboratory diagnosis of rickettsiosis, chlamydiosis and mycoplasmosis.
- To study the morphological and tynktorial properties of rickettsia and chlamydia in the demonstration smears, stained by Zdrodovskiy and Romanovsky-Himza methods.
- To study methods of serological diagnosis of typhus and Q-fever. To read the results of complement fixation test CFT, Rickettsia agglutination test (RAT), Indirect haemagglutination test (IHAT) for serological diagnosis of rickettsiosis.
- Familiarised with the medicine for the diagnosis, specific prevention and treatment of rickettsiosis.

Content topics:

In practice, students studed classification and biological properties of rickettsiae, chlamydia and mycoplasma, familiarised with the pathogenesis and immunogenesis of diseases that these bacteria cause; studied smerses of rickettsiae (stained by Zdrobovskiy) with microscope; learn to detect inclusion of chlamydia in the cylindrical epithelium cells of the urethra in the smears, obtained from patients with urogenital chlamydiosis. Students analyze methods of laboratory diagnosis of rickettsiosis, chlamydiosis and mycoplasmosis, read the results of rickettsia agglutination test (RAT), complement fixation test (CFT), indirect hemagglutination test (IHAT), for serological diagnosis of rickettsiosis; familiarised with medicine, that are used for diagnosis, specific prevention and treatment of rickettsiosis.

Completed tasks students write in the protocol and sign it with a teacher.

5.1. Recommendations for design of the protocol.

Methods of laboratory diagnosis of chlamydia.

Pathological specimens:

swabs from urethral mucosa, conjunctiva, deposit of urine, biopsy tissue, pus, etc.

1. ***Microscopic method*** used in practical laboratories for diagnosis of trachoma, urogenital chlamydia. Smears from urethra, conjunctiva, etc., stained by Romanovsky-Himza method and study with microscopic method. Detection of 5-10 typical reticular or elementary bodies in the cytoplasm of infected cells has diagnostic value.

2. ***Express methods*** (direct and indirect RIF, ELISA) with monoclonal chlamydial antibodies are usd for detection of morphological structures and antigens of *Chlamydia* in tested specimens. The sensitivity of ELISA for detection of *Chlamydia* ranges 50-70% in men and 88-100% in women.

3. ***Methods for isolation and identification*** of *Chlamydia*. *Chlamydia* are isolated by infection of chicken embryos or cell culture (L-929, McCoy, HeLa). This is the most accurate and provable method of diagnosis of chlamydiosis (gold standard). After 48-96 hours infected cells stained by Romanovsky-Himza method, or treated with fluorescent antibodies and detect the presence of cytoplasmic inclusions. The method is highly specific (100%), sensitive (75%), the result is known by 42-96 hours, but inaccessible for practical labs and is a great risk of contracting personnel.

4. ***Serological diagnosis.*** Use indirect haemagglutination test (Indirect HAT), indirect RIF (mainly for the diagnosis of zoonose chlamydia diseases) CFT (not used for diagnosis of urogenital chlamydiosis), ELISA (highly specific and available, use diagnostic test kits). Examined paired serum where are determined increase antibody titers in 4 or more times.

5. ***Allergic test with ornithine*** (inactivated alantoyis culture of *Chl.psittaci*) refers as early diagnosis of *Chlamydia ornithosis* (2-9 day illness) and for the retrospective diagnosis of *Chlamydia ornithosis*.

6. ***Biological method*** of diagnosis are used only for *Chlamydia ornithosis*. White mice inoculat (intracerebral, intranasal, or into the abdominal cavity) by ifected specimens. Includes of chlamydia detect in the cytoplasm of mononuclear cells into the smears-imprints.

Microbiological diagnosis of Mycoplasma and Ureaplasma infections.

Pathological specimens:

blood, sputum, nasal mucus, discharge and swabs from the urethra, vagina, cervix, urine sediment, semen, and more.

Mycoplasmosis

1. ***Bacteriological methods.*** The most demonstrative. For isolation of pure cultures of mycoplasma is needed to inoculate tested specimens into the solide ore two-phase nutrient media (selective agar with pour over it a serum-glucose broth with the addition of penicillin and acetate Tallium). Signs of growth appear from one to seven weeks. Identification of colonies may be faster with use epifluorestsent. Cultures are identified according morphological, cultural, and enzymatic properties.

2. ***Express method.*** Mycoplasma or their antigens detect in clinical spacimens with RIF, Indirect HAT, ELISA, and agregat-agglutination.

3. ***Serological diagnosis.*** For detection of specific antibodies in the blood of the patient serum are used CFT, ELISA, agregat-agglutination, indirect RIF. Respiratory mycoplasmosis in practical laboratories detect with serological methods.

4. ***Polymerase chain reaction*** (PCR) - is the most sensitive method of diagnosis of mycoplasmosis.

Ureaplasmosis

1. ***Bacteriological method*** – is more used in practice.

They carry by inoculation tested specimens into the solide and liquide nutrient media. The results of inoculation to read after 24-48 hours according color changing in

media with absence of turbidity, - beacause of ability of U.urealiticum to ferment urea. The method of determination of urea activity is the main in the diagnosis of ureaplasmosis.

2. ***Express method.*** Microscopic detection of Ureaplasma in semen smears, treated with fluorohromom (olivomitcyn).

3. ***Serological diagnosis*** in CFT, indirect RIF, indirect HAT, ELISA, agregat-agglutination. But reagents for these reactions are difficult to access for practical laboratories.

Laboratory diagnosis of rickettsiosis.

Pathological specimences:

blood (the source of pathogens), while Q-fever - sputum, urine, spinal cerebrospinal fluid, milk, meat animals.

1. ***Microscopic method.*** Detection of rickettsiae in the studied specimences, in the body of infected animals, in the chicken embryos,in the body of lice, fleas, ticks. Smesarses are stained by Zdrodovsky method, Romanovsky-Himza method.

Isolation of *Rickettsiae*

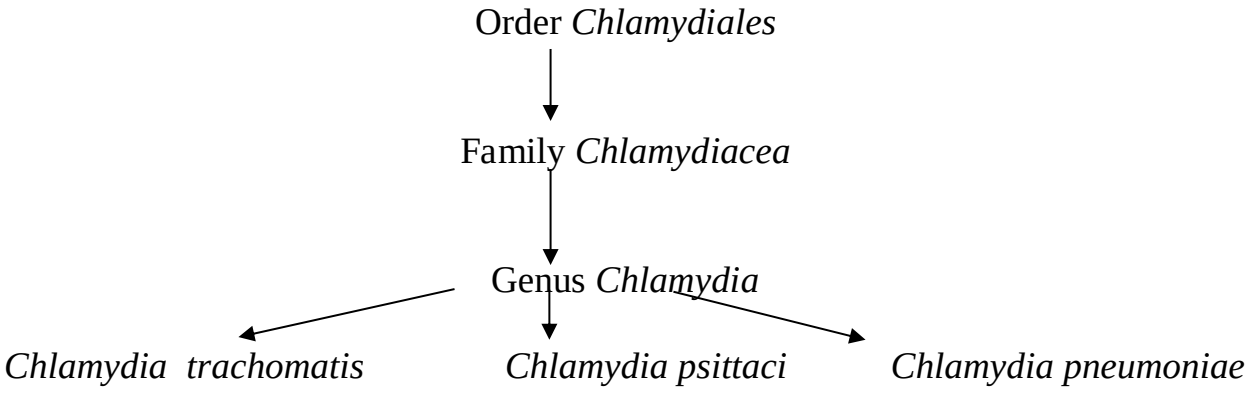
2. ***Methods of cultivation.*** *Rickettsiae* not multiply in cell-free environments, they are obligate intracellular parasites.They cultivated in chicken embryos, chicken embryo fibroblasts, in some cell cultures (McCoy).

3. ***Biological diagnosis.*** Biotests is ineffective for the diagnosis of epidemic typhus (isolation of *R.prowazekii*). Infected animals have diagnostic value for Q-fever, endemic typhus (guinea pigs infect by patients blood), for Tsutsuhimushi fever (infect white mice, hamsters, cotton rats). But any manipulations with rickettsiae are very dangerous (is high risk), because, *Rickettsiae* isolation make only for scientific researchs only in laboratories with special regime.

4. ***Serological diagnosis*** - the basis of modern laboratory diagnostics. Apply Weil-Felix reaction, agglutination of rickettsiae, indirect HAT, CFT, indirect RIF (all of them allow to identify the IgM and IgG for early diagnosis and determination the stage of disease).Reliable results are possible only at the end of the first week of deasase. In determining diagnostic titers should also focus on the arise of antibody titers, which has more epidemiological significance.

5. ***Polymerase chain reaction*** was developed for the diagnosis of reactive erlihiozis.

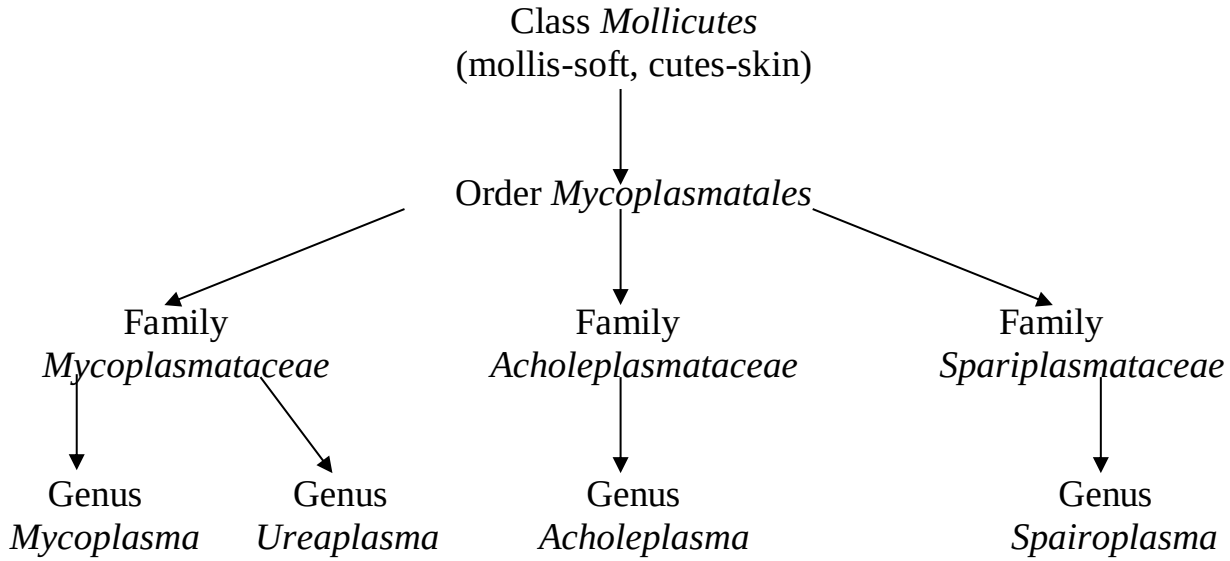
Chlamydia classification



Causative agents of Chlamidiosis

Species	Diseases	Serological variants
Chlamydia trachomatis	Trachoma, paratrachoma	A, B, B_a, C
	Urogenital chlamidiosis, infant pneumonia	D, F, G, H, I, J, K
	Lymphorganuloma venereum	L_1, L_2, L_3
Chlamydia psittaci	Ornithosis (animals are primary pathogens)	13 (?)
Chlamydia pneumoniae	Pneumoniae, ГРЗ, arterial sclerosis, sarcoidosis, brochitic asthma	TWAR, AR, KA, CWL

Mycoplasma classification



The main causative agents of human mycoplasmosis

Species of *Mycoplasma*	Diseases
M.pneumoniae (Iton agent)	Atypical pneumonia, pharyngitis, acut respiratory infection
M.hominis, Ureaplasma urealyticum	Urethritis, cervicitis, prostatitis, vulvovaginitis, pyelonephritis, pregnancy and fetal pathology, other men's fertility
M.hominis, M.fermentans, M.arthritidis	Rheumatoid arthritis

The main biochemical features of mycoplasma, pathogenic for human

Species	Arginine hydrolyse	Acid production due to glucose fermentation	Acid production due to mannose fermentation	Restoration of tetrasoliume salt (a.c.*/an.c.*)
M.pneumoniae	-	+	+	+/+
M.hominis	+	-	-	-/-

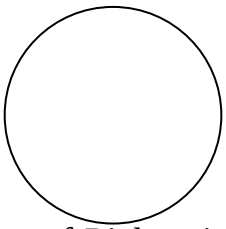
M.fermentans	+	+	-	-/+
M.genitalium	-	+	±	+/-

* **a.c.** – *aerobic conditions; an.c. - anaerobic conditions*

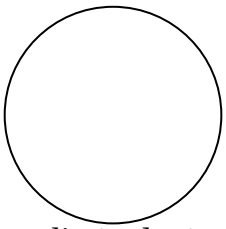
Pathogenic for human *Rickettsia* Classification

Order	*Rickettsiales*				
Family	Rickettsiaceae				
Trib	Rickettsiaea				
Genus	*Rickettsia*	*Orientia*	*Erlichia*	*Coxiella*	*Bartonella*
Spotted fever group	*R.prowazekii* *R.mooseri*	*O.tsutsugamushi*	*E.sennetsu* ***(Japan, lympoi*****d hyperplasy)**	*C.brunetti*	*B.quintana* *B.henselae*
Rocky Mountain spotted fever	*R.rickettsii* *R.conorii* *R.australis* *R.sibirica* *R.acari*		*E.chaffensis* ***(North America, Europe, Asia; vasculitis, granulomatosis)***		

Work 1. To determine morphological properties of *Rickettsiaea* and *Chlamydia* in demostrate smearses.

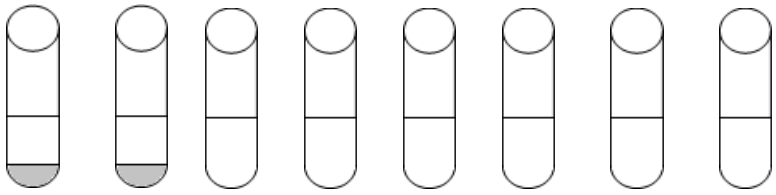


1. Smers of *Rickettsiaea* (by Zdrodovskiy)



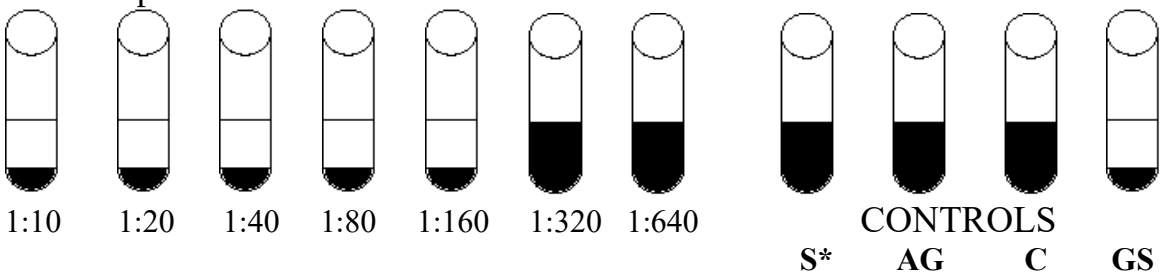
2. *Chlamydia* inclusions in the uretral epitelium cells in Patient with Chlamydiosis (Romanovsky-Himza method)

Work 2. To read the results *Rickettsia* agglutination test (RAT) for serological diagnosis of rickettsiosis.



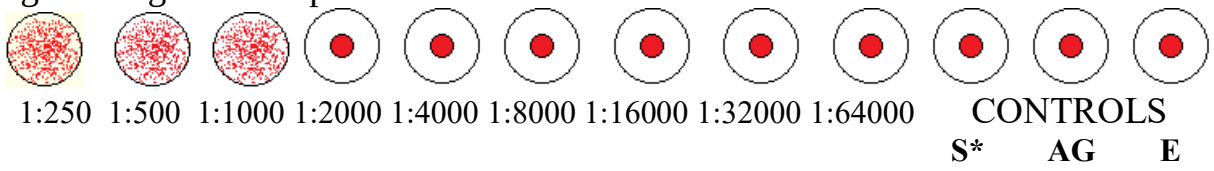
*** S – serum**
AG - antigen

Work 3. To read the results of complement fixation test CFT for serological diagnosis of epidemic fever.



*** S – serum**
AG - antigen
C – complement
GS – gemolitik system

Work 4. To read the results of Indirect haemagglutination test (IHAT) for serological diagnosis of epidemic fever.



*** S – serum**
AG - antigen
E – erythrocyte

Work 5. Examine samples of medicine that are used for diagnosis, specific prevention and treatment of rickettsiosis.

Questions for self-control.

- Taxonomic position and basic biological properties of rickettsiae, chlamydia and mycoplasma. What is the diference these microrganisms from bacteria and viruses? What are the similsr these microrganisms from bacteria and viruses?
- Classification of rickettsiae. History of discovery. *Rickettsiae* cultivation.
- The role of rickettsia in human pathology. Pathogenesis and immunogenesis epidemic fever, Brill disease. Features of pathogenesis Q-fever.
- Methods of laboratory diagnosis of epidemic fever and Q-fever and their evaluation. Serological diagnosis of rickettsiosis.
- What medicine are used for diagnosis, treatment and specific prevention of epidemic fever and Q-fever?
- What are the diseases caused by chlamydia? Classification and biological properties of chlamydia. Pathogenesis, immunity, features of the laboratory diagnosis of chlamydia.
- What are the Mycoplasma- and Ureaplasma-infections? Characteristics of pcausative agents, pathogenesis, diagnosis, treatment of these infections.

Class №36

Topic: “Spirochete. Microbiological diagnosis spirohetosis”

Relevance of the topic.

Pathogenic spirochetes belonging to the family Spirochetaceae, order *Spirochaetales*. The family consists of 5 genera. Human Pathogens belonging to 3 genera: *Treponema* (causative agent of syphilis - *T.pallidum*, causative agent of yaws - *T.pallidum* subsp. perenue, causative agent of pinta - *T.carateum*); *Borrelia* (causative agent of relapsing fever epidemic - *B.recurrentis*, causative agents of tick-borne relapsing fever - *B.persica*, *B.hispanica* et al.) and the genus *Leptospira* family *Leptospiraceae* (causative agent of leptospirosis - *Leptospira interrogans*). Diseases that cause spirochete called spirohetosis. They have some common features in the pathogenesis, epidemiology and clinical symptoms. Thus, for all spirohetosis are typical cyclic course of diseases, especially for relapsing fever with repeated bouts of fever. Syphilis also runs as a series of stages. The presence of recurrence observed in leptospirosis.

Causative agents of spirohetosis are founded in blood and tissues. Relapsing fever is a typical blood infection, transmission of which is possible only through the bloodsuckers (lice, mites). Due to syphilis spirochetes multiply mainly in the tissues, and some stages are in the blood. Due to leptospirosis spirochete also found in blood and tissues. According to the World Health Organization each year in the world infected with syphilis 50 million people, in Europe - 40,000, and the number of unregistered cases is much more. Syphilis is a marker of AIDS, which causes human immunodeficiency virus. At last time boreliosis (Lyme disease) has been extended – causative agent is *B.burgdorferi*. The sporadic cases of relapsing fever is registered, leptospirosis is widespread in Ukraine.

Practice class is devoted to the familiarization and studing pathogenesis, principles of laboratory microbiological diagnostics of spirohetosis (relapsing fever, leptospirosis and syphilis) by students is need and exedient. All the above leads to the relevance of topics and sessions aimed at building up a positive motivation to learn.

Specific objectives:

- To learn the biological properties of spirochetes; to learn a pathogenesis and immunity characteristics, diagnostic methods of campylobacteriosis, helicobacteriosis, syphilis, relapsing fever and leptospirosis.
- Master the methods of laboratory diagnosis of syphilis, relapsing fever and leptospirosis.
- Examine the preparations used for diagnosis, specific preventions and therapy of campylobacteriosis, helicobacteriosis, spirohetosis.

Basic knowledge, skills, skills needed to study topics (interdisciplinary integration). See a class №34.

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

Terms	Definition
Pathogenic spirochetes	Pathogenic spirochetes – is a thin, winding, moving microorganisms that differ in size, depth of spiral, amplitude and number of bends. *Borrelia* well stained by Romanovsky-Himza method in blue and purple; *Treponema* very little stained by Romanovsky-Himza in pale pink. Pathogenic spirochets (in living condition) can be detected in tested specimence with dark field microscopy. Spirochets have 4 kinds of motion: progressive, bending, twisting and like pendulum.
Syphilis	Syphilis - a sexually transmitted infectious disease in which affected the skin, mucous membranes, internal organs and central nervous system. The word "syphilis" first appeared in the poem of famous Italian scientist, physician, philosopher and poet from Verona-Girolamo Frakastoro "Syphilis", or "French disease" which was published in Venice in 1530. According the name of the hero of the poem shepherd Sifila, whom the gods punished by genital disease (*Suis* - pig, *philos* - loving), this disease called as "syphilis."
Causative agent of syphilis	The causative agent of syphilis - is Treponema pallidum, which belongs to the genus Treponema (from Lat. Trepo - return, nemo - thread), opened in 1905 by Shaudinom F. and E. Hofmann. The old name of this pathogen – is pale pallidum due to the fact that the microorganism badly painted dyes beacause a low content of nucleoprotein. *Treponema pallidum* – is a spiral form thin cells with 12-14 scrolls.
Relapsing fevers	Relapsing fevers - acute, blood-borne infections that caused by *Borellia*. They characterized as well intoxication periods with repeated bouts of fevers, as periods without temperature (days looks like convalescence). Due to this deseases may be varying degrees of liver and spleen lesions, and sometimes jaundice, meningitis, lesions of other organs. Infections featurs of patients blood was proved in autoinfection experiments by G.M. Minchen from Odessa in 1874.
Causative agents of relapsing fever	*Borrelia* of relapsing fever – are causative agents of borreliosis. Epidemic (lousy) relapsing fever causing *Borrelia* recurrentis, and endemic - *B.persica*, *B.duttoni*, *B.caucasica*. Causative agents of relapsing fever differ from Treponema by large flat irregular curls, whose number ranges from 3 to 10.
Leptospirosis	Leptospirosis –is a acute zoonoz infectious disease that characterized by leptospiremia, fever, intoxication, lesions of kidney and the liver capillaries, muscle, cardiovascular and central nervous systems.
Causative agents of leptospirosis	Leptospiras are causative agents of acute infectious disease - leptospirosis, which harm man, rodents, cattle, sheep, pigs,

	dogs and other animals. Leptospirosis caused by *Leptospira interrogans* (Greek leptos - long, thin). Causative agents of leptospirosis are often *L.pomona, L.monijakov, L.grippotyphosa, L.tarassovi, L.canicola, L.icterohaemorragiae.* Leptospiras are long, thin spiral bacteria with 12-18 small scrolls. Their ends are bent in the form of hooks.

Theoretical questions to studies.

- Pathogenic Spirochetes. Causative agents of fever, transmissed by the rat bites (Sodoku disease). Microbiological diagnosis of disease.
- General characteristics of the Spirochetes family. Classification.
- Historical data about causative agents of relapsing fever, syphilis, leptospirosis.
- The causative agent of syphilis. Biological properties. Pathogenesis and immunity. Microbiological diagnosis and specific treatment of syphilis.
- Leptospiras, their characteristics, classification. Pathogenesis, immunity and microbiological diagnosis of leptospirosis. Specific prevention and therapy.
- Borrelia, biological properties. Role in human pathology. Pathogens of epidemic and endemic relapsing fevers, Lyme disease. Pathogenesis, immunogenesis, microbiological diagnosis of relapsing fever, Lyme disease. Specific prevention and therapy.

Practical tasks performed in class:

- To make the schemes of microbiological diagnosis of syphilis, relapsing fever and leptospirosis.
- Familiar with methods bacterioscopic diagnosis of syphilis and relapsing fever.
- Master technique serological diagnosis of syphilis with Wasserman test.
- Examine the medicines that are used for diagnosis, specific preventions and therapy of spirohetosis

Content topics:

In practice, students study the evolution and properties of the winding forms of bacteria; history of discovery and study, biological properties, patterns of pathogenesis and immunity characteristics of Spirochetes; methods of diagnostic campilobacteriosis, helicobacteriosis, syphilis, relapsing fever and leptospirosis; preparations for diagnosis, specific preventions and therapy of spirohetosis. Completed tasks students write in the protocol and sign it with a teacher.

Recommendations for design of the protocol

In the protocol should be made:

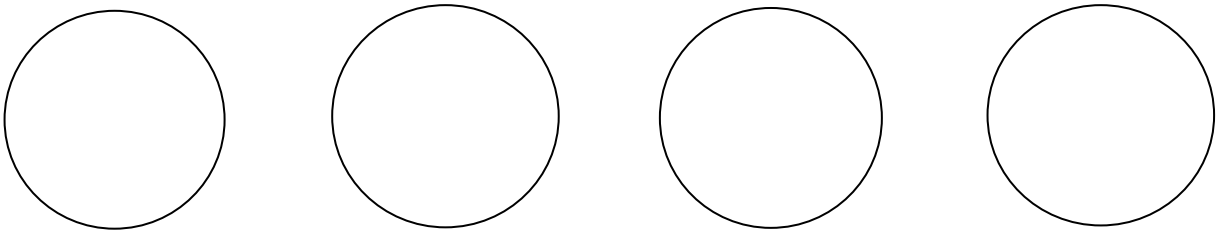
- Classification of pathogens from the family *Spirochetaceae*
- Morphology propertis of spirochetes
- Schemes of microbiological diagnosis of syphilis, relapsing fever and leptospirosis.
- The schematical representation of the main reactions Wasserman test.

- Table differentiation of epidemic and endemic relapsing fever.
- Preparations which are used for diagnosis, treatment and specific prevention of syphilis, relapsing fever and leptospirosis.

Classification of pathogens from the family *Spirochetaceae*

Рід	**Вид**	**Підвид**	**Захворювання**
Treponema	*pallidum* *pallidum* *carateum* *vincentii*	pallidum pertenue	Syphilis Yaws Pinta Tonsilatis Vincent-Plaut
Borrelia *Borrelia*	*recurrentis* *caucasica, duttoni, persica etc.*		Epidemic (louse-born) relapsing fever Endemic (tick-born) relapsing fever
Borrelia	*burgdorferi*		Lyme desease
Leptospira	*interrogans*		Leptospirosis

Task 1. The morphology features of spirochetes.



Treponema pallidum		*Borrelia spp.*	*Leptospira interrogans*
Romanovsky-Himza method	**Dark field microscopy**	**Romanovsky-Himza method**	**Silver method**

Features of microbiological diagnosis of spirohetosis (syphilis, leptospirosis, relapsing fever)

Microbiological diagnosis of syphilis

1. Microscopy (native and fixed material):
- *Phase contrast*
- *Dark field*

2. Serological diagnosis:
- *Enzyme immunoassay (ELISA)*
- *Indirect hemagglutination test (IHAT)*
- *Wassermann test*

- *Sedimentation test (Kan and Zax-Vitebsk)*
- *Immobilization test of Treponema pallidum (IT)*
- *Immunofluorescence (IF) test*

3. Polimeraz chain reaction (PCR)

Microbiological diagnosis of relapsing fever: bacterioscopic and serological principles.

Differentiation of epidemic and endemic relapsing fever

Features	***Epidemic relapsing fever***	***Endemic (tick-borne) relapsing fever***
Causative agent	*B.recurrentis*	*B.persica, B.caucasica*
Carrier	*Pediculus vestimenti* and sometimes *P.capitis*	*Alectorobius papillipes Alectorobius verrucosus*
Pathogenicity for guinea pig (Biological method)	Do not suffer	The disease occurs after 5-7 days (subcutaneously injection of 0.5 cm^3 blood or 1-2 drops in the eye conjunctiva).
The presence of the causative agent in the "thick" blood drop during the febrile period	Large number of spirochetes are detected	Isolated little numbe of spirochetes, but their detection is possible both during attacks and between them.
With dark field microscope	presence of one contour in spirochetes	presence of two contour in spirochetes
Dissemination	Absent in practical	Central, Midl Asia and the Mediterranean Sea (*B.persica*); Caucasus, Ukraine (*B.caucasica*).

Microbiological diagnosis of leptospirosis:

1. ***Bacterioscopic*** (poor sensitivity) ***methods***
2. ***Bacteriological methods***
3. ***Serological methods***
4. ***PCR.***

Task 2. To read the result of Waserman test, for serological diagnosis of syphilis (the scheme).

For test to get the serum, inactivated it in a water heater at 56^0C 30 min and poured into four test tubes.

In Wasserman test are used: 1) specific antigen, which contains the causative agents antigens - Treponema destroyed by ultrasound; 2) non-specific antigen – is a lipoid extract

from bovine heart with lecithin and cholesterol (kardiolipoid antigen); 3) non-specific antigen - alcoholic lipoid extract of bull muscle heart with cholesterol.

Scheme of the main Wasserman test

Ingredients	**tube №1**	**tube №2**	**tube №3**	**tube №4**
Blood serum of the patient,inactivated and diluted 1:5	0,5cm^3	0,5 cm^3	0,5 cm^3	0,5 cm^3
Antgen№1(specific)	0,5 cm^3	-	-	-
Antigen№2(nonspecific)	-	0,5 cm^3	-	-
Antigen№3(nonspecific)	-	-	0,5 cm^3	-
Complement (working dose)	0,5 cm^3	0,5 cm^3	0,5 cm^3	0,5 cm^3
Isotonic solution of NaCl (sodium chloride)	-	-	-	0,5 cm^3
Thermostat at a temperature of 37^0 C during 45 min.				
Haemolitic sencibilized system	1,0 cm^3	1,0 cm^3	1,0 cm^3	1,0 cm^3
Thermostat at a temperature of 37^0 C during 1 hour				
Result:	**tube №1**	**tube №2**	**tube №3**	**tube №4**
serum from a patient with syphilis	-G	-G	-G	+ G
with normal serum	+ G	+ G	+ G	+ G

Note:

- G - no hemolysis,

+ G – hemolysis is presence,

"+" - positive result,

"-" - negative result.

Accounting for results is performed after hemolysis in all control.

In 50% of patients the test is positive after the chancre. In the second and third periods of syphilis 75-90% positive reactions is presence. After treatment Wasserman test is negative.

Questions for self-control.

- What is winding bacteria position in systematic of pathogenic microorganisms?
- What groups subdivided the spirochete which are pathogenic to humans?
- What are the historical data about causative agents of relapsing fever, syphilis and leptospirosis?
- What are the biological characteristics causative agents of spirohetosis. What are the criteria for differentiating them from saprophytic microorganisms of the same morphology?
- What are the forms of relapsing fever, vectors and disease pathogenesis?
- Pathogenesis, clinical forms of syphilis, immunity.

- What are the biological properties of leptospiras?
- Pathogenesis, clinical forms, immunity and specific prevention of leptospirosis, immunity and specific prevention.
- What is the mechanism of immunity in diseases caused by *Treponema*, *Borrelia* and *Leptospira*?
- How can you grounded the principles of microbiological diagnostics of spirohetosis, leptospirosis, helikobakteriosis, campilobacteriosis and diagnostic methods are used?
- What is the theoretical basis for treatment and prevention spirohetosis, leptospirosis, helicobacteriosis and campilobacteriosis?

Class 37

Topic: "Pathogenic fungi and actinomycetes. Microbiological diagnosis of mycoses"

Topic relevance

Fungi or the *Eumycota* are a distinct class of microorganisms, most of which are free-living in nature where they function as decomposers in the energy cycle. Of the more than 90 000 known species, fewer than 200 have been reported to produce disease in humans. These diseases have unique clinical and microbiologic features and are increasing in immunocompromised patients.

Fungi are eukaryotes with a higher level of biologic complexity than bacteria. They are spore bearing; reproducing both sexually and asexually. Fungi may be unicellular or may differentiate and become multicellular by the development of long-branching filaments. They acquire nutrients by absorption but lack the chlorophyll of plants. The diseases caused by fungi are called mycoses or fungal pathogenesis. They vary greatly in their manifestations but tend to be subacute to chronic with indolent, relapsing features. Acute disease, such as that produced by many viruses and bacteria, is uncommon with fungal infections.

Fungal pathogens can be divided into two general classes' primary pathogens and opportunistic pathogens. Currently, there has been a dramatic increase in fungal infections of this type, in particular candidiasis, cryptococcosis, aspergillosis, and zygomycosis. More recently described mycoses of this category include hyalohyphomycosis and phaeohyphomycosis.

Practice class is devoted to the familiarization and studing pathogenesis, principles of laboratory microbiological diagnostics of mycoses by students is need and exedient. All the above leads to the relevance of topics and sessions aimed at building up a positive motivation to learn.

Concrete objectives:

- Identify taxonomic position and biological properties of fungi.
- Study the epidemiology and pathogenesis of diseases caused by fungi.
- Master the techniques of laboratory diagnosis of mycoses.

Basic knowledge, skills, skills needed to study topics (interdisciplinary integration). See a class 34.

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

Terms	Definition
Mycoses	Mycoses are classified as superficial, cutaneous, subcutaneous, or systemic (deep) infections depending on the type and degree of tissue involvement and the host response to the pathogen. Superficial mycoses are limited to the stratum corneum and essentially elicit no inflammation. Cutaneous infections involve

	the integument and its appendages, including hair and nails. Infection may involve the stratum corneum or deeper layers of the epidermis. Inflammation of the skin is elicited by the organism or its products. Subcutaneous mycoses include a range of different infections characterized by infection of the subcutaneous tissues usually at the point of traumatic inoculation. An inflammatory response develops in the subcutaneous tissue frequently with extension into the epidermis. Deep mycoses involve the lungs, abdominal viscera, bones and or central nervous system. The most common portals of entry are the respiratory tract, gastrointestinal tract, and blood vessels.
Superficial Mycoses	Superficial Mycoses include the following fungal infections and their etiological agent: black piedra (*Piedraia hortae*), white piedra (*Trichosporon beigelii*), pityriasis versicolor (*Malassezia furfur*), and tinea nigra (*Phaeoannellomyces werneckii*). *Pityriasis versicolor* is a common superficial mycosis, which is characterized by hypopigmentation or hyperpigmentation of skin of the neck, shoulders, chest, and back. *Pityriasis versicolor* is due to *Malassezia furfur* which involves only the superficial keratin layer. Black piedra is a superficial mycosis due to *Piedraia hortae* which is manifested by a small firm black nodule involving the hair shaft. By comparison, white piedra due to *T beigelii* is characterized by a soft, friable, beige nodule of the distal ends of hair shafts. Tinea nigra most typically presents as a brown to black silver nitrate-like stain on the palm of the hand or sole of the foot.
Cutaneous Mycoses	Cutaneous Mycoses may be classified as dermatophytoses or dermatomycoses. Dermatophytoses are caused by the agents of the genera *Epidermophyton*, *Microsporum*, and *Trichophyton*. Dermatomycoses are cutaneous infections due to other fungi, the most common of which are Candida spp. The dermatophytoses are characterized by an anatomic site-specificity according to genera. For example, *Epidermophyton floccosum* infects only skin and nails, but does not infect hair shafts and follicles. Whereas, *Microsporum spp.* infect hair and skin, but do not involve nails. *Trichophyton spp*. may infect hair, skin, and nails.
Subcutaneous mycoses	There are three general types of subcutaneous mycoses: chromoblastomycosis, mycetoma, and sporotrichosis. All appear to be caused by traumatic inoculation of the etiological fungi into the subcutaneous tissue. Chromoblastomycosis is a subcutaneous mycosis characterized by verrucoid lesions of the skin (usually of the lower extremities); histological examination reveals muriform cells (with perpendicular septations) or so-called “copper pennies” that are characteristic of this infection. Chromoblastomycosis is generally limited to the subcutaneous tissue with no involvement of bone, tendon, or muscle. By

	comparison, mycetoma is a suppurative and granulomatous subcutaneous mycosis, which is destructive of contiguous bone, tendon, and skeletal muscle. Mycetoma is characterized by the presence of draining sinus tracts from which small but grossly visible pigmented grains or granules are extruded. These grains are microcolonies of fungi causing the infection.
Deep mycoses	Deep mycoses are caused by primary pathogenic and opportunistic fungal pathogens. The primary pathogenic fungi are able to establish infection in a normal host; whereas, opportunistic pathogens require a compromised host in order to establish infection (e.g., cancer, organ transplantation, surgery, and AIDS). The primary deep pathogens usually gain access to the host via the respiratory tract. Opportunistic fungi causing deep mycosis invade via the respiratory tract, alimentary tract, or intravascular devices.
Candidiasis	Candidiasis (due to *C albicans* and other *Candida* spp.) is the most common opportunistic fungal infection. *Candida albicans* is the most common cause of candidiasis. Candidiasis may be classified as superficial or deep. Superficial candidiasis may involve the epidermal and mucosal surfaces, including those of the oral cavity, pharynx, esophagus, intestines, urinary bladder, and vagina. The alimentary tract and intravascular catheters are the major portals of entry for deep (or visceral) candidiasis. The kidneys, liver, spleen, brain, eyes, heart, and other tissues are the major organ sites involved in deep or visceral candidiasis. The principal risk factors predisposing to deeply invasive candidiasis are protracted courses of broad spectrum antibiotics, cytotoxic chemotherapy, corticosteroids, and vascular catheters.
Aspergillosis	Invasive aspergillosis most frequently involves the lungs and paranasal sinuses. This fungus may disseminate from the lungs to involve the brain, kidneys, liver, heart, and bones. The main portal of entry for aspergillosis is the respiratory tract, however, injuries to the skin may also introduce the organism into susceptible hosts. Quantitative and functional defects in circulating neutrophils are key risk factors for development of invasive aspergillosis. For example, neutropenia due to cytotoxic chemotherapy and systemic corticosteroids are common predisposing factors for invasive aspergillosis.
Zygomycosis	Zygomycosis due to *Rhizopus, Rhizomucor, Absidia, Mucor* species, or other members of the class of *Zygomycetes*, also causes invasive sinopulmonary infections. An especially life-threatening form of zygomycosis (also known as mucormycosis), is known as the rhinocerebral syndrome, which occurs in diabetics with ketoacidosis. In addition to diabetic ketoacidosis, neutropenia and corticosteroids are other major risk factors for zygomycosis.

	Aspergillus spp. and the *Zygomycetes* have a strong propensity for invading blood vessels.
Actinomycetes	Species, which are non-motile, filamentous, branching, gram-positive and predominantly anaerobic bacteria. These organisms belong to the phylum, *Actinobacteria*, in the order *Actinomycetales*, and family, *Actinomycetaceae*.
Actinomycosis	Actinomyosis is a subacute to chronic bacterial disease that is characterized by slowly progressing suppurative fibrosing inflammation, development of draining sinus tracts that may discharge characteristic "sulfur granules," and direct dissemination via contiguous tissues. It most commonly involves the cervicofacial area, thorax or abdomen, including the pelvis, but rarely also the central nervous system (CNS), skin or bone. The disease is worldwide in distribution and more common in males.

Theoretical questions to the class:

- Classification of fungi. Morphology and cultural properties of fungi.
- Fungi that are pathogenic to humans. The diseases they cause.
- Principles of microbiological diagnosis of mycoses.
- Candidiasis. Pathogenesis, diagnosis and treatment.
- Causes of dermatomycosis. Pathogenesis, laboratory diagnostics. Treatment.
- Taxonomic position and basic biological properties of actinomycetes.
- Bringing actinomycetes closer to mushrooms.
- The role of actinomycetes in human pathology. Pathogenesis, diagnosis, treatment of actinomycetes.

Practical tasks performed in the class:

- To study the classification and basic biological properties of fungi and actinomycetes.
- To study the morphological and cultural properties of fungi and actinomycetes on live cultures, Gram staining and methylene blue.
- Familiarize yourself with the epidemiology and pathogenesis of mycosis. To analyze the schemes of microbiological diagnostics of dermatomycosis and candidiasis.
- Familiarize yourself with preparations for the diagnosis and treatment of mycoses and actinomycoses.

Content topics:

In the practical training students study the classification and biological properties of fungi and actinomycetes, get acquainted with the pathogenesis, diagnosis and treatment of diseases caused by pathogenic representatives of these microorganisms; study under the microscope native and colored preparations of actinomycetes, fungi of the genus

Candida, Aspergillus, Penicillium, Mucor. Students analyze methods of laboratory diagnostics. Students complete the tasks in the protocol and sign it with the teacher.

Recommendations for the protocol design

Laboratory diagnosis of mycoses

Culture, direct microscopy, and histopathology have been the foundation for diagnosis of fungal infection for many decades. Microscopy, histopathology, and use of fungal-specific stains play important roles in diagnosis of infection by *C. neoformans, P. jirovecci, Candida spp., Aspergillus spp., H. capsulatum, Blastomyces dermatitidis, Coccidioides immitis, Sporothrix schenckii, Paracoccidioides brasiliensis,* and the *Mucorales*. Sensitivity of microscopy for diagnosis of fungal infection varies with the individual agent, the source and quality of the specimen, and the skills and experience of the laboratorian. Finally, diagnosis of invasive fungal infection by direct microscopy and histopathology may require the use of biopsies of deep tissues, which poses a risk to those patients who are most susceptible to invasive disease.

Culture from a clinical sample is the gold standard for diagnosis of fungal infection. Culture has the advantage of yielding the specific etiological agent if positive. Moreover, culture allows for susceptibility testing. However, use of culture for diagnosis of IFD has significant limitations. Culture may take many days to a result with several of the filamentous fungi. Identification of less common fungi that may cause opportunistic infections requires a high level of expertise on the part of laboratory personnel.

Serologic tests for patient antibodies have been useful for non-culture-based diagnosis of fungal infection since the middle of the last century. Serology is of greatest value in diagnosis of endemic mycoses. Available technologies include immunodiffusion (ID), complement fixation (CF), and enzyme immunoassay (EIA).

CF and immunodiffusion are the most common serologic tests for diagnosis of histoplasmosis. The ID test detects precipitating antibodies to Histoplasma H and M antigens. Serologic testing for histoplasmosis is most useful if an increase in CF titer is observed between acute and convalescent sera in acute histoplasmosis.

MOLECULAR DIAGNOSTICS

PCR is a central component for many molecular methods, either as the main diagnostic strategy or as one of the preliminary steps in the diagnostic assay. Consequently, diagnostic PCR encompasses a number of different approaches. The simplest consists of conventional PCR in which species-specific primers that have been designed based on existing sequence or data, are used to amplify fungal DNA from clinical specimens.

ANTIGEN DETECTION

Fungal polysaccharides or proteins may be shed into body fluids during the course of infection. If an antibody can be raised against such a shed antigen, an immunoassay can be constructed for antigen detection.

Laboratory diagnosis of actinomycosis

A definitive diagnosis cannot be made solely on clinical grounds. The diagnosis of actinomycosis may be difficult and often depends on a heightened level of clinical

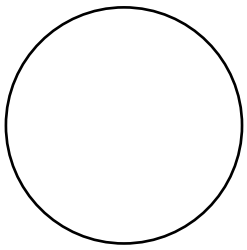
suspicion and prior notification of the clinical laboratorian and pathologist. Bronchoscopy and bronchoalveolar lavage fluid examination and culture may be useful in thoracic actinomycosis, but may also produce misleading results due to contamination of the specimens with the normal oropharyngeal microflora. Detection of the causative agents by Gram stain and culture from an appropriately obtained specimen is needed. Exudates and biopsy material are particularly suitable for examination, and should be cultured promptly under anaerobic conditions.

After a sample is obtained, it should be examined by standard histologic methods, anaerobic culture for 2 weeks, and immunofluorescence if available. Culture is the least reliable method of verifying infection when standard aerobic and anaerobic culture techniques are used. Results can be better for samples cultured in the presence of metronidazole, which inhibits the growth of faster growing anaerobes, and even better results may be obtained when transparent agar media, Fortner's method for producing a semianaerobic atmosphere, and microscopic examination of the cultures for up to 14 days of incubation at 36 ± 1°C are used. The last procedure also facilitates the detection of actinomycete colonies among a usually large amount of various colony types of concomitant microbes.

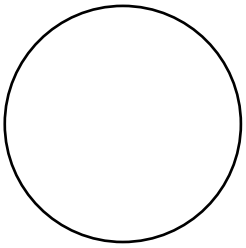
Serodiagnosis of actinomycosis by detection of precipitating or other antibodies has not been a particularly useful diagnostic test. Demonstration of pathogenic actinomycetes from smears of lesions by immunofluorescence is a more promising technique. More recently, molecular genotypic techniques have been utilized for identification of Actinomyces species. 16 S ribosomal RNA (rRNA) gene sequencing is now becoming the standard method of identification in most academic and reference laboratories.

Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry is a newer, promising technology that can accurately identify Actinomyces at the genus level, although precise species identification can sometimes be difficult.

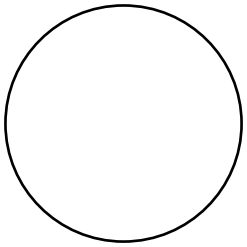
Practical activity 1. Examine under the microscope museum preparations of pathogenic fungi and actinomycetes and draw.



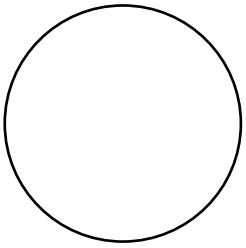
***Candida albicans* (by Gram)**



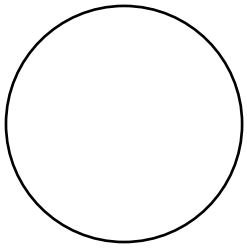
***Actinomyces israelii* (by Gram)**



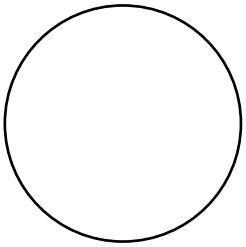
***Actinomycetes* in tissue**



Mucor



Penicillinum



Aspergillus

Control questions:

- Classification and biological authority of mushrooms.
- How can I catch mushrooms?
- Morphological and biological characteristics of zbudnik dermatomycosis, name zhvoryuvan?
- Candidiasis. Characteristics of Candidiasis zabudnikov, pathogenesis, diagnosis and diagnosis.
- Methods of laboratory diagnostics of mikoziv.
- What are the preparations for the diagnosis and diagnosis of dermatomycosis, candidiasis and systemic infections?
- Taxonomic position and basic biologic power of actinomycet?
- The role of actinomycetes in the pathology of people. See pathogenic actinomycetes.

Class 38

Topic: “Pathogenic Protozoa. Microbiology diagnostic of protozoan diseases”

Topic revalence:

The *Protozoa* are considered to be a subkingdom of the kingdom Protista, although in the classical system they were placed in the kingdom *Animalia*. More than 50,000 species have been described, most of which are free-living organisms; protozoa are found in almost every possible habitat. Virtually all humans have protozoa living in or on their body at some time, and many persons are infected with one or more species throughout their life. Some species are considered commensals, i.e., normally not harmful, whereas others are pathogens and usually produce disease. Protozoan diseases range from very mild to life-threatening. Individuals whose defenses are able to control but not eliminate a parasitic infection become carriers and constitute a source of infection for others. In geographic areas of high prevalence, well-tolerated infections are often not treated to eradicate the parasite because eradication would lower the individual's immunity to the parasite and result in a high likelihood of reinfection.

Many protozoan infections that are inapparent or mild in normal individuals can be life-threatening in immunosuppressed patients, particularly patients with acquired immune deficiency syndrome (AIDS). The lack of effective vaccines, the paucity of reliable drugs, and other problems, including difficulties of vector control, prompted the World Health Organization to target six diseases for increased research and training. Three of these were protozoan infectionsmalaria, trypanosomiasis, and leishmaniasis. Although new information on these diseases has been gained, most of the problems with control persist.

Concrete objectives:

- Identify taxonomic position and biological properties of protozoa.
- Study the epidemiology and pathogenesis of diseases caused by protozoa.
- Master the techniques of laboratory diagnosis of diseases caused by protozoa (malaria, leishmaniasis, trypanosomiasis).
- Examine the drugs used in medical protistology.

Basic knowledge, skills, skills needed to study topics (interdisciplinary integration). See a class 34.

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

Terms	Definition
Protozoa	*Protozoa* are one-celled animals found worldwide in most habitats. Most species are free living, but all higher animals are infected with one or more species of protozoa. Infections range from asymptomatic to life threatening, depending on the species and strain of the parasite and the resistance of the host.

Malaria	Malaria is a mosquito-borne infectious disease affecting humans and other animals caused by parasitic protozoans (a group of single-celled microorganisms) belonging to the *Plasmodium* type.
Causative agents of malaria	Five species of *Plasmodium* can infect and be spread by humans. Most deaths are caused by P. falciparum, because *P. vivax*, *P. ovale*, and *P. malariae* generally cause a milder form of malaria. The species *P. knowlesi* rarely causes disease in humans. Malaria is typically diagnosed by the microscopic examination of blood using blood films, or with antigen-based rapid diagnostic tests. Methods that use the polymerase chain reaction to detect the parasite's DNAhave been developed, but are not widely used in areas where malaria is common due to their cost and complexity
Toxoplasmosis	Toxoplasmosisis a parasitic disease caused by *Toxoplasma gondii*. Infections with toxoplasmosis usually cause no symptoms in adult humans. Occasionally there may be a few weeks or months of mild flu-like illness such as muscle aches and tender lymph nodes. In a small number of people, eye problems may develop. In those with a weak immune system, severe symptoms such as seizures and poor coordination may occur. If infected during pregnancy, a condition known as congenital toxoplasmosis may affect the child.
Life forms of *T. gondii*	In its life cycle, *T. gondii* adopts several forms. Tachyzoites are responsible for acute infection; they divide rapidly and spread through the tissues of the body. After proliferating, tachyzoites convert into bradyzoites, which take the form of latent intracellular tissue cysts that form mainly in the tissues of the muscles and brain. The transformation into cysts is in part triggered by the pressure of the host immune system. The bradyzoites are not responsive to antibiotics. The bradyzoites, once formed, can remain in the tissues for the lifespan of the host. In a healthy host, if some bradyzoites convert back into active tachyzoites, the immune system will quickly destroy them. However, in immunocompromised individuals, or in fetuses, which lack a developed immune system, the tachyzoites can run rampant and cause significant neurological damage
Leishmaniasis	Leishmaniasis, also spelled leishmaniosis, is a disease caused by protozoan parasitesof the genus *Leishmania* and spread by the bite of certain types of sandflies. The disease can present in three main ways: cutaneous, mucocutaneous, orvisceral leishmaniasis. The cutaneous form presents with skin ulcers, while the mucocutaneous form presents with ulcers of the skin, mouth, and nose, and the visceral form starts with skin ulcers and then later presents with fever, low red blood cells, and enlarged spleen and liver. Infections in humans are caused by more than 20 species of *Leishmania*. Risk factors include poverty, malnutrition, deforestation, and urbanization. All three types can be diagnosed

	by seeing the parasites under the microscope. Additionally, visceral disease can be diagnosed by blood tests.
Causative agents of leishmaniasis	Leishmaniasis is transmitted by the bite of infected female phlebotomine sandflies which can transmit the protozoa *Leishmania*. The sandflies inject the infective stage, metacyclicpromastigotes, during blood meals (1). Metacyclic promastigotes that reach the puncture wound are phagocytized by macrophages (2) and transform into amastigotes (3). Amastigotes multiply in infected cells and affect different tissues, depending in part on which *Leishmania* species is involved (4). These differing tissue specificities cause the differing clinical manifestations of the various forms of leishmaniasis. Sandflies become infected during blood meals on infected hosts when they ingest macrophages infected with amastigotes (5, 6). In the sandfly's midgut, the parasites differentiate into promastigotes (7), which multiply, differentiate into metacyclic promastigotes, and migrate to the proboscis (8). The genomes of three *Leishmania* species (*L. major*, *L. infantum*, and *L. braziliensis*) have been sequenced, and this has provided much information about the biology of the parasite. For example, in *Leishmania*, protein-coding genes are understood to be organized as largepolycistronic units in a head-to-head or tail-to-tail manner; RNA polymerase II transcribes long polycistronic messages in the absence of defined RNA pol II promoters, and *Leishmania* has unique features with respect to the regulation of gene expression in response to changes in the environment. The new knowledge from these studies may help identify new targets for urgently needed drugs and aid the development of vaccines.

Theoretical questions:

- Classification of protozoa. Morphological and biological properties of protozoa.
- Differences between prokaryotes and protozoa.
- Pathogenic protozoa as causative agents of human diseases.
- Malaria. The source and mechanism of transmission of malaria. The pathogenesis of the disease and life cycle of malaria plasmodium. Laboratory diagnosis of malaria.
- Toxoplasmosis. Morphological features of toxoplasma, pathogenesis. The source of infection, the mechanism of transmission, the main clinical manifestations. Methods of laboratory diagnosis.
- Leishmaniasis. Morphological features of the pathogen. The source of infection, the mechanism of transmission, the main clinical manifestations. Methods of laboratory diagnosis.

Practical activities performed in class:

- To create the schemes of microbiological diagnosis of malaria, toxoplasmosis and leishmaniasis.

- To check the results of laboratory diagnosis of malaria, toxoplasmosis and leishmaniasis.
- To study the methods of preparations of smears from material of patients with malaria, toxoplasmosis and leishmaniasis.
- To study the drugs used for treatment of diseases caused by protozoa.

Topic content:

In practice, students study the evolution and properties of the to create the schemes of microbiological diagnosis of malaria, toxoplasmosis and leishmaniasis. To study the methods of preparations of smears from material of patients with malaria, toxoplasmosis and leishmaniasis. To study the drugs used for treatment of diseases caused by protozoa. Completed tasks students write in the protocol and sign it with a teacher.

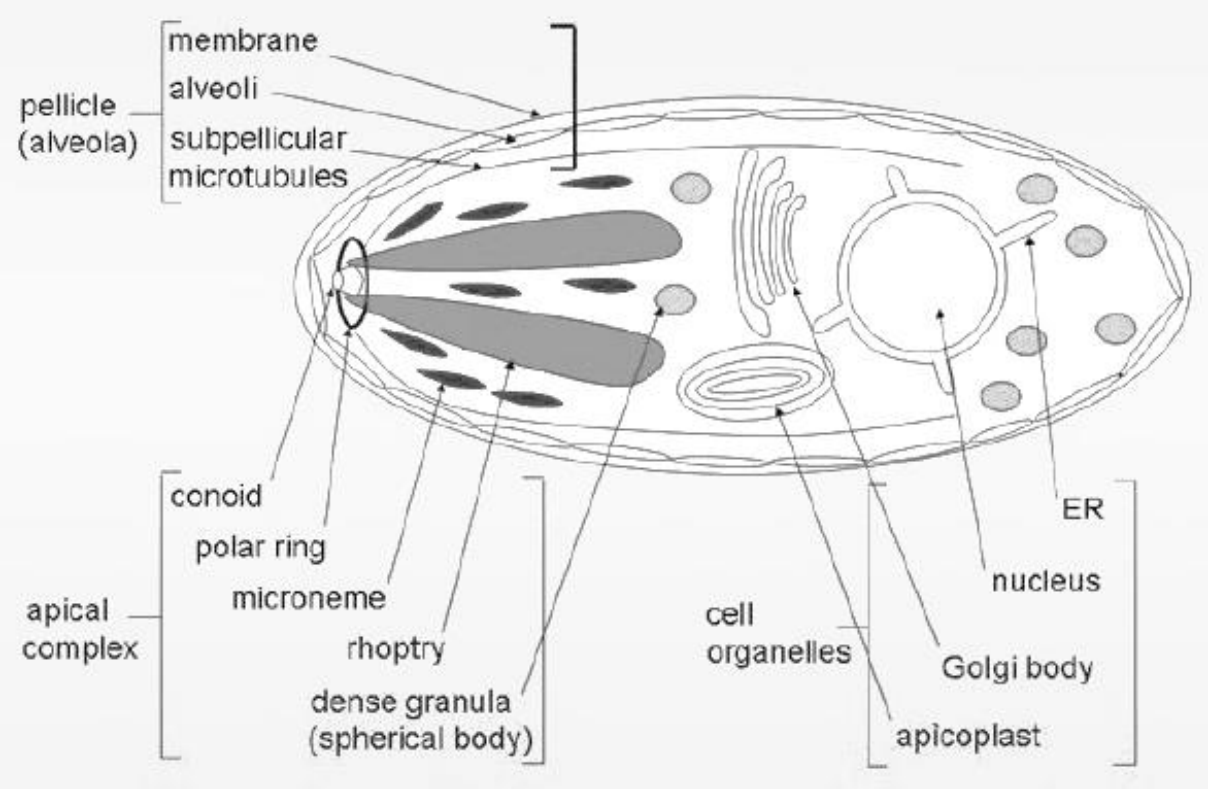
Recommendations for the protocol design

Differentiation of Malaria Parasites

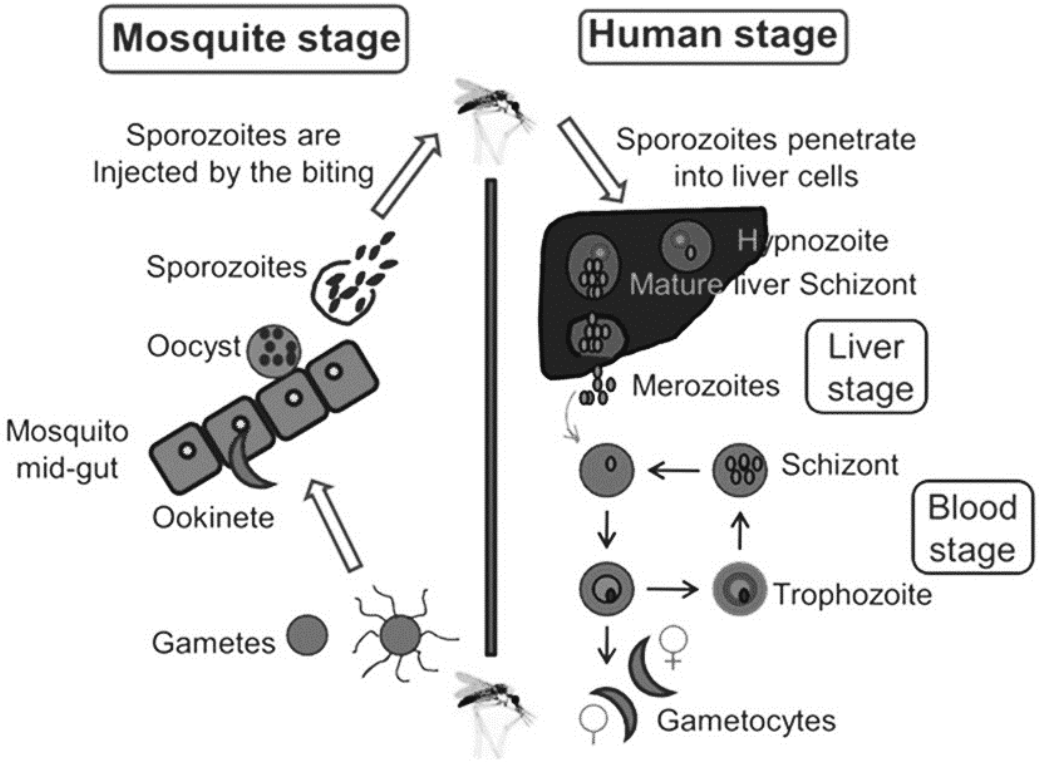
Finding	*P. falciparum*	*P. vivax*	*P. malariae*	*P. ovale*
RBC Size	Not enlarged	Enlarged	Not enlarged	Enlarged
RBC Shape	Round, sometimes crenated	Round or oval, frequently bizarre	Round	Round or oval, often fimbriated
RBC Colour	Normal, but may become darker; may have a purple rim	Normal to pale	Normal	Normal
Stipling	Maurer's spots, appear as large red spots, loops and clefts; up to 20 or fewer.	Schuffner's dots, appear as small red dots, numerous.	Ziemann's dots, few tiny dots, rarely detected	Schuffner's dots (James's dots). Numerous small red dots.
Pigment	Black or dark brown; in asexual forms as one or two masses; in gametocytes as about 12 rods	Seen as a haze of fine golden brown granules scattered through the cytoplasm	Black or brown coarse granules; scattered	Intermediate between P. vivax and *P. malariae*
Early trophozoite (ring)	Smallest, delicate; sometimes two chromatin dots; multiple rings commonly found	Relatively large; one chromatin dot, sometimes two; often two rings in one cell	Compact; one chromatin dot; single	Compact; one chromatin dot; single
Schizont	Medium size; compact; numerous chromatin masses; coarse pigments; rarely seen in peripheral blood	Large; amoeboid; numerous chromatin masses; fine pigments	Small; compact; few chromatin masses; coarse pigments	Medium size; compact; few chromatin masses; coarse pigments

Gametocyte	Crescent shaped, larger and slender; central chromatin	Spherical; compact	Similar to *P. vivax*, but smaller and less numerous	Like *P. vivax*, but smaller

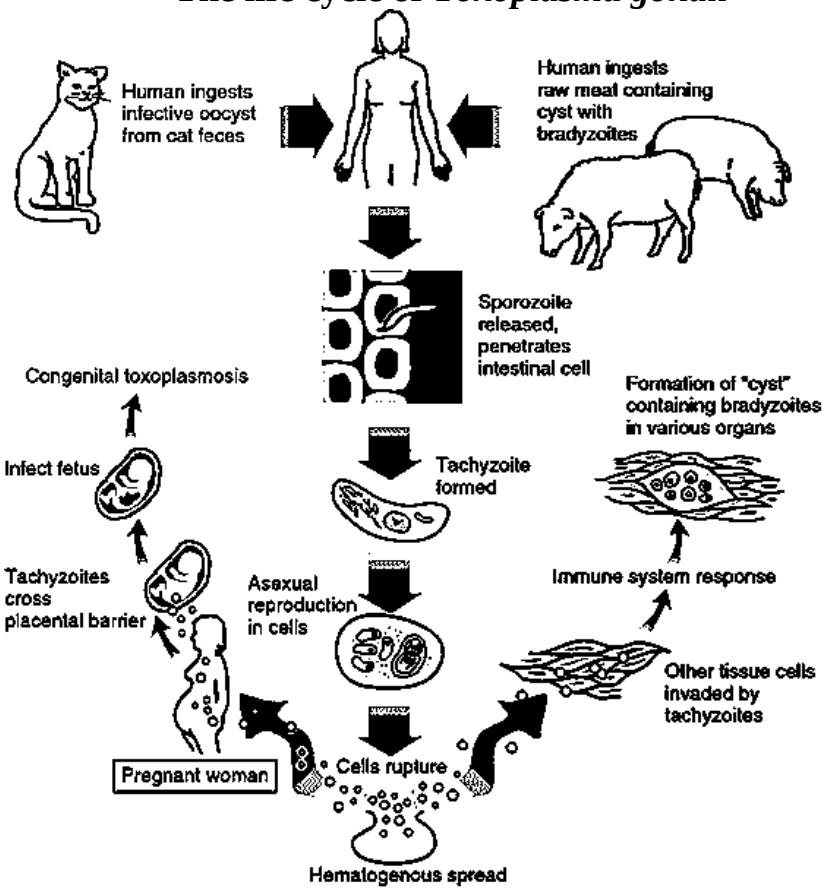
Morphology of *Apicomplexan*



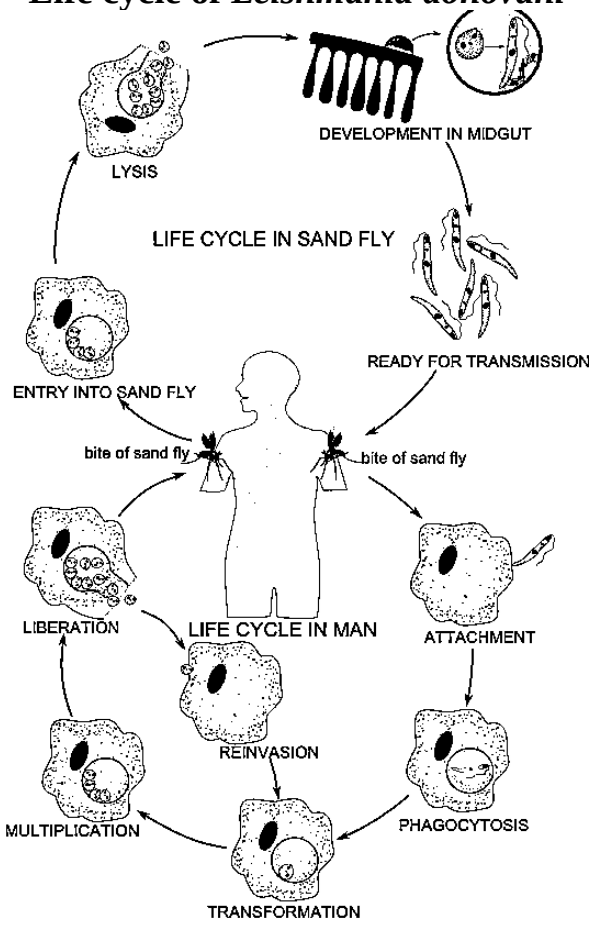
The life cycle of malaria parasite



The life cycle of *Toxoplasma gondii*

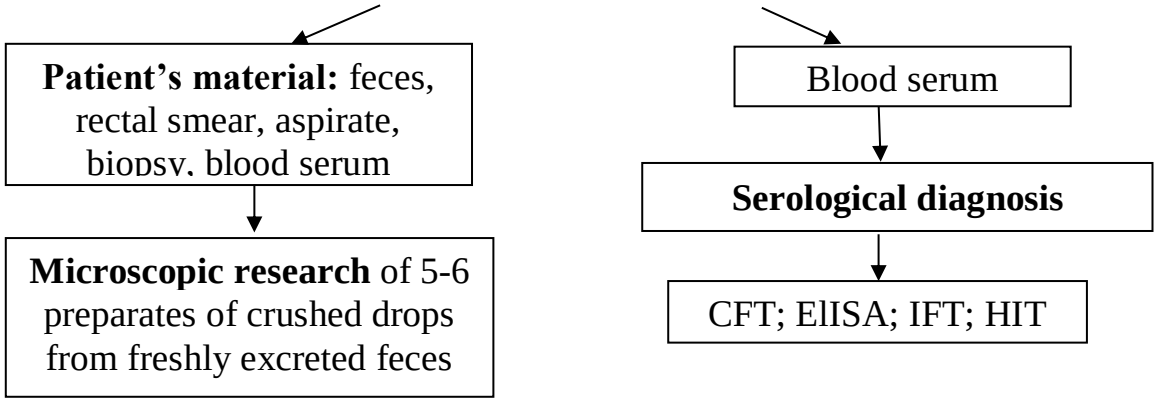


Life cycle of *Leishmania donovani*

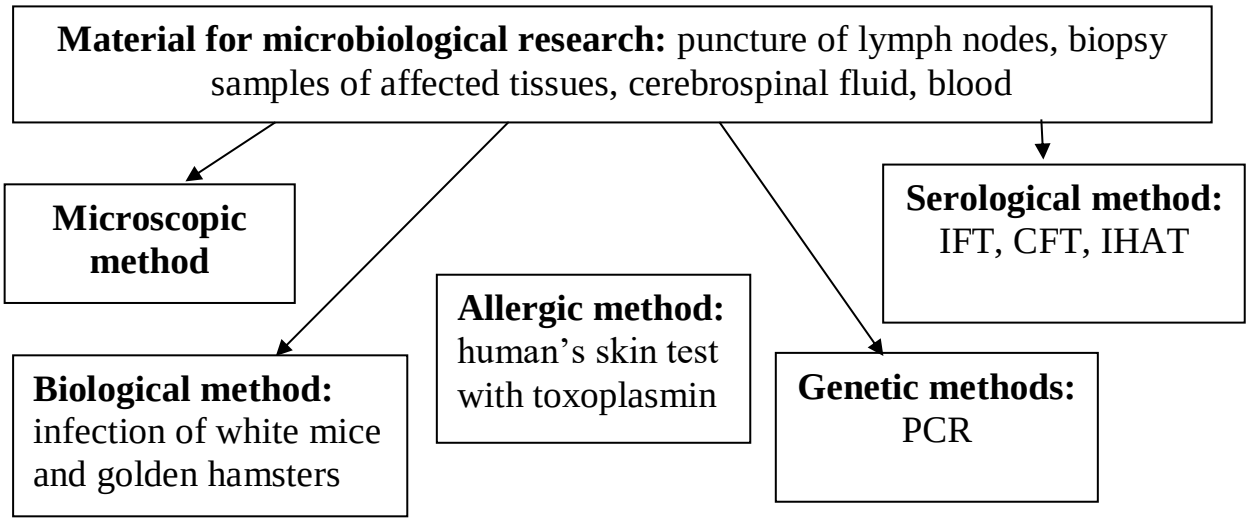


Practical activity № 3. To create the scheme of laboratory diagnosis of toxoplasmosis and amebiasis.

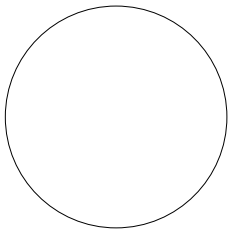
Laboratory diagnosis of amoebiasis



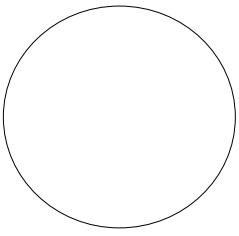
Laboratory diagnosis of toxoplasmosis



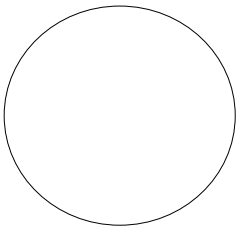
Practical activity № 1. To examine the smears with pathogenic protozoa in patient's material stained by Romanowsky-Giemsa.



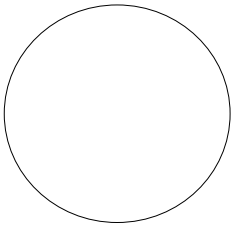
Entamoeba histolytica



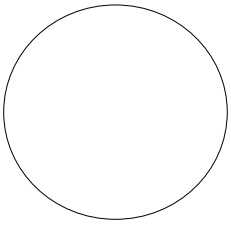
Leishmania donovani



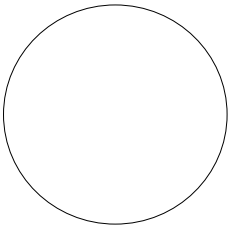
Trypanosoma brucei



Lamblia intestinalis

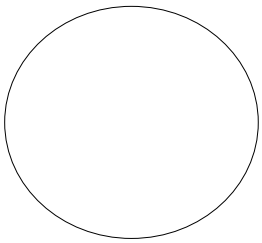


Plasmodium vivax



Toxoplasma gondii

Practical activity № 2. To check the results of immune fluorescence essay for diagnostics of toxoplasmosis.



Lymph node punctate

Practical activity № 4. To write down the medicines for treatment of deseases caused by protozoa.

- **The medicines for treatment of leishmaniasis:** *solyusurmin, hlyukantym, monomitsin, aminohinol*.
- **The medicines for prevention and treatment of malaria:** *quinine, mefloquine, mepacrine, prymahin, bimuhal.*
- **The medicines for treatment of toxoplasmosis**: *pyrimethamine, sulfonamides, spiramycin.*

Control questions:

- Morphology of malaria parasites.
- Epidemiology and pathogenesis of malaria, immunity.
- Methods of laboratory diagnosis of malaria. Differentiation of malaria parasites.
- Major trends in the prevention and treatment of malaria.
- Morphology and development cycle of causative agents of toxoplasmosis.
- The pathogenesis of toxoplasmosis.
- Methods of laboratory diagnosis of toxoplasmosis, prevention of disease.
- The causative agent of amebiasis. Morphology. The pathogenesis of the disease.
- Laboratory diagnosis and prevention of amebiasis.
- Trichomonas. Morphology. Cultivation.
- Pathogenesis, laboratory diagnostics, prevention and treatment of trichomoniasis.
- Leishmania. Morphology.
- Pathogenesis, laboratory diagnostics, prevention and treatment of cutaneous leishmaniasis.
- Trypanosoma. Morphology. Pathogenesis, laboratory diagnostics, prevention and treatment of African trypanosomiasis.
- Pathogenesis, laboratory diagnostics, prevention and treatment of American trypanosomiasis.

RECOMMENDED LITERATURE.

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