

GENERAL MICROBIOLOGY WITH MICROBIOLOGY OF THE ORAL CAVITY

DENTISTRY, the academic year 2024-2025

Meeting Times and Room Assignments

Laboratory Exercises (30 hours) :

Wednesday: GROUP 1 (12.00-13.30)

lab #209, the Department of Microbiology, Street Chalubińskiego 4

Exercise Schedule

Date	#	Topic
9.10.2024	1	Principles of laboratory work. Morphology of bacteria. Staining methods. Methods of cultivation and differentiation of microorganisms.
16.10.2024	2	Gram positive cocci of <i>Staphylococcus</i> , <i>Micrococcus</i>
23.10.2024	3	Gram positive cocci of <i>Streptococcus</i> , <i>Enterococcus</i>
30.10.2024	4	Gram-positive bacilli of <i>Corynebacterium</i> , <i>Rothia</i> , <i>Lactobacillus</i> and acid-fast <i>Mycobacterium</i> .
6.11.2024	5	TEST 1 (topics 1-4) Gram-negative bacilli of <i>Haemophilus</i> , <i>Bordetella</i> , <i>Legionella</i> . Gram-negative cocci of <i>Neisseria</i> , <i>Moraxella</i> .
13.11.2024	6	Gram-negative bacilli of <i>Enterobacterales</i> and nonfermenters of <i>Pseudomonas</i> , <i>Acinetobacter</i> <i>Stenotrophomonas</i> .
20.11.2024	7	Gram positive sporulating anaerobic bacilli of <i>Clostridium</i> and <i>Clostridioides</i> .
27.11.2024	8	Gram-positive and Gram-negative non-sporulating anaerobic bacteria.
4.12.2024	9	TEST 2 (topics 5-8) Antibiotics and chemotherapeutics. Laboratory methods of sensitivity testing.
11.12.2024	10	Mechanisms of antimicrobial resistance
18.12.2024	11	TEST 3 (topics 9-10) Fungal infections of the oral cavity.
8.01.2025	12	Oral microbiota, part 1. Microbiology of dental caries and gingivitis.
15.01.2025	13	Oral microbiota, part 2. Microbiology of periodontal diseases.
22.01.2025	14	TEST 4 (topics 11-14). Infection control. Sterilization, disinfection and antisepsis.
29.01.2025	15	PRACTICAL SKILLS TEST.

Single-choice test (**10 questions / 1 point**; only a single correct answer) and **5 open-ended questions / 2 points** (cannot be answered with a simple 'yes' or 'no', and instead require to elaborate on their points).

Evaluation criteria:

- 0 - 11 points (55%) - insufficient
- 12 - 13 points (60-65%) - sufficient
- 14 - 15 points (70-75%) - a sufficient plus
- 16 - 17 points (80-85%) - good
- 18 - 19 points (90-95%) - a good plus
- 20 points (100%) - very good

A DETAILED PROGRAM OF LABORATORY EXERCISES

EXERCISE # 1. PRINCIPLES OF LABORATORY WORK MORPHOLOGY OF BACTERIA. STAINING METHODS. METHODS OF CULTIVATION AND DIFFERENTIATION OF MICROORGANISMS

The theoretical part:

Organization, rules and regulations of laboratory work

Staining methods (simple, differentiation, positive, negative, negative-positive)

The culture conditions - effects of temperature, pH, oxidation-reduction potential, nutrients, exogenous compounds (growth factors).

Type of microbiological media (simple, enriched, selective-differential, differential).

Practice:

Preparation of slides (Gram staining) and microscopy analysis of microbial morphology and Gram reaction
Demonstration of bacteriological media and the morphology of microbial colonies tests for identification of microorganisms

EXERCISE # 2. GRAM-POSITIVE COCCI OF *STAPHYLOCOCCUS*, *MICROCOCCUS*

Theoretical part:

Medical importance

Phenotypic characteristics of:

Staphylococcus aureus, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Staphylococcus lugdunensis* and *Micrococcus* spp.

Principles of laboratory diagnostics

Practice:

Demonstration of cultures on blood agar, characterization of colony morphology, haemolysis.

Identification tests (detection of: catalase, CF, free coagulase, ID32 Staph, Crystal GP)

Preparation and analysis of microscopic slides (from selected cultures).

EXERCISE # 3. GRAM-POSITIVE COCCI OF *STREPTOCOCCUS* AND *ENTEROCOCCUS*

The theoretical part:

Medical importance

Phenotypic characteristics of *Streptococcus* groups (oral streptococci):

1. MUTANS group (*S. mutans*, *S. sobrinus*, *S. rattii*)
2. SALIVARIUS group (*S. salivarius*, *S. vestibularis*)
3. MITIS group (*S. mitis*, *S. oralis*, *S. cristatus*, *S. pneumoniae*, *S. pseudopneumoniae*, *S. sanguinis*)

4. ANGINOSUS group (*S. anginosus* *S. intermedius* *S. constellatus*)
Streptococcus pyogenes, *S. agalactiae*,
Enterococcus faecalis, *Enterococcus faecium*
Principles of laboratory diagnostics.

Practice:

Laboratory methods of *Streptococcus* spp. and *Enterococcus* spp. identification
Preparation and analysis of microscopic slides (from selected cultures).

**EXERCISE # 4. GRAM-POSITIVE BACILLI OF *CORYNEBACTERIUM*, *ROTHIA*,
LACTOBACILLUS and ACID-FAST *MYCOBACTERIUM*.**

The theoretical part:

Medical importance

Phenotypic characteristics of: *Corynebacterium diphtheriae*, *Rothia dentocariosa*, *Lactobacillus* spp. and
Mycobacterium tuberculosis)

Principles of laboratory diagnostics

Practice

Laboratory methods of identification

Preparation and analysis of microscopic slides (from selected cultures).

EXERCISE # 5. TEST 1 (topics 1-4)

**GRAM-NEGATIVE BACILLI OF *HAEMOPHILIS*, *BORDETELLA*,
LEGIONELLA. GRAM-NEGATIVE COCCI OF *NEISSERIA*, *MORAXELLA*.**

The theoretical part:

Medical importance

Phenotypic characteristics of:

- cocci: *Neisseria*, *Moraxella*

- fastidious bacilli: *Haemophilus*, *Bordetella* and *Legionella*

Principles of laboratory diagnostics

Immunoprophylaxis (vaccination)

Practice

Clues to *Neisseria gonorrhoe*, *Neisseria meningitidis*, *Haemophilus influenzae*, *Bordetella pertussis* and
Legionella pneumophila culturing and identification.

Preparation and analysis of microscopic slides (from selected cultures).

**EXERCISE # 6. GRAM-NEGATIVE BACILLI OF *ENTEROBACTERALES* AND
NONFERMENTERS OF *PSEUDOMONAS*, *ACINETOBACTER*, *STENOTROPHOMONAS***

The theoretical part:

Medical importance

Phenotypic characteristics of members of *Enterobacterales* (*Escherichia*, *Proteus*, *Klebsiella*, *Salmonella*,
Shigella) and nonfermentative bacilli (*Pseudomonas*, *Acinetobacter*, *Stenotrophomonas*)

Principles of laboratory diagnostics

Practice

Laboratory methods (media, identification tests) used to isolate and identify members of *Enterobacterales*
and nonfermentative bacilli. Preparation and analysis of microscopic slides (from selected cultures).

EXERCISE # 7. GRAM-POSITIVE ANAEROBIC SPORULATING BACILLI OF *CLOSTRIDIUM*, *CLOSTRIDIODES*.

The theoretical part:

Medical importance

Phenotypic characteristics of *B. anthracis*, *Bacillus cereus*,

Clostridium tetani, *Clostridium botulinum*, *Clostridioides difficile*, *Clostridium perfringens*

Immunoprophylaxis (vaccination)

Practice

Laboratory methods of identification.

Preparation and analysis of microscopic slides (from selected cultures).

EXERCISE # 8. GRAM-POSITIVE AND GRAM-NEGATIVE ANAEROBIC NON-SPORULATING BACTERIA

The theoretical part:

Clinical significance

Phenotypic characteristics of anaerobes:

a) Gram-positive bacteria of: *Actinomyces*, *Cutibacterium*, *Eubacterium*, *Peptostreptococcus*

b) Gram-negative bacteria of: *Bacteroides*, *Tannerella*, *Porphyromonas*, *Prevotella*, *Veillonella*,
Fusobacterium, *Leptotrichia*, *Treponema*, *Selenomonas*

Principles of laboratory diagnostics

Practice

Methods for cultivation and identification of anaerobic isolates (presumptive identification, simple observation, tests, definitive identifications)

Preparation and analysis of microscopic slides (from selected cultures).

EXERCISE # 9. TEST 2 (topics 5-8)

ANTIBIOTICS AND CHEMOTHERAPEUTICS. LABORATORY METHODS OF SENSITIVITY TESTING

The theoretical part:

1. General mechanisms of antimicrobial action, antimicrobial spectrum of: β -lactams, aminoglycosides, macrolides and ketolides, lincosamides, glycopeptides, tetracyclines, oxazolidinones, quinolones, nitroimidazoles, sulfonamides.

2. Susceptibility testing:

- disc diffusion method,
- serial dilutions method (MIC, MBC determination)
- E-test
- ATB - susceptibility testing for anaerobic bacteria - demonstration

Practice:

Students prepare susceptibility tests for: *Staphylococcus aureus* MRSA, *Enterococcus faecalis* HLAR, *Klebsiella pneumoniae* ESBL

EXERCISE # 10. MECHANISMS OF ANTIMICROBIAL RESISTANCE

The theoretical part:

Overview of selected mechanisms of bacterial resistance to antibiotics: MRS (MRSA, MRCNS), VISA,

VRSA, VRE, GRE, HLAR, ESBL, MBL, KPC, MLS_B based on the results of the previous exercise.

Practice:

Reading of susceptibility tests (from the previous exercise)

Demonstration of resistance phenotypes: ESBL, MBL, MRS, MLS_B, VRE, GRE, HLAR

Detection of β -lactamase producing strains (cefinaise method)

EXERCISE # 11. TEST 3 (topics 9-10)
FUNGAL INFECTIONS OF THE ORAL CAVITY

The theoretical part:

collection and transportation of specimens; laboratory testing; principles of the culture of pathogenic fungi:

- Characteristics of yeasts: *Candida* spp. and *Cryptococcus* pp.
 - a) **Candidiasis:**
 - acute pseudomembranous candidiasis (thrush)
 - acute atrophic candidiasis
 - chronic atrophic candidiasis
 - chronic hyperplastic candidiasis
 - inflammation of the mouth corner
 - prosthetic stomatopathy
 - b) **Cryptococcosis**
- Characteristics of molds and dimorphic fungi (mouth ulcers in AIDS patients)

Practice:

- a) Identification of yeast (demonstration):
 - morphology of *Candida* colonies on Sabouraud agar and CHROMagar
 - germ tube test (microscopy)
 - *Candida* chlamydospores in microcultures,
 - ID 32 C test.
- b) molds
 - demonstration of slides (lactophenol Blue) and cultures
 - Preparation and analysis of microscopic slides (from selected cultures of yeasts).

EXERCISE # 12. ORAL MICROBIOTA, PART 1.
MICROBIOLOGY OF DENTAL CARIES AND GINGIVITIS.

Theoretical part:

positive and negative role of microbiota;

oral microbiota and systemic endogenous infections;

etiologial factors and microbiological diagnostics of :

- a) dental caries
- b) gingivitis - associated / not associated with dental plaque
- c) acute necrotizing ulcerative gingivitis (ANUG) and its aggressive form - NOMA

Practice:

inoculation of oral specimens (swabs from the cheek, tongue, plaque, gingival pockets) on selected media

EXERCISE # 13. ORAL MICROBIOTA, PART 2.
MICROBIOLOGY OF PERIODONTAL DISEASES.

Theoretical part:

etiological factors and microbiological diagnostics of:

- chronic periodontitis (local and generalized)
- aggressive periodontitis (local and generalized)
- necrotizing ulcerative periodontitis (NUP)
- abscesses of the periodontium
- *peri-coronitis, peri-implantitis, peri-mucositis*

Selection, collection and transportation of samples for microbiological and serological testing; oral cavity specimen processing; aerobic / anaerobic incubation

Practice:

analysis of the prepared cultures (oral specimens)

Preparation and analysis of microscopic slides (from selected cultures).

EXERCISE # 14. TEST 4 (topics 11-14)
INFECTION CONTROL.
DISINFECTION, STERILIZATION AND ANTISEPSIS

The theoretical part:

Physical and chemical methods of sterilization and disinfection (with particular emphasis on sterilization of dental equipment).

Demonstration:

- Tests for biological control of sterilization,
- equipment for sterilization: packages, sleeves, etc.

Practice: students perform 2 experiments -

1. The bactericidal effect of UV radiation.
2. Effect of disinfectants on the human microbiota.

EXERCISE # 15. PRACTICAL SKILLS TEST.

The test of skills in the preparation of microbiological slides and the identification of the observed microorganisms.

Two slides should be evaluated:

1. Prepared by the teacher
2. Prepared by a student from the given solid culture

Both preparations should specify (written answers):

1. Staining technique used
2. Morphology and arrangement of the observed cells
4. Suspected group of microorganisms (Latin names)