

Taras Shevchenko National University of Kyiv
Educational and Scientific Centre “Institute of Biology and Medicine”

**Laboratory Notebook on
the discipline
“MICROBIOLOGY, VIROLOGY, AND IMMUNOLOGY”
for foreign student of specialization “Medicine”
Fifth (V) semester:
“SPECIAL, CLINICAL AND SANITARY MICROBIOLOGY”**

Student of the third academic year

(Student's full name)

(Student's group number)

Kyiv – 2024

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The Laboratory Notebook was considered and approved at the meeting of the methodical commission of the Educational and Scientific Centre “Institute of Biology and Medicine”, Taras Shevchenko National University of Kyiv.

Protocol № _____, «_____» _____ 2024

PROCEDURE OF PRACTICAL CLASS 1. STAPHYLOCOCCI AND STREPTOCOCCI

Main goal 1. Determine the stages of microbiological diagnosis of diseases caused by staphylococci

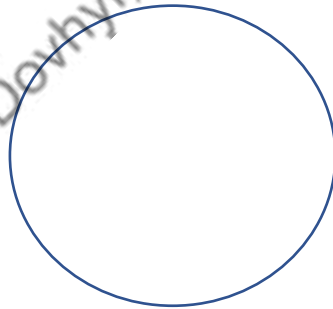
Task1. Study the rules of material sampling and procedure of its preparation for microbiological study:

1. Sampling of pathologic material with the swab from pharynx and wounds.
2. Sampling of cerebrospinal fluid by the puncture and obtaining blood from the ulnar vein.
3. Sampling of other pathological material from patient (vomit, gastric lavage, feces) and food leftovers for microbiological examination.

Task 2. Study the procedure of bacterial smear preparation from pathological material and its examination.

- 1) Make notes about the morphology and tinctorial properties of staphylococci.

- 2) Draw the preparation of staphylococci under the microscope.



Task 3. Study the nutrient media for the isolation of staphylococci from pathological material and cultural properties of staphylococci colonies.

- 1) Make notes about main components for preparation of elective-differential media and purpose of using these media.

2) Fill in the Table 1 with the main characteristics of the colonies.

Table 1. Morphological characteristics of isolated colonies and cells of microorganisms.

№	Biological material	Morphological characteristics of the colonies						Cell shape
		Size	Shape	Color	Margin	Surface	Consistency	

Task 4. Study the main biochemical tests for identification of pathogenic staphylococci from colonies on elective-differential media or obtained from the pure culture.

1) Make notes about principle of the test for determination the **catalase activity** of staphylococci and the expected results.

2) Make notes about principle of the test for determination the **coagulase activity** of staphylococci and the expected results.

3) Make notes about principle of the test for determination **DNase activity** of staphylococci and the expected results.

4) Make notes about principle of the test for determination the ability **to ferment glucose and mannitol under anaerobic conditions.**

5) Make notes about additional signs of pathogenicity of staphylococci.

Task 5. Study the main steps of staphylococci isolation from blood and their following examination.

Task 6. Study the main steps of identification of the staphylococcal enterotoxins.

Task 7. Study the main steps of identification of *S. aureus* carriers.

Task 8. Study the purpose and principles of the phagovar determination of *Staphylococcus aureus*.

Task 9. Study the main serological tests used for the diagnosis of staphylococcal infections. Make notes about the principles of these tests.

Task 10. Study the strategies for treatment of infections caused by pathogenic staphylococci. Make notes about effective antibiotics which are used for treatment.

Main goal 2. Determine the stages of microbiological diagnosis of diseases caused by streptococci

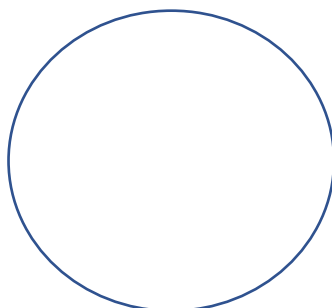
Task1. Study the rules of material sampling and procedure of its preparation for microbiological study:

- 1) Sampling of pathologic material with the swab from **pharynx and nous**. Note the pathogenic material: _____
- 2) Sampling of **cerebrospinal fluid** by the puncture and obtaining **blood** from the ulnar vain
- 3) Sampling of other pathological material from patient (**from skin vesicles, tonsils**) using different techniques.

Task 2. Study the procedure of bacterial smear preparation from pathological material and its examination.

1) Make notes about the morphology and tinctorial properties of streptococci.

2) Draw the preparation of streptococci under the microscope (*Streptococcus pyogenes*)



Task 3. Study the nutrient media for the isolation of streptococci from pathological material and cultural properties of streptococci colonies.

- 1) Make notes about main components for preparation of elective-differential media and purpose of using these media. Write the 3 types of hemolysis caused by different strains of *Streptococcus pyogenes*. Note the morphological differences between streptococci in smears from different media.

- 2) Fill in the Table 2 with the main characteristics of the colonies.

Table 2. Morphological characteristics of isolated colonies and cells of microorganisms.

№	Biological material	Morphological characteristics of the colonies						Cell shape
		Size	Shape	Color	Margin	Surface	Consistency	

Task 4. Study the main biochemical tests for identification of pathogenic streptococci from colonies on elective-differential media or obtained from the pure culture.

- 1) Make notes about principle of the test for determination the **catalase activity** of streptococci and the expected results.

- 2) Make notes about principle of the test for determination the sensitivity of streptococci to bacitracin and sulfonamides and the expected results.

- 3) Make notes about principle of the CAMP test and the expected results.

4) Make notes about principle of the test for **hydrolysis of sodium hippurate**.

5) Make notes about bile-esculin test for identification of pathogenic streptococci.

6) Make notes about determination of the pyrolydonylarylamidase enzyme (PYR test)

7) Make notes about **growth of pathogenic streptococci in broth containing 6.5% sodium chloride**.

8) Fill in the table 3 with the main biological properties of pathogenic streptococci

Table 3. The main biological properties of pathogenic streptococci

Properties	<i>S. pyogenes</i>	<i>S. agalactiae</i>	Group C and G streptococci	<i>S. bovis</i>
Type of hemolysis on blood agar				
Hydrolysis of sodium hippurate				
CAMP-test				
PYR-test				
Bile-esculin test				
Growth in broth supplemented with 6.5% sodium chloride				
Sensitivity to bacitracin				
Sensitivity to sulfamethoxazole and trimethoprim				

Task 5. Study the principles of determination of the group and serovar of streptococci. Make notes about the reactions are used for this purpose.

Task 6. Study the main serological tests used for the diagnosis of streptococcal infections. Make notes about the principles of these tests.

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Literature to get prepared for CLASS 1. Yi-Wei Tang, Max Sussman, Dongyou Liu, Ian Poxton, Joseph Schwartzman. Molecular Medical Microbiology. Volume 2. 2nd edition. – Copyright © 2015, 2002 Elsevier Ltd. – p.p. 655-673; 675-715.

PROCEDURE OF PRACTICAL CLASS 2. MENINGOCOCCI AND GONOCOCCI

Main goal 1. Determine the stages of microbiological diagnosis of diseases caused by meningococci

Task1. Study the rules of material sampling and procedure of its preparation for microbiological study:

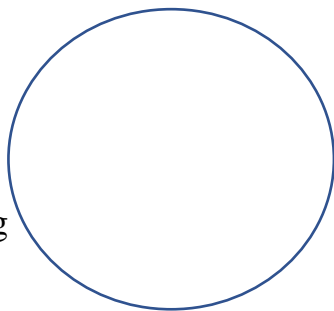
1. Sampling of pathological material (mucus) with the swab from **nasopharynx**
2. Sampling of **cerebrospinal fluid** by the puncture and obtaining **blood** from the ulnar vein

Task 2. Study the procedure of bacterial smear preparation from pathological material, staining, and its examination.

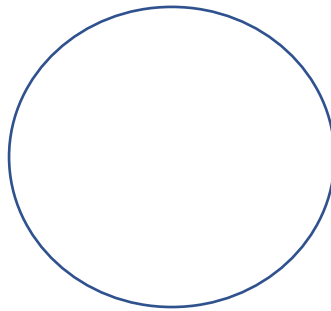
1) Make notes about the morphology and tinctorial properties of meningococci.

2) Draw the preparation of meningococci under the microscope (*Neisseria meningitidis*) stained by different techniques.

Gram staining



Methylene blue staining



Task 3. Study the nutrient media for the isolation of meningococci from pathological material and cultural properties of meningococcal colonies.

- 1) Make notes about main components for preparation of selective media and fastidious growth requirements of meningococci.

- 2) Fill in the Table 1 with the main characteristics of the colonies.

Table 1. Morphological characteristics of isolated colonies and cells of meningococci.

№	Biological material	Morphological characteristics of the colonies						Cell shape
		Size	Shape	Color	Margin	Surface	Consistency	

Task 4. Study the main biochemical tests for identification of pathogenic meningococci from colonies on selective media or obtained from the pure culture.

- 1) Make notes about principle of the test for determination the **catalase activity** of meningococci and the expected results.

2) Make notes about principle of the test for determination the **oxydase activity** of meningococci and the expected results.

3) Make notes about principle of the test for determination the ability **to oxidize glucose and maltose**.

4) Make notes about additional differential characteristics of meningococci.

Task 5. Study serological test used for the determination the serogroup of meningococci. Make notes about the principle of the test.

Task 6. Study serological tests used for the determination of specific antibodies in the serum of patient with meningitis. Make notes about serum level of immunoglobulins in different stages of infection.

Task 7. Study antibiotic susceptibility of meningococci. Make notes about resistance of Meningococcus to antibiotics and method used to determine it.

Main goal 2. Determine the stages of microbiological diagnosis of diseases caused by gonococci

Task1. Study the rules of material sampling from male and female patients, procedure of preparation of the pathological material for microbiological study:

- 1) Sampling of pathological material from **oropharynx** in patients with oropharyngeal gonorrhoea.

- 2) Sampling of the pathological material from **the urethra, its passage, the prostate gland, rectum** in male patients using different techniques.
 - 3) Sampling of the pathological material from **the urethra, paraurethral ducts, cervix, vagina, rectum** in female patients using different techniques.
 - 4) Name the diseases during which **blood** and **synovial fluid** are taken from patients with complicated gonococcal infections.
-

Task 2. Study the procedure of bacterial smear preparation from pathological material, different types of staining techniques, and its examination.

- 1) Make notes about the morphology and tinctorial properties of gonococci.
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- 2) Draw the preparation of gonococci (*Neisseria gonorrhoeae*) under the microscope stained by different techniques.



Task 3. Study the nutrient media for the isolation of gonococci from pathological material and cultural properties of gonococcal colonies.

- 1) Make notes about the main selective media used for identification of the gonococcus and their fastidious growth requirements.
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- 2) Fill in the Table 2 with the main characteristics of the colonies.

Table 2. Morphological characteristics of isolated colonies and cells of gonococci.

№	Biological material	Morphological characteristics of the colonies						Cell shape
		Size	Shape	Color	Margin	Surface	Consistency	

Task 4. Study the main biochemical tests for identification of gonococci from colonies on selective media or obtained from the pure culture.

- 1) Make notes about principle of the test for determination the **catalase activity** of gonococci and the expected results.

- 2) Make notes about principle of the test for determination the **oxidase activity** of gonococci and the expected results.

- 3) Make notes about principle of the test for determination the ability **to oxidize glucose**, maltose and sucrose.

Task 5. Study serological tests used for the determination of specific antibodies in the serum of patient with gonorrhoea. Make notes about the principle of the tests.

Task 6. Fill in the table 3 with the main biological properties of pathogenic *Neisseria* species.

Table 3. Differential biochemical characteristics of *Neisseria* species

Species	Growth on		Growth dependence on CO ₂ in the first generations	Pigment	Catalase	Oxidase	Carbohydrate fermentation					Reduction	
	MPA	Serum agar					Glucose	Maltose	Saccharose	Fructose	Lactose	NO ₂	NO ₃
<i>N. meningitidis</i>													
<i>N. gonorrhoeae</i>													
<i>N. subflava</i>													

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Instructor's Full name and Signature: _____

Literature to get prepared for CLASS 2. Yi-Wei Tang, Max Sussman, Dongyou Liu, Ian Poxton, Joseph Schwartzman. Molecular Medical Microbiology. Volume 3. 2nd edition. – Copyright © 2015, 2002 Elsevier Ltd. – p.p. 1471-1484.

PROCEDURE OF PRACTICAL CLASS 3. ESCHERICHIA

Main goal 1. Determine the stages of microbiological diagnosis of diseases caused by Escherichia

Task1. Study the rules of material sampling and procedure of its preparation for microbiological study:

1. Sampling of pathological material (**feces, vomit, gastro-intestinal lavage**) in case of food poisoning (enteritis);

2. Sampling of **cerebrospinal fluid, blood, pus, cerebrospinal fluid, discharge from the mucous membranes, urine, sputum** in different cases of escherichiosis.
3. Note about other materials which can be taken for microbiological examination in case of food poisoning

Task 2. Study the nutrient media for the isolation of *E. coli* from pathological material and cultural properties of their colonies.

- 1) Make notes about main components of selective and differential diagnostic media for the diagnosis of *E. coli* infections.

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- 2) Fill in the Table 1 with the main characteristics of the colonies on different media

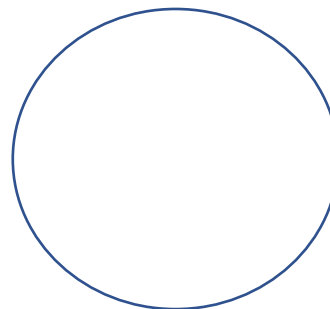
Table 1. Morphological characteristics of isolated colonies and cells of *E. coli*

Medium	Biological material	Morphological characteristics of the colonies						Cell shape
		Size	Shape	Color	Margin	Surface	Consistency	
Endo agar								
EMB/ Levine								
MacConkey agar								

Task 3. Study the procedure of bacterial smear preparation from *E. coli* colonies, staining, and its examination.

- 1) Make notes about the morphology and tinctorial properties of *E. coli*.

2) Draw the preparation of *E. coli* stained by Gram.



Task 4. Study the procedure of the pure culture isolation. Make notes about the media used to isolate *E. coli* pure culture and to examine its biochemical properties.

Task 5. Study the main biochemical tests for identification of pathogenic *E. coli* from colonies on selective media or obtained from the pure culture. Make notes about the ability to ferment **glucose, lactose**, sucrose, to form **indole, gas** and **acids**.

Task 6. Study serological test used for the determination the pathogenic serogroup of *E. coli*.

- 1) Make notes about the principle of the serotyping and the typing sera which are used to examine antigenic types of *E. coli* isolates.

- 2) Make notes about serogroups which are often detected in bacteremia and urinary tract infections.

Task 7. Study serological tests used for the determination of heat-labile enterotoxin production by the pathogenic *E. coli*. Make notes about the principle of the test.

Task 8. Fill in the table 2 with the main biological properties of pathogenic *E. coli*

Table 2. Differential biochemical characteristics of *E. coli*

Species	Carbohydrate fermentation					PP	
	Glucose	Maltose	Saccharose	Mannitol	Lactose	H ₂ S	Indole
<i>E. coli</i>							

Task 9. Study the groups of the pathogenic *E. coli* that cause gastroenteritis in humans.

Match the group with its characteristic properties.

1)	Enterotoxigenic <i>Escherichia coli</i> (ETEC)	aggregation of the bacteria in a “stacked-brick” manner
2)	Enteropathogenic <i>Escherichia coli</i> (EPEC)	cause dysentery-like diseases, ipaH gene presence
3)	Enteroinvasive <i>Escherichia coli</i> (EIEC)	cause bloody diarrhea, shiga toxin-producing
4)	Enterohemorrhagic <i>Escherichia coli</i> (EHEC)	cause cholera-like diseases, the heat-labile toxin (LT) gene presence
5)	Enteroadhesive <i>Escherichia coli</i> (EAEC)	cause classic <i>E. coli</i> enteritis, eae gene presence

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PROCEDURE OF PRACTICAL CLASS 4. SALMONELLA

Main goal 1. Determine the stages of microbiological diagnosis of diseases caused by *Salmonella*

Task1. Study the rules of material sampling and procedure of its preparation for microbiological study:

- 1) Sampling of pathological material (**feces, vomiting, washing waters, blood and urine**) from patients with typhoid fever and paratyphoid fever;

Note the about other materials which can be taken for microbiological examination in case of typhoid fever.

- 3) Sampling of **cerebrospinal fluid, blood, bone marrow, bile, urine** in different cases of salmonellosis.

- 4) Note about other materials which can be taken for microbiological examination in case of food poisoning and bacterial carriage.
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Task 2. Study the nutrient media for the isolation of *Salmonella* from pathological material and cultural properties of their colonies.

- 1) Make notes about main components of selective and differential diagnostic media for the diagnosis of *Salmonella* infections.
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- 2) Study the procedure of **blood culture, bone marrow culture, and bile culture** cultivation. Make notes about the selective medium which can be used for this purpose.

- 3) Fill in the Table 1 with the main characteristics of the colonies on different media.

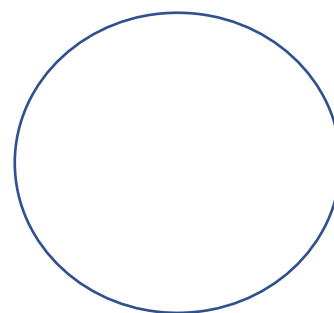
Table 1. Morphological characteristics of isolated colonies and cells of *Salmonella*

Medium	Morphological characteristics of the colonies						Cell shape
	Size	Shape	Color	Margin	Surface	Consistency	
Endo agar							
Ploskirev agar							
Bismuth sulfite agar							
Salmonella-shigella agar (SS agar)							
MacConkey agar							

Task 3. Study the procedure of bacterial smear preparation from *Salmonella* colonies, staining, and its examination.

1) Make notes about the morphology and tinctorial properties of *Salmonella*.

2) Draw the preparation of *Salmonella* stained by Gram.



Task 4. Study the procedure of the pure culture isolation. Make notes about the media used to isolate *Salmonella* pure culture and to examine its biochemical properties.

Task 5. Study the main biochemical tests for identification of *Salmonella* species from colonies on selective media or obtained from the pure culture. Make notes about

the ability to ferment **glucose**, lactose, sucrose, to produce indole, to form **gas** and **acids**, to release **hydrogen sulfide**.

Task 6. Study serological test used for the determination the antigenic structure of *Salmonella* and for identification *Salmonella* serogroups.

1) Make notes about the principle of the serotyping and the typing sera which are used to examine antigenic types of *Salmonella* isolates (*S. typhi*, *S. paratyphi A*, *S. schottmuelleri*).

2) Make notes about *Salmonella* serovar identification and the sera which are used for this purpose.

3) Study the Widal agglutination test and make notes about the components of the reaction.

4) Study Vi-hemagglutination reaction and make notes about the components of the reaction

5) Study phagotyping of typho-paratyphoid *Salmonella* group and make notes about the observation of positive results of the test.

Task 7. Study the main *Salmonella* species that cause **food poisoning infections (salmonellosis)**. Write down the Latin names for those species.

Task 8. Fill in the table 2 with the main antigenic properties of **Salmonella** species on which classification of *Salmonella* according to Kauffman – White is based.

Table 2. Serological classification of *Salmonella* according to Kauffman - White

Group	Species	O-antigen	H-antigen	
			2 phase	1 phase
A	<i>S. paratyphi A</i>			
B	<i>S. typhimurium</i> <i>S. derby</i> <i>S. haifa</i> <i>S. heidelberg</i>			
C	<i>S. choleraesuis</i>			
D	<i>S. enteritidis</i>			
E	<i>S. anatum</i>			

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Instructor's Full name and Signature: _____

Literature to get prepared for CLASS 4. Yi-Wei Tang, Max Sussman, Dongyou Liu, Ian Poxton, Joseph Schwartzman. Molecular Medical Microbiology. Volume 2. 2nd edition. – Copyright © 2015, 2002 Elsevier Ltd. – p.p. 1275-1305; 1307-1317.

PROCEDURE OF PRACTICAL CLASS 5. SHIGELLA

Main goal 1. Determine the stages of microbiological diagnosis of diseases caused by Shigella

Task1. Study the rules of material sampling and procedure of its preparation for microbiological study:

- 1) Sampling of pathological material (**feces, vomit, gastro-intestinal lavage**) in patient with dysentery;

- 2) Note about other materials which can be taken for microbiological examination in case of dysentery outbreaks. What pathological material can be taken at autopsy?
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Task 2. Study the nutrient media for the isolation of *Shigella* from pathological material and cultural properties of their colonies.

- 1) Make notes about main components of selective and differential diagnostic media used in laboratory diagnosis of dysentery.
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- 2) Fill in the Table 1 with the main characteristics of the colonies on different media

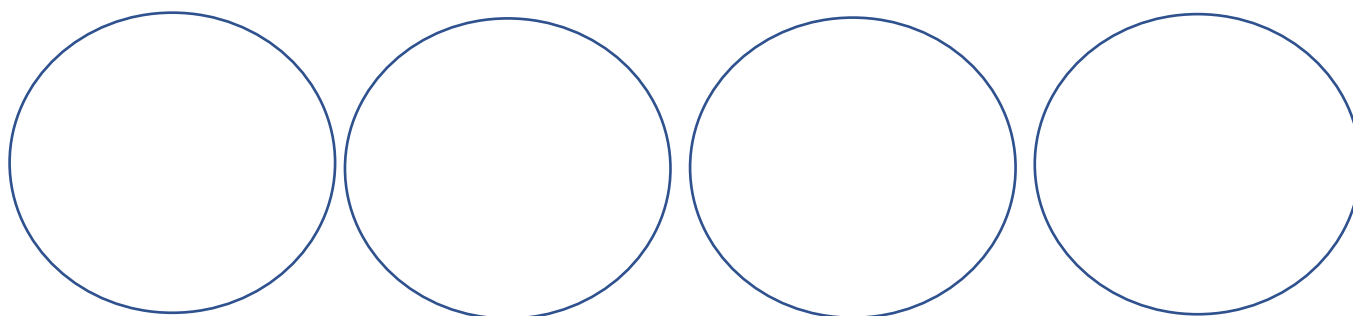
Table 1. Morphological characteristics of isolated colonies and cells of *Shigella*

Medium	Biological material	Morphological characteristics of the colonies						Cell shape
		Size	Shape	Color	Margin	Surface	Consistency	
Endo agar								
Levine								
SS media								

Task 3. Study the procedure of bacterial smear preparation from *Shigella* colonies, staining, and its examination.

- 1) Make notes about the morphology and tinctorial properties of *Shigella*.
-
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2) Draw the preparations of *Shigella* stained by Gram.



S. flexneri

S. dysenteriae

S. boydii

S. sonnei

Task 4. Study the procedure of the pure culture isolation. Make notes about the media used to isolate *Shigella* pure culture and to examine its biochemical properties.

Task 5. Study the main biochemical tests for identification of *Shigella* grown on selective media or obtained from the pure culture. Make notes about the ability of *S. dysenteriae*, *S. flexneri*, *S. sonnei*, *S.boydii* to ferment mannitol, glucose, lactose, maltose, sucrose, to form indole, H₂S, gas, and **acids**.

Task 6. Fill in the table 2 with the main biological properties of *Shigella*

Table 2. Biochemical properties of *Shigella* strains

Shigella species	Fermentation					Production		
	lactose	glucose	maltose	mannitol	sucrose	Hydrogen sulfide	Indole	Catalase
<i>S.dysenteriae</i>								
<i>S.flexneri</i>								
<i>S.boydii</i>								
<i>S.boydii</i>								

Note: "+" - positive reaction, "-" negative reaction, [+]
- late reaction, occurs no earlier than 2-3 days, "a" - fermentation to acid, "ag" - fermentation to acid and gas, "±" - a greater number of strains gives a positive reaction, "—, +" - a greater number of strains gives a negative reaction.

Task 7. Study serological test used for the determination the antigenic structure of *Shigella* and for identification of *Shigella* serotype (subserotype).

- 1) Make notes about the principle of the serotyping and the typing sera which are used to examine antigenic types of *Shigella* isolates (*S. flexneri*, *S. sonnei*).

- 2) Make notes about passive (indirect) hemagglutination assay (IHA) used in diagnosis of dysentery.

Task 8. Study the different methods for the diagnosis of shigellosis.

- 1) Make notes about necessary reagents and equipment to perform immunofluorescence essay.

- 2) Study the serodiagnosis of shigellosis and make notes about the diagnostics which are used for that purpose.

- 3) Study phagotyping of *Shigella* and make notes about the diagnostic preparation.

Task 9. Study the treatment of shigellosis. Make notes about the types of preparations which are used for prevention and treatment of shigellosis.

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Instructor's Full name and Signature: _____

Literature to get prepared for CLASS 5. Yi-Wei Tang, Max Sussman, Dongyou Liu, Ian Poxton, Joseph Schwartzman. Molecular Medical Microbiology. Volume 2. 2nd edition. – Copyright © 2015, 2002 Elsevier Ltd. – p.p. 1147-1167.

PROCEDURE OF PRACTICAL CLASS 6. VIBRIO

Main goal 1. Determine the stages of microbiological diagnosis of cholera

Task1. Study the rules of material sampling and procedure of its preparation for microbiological study:

- 1) Sampling of pathological material (**feces, vomit, gastro-intestinal lavage**) in patient with cholera;
- 2) Make notes about different types of material sampling in case of cholera outbreaks. What pathological material can be taken at autopsy?

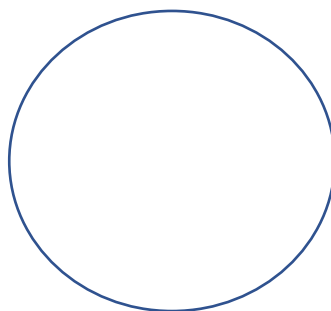
3) Make notes about material which should be examined for the purpose of epidemiological investigation.

4) Make notes about the media used for storage and transportation of the pathological material.

Task 2. Study the procedure of bacterial smear preparation from material contaminated with *Vibrio cholerae*, staining, and its examination.

1) Make notes about the morphology and tinctorial properties of *Vibrio cholerae*.

2) Draw the preparations of *Vibrio cholerae* stained by Gram.



Task 3. Study the nutrient media for the isolation of *Vibrio cholerae* from pathological material and cultural properties of their colonies.

1) Make notes about main components of selective and differential diagnostic media used in laboratory diagnosis of cholera.

2) Make notes about the procedure of the enrichment of *Vibrio cholerae* and medium used for that purpose.

3) Fill in the Table 1 with the main characteristics of the colonies on different media

Table 1. Morphological characteristics of isolated colonies and cells of *V. cholerae*

Medium	Biological material	Morphological characteristics of the colonies						Cell shape
		Size	Shape	Color	Margin	Surface	Consistency	
Aronson's agar								

Alkaline MPA								
TCBS medium								
Monsour medium								

Task 4. Study the procedure of the pure culture isolation. Make notes about the media used to isolate of *Vibrio cholerae* pure culture and to examine its biochemical properties.

Task 5. Study the main biochemical tests for identification of *Vibrio biovars* grown on selective media or obtained from the pure culture.

- 1) Make notes about the ability to produce oxidase, to ferment **glucose**, lactose, **sucrose**, to form acetoin, hydrogen sulfide, gas, and **acids**.
-
-
-

- 2) Make notes about the principle of the String test used for identification of *Vibrio cholera*.
-

- 3) Fill in the table 2 with the biochemical properties of cholera vibrio and study the groups of *Vibrio* according to Heiberg

Table 2. Biochemical characterization of *Vibrio* according to Heiberg

Sugars	Vibrions group							
	1	2	3	4	5	6	7	8
Mannose								
Saccharose								
Arabinose								

Task 7. Study the test used for the determination the antigenic structure of *Vibrio*, their pathogenic properties, resistance to antibiotic and phages in procedure for distinguishing *Vibrio* biovars.

1) Make notes about the principle of pathogenic properties determination.

2) Make notes about the principle of antigenic structure determination.

3) Make notes about the principle of phage sensitivity and antibiotic resistance determination.

Task 8. Study the different serological methods for the diagnosis of cholera.

1) Make notes about the determination of vibriocidal antibodies in patient serum.

2) Make notes about the determination of anti-cholera antibodies by detailed agglutination reaction.

3) Make notes about the determination of antitoxins in sera by indirect hemagglutination assay.

4) Make notes about the express method of cholera diagnosis.

Task 9. Study the environmental and ecological investigation. Make notes about the principle of differentiation cholera and cholera-like vibriions from other related genera (fill in the table 3).

Table 3. Distinguish of cholera vibriions from other related genera

Genera	Oxidase	Gelatinase	Break down				«String test»
			Glucose	Mannose	Mannitol	Inositol	
<i>Vibrio</i>							

<i>Aeromonas</i>							
<i>Pseudomonas</i>							
<i>Plesiomonas</i>							

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Instructor's Full name and Signature: _____

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PROCEDURE OF PRACTICAL CLASS 7. CORYNEBACTERIA

Main goal 1. Determine the stages of microbiological diagnosis of diphtheria

Task1. Study the rules of material sampling and procedure of its preparation for microbiological study:

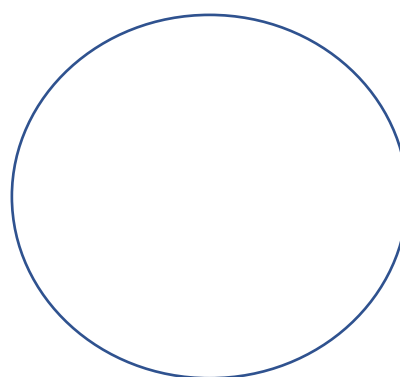
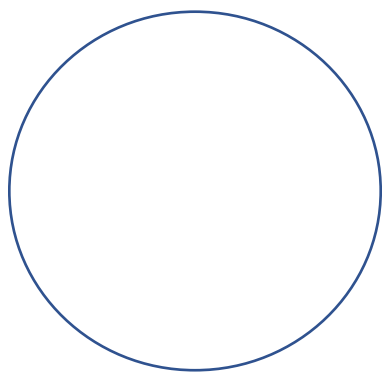
- 1) Sampling of pathological material (film from the tonsils, palate, uvula, mucus from the pharynx and nose) in patient with diphtheria;
- 2) Sampling of other pathological material (discharge from the eyes, ears, wounds, vagina, and the affected area of the skin). Make notes about different types of food and other item sampling.

-
- 3) Make notes about the storage and transportation (transport medium) of the pathological material.
-

Task 2. Study the procedure of **bacterioscopic analysis**, bacterial smear preparation from material contaminated with *C. diphtheriae*, staining, and its examination.

- 1) Make notes about the morphology and tinctorial properties of *C. diphtheria*.

2) Draw the preparations of *C. diphtheriae* stained by Loeffler and by Neisser method.



Task 3. Study the nutrient media for the isolation of *C. diphtheria*. from pathological material and cultural properties of their colonies.

- 1) Make notes about main components of selective and differential diagnostic media used in laboratory diagnosis of diphtheria.

- 2) Fill in the Table 1 with the main characteristics of the colonies on different media

Table 1. Morphological characteristics of isolated colonies and cells of *C. diphtheria* biovars

Medium	Biovar	Morphological characteristics of the colonies						Cell shape
		Size	Shape	Color	Margin	Surface	Consistency	
Blood agar	<i>C. d. gravis</i>							
	<i>C.d. mitis</i>							
	<i>C.d. belfanti</i>							
	<i>C.d. intermedius</i>							

Tellurite blood medium	<i>C. d. gravis</i>							
	<i>C.d. mitis</i>							
	<i>C.d. belfanti</i>							
	<i>C.d. intermedius</i>							

Task 4. Study the main biochemical tests and media used to examine biochemical properties of *Corynebacteria* grown on selective media or obtained from the pure culture.

1) Make notes about the principle of determination of **cystinase** (Pizu`s test).

Make notes about the principle of determination of **urease**.

2) Make notes about the medium used to study the ability to ferment glucose, sucrose, and soluble starch.

3) Make notes about the determination of nitrate reductase to identify *Corynebacteria* species.

4) Fill in the table 2 and 3 with the main biochemical properties of *Corynebacteria*

Table 2. Biochemical properties of *Corynebacteria*

<i>Corynebacteria</i> species	Toxigenic properties	Cystinase	Urease
<i>C. diphtheriae</i> var. <i>gravis</i>			
<i>C. diphtheriae</i> var. <i>mitis</i>			
<i>C. diphtheriae</i> var. <i>intermedius</i>			
<i>C. diphtheriae</i> var. <i>belfanti</i>			
<i>C. ulcerans</i>			
<i>C. pseudotuberculosis</i>			
<i>C. pseudodiphtheriticum</i> (<i>C. hofmani</i>)			
<i>C. xerosis</i>			

Note: “-” the reaction is negative, “+” the reaction is positive, “+/-” some strains give a positive reaction, other strains of this species are negative.

Table 3. The main differential diagnostic properties of *Corynebacteria*

<i>Corynebacteria</i> species	Catalase	Fermentation						Nitrate reduction
		glucose	maltose	sucrose	starch	cystine	urea	
<i>C. diphtheriae gravis</i>								
<i>C. d. mitis</i>								
<i>C. d. intermedius</i>								
<i>C. d. belfanti</i>								
<i>C. ulcerans</i>								
<i>C. pseudodiphtheriticum</i>								
<i>C. xerosis</i>								
<i>C. pseudotuberculosis</i>								
<i>C. jeikeium</i>								

Note: “-” the reaction is negative, “+” the reaction is positive, “+/-” some strains give a positive reaction, other strains of this species are negative.

Task 5. Study the test used for the determination the **toxigenic properties** of *Corynebacteria*. Make notes about the principle of Elek's test.

Task 6. Study the use of **polymerase chain reaction** and its advantages in diagnosis of diphtheria. Make notes about the genes detection of which in this reaction is important for the diagnosis.

Task 7. Study the methods and preparations for the prevention and treatment of diphtheria.

- 1) Make notes about composition of **adsorbed diphtheria toxoid (AD-M-diphtheria toxoid)** used to prevent diphtheria.

2) Make notes about composition of **adsorbed diphtheria-tetanus-pertussis vaccine (DTP)** used to prevent three infectious diseases.

3) Make notes about composition of **adsorbed diphtheria-tetanus toxoid (ADT-toxoid)**.

4) Make notes about composition of **diphtheria-tetanus toxoid (ADT-M-toxoid)**.

5) Make notes about the use of **anti-diphtheria immunoglobulin**.

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PROCEDURE OF PRACTICAL CLASS 8. MYCOBACTERIA

Main goal 1. Determine the stages of microbiological diagnosis of tuberculosis

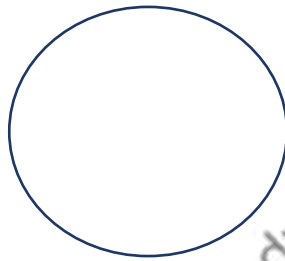
Task1. Study the rules of material sampling and procedure of its preparation for microbiological study:

Sampling of pathological material (sputum, mucus from the pharynx, pleural exudates, pus) in patient with tuberculosis. Make notes about other material sampling in rare cases.

Task 2. Study the procedure of **bacterioscopic analysis**, bacterial smear preparation from material contaminated with *Mycobacterium tuberculosis*, staining, and its examination.

1) Make notes about the morphology and tinctorial properties of *M. tuberculosis*.

2) Draw the preparations of *Mycobacterium tuberculosis* stained by Ziehl – Neelsen.



3) Make notes about the appearance of *M. tuberculosis* in smears stained with auramine-rhodamine. What other types of fluorescent staining is used?

4) Make notes about the homogenization method for improvement the detection of *M. tuberculosis* in sputum.

5) Make notes about the flotation method for improvement the detection of *M. tuberculosis* in sputum.

Task 3. Study the nutrient media for the isolation of *M. tuberculosis* from pathological material and cultural properties of their colonies.

1) Make notes about main components of selective and differential diagnostic media used in laboratory diagnosis of tuberculosis.

2) Make notes about the solutions used in procedure of normal microbiota elimination in patient material.

3) Fill in the Table 1 with the main characteristics of the colonies of *M. tuberculosis*

Table 1. Morphological characteristics of isolated colonies and cells of *M. tuberculosis*

Medium	Morphological characteristics of the colonies						Cell shape
	Size	Shape	Color	Margin	Surface	Consistency	
Lowenstein-Jensen medium							
Finn-II medium							

4) Make notes about the growth of *M. tuberculosis* in liquid media and the composition of these media.

5) Make notes about the principle of Price and Shkolnikova methods of microcultures in identification of *M. tuberculosis*.

Task 4. Study the main biochemical tests and media used to examine biochemical properties of *M. tuberculosis* grown on selective media.

1) Make notes about the main properties of *Mycobacterium* which are examined for the detection of causative agent of tuberculosis.

2) Make notes about the principle of Niacin test.

3) Make notes about the principle of *Mycobacterium* detection on sodium salicylate containing media.

4) Fill in the table 2 with the main biological and biochemical properties of *Mycobacterium*.

Table 2. Biochemical properties of different mycobacteria for their distinguishing

Species	Growth time, days	Catalase, 68 °C	Urease	Nicotinamidase	Niacinase	Nitrate reductase	Pigment
<i>M. tuberculosis</i>							
<i>M. bovis</i>							
<i>M. africanum</i>							
<i>M. kansasii</i>							
<i>M. avium</i>							
<i>M. smegmatis</i>							

5) Make notes about the principle of the virulence determination of mycobacteria.

Task 5. Study the tests used for the determination of **mycobacteria resistance to antibiotics**. Make notes about the direct and indirect methods.

Fill in the table 3 with the scale for evaluating the resistance of mycobacteria to different drugs.

Table 3. The scale for evaluating the resistance of mycobacteria to drugs

Medicines	Resistant at growth on drug-containing media, mg / ml	
	Solid	Liquid
Streptomycin		
Tubazide		
PAS		
Cycloserine		
Kanamycin		
Ethionamide		
Biomycin		

Task 6. Study the use of **biological method** for the diagnosis of tuberculosis. Make notes about the principle of the method.

Task 7. Study the methods of serological diagnosis of tuberculosis and novel methods of detection of tuberculosis infection.

1) Make notes about the main tests used for detection of specific antibodies in patient with tuberculosis.

2) Indicate the diagnostic titer of the serum of the patient.

3) Make notes about the PCR test and ELISA used for detection of causative agent.

Task 8. Study the **skin-allergic test** for the diagnosis of tuberculosis. Make notes about the tuberculin preparation and its use in the Mantoux tuberculin skin test, and possible results of the test.

Task 9. Study the methods and preparations for the prevention of tuberculosis.

1) Make notes about composition of BCG vaccine used to prevent tuberculosis.

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PROCEDURE OF PRACTICAL CLASS 9. CLOSTRIDIA

Main goal 1. Determine the stages of microbiological diagnosis of botulism

Task1. Study the rules of material sampling and procedure of its preparation for microbiological study:

Sampling of pathological material (gastric lavage, vomit, blood, feces, urine, suspicious food leftovers) in patient with botulism. Make notes about material sampling in autopsy.

Task 2. Study the nutrient media for the isolation of *Clostridium botulinum* from pathological material and cultural properties of their colonies.

Make notes about main components of selective and differential diagnostic media used in laboratory diagnosis of botulism.

1) Make notes about the cultivation conditions to growth of *C. botulinum* in liquid media.

2) Study the procedure of *C. botulinum* pure culture isolation. Make notes about the media used to isolate *C. botulinum* pure culture and to examine its biochemical properties.

3) Fill in the Table 1 with the main characteristics of the colonies of *Clostridium botulinum*

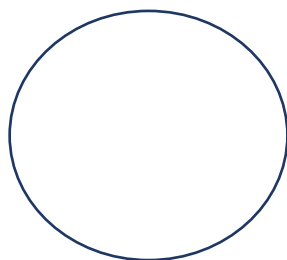
Table 1. Morphological characteristics of isolated colonies and cells of *Clostridium botulinum*

Medium	Morphological characteristics of the colonies						Cell shape
	Size	Shape	Color	Margin	Surface	Consistency	
Blood agar							
Sugar agar							

Task 3. Study the procedure of bacterial smear preparation from colonies of *Clostridium botulinum*, staining, and its examination.

1) Make notes about the morphology and tinctorial properties of *C. botulinum*.

2) Draw the preparations of *C. botulinum* stained by Gram.



Task 4. Study the main biochemical tests and media used to examine biochemical properties of *C. botulinum* obtained from the pure culture.

1) Make notes about the ability of *C. botulinum* to ferment glucose, galactose, glycerin, maltose, sucrose, fructose, to produce acid, gas, indole, and H₂S, to digest gelatin.

2) Fill in the table 2 with the main biochemical properties of *Clostridium*

Table 2. Differential characteristics of *Clostridium*

Species	Motility	Lecithinase	Lipase	Fermentation			Indole
				Glucose	Lactose	Mannitol	
<i>C. botulinum</i>							
<i>C. difficile</i>							
<i>C. histolyticum</i>							
<i>C. novyi</i>							
<i>C. perfringens</i>							
<i>C. septicum</i>							
<i>C. sordellii</i>							
<i>C. sporogens</i>							
<i>C. tetani</i>							

Task 5. Study the use of **biological method** for the diagnosis of botulism.

1) Make notes about the principle of **toxin neutralization assay**.

2) Make notes about the principle of determination of the **botulinum toxin type**.

Main goal 2. Determine the stages of microbiological diagnosis of tetanus

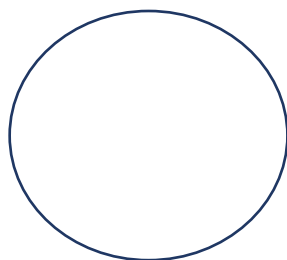
Task1. Study the rules of material sampling and procedure of its preparation for microbiological study:

1) Sampling of pathological material (pus, wound contents, exudates, pieces of necrotic tissue, wound swabs, foreign bodies) in patient with suspected tetanus. Make notes about material sampling in autopsy and after abortion or childbirth.

Task 2. Study the procedure of bacterial smear preparation from material contaminated with *Clostridium tetani*, staining, and its examination.

1) Make notes about the morphology and tinctorial properties of *C. tetani*.

2) Draw the preparations of *C. tetani* stained by Gram or by Ozheshko method.



Task 3. Study the nutrient media for the isolation of *Clostridium tetani* from pathological material and cultural properties of their colonies.

1) Make notes about main components of selective and differential diagnostic media used in laboratory diagnosis of tetanus and the cultivation conditions.

2) Study the procedure of *C. tetani* pure culture isolation. Make notes about the media used and antibiotics added to isolate *C. tetani*.

3) Fill in the Table 3 with the main characteristics of the colonies of *C. tetani*.

Table 3. Morphological characteristics of isolated colonies and cells of *Clostridium tetani*

Medium	Morphological characteristics of the colonies						Cell shape
	Size	Shape	Color	Margin	Surface	Consistency	
Blood agar							
Sugar agar							

Task 4. Study the use of **biological method** for the diagnosis of tetanus.

1) Make notes about the principle of **determination of toxigenicity**.

Task 5. Study **serological** test used for the diagnosis of tetanus. Make notes about the main tests used for that purpose.

Main goal 3. Study the cultivation conditions for causative agent of anaerobic infections.

Task 1. Inoculate the culture of *Clostridium sp.* in thioglycollate broth.

Thioglycollate broth is the enrichment broth which supports growth of anaerobes, aerobes, microaerophilic, and fastidious microorganisms. It contains many nutritive factors, including casein, yeast, beef extracts, and vitamins to enhance the growth of most medically important bacteria and other nutrient supplement. This medium also contains 0.075% agar to prevent convection currents from carrying atmospheric oxygen throughout the broth. This agar supplement and the presence of reducing agent thioglycolic acid create an anaerobic environment deeper in the tube which allows anaerobic bacteria to grow.

When anaerobic infection is suspected, thioglycollate medium is recommended to isolate strict anaerobes from blood. Sufficient volume of broth must be used to prevent the blood from clotting and to dilute out the blood's natural bactericidal substances. The blood should be diluted at least 1 in 10 with broth (SPS, anticoagulant, is inhibitory to anaerobic streptococci, so is not added in thioglycollate broth).

Thioglycollate broth is used to find out growth characteristics of various bacteria based on their oxygen requirements.

Principles:

Cystine and casein: They supply carbon and nitrogenous compounds,

Dextrose: It is added as another energy source

Yeast extract or papaic digest of soybean meal are added as growth enhancers.

Sodium chloride: It maintains osmotic equilibrium.

Sodium Thioglycollate: Sodium thioglycollate is a reducing agent which maintains a low oxygen tension by removing molecular oxygen from the environment i.e., it creates anaerobic conditions when it reduces molecular oxygen to water. Peroxides, which may be lethal to many anaerobic organisms, are not formed under this condition.

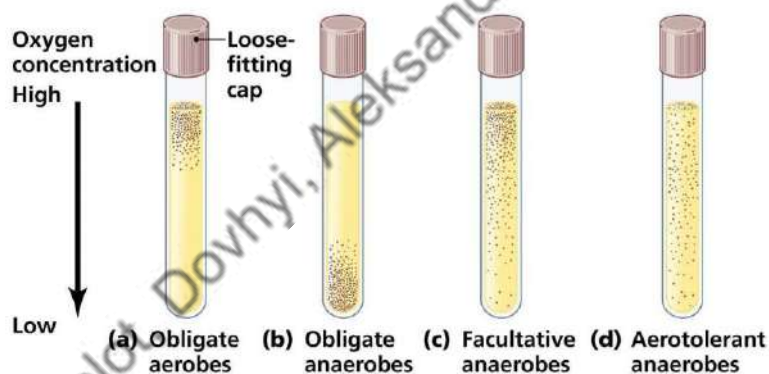
Resazurin is an oxidation-reduction indicator that turns pink when increased oxidation has occurred, it is colorless when reduced.

Agar: The addition of a small amount of agar in Thioglycollate medium aids in the initiation and growth of small inocula and anaerobes by impeding the diffusion of oxygen into the medium. It also retards the dispersion of CO₂ and the reducing substance from the microenvironment surrounding the inoculum.

Inoculation:

Inoculate the medium following aseptic technique.

Once inoculated, the broth should be incubated at 35-35°C as soon as possible.



Results are read after 48 hours incubation of the inoculated test tubes. When oxygen diffuses near the top of the broth a pink band develops (**Remember:** *Oxidation-Reduction indicator Resazurin is pink when oxidized and colorless when reduced*). The absence of pink in the rest of the tube indicates the absence of oxygen and a suitable environment for strict anaerobes. Growth of the organisms in different regions of the test tubes will depend on the oxygen requirement characteristics of those organisms:

1. Strict aerobes will grow only in the pink band,
2. Microaerophiles will grow near the bottom of the band where the concentration of oxygen is lower.
3. Both facultative anaerobes and aerotolerant anaerobes will grow throughout the tube. Facultative anaerobes grow throughout the media but heavier at the bottom and facultative aerobes grow throughout but more at the top. The facultative grow most densely where oxygen is present.

4. The aerotolerants grow equally well throughout the tube. Usually this test should be read at about 48 hrs although some slower growing microbes may take more incubation time.

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PROCEDURE OF PRACTICAL CLASS 10. ZOOANTROPONOUS INFECTION AGENTS

Main goal 1. Determine the stages of microbiological diagnosis of plague

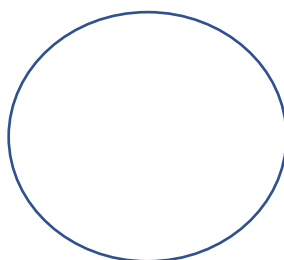
Task1. Study the rules of material sampling and procedure of its preparation for microbiological study:

Sampling of pathological material in patient with plague. Make notes about material sampling in case of skin form, bubonic form, in all forms, and in case of damage to the intestines or meninges.

Task 3. Study the procedure of bacterial smear preparation from material contaminated *Yersinia pestis*, staining methods, and its examination.

1) Make notes about the morphology and tinctorial properties of *Yersinia pestis*.

2) Draw the preparations of *Yersinia pestis* stained by Gram or with methylene blue.



3) Make notes about other method used for specific revealing *Y. pestis* in smears.

Task 3. Study the nutrient media for the isolation of *Y. pestis* from pathological material and cultural properties of their colonies.

1) Make notes about main components of selective and differential diagnostic media used in laboratory diagnosis of plague.

2) Make notes about the growth of *Y. pestis* in liquid media.

3) Fill in the Table 1 with the main characteristics of *Y. pestis* colonies

Table 1. Morphological characteristics of colonies on solid media and cells of *Y. pestis*

Hottinger media	Morphological characteristics of the colonies						Cell shape
	Size	Shape	Color	Margin	Surface	Consistency	
10-12 h							
20-24 h							
40-48 h							

4) Study the procedure of *Y. pestis* pure culture isolation. Make notes about the media used to isolate *Y. pestis* pure culture and to examine its biochemical properties.

Task 4. Study the main biochemical and serological tests used to examine biochemical and antigenic properties of *Y. pestis* obtained from the pure culture.

1) Make notes about tests used for examination of antigenic properties of *Y. pestis*.

2) Make notes about the media used to determine biochemical properties of *Y. pestis* and the ability of *Y. pestis* to ferment **glucose, mannitol, galactose, fructose, xylose**, arabinose and glycerol, adonite, rhamnose and sucrose, to produce **acid, gas, H₂S**, indole, oxidase, urease, **catalase, plasmocoagulase**.

3) Fill in the table 2 with the main biochemical properties of *Y. pestis*

Table 2. Differentiation of plague, pseudotuberculosis, and intestinal yersiniosis agents

Features	<i>Yersinia pestis</i>	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia enterocolitica</i>
Motility			
Adonite fermentation			
Rhamnose fermentation			
Saccharose fermentation			
Lysis by plague phage			
H ₂ S production			
Plasmocoagulase			
Catalase			
Urease			
Colonies on citrate			
Deoxycholate agar			

Note: "+" is a positive reaction, "-" is a negative reaction.

4) Make notes about the tests used for determination of sensitivity to antibiotics.

Task 5. Study the use of **biological and serological methods** for the diagnosis of plague.

1) Make notes about the principle of **biological assay**.

2) Make notes about the principle of **serological diagnosis** of plague.

3) Make notes about the **express methods** for the detection of the plague pathogen

Main goal 2. Determine the stages of the diagnosis of tularemia

Task1. Study the rules of material sampling and procedure of its preparation for microbiological study:

1) Sampling of pathological material (oropharyngeal mucosa, bubonic puncture, sputum, blood, conjunctival pus, material of pustules and ulcers) in patient with tularemia. Make notes about other material sampling.

Task 2. Study the use of **biological method** for the diagnosis of tularemia and make notes about it.

Task 3. Study selective nutrient media for the isolation of *Francisella tularensis* from pathological material and cultural properties of their colonies.

1) Make notes about main components of selective and differential diagnostic media used in laboratory diagnosis of tularemia and the cultivation conditions.

2) Fill in the Table 3 with the main characteristics of the colonies of *Francisella tularensis*.

Table 3. Morphological characteristics of isolated colonies and cells of *Francisella tularensis*

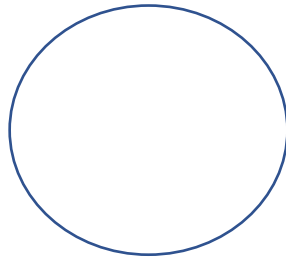
Medium	Morphological characteristics of the colonies						Cell shape
	Size	Shape	Color	Margin	Surface	Consistency	
McCoy and Chapin medium							

Francis agar							
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Task 3. Study the procedure of bacterial smear preparation from material contaminated with *Francisella tularensis*, staining, and its examination.

1) Make notes about the morphology and tinctorial properties of *F. tularensis*.

2) Draw the preparations of *F. tularensis* stained by Romanovsky-Giemsa method.



Task 4. Study biochemical properties of *Francisella tularensis* and **serological** tests used for the diagnosis of tularemia. Make notes about the main tests used for that purpose.

1) Biochemical properties

2) Antigenic properties and serological reactions

Task 5. Study the allergic test used for the diagnosis of tularemia. Make notes about administration of *tularin* for that purpose and the sign of positive reaction.

Main goal 3. Determine the stages of the diagnosis of brucellosis

Task1. Study the rules of material sampling and procedure of its preparation for microbiological study:

1) Sampling of pathological material (blood, bone marrow, feces, bile, urine, cerebrospinal fluid, sputum, lymph nodes punctate) in patient with brucellosis. Make notes about animal material sampling.

Task 2. Study selective nutrient media for the isolation of *Brucella* from pathological material and cultural properties of their colonies.

1) Make notes about main components of selective and differential diagnostic media used in laboratory diagnosis of brucellosis and the cultivation conditions.

2) Make notes about Ruiz-Castaneda method to isolate *Brucella*.

3) Make notes about myelo-culture and bile-culture isolation.

4) Make notes about the use of chicken embryo to isolate *Brucella*

5) Fill in the Table 4 with the main characteristics of the colonies of *Brucella*.

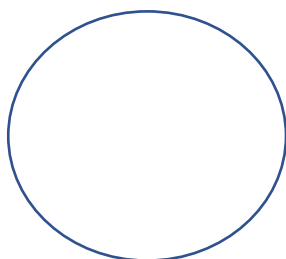
Table 4. Morphological characteristics of isolated colonies and cells of *Brucella*

Medium	Morphological characteristics of the colonies						Cell shape
	Size	Shape	Color	Margin	Surface	Consistency	
<i>Serum dextrose agar</i>							

Task 3. Study the procedure of bacterial smear preparation from colonies of *Brucella*, staining, and its examination.

1) Make notes about the morphology and tinctorial properties of *Brucella*.

2) Draw the preparations of *Brucella* stained by Gram method.



Task 4. Study the main distinguishing properties of *Brucella* species. Fill in the Table 5.

Table 5. Distinguish between different brucella biovars and species

Species	Biovars	Requirem. CO ₂	H ₂ S producing	Growth on agar with dye		Agglutination by monoreceptor sera		Lysis by phage
				Thionin	Fuchsin	<i>Brucella abortus</i>	<i>Brucella melitensis</i>	
<i>Brucella melitensis</i>	1							
	2							
	3							
<i>Brucella abortus</i>	1							
	2							
	3							
	4							
	5							
	6							
	7							
	8							
	9							
	8							
9								

Note: «+» - positive reaction, «-» - negative reaction.

Task 5. Study the use of **biological method** for the diagnosis of brucellosis and make notes about it.

Task 6. Study the use of **serological methods** for the diagnosis of brucellosis and make notes about Wright agglutination test and agglutination reaction on the glass (Hedderson reaction).

Task 7. Study the use of the Burne allergy test for the diagnosis of brucellosis and make notes about the positive reaction of the test.

Main goal 4. Determine the stages of the microbiological diagnosis of anthrax

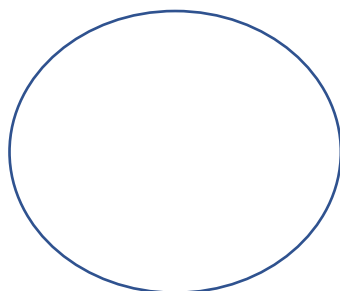
Task1. Study the rules of material sampling and procedure of its preparation for microbiological study:

1) Sampling of pathological material in patient with anthrax. Make notes about material sampling in case of skin form, intestinal form, pulmonary form, and in all clinical forms.

Task 2. Study the procedure of bacterial smear preparation from material contaminated with *Bacillus anthracis*, staining, and its examination.

1) Make notes about the morphology and tinctorial properties of *Bacillus anthracis*.

2) Draw the preparations of *B. anthracis* stained by Rebigers method.



3) Make notes about the different staining methods of smears with *B. anthracis*.

Task 3. Study selective nutrient media for the isolation of *B. anthracis* from pathological material and cultural properties of their colonies.

1) Make notes about main components of selective and differential diagnostic media used in laboratory diagnosis of anthrax and the cultivation conditions.

2) Fill in the Table 6 with the main characteristics of the colonies of *B. anthracis*.

Table 6. Morphological characteristics of isolated colonies and cells of *B. anthracis*

Medium	Morphological characteristics of the colonies						Cell shape
	Size	Shape	Color	Margin	Surface	Consistency	
Hottinger`s agar							

Task 4. Study the main biochemical and biological tests used to examine biochemical and antigenic properties of *B. anthracis* obtained from the pure culture. Fill in the Table 7 with the main biochemical properties of *B. anthracis*.

Table 7. Differential features of the causative agent of anthrax and non-pathogenic species of Bacillus

Species	Capsule	Pathogenicity	Lysis by phage	Pearl necklace test	IF-serological test	Motility	Haemolysis	Lecithinase (lipase)	Phosphatase
<i>B. anthracis</i>									
<i>B. subtilis</i>									

Note: «+» - positive reaction, «-» - negative reaction.

Make notes about the "pearl necklace" test.

Task 5. Study the use of **biological method** for the pathogenicity determination in diagnosis of anthrax and make notes about it.

Task 6. Study the principle of **serological reaction** for the detection of *B. anthracis*. Make notes about **Ascoli** ring precipitation.

Task 7. Study the principle of **the allergic method** and make notes about its use.

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Literature to get prepared for CLASS 10. Yi-Wei Tang, Max Sussman, Dongyou Liu, Ian Poxton, Joseph Schwartzman. Molecular Medical Microbiology. Volume 3. 2nd edition. – Copyright © 2015, 2002 Elsevier Ltd. – p.p. 1771-1865.

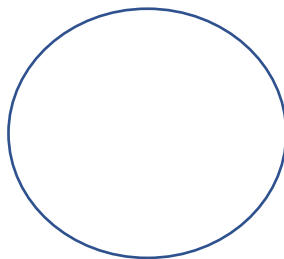
PROCEDURE OF PRACTICAL CLASS 11. RICKETTSIA, CHLAMYDIA, MYCOPLASMA

Main goal 1. Determine the stages of diagnosis of rickettsiosis

Task1. Study the microscopic methods of identification of *Rickettsia prowazekii* in bacterial smear prepared from contaminated material for the diagnosis of **epidemic typhus fever**.

1) Make notes about smear fixation and staining methods, and the morphology of *R. prowazekii*.

2) Draw the preparation of *R. prowazekii* stained by Zdrodovski.



3) Make notes about material contaminated with *R. prowazekii* that can be examined using fluorescent serum.

Task 2. Study the serological methods used for diagnosis of infections caused by *R. prowazekii*.

1) Make notes about the principle of Weigl's reaction of agglutination and its components, and diagnostically important serum dilution.

2) Make notes about other serological methods used for diagnosis of epidemic typhus fever.

3) Make notes about molecular biology method of rickettsiae detection in pathological material.

Task 3. Study the **serological** methods of identification of specific antibodies against *Rickettsia typhi* for the diagnosis of **endemic (rat) typhus fever = Flea-borne (murine) typhus**.

Make notes about differential identification of infections caused by *R. typhi* and *R. prowazekii* using serological reactions.

Task 4. Study the **biological** method of identification *Rickettsia typhi* and make notes about its principle.

Task 5. Study the methods of identification of *Coxiella burnetii* for the diagnosis of Q-fever.

1) Make notes about pathological material sampling in patient with Q-fever and isolation of *Coxiella burnetii* from it.

2) Make notes about microscopic method of *Coxiella burnetii* examination.

3) Make notes about serological methods of diagnosis of Q-fever.

4) Make notes about Q-fever skin test.

Task 6. Study the methods of identification of *Rickettsia sibirica* for the diagnosis of Siberian or North Asian tick typhus.

1) Make notes about the principle of the **biological** method of *R. sibirica* identification.

2) Make notes about localization of *R. sibirica* in eukaryotic cells stained by Zdrodovski.

3) Make notes about serological methods of diagnosis of infections caused by *R. sibirica* (CFT, indirect hemagglutination (IHA) test, Weil-Felix test, immunofluorescence, indirect hemolysis).

Main goal 2. Determine the stages of the diagnosis of chlamydiosis

Task 1. Study the rules of material sampling and procedure of its preparation for microbiological study. Make notes about causative agents and sampling of pathological

material in patient with trachoma, ornithosis (psittacosis, avian chlamydiosis), pneumonia, urogenital chlamydiosis.

Task 2. Study the bacterioscopic method of *Chlamydia* identification in bacterial scrape preparations from patients diagnosed with chlamydiosis.

1) Make notes about preparation fixation and staining methods, and the morphology of *Chlamydia*.

2) Draw the preparation of *Chlamydia* (elementary body and reticulate body) in eukaryotic cells stained by Romanowsky-Giemsa.



Task 3. Study the use of **biological method** for the diagnosis of *chlamydiosis* and make notes about it.

Make notes about the eukaryotic cell cultures used for isolation of *Chlamydia trachomatis* and *Chlamydia psittaci* and principle of the method.

Task 4. Fill in the Table 1 with the main properties of *Chlamydia* species.

Table 4. Differential characteristics of pathogenic *Chlamydia*

	Form of elementary bodies	Microcolonies in eukaryotic cells	Glycogen synthesis	Resistance to sulfadiazine	Growth in chicken embryos
<i>C. trachomatis</i>					

<i>C. psittaci</i>					
<i>C. pneumoniae</i>					

Task 5. Study the immunology and molecular biology methods for the diagnosis of chlamydiosis. Make notes about the main tests used for that purpose.

C. trachomatis _____

C. psittaci _____

C. pneumoniae _____

Task 6. Study the biological and allergic tests used for the diagnosis of ornithosis. Make notes about animals used in the biological test and method of administration of test material.

Make notes about type of allergen used in skin test and method of its result evaluation.

Main goal 3. Determine the stages of the diagnosis of mycoplasma infections.

Task1. Study the rules of material sampling and procedure of its preparation for microbiological study:

- 1) Make notes about sampling of pathological material in patients with different forms of mycoplasmosis: respiratory, urogenital and arthritis.

Task 2. Study selective nutrient media for storage of tested material and isolation of *Mycoplasma* from pathological material, cultural properties of their colonies.

1) Make notes about main components of selective and differential diagnostic media used in laboratory diagnosis of infections caused by *Mycoplasma* and *Ureaplasma*.

2) Make notes about the growth of *Mycoplasma* and *Ureaplasma* in liquid selective broth.

3) Make notes about the growth of *Mycoplasma* and *Ureaplasma* colonies on agar.

Task 3. Study the main biochemical tests and media used to examine biochemical properties of *Mycoplasma*.

1) Make notes about the ability of *Mycoplasma* to hemolysis and hemadsorption.

2) Make notes about the ability of *Mycoplasma* to utilize arginine, urea, glucose.

Task 4. Study the main distinguishing properties of *Mycoplasma* species.

Fill in the Table 2.

Table 2. **Differential characteristics of *Mycoplasma***

Species	Produce			Ferment		Resistance to erythromycin
	urease	arginase	phosphatase	glucose	mannose	
<i>M. pneumoniae</i>						
<i>M. hominis</i>						
<i>M. arthritidis</i>						
<i>M. fermentans</i>						
<i>U. urealyticum</i>						

Task 5. Study the use of **serological methods and molecular biology method** for the diagnosis of *Mycoplasma* infections.

1) Make notes about immunofluorescence assay (IFA).

2) Make notes about a reaction of passive hemagglutination (RPHA)

3) Make notes about a reaction of aggregate-hemagglutination (RAHA)

4) Make notes about an enzyme-linked immunosorbent assay (ELISA)

5) Make notes about the use of PCR for the diagnosis of *Mycoplasma* infections.

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Literature to get prepared for CLASS 11. Yi-Wei Tang, Max Sussman, Dongyou Liu, Ian Poxton, Joseph Schwartzman. Molecular Medical Microbiology. Volume 3. 2nd edition. – Copyright © 2015, 2002 Elsevier Ltd. – p.p. 1449-1469; 1587-1609: 2043-2056.

PROCEDURE OF PRACTICAL CLASS 12. SPIROCHETES

Main goal 1. Determine the stages of the diagnosis of borreliosis

Task1. Study the rules of material sampling and procedure of its preparation for microbiological study. Make notes about pathological material taken from patient infected with *Borrelia recurrentis*.

Task2. Study the identification of *Borrelia* in bacterial smear prepared from contaminated material for the diagnosis of borreliosis (epidemic relapsing fever).

1) Make notes about types of microbiological preparations and staining methods used for identification of bacteria in blood.

2) Draw the preparation of *Borrelia recurrentis* stained by Giemsa method.



Task 3. Study the serological methods used for diagnosis of infections caused by *Borrelia*.

1) Make notes about the principle of determination of bactericidal activity of patient's serum.

2) Make notes about the principle of immobilization reaction.

3) Make notes about other serological reactions used for diagnosis of borreliosis.

4) Study the **biological** method of *Borrelia recurrentis* identification.

5) Make notes about molecular biology method of *Borrelia* detection in pathological material.

Task 4. Study the diagnostics of endemic relapsing fever. Make notes about the causative agents of the disease and vectors for transmission.

1) Make notes about the patient pathological material taken for analysis.

2) Make notes about staining methods for prepared smears and dark field microscopy.

3) Study the **biological** method for endemic relapsing fever diagnosis.

Task 5. Study the diagnostics of **Lyme borreliosis** caused by *B. burgdorferi*.

1) Make notes about the patient pathological material taken for analysis and nutrient medium used for bacteria isolation.

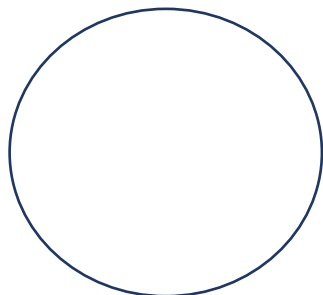
2) Make notes about **serological** methods of identification of specific antibodies against *B. burgdorferi*.

Main goal 2. Determine the stages of the diagnosis of syphilis

Task 1. Study the rules of material sampling and procedure of its preparation for microbiological study. Make notes about sampling of pathological material in patient with different stages of syphilis: in the early period (primary stage) of the disease and in the secondary and tertiary stages.

Task 2. Study the bacterioscopic method of *Treponema pallidum* identification in microbiological preparations from material of patients diagnosed with syphilis.

1) Make notes about microscopy type used for examination of *T. pallidum* contaminated material and the morphology of bacteria (draw the preparation).



2) Make notes about tinctorial properties of *T. pallidum*, fixation and staining techniques used.

Task 3. Study the use of **serological methods** in diagnosis of syphilis.

1) Make notes about non-treponemal test and antigen used for it.

2) Make notes about treponemal tests and antigen used for these.

3) Make notes about *Treponema pallidum* immobilization (TPI) assay and **stages of disease** it can be used for.

4) Make notes about microreaction of precipitation (MRP) with cardiolipin antigen.

5) Make notes about indirect immunofluorescence reaction (RIF) in diagnosis of syphilis.

6) Make notes about Wasserman complement-binding reaction.

Find and correct errors in the proposed Wasserman reaction procedure description.

Next, place the following steps in order they appear in this complement fixation test.

Number of the step (from 1 to 9)	Description of the step
	Heat the serum at 56°C for 30 min.
	Use whole blood sample (contain antibody of interest).
	Isolate the serum from blood sample.
	Mix a known antigen with patient's serum.
	Incubate test system and indicator system at 37°C for 45 min.
	Incubate the test system at +4°C for about 1 hour (30 min to 1 h).
	Observed result: no hemolysis occurs.
	Add sheep BRC and antibodies against sheep RBC to the test system.
	Add Guinea pig's complement to the test system.

7) Make notes about enzyme-linked immunosorbent assay and its importance in diagnosis of syphilis.

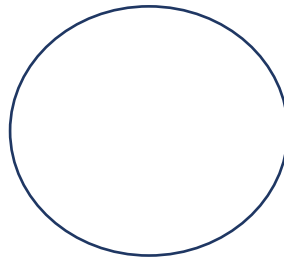
Main goal 3. Determine the stages of the diagnosis of leptospirosis.

Task1. Study the rules of material sampling and procedure of its preparation for microbiological study:

1) Make notes about sampling of pathological material in patients with leptospirosis at the different stages of the disease.

Task 2. Study the bacterioscopic method of *Leptospira interrogans* identification in microbiological preparations from material of patients diagnosed with leptospirosis.

1) Make notes about microscopy type used for examination of *L. interrogans*, tinctorial properties and the morphology of bacteria (draw the preparation).



Task 3. Study selective nutrient media for isolation of *Leptospira* pure culture.

Make notes about the main components of selective and differential diagnostic media used in laboratory diagnosis of leptospirosis and time needed for cultivation.

Task 4. Study the main biochemical properties of *Leptospira*.

1) Make notes about the principle of the tests for hemolytic activity examination.

Method of "*wells*" _____

The method of agar layers _____

2) Make notes about the principle of the tests for *phospholipase* activity examination.

3) Make notes about the principle of the tests for *esterase* activity examination.

4) Make notes about the principle of the tests for **DNAase** activity examination.

Task 5. Study the **biological** method for leptospirosis diagnosis.

Task 6. Study the use of **serological methods and molecular biology method** for the diagnosis of leptospirosis.

1) Make notes about microagglutination test and serum diagnostic titer.

2) Make notes about an enzyme-linked immunosorbent assay (ELISA).

5) Make notes about the use of PCR for the diagnosis of leptospirosis.

Main goal 4. Using blood agar in the bacteriological diagnosis of infectious disease (stage II). Bacterial capsule examination.

Task 1. Estimate the type of hemolysis on blood agar with different bacterial cultures. Make a conclusion about these properties of isolates.

Task 2. Study staining technique by Burri for capsule revealing.

1. Put a drop of black ink onto a fat-free glass slide and resuspend the culture of *Bacillus megaterium* and *B. subtilis* in it.
2. Make a smear according to “blood smear” preparation technique and air dry it.
3. Add 2-3 drops of ethanol onto the smear and burn it on the smear (to fix the cells).
4. Add fuchsin solution onto the smear for 2-3 minutes
5. Rinse smear thoroughly with water, air dry and analyze microscopically using the oil immersion lens.

In the field of view: bright red cells surrounded by a transparent capsule.

Conclusion _____

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Literature to get prepared for CLASS 12. Yi-Wei Tang, Max Sussman, Dongyou Liu, Ian Poxton, Joseph Schwartzman. Molecular Medical Microbiology. Volume 3. 2nd edition. – Copyright © 2015, 2002 Elsevier Ltd. – p.p. 1867-1909; 1973-1990.

Task2. Study the procedure of diagnosis of candidiasis.

1) Study the rules of material sampling and procedure of its preparation for microbiological study. Make notes about pathological material taken from patient infected with *Candida albicans*.

2) Study the microscopic picture of *Candida albicans* in pathological material preparations. Make notes about morphology of the fungi and draw the preparation.



3) Study the procedure of the pure culture isolation from pathological material contaminated with *C. albicans*. Make notes about the media used for candidiasis diagnosis.

4) Study the assimilation test for the identification of *Candida*. Make notes about the principle of the test.

5) Study *Candida Albicans* Skin Test. Make notes about the principle of the test.

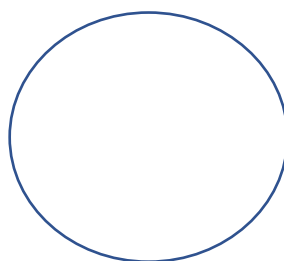
6) Study serological tests and molecular biology test for the identification of *Candida albicans* infection. Make notes about the principle of indirect immunofluorescence test, latex agglutination test, immunoelectrophoresis, PCR.

7) Study the etiological therapy of candidiasis. Make notes about the main drugs and their active ingredients used for systemic and local treatment of *Candida albicans* infection.

Task 3. Study the procedure of diagnosis of aspergillosis.

1) Study the rules of material sampling and procedure of its preparation for microbiological study. Make notes about pathological material taken from patient infected with *Aspergillus*.

2) Study the microscopic picture of *Aspergillus fumigatus* in pathological material preparations. Make notes about morphology of the fungi and draw the preparation.



3) Study the procedure of the pure culture isolation from pathological material contaminated with *Aspergillus*. Make notes about the purpose of cultural method.

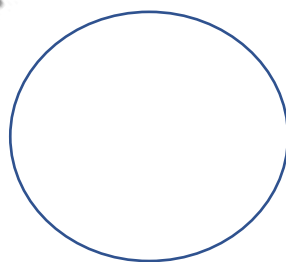
4) Study serological test and skin test for the identification of *Aspergillus* infection. Make notes about the principle of the test.

5) Study the standard treatment of aspergillosis. Make notes about the main drugs and methods of treatment of *Aspergillus* infection.

Task 4. Study the procedure of diagnosis of pneumocystosis.

1) Study the rules of material sampling and procedure of its preparation for microbiological study. Make notes about pathological material taken from patient infected with *Pneumocystis jiroveci*.

2) Study the microscopic picture of *Pneumocystis jiroveci* in pathological material preparations. Make notes about the methods of staining, morphology of the fungi and draw the preparation.

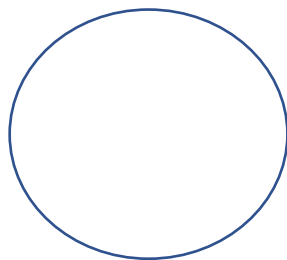


3) Study serological tests and histological study for the identification of *P. jiroveci* infection. Make notes about the principle of the tests.

Task 5. Study the procedure of diagnosis of zygomycosis.

1) Study the rules of material sampling for microbiological study. Make notes about pathological material taken from patient with zygomycosis.

2) Study the microscopic picture of *Cunninghamella bertholletiae* in pathological material preparations. Make notes about the morphology of the fungi and draw the preparation.

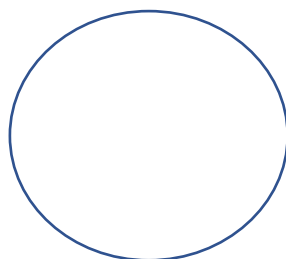


3) Study the main clinical diagnostic tests for zygomycosis and methods of therapy. Indicate the clinical tests and names of the drug used for the therapy.

Task 6. Study the procedure of diagnosis of fusariosis.

1) Study the rules of material sampling for microbiological study. Make notes about pathological material taken from patient with mycotoxicosis caused by *Fusarium sporotrichiella*.

2) Study the microscopic picture of *Fusarium* in pathological material preparations. Make notes about the morphology of the fungi and draw the preparation.



3) Study the procedure of the pure culture isolation from pathological material contaminated with *Fusarium*. Make notes about the medium used for the causative agent isolation and morphology of their colonies.

4) Study serological tests for fusariosis diagnosis and methods of the therapy. Indicate the tests and names of the drug used for the therapy.

Task 7. Study the procedure of diagnosis of penicilliosis.

1) Study the rules of material sampling for microbiological study. Make notes about pathological material taken from patient with penicilliosis marneffeii caused by *Penicillium marneffeii*.

2) Study the microscopic picture of *Penicillium marneffeii* in pathological material preparations. Make notes about the staining techniques, morphology of the fungi, and draw the preparation.



3) Study the procedure of the pure culture isolation from pathological material contaminated with *Penicillium marneffeii*. Make notes about the medium used for the causative agent isolation and the growth conditions for their colonies.

4) Study serological and cytological tests for penicilliosis diagnosis and methods of the therapy. Indicate the tests and names of the drug used for the therapy.

Main goal 2. Preparation of Fungi for Microscopic Examination.

Task 1. Study unstained wet mount (“crushed drop”) technique.

1) Clean a plain microscope slide thoroughly: remove fat applying a soap and then cleaning it off with a fiber-free cloth.

- 2) Transfer one or two loopfuls of sterile physiological solution on to the centre of the slide.
- 3) Using aseptic technique, transfer a very small part of a single fungal (yeast) colony from a plate or slope of agar medium into the sterile physiological solution on the slide.
- 4) Cover the preparation with a cover slip applying it at a 45 degree angle. Push the air to the side and therefore minimize the risk of air bubbles. The excess liquid can be drained from the edges of the cover slip by filter paper.
- 5) Examine the wet preparation under the x40 objective or using immersion oil objective. Condenser must be lowered and illumination must be reduced for the examination of living microorganisms.

Task 2. Study simple staining technique for examination of fixed smears from yeasts.

- 1) Make a smear preparation of yeast (*Candida albicans*) as you do for bacteria. Fix it by Bunsen burner flame.
- 2) Thoroughly cover the smear with methylene blue dye and leave for 1-2 min.
- 3) Rinse off the stain with tap water.
- 4) Blot-dry the smear with filter/fibre-free blotting paper using firm pressure, but not sideways movements that might remove the smear.
- 5) Examine under oil immersion.

Draw the preparations:



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Literature to get prepared for CLASS 13. F.H. Kayser, K.A. Bieiz, J. Eckert, R.M. Zinkernagel. Medical microbiology. Thieme. 2005. – 724 p.

PROCEDURE OF PRACTICAL CLASS 14. MICROBIOLOGICAL DIAGNOSTICS OF NOSOCOMIAL INFECTIONS

Main goal 1. Determine the stages of the diagnosis of nosocomial infections

Task1. Study the procedure of diagnosis of infections caused by *Pseudomonas aeruginosa*.

1) Study the main diseased condition caused by *P. aeruginosa*. Note them below:

2) Study the rules of material sampling and procedure of its preparation for microbiological study. Make notes about pathological material taken from patient infected with *P. aeruginosa*.

3) Study the main nutritional media for *P. aeruginosa* isolation. Make notes about differential diagnostic media used for the bacteriological examination and growth conditions.

4) Study the growth type of *P. aeruginosa* in liquid and on solid media. Fill in the Table 1 with the main characteristics of the colonies.

**Table 1. Morphological characteristics of isolated colonies and cells of
*P. aeruginosa***

Medium	Morphological characteristics of the colonies						Cell shape
	Size	Shape	Color	Margin	Surface	Consistency	
MPA							
Ploskirev's agar							

5) Study procedure of the pigment *pyocyanin* production examination. Make notes about medium and reagents needed for this study.

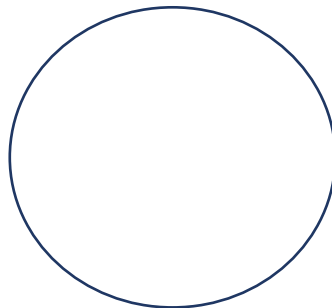
6) Study the main biochemical tests for identification of *P. aeruginosa* and media used for that purpose. Make notes about the proteolytic activity, nitrate reduction, the ability to ferment **glucose**, to produce indole, hydrogen sulfide, and **oxidase**, to form **acid**, Voges-Proskauer (acetoin) test.

Make notes about growth at 42° C, the phenomenon of spontaneous iridescent lysis, type of hemolysis on blood agar.

7) Study the procedure of bacterial smear preparation from *P. aeruginosa* colonies, staining, and its examination.

Make notes about the morphology and tinctorial properties of *P. aeruginosa*.

Draw the preparation of *P. aeruginosa* stained by Gram.

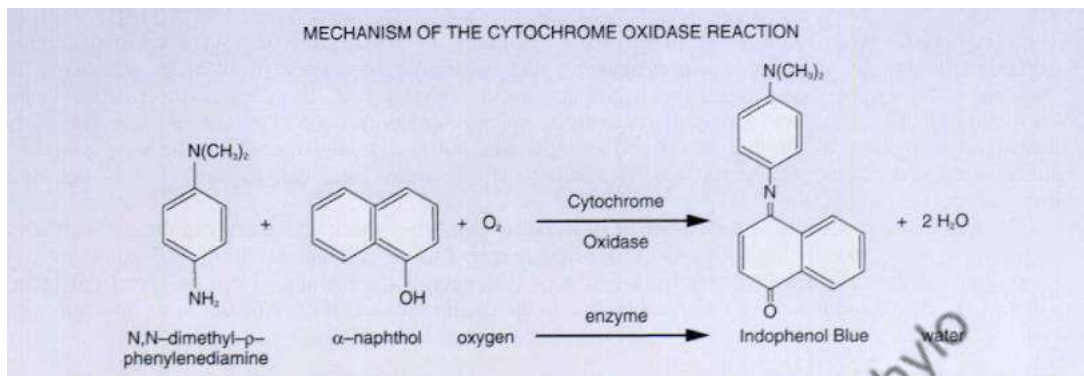


8) Study serological test for the identification of *P. aeruginosa* strains. Make notes about the principle of the test.

Main goal 2. Oxidase test performing.

Task 1. Study oxidase test: principle, procedure and oxidase positive organisms.

The oxidase test is used to identify bacteria that produce cytochrome c oxidase, an enzyme of the bacterial electron transport chain. When present, the cytochrome c oxidase oxidizes the reagent (tetramethyl-p-phenylenediamine) to (indophenols) purple color end product. When the enzyme is not present, the reagent remains reduced and is colorless.



Mechanism of the Cytochrome Oxidase Reaction

All bacteria that are oxidase positive are aerobic, and can use oxygen as a terminal electron acceptor in respiration. This does NOT mean that they are strict aerobes. Bacteria that are oxidase-negative may be anaerobic, aerobic, or facultative; the oxidase negative result just means that these organisms do not have the cytochrome c oxidase that oxidizes the test reagent. They may respire using other oxidases in electron transport.

Oxidase test is most helpful in screening colonies suspected of being one of the *Enterobacteriaceae* (all negative) and in identifying colonies suspected of belonging to other genera such as *Aeromonas*, *Pseudomonas*, *Neisseria*, *Campylobacter*, and *Pasteurella* (positive).

Bacterial species showing positive and negative reactions should be run as controls at frequent intervals. The following are suggested:

A. Positive control: *Pseudomonas aeruginosa*

B. Negative control: *Escherichia coli*

Procedure of Oxidase test:

- 1) Take a filter paper soaked with the substrate tetramethyl-p-phenylenediamine dihydrochloride;
- 2) Moisten the paper with a sterile distilled water;
- 3) Pick the colony to be tested with wooden or platinum loop and smear in the filter paper;
- 4) Observe inoculated area of paper for a color change to deep blue or purple within 10-30 seconds.

Precaution to be taken while performing oxidase test:

Do not use Nickel-base alloy wires containing chromium and iron (nichrome) to pick the colony and make smear as this may give false positive results.

Interpret the results within 10 seconds, timing is critical.

Note:

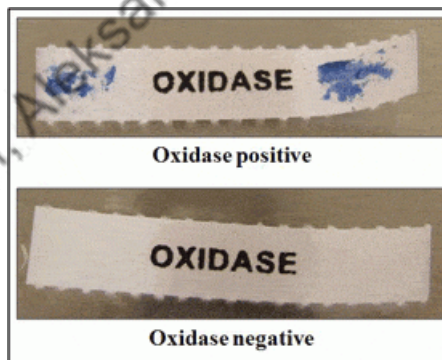
The oxidase test must be performed from 5% sheep blood agar or another medium without a fermentable sugar. Fermentation of carbohydrate results in acidification of the medium (e.g., lactose in MacConkey Agar or Sucrose in TCBS), and a false negative oxidase test may result if the surrounding pH is below 5.1. Subinoculation on Nutrient Agar is required before the oxidase test can be performed.

During the identification of suspected *Vibrio cholerae* isolate, it is not possible to perform an oxidase test directly from a TCBS culture because the acid produced by the sucrose fermenting colonies will inhibit the oxidase reaction.

Oxidase test results

Bacterial genera characterized as oxidase positive include *Neisseria* and *Pseudomonas* etc. Genera of the *Enterobacteriaceae* family are characterized as oxidase negative. Name of Oxidase positive bacteria are: Mneomoics for Oxidase Positive Organisms- PVNCH (It's just an acronym inspired by the famous mneemonic for Urease Positive organisms- PUNCH)

1. P: *Pseudomonas spp*
2. V: *Vibrio cholerae*
3. N: *Neisseria spp*
4. C: *Campylobacter spp*
5. H: *Helicobacter spp/ Haemophilus*
6. A: *Aeromonas spp*
7. Alcaligens



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Literature to get prepared for CLASS 14. Yi-Wei Tang, Max Sussman, Dongyou Liu, Ian Poxton, Joseph Schwartzman. Molecular Medical Microbiology. Volume 2. 2nd edition. – Copyright © 2015, 2002 Elsevier Ltd. – p.p. 753-767.

PROCEDURE OF PRACTICAL CLASS 15. ISOLATION AND IDENTIFICATION OF SANITARY- IMPORTANT MICROORGANISMS FROM WATER AND AIR IN ENCLOSED SPACES

Main goal 1. Determine the stages the sanitary and microbiological quality control of drinking water.

Task 1. Study the procedure of the collection of material and its preparation for microbiological study.

1) Study the main rules of tap water sample collection. Note them below:

Task 2. Study the determination of the total microbial count (TMC) when assessing the quality of drinking water. Make notes about it

1) Study the **deep inoculation** into solid nutrient media. Make notes about the method.

2) Make notes about TMC for drinking water and for the water of wells according to **State sanitary rules and norms No. 383**

Task 3. Study procedure of “coliform bacteria” determination. Make notes about these bacteria.

1) Study the **method of membrane filters**. Make notes about the medium used and necessary volume of the tested water.

2) Make notes about tinctorial and biochemical properties which are studied to determine the coliform bacteria in water.

3) Make notes about the standard requirements for coliform index and coli-titer of water

4) Study the **tube fermentation method**. Make notes about the principle of the method.

5) Study the procedure detection of *Escherichia coli* phage. Make notes about the principle of the method. Fill the table 1 with hygienic requirements for water.

Table 1

Indicators of microbial safety of drinking water

Indicators	Units of Measurement	Standards
The number of bacteria in 1 cm ³ of water (TMC)		
The number of coliform bacteria in 1 dm ³ of water (coliform index)		
The number of thermostable coliform bacteria in 100 cm ³ of water (FC index)		
The number of pathogenic microorganisms in 1 dm ³ of water		
The number of coliphages in 1 dm ³ of water		

Notes: CFU – colony-forming units (microorganisms); PFU – plaque-forming units (viruses).

Main goal 2. Determine the stages the sanitary and microbiological quality control of air.

Task 1. Study the **sanitary and microbiological indicators of air**.

1) Make notes about bacteria species including pathogenic, determinations of which are carried out for air sanitary condition examination.

Task 2. Study **Koch sedimentation method**.

1) Make notes about the principle of the method and media used for different microorganism isolation from air.

2) Make notes about the determination of the microbial number of air according Omelyansky formula.

Task 3. Study aspiration method with the use of the Krotov's apparatus.

1) Make notes about TMC determination using this method.

2) Study the procedure of examination of air for the presence of fungi. Make notes about the principle of their isolation.

3) Make notes about the index of sanitary-indicative microorganisms.

Main goal 3. SANITARY EXAMINATIONS OF WATER AND AIR.

Task 1. Procedure of the water examination.

Note: Aseptic technique must be used.

1. Petri dishes with MPA and Endo medium should be prepared in advance and stored in a refrigerator.
2. Volume of 100 ml of tap water samples are obtained in sterile tubes after flaming of the fringe of water tap and 10 min water bleeding.
3. Using a sterile pipette, place 0.1 ml of water samples on the media in Petri dishes.
4. Mark the Petri dishes with the sample number or other identification. The sample volume should also be recorded. Use a wax pencil or waterproof pen when writing on Petri dishes.
5. Maintain the Petri dishes in an incubator and incubate them at 37°C, for 24 hrs.
6. After incubation, count the colonies. Express the results as number of colonies per 100 ml of sample.
7. The colonies with green metallic sheen counted at Endo medium are presumed to be coliform bacteria.

Task 2. The settle plate technique using Koch's sedimentation method. Determination of total airborne bacterial (TAB) counts (CFU/m³ of air).

1. Label Blood agar plates and MPA plates with the respect to the date, time and period of exposure.
2. Place them at different areas in the room with the lids open for a period of 30 min and later close.
3. Incubate plates at 37 °C for 24 hrs.
4. After incubation, the colonies on the plates are counted and recorded as a number of bacteria carrying particles settling over the area of the plate in a given period of time. The level of bacterial contamination of air is expressed as the number of bacteria carrying particles per cubic millimeter.

$CFU/m^3 = a \times 1000 / p \times t \times 0.2$ where

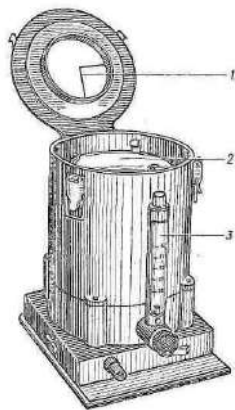
a= the number of colonies on the Petri plate

p = the surface measurement of the plate

t = the time of exposure of the Petri plate

Task 3. Aspiration method procedure.

Krotov apparatus is used for bacteriological air research. It gives possibility to let pass 50-100 L of air with a speed of 25 L per min through clinoid chink in the special glass above the open dish with medium (MPA, Egg-yolk agar, Sabouraud). The rotation of Petri dish (1 rotation/sec) provides uniform dispersion of microorganisms on all surface of a medium. Then dish is incubated in a thermostat at 37 °C for 18-24 hrs.



1–WEDGE-SHAPED SLOT 2 –ROTATING DISC 3 –RHEOMETER

With help of the ventilator the air is sucked through the wedge-shaped slot, which is placed along a radius over the Petri dish.

It is necessary to place the Petri dish with a nutrient medium onto the device disc. The device is connected to electric mains with help of its rheometer. Air speed is adjusted: 25l/ml. The amount of the air passed for counting the total number of bacteria should be 100 L.

The disc rotates. As a result, microorganisms cover the surface of the nutrient medium.

Meat-peptone agar is used for determination of the count of bacteria. Sabouraud agar – for determination of the count of fungi. Temperatures during incubation in the thermostat are kept at the level of:

for bacteria – $(32.5 \pm 2.5)^\circ\text{C}$ (2 days)

for fungi – $(22.5 \pm 2.5)^\circ\text{C}$ (5 days).

1. Using Krotov apparatus 250L (10 min of rotation) of air are sampled on the surface of open Petri dish with yolk-salt agar for staphylococci and with blood agar for streptococci.
2. The dishes are incubated in a thermostat at 37°C for 18-24 hrs.
3. After incubation growing up colonies are accounted and the number of staphylococci or streptococci in 1m³ of the air is calculated.

Student's Full name and Signature: _____

Instructor's Full name and Signature: _____

Literature to get prepared for CLASS 15.

1. Larry L. Barton, Robert J. C. McLean. Environmental Microbiology and Microbial Ecology. Wiley-Blackwell publishing. – 2019. – 464 p.
2. Sanitary microbiology: learning guide for the 2nd and 3rd year English medium students of the Faculty of Medicine and the Faculty of Dentistry (Microbiology, virology and immunology) / comp. N. I. Kovalenko, T. M. Zamazyi. – Kharkiv: Kharkiv National Medical University, 2021. – 48 p.