DANYLO HALYTSKY LVIV NATIONAL MEDICAL UNIVERSITY

Department of Microbiology

«APPROVED»
First vice-rector for scientific and pedagogical work
Assos. Prof. I.I. Solonynko
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WORKING CURRICULUM OF DISCIPLINE
MICROBIOLOGY, VIROLOGY AND IMMUNOLOGY

preparation of specialists of the second (master's) level of higher education

in the field of knowledge 22 "Healthcare"

in the specialty 222 «Medicine»

Discussed and approved
on the methodical meeting
of the department of microbiology
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Head of the department
O.P. Korniychuk, MD

«APPROVED» at the sitting of the cyclic methodical commission
on the preventive medicine
Protocol No 4
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Head of the commission
Professor V.I. Fedorenko

Methodical guide was composed by:O.Korniychuk, MD, Head of the Microbiology Department, assoc. prof., PhD S.Pavliy, assoc. prof. PhD M.Panas, assoc. prof. PhD I.Tymchuk, ass. A. Hural, ass. Y. Konechnyi

Methodical guide was discussed and approved at the sitting of the cyclic methodical commission on preventive medicine
protocol_____.

THEMATIC PLAN

Practical class 1

I. Subject: Microbiological aspects of COVID-19. Staphylococci. Streptococci. Microbiological diagnosis of staphylococcal and streptococcal infections.

II. Purpose and tasks: mastering of the methods of investigation of morphological, culture, biochemical, toxigenic and pathogenic properties of staphylococci and streptococci. Study pathogenesis of diseases and drugs employed for diagnosis, specific prevention and treatment of gram- positive diseases caused by pathogenic gram-positive cocci.

III. Test questions to the theoretical part of the class: a) classification of pathogenic cocci; b) morphological, culture, biochemical, toxigenic and antigenic properties of pathogenic staphylococci and streptococci; c) pathogenesis of diseases caused by pathogenic staphylococci and streptococci; d) drugs for specific prevention; e) drugs for specific treatment.

IV. MCQ Tests

1. Ability of some kinds of staphylococcus to cause in people food poisoning it is connected to production:
A. Endotoxin
B. Alfa-toxin
C. Enterotoxin
D. Exfoliatin
E. Hemolysin

2. Staphylococcus aureus can best be differentiated from *Staphylococcus epidermidis* by:
A. Gram's stain
B. Morphology of colonies
C. Coagulase production
D. Catalase production
E. Cell diameter

3. The toxin produced by *Staphylococcus aureus* that is responsible for the staphylococcal scalded skin syndrome is:
A. The Panton-Valentine leukocidin
B. Exfoliatin
C. Protein A
D. Alpha toxin
E. Beta toxin

4. Which of the following is a serious complication that may develop after recovery from strept. throat :
A. Scarlet fever
B. Acute glomerulonephritis
C. Rheumatic fever
D. A and B are correct
E. B and C are correct

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5. Acute glomerulonephritis, a consequence of throat infections, is actually caused by:

A. Immune complexes in the kidneys
B. Streptococcus pyogenes endotoxins
C. Immune complexes in throat
D. Streptococcus pyogenes exotoxins
E A and D correct

KROK tests

1. Examination of a patient with pustular skin lesions allowed to isolate a causative agent that forms in the blood agar roundish yellow middle-sized colonies surrounded by haemolysis zone. Smears from the colonies contain irregular-shaped clusters of gram-positive cocci. The culture is oxidase and catalase-positive, ferments mannitol and synthesizes plasmocoagulase. What causative agent was isolated?

A. Staphylococcus aureus
B. Streptococcus agalactiae
C. Streptococcus pyogenes
D. Staphylococcus epidermidis
E. Staphylococcus saprophyticus

2. Blood of a patient with presumable sepsis was inoculated into sugar broth. There appeared bottom sediment. Repeated inoculation into blood agar caused growth of small transparent round colonies surrounded by hemolysis zone. Examination of a smear from the sediment revealed gram-positive cocci in form of long chains. What microorganisms are present in blood of this patient?

A. Streptococci
B. Micrococci
C. Staphylococci
D. Tetracocci
E. Sarcina

V. Course of the practical class:

Stage 1 work. Study the morphology of staphylococci and streptococci. Examine preparations from staphylococci and streptococci pure cultures and smears from pathologic material under the microscope. Focus on the coloration of microorganisms stained by Gram's technique, on the placement of bacterial cells and presence of capsules.

Stage 2 work. Comparative study of the culture and biochemical properties of staphylococci, pneumonic and pyogenic streptococci. Focus on pigment formation of staphylococci. Focus on biochemical differentiation of *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Staphylococcus saprophyticus*. Draw a table of characteristic biochemical properties of staphylococci and streptococci by the following scheme:

Species	Lactose	Glucose	Mannitol	Mannitol in anaerobic condition.
S.aureus				
S. epidermidis				

Species	Glycerin	Inuline	Mannitol	Growth in 40% bile
S. pyogenes				
S. pneumonia				
S. faecalis				

Stage 3 work. Comparative study of presence or absent of virulent factors of staphylococci. Draw a table of characteristic virulent factors of staphylococci by the following scheme:

Species	Hemo-lysins	Plasmo-coagulase	Lecithinnase	Hyaluronidase	Toxins
S.aureus					
S.epidermidis					

Stage 4 work. Draw a table of characteristic virulent factors and their functions of streptococci by the following scheme:

Virulent factor	Function
Capsule	
M-protein	
Lipoteichoic acid	
Hyaluronidase	
Streptolysin O	
Streptolysin S	
Erythrogenic toxin	

Stage 5 work. Microbiological diagnosis of purulent processes. Inoculate the tested material (pus) on meat peptone agar, blood agar and another differential media for isolation of pure culture. Use the rules of isolation of bacterial pure culture.

Stage 6 work. Study the phagotype and antibiotic sensitivity of the staphylococci and streptococci. Determine the phagotype of staphylococcal strain on demonstration cultures and study antibioticograms.

Stage 7 work. Microbiological diagnosis of staphylococcal and streptococcal carriage. Inoculate the swabs, taken by the students from the throat of each other, into sugar broth.

Stage 8 work. Microbiological diagnosis of the diseases caused by Streptococcus pneumoniae. Make the scheme of microbiological investigation of the pneumonia case including into the scheme tests on animals and serological methods of identification.

Case studies

A 36-year-old male patient has an buccal abscess with a strain of Staphylococcus aureus that is beta-lactamase positive.

A. Which antibiotics does it indicate the body is resistant to?

B. What is the effect of beta-lactamase?

C. Describe methods to determine sensitivity to antibiotics.

VI. Tasks. Drawing up of the protocols. Make micrographs of the preparations (work 1). Draw the table (work 2). Make conclusion (work 3, 4, 5). Make the scheme of microbiological investigation (work 7, 8). Answer on the case studies.

Recommended literature:

1. Medical microbiology,virology and immunology=Медична мікробіологія, вірусологія та імунологія : a textbook for English-speaking students of higher medical schools: translation from ukr.Published / [T.V. Andrianova, V.V. Bobyr, V.V. Danyleichenko, ect.] ed. by V. P. Shyrobokov. Vinnytsia: Nova Knyha, 2019. - 744 p. : ill.

2. Medical microbiology and immunology = Медична мікробіологія та імунологія : підручник / Тимків М. З. , Корнійчук О. П., Павлій С. Й. [та ін.]. – Вінниця : Нова Книга, 2019. – 416 с.

3. Medical Microbiology. Patrick R. Murray. - 9th edition, Elsevier Inc. 2021. – 987p.

4. Ananthanarayan and Paniker's Textbook of Microbiology.- 10th ed.-N.Y., 2017.- P. 725.
5. Medical Microbiology. Jawetz, Melnick, Adelbergs – The McGraw-Hill Companies, Inc, 2013. – P. 877.

Practical class 2

I. Subject: Meningococci and gonococci. Microbiological diagnosis of diseases caused by meningococci and gonococci.

II. Purpose and tasks: study the biological properties of causative agents of meningitis and gonorrhea. Mastering of the methods of investigation of morphological, culture, biochemical, toxigenic and pathogenic properties of *Neisseria*. Study the drugs for diagnosis, specific prevention and treatment of diseases caused by meningococci and gonococci.

III. Test questions to the theoretical part of the class: a) classification of Neisseria spp.; b) morphological, culture, biochemical, toxigenic and antigenic properties of *Neisseria*; c) pathogenesis, clinical symptoms of diseases, cause by *Neisseria*, d) drugs for treatment and specific prevention of diseases caused by meningococci and gonococci.

IV. MCQ Tests

1. After seeding pus from a urethra on a special nutrient medium bluish colonies have grown. At microscopy of preparations from them revealed gram negative diplococci. The causative agent of what disease they are?
A. Tularemia
B. Syphilis
C. Gonorrhea
D. Meloidosis
E. Chlamidiosis
2. Certain way of contaminations of N. meningitis:
A. Sexualy
B. Respiratory
C. Alimentary
D. Bloodly
E. Contactly
3. The clinical materials for laboratory diagnostic for meningitis:
A. Urine
B. Blood
C. CSF
D. Smear from skin
E. Feces
4. *N.gonorrhea* is causative agent of:
A. Meningitis
B. Pneumoniae
C. Influensa
D. Vulvovaginitis
E. Angine

5. In diagnosis the causative organism of this disease is seen as pairs of gram-negative cocci contained within phagocytic leucocytes
A. Syphilis
B. Trichomoniasis.
C. Chlamidious
D. Genital herpes
E. Gonorrhea

KROK tests

1. Bacteriological examination of purulent discharges from the urethra revealed gram-negative bacteria looking like coffee beans. They were localized in the leukocytes and could decompose glucose and maltose to acid. These are the causative agents of the following disease:
A. Gonorrhoea
B. Syphilis
C. Veneral lymphogranulomatosis
D. Soft chancre
E. Melioidosis
2. On autopsy it is revealed: soft arachnoid membrane of the upper parts of cerebral hemisphere is plethoric, it is of yellowish-green color, soaked with purulent and fibrose exudate, it lookes like cap. For what disease is it characteristical picture
A. Meningococcal meningitis
B. Meningitis at typhus
C. Influenza meningitis
D. Meningitis at anthrax
E. Tuberculosis meningitis

V. Course of the practical class:

Stage 1 work. Study the morphology of pathogenic *Neisseria*. Examine preparations from *Neisseria meningitidis* and *Neisseria gonorrhea* pure cultures and smears from pathologic material under the microscope. Focus on the coloration of microorganisms stained by Gram's technique and on the placement of bacterial cells.

Stage 2 work. Comparative studying of the culture and biochemical properties of gonococci and meningococci. Draw the table of characteristic morphological, tinctorial, culture and biochemical properties of *Neisseria* by the following scheme:

Species	Morphology	Shape of colony	Pigment	Fermentation of sugars glucose	Maltose	Saccharose	Requirements to the factors of growth

Stage 3 work. Draw a table of characteristic virulent factors and their functions of meningococci and gonococci by the following scheme:

Virulent factor	Function
Capsule	
Pili	
outer-membrane protein	

LPS	
IgA protease	

Stage 4 work. Microbiological diagnosis of cerebrospinal meningitis. Make the scheme of diagnosis of cerebrospinal meningitis.

Stage 5 work. Microbiological diagnosis of gonorrhea. Detect the presence of gonococci in the smears stained with methylene blue. The causative agents are usually placed within leucocytes in clusters.

Stage 6 work. Study the drugs for diagnosis, treatment and prevention of diseases caused by *Neisseria*. Study the demonstration show cases and sets of drugs.

Case studies

A family brings their youngest child to the emergency room because of fever and a stiff neck. The 18-month-old child is acutely ill with a temperature of 40℃ (104.0 F). CSF is Gram stained, examined in a rapid test, and also cultured. A Gram stain shows pleomorphic, gram-negative bacteria looking like coffee beans.

A. What laboratory test could confirm the identity of the isolate?
B. What growth factors are required to grow the isolate on blood agar?
C. What is the drug of choice?

VI. Tasks. Drawing up of the protocols: draw micrographs and complete the table (works 1, 2, 3). Make the scheme (works 3, 4). Make the table "Characteristics of drugs for diagnosis, treatment and prevention of meningococcal and gonococcal infections" (work 6).

Recommended literature:

1. Medical microbiology,virology and immunology=Медична мікробіологія, вірусологія та імунологія : a textbook for English-speaking students of higher medical schools: translation from ukr.Published / [T.V. Andrianova, V.V. Bobyr, V.V. Danyleichenko, ect.] ed. by V. P. Shyrobokov. Vinnytsia: Nova Knyha, 2019. - 744 p. : ill.
2. Medical microbiology and immunology = Медична мікробіологія та імунологія : підручник / Тимків М. З. , Корнійчук О. П., Павлій С. Й. [та ін.]. – Вінниця : Нова Книга, 2019. – 416 с.
3. Medical Microbiology. Patrick R. Murray. - 9th edition, Elsevier Inc. 2021. – 987p.
4. Ananthanarayan and Paniker's Textbook of Microbiology.- 10th ed.-N.Y., 2017.- P. 725.
5. Medical Microbiology. Jawetz, Melnick, Adelbergs – The McGraw-Hill Companies, Inc, 2013. – P. 877.

Practical class 3

I. Subject: Common characteristics of family Enterobacteriaceae. Escherichia. Microbiological diagnosis of diseases caused by Escherichia coli.

II. **Purpose and tasks:** study the biological properties of enterobacteria, pathogenesis of escherichiosis and mastering of the microbiological method of diagnosis of enteritis caused by enteropathogenic *E.coli.*

III. Test questions to the theoretical part of the class: a) general characteristics of Enterobacteriaceae, characteristic of Escherichia coli (morphological, culture properties); b) basic biochemical characteristics of pure culture of *E.coli* to be considered at their identification; c) sources and ways of *E.coli* infection; d) antigenic structure of *E.coli*; e) factors of pathogenecity of *E.coli*, their genetic determinants, methods of microbiological diagnosis and drugs for treatment.

IV. MCQ Tests

1. Oxygen requirement of *Escherichia coli* is:
A. Obligate anaerobe
B. Aerobic
C. Facultative anaerobe
D. Microaerophilic
E. Capnophiles

2. The identification of bacteria by serologic tests is based on the presence of specific antigens. Which one of the following antigens presents in *E.coli*?
A. O-somatic, K-capsular, M-protein
B. K-capsular H-flagellar, O-somatic
C. H-flagellar and O-somatic, Vi-virulent
D. Vi-virulent and M-protein
E. Non about

3. A lactose- fermenting nosocomial pathogen that resides exclusively in the intestinal tract:
A. Escherichia coli
B. Klebsiella pneumoniae
C. Proteus mirabilis
D. Pseudomonas aeruginosae
E. Proteus vulgaris

4. The bacterium that produces a toxin that activates adenylate cyclase, resulting in an accumulation of cyclic AMP in the epithelial cells of the mucosal lining , is:
A. Escherichia coli
B. Klebsiella pneumoniae
C. Streptococcus pneumoniae
D. Staphylococcus aureus
E. Streptococcus pyogenes

5. Media for cultivation of *E.coli* are:
A. Nutrient media
B. Special media
C. Differential –diagnostic media
D. Simple media
E. Reducing media

KROK test

1. On bacteriological examination of the defecation of a 4-months-old baby with the symptoms of acute bowel infection there were revealed red colonies spread in the large quantity in the Endo environment. What microorganism can it be?
A. Escherichia

B. Salmonella
C. Staphylococcus
D. Streptococcus
E. Shigell

2. Among junior children of an orphanage an outbreak of intestinal infection with signs of colienteritis was registered. In order to identify isolated causative agent it is necessary to:
A. Study antigenic properties of the causative agent
B. To determine sensitivity to antibiotics
C. To study sensitivity to bacteriophages
D. To study biochemical properties of the causative agent
E. To study virulence of the causative agent

V. Course of the practical class:

Stage 1 work. Study morphological properties of *Echerichia coli*. Examine by microscopy the demonstration smears prepared from pure cultures of some *E.coli* species stained by Gram's method.

Stage 2 work. Study the kinds and general properties of basic nutrient media used for diagnosis of escherichiosis and other intestinal infections. Study the standard nutrient media manufactured and dispensed at the factories and demonstrative dishes with simple, elective and differential-diagnostic nutrient media, make their list, characterize their composition and peculiarities of employment.

Examine demonstrative cultures and characterize the colonies cultivated on the following media: meat-peptone agar, Endo's medium, Levin's medium, Ploskirev's agar, Mac-Conkey. Complete the table.

Species	Meat-peptone agar	Endo's	Ploskirev's	Levin's	Mac-Conkey
E.coli					

Stage 3 work. Study the biochemical differential-diagnostic properties of *E.coli*. Estimate the biochemical activity of *E.coli* in the demonstrative media of motley row (Hiss' medium). Complete the table.

Species	Lactose	Glucose	Mannitol	Saccharose	H_2S	Indole
E.coli						

Stage 4 work. Serological diagnosis of escherichiosis: examine a demonstration agglutination test.

Presumptive AT is performed on glass slides. Using a Pasteur pipette, transfer several drops of serum of low dilutions (1:10 -1:20) and a drop of isotonic saline for control on a grease-free glass slide. Into each drop of the serum as well as in the control drop, inoculate a loopful of 24-hour living culture of microorganisms from escherichiosis picked from the surface of a solid nutrient medium or pipette one drop of the suspension of dead microorganisms. The inoculated culture is to be thoroughly mixed until the drop of liquid becomes uniformly turbid.

The reaction is performed at room temperature. Inspect visually the results in 5-10 min; occasionally one may use a 5 x magnifying lens for this purpose. If the glass slides are placed into a humid closed chamber to prevent evaporation, the results of the test may be read in 30-40 min as well.

A positive test is indicated by the appearance of large or small flakes in the drop with serum, which are readily visible upon rocking of the cover-slip. In a negative test, the fluid remains uniformly turbid. In cases where the number of microorganisms is small and the results of the test are difficult to interpret, dry a drop of inoculated serum, fix the preparation, stain it with Pfeiffer's fuchsine, and study under the microscope. In a positive test, a microscopic field is largely free of microorganisms but they are accumulated in some places. In a negative test, microorganisms are uniformly distributed throughout the microscopic field. This test is known as microagglutination.

Stage 5 work. Study the kinds of *Escherichia coli* strains that cause enteric infections.

Strains	Pathogenic mechanism	Site of infection	Disease
Enterotoxigenic E.coli (ETEC)	Enterotoxin production	Small bowel	Diarrhea
Enteropathogenic E.coli (EPEC)	Cytotoxin production	Small bowel	Diarrhea
Enteroinvasive E.coli (EIEC)	Enterocyte invasion	Colon	Dysentery
Enterohemorrhagic E.coli(EHEC or VTEC)	Cytotoxin (verotoxin) production	Colon	Hemorrhagic colitis

Case studies

A 20-year-old man presents to the emergency department complaining of profuse bloody diarrhea of two days duration. On examination he has a purpuric rash over a large portion of his body, although his temperature is normal. The patient is dehydrated and weak, and lab values reveal an elevated blood urea nitrogen and creatinine, with thrombocytopenia. PT and PTT are within normal limits. Culture of the feces grew organ-isms which produced both colorless and colored colonies on sorbitol MacConkey medium.

A. What is your diagnosis?
B. What is the most likely source of his infection?
C. What is the mechanism of pathogenesis?

VI. Tasks. Drawing up of the protocols: make micrographs (work1). Describe the colonies of *Escherichia* on meat peptone agar and differential-diagnostic nutrient media (work 2). Make the table of biochemical properties of *Escherichia* (work 3). Describe the course of fulfilments of work about serological diagnosis (works 4).

Recommended literature:

1. Medical microbiology,virology and immunology=Медична мікробіологія, вірусологія та імунологія : a textbook for English-speaking students of higher medical schools: translation from ukr.Published / [T.V. Andrianova, V.V. Bobyr, V.V. Danyleichenko, ect.] ed. by V. P. Shyrobokov. Vinnytsia: Nova Knyha, 2019. - 744 p. : ill.
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5. Medical Microbiology. Jawetz, Melnick, Adelbergs – The McGraw-Hill Companies, Inc, 2013. – P. 877.

Practical class 4

I. Subject: Salmonella. Microbiological diagnosis of typhoid, paratyphoid fevers and gastroenteritis, caused by Salmonella spp.

II. **Purpose and tasks:** study the biological properties of *Salmonella*, pathogenesis of typhoid fever, paratyphoid fever and salmonellosis. Mastering of the microbiological method of diagnosis of diseases caused by *Salmonella.*

III. Test questions to the theoretical part of the class: a) comparative characteristic of *Salmonella* (morphological, culture properties); b) basic biochemical characteristics of *Salmonella* to be considered at their identification; c) sources and ways of diseases caused by *Salmonella*; d) antigenic structure of *Salmonella*; e) methods of typhoid fever microbiological diagnosis depending on the stage of the disease; f) methods of laboratory diagnosis of salmonellosis; g) preparations employed for diagnosis, treatment and prevention of diseases caused by *Salmonella*.

IV. MCQ Tests

1. Morpho-tinctorial properties of *Salmonella*:
A. Gram negative coccobacteria
B. Gram negative the big bacteria.
C .Gram negative streptobacilli.
D. Gram positive rods.
E. Gram negative rods, perytrichous
2. Media for cultural characteristics of *S. paratyphi A*:
A. Meat peptone agar
B. Blood agar
C. Nutrient agar
D. Serum agar
E. Mac Conkey`s medium
3. Methods of microbiological diagnosis of *Salmonella typhi*:
A. Biological
B. Bacterioscopical
C. Bacteriological
D. PCR
E. Mycological
4. Phenotypic characteristic of *Salmonella* spp. is:
A. Catalase "-"
B. Enveloped
C. Non-glucose fermented
D. Motility "+"
E. Lancefield nongroupable

5. When symptoms of typhoid fever first become apparent *S.typhi* is more frequently isolated from:
A. The feces
B. Purulent exudates
C. The sputum
D. The blood
E. The urine

KROK test

1. Bacteriological examination of a patient with food poisoning required inoculation of a pure culture of bacteria with the following properties: gram-negative movable bacillus that grows in the Endo's medium in form of colourless colonies. A representative of which species caused this disease?
A. Salmonella
B. Shigella
C. Iersinia
D. Esherichia
E. Citrobacter
2. Reaction of passive hemagglutination conducted with erythrocytic typhoid Vi-diagnosticum helped to reveal some antibodies in the dilution of the patient's serum at a ratio of 1:80 that exceeds the diagnostic titer. Such result witnesses of:
A. Being a potential carrier of typhoid bacilli
B. Being ill with acute typhoid fever
C. Typhoid fever recurrence
D. Incubation period of typhoid fever
E. Reconvalescence of a patient ill with typhoid fever

V. Course of the practical class:

Stage 1 work. Study morphological properties of Salmonella. Examine by microscopy the demonstration smears prepared from pure cultures of some Salmonella species stained by Gram's method. Compare morphological properties of investigated enterobacteria.

Stage 2 work. Study the kinds and general properties of basic nutrient media used for diagnosis of salmonellae. Examine demonstrative cultures and characterize the colonies cultivated on the following media: meat-peptone agar, Endo's medium, Levin's medium, Ploskirev's, bismuth-sulphate agar. Complete the table.

Species	Meat-peptone agar	Endo	Ploskirev	Levin	Mac-Conkey	Bismuth-sulphate agar
S.typhi						
S.paratyphi A						
S.paratyphi B						

Stage 3 work. Study the biochemical differential-diagnostic properties of *Salmonella*. Estimate the biochemical activity of salmonellae in the demonstrative media of motley row (Hiss' medium). Complete the table.

Species	Lactose	Glucose	Mannitol	Saccharose	H_2S	Indole
S.typhi						
S.paratyphi A						
S.paratyphi B						

Stage 4 work. Early diagnosis of typhoid and paratyphoid fevers – isolation of blood culture. Before hand have made inoculated 10 ml of blood of sick person into 100 ml of Rappoport's medium (the first stage). The second stage: describe macroscopical changes in the medium after 24 hrs incubation (turbidity, gas production). Prepare smear and stain it by Gram's technique. Reinoculate the material from Rappoport's medium on Wilson and Blair bismuth sulfite agar if monoculture of gram-negative rods is present in the smear. Continue the scheme of isolation of blood culture.

Stage 5 work. Isolation of a fecal and urine cultures. Feces and urine are preliminary inoculated into enrichment medium – selenite broth (first stage). The second stage: inoculate the material from selenite broth onto Wilson and Blair bismuth sulfite agar if monoculture of Gram negative rods is present in the smear. Continue the scheme of isolation of a fecal and urine cultures.

Stage 6 work. Serological diagnosis of typhoid and paratyphoid fevers. A) examine a demonstration Widal's test. Into three rows of test tubes are made the dilutions of serum of sick person (1:100 – 1:1600). The diagnosticum of typhoid fever is added into the first row of the test tubes, the diagnosticum of paratyphoid A is added into the second row of test tubes and the diagnosticum of paratyphoid B is added into third row of the test tubes. Determine a titer of an antibody to antigens of the causative agents of typhoid and paratyphoid fevers; b) indirect (passive) haemagglutination test. Serum of the sick person is diluted in the holes of a polyester plate (1:10 – 1:320) in the volume of 0.5 ml in 2 rows. Somatic "0_4"salmonella group B antigen is added into the test tubes of the first row, to the second row – somatic "0_9" salmonella group D antigen is added into the test tubes of the second row. Estimation of the result after 45 min. Determine the antibody's titer to antigens 0_4 and 0_9. Make conclusion about the role of Vidal's and passive (indirect) haemagglutination tests in diagnosis of typhoid and paratyphoid fevers.

Stage 7 work. Draw the table "Antigenic structure of *Salmonella*" (Kaufmann- White scheme)

Name of bacteria	Group	Antigen		
		O-somatic	H-flagellar	
			I phase (specific)	II phase (non-specific)
S. paratyphi A	A	1,2,12	a	-
S. paratyphi B	B	1,4,5,12	b	1,2
S. typhosa	D	9,12,vi	d	
S. typhimurium	B	1,4,5,12	i	1,2
S. enteritica	C	6,7	c	1,5
S. enteritidis	D	1,9,12	g, m	-

Stage 8 work. Make the scheme of microbiological diagnosis of gastroenteritis, caused by *Salmonella***.**

Stage 9 work. Make the table "Drugs for diagnosis, prevention and treatment of typhoid, paratyphoid fevers and salmonellosis".

Case studies

A 12-year-old boy with sickle cell disease presents to the emergency department with complaints of severe pain in the area of his right humerus. His temperature is 37.1°C (98.8°F), blood pressure is 100/60 mm Hg, pulse is 89/min, and respiratory rate is 22/min. Physical examination shows no other abnormalities. A radiograph is obtained that shows lytic changes and periosteal elevation in the middle and distal humeral shaft. Bacteriological examination of a patient required inoculation of a pure culture of bacteria with the following properties: gram-negative movable bacillus that grows in the Endo's medium in form of colourless colonies

A. What is your diagnosis?
B. What is the most likely pathogen responsible for this patient's condition?
C. What is the drug for treatment?

VI. Tasks. Drawing up of the protocols: make micrographs (work1). Describe the colonies of *Salmonella* on meat peptone agar and differential-diagnostic nutrient media (work 2). Make the table of biochemical properties of *Salmonella* (work 3). Describe the course of fulfilments of works about isolation of pure culture and serological diagnosis (works 4, 5, 6, 8). Make the tables (works 7, 9).

Recommended literature:

1. Medical microbiology,virology and immunology=Медична мікробіологія, вірусологія та імунологія : a textbook for English-speaking students of higher medical schools: translation from ukr.Published / [T.V. Andrianova, V.V. Bobyr, V.V. Danyleichenko, ect.] ed. by V. P. Shyrobokov. Vinnytsia: Nova Knyha, 2019. - 744 p. : ill.
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5. Medical Microbiology. Jawetz, Melnick, Adelbergs – The McGraw-Hill Companies, Inc, 2013. – P. 877.

Practical class 5

I. Subject: Shigella. Microbiological diagnosis of shigellosis. Vibrio. Microbiological diagnosis of cholera.

II. Purpose and tasks: study the biological properties of *Shigella*. Mastering of the microbiological methods of diagnosis of dysentery. Study the drugs for treatment of shigellosis. study the biological and pathogenic properties of *Vibrio cholerae,* epidemiology and methods of microbiological investigation of cholera. Study the drugs for diagnosis, specific prevention and treatment of cholera.

III. Test questions to the theoretical part of the class: a) classification, morphological, culture and biochemical properties of *Shigella*; b) sources and ways of transmission of dysentery; c) differentiation of shigella from others enterobacteria; d) methods of dysentery microbiological diagnosis; e) peculiarities of application and storage of the drugs used for diagnosis, prevention and treatment of dysentery. f) classification and characteristics of Vibrio cholerae and cholera-like vibrios: morphological, culture and biochemical properties, antigenic structure, biological and serological variations; g) sources and ways of transmission of cholera infection; h) microbiological diagnosis of cholera, express techniques of diagnosis; i) specific drugs for prevention and treatment of cholera.

IV. MCQ Tests

1. Phenotypic characteristic of *Shigella* spp. is:

A. Indole "-"
B. Lancefield nongroupable
C. Incomplete dsDNA
D. Urease "+"
E. Eggs have a small lateral spine

2. Therapy of *Shigella* spp. is:

A. Sodium stibogluconate
B. Chloramphenicol
C. Ceftriaxone
D. Cefazolin
E. Clindamycin

3. A person was admitted to a hospital with diarrhea and fever. Stools were grossly bloody and mucoid. Cultures of the stools subsequently grew *Shigella flexneri*. Which properties were basis for identification inoculated cultures?

A. Fermentative and antigenic
B. Morphological and tinctorial
C. Fermentative and toxicity
D. Morphological and antigenic
E. Cultural and morphological

4. Which of these is NOT a common enteric (stool) pathogen?

A. Enterobacter species
B. Salmonella species
C. Campylobacter species
D. Shigella species
E. Vibrio species

5. Shigella virulence factors include:

A. Low infectious dose
B. Shiga toxin
C. Bacterial replication inside of intestinal epithelial cells.
D. All of the above.
E. None of the about.

6. Stain of Vibrio cholerae is:

A. Modified acid fast "+"

B. Gram "+"
C. Gram "-"
D. Acid fast "+"
E. None of the above
7. Morbidity of Vibrio cholerae is:
A. Cholera gravis
B. Intestinal ulcers
C. Meningitis
D. Urethritis
E. Hepatoma
8. Comma-shaped rod, causes high-volume watery diarrhea:
A. Yersinsa pestis
B. Vibrio cholera
C. Bordetella pertussis
D. Sallmonela typhi
E. Escherechia coli
9. The clinical materials for laboratory diagnosis for cholera:
A. Urine
B. Blood
C. CSF
D. Smear from skin
E. Feces
10. Flagella surrounding a *Vibrio cholera* spp. are termed:
A. Monotrichous
B. Diplotrichous
C. Lophotrichous
D. Peritrichous
E. Amphitrichous

KROK test

1. Autopsy of a 46-year-old man revealed multiple brown-and-green layers and hemmorhages on the mucous membrane of rectum and sigmoid colon; slime and some blood in colon lumen; histologically - fibrinous colitis. In course of bacteriological analysis of colon contents S.Sonne were found. What is the most probable diagnosis?
A. Dysentery
B. Yersiniosis
C. Cholera
D. Crohns disease
E. Salmonellosis
2. Patient with diarrhoea was admitted to the infection unit. Gramnegative curved rod-like bacteria were founded on bacterioscopic examination of faecal masses. What is the most likely disease in this patient?
A. Cholera
B. Typhoid fever
C. Salmonellosis gastroenteritis

D. Diphtheria
E. Intestinal form of plague

V. Course of the practical class:

Stage 1 work. Comparative study of morphological properties of various types of Shigella and Vibrio. Examine by microscopy the demonstration smears of different types of Shigella and Vibrio stained by Gram's method.

Stage 2 work. Study the character of Shigella colonies on simple and differential-diagnostic media employed for dysentery diagnosis (meat-peptone agar, Endo's, Levin's, Ploskirev's media). Complete the table.

Species	Meat-peptone agar	Endo's	Ploskirev's	Levin's	Mac-Conkey
Shigella					

Stage 3 work. Study the biochemical properties of Shigella. Estimate biochemical activity of different types of Shigella in the demonstration Hiss's media. Draw the table of tests employed for determination of Shigella biochemical properties.

Species	Lactose	Glucose	Mannitol	Saccharose	H_2S	Indole
S.dysenteriae						
S.flexneri						
S.boydii						
S. sonnei						

Stage 4 work. Study the biochemical activity of cholera and cholera-like vibrios in Hiss's media and other differential-diagnostic media. Complete the table.

Species	Lactose	Saccharose	Mannose	Arabinose	H_2S	Indole
V.cholera classic						
V.El.Tor						
Cholera-like vibrio						

Stage 5 work. Study the differential signs of cholera causative agents and cholera-like vibrio.

Tests	V.cholera classic	V.El.Tor	Cholera-like vibrio
Hemolysis			
Voges Proskauer			
Agglutination of chick erythrocytes			
Agglutination with O-antiserum			
Polymyxin B sensitivity			
Group IV phage susceptibility			

Stage 6 work. Serological diagnosis of dysentery. Estimate of indirect haemagglutination test. The serum of sick person is diluted in two rows of polis tyrol holes (1:10 – 1:320) in the volume of 0.5 ml. Sonnei's erythrocytic diagnosticum was added into the first row of the holes and Flexneri's erythrocytic diagnosticum was added into the second row.

Stage 7 work. Study the scheme of microbiological diagnosis of cholera. Draw the scheme of cholera microbiological investigation with the use of a demonstrative table. Indicate differential properties of vibrios, stages of investigation and rapid methods of diagnosis.

Stage 8 work. Study the drugs for diagnosis, prevention and treatment of shigellosis and cholera. Familiarize with a set of specific drugs displayed in demonstrative show cases; indicate the purpose, method and efficiency of their application.

Case studies

A 4-year-old girl is brought to the pediatrician after experiencing abdominal pain, vomiting, and diarrhea containing mucus and blood for the past 3 days. Several children in her daycare class have similar symptoms. Her temperature is 39.4°C. On examination she has lower abdominal tenderness and decreased bowel sounds. Stool culture and Gram stain reveal a straight, Gram-negative, oxidase-negative rod that produces white colonies on MacConkey agar, and does not cause blackening on triple sugar iron (TSI) slant.

A. What is your diagnosis?
B. What is the most likely pathogen responsible for this patient's condition?
C. What is the drug for treatment?

VI. Tasks. Drawing up of the protocols: make micrographs of some strains of Shigella and vibrio (work 1). Describe the characteristics of Shigella colonies on the nutrition and differentiation media (work 2). Make the table of tests employed for determination of Shigella biochemical properties (work 3). Make the scheme of microbiological diagnosis of cholerae Estimate of indirect haemagglutination test and make conclusion about antibodies presence (work 4). Make the table "Drugs for diagnosis, prevention and treatment of typhoid, paratyphoid fevers and dysentery" Make the table of the drugs for specific prevention, diagnoses and treatment of cholera (work 8).

Recommended literature:

1. Medical microbiology,virology and immunology=Медична мікробіологія, вірусологія та імунологія : a textbook for English-speaking students of higher medical schools: translation from ukr.Published / [T.V. Andrianova, V.V. Bobyr, V.V. Danyleichenko, ect.] ed. by V. P. Shyrobokov. Vinnytsia: Nova Knyha, 2019. - 744 p. : ill.
2. Medical microbiology and immunology = Медична мікробіологія та імунологія : підручник / Тимків М. З. , Корнійчук О. П., Павлій С. Й. [та ін.]. – Вінниця : Нова Книга, 2019. – 416 с.
3. Medical Microbiology. Patrick R. Murray. - 9th edition, Elsevier Inc. 2021. – 987p.

4. Ananthanarayan and Paniker's Textbook of Microbiology.- 10th ed.-N.Y., 2017.- P. 725.
5. Medical Microbiology. Jawetz, Melnick, Adelbergs – The McGraw-Hill Companies, Inc, 2013. – P. 877.

Practical class 6

I. Subject: Corynebacteria and Bordetella. Microbiological diagnosis of diphtheria and pertussis. Mycobacteria. Microbiological diagnosis of tuberculosis.

II. Purpose and tasks: study the biological properties of *Corynebacterium and Bordetella*); sources and ways of transmission of diseases. Mastering of the methods of microbiological diagnosis of diphtheria and pertussis; drugs for diagnosis, specific prevention, and etiotropic treatment. Study the biological properties of pathogenic mycobacteria, pathogenesis of disease caused by them, drugs for specific diagnosis, prevention and treatment. Mastering of the methods of microbiological diagnosis of tuberculosis.

III. Test questions to the theoretical part of the class: a) morphological peculiarities of diphtheriae rod to differentiate it with other Corynebacteria; b) the main biovars of causative agent of diphtheriae; c) media for cultivation of Corynebacterium diphtheriae; d) characteristics of the toxin of Corynebacterium diphtheria and methods of is determination; e) morphological, culture and biochemical properties of haemophilic bacteria. f) the methods of microbiological diagnosis of a diphtheria and pertussis; g) drugs for diphtheria and pertussis specific prevention and treatment. h)classification of mycobacteria , causative agents of tuberculosis; i) basic biological properties of mycobacteria; media for cultivation and their culture properties; j) factors of mycobacteria tuberculosis pathogenecity; k) sources and ways of infection, peculiarities of tuberculosis pathogenesis; l) significance of microscopic, bacteriological and experimental investigations for the diagnosis of tuberculosis ; m) allergy tests employed in tuberculosis; n) express methods of tuberculosis diagnosis; o) drugs for specific prevention and treatment of tuberculosis.

IV. MCQ tests

1. Media for *C.diphtheriae* cultivation:
A. Bordet-Gengou, coalcasein agar
B. Lowenshtein-Yensen, potato agar
C. Kitt-Tarozzi, milk
D. Tellurite agar, Loeffler serum
E. Endo, Levin
2. The primary virulence factor for *Corynebacterium diphtheria* is:
A. Enterotoxin
B. Endotoxin
C. Exotoxin
D. Capsule
E. Spore
3. For detection of diphtheriae toxin use:

A. Neutralization test
B. Indirect hemagglutination test
C. Precipitation test
D. Complement fixation test
E. Agglutination test.
4. Sputum smears of the patient with chronic pulmonary disease were stained by Ziel-Neelsen method and analysed in the bacteriological laboratory. Microscopy revealed red bacillus. What property of tuberculosis mycobacteria was found?
A. Alcohol resistance
B. Acid resistance
C. Alkali resistance
D. Spore formation
E. Encapsulation
5. Diagnosis of tuberculosis is by:
A. X ray
B. Mantoux
C. Acid-fast stain
D. Biological test
E. All of these

KROK test

1. While examining a patient an otolaryngologist noticed hyperaemia and significantly edematous tonsils with a grayish film upon them. Microscopical examination of this film revealed some gram-positive bacilli placed at an angle with each other. What disease might be suspected?
A. Diphtheria
B. Angina
C. Scarlet fever
D. Meningococcal nasopharyngitis
E. Epidemic parotitis
2. Microscopy of stained (Ziehl-Neelsen staining) smears taken from the sputum of a patient with chronic pulmonary disease revealed red bacilli. What property of tuberculosis bacillus was shown up?
A. Acid resistance
B. Alkali resistance
C. Alcohol resistance
D. Capsule formation
E. Sporification

V. Course of the practical class:

Stage 1 work. Study the morphology of diphtheria causative agents. Examine by microscopy: a) the smears of *Corynebacterium diphtheriae* and pseudodiphtheriae stained by Gram, Neisser and Loeffler techniques. Focus on their arrangements in the smear: they resemble clusters of matches or pins and may look like the letter V. *Corynebacterium pseudodiphtheriae* are arranged as a palisade or a stack. Bodies of diphtheria rods stained by Neisser's technique turn yellow and their volutine

inclusions – dark blue. Bodies of diphtheria rods stained by Loeffler's method turn light blue while their inclusions - dark blue.

Stage 2 work. Comparative study of the culture, biochemical and toxigenic properties of *C.diphtheriae:* a) study the demonstrative cultures of *C.diphtheriae* on differential-diagnostic media. Colonies cultivated on blood tellurite agar (0.04%) are convex; 1-2mm in diameter with the entire or ragged edges, typical malted sheen, their coloration may be dark grey or black due to the reduction of tellurite. On Buchin's medium, they are small, dark blue. Colonies cultivated on Loeffler's agar (slant serum agar), are small, granular with irregular edges, moist, creamy, glistening.

b) study the differential signs of Corynebacteria. Complete the table.

Tests	*C.diphtheriae*	*C. ulcerans*	*C. pseudodiphteriticum*
cystinase			
urease			
nitrate			
Gelatin liquefaction			
Fermentation of starch			

c) Detect toxigenicity of diphtheria strains by the method of diffuse precipitation in gel (Ouchterlony's method). Add 20% of normal horse serum into the melted agar precooled to 50^0C and pour the admixture into sterile Petri dishes. Soak a strip of filter paper (1.5 x 6 cm) with diluted antitoxic diphtheria serum (5000 AU in 1 ml). Place this paper onto the surface of solidified agar and dry a dish at 37^0C for 30 min. Inoculate the tested cultures in horizontal streaks on both sides of the filter paper. Control inoculation of pathogenic culture is compulsory to compare with the obtained results. Inoculations are incubated in the thermostat at 37^0 C for 24-48 hrs. If the culture possesses a property of toxin-production it spreads into the gel and meets antitoxic serum diffused in the gel. The precipitate which looks like a white line is formed at the site where the antigen and antibodies meet.

Stage 3 work. Serological method of diagnosis of diphtheria. Estimate the indirect haemagglutination test in diphtheria.

Stage 4 work. Microbiological diagnosis of the diseases caused by *Corynebacterium diphtheriae.* Make the schemes of microbiological diagnosis of diphtheria.

Stage 5 work. Study the drugs for specific diagnosis, prevention and treatment of diphtheria. Study the demonstration show cases and sets of drugs. Draw a table of the characteristics of drugs.

Stage 6 work. Comparative study of the culture and biochemical properties of haemophilic bacteria. Study the growth of *Bordetella pertussis* and *Bordetella parapertusis* on the media: casein-charcoal agar (brownish pigment is produced by B.pertussis), Bordet-Gengou glycerine potato blood agar (typical tiny colonies of *B. pertussis* are convex, smooth, raised, moist, shiny, glistening, with a slightly metalic or pearl-like luster, grey, resembling mercury drops. Colonies of *B. parapertussis* are somewhat larger and more opaque. They produce haemolyses zones around the

colonies). Study motley sets; focus on urease test of pertussis and parapertussis rods. Study antibioticograms, detect strains of haemophilic bacteria responsive and resistant to antibiotics. Complete the table of differential signs of *Bordetella*.

Tests	*B. pertussis*	*B. parapertussis*	B. bronchiseptica
Motility			
Growth on the Bordet-Gengou medium			
Growth on the MPA			
Urease			
Utilization of citrate			
Nitrate reduction			

Stage 7 work. Microbiological diagnosis of the diseases caused by haemophilic bacteria. *Task:* Make the schemes of microbiological diagnosis of pertussis.

Stage 8 work. Study morphological properties of mycobacteria. Examine by microscopy and make micrographs of the smears from pure cultures of Mycobacteria tuberculosis. Focus on their properties: long, slender, straight, or curved rods with a slight tendency to be filamentous or branching.

Stage 9 work. Study the culture properties of different types of tuberculosis rods and non-pathogenic mycobacteria. Study the demonstration cultures on Lowenstein-Jensen medium. Focus on the types of colonies and their pigmentation. Differentiate pathogenic mycobacteria from non-pathogenic ones taking into consideration their requirements to the nutrient media, temperature regimen and time of cultivation.

Stage 10 work. Microscopic investigation of the sample of sputum of a tuberculosis patient. Stain the smear by Ziehl-Nilssen technique. Detect causative agents in the preparation (bright red acid-fast bacilli against a blue background), estimate their quantity in the field of vision and make a micrograph of the smear.

Stage 11 work. Bacteriological diagnosis of tuberculosis. Study the scheme of bacteriological investigation of tuberculosis. Focus on the methods of microscopic investigations, as: enrichment methods (*homogenization and flotation*), preliminary treatment of the smear with 6 % acid, methods of identification of the isolated pure culture of causative agent with determination of morphological, culture, biochemical (niacin, catalase, peroxidases tests), its pathogenic properties, sensitivity to antibiotics and chemotherapeutic preparations by the dilution series method.

Complete the table of differential signs of Mycobacteria.

Tests	*M. tuberculosis*	*M. bovis*	*M. kansasii*
Niacin			
Catalase			
Nitrate reduction			
Peroxidase			
Urease			
Growth on the LJ medium			

Stage 12 work. Express diagnosis of tuberculosis *by Prise's technique*. Study by microscope the painted microcolonies on the glass slides; make micrograph and conclusion about important of the method.

Stage 13 work. Study the drugs for specific diagnosis, prevention and treatment of mycobacteriosis with the employment of demonstration show-cases and sets of drugs.

Case studies

Profilactic toxoid vaccination of a student's group was necessary because of a case of diphtheria.

A. Describe method of toxoid production.

B. Which type of immunity toxoid injection creates?

VI. Tasks. Drawing up of the protocols. Make micrographs of Corynebacteria diphtheriae, pseudodiphtheriae, *Bordetella pertussis* and *parapertusis* (works 1, 6). Write the results of comparative study of the culture, biochemical and toxigenic properties of Corynebacteria and Bordetella (works 2, 6). Write the result of the the indirect haemagglutination test (work 3). Draw: a) the schemes of laboratory diagnosis of diphtheria and pertussis; b) the table "Characteristics of drugs for diagnosis, treatment and prevention of diphtheria, pertussis" (works 4, 5, 7).

Recommended literature:

1. Medical microbiology,virology and immunology=Медична мікробіологія, вірусологія та імунологія : a textbook for English-speaking students of higher medical schools: translation from ukr.Published / [T.V. Andrianova, V.V. Bobyr, V.V. Danyleichenko, ect.] ed. by V. P. Shyrobokov. Vinnytsia: Nova Knyha, 2019. - 744 p. : ill.
2. Medical microbiology and immunology = Медична мікробіологія та імунологія : підручник / Тимків М. З. , Корнійчук О. П., Павлій С. Й. [та ін.]. – Вінниця : Нова Книга, 2019. – 416 с.
3. Medical Microbiology. Patrick R. Murray. - 9th edition, Elsevier Inc. 2021. – 987p.
4. Ananthanarayan and Paniker's Textbook of Microbiology.- 10th ed.-N.Y., 2017.- P. 725.
5. Medical Microbiology. Jawetz, Melnick, Adelbergs – The McGraw-Hill Companies, Inc, 2013. – P. 877.

Practical class 7

I. Subject: Causative agents of anaerobic diseases. Microbiological diagnosis of anaerobic diseases.

II. Purpose and tasks: Study the properties of pathogenic anaerobes, its role in pathology of human. Mastering of the methods of microbiological investigation in tetanus, gas gangrene and botulism. Study the principles and measures of specific prevention and treatment of its diseases.

III. Test questions to the theoretical part of the class: a) pathogenic anaerobes: their morphological, culture, biochemical, antigenic properties; b) toxins and others factors of pathogenecity of *Clostridium*; c) differentiation of gas gangrene

causative agents by biochemical properties; d) epidemiology, pathogenesis and clinical symptoms of clostridial infection, specificity of immunity; e) basic methods of laboratory diagnosis of tetanus, gas gangrene, and botulism: bacteriological and biological tests, express tests; f) drugs for prevention and treatment of tetanus, gas gangrene, and botulism; g) meaning of *Cl.difficile* in antibiotic-associated colitis.

IV.MCQ Tests

1. An organism is found to be a gram-positive, anaerobic, endospore-forming rod. It probably belongs to the genus:
A. Actinomyces
B. Clostridium
C. Bacillus
D. Listeria
E. Escherichia
2. The primary toxin associated with invasive *Cl.perfringens* is:
A. Neuraminidase
B. Alpha toxin
C. Hyaluronidase
D. Theta toxin
E. Collagenase
3. The person was selling “homemade” pork’s sausages on market. State sanitary inspector suspected falsifications of sausage. With help of what serological immune reaction can food substance be identified?
A. Precipitation test
B. Indirect hemagglutination test
C. Immunofluorescence test
D. Complement fixation test
E. Agglutination test.
4. Stain of *Clostridium tetani* is:
A. Gram "-"
B. Acid fast "+"
C. Gram "+"
D. Modified acid fast "+"
E. Acid fast
5. For definition of serotype of botulinum toxin can use:
A. Neutralization test
B. Indirect hemagglutination test
C. Immunofluorescence test
D. Complement fixation test
E. Agglutination test.

KROK test

1. Microscopical examination of a microbal culture revealed fusiform spore-forming microorganisms that get violet-blue Gram's stain. What microorganisms were revealed?
A. *Clostridia

B. Streptococci
C. Spirochaete
D. Actinomycete
E. Diplococci
2. A patient has food poisoning. Laboratory analysis revealed a culture of anaerobic gram-positive spore-forming bacteria. What is the most likely kind of the isolated causative agent?
A. *C. perfringens
B. P. mirabilis
C. Proteus vulgaris
D. Vibrio parahemolyticus
E. Esherichia coli

V. Course of the practical class:

Stage 1 work. Study morphological properties of the causative agents of anaerobic infections. Examine the smears of pure cultures from the sick persons and organs of experimental animals by microscope. Focus on the morphological characteristics: location, shape, size, Gram staining variations, spore- and capsule-formation.

Stage 2 work. Comparative study of the culture properties of pathogenic anaerobes. Study the culture properties of pathogenic anaerobes with the employment of demonstrative inoculations on the nutrient media and educational tables. Note the differences in the character of anaerobe's growth into Kitt-Tarozzi, Vinial-Veillon's tubes, onto Zeissler's medium, into milk, Wilson and Blair medium (the shapes of colonies, hemolytic properties, peculiarities of the changes in milk, saccharolytic properties, and ability to decompose ethers). Make the table of express diagnosis of gas gangrene on the basis of differences of the growth of the causative agents in the listed media.

	Kitt-Tarozzi	Vinial-Veillon	Wilson and Blair	Milk
C.perfringens				
C. novyi				
C. septicum				
C. histolyticum				

Stage 3 work. Study the stages of microbiological diagnosis of the diseases caused by pathogenic anaerobes. With the employment of charts, educational tables and demonstrative cultures, study the stages of microbiological diagnosis of tetanus, gas gangrene and botulism: a) ways of collection and preparation of the investigation material; b) inoculation into Kitt-Tarozzi medium to obtain greater numbers of bacteria; c) inoculation onto Zeisler's medium and subsequent subinoculation of a separate colony into the Kitt-Tarozzi medium for pure culture isolation; d) determination of biochemical and antigenic properties of isolated pure culture and its pathogenecity in experimental animals; e) neutralization test for definition of serotype of toxin. The toxin containing specimen inject into the 4 mouses. Then

antitoxic sera A is added for the first mouse, type B – for the second mouse, type C – for the third mouse, type D – for the fourth mouse. Test is positive (antitoxic sera is specific with toxin and neutralized this toxin) if mouse remain alive.

Stage 4 work. Study the drugs for laboratory diagnosis (polyvalent and type-specific toxin antisera, diagnostic test system for indirect haemagglutination test), prophylaxis and treatment of anaerobic infections (toxoids, botulinic, gangrene and tetanus antisera). Study the employment of chemotherapeutic preparations and their combinations taking into consideration their synergetic action on the causative agents of anaerobic infections. Conducting this work, use the show cases with preparations.

Case studies

VI.Tasks. Drawing up of the protocols. Make micrographs of the smears (work 1); draw the schemes of microbiological diagnosis (works 2, 3); make a list of drugs for specific prevention and treatment of anaerobic infections and characterize them (work 4).

Recommended literature

David J.Hentges, Ph.D. Microbiology & Immunology: an illustrated review with questions and explanations. – 2hd ed. – Boston: Little, Brown and Company, 1995. – P. 104 – 108.

Nester E., Roberts E., Nester M. Microbiology. A human perspective. – Dubuque, Wm.C.Brown Publishers, 1995. – P. 19, 82 – 83, 95, 225, 256, 517, 528 – 530, 595, 600 – 604,719 – 722, 727.

Patrick R. Murray, Ken S. Rosenthal, George S. Kobayashi, Michael A. Pfaller Medical microbiology. - 3 rd ed. – St. Louis: Mosby, 1998. – P. 175 – 205.

Satish Gupte, MD The short textbook of Medical microbiology. – New Delhi: Jaypee Brothers, 1989. – P. 177 – 203.

Topley & Wilson's Principles of bacteriology, virology and immunity. – 8 th ed. – Philadelphia Hamilton: B.C. Decker Inc, 1992. – P. 120 – 136, 139 – 141.

Practical class 8

I. Subject: Causative agents of zoonotic infections. Microbiological diagnosis of zoonotic infections.

II. Purpose and tasks: Study the biological and pathogenic properties of causative agents of plague and anthrax, pathogenesis of diseases in human, epidemiology and the methods of microbiological diagnosis. Study the drugs for specific prevention and treatment of plague and anthrax.

III. Test questions to the theoretical part of the class: a) zoonotic infection – essentially dangerous diseases; b) the rules and precautions of taking clinical samples for microbiological investigations; c) morphological, culture and biochemical properties, antigenic structure of *Yersinia pestis*; d) sources and ways of transmission of plague; e) methods of microbiological diagnosis of plague, express methods (ELISA test, PCR); f) morphological, culture and biochemical properties, antigenic structure of *Bacillus anthracis*; g) sources and ways of transmission of anthrax; h)

methods of microbiological diagnosis of anthrax, express methods (ELISA test, PCR); i) drugs for specific diagnosis, prevention and treatment of zoonotic infections.

IV. MCQ tests

1. The bubo of bubonic plague is a:
A. Ulcer where the flea bite occured
B. Granuloma in the skin
C. Enlarge lymph node
D. Infected sebaceous gland
E. None the above
2. Transmission of *Yersinia pestis* (pneumonic form) is by:
A. Eat to flea
B. Flea to human
C. Human to human
D. Rat to human
E. None the above
3. Morpho-tinctorial properties of *B. anthracis:*
A. Gram negative coccobacteria
B. Gram negative the big bacteria.
C. Gram positive streptobacilli.
D. Gram positive rods.
E. Gram negative rods, peritrichous.
4. Anthrax results from exposure to:
A. Exotoxin
B. Spores
C. Endotoxin
D. Hyaluronidase
E. Lecithinase
5. Which infection would be categorized as a zoonosis?
A. Anthrax
B. Gas gangrene
C. Diphtheria
D. Typhoid fever
E. Cholera

V. Course of the practical class:

Stage 1 work. Study the rules of work in bacteriological laboratory, collection of infected material, its transportation and registration.

Stage 2 work. Study the morphological properties of the causative agents of zoonotic infections. Microscopy of the preparations from pure cultures of Y.pestis and B. anthracis and smears-imprint from animals' organs with plague and anthrax. Focus on the shape, size of cells, its placements after replication, relation to the Gram's stain technique, presence of capsules in bacillus from organs and spores in the smears from pure cultures.

Stage 3 work. Study the culture properties of *Yersinia pestis* and *Bacillus anthraci.* To examine and describe the demonstrative cultures of *Yersinia pestis* and Bacillus anthracis on the Petri dishes with meat peptone agar and in liquid media.

Stage 4 work. Study the differential signs of causative agents of plague and pseudotuberculosis. Make the scheme of microbiological diagnosis of plague with the use of express methods and differentiation with causative agent of pseudotuberculosis.

Tests		*Yersinia pestis*	*Yersinia pseudotuberculosis*
motility			
Fermentation of sugar	adonit		
	arabinose		
	xylose		
H_2S			

Stage 5 work. Study the differential sings of causative agents of Bacillus anthracis and Bacillus anthracoides. Make the scheme of microbiological diagnosis of anthrax.

Tests	*B.anthracis*	*B.anthracoides*	*B.subtilis*
Motility			
Capsule			
Blood agar			
Litmus milk			

Stage 6 work. Study the drugs for diagnosis, prevention and treatment of plaque and anthrax. Familiarize with a set of specific drugs displayed in demonstrative show cases, give characteristics of vaccines, antibiotics; indicate the purpose, method and efficiency of their application.

VI. Tasks. Drawing up of the protocols: Write down the rules (work 1). Make micrographs (work 2). Describe of cultural properties (work 3). Make the schemes of diagnosis (works 4, 5). Make the table of drugs for diagnosis, prevention and treatment of plague and anthrax (work 6).

Recommended literature:

David J.Hentges, Ph.D. Microbiology & Immunology: an illustrated review with questions and explanations. – 2hd ed. – Boston: Little, Brown and Company, 1995. – P. 103, 112 – 116.

Nester E., Roberts E., Nester M. Microbiology. A human perspective. – Dubuque, Wm.C.Brown Publishers, 1995. – P. 7, 79, 258, 338, 620 – 621, 748, 780.

Patrick R. Murray, Ken S. Rosenthal, George S. Kobayashi, Michael A. Pfaller Medical microbiology. - 3 rd ed. – St. Louis: Mosby, 1998. – P. 209 –210, 241, 269 – 274, 276 – 283.

Satish Gupte, MD The short textbook of Medical microbiology. – New Delhi: Jaypee Brothers, 1989. – P. 287 – 295.

Topley & Wilson's Principles of bacteriology, virology and immunity. – 8 th ed. – Philadelphia Hamilton: B.C. Decker Inc, 1992. – Vol. 3. - P. 365 – 423, 547 - 573.

Practical class 9

I. Subject: Spirochetes. Microbiological diagnosis of spirochetosis.

II. Purpose and tasks: Study the morphological properties, classification and methods of spirochetes cultivation; sources of infections and routes of transmission of the recurrent fever, syphilis, leptospirosis causative agents; basic methods of microbiological and serological diagnosis; drugs for treatment, prevention and diagnosis of these diseases.

III. Test questions to the theoretical part of the class: a) classification of Spirochetes, their biological properties, role in pathology of human; b) peculiarities of pathogenesis of syphilis – anthroponosis infection; c) important of microscopical method of investigation in diagnosis of syphilis (differentiation between pathogenic treponema and saprophytic representatives of this species); d) peculiarities of cultivation of treponema (culture and tissues forms); e) serological diagnosis of syphilis (seropositive and seronegative periods of syphilis); f) serological tests for diagnosis of syphilis with specific and non specific antigens; g) characteristics of the causative agent of recurrent fever, transmission routes, methods of detection of the causative agent; h) properties of leptospirosis causative agent, methods of microbiological investigation in leptospirosis; l) etiology, epidemiology and pathogenesis of Lyme disease, methods of microbiological diagnosis; j) drugs for the diagnosis, treatment and prevention of spirochetosis,.

IV. MCQ tests

1. A 50-year-old man develops an acute ulcerative gingivitis. Gram stain showed spirochetes. How do spirochetes typically behave in a Gram stain?

A. As gram negatives
B. As gram variables
C. As acid fast
D. As gram positives
E. Native smear

2. Diagnostic test of *Borrelia burgdorferi* is:

A. Visual stool examination
B. Antibody detection
C. Toxin detection
D. Direct antibody staining
E. Culture

3. Mode of transmission of *Leptospira interrogans* is:

A. Ingestion of infected beef
B. Ingestion of urine infected water
C. Ingestion of infected feline feces
D. Skin contact with larvae
E. Blackfly bites

4. Lyme disease is caused by:

A. Borrelia reccurents
B. Borrelia hermisii

C. Borrelia burgdorferi
D. Borrelia humanus
E. Borrelia bovis
5. The vector for transmission of Lyme disease is:
A. Mosquito
B. Flea
C. Tick
D. Louse
E. Rodents

V. Course of the practical class:

Stage 1 work. Study the morphological properties of spirochetes. Study the demonstrative smears painted by "negative method", Gram's and Romanovsky-Giemsa techniques. Focus on: the character of helixes, their quantity and tips of spirochetes.

Stage 2 work. Study the scheme of microbiological diagnosis of syphilis. Make the analysis of the scheme of microbiological diagnosis of syphilis, consideration of the methods of detection of causative agent and of the level of specific and non-specific antibodies.

Stage 3 work. Performance and estimation of the results of Wassermann reaction (with non-specific antigen). Ingredients: investigative serum; blood serum of syphilitic; blood serum of healthy person; antigen (the extract from lipids of the bull's heart); complement; haemolytic system (erythrocyte of ram + haemolytic serum). Reaction is performing in the 3-rd test tubes and final volume of all ingredients is 2.5 ml. Test tubes contain: №1 – investigative serum (0.5 ml), non-specific antigen (0.5 ml), complement (0.5 ml); №2 – blood serum of sick person (0.5 ml), non-specific antigen (0.5 ml), complement (0.5 ml); №3 – blood serum of healthy person (0.5 ml), antigen (0.5 ml), complement (0.5 ml). The test tubes are incubated at $37^{\circ}{}^{0}$C for 45 min. Then 10 ml of haemolytic serum were added into each of 3 test tubes. The test tubes are incubated again into the incubator at 37°C for 40 - 60 min. Estimation of reaction conduct in appear of complete haemolysis into test tube №3. Determine the delay of haemolysis (if complement is fixed with the complex of "antigen-antibody" in the first phase of reaction) in pluses: ++++, +++, ++, +. Make conclusion about presence or non-presence antibodies (Ig M) into investigative serum.

Stage 4 work. Study the methods of microbiological diagnosis of recurrent fever. Make the scheme of microbiological diagnosis of epidemic and endemic recurrent fevers according the methods of differentiation of the causative agents.

Stage 5 work. Study the method of microbiological diagnosis of leptospirosis. Make the scheme of microbiological diagnosis of leptospirosis with the employment of an educational table.

Stage 6 work. Study the drugs for diagnosis, treatment and prevention of spirochetosis. Study the show cases with the sets of preparations; draw the table of characteristics of the drugs for spirochetosis according to the following scheme: drugs for the diagnosis of syphilis; drugs for the treatment of syphilis.

Stage 7 work. Make the scheme of pathogenesis of Lyme disease.

VI. Tasks: Drawing up of the protocols. Draw the scheme of classification of Spirochetes and morphological peculiarities of Borellia, Leptospira, Treponema; make micrographs (work 1); make the analysis of the scheme (work 2); describe the Wassermann reaction and estimation of its results (work 3), make the scheme of microbiological diagnosis of epidemic and endemic recurrent fevers (work 4); make the scheme of microbiological diagnosis of leptospirosis (work 5); draw the table of characteristics of the drugs for diagnosis, treatment and prevention of the studied diseases (work 6).

Recommended Literature:

David J.Hentges, Ph.D. Microbiology & Immunology: an illustrated review with questions and explanations. – 2hd ed. – Boston: Little, Brown and Company, 1995. – P. 128 – 133.

Nester E., Roberts E., Nester M. Microbiology. A human perspective. – Dubuque, Wm.C.Brown Publishers, 1995. – P. 49, 221, 238 – 240, 433 – 435, 544, 547 – 548, 554 – 558, 745, 749 - 751, 761.

Patrick R. Murray, Ken S. Rosenthal, George S. Kobayashi, Michael A. Pfaller Medical microbiology. - 3 rd ed. – St. Louis: Mosby, 1998. – P. 331 – 347.

Satish Gupte, MD The short textbook of Medical microbiology. – New Delhi: Jaypee Brothers, 1989. – P. 304 - 314.

Topley & Wilson's Principles of bacteriology, virology and immunity. – 8 th ed. – Philadelphia Hamilton: B.C. Decker Inc, 1992. – Vol. 2. - P. 604 – 623.

Practical class 10

I. Subject: Rickettsia. Chlamydia, Mycoplasma. Microbiological diagnosis of chlamydiosis and mycoplasmosis.

II. Purpose and tasks: Study the morphological properties, classification, and methods of Rickettsia cultivation; sources of infections and routes of transmission of the rickettsiosis; basic methods of microbiological and serological diagnosis; drugs for treatment, prevention and diagnosis of these diseases. Study the morphological properties, classification, and methods of Chlamydia and Mycoplasma cultivation; sources of infections and routes of transmission of the chlamydiosis and mycoplasmosis causative agents; basic methods of microbiological and serological diagnosis; drugs for treatment, prevention and diagnosis of these diseases.

III. Test questions to the theoretical part of the class: a) classification of *Rickettsia*, their biological properties, role in pathology of human; b) the role of arthropod vectors in the spreading of rickettsia to humans; c) epidemiology, pathogenesis of typhus, Brill's disease; d) methods of microbiological diagnosis of epidemic typhus. e) biological properties of Chlamydia; f) epidemiology, pathogenesis of chlamydiosis; g) methods of diagnosis of chlamydiosis; h) biological properties of Mycoplasma; i) methods of microbiological diagnosis of mycoplasmosis; j) drugs for the diagnosis, treatment and prevention of chlamydiosis and mycoplasmosis.

IV. MCQ tests

1. Rickettsia are classified as obligate parasite because they require from the host:

A. ATP
B. NADH
C. Sterols
D. Electron transport chain
E. An anaerobe condition
2. Rocky Mountain Spotted Fever is caused by:
A. Rickettsia rickettsia
B. Rickettsia prowazekii
C. Coxiella burnetii
D. Rickettsia typhi
E. Rickettsia akari
3. Choose the correct statement about Rickettsia:
A. They are stained with Gram (gram-negative)
B. They form spores into the environment
C. Rickettsia are facultative parasites
D. Their cell wall include a lot amount of peptidoglycane
E. They are motile
4. All of the following are true about Weil-Felix test EXCEPT:
A. Utilizes cross reacting antibodies to *Treponemal* spp.
B. Is used in the diagnosis of rickettsial disease
C. Is an agglutination test
D. Is highly sensitive
E. Is highly specific
5. Miscellaneous bacterial agents such as Mycoplasma, Chlamydia are most often treated with:
A. Gentamicin
B. Chloramphenicol
C. Erythromycin
D. Cephalosporin
E. Quinolon
6. Mycoplasma have all of the following characteristics except:
A. Possession of both DNA and RNA
B. Capabiliy for cell-free growth
C. Susceptibility to penicillin G
D. Extracellular parasitism in vivo
E. Cause pneumonia
7. Each of the following statements concerning Chlamydia EXCEPT:
A. Contain muramic acid in the cell wall
B. Possess a Gram negative cell wall
C. Are obligate intracellular parasites
D. Can not grow in inanimate media
E. Appear as basophilic intracytoplasmic inclusion bodies on Giemsa stain

V. Course of the practical class:

Stage 1 work. Study the morphological properties of *Rickettsia*, its morphological variants.

Stage 2 work. Weil-Felix test for serological diagnosis of rickettsiosis. Slide method. On a glass slide a small amount (50–100 μL) of the patient's serum is placed. A single drop of the desired antigen is added, and the resulting suspension is mixed and then rotated for one minute. Visible agglutination is indicative of a positive result, and corresponds roughly to a titer of 1:20. Positive results can be further titrated using the tube method, which is more labour-intensive.

Tube method. Using 0.25% phenol saline as a diluent, a series of tubes containing twofold dilutions of patient serum are made with a final volume of 1 mL. A drop of antigen suspension is added to each tube, and the mixture is incubated at 50–55 °C for 4–6 hours. A positive tube would show visible flocculation or granulation, which is accentuated when the tube is gently agitated. The titer corresponds to the most dilute tube in the series that still shows positivity. Generally, a titer of ≥1:320 is considered diagnostic.

Stage 3 work. Study the drugs for diagnosis, treatment and prevention of rickettsiosis. Study the show cases with the sets of preparations. Write the list of the drugs for rickettsiosis tratment.

Stage 4 work. Study the morphological properties of Chlamydia, its morphological variants and course of development. Focus on Chlamydia inclusions in contaminated cells.

Stage 5 work. Study the method of express diagnosis of chlamydiosis. Detect the Chlamydia antigens in native clinical samples by direct and indirect immunofluorescence tests. Focus on typical green shine of Chlamydia inclusions in luminescent microscopy of contaminated cells.

Stage 6 work. Study the morphological properties of Mycoplasma, its pleomorphic shape

Stage 7 work. Study the culture properties of Mycoplasma. Estimate the character of growth of pure cultures of Mycoplasma onto solid medium by microscope (ocular x10, objective x7). Focus on the character of colonies' surface.

Stage 8 work. Serological diagnosis of mycoplasmosis. Estimate the results of complement fixation test with pair sera of a pneumonia patient using antigen of Mycoplasma pneumonia. Determine the titer and diagnostic importance of anti-mycoplasma antibodies.

Stage 9 work. Study the method of serological identification of Mycoplasma by the reaction of inhibition its growth by hyperimmune sera. Detect a zone of inhibition of mycoplsma growth around the disks with homological hyperimmune serum and presence of the growth near the discs with heterological serum.

Stage 10 work. Study of the drugs for diagnosis, treatment and prevention of chlamydiosis and mycoplasmosis. Study the show cases with the sets of preparations. Draw the list of the drugs for chlamydiosis and mycoplasmosis.

VI. Tasks: Drawing up of the protocols. Draw morphological peculiarities of *Rickketsia*, make micrographs (work 1); write the result of a serological test (work 2); draw the table of characteristics of the drugs for diagnosis, treatment and prevention of the studied diseases (work 3).

Recommended Literature:

David J.Hentges, Ph.D. Microbiology & Immunology: an illustrated review with questions and explanations. – 2hd ed. – Boston: Little, Brown and Company, 1995. – P. 128 – 133.

Nester E., Roberts E., Nester M. Microbiology. A human perspective. – Dubuque, Wm.C.Brown Publishers, 1995. – P. 49, 221, 238 – 240, 433 – 435, 544, 547 – 548, 554 – 558, 745, 749 - 751, 761.

Patrick R. Murray, Ken S. Rosenthal, George S. Kobayashi, Michael A. Pfaller Medical microbiology. - 3 rd ed. – St. Louis: Mosby, 1998. – P. 331 – 347.

Satish Gupte, MD The short textbook of Medical microbiology. – New Delhi: Jaypee Brothers, 1989. – P. 304 - 314.

Topley & Wilson's Principles of bacteriology, virology and immunity. – 8 th ed. – Philadelphia Hamilton: B.C. Decker Inc, 1992. – Vol. 2. - P. 604 – 623.

Practical class 11

I. Subject: Pathogenic fungi and actinomycetes.

II. Purpose and tasks: Study biological properties of fungi and *Actinomycetes*, pathogenesis of mycosis and actinomycosis, drugs for diagnosis, prevention and treatment of these diseases. Mastering of the methods of microbiological diagnosis of mycoses.

III. Control questions to the theoretical part of the practical class:

a) place of Actinomycetes and Fungi among the microorganisms;

b) classification of Fungi, their general biological characteristics and significance in microbiological industry; role of Fungi in the pathologies of human;

c) differential morphological features of Fungi, in particular, Yeast and yeast-like fungi;

d) causative agents of candidosis, conditions of their development, pathogenesis and microbiological diagnosis of candidosis;

e) characteristic features of mold fungi and pathologies which they cause;

i) differentiative signs of dermatomycetes, their role in the development of skin diseases and methods of their diagnosis;

j) causative agents of actinomycosis and deep mycoses (histoplasmosis, coccidioidosis, blastomycosis); importance of microbiological methods of investigation

IV. MCQ Tests

1. Fungi differ from bacteria :

A. They are eukaryotic

B. Contain both DNA and RNA

C. Contain cell walls

D. Can reproduce sexually

E. Can form spores.

2. Which of the following antibiotics are selectively toxic to fungi?

A. Amphotericin B

B. Erythromycin

C. Augmentin

D. Ceclor
E. Ampicillin.
3. The dermatomycoses are IMPOPTANT fungal diseases because the:
A. Infections tend to resist most forms of therapy.
B. Organisms are primarily found in the soil.
C. Organisms frequently cause epidemics.
D. Infections are often fatal.
E. All of the above
4. Stains useful for identifying fungus include:
A. Gram stain.
B. Haematoxylin and eosin.
C. Gomori methanamine silver .
D. PAS (periodic acid-Schiff.)
E. Giemsa.
5. True statements about Actinomyces israeli include:
A. It is a strict anaerobe
B. It is a bacteria
C. It causes chronic canaliculitis
D. It is usually sensitive to penicillin
E. Infection is associated with discharges containing yellow sulphur granules.

V. Course of the practical class:

Stage 1 work. Study the morphology of Fungi. *Task:* Prepare the smear by hanging drop technique in physiological solution from mycelium of cultures: aspergillus, penicillus and mucor fungi; Focus on: non separated mycelium and endospores of mucor; separated mycelium and exospores (conidia) of aspergillus and penicillus fungi – differential features of its mould fungi in microscopy.

Stage 2 work. Study the culture properties of aspergillus, penicillus, and mucor . *Task:* Study culture properties of aspergillus, penicillus, and mucor fungi on demonstrative cultures onto Saubourad's medium. Focus on presence of hyphae, got together in mycelium, and pigmentation of the spores.

Stage 3 work. Study laboratory diagnosis of aspergillosis, penicillosis, and mucorosis. *Task:* Make the scheme of laboratory diagnosis of aspergillosis, penicillosis, and mucorosis with the use of educational tables.

Stage 4 work. Study the morphology of yeast-like fungi. *Task:* Prepare the smear from the pure culture of yeast-like fungi and stain it by methylene blue. Focus on a shape, value of the cells and its arrangements after budding, pseudomicelium formation (stretching of separate cells, which don't unite of mutual envelope).

Stage 5 work. Study the smear from the mucus of urinogenital organs of candidosis patient. *Task:* Study the smear from the mucus of urinogenital organs of candidosis patient by microscope. Focus on all possible present elements of the smear: epithelial cells, bacteria, leucocytes, fungi.

Stage 6 work. Study the culture properties of Candida. *Task:* Study the culture of Candida on Sabouraud's medium. Study the methods of microbiological diagnosis of candidosis. *Task:* Make the scheme of microbiological diagnosis of candidosis.

Stage 7 work. Study the peculiarities of tissue forms of dermatomycetes. *Task:* Study the demonstrative "hanging drop" smears (in 20 percent solution of sodium oxygen hydrogen), prepared from the scales and hair damaged by dermatomycosis

Stage 8 work. Study the culture properties of dermatomycetes. *Task:* Study of the demonstrative cultures of dermatomycetes genuses: Trichophyton, Microsporum, and Achorion on Sabouraud's media.

Stage 9 work. Study the methods of microbiological diagnosis of dermatomycoses. *Task:* Make the scheme of microbiological diagnosis of dermatomycosis.

Stage 10 work. Study the morphology of Actinomycetes. Study the smear from the patient with actinomycosis. Focus on the "druza" formation. Study the methods of microbiological diagnosis of actinomycosis. Make the scheme of microbiological diagnosis of dermatomycosis.

Stage 11 work. Study the drugs for treatment of mycosis and actinomycosis. *Task:* Study the show cases with the sets of preparations; draw the table of characteristics of the drugs.

VI. Task. Drawing up the records . Describe the culture properties of aspergillus, penicillus, and mucor fungi (works 1-3). Make the scheme of laboratory diagnosis of aspergillosis, penicillosis, and mucorosis (work 3). Describe the culture properties of Candida (work 6) . Make the scheme of microbiological diagnosis of candidosis (work 6). Describe the dermatomycetes genuses: Trichophyton, Microsporum, and Achorion on Sabouraud's media. Make the scheme of microbiological diagnosis of dermatomycosis (work 9). Make the scheme of microbiological diagnosis of actinomycosis (work 10) . Draw the table of characteristics of the drugs (work 11).

Recommended literature

1. Ananthanarayan and Paniker's Textbook of Microbiology.- 7th ed.-N.Y., 2005.- P. 400 – 403, 610 - 628.
2. Fritz H. Kayser, Kurt A. Bienz, Johanenes Eckert, Rolf M. Zinkernagel Medical Microbiology. – Thieme, 2010. – P. 348 - 375.
3. Lakshman P. Samaranayake Essential Microbiology for Dentistry. – 3ed ed. – Elevier Limitid, 2006. – P. 127 – 132, 177 - 184.
4. Richard J. Lamont and Howard F. Wiley Oral Microbiology at a Glance, 1st ed.. – Blackwell Jenkinson, 2010.
5. Philip D. Marsh, Michael V. Martin Oral Microbiology Text and Evolve eBooks Package, 5th ed. – FRCPath FFGDPDCS (UK), 2009.
6. Jawetz Melnick & Adelbera's Medical Microbiology.- 25th ed.- Mc Graw Hill Medical, 2010. – P. 220 – 222, 621 - 658.
7. Michael J.Pelczar, JR, E.C.S. Chan, Noel R. Krieg Microbiology. – 5th ed. – Tata McGraw-Hill Publishing Company Limited, 2002.- P.850 - 877.
8. Kathleen Park Talaro, Arthur Talaro Fundations in Microbiology.- 4th ed.- Mc Graw Hill, 2002.- P. 597 – 598, 663 - 666.

Practical class 12

I. Subject: **Clinical microbiology. Features of diagnosis of COVID-19 as a nosocomial infection.**

II. Purpose and tasks: Estimation of the role of clinical microbiology in medical practice. Study the modern methods of performance of microbiological investigation. Study the main stages of investigation of clinical samples for the determination of etiology of an infectious process and means of its adequate medication.

III. Test questions to the theoretical part of the class: a) rules of the taking clinical material for microbiological investigations of opportunistic infections; b) representative genuses of causative agents of opportunistic infections; c) main properties of opportunistic bacteria; d) sources of specimens and its collection; e) methods of diagnosis of bacterial infections; f) importance of laboratory findings for a clinician and an epidemiologist; g) basic methods of investigation employed in clinical microbiology; h) methods of rapid diagnosis, indications for employment and their diagnostic mean; i) gram-positive and gram-negative causative agents of hospital infections; g) role of microbiological investigation in the choice of a rational antimicrobial therapy, make the schemes of microbial investigation of the sputum, faeces, urine, blood .

IV. MCQ tests

1. Clinical microbiology deals with the diseases:
A. Opportunist
B. Disbacteriosis
C. Hospital infection
D. Dangerous infection
E. Virulence infection
2. Laboratory test used for diagnosis of hospital disease:
A. Stain
B. Culture
C. Animal inoculation
D. Serologic
E. All of these
3. Microbiologic specimens for nosocomial infection diagnosis may be collected from:
A. Urine
B. Sputum
C. Feces
D. Blood
E. All of these
4. Members of the normal flora that are able to cause disease under special circumstances name:
A. Parasite
B. Pathogens
C. Opportunists
D. Protozoa
E. Virus

5. Opportunistic pathogens cause disease when:
A. Host's defences are compromised
B. They become established in part of the body that is not natural to them
C. They are capable of causing disease in healthy person
D. They are capable of causing disease in in persons with normal immune defences
E. A and B are correct

V. Course of the practical class:

Stage 1 work. Study the rules and precautions of taking clinical samples for microbiological investigations, ways of their transportation, storage and their preliminary treatment. Make the scheme of performance of microbiological investigations of the main kinds of clinical material (blood, feces, phlegm, pus, excreta from genitalia and urinary organs).

Septic condition
↓
Blood — Material
↓ ↓
The take way
↓
Cirsopunction — Cirsotomy — Fixed catheter
↓ ↓ ↓
Factors effecting on the possibility of contamination with foreign microflora:
↓

1. Following the rules of the treatment of skin.
2. Sterility of syringes and instruments.
3. Condition of the fixed catheter.
4. Following the rules and technique of inoculation.

Factors effecting the efficacy of haemoculture isolation:

1. Proper election of a nutrient medium by its composition and purpose.
2. Correspondence of blood amount to the amount of liquid medium.
3. Sufficient amount of the taken blood.
4. Proper moment of the blood take (peak of the temperature curve).
5. Preliminary chemo- and antibioticotherapy.
6. Time from the moment of taking blood to inoculation.
7. Prevention of the contact of tested material with disinfectants.

Stage 2 work. Study the modern methods of performance of microbiological investigation. Familiarise with the test-systems for isolation and identification of microorganisms, equipment for registering of obtained findings and methods of investigation (devices for ELISA, labelled antisera, and monoclonal antibodies). Study the patterns of answers received from modern laboratories. Make a conclusion on the diagnostic significance of the investigation data.

Stage 3 work. Study the main causative agents found in the clinical material. See a table.

Microorganism	Microscopy of	Media for primary	Methods of

material for investigation.	investigation material	inoculation	identification
1	2	3	4
Staphylococcus Blood Sputum Urine Liquor	 - + + +	Sugar broth (blood culture), Blood agar, milk salt agar, yolk salt agar	Rabbit citrate plasma, manitole (anaerobic conditions)
Streptococcus Blood Sputum Urine Liquor	 - + + +	Sugar broth, blood broth, Haro's medium	Precipitation test with groups antisera (A,B,C etc.) Presumptive agglutination test with typical antisera (detection of serovar)
Streptococcus pneumonia Sputum	+	Sugar broth, blood broth, Haro's medium, serum broth	Lysis into 20-40% bile, Hiss's row, inuline unlink (acid), arginine unlink (NH_3)
Meningococcus Blood Liquor	+ +	Serum broth, serum agar	Agglutination test with meningococcal antisera
Salmonella Blood Feces Urine Liquor (sometimes)	- - - -	10% bile broth, Rapopport's medium, selenite broth, Endo's agar, Levine's agar, Ploskirev's agar, Wilson and Blair bismuth sulphite medium	Hiss's row, Agglutination test with agglutination adsorpted antisera – A,B,C,D,E, and with 0-1 H-antisera.
Shigella Feces	 -	Ploskirev's agar	Hiss's row, Precipitation test with monoreseptor species antisera
Proteus Feces Urine	- -	Nutrient agar	Hiss's row, inoculation by Shukevich
Klebsiella Sputum Blood	 + +	Nutrient agar	Hiss's row, bile broth, capsule agglutination test on a glass, O-agglutination test on a glass
Bordetella		Bordet-Gengou's	Tyrosine medium, urea

Sputum	+	glycerine potato blood agar medium, caseino-charcoal medium	unlink, Agglutination test with pertussis and parapertussis antisera
Hemophilus Sputum Blood Liquor	 - - -	Blood agar, chocolate agar	Hiss's row, Agglutination test with type specific poli- and monoantisera
Pseudomonas aeruginosa Blood Feces Urine Liquor	 - - - -	Nutrient agar, 1% peptone water, Ploskirev's medium	Oxydase activity, catalase activity, glucose oxidation and fermentation
Food poisoning (Causative agent Clostridium perfringens) Feces Blood	 - -	Wilson and Blair bismuth sulphite medium, blood agar, nutrient agar, Kitt-Tarozzi medium	Litmus milk, Neutralization test for toxoid detect and it type

Stage 4 work. Make a scheme of microbiological investigation of the material to be tested according to the task and define the stages of investigation. Perform the investigation in subsequent stages (one stage at one practical class), then report obtained results of the investigation according to accepted form (see work 1).

Stage 5 work. Make a scheme of blood examination in septic process. Take into consideration a possibility of isolation of gram-positive and gram-negative bacteria – causative agents of septic process. Choose proper media. Describe characteristics of the culture on enrichment medium and other media. Make a schedule of the subsequent investigation corresponding to the type of a detected agent.

Stage 6 work. Make a scheme of microbiological investigation of sputum (purulent inflammation in the lungs). The scheme suggests quantitative study of the coccal microflora, microscopy of the smears, substantiated choice of the kinds and means of examination for the isolation of streptococci, staphylococci, haemophilic bacteria, *Klebsiella*, *Neisseria*, *Corynebacteria* and other possible causative agents of the process. On the basis of examination of demonstrative cultures, make a conclusion on etiological agents of the purulent inflammatory process.

Stage 7 work. Make a scheme of microbiological investigation of the discharge from the urogenital organs of a parturient female. The investigation suggests detection of coccal microflora, including *Neisseria*, gram-negative aerobic microflora and non-clostridial anaerobes. On the basis of examination of demonstrative culture, make a conclusion on etiological agents of the process; make a schedule of the subsequent investigation.

Stage 8 work. Make a scheme of microbiological investigation of urine in pyelonephritis. The investigation suggests a quantitative study of microflora and substantiated choice of methods and means of the detection of gram-positive and gram-negative bacteria as possible causative agents of the pathologic process. Having studied the demonstrative cultures, make a scheme of subsequent investigations.

Stage 9 work. Make a scheme of microbiological investigation of faeces. The investigation suggests inoculations in order to isolate *Salmonella, Shigella,* enteropathogenic *E. coli,* conditional pathogenic microorganisms and other possible causative agents. Make a scheme of a quantative study of microflora (*E. coli, B. bifidum, Lactobacilli, E.coli* with low fermentative activity and haemolytic *E. coli,* staphylococci, fungi). Make a conclusion on the presence of disbacteriosis taking into consideration the results of demonstrative cultures examination.

Stage 10 work. Study the role of microbiological investigation in the choice of a rational antimicrobial therapy. Examine and compare the results of antibioticograms of different strains of one kind of bacteria isolated from the patients. Choose proper antibiotics and chemopreparations for the treatment of these patients.

VI. Tasks. Drawing up of the protocols. Write a scheme of specimen collection rules and conditions for bacteriological investigation of different kinds of material (work 1); scheme of performance of rapid modern methods of microbiological investigation (work 2); write a scheme of the further investigation according to the found of microorganisms (works 3, 5, 6); write the scheme of investigation (work 4). Write the schemes of microbiological investigation (works 7-9). Write the results of examine of antibioticograms (work 10).

Recommendated literature:

Danileychenko V.,Bobrovnik A.,Korniychuk O Methodical guide to practical classes in clinical microbiology for the students of Medical and Dentistry faculties. – Lviv, 2002.

David J.Hentges, Ph.D. Microbiology & Immunology: an illustrated review with questions and explanations. – 2hd ed. – Boston: Little, Brown and Company, 1995. – P. 37 – 38.

Fischbach, FrancesTalaska A manual of Laboratory & Diagnostic tests. – 4 th ed. – Philadelphia: J.B. Lippincott Company, 1992. – P. 394 – 457.

Nester E., Roberts E., Nester M. Microbiology. A human perspective. – Dubuque, Wm.C.Brown Publishers, 1995. – P. 173, 408 – 409, 414 – 416.

Patrick R. Murray, Ken S. Rosenthal, George S. Kobayashi, Michael A. Pfaller Medical microbiology. - 3 rd ed. – St. Louis: Mosby, 1998. – P. 144 – 152, 169 – 175.

Practical class 13

I. Subject: **Ecology and microecology. Sanitary microbiology.**

II. Purpose and tasks: Study the methods of microbiological investigations of environmental objects Study the methods of human microflora investigation. Study the microflora of human body (the oral cavity, the respiratory tract, the ingestive tract, the genitourinary organs).

III. Test questions to the theoretical part of the class: a) type of ecological communications of microorganisms;
b) definition of microbiocenosis, symbiosis, commensalisms, satellitism, antagonism, parasitism;
c) normal microflora of the human body and its importance;
d) microflora of the main biotops of human body (representatives, peculiarities);
e) dysbacteriosis, mechanism of development, drugs for correction;
f) sanitary indicative microorganisms of the water, air and soil

IV. MCQ Tests

1. The largest density (greatest number) of bacteria on the surfaces of the human body is found on (in) the:
A. Skin
B. Lower gastrointestinal tract
C. Stomach
D. Lower respiratory tract
E. Mouth
2. The main carrier site on the human body for strains of potentially pathogenic Staphylococcus aureus is the :
A. Oral cavity
B. Throat (posterior nasopharynx)
C. Nasal membranes
D. Gastrointestinal tract
E. Vagina
3. The microorganism which has been isolated most frequently from infected tooth pulps is:
A.Yeast
B.Antinomyces
C.Streptococci
D.Lactobacilli
E.Staphylococci
4.Exotoxins:
A. Produce general symptoms
B. Are proteins
C. Are produced mainly by gram-negative organisms
D. Are heat stable
E. Are part of the bacterial cell wall
5. Which of the following describes an infection that does not cause noticeable illness?
A.Superinfection
B.Asymptomatic infection
C.Subclinical infection
D.Acute infection
E.Reinfection
1. Amount of microorganism in 1ml of water is:
A. Coli-titre

B. Coli-index
C. Microbial number
D. Perfringens titre
E. All of these
2. Sanitary indicate microorganisms of air are:
A. Hemolytic staphylococci
B. Escherichia coli
C. Enterobacteria
D. Clostridia perfringens
E. Candida albicans
3. An oligotrophic ecosystem would be most likely to exist in a:
A. Ocean
B. High mountain lake
C. Tropical pond
D. Polluted river
4. Which of the following would be accurate in detecting coliform bacteria in a water sample?
A. The standart plate count
B. Aspiration test
C. Sedimentation test
D. Fermentation test
E. All of these
5.In a relationship of symbiosis when the member receives benefits, while its coinhabitant is neither harmed nor benefited is:
A. Mutualism
B. Commensalism
C. Parasitism
D. Antagonism
E. Synergism

KROK tests

1. Sanitary bacteriological research on water by the membrane filter method revealed two red colonies on a membrane filter (Endo agar) through which 500 ml of analyzed water were passed. Calculate the coli index and coli titer of the analyzed water:
A. *4 and 250
B. 2 and 500
C. 250 and 4
D. 50 and 2
E. 250 and 2
2. As a result of durative antibiotic therapy a 37-year old patient developed intestinal dysbacteriosis. What type of drugs should be used in order to normalize intestinal microflora?
A. *Eubiotics
B. Sulfanilamides
C. Bacteriophages
D. Autovaccines

E. Vitamins

3. During the regular sanitary-epidemiological inspection of a pharmacy, the bacteriological analysis of air was performed. The air was found to have bacilli, yeast fungi, hemolytic streptococci, micrococci. Which of the detected microorganisms indicate the direct epidemic danger?

A. *Haemolytic streptococci

B. Micrococci

C. Yeast fungi

D. Bacilli

V. Course of the practical class:

Stage 1 work. Study microflora of human intestine. Study the following preparations: feces of a child and an adult; make micrographs.

Stage 2 work. Study microflora of the genitourinary tract: a) smear from mucus of man's urinary tract, stained by Zeihl-Neelsen method (Smegmatis rods); b) smear from vagine excretion, stained by Gram's method (Doderlein's bacillus).

Stage 3 work. Microflora of dental plaque. This is a general term for the diverse microbial community (predominantly bacteria) found on the tooth surface, embedded in a matrix of polymers of bacterial and salivary origin. Plaque develops naturally on teeth, and forms part of the host's defense systems by helping prevent colonization of the enamel by exogenous (and often pathogenic) microorganisms (colonization resistance). Plaque is an example of a biofilm; current research shows that the properties of bacteria associated with a surface in a biofilm can be markedly different from those of the same cells growing in liquid broth (planktonic cells). Plaque is found preferentially on protected and stagnant surfaces, and these are at the greatest risk of the disease.

Stage 4 work. Determination of *a microbial number* of potable water: Pour 1 ml of the examined water with a sterile pipette into 2 sterile Petri dishes. Add 15 ml of melted and precooled meat-peptone agar into each dish. Mix the contents of the dishes by slight rotating movement. Allow the dishes to remain in a horizontal position until the agar has solidified. Then place the dishes into the thermostat at 37°C for 24 hrs. Count the colonies which have grown up on the nutrient media, determine the average number of the colonies on one dish and compare it with the standard of maximum permissible contamination of water.

Stage 5 work. *Estimation of the Coli-titer of water by a fermentative method.* Pour 100 ml of water with a sterile pipette into 3 sterile flasks with concentrated *Eichman's medium*. Add 10 ml of the examined water into each of 3 test tubes containing 1 ml of concentrated Eichman's medium. Pour 1 ml of examined water into 3 test tubes with 10 ml of diluted Eichman's nutrient medium. Place all containers into the thermostat to incubate the inoculations at 42-43°C for 24 hrs. Then reinoculate those samples of water which showed turbidity and gas formation on the sectors of Petri dishes with Endo's nutrient medium. After the cultivation on Endo's medium, detect colonies with metallic luster, examine the smears from these colonies under the microscope and detect gram-negative rods. Check up the colonies by oxidation test the result of which is supposed to be negative. Determine Coli-titer

by means of an educational table. Make conclusions on the correspondence of water samples to the standards of potable water quality.

Stage 6 work. *Bacteriological study of the air. Inoculation of the air sample by a sedimentation method*. Take 3 Petri dishes (one with blood agar and 2 with meat-peptone agar), place a dish with meat-peptone agar on the floor and the dishes with meat-peptone agar and blood agar 1.5 m above the level of the floor, and allow them to stand open for 5 min. Then cover the dishes and place them into the thermostat at 36^0 C for 24 hrs. After cultivation, count the total number of colonies which have grown up on meat-peptone agar and the colonies with haemolysis on blood agar. Determine the number of microorganisms in 1 m^3 of air by Omelyansky's formula:

$$X = \frac{a \times 100 \times 1000 \times 5}{B \times 10 \times T}$$

In which X – is the number of microorganisms in 1 m^3 of the air; a – the number of colonies on the dish; B – the area of the dish; T – the time during which the dish remained open; 5 – the time in minutes in Omelyansky's formula; 10 – volume of the air in liters from which sedimentation of microorganisms occurs; 1000 – volume of the air in liters to be determined.

Using educational tables make conclusions on the degree of air pollution with microorganisms according to the standards for closed rooms.

Stage 7 work. Estimate a microbial number of the soil. Demonstrative inoculation: inoculate 1 ml of diluted soil from 1:1000 to 1:1000000 into 4 Petri dishes with melted nutrient agar. Place the material into the thermostat at 36^0C for 24 hrs. After cultivation, count the total number of colonies, count the average number of colonies taking into consideration the degree of dilution and compare it to the standards of permissible contamination of the soil.

Stage 8 work. Determination of Coli-titer of soil. Demonstrative growth: inoculated 1 ml of suspension from diluted of soil from 1 : 10 to 1 : 1000000 *on Kesler's medium* and cultivated into incubate at 37^0 C for 48 hrs. Performed reinoculation those samples of water which showed turbidity and gas formation on the sectors of Petri dishes with Endo's nutrient medium. After cultivation on Endo's medium, detect colonies with metallic luster, examine the smears from these colonies under the microscope and detect gram-negative rods. Determine Coli-titer by means of an educational table. Make conclusion.

Stage 9 work. Determination of Perfringens-titer of soil. Demonstrative material: inoculated 1 ml of suspension from diluted of soil from 1 : 10 to 1 : 1000000 into test-tubes with melted Wilson-Blair's medium and cultivated into the thermostat at 42 – 43^0 C for 24 hrs. Count the cultures and examine the smears prepared from round black colonies, which tear up of agar in the places of gas formation. Detection of characteristic gram-positive rods. Give sanitary-microbiological character of soil about perfringens-titre using the educational table.

VI. Tasks. Drawing up the records. Describe the course of work, make micrographs (works 1,2,3,4).

Recommended literature:

David J.Hentges, Ph.D. Microbiology & Immunology: an illustrated review with questions and explanations. – 2hd ed. – Boston: Little, Brown and Company, 1995. – P. 87 - 90, 100 – 102, 133 – 135.

Nester E., Roberts E., Nester M. Microbiology. A human perspective. – Dubuque, Wm.C.Brown Publishers, 1995. – P. 111, 123 – 128, 334 – 337, 668 – 702,

Patrick R. Murray, Ken S. Rosenthal, George S. Kobayashi, Michael A. Pfaller Medical microbiology. - 3 rd ed. – St. Louis: Mosby, 1998. – P. 152 - 159.

Satish Gupte, MD The short textbook of Medical microbiology. – New Delhi: Jaypee Brothers, 1989. – P. 77 - 81.

Topley & Wilson's Principles of bacteriology, virology and immunity. – 8 th ed. – Philadelphia Hamilton: B.C. Decker Inc, 1992. – Vol. 2. - P. 13.