**GENERAL MICROBIOLOGY WITH MICROBIOLOGY OF THE ORAL CAVITY**

**DENTISTRY, the academic year 2023-2024**

**Meeting Times and Room Assignments**

**Laboratory Exercises** (30 hours):

Wednesday: GROUP 1 (12.00-13.30) / GROUP 2 (13.45-15.15)

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

**Exercise Schedule**

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| --- | --- | --- |
| **Date** | **#** | **Topic** |
| **04.10.23** | **1** | Principles of laboratory work. Morphology of bacteria. Staining methods. Methods of cultivation and differentiation of microorganisms. |
| **11.10.23** | **2** | Gram positive cocci of *Staphylococcus, Micrococcus* |
| **18.10.23** | **3** | Gram positive cocci of *Streptococcus, Enterococcus* |
| **25.10.23** | **4** | Gram-positive bacilli of *Corynebacterium, Rothia, Lactobacillus* and acid-fast *Mycobacterium.* |
| **8.11.23** | **5** | Gram-negative bacilli of *Haemophilus, Bordetella, Legionella.*Gram-negative cocci of *Neisseria, Moraxella.* |
| **15.11.23** | **6** | **TEST 1 (topics 2-5)** Gram-negative bacilli of *Enterobacterales* and nonfermenters of *Pseudomonas, Acinetobacter Stenotrophomonas.*  |
| **22.11.23** | **7** | Gram positive sporulating anaerobic bacilli of *Clostridium* and *Clostridioides.* |
| **29.11.23** | **8** | Gram-positive and Gram-negative non-sporulating anaerobic bacteria.  |
| **6.12.23** | **9** | **TEST 2 (topics 6-8)**Antibiotics and chemotherapeutics. Laboratory methods of sensitivity testing.  |
| **13.12.23** | **10** | Mechanisms of antimicrobial resistance |
| **20.12.23** | **11** | **TEST 3 (topics 9-10)**Infection control. Sterilization, disinfection and antisepsis.  |
| **10.01.24** | **12** | Fungal infections of the oral cavity. |
| **17.01.24** | **13** | Oral microbiota, part 1. Microbiology of dental caries and gingivitis.  |
| **24.01.24** | **14** | **TEST 4 (topics 11-14).**Oral microbiota, part 2. Microbiology of periodontal diseases. |
| **31.01.24** | **15** | **PRACTICAL EXAM.** |

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 coloniestests 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. sorbinus S. ratti)*
2. SALIVARIUS group *(S. salivarius, S. vestibularis)*
3. MITIS group *(S. mitis, S. oralis, S. cristatus, S. pneumoniae, S. pseudopneumoniae, S. sanquinis)*
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 diphteriae, 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 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 TEST 1 (topics 2-5)**

 **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,***

 ***CLOSTRIDIOIDES.***

**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 6-8)**

**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, MLSB based on the results of the previous exercise.

**Practice**:
Reading of susceptibility tests (from the previous exercise)
Demonstration of resistance phenotypes: ESBL, MBL, MRS, MLSB, VRE, GRE, HLAR
Detection of β-lactamase producing strains (cefinase method)

**EXERCISE # 11. TEST 3 (topics 9-10)**

 **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 # 12. FUNGAL INFECTIONS OF THE ORAL CAVITY**

**The theoretical part:**

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

a) *Candida (C. albicans, C. auris, C. glabrata, C. tropicalis, C. krusei, C. dubliniensis, C. kefyr,*

 *C. guilliermondii, C. parapsilosis)* involved in:

* acute pseudomembranous candidiasis (thrush),
* acute atrophic candidiasis,
* chronic atrophic candidiasis,
* chronic hyperplastic candidiasis,
* inflammation of the mouth corner;
* prosthetic stomatopathy

b) *Cryptococcus spp.* (cryptococcosis)

c)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) moulds
 demonstration of slides (lactopheol Blue) and cultures

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

**EXERCISE # 13.** **ORAL MICROBIOTA, PART 1. MICROBIOLOGY OF**

 **DENTAL CARIES AND GINGIVITIS.**

**Theoretical part:**

positive and negative role of microbiota;

oral microbiota and systemic endogenous infections;

etiological 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 # 14. TEST 4 (topics 11-14)**

 **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-implantistis, peri-mucositis*

**S**election, 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 # 15. PRACTICAL EXAM**

The practical exam is a 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 microbial cells

4. Suspected group of microorganisms (Latin names)