

Ministry of Health Care, Republic of Belarus
Vitebsk State Medical University
Department of Clinical Microbiology

INSTRUCTIONS
FOR LABORATORY TRAINING

in Special Microbiology & Virology
for Students of Faculty of Dentistry

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Instructions for laboratory training in Special Microbiology and Virology were elaborated according to basic educational plan and program, approved by Ministry of Health Care of Republic of Belarus. This workbook comprises the plan, schedule of practical training and basic practical skills in Special Microbiology and Virology.

The instructions are assigned for students of dentistry faculties of medical universities.

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Laboratory class №1

The topic: *Causative agents of suppurative and wound infections: Streptococci and Clostridia*

The main aim and the tasks of the work:

1. To learn the taxonomy of streptococci, and the role of various streptococcal species in human pathology.
2. To know the methods of laboratory diagnosis of streptococcal infections.
3. To know the basic properties of clostridia and their role in the development of gas gangrene.
4. To learn the principles of laboratory diagnosis of gas gangrene.
5. To know etiology, pathogenesis, clinical findings, laboratory diagnosis, specific prophylaxis and treatment of tetanus.

The questions to the topic:

1. Classification, structure and properties of streptococci.
2. Virulence factors of streptococci.
3. Pathogenesis and clinical findings in streptococcal infections.
4. Laboratory diagnosis of streptococcal infections. Differential diagnosis of *S. pyogenes*, *S. agalactiae*, *S. pneumoniae*, *Enterococcus spp.* Specific prophylaxis and treatment of streptococcal infections.
5. Classification, structure and properties of clostridia.
6. Virulence factors of clostridia – causative agents of gas gangrene.
7. Pathogenesis and clinical findings in gas gangrene. Laboratory diagnosis, prophylaxis and treatment.
8. Structure and properties of *C. tetani*.
9. Pathogenesis and clinical findings in tetanus. Laboratory diagnosis, specific prophylaxis and treatment.

The Literature:

1. Lecture material.
2. I.I. Generalov “Special Microbiology & Medical Virology: Lecture Course”. 2005, p. 18-34.

Personal work of students:

1. **Demonstration:** microscopy of slides with *S. pneumoniae*, *C. tetani*, and *C. perfringens* (Gram stain).

2. Demonstration: the medicines for diagnosis, specific prophylaxis and treatment of tetanus and gas gangrene – DPT vaccine, tetanus toxoid, anti-tetanus donor's immunoglobulin.

3. Evaluation of ELISA test for detection of tetanus toxin.

	1	2	3	4	5	6	7	8	9	10	11	12
A											K₁(-)	K₂(-)
B											K₁(+))	K₂(+)
C												
D												

Conclusion:

Reading of results is performed by microplate colorimetric reader at $\lambda=492\text{ nm}$.

The optical density (OD) for positive results must exceed previously established cut-off values.

Cut-off values are calculated as $(\text{mean } OD_{K(-)} + 0,5 OD)$ where $OD_{K(-)}$ indicates the values of optical density of negative controls (wells A11 and A12).

Mean value of optical density of negative controls (wells A11 and A12) should be equal or less than 0.3 OD units.

Mean value of optical density of positive controls (wells B11 and B12) should be equal or more than 0.8 OD units

Laboratory class №2

The topic: *Infection-associated diseases of oral cavity.*

Causative agents of caries, pulpitis, gingivitis, and periodontitis.

Suppurative and inflammatory infections of orofacial and neck areas

The main aim and the tasks of the work:

1. To learn common principles of pathogenesis of infection-associated diseases of oral cavity.
2. To know major microbial causative agents of dental, periodontal, and orofacial suppurative diseases.
3. To know the main principles of bacteriological testing in dental practice.

The questions to the topic:

1. Infections of oral cavity, their classification. Common steps of pathogenesis of dental, periodontal, and gum diseases.

2. Dental caries: major microbial causative agents, pathogenesis, steps of caries development, clinical classification, prophylaxis and treatment.
3. Pulpitis: microbial pathogenesis, clinical findings, treatment and prophylaxis of pulpitis.
4. General characteristics of periodontal diseases. Gingivitis: pathogenesis of various clinical forms. Acute necrotizing ulcerative gingivitis. Treatment and prophylaxis of gingivitis.
5. Characteristics of microbial clusters (or “complexes”) comprising major periodontal pathogens. Pathogenesis of periodontitis.
6. Clinical variations of periodontitis (local juvenile periodontitis, early-onset periodontitis, adult or chronic periodontitis, peri-implantitis); the role of microbial factors in their etiology and pathogenesis.
7. Suppurative and inflammatory infections of orofacial and neck areas: (periostitis, osteomyelitis, abscesses and phlegmonas, odontogenic sinusitis, facial and neck lymphadenitis).
8. Odontogenic bronchial and pulmonary infections.
9. Severe inflammatory response syndrome (SIRS) and sepsis in dental practice. Pathogenesis, laboratory diagnosis, prophylaxis and treatment of sepsis.
10. Principles of bacteriological testing in dental practice.

The Literature:

1. Lecture material.
2. I.I. Generalov “Medical Microbiology in Dentistry: Lecture Course”. Vitebsk, 2014, p. 31-54.

Personal work of students:

1. Microscopy of specimen from dental pocket (Gram stain).

Laboratory class №3

The topic: *Pathogenic Enterobacteria: Escherichia coli and Shigellae*

The main aim and the tasks of the work:

1. To learn general characteristics of *Enterobacteriaceae* family.
2. To know the main properties of *Escherichia coli* and its role in human pathology.
3. To know the basic properties of shigellae, their role in human pathology.
4. To get skills of laboratory diagnosis of escherichiosis.

The questions to the topic:

1. Classification, structure and properties of *Escherichia coli*.
2. Specific and non-specific *E. coli* infections.
3. Enteropathogenic and enterotoxigenic *E. coli* infections: pathogenesis and clinical findings.
4. Enteroaggregative, enteroinvasive and enterohemorrhagic *E. coli* infections: pathogenesis and clinical findings of diseases.
5. Laboratory diagnosis of escherichioses, their specific prophylaxis and treatment.
6. Classification, structure and properties of shigellae.
7. Virulence factors of shigellae.
8. Pathogenesis and clinical findings in shigellosis.
9. Laboratory diagnosis, prophylaxis and treatment of shigellosis.

Personal work of students:***Laboratory investigation of coli-enteritis.***

Day of investigation	Material for investigation	Steps of investigation	Results						
1.	Patient's feces	Plating of material onto EMB (eosin-methylene blue) medium.	—						
2.		Evaluation of microbial growth on EMB agar. Slide tentative agglutination test with polyspecific OK(B) antiserum. Inoculation of material from positive colony on the slant agar.							
3.		Evaluation of microbial growth on slant agar. Slide tentative agglutination reaction with monospecific OK(B) antisera: O ₁₁₁ K ₅₈ , O ₅₅ K ₅₉ , O ₂₀ K ₈₄ , O ₂₆ K ₆₀ . Extended agglutination test with monospecific OK(B) antiserum. Inoculation of isolated culture into Hiss media							
4.		Evaluation of biochemical properties of isolated culture	G	L	Mn	S	M	Indole	H ₂ S

Conclusion:

The Literature:

1. Lecture material.
2. I.I. Generalov “Special Microbiology & Medical Virology: Lecture Course”. 2005, p. 35-47.

Laboratory class №4
The topic: *Pathogenic Salmonellae*

The main aim and the tasks of the work:

1. To learn the common properties of salmonellae.
2. To know the main steps of pathogenesis of enteric typhoid fever and salmonellosis.
3. To get skills of laboratory diagnosis of food poisoning (salmonella toxoinfection).

The questions to the topic:

1. Classification, structure and main properties of salmonellae.
2. Antigenic structure of salmonellae. Kauffmann and White classification.
3. Virulence factors of salmonellae.
4. Pathogenesis and clinical findings in enteric fever.
5. Pathogenesis and clinical findings of salmonellosis.
6. Laboratory diagnosis of enteric typhoid fever.
7. Laboratory diagnosis of food poisoning and other salmonellosis.
8. Specific prophylaxis and treatment of enteric typhoid fever and salmonellosis.

The Literature:

1. Lecture material.
2. I.I. Generalov “Special Microbiology & Medical Virology: Lecture Course”. 2005, p. 48-56.

Personal work of students:***Hemoculture investigation in enteric typhoid fever.***

Day of investigation	Material for investigation	Steps of investigation	Results
1.	Patient's blood	Inoculation of 5 ml of blood into 50 ml of bile salt broth.	—

2.		Evaluation of microbial growth on bile salt broth. Planting of material from bile salt broth onto EMB agar.							
3.		Evaluation of microbial growth on EMB agar. Planting of material from lactose-negative colorless colony into Russel medium.							
4.		Evaluation of microbial growth on Russel medium. Slide tentative agglutination reaction with specific H-antisera to <i>S. typhi</i> and <i>S. paratyphi B</i> (<i>S. schottmuelleri</i>). Inoculation of isolated culture into Hiss media.							
5.		Evaluation of biochemical properties of isolated culture	G	L	Mn	S		In do le	H ₂ S

Conclusion:

Laboratory class №5

The topic: *Pathogenic Vibrios – causative agents of cholera. Pathogenic Yersiniae. Causative agent of botulism*

The main aim and the tasks of the work:

1. To know the common properties of *Vibrios*.
2. To learn the main steps of cholera pathogenesis.
3. To know the methods of laboratory diagnosis of cholera.
4. To known general properties of *Yersiniae*.
5. To learn pathogenesis and laboratory diagnosis of yersinioses.
6. To get skills of serological testing in yersiniosis.
7. To know the properties of botulism causative agent, pathogenesis, laboratory diagnosis, prophylaxis and treatment of botulism

The questions to the topic:

1. Classification, structure and properties of vibrios.
2. Virulence factors of cholera vibrios.
3. Pathogenesis and clinical findings in cholera.
4. Laboratory diagnosis of cholera, specific prophylaxis and treatment of the disease.
5. Classification, structure and basic properties of yersiniae.
6. Pathogenesis and clinical findings in yersinioses.
7. Laboratory diagnosis of yersinioses, specific prophylaxis and treatment.
8. Classification and properties of *C. botulinum*.
9. Pathogenesis and clinical findings in botulism.
10. Laboratory diagnosis of botulism, prophylaxis and treatment.

The Literature:

1. Lecture material.
2. I.I. Generalov "Special Microbiology & Medical Virology: Lecture Course". 2005, p. 57-71.

Personal work of students:**1. Indirect hemagglutination test for serological diagnosis of yersiniosis.**

Reagents	Patient's serum dilutions					
	1:20	1:40	1:80	1:160	1:320	K
	1	2	3	4	4	5
Saline	0,1	0,1	0,1	0,1	0,1	0,1
Patient's serum, diluted 1:10	0,1 →	0,1 →	0,1 →	0,1 →	0,1	-
Erythrocyte antigenic yersinia diagnosticum	0,1	0,1	0,1	0,1	0,1	0,1
<i>Incubation at 37°C for 2 h</i>						↓
Results						

Conclusion:**2. Demonstration:** microscopy of slides with *V. cholerae* (Gram stain).

Laboratory class №6

The topic: *Causative agents of bacterial respiratory infections: Meningococci, Bordetellae, Mycoplasmas*

The main aim and the tasks of the work:

1. To learn the theoretical knowledge of the topic.
2. To learn the methods of laboratory diagnosis of meningococcal infections.
3. To renew the skills of precipitation reaction technique for determination of meningococcal antigen in cerebrospinal fluid.
4. To learn the methods of laboratory diagnosis of whooping cough disease.
5. To learn the role of mycoplasmas in human pathology, clinical findings and laboratory diagnosis of mycoplasmal pneumonia.

The questions to the topic:

1. Meningococci, taxonomy, properties.
2. Virulence factors of meningococci. Pathogenesis of meningococcal diseases. Immunity.
3. Laboratory diagnosis of meningococcal infections. Specific prophylaxis and treatment.
4. Classification, structure and properties of bordetellae.
5. Bordetellae virulence factors. Pathogenesis of whooping cough. Immunity.
6. Laboratory diagnosis, prophylaxis and treatment of whooping cough.
7. Pathogenic mycoplasmas, classification and properties.
8. Virulence factors of mycoplasmas. Pathogenesis of mycoplasmal pneumonia. Laboratory diagnosis, prophylaxis and treatment of the disease.

The Literature:

1. Lecture material.
2. I.I. Generalov "Special Microbiology & Medical Virology: Lecture Course". 2005, p. 72-84.

Personal work of students:

1. Ring precipitation test for determination of meningococcal antigens in cerebrospinal fluid.

- Reagents:
- 1) Patient's cerebrospinal fluid.
 - 2) Meningococcal precipitating serum.
 - 3) Pneumococcal precipitating serum.

Reaction steps:

1. 1 ml of serum for precipitation of meningococcal antigens is dropped into the test tube №1.
2. 1 ml of cerebrospinal fluid is layered carefully on the serum surface.
3. The same manipulation is made with test tube №2, where the serum for precipitation of pneumococcal antigens is used.
4. Incubation for 10 min at room temperature. Ring of precipitation is to be formed.
5. Drawing of the results.

2. Demonstration: microscopy of patient's smear of cerebrospinal fluid with meningococci (Gram stain).

Laboratory class №7**The topic: *Mycobacterium tuberculosis. Corynebacterium diphtheria*****The main aim and the tasks of the work:**

1. To learn the theoretical knowledge of the topic
2. To be able to detect pathogenic mycobacteria and corynebacteria in smears from clinical specimens.
3. To know the methods of laboratory diagnosis of tuberculosis and diphtheria.
4. To get skills of *C. diphtheriae* toxigenicity determination.

The questions to the topic:

1. Classification of mycobacteria. Epidemiology of tuberculosis.
2. Structure and properties of pathogenic mycobacteria.
3. Pathogenesis and clinical findings in tuberculosis.
4. Laboratory diagnosis of tuberculosis.
5. Specific treatment and prophylaxis of tuberculosis.
6. Classification, structure and properties of *C. diphtheriae*.
7. Virulence factors of *C. diphtheriae*, mechanism of action of diphtheria exotoxin.
8. Pathogenesis and clinical findings in diphtheria.
9. Laboratory diagnosis of diphtheria.
10. Specific treatment and prophylaxis of diphtheria.

The Literature:

1. Lecture material.
2. I.I. Generalov "Special Microbiology & Medical Virology: Lecture Course". 2005, p. 85-101.

Personal work of students:**1. Demonstration:**

- a) microscopy of slides with *Corynebacterium diphtheriae* (Neisser stain);
- b) microscopy of slides with *M. tuberculosis* (Ziehl-Neelsen stain);
- c) microscopy of slides with *M. tuberculosis* microcolonies – cord-factor detection (Ziehl-Neelsen stain).

2. Demonstration: biological products and reagents for diagnosis, specific prophylaxis and treatment of diphtheria and tuberculosis.

Laboratory class №8

The topic: *Causative agents of sexually transmitted diseases (syphilis, gonorrhoea, urogenital chlamydioses and mycoplasmoses)*

The main aim and the tasks of the work:

1. To learn the theoretical knowledge of the topic.
2. To know the properties of *T. pallidum*, and the methods of laboratory diagnosis, treatment and prophylaxis of syphilis.
3. To be able to apply Wasserman's complement fixation test for serological diagnosis of syphilis.
4. To reproduce the skills of immersion microscopy for detection of treponemas, gonococci and chlamydiae in patient's specimens.

The questions to the topic:

1. Classification, structure and properties of *Treponemas*.
2. Pathogenesis and clinical findings in syphilis.
3. Laboratory diagnosis of syphilis, treatment and prophylaxis of the disease.
4. Classification, structure and properties of *Neisseria gonorrhoeae*.
5. Pathogenesis and clinical findings in gonorrhoea, laboratory diagnosis, treatment and prophylaxis of the disease.
6. Classification, structure and properties of pathogenic chlamydiae.
7. Pathogenesis and clinical findings in chlamydial urogenital infections, laboratory diagnosis, prophylaxis and treatment of the diseases.
8. Classification, structure and properties of uropathogenic mycoplasmas.
9. Pathogenesis and clinical findings in mycoplasmal urogenital infections, laboratory diagnosis, prophylaxis and treatment of the diseases.

The Literature:

1. Lecture material.
2. I.I. Generalov "Special Microbiology & Medical Virology: Lecture Course". 2005, p. 121-136.

Personal work of students:**1. Wasserman's reaction for serological diagnosis of syphilis.**

Reagents	Serum dilutions							Controls		
	1:10	1:20	1:40	1:80	1:160	1:320	1:640	Hemol. system	Ag	Compl.
	1	2	3	4	5	6	7	8	9	10
Saline	0,05	0,05	0,05	0,05	0,05	0,05	0,05	0,15	0,05	0,1
Patient serum	0,05	0,05	0,05	0,05	0,05	0,05	0,05	-	-	-
Specific treponemal antigen in working dose	0,05	0,05	0,05	0,05	0,05	0,05	0,05	-	0,05	-
Complement in working dose	0,05	0,05	0,05	0,05	0,05	0,05	0,05	-	0,05	0,05
<i>Incubation at 37°C for 1 h or at 4°C for 18-20 h</i>										
Hemolytic system	0,1	0,1	0,1	0,1	0,1	0,1	0,1	0,1	0,1	0,1
<i>Incubation at 37°C for 1 h</i>										
Results*										

Conclusion:

*Results are tested after control probes evaluation (test tubes №№ 8, 9, 10).
Hemolysis absence means *positive* complement fixation test result.

2. Demonstration:

- a) microscopy of slides with *T. pallidum* (Giemsa stain);
- b) microscopy of slides with *C. trachomatis* (Giemsa stain);
- c) microscopy of slides with incomplete phagocytosis of *N. gonorrhoeae* (methylene blue stain).

Laboratory class №9
Final control study of the section “Medical bacteriology”

The main aim and the tasks of the work:

To summarize the data of most significant bacterial infections, to learn major diseases of bacterial origin and methods of their laboratory diagnosis, prophylaxis and treatment.

The questions to the topic:

1. Staphylococci: classification, structure and properties. Virulence factors of staphylococci.

2. Pathogenesis and clinical findings in staphylococcal infections. Laboratory diagnosis, specific prophylaxis and treatment.

3. *Pseudomonas aeruginosa*: classification, structure and properties. Pathogenesis and clinical findings in *Pseudomonas aeruginosa* infections. Laboratory diagnosis, prophylaxis and treatment.

4. Classification of pathogenic gram-negative non-sporeforming anaerobes. Structure and properties of bacteroids, prevotellae, porphyromonads. Bacteroidal infections. Laboratory diagnosis, prophylaxis and treatment.

5. Streptococci: classification, structure and properties. Virulence factors of streptococci.

6. Pathogenesis and clinical findings in streptococcal infections. Laboratory diagnosis. Differential diagnosis of *S. pyogenes*, *S. agalactiae*, *S. pneumoniae*, and *Enterococcus spp.* Specific prophylaxis and treatment of streptococcal infections.

7. Classification, structure and properties of clostridia. Virulence factors of clostridia – causative agents of gas gangrene.

8. Pathogenesis and clinical findings in gas gangrene. Laboratory diagnosis, prophylaxis and treatment.

9. Structure and properties of *C. tetani*. Virulence factors.

10. Pathogenesis and clinical findings in tetanus. Laboratory diagnosis, specific prophylaxis and treatment.

11. Classification and properties of *C. botulinum*. Botulotoxin – properties, mechanism of action.

12. Pathogenesis and clinical findings in botulism. Laboratory diagnosis, prophylaxis and treatment.

13. Classification, structure and properties of *Escherichia coli*. Non-specific *Escherichia coli* infections.

14. Enteropathogenic, enterotoxigenic, enteroaggregative, enteroinvasive and enterohemorrhagic *E. coli*: pathogenesis and clinical findings of the diseases. Laboratory diagnosis of escherichioses. Prophylaxis and treatment.

15. Shigellae: classification, structure and properties. Virulence factors.

16. Pathogenesis and clinical findings in shigelloses. Laboratory diagnosis, prophylaxis and treatment.

17. Salmonellae: classification, structure and properties. Antigenic structure. Kauffmann and White scheme of salmonella typing. Virulence factors.

18. Pathogenesis and clinical findings in enteric typhoid fever. Laboratory diagnosis, specific prophylaxis and treatment.

19. Pathogenesis and clinical findings in salmonellosis. Laboratory diagnosis of salmonellosis, specific prophylaxis and treatment.

20. Classification, structure and properties of vibrios. Virulence factors of *Vibrio cholerae*.

21. Pathogenesis and clinical findings in cholera. Laboratory diagnosis, specific prophylaxis and treatment of cholera.

22. Classification, structure and basic properties of yersiniae.

23. Pathogenesis and clinical findings in yersiniosis. Laboratory diagnosis, prophylaxis and treatment.

24. Meningococci, taxonomy, properties. Virulence factors of meningococci.

25. Pathogenesis of meningococcal diseases. Immunity. Laboratory diagnosis of meningococcal infections. Specific prophylaxis and treatment.

26. Classification, structure and properties of bordetellae. Virulence factors. Pathogenesis of whooping cough. Laboratory diagnosis, prophylaxis and treatment of whooping cough.

27. Pathogenic mycoplasmas, classification and properties. Virulence factors of mycoplasmas.

28. Pathogenesis of mycoplasmal pneumonia. Laboratory diagnosis, prophylaxis and treatment of mycoplasmal pneumonia. Pathogenesis and clinical findings in mycoplasmal urogenital infections, laboratory diagnosis, prophylaxis and treatment.

29. Classification of pathogenic mycobacteria. Epidemiology of tuberculosis. Structure and properties of *M. tuberculosis*.

30. Pathogenesis and clinical findings in tuberculosis. Laboratory diagnosis of tuberculosis. Specific prophylaxis and treatment of tuberculosis.

31. Classification, structure and properties of *C. diphtheriae*. Virulence factors of *C. diphtheriae*, mechanism of action of diphtheria exotoxin.

32. Pathogenesis and clinical findings in diphtheria. Laboratory diagnosis of diphtheria. Specific treatment and prophylaxis of diphtheria.

33. Classification, structure and properties of treponemas. Pathogenesis and clinical findings in syphilis.

34. Laboratory diagnosis of syphilis. Serological tests for syphilis diagnosis. Treatment and prophylaxis of the disease.

35. Classification, structure and properties of *Neisseria gonorrhoeae*. Pathogenesis and clinical findings in gonorrhoea, laboratory diagnosis, treatment and prophylaxis of the disease.

36. Classification, structure and properties of pathogenic chlamydiae. Pathogenesis and clinical findings in chlamydial urogenital infections, laboratory diagnosis, prophylaxis and treatment of the diseases.

37. Infections of oral cavity, their classification. Common steps of pathogenesis of dental, periodontal, and gum diseases.

Pulpitis: microbial pathogenesis, clinical findings, treatment and prophylaxis of pulpitis.

38. Dental caries: major microbial causative agents, pathogenesis, steps of caries development, clinical classification, prophylaxis and treatment.

39. General characteristics of periodontal diseases. Gingivitis: pathogenesis of various clinical forms. Acute necrotizing ulcerative gingivitis. Treatment and prophylaxis of gingivitis.

40. Characteristics of microbial clusters (or “complexes”) comprising major periodontal pathogens. Pathogenesis of periodontitis.

41. Clinical variations of periodontitis (local juvenile periodontitis, early-onset periodontitis, adult or chronic periodontitis, peri-implantitis); the role of microbial factors in their etiology and pathogenesis.

42. Suppurative and inflammatory infections of orofacial and neck areas: (periostitis, osteomyelitis, abscesses and phlemonas, odontogenic sinusitis, facial and neck lymphadenitis). Odontogenic bronchial and pulmonary infections.

43. Severe inflammatory response syndrome (SIRS) and sepsis in dental practice. Pathogenesis, laboratory diagnosis, prophylaxis and treatment of sepsis.

44. Principles of bacteriological testing in dental practice.

The Literature:

1. Lecture material.

2. I.I. Generalov “Special Microbiology & Medical Virology: Lecture Course”. 2005, p. 5-101; 121-136.

3. I.I. Generalov “Medical Microbiology in Dentistry: Lecture Course”. Vitebsk, 2014, p. 31-54.

Demonstration material: Vi-hemagglutination test for enteric fever carriage detection, nutrient media for pathogenic bacteria cultivation, biological products and reagents for immunodiagnostics, immunoprophylaxis and immunotherapy of bacterial infections.

Laboratory class №10

The topic: *Causative agents of bacterial zoonoses (plague, anthrax, brucellosis, and tularemia)*

The main aim and the tasks of the work:

1. To learn the theoretical knowledge of the topic
2. To be able to detect pathogenic yersiniae, brucellae, francisellae and anthracoides bacilli in smears from clinical specimens.
3. To know the methods of laboratory diagnosis of plague, anthrax, brucellosis, and tularemia.
4. To get skills of serological diagnosis of brucellosis (Huddleson's and Wright's agglutination tests).

The questions to the topic:

1. Classification, structure and properties of *Y. pestis*.
2. Virulence factors of *Y. pestis*. Pathogenesis and clinical findings in plague.
3. Laboratory diagnosis of plague, specific prophylaxis and treatment of the disease.
4. Classification, structure and properties of *B. anthracis*.
5. Virulence factors of *B. anthracis*. Pathogenesis and clinical findings in anthrax.
6. Laboratory diagnosis of anthrax, specific prophylaxis and treatment.
7. Structure and properties of brucellae.
8. Pathogenesis and clinical findings in brucellosis.
9. Laboratory diagnosis of brucellosis, specific prophylaxis and treatment.
10. Structure and properties of *F. tularensis*.
11. Pathogenesis and clinical findings in tularemia.
12. Laboratory diagnosis of tularemia, specific prophylaxis and treatment.

The Literature:

1. Lecture material.
2. I.I. Generalov "Special Microbiology & Medical Virology: Lecture Course". 2005, p. 102-120.

Personal work of students:

1. Slide agglutination test (Huddleson's reaction) for serological diagnosis of brucellosis.

Reagents	Specimen			Control	
				Serum	Antigen
Patient's serum	0,04	0,02	0,01	0,02	-
Brucella microbial diagnosticum	0,03	0,03	0,03	-	0,03
Saline	-	-	-	0,03	0,03
Results					

Conclusion:

2. Extended agglutination test (Wright's reaction) for serological diagnosis of brucellosis.

Reagents	Serum dilutions					
	1:100	1:200	1:400	1:800	1:1600	K
Saline	1,0	1,0	1,0	1,0	1,0	1,0
Patient's serum	1,0	→ 1,0 →	1,0 →	1,0 →	1,0	-
Brucella microbial diagnosticum	3 drops into every test tube					↓
Results						

Conclusion:

3. Demonstration:

- a) microscopy of slides with *Y. pestis* (methylene blue stain);
- b) microscopy of slides with *B. anthracis* (Gram stain);
- c) microscopy of slides with *F. tularensis* (Gram stain);
- d) microscopy of slides with *B. abortus* (Gram stain).

4. Demonstration: medicines and reagents for diagnosis, specific prophylaxis and treatment of plague, anthrax, brucellosis, and tularemia.

Laboratory class №11

The topic: *Pathogenic Borreliae, Leptospirae, Rickettsiae and Coxiellae.*
Laboratory diagnosis of relapsing fever, Lyme disease,
leptospirosis, epidemic typhus, Q fever

The main aim and the tasks of the work:

1. To learn the theoretical knowledge of the topic
2. To know the properties of borreliae, leptospirae, rickettsiae and coxiellae.
3. To know the methods of laboratory diagnosis, treatment and prophylaxis of relapsing fever, Lyme disease, leptospirosis, epidemic typhus fever, Q fever.

The questions to the topic:

1. Classification, structure and common properties of borreliae.
2. Pathogenesis, clinical findings and laboratory diagnosis of relapsing fevers.
3. Pathogenesis, clinical findings and laboratory diagnosis of Lyme disease.
4. Classification, structure and properties of leptospirae.
5. Pathogenesis, clinical findings and laboratory diagnosis of leptospirosis.
6. Classification, structure and properties of rickettsiae.
7. Pathogenesis and clinical findings in rickettsioses.
8. Laboratory diagnosis, prophylaxis and treatment of epidemic typhus.
9. Classification, structure and properties of *Coxiella burnetii*.
10. Pathogenesis and clinical findings in Q fever. Laboratory diagnosis, prophylaxis and treatment of the disease.

The Literature:

1. Lecture material.
2. I.I. Generalov "Special Microbiology & Medical Virology: Lecture Course". 2005, p. 137-160.

Personal work of students:

1. Evaluation of ELISA test for serological diagnosis of Lyme disease.

	1	2	3	4	5	6	7	8	9	10	11	12
A											K ₁ (-)	K ₂ (-)
B											K ₁ (+)	K ₂ (+)
C												
D												

Conclusion:

Reading of results is performed by microplate colorimetric reader at $\lambda=492\text{ nm}$.

The optical density (OD) for positive results must exceed previously established cut-off values.

Cut-off values are calculated as ($\text{mean OD}_{K(-)} + 0,3\text{ OD}$) where $\text{OD}_{K(-)}$ indicates the values of optical density of negative controls (wells A11 and A12).

Mean value of optical density of negative controls (wells A11 and A12) should be equal or less than 0.3 OD units.

Mean value of optical density of positive controls (wells B11 and B12) should be equal or more than 0.8 OD units.

2. Demonstration:

- a) microscopy of slides with *B. recurrentis* in patient's blood (Giemsa stain);
- b) microscopy of slides with *R. provazekii* in infected tissues (Zdrodovsky stain).

Laboratory class №12

The topic: *General virology: morphology and physiology of viruses.* *Bacteriophages*

The main aim and the tasks of the work:

1. To learn the theoretical knowledge of the topic.
2. To know the structure, basic properties, and replication cycle of major viral groups.
3. To know the methods of virus determination in embryonated chicken eggs and cell cultures.
4. To get skills of hemagglutination testing for diagnosis of viral infections.
5. To get acquaintance with the methods of viral detection in the cell cultures by hemadsorption, symplast formation and by "color reaction".
6. To get the basic knowledge of bacteriophages, to learn the main practical applications of bacteriophages in biology and medicine.

The questions to the topic:

1. General characteristics of viruses.
2. Classification of viruses.
3. Structure of viruses.
4. Viral genomic organization.
5. Virus replication cycle.
6. Outcomes of viral infections

7. Laboratory diagnosis of viral infections. Detection (indication) and identification of viruses in the embryonated chicken eggs.
8. Different types of cell cultures. Indication and identification of viruses in the cell cultures.
9. Bacteriophages, their characteristics. The morphology of bacteriophages. Interaction of phages with bacterial cells.
10. Outcomes of phage-bacterial interaction. Lysogenic conversion. Production of the phage culture.
11. Laboratory determination of bacteriophage activity. Bacteriophage titration in liquid and solid nutrient medium. Practical applications of phages in biology and medicine.

The Literature:

1. Lecture material.
2. I.I. Generalov "Special Microbiology & Medical Virology: Lecture Course". 2005, p. 162-174.
3. I.I. Generalov "General Microbiology & Essential Immunology: Lecture Course". 2004, p. 77-82.

Personal work of students:

1. Hemagglutination test for the indication and titration of the virus.

Reagents	Allantoic fluid dilutions						Control
	1:5	1:10	1:20	1:40	1:80	1:160	
	1	2	3	4	5	6	
Saline	0,1	0,1	0,1	0,1	0,1	0,1	0,1
Allantoic fluid	0,1	→ 0,1 →	0,1 →	0,1 →	0,1 →	0,1	-
Erythrocyte suspension	0,1	0,1	0,1	0,1	0,1	0,1	0,1
<i>Incubation at 37° C for 1 h</i>							
Results							↓

Conclusion:

3. Demonstration:

- a) microscopy of infected cell cultures with symplasts in measles;
- b) microscopy of hemadsorption test;
- c) titration of bacteriophage on solid and liquid medium;
- d) test of *S. aureus* phagotyping.

Laboratory class №13

The topic: *Orthomyxoviruses. Paramyxoviruses. Coronaviruses*

The main aim and the tasks of the work:

1. To learn the theoretical knowledge of the topic.
2. To get acquaintance with the methods of laboratory diagnosis of influenza, parainfluenza, mumps, measles, respiratory syncytial viral infections.
3. To know the methods of specific prophylaxis and treatment of respiratory viral infections.

The questions to the topic:

1. Influenza viruses, classification, structure and properties, viral replication cycle.
2. Pathogenesis and clinical findings in influenza. Laboratory diagnosis of disease.
3. Specific prophylaxis and treatment of influenza.
4. Paramyxoviruses. Classification, general characteristics, replication cycle.
5. Pathogenesis and clinical findings in parainfluenza. Laboratory diagnosis of disease, specific prophylaxis and treatment.
6. Pathogenesis and clinical findings in measles and mumps. Laboratory diagnosis of the diseases, specific prophylaxis and treatment.
7. Pathogenesis and clinical findings in respiratory syncytial infections. Laboratory diagnosis, specific prophylaxis and treatment.
8. Coronaviruses, classification, structure and properties.
9. Coronaviral infections in humans: pathogenesis, clinical findings, laboratory diagnosis, prophylaxis and treatment.
10. Structure and properties of SARS-associated coronavirus.
11. Pathogenesis and clinical findings in SARS. Laboratory diagnosis of SARS, prophylaxis and treatment.

The Literature:

1. Lecture material.
2. I.I. Generalov "Special Microbiology & Medical Virology: Lecture Course". 2005, p. 175-194.

Personal work of students:**1. Hemagglutination inhibition test for the identification of influenza virus.**

Reagents	Serum dilutions						Control
	1:10	1:20	1:40	1:80	1:160	1:320	
	1	2	3	4	5	6	
Saline	0,1	0,1	0,1	0,1	0,1	0,1	0,1
Anti-influenza serum (H ₂ N ₂), 1/5	0,1 →	0,1 →	0,1 →	0,1 →	0,1 →	0,1	
Anti-influenza serum (H ₃ N ₂), 1/5	0,1 →	0,1 →	0,1 →	0,1 →	0,1 →	0,1	
Allantoic fluid (4 HAU)	0,1	0,1	0,1	0,1	0,1	0,1	0,1
<i>Incubation at 37°C for 1 h</i>							
Erythrocyte suspension	0,1	0,1	0,1	0,1	0,1	0,1	0,1
<i>Incubation at 37°C for 1 h</i>							
Results							
Anti-influenza serum (H ₂ N ₂), 1/5							
Anti-influenza serum (H ₃ N ₂), 1/5							

Conclusion:**2. Demonstration:**

- a) microscopy of the cell cultures with measles virus symplasts;
- b) microscopy of adenoviral inclusions.

Laboratory class №14

The topic: Picornaviruses (Enteroviruses: polioviruses, Coxsackie viruses, ECHO viruses). Reoviruses and Rotaviruses. Adenoviruses

The main aim and the tasks of the work:

1. To learn the theoretical knowledge of the topic.
2. To get acquaintance with laboratory diagnosis of poliomyelitis, enteroviral infections, rotaviral infection; infections, caused by adenoviruses.
3. To know the methods of specific prophylaxis and treatment of enteroviral, rotaviral and adenoviral infections.

The questions to the topic:

1. Picornaviruses: classification and general characteristics.
2. Polioviruses. Pathogenesis and clinical findings in poliomyelitis. Laboratory diagnosis and specific prophylaxis of disease.
3. Viruses Coxsackie of A and B groups. Clinical forms of coxsackievirus infection. Laboratory diagnosis and prophylaxis of Coxsackie virus infection.
4. ECHO viruses and other pathogenic enteroviruses. Clinical findings, laboratory diagnosis and prophylaxis of echovirus infections.
5. Reoviruses and rotaviruses. Classification and general characteristics.
6. Laboratory diagnosis and prophylaxis of rotaviral infections.
7. Adenoviruses: classification, structure and properties, replication cycle.
8. Pathogenesis and clinical findings in adenoviral infections. Laboratory diagnosis of adenoviral diseases, specific prophylaxis and treatment.

The Literature:

1. Lecture material.
2. I.I. Generalov “Special Microbiology & Medical Virology: Lecture Course”. 2005, p. 195-209.

Personal work of students:

1. Evaluation of RT-PCR test for diagnosis of rotaviral infection.
2. Identification of enteroviruses by neutralization test of viral cytopathic effect in cell culture.

Laboratory class №15

**The topic: *Retroviruses and Human Immunodeficiency Virus (HIV).
Hepatotropic viruses (causative agents of hepatitis A, B, C, D, E)***

The main aim and the tasks of the work:

1. To learn the theoretical knowledge of the topic.
2. To get acquaintance with laboratory diagnosis of HIV-infection (ELISA test, western blot analysis).
3. To get acquaintance with laboratory diagnosis of viral hepatitis.
4. To get skills of HBV laboratory diagnosis by PCR test.

The questions to the topic:

1. Retroviruses, their classification. Structure of HIV. Virion resistance.
2. HIV replication cycle. Pathogenesis of HIV infection.
3. Epidemiology and clinical findings in HIV infection. AIDS progressing.
4. Laboratory diagnosis, specific treatment and prophylaxis of HIV infection.
5. Hepatitis A virus. Pathogenesis and clinical findings in hepatitis A. Laboratory diagnosis of disease, specific prophylaxis and treatment.
6. Hepatitis B virus. Classification, structure and properties, HBV replication cycle. Pathogenesis and clinical findings in hepatitis B. Laboratory diagnosis, specific prophylaxis and treatment.
7. Hepatitis D virus. Classification, structure and properties. Clinical findings in delta-infection. Laboratory diagnosis of hepatitis D.
8. Hepatitis C virus. Classification, structure and properties. Clinical findings in hepatitis C. Prognosis of HCV infection. Laboratory diagnosis, prophylaxis and treatment.
9. Hepatitis E virus. Structure and properties. Clinical findings in hepatitis E. Laboratory diagnosis of HEV infection.

The Literature:

1. Lecture material.
2. I.I. Generalov "Special Microbiology & Medical Virology: Lecture Course". 2005, p. 210-231.

Personal work of students:

1. *Demonstration* of western blotting analysis for serological diagnosis of HIV infection.
2. Evaluation of PCR test for diagnosis of hepatitis B.

Laboratory class №16

The topic: *Herpesviruses. Rubella virus. Rabdoviruses. Prions and prion diseases*

The main aim and the tasks of the work:

1. To learn the the theoretical knowledge of the topic.
2. To get acquaintance with laboratory diagnosis of herpes viral infection, rabies and rubella.

3. To get skills of rabies diagnosis by microscopy.
4. To get skills of PCR practical application for diagnosis of viral infections.
5. To get acquaintance with main groups of prion diseases.

The questions to the topic:

1. Herpesviruses: classification and general characteristics.
2. Herpes simplex viruses of 1 and 2 type. Pathogenesis and clinical findings in herpetic infection. Laboratory diagnosis, treatment and prophylaxis of the diseases.
3. Varicella-zoster herpesvirus infections. Pathogenesis and clinical findings in varicella and shingles. Laboratory diagnosis, prophylaxis and treatment of infections.
4. Cytomegalovirus infection. Pathogenesis and clinical findings in CMV infection. Laboratory diagnosis, prophylaxis and treatment.
5. Epstein-Barr virus infection. Clinical findings in EBV infection. Laboratory diagnosis, prophylaxis and treatment. Burkitt's lymphoma and nasopharyngeal carcinoma.
6. Herpesvirus infection of HV types 6, 7 and 8. General characteristics. Laboratory diagnosis.
7. Rubella virus: classification, structure and properties.
8. Clinical findings in rubella. Congenital rubella syndrome. Laboratory diagnosis, specific prophylaxis and treatment of rubella.
9. Rabdoviruses: classification and general characteristics. Rabies virus. Structure and properties.
10. Pathogenesis and clinical findings in rabies. Laboratory diagnosis, specific passive and active prophylaxis of rabies.
11. Prions and prion diseases. Classification and general characteristics. Pathogenesis of prion infections: the role of PrP^{Sc} proteins.
12. Clinical finding in human prion diseases. Laboratory diagnosis and prophylaxis of human prion diseases.

The Literature:

1. Lecture material.
2. I.I. Generalov "Special Microbiology & Medical Virology: Lecture Course". 2005, p. 232-249.

Personal work of students:

1. Evaluation of PCR test for diagnosis of cytomegalovirus infection.

2. Demonstration:

- a) microscopy of Babes-Negri bodies in hippocampus section in rabies;
- b) microscopy of cytoplasmic inclusions in epithelial cells of salivary glands in CMV infection.

Laboratory class №17
Final control study of the section “Medical virology”

The main aim and the tasks of the work:

To summarize essential data of medical virology, to revise the knowledge of major viral infections and methods of their laboratory diagnosis, prophylaxis and treatment.

The questions to the topic:

1. Classification and structure of viruses. Viral genomic organization.
2. Virus replication cycle. Outcomes of viral infections.
3. Laboratory diagnosis of viral infections. Different types of cell cultures. Indication and identification of viruses in cell cultures.
4. Influenza viruses, classification, structure and properties, replication cycle. Pathogenesis and clinical findings in influenza.
5. Laboratory diagnosis of influenza. Specific prophylaxis and treatment of the disease.
6. Paramyxoviruses. Classification, general characteristics, replication cycle. Pathogenesis and clinical findings in parainfluenza. Laboratory diagnosis of disease, specific prophylaxis and treatment.
7. Measles virus, classification, structure and properties. Pathogenesis and clinical findings in measles. Laboratory diagnosis of disease, specific prophylaxis and treatment.
8. Mumps virus, classification, structure and properties. Pathogenesis and clinical findings in mumps. Laboratory diagnosis of disease, specific prophylaxis and treatment.
9. Respiratory syncytial virus, classification, structure and properties. Pathogenesis and clinical findings in respiratory syncytial infections. Laboratory diagnosis of diseases, specific prophylaxis and treatment.
10. Human coronaviruses, classification, structure and properties. SARS-associated virus. Pathogenesis and clinical findings in severe acute respiratory syndrome. Laboratory diagnosis, prophylaxis and treatment.

11. Adenoviruses, classification, structure and properties. Pathogenesis and clinical findings in adenoviral infections. Laboratory diagnosis of adenoviral diseases, specific prophylaxis and treatment.

12. Picornaviruses: classification and general characteristics. Polioviruses. Pathogenesis and clinical findings in poliomyelitis. Laboratory diagnosis and specific prophylaxis of disease.

13. Coxsackieviruses of A and B groups. Classification and general characteristics. Clinical forms of coxsackieviral infection. Laboratory diagnosis and prophylaxis of coxsackieviral infection

14. Reoviruses and rotaviruses. Classification and general characteristics. Laboratory diagnosis and prophylaxis of rotaviral infections.

15. Hepatitis A virus. Pathogenesis and clinical findings in hepatitis A. Laboratory diagnosis of disease, specific prophylaxis and treatment.

16. Hepatitis B virus. Classification, structure and properties, HBV replication cycle. Pathogenesis and clinical findings in hepatitis B. Laboratory diagnosis, specific prophylaxis and treatment.

17. Hepatitis D virus. Structure and properties. Clinical findings in hepatitis D. Laboratory diagnosis of delta-infection.

18. Hepatitis C virus. Classification, structure and properties. Clinical findings in hepatitis C. Prognosis of HCV infection. Laboratory diagnosis, prophylaxis and treatment.

19. Hepatitis E virus. Structure and properties. Clinical findings in hepatitis E. Laboratory diagnosis of HEV infection.

20. Retroviruses, their classification. Structure of HIV. Virion resistance. HIV replication cycle. Pathogenesis of HIV infection.

21. Epidemiology and clinical findings in HIV infection. AIDS progression. Laboratory diagnosis, prophylaxis and specific treatment of HIV infection.

22. Herpesviruses. Classification and general characteristics. Herpes simplex viruses of 1 and 2 type. Pathogenesis and clinical findings in herpetic infection. Laboratory diagnosis, treatment and prophylaxis of disease.

23. Varicella-zoster herpesvirus infections. Pathogenesis and clinical findings in varicella and shingles. Laboratory diagnosis, prophylaxis and treatment of varicella and shingles.

24. Cytomegalovirus infection. Pathogenesis and clinical findings in CMV infection. Laboratory diagnosis, prophylaxis and treatment. Herpesvirus infection of HV types 6, 7 and 8. General characteristics. Laboratory diagnosis.

25. Epstein-Barr virus infection. Clinical findings in EBV infection. Laboratory diagnosis, prophylaxis and treatment. Burkitt's lymphoma and nasopharyngeal carcinoma.

26. Rubella virus. Classification, structure and properties. Clinical findings in rubella. Congenital rubella syndrome. Laboratory diagnosis, specific prophylaxis and treatment of rubella.

27. Rabdoviruses, classification and general characteristics. Rabies virus. Structure and properties. Pathogenesis and clinical findings in rabies. Laboratory diagnosis, specific passive and active prophylaxis of rabies.

28. Bacteriophages, their characteristics. The morphology of bacteriophages. Interaction of phages with bacterial cells. Outcomes of phage-bacterial interaction. Lysogenic conversion.

29. Production of the phage culture. Laboratory determination of bacteriophage activity. Bacteriophage titration in liquid and solid nutrient medium. Practical applications of phages in biology and medicine.

30. Prions and prion diseases. Classification and general characteristics. Pathogenesis of prion infections: the role of PrP^{Sc} proteins.

31. Clinical finding in human prion diseases. Laboratory diagnosis and prophylaxis of prion diseases.

The Literature:

1. Lecture material.

2. I.I. Generalov “Special Microbiology & Medical Virology: Lecture Course”. 2005, p. 162-249.

3. I.I. Generalov “General Microbiology & Essential Immunology: Lecture Course”. 2004, p. 77-82.

Demonstration of tests, reagents and biological products: hemagglutination inhibition test and enzyme-linked immunosorbent assay (ELISA) for serological diagnosis of viral infections, anti-rabies gamma-globulin, anti-measles gamma-globulin; anti-influenza type A serum (sub-type H₃N₂), influenza viral diagnosticum type A (sub-type H₃N₂), HBs-vaccine, live trivalent poliovaccine, measles-parotitis-rubella vaccine.

Laboratory class №18

The topic: *Infectious diseases with specific lesions in oral cavity*

The main aim and the tasks of the work:

1. To get acquaintance with typical oral manifestations of common bacterial, fungal, and viral diseases.

2. To know the principles of laboratory testing of bacterial, fungal, and viral infections, demonstrating specific oral lesions.

The questions to the topic:

1. Characteristics of oral manifestations of tuberculosis and actinomycosis, principles of laboratory diagnosis.
2. Oral manifestations of diphtheria, laboratory diagnosis of the disease.
3. Characteristics of oral lesions in sexually transmitted diseases (syphilis and gonorrhoea). Laboratory diagnosis of venereal diseases with specific oral manifestations.
4. Oral manifestations, typical for certain bacterial infections (scarlet fever, anthrax). Principles of laboratory diagnosis.
5. Oral candidiasis: characteristics of causative agent. Clinical forms of oral candidiasis. Candida-associated denture-induced stomatitis. Laboratory diagnosis, treatment, and prophylaxis of oral candidiasis.
6. General characteristics of viral infections, affecting oral cavity. Oral manifestations of infections, caused by herpesviruses. Laboratory tests for diagnosis of oral herpetic lesions.
7. Oral lesions specific for certain viral infections (measles, enteroviral stomatitis and herpangina).
8. Oral manifestations, associated with HIV infection and AIDS. Laboratory diagnosis of opportunistic AIDS-related infections.

The Literature:

1. Lecture material.
2. I.I. Generalov. Medical Microbiology in Dentistry: Lecture Course. Vitebsk, 2014, p. 55-72.

Personal work of students:***Demonstration:***

- a) microscopy of specimen with *M. tuberculosis*, Ziehl-Neelsen stain.
- b) microscopy of *C. albicans* fungi, methylene blue stain.
- c) *C. albicans* growth on Sabouraud agar.

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in Special Microbiology & Virology
for Students of Faculty of Dentistry
(Russian and English Medium)

МЕТОДИЧЕСКИЕ УКАЗАНИЯ К ЛАБОРАТОРНЫМ ЗАНЯТИЯМ
ПО ЧАСТНОЙ МИКРОБИОЛОГИИ

для студентов стоматологического факультета
с русским и английским языком обучения
высших медицинских учебных заведений

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