

<b>MEDICAL MICROBIOLOGY WITH LABORATORY TECHNOLOGIES I</b>	
<b>GENERAL INFORMATIONS</b>	
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Assistant/Associate	Asst. Prof. Arlen Antolović-Požgain, MD, PhD Asst. Prof. Maja Bogdan, MD, PhD Maja Tomić-Paradžik, MD, PhD Marijan Orlović, MD Marko Živkov, MD Ivana Roksandić- Križan, MD Sara Kibel, MD Dinko Paulić, MD
Study Programme	University Undergraduate Study of Medical Laboratory Diagnostics
Status of the course	mandatory
Year of study, semester	3 <sup>rd</sup> year, 5 <sup>th</sup> semester
ECTS	<b>5</b>
Workload (hours)	Lecture: 45 ; seminars: 15; Laboratory exercise:15
Expected number of students	30-35
<b>COURSE DESCRIPTION</b>	
<b>Course objectives</b>	
<p>Get to know, understand and independently interpret the basic biological characteristics of microorganisms (bacteria and viruses) that cause infections in humans as well as pathogenic properties of these microorganisms, their prevalence and resistance to environmental conditions, ways of their transmission among humans, their sensitivity to antimicrobial drugs and the basics of persons defense from infection. Students will also get to know and understand the types of vaccines with individual microorganisms, as well as the role of vaccination in protecting the population from infectious diseases. The special goal is for students to get acquainted with the basic groups of antimicrobial drugs from the point of view of spectrum of action, mechanism of action and mechanism of resistance of microorganisms to antimicrobial drugs, understand their action and critically interpret their application. The aim of the course is also to acquire knowledge about diagnostic procedures in bacteriology and virology, namely: direct diagnosis, cultivation, serological diagnosis, molecular diagnostics and rapid diagnostic procedures.</p>	
<b>Enrolment requirements and entry competencies</b>	
No additional conditions	
<b>Learning outcomes at the Programme level</b>	
<b>1.1, 1.2, 2.1, 2.2, 2.3, 2.6, 3.1</b>	
<b>Learning outcomes at the course level</b>	
<p>After attending lectures, completing seminars and exercises, independent study and passing the exam, students will be able to:</p> <ol style="list-style-type: none"> <li>1. interpret the basic biological characteristics of microorganisms, bacteria and viruses, which cause infections in humans and the pathogenic properties of these microorganisms.</li> <li>2. independently determine the type of the most common microorganisms.</li> <li>3. according to the microscopic preparation or other characteristics, read the sensitivity test, and determine the method of transmission and the method of human defense against the specific microorganism.</li> <li>4. independently take nasal and pharyngeal swabs, and inoculate biological materials on microbiological substrates.</li> </ol>	

5. adopt the rules of work in the microbiological laboratory, the concept of hygiene in laboratories.
6. became familiar with cell cultures as a diagnostic medium in bacteriology and virology.
7. independently inoculate the rootstocks and describe the grown culture;
8. justify the methods of testing the biochemical activity of microorganisms and their application in identification.
9. test the sensitivity of the isolated bacterial strain to antibiotics (dilution and diffusion method of antibiogram, E test).
10. explain the most important diagnostic procedures in bacteriology and virology.

### Course content

#### Lectures:

L1: Introduction to medical microbiology. Nomenclature and classification of microorganisms. General bacteriology. Introduction to medical microbiology. Structure, physiology, genetics and metabolism of bacteria. Bacterial morphology and cell wall synthesis. Preparation and microscopy. Basic staining in bacteriology: simple and complex staining. Immune response to bacterial infections. Pathogenesis of bacterial infections. Bacterial toxins.

L2: General diagnostic principles in microbiology. Diagnostic procedures in bacteriology. Diagnostic procedures in virology. Diagnostic procedures in parasitology. Diagnostic procedures in mycology. Sampling of materials for microbiological tests. Direct and indirect diagnostics. Cultivation and isolation of microorganisms. Serological methods in microbiological diagnostics. Molecular diagnostics.

L3: Antimicrobial chemotherapeutics, disinfection and sterilization. Antimicrobial chemotherapeutics: types, division, mechanisms of action, therapeutic spectrum, testing of bacterial susceptibility to antibiotics, bacterial resistance and mechanisms of resistance. Disinfection and sterilization: types of disinfectants and mechanism of action, selection of disinfectant. Sterilization procedures: types of sterilization, control of sterilization procedure.

L4: Special bacteriology I. Gram-positive cocci: genus *Staphylococcus*. Description and characteristics of the genus *Staphylococcus*. Cultivation characteristics and nutrients. The most important representatives and the infectious diseases they cause. Samples for microbiological research and microbiological laboratory diagnostics: isolation, description of colonies and identification. Sensitivity to antibiotics. Treatment, prevention and control of infections caused by species of the genus *Staphylococcus*.

L5: Special bacteriology II. Gram-positive cocci: genus *Streptococcus*, genus *Enterococcus*. Description and characteristics of the genera *Streptococcus* and *Enterococcus*. Cultivation characteristics and nutrients. The most important representatives and the infectious diseases they cause. Samples for microbiological research and microbiological laboratory diagnostics: isolation, description of colonies and identification. Sensitivity to antibiotics. Treatment, prevention and control of infections caused by species of the mentioned genera.

L6: Special bacteriology III. Gram negative cocci: genera *Neisseria*, *Moraxella*; Gram-negative coccobacilli: genera *Haemophilus*, *Bordetella*, *Brucella*, *Francisella*. Description and characteristics of genera: *Neisseria*, *Moraxella*, *Haemophilus*, *Bordetella*, *Brucella*, *Francisella*. Cultivation characteristics and nutrients. The most important representatives and the infectious diseases they cause. Samples for microbiological research and microbiological laboratory diagnostics: isolation, description of colonies and identification. Sensitivity to antibiotics. Treatment, prevention and control of infections caused by species of the mentioned genera.

L7: Special bacteriology IV. Gram negative rods (fermenting): *Enterobacteriaceae* family: *Salmonella*, *Shigella*, *Yersinia*. Description and characteristics of the *Enterobacteriaceae* family. Genera: *Salmonella*, *Shigella*, *Yersinia*. Cultivation characteristics and nutrients. The most important representatives and the infectious diseases they cause. Samples for microbiological research and microbiological laboratory diagnostics: isolation, description of colonies and identification. Sensitivity

to antibiotics. Treatment, prevention and control of infections caused by species of the mentioned genera.

L8: Special bacteriology V. Gram-negative rods (fermenting): family *Enterobacteriaceae*: genera *Escherichia*, *Klebsiella*, *Proteus* and other enterobacteria. Description and characteristics of the family *Enterobacteriaceae* - genera: *Escherichia*, *Klebsiella*, *Proteus*, *Enterobacter*, *Serratia*, *Citrobacter*, *Providentia*, *Morganella*. Cultivation characteristics and nutrients. The most important representatives and the infectious diseases they cause. Samples for microbiological research and microbiological laboratory diagnostics: isolation, description of colonies and identification. Sensitivity to antibiotics. Treatment, prevention and control of infections caused by species of the mentioned genera.

L9: Special bacteriology VI. Gram-negative rods (non-fermenting): genera *Pseudomonas*, *Acinetobacter*, *Stenotrophomonas*. Genus *Legionella*. Description and characteristics of non-fermenting gram negative bacteria - genera: *Pseudomonas*, *Acinetobacter*. Genus *Legionella*. Cultivation characteristics and nutrients. The most important representatives and the infectious diseases they cause. Samples for microbiological research and microbiological laboratory diagnostics: isolation, description of colonies and identification. Sensitivity to antibiotics. Treatment, prevention and control of infections caused by species of the mentioned genera.

L10: Special bacteriology VII. Gram-negative coiled coccobacilli: genera *Vibrio*, *Campylobacter*, *Helicobacter* Spiral bacteria: genera *Treponema*, *Borrelia*, *Leptospira*. Description and characteristics of the genera *Vibrio*, *Campylobacter*, *Helicobacter*. Cultivation characteristics and nutrients. Spiral bacteria - genera *Treponema*, *Borrelia*, *Leptospira*. The most important representatives and the infectious diseases they cause. Samples for microbiological research and microbiological laboratory diagnostics. Sensitivity to antibiotics. Treatment, prevention and control of infections caused by species of the mentioned genera.

L11: Special bacteriology VIII. Gram positive rods: genus *Corynebacterium*, genus *Listeria*. Description and characteristics of genera: *Corynebacterium*, *Listeria*. Cultivation characteristics and nutrients. The most important representatives and the infectious diseases they cause. Samples for microbiological research and microbiological laboratory diagnostics. Sensitivity to antibiotics. Treatment, prevention and control of infections caused by species of the mentioned genera.

L12: Special bacteriology IX. Sporogenic bacteria: genus *Clostridium*, genus *Bacillus*. Anaerobic asporogenous bacteria. Sporogenic bacteria. Sporogenesis. Description and characteristics of sporogenous and asporogenous anaerobic bacteria. Cultivation characteristics, nutrient media and anaerobic cultivation. The most important representatives and the infectious diseases they cause. Description and characteristics of bacterial species of the genus *Clostridium*: *C. perfringens*, *C. tetani*, *C. botulinum*, *C. difficile*. Pathogenesis of diseases caused by species from the genus *Clostridium*. Cultivation features: anaerobic cultivation. Sporogenesis and germination. Exotoxins. Vaccines. Genus *Bacillus*: *B. anthracis*, *B. cerus*, other species. Samples for microbiological research and microbiological laboratory diagnostics. Sensitivity to antibiotics. Treatment, prevention and control of infections caused by species of the mentioned genera.

L13: Special bacteriology X. Acid-resistant and branched bacteria: genus *Mycobacterium*, families *Actinomycetaceae*, *Nocardiaceae*. Genus *Mycobacterium*. *Mycobacterium tuberculosis* - micromorphology and macromorphology of mycobacteria, laboratory diagnostics (cultivation, sensitivity testing, rapid diagnostic methods, clinical samples, sensitivity testing to antituberculosis drugs. Specific staining: Ziehl Nielsen, Kinyoun, auramin. Families *Actinomycetaceae*, *Nocardiaceae*: cultivation characteristics and nutrient media. The most significant representatives and the infectious diseases they cause Samples for microbiological examination and microbiological laboratory diagnostics Susceptibility to antibiotics. Treatment, prevention and control of infections caused by species of the above genera.

L14: Special bacteriology XI. Intracellular bacteria: *Chlamydia*, *Mycoplasma*, *Rickettsia*, *Coxiella*. Description and characteristics of intracellular bacteria. Mycoplasmas: *M. pneumoniae*, *M. hominis*, *Ureaplasma urealyticum*; *Chlamydia*: *C. trachomatis*, *C. pneumoniae*, *C. psittaci*. Genus

*Rickettsia: rickettsiae* from the spotted typhus group, *rickettsiae* from the spotted fever group. Genus *Coxiella*. Samples for microbiological research and microbiological laboratory diagnostics. Sensitivity to antibiotics. Treatment, prevention and control of infections caused by species of the mentioned genera.

L15: General virology. Methods of virological diagnostics. Structure and definition of viruses. Taxonomy of viruses. Virus cultivation in cell culture, on fertilized hen's egg and in an experimental animal. Preparation of cell cultures - primary and continuous cell cultures, diploid stock cultures. Cytopathic effects of viruses in cell culture. Viral inclusions. Viral vaccines - types, production and application. Antiviral drugs: chemoprophylaxis and chemotherapy of viral diseases. highly effective antiretroviral therapy (HAART). Laboratory diagnostics of viral diseases: samples, direct detection of viruses, isolation, molecular diagnostics, serology.

L16: Special virology I. DNA viruses: families *Adenoviridae*, *Papovaviridae* (families *Papillomaviridae* and *Polyomaviridae*), *Poxviridae*. Structure, replication, pathogenesis, epidemiology, clinical syndromes, laboratory diagnostics, treatment, prevention and control of adenoviruses, papillomaviruses and polyomaviruses. *Poxviridae*.

L17: Special virology II: DNA viruses: families *Herpesviridae*, *Parvoviridae*. Structure, replication, pathogenesis, epidemiology, clinical syndromes, laboratory diagnostics, treatment, prevention and control of *Parvoviridae* viruses (parvovirus B-19) and from the *Hepresviridae* family: herpes simplex virus (VHS), varicella zoster virus (VZV), Epstein-Barr virus (EBV), Cytomegalovirus (CMV), human herpes virus 6,7,8 (HHV-6, HHV-7 and HHV-8). TORCH – serological testing of pregnant women.

L18: Special virology III. RNA viruses: families *Picornaviridae*, *Rhabdoviridae*. Structure, replication, pathogenesis, epidemiology, clinical syndromes, laboratory diagnostics, treatment, prevention and control of viruses from the family *Picronaviridae* and *Rhabdoviridae*. Rabies virus.

L19: Special virology IV. RNA viruses: families *Orthomyxoviridae*, *Paramyxoviridae*, *Coronaviridae*. Structure, replication, pathogenesis, epidemiology, clinical syndromes, laboratory diagnostics, treatment, prevention and control of orthomyxovirus, paramyxovirus, coronavirus. Influenza virus - "shift" and "drift". Flu pandemics and epidemics. Respiratory syncytial virus and human metapneumovirus. Measles virus. Parotitis virus. Viruses causing respiratory diseases. The SARS virus.

L20: Special virology V. RNA viruses: *Togaviridae* family. Arboviruses. Viruses that cause gastrointestinal infections. Structure, replication, pathogenesis, epidemiology, clinical syndromes, laboratory diagnostics, treatment, prevention and control of viruses from the *Togaviridae* family. Structure, replication, pathogenesis, epidemiology, clinical syndromes, laboratory diagnostics, treatment, prevention and control of arthropod-borne viruses: family *Togaviridae* (*Alphavirus*), family *Flaviviridae*, family *Bunyaviridae* (genera: *Bunyavirus*, *Phlebovirus*, *Nairovirus*, *Hantavirus*). Structure, replication, pathogenesis, epidemiology, clinical syndromes, laboratory diagnostics, treatment, prevention and control of viruses causing gastrointestinal infections: rotaviruses, adenoviruses, astroviruses, caliciviruses - norovirus.

L21: Special virology VI. Hepatitis viruses. Retroviruses. Structure, replication, pathogenesis, epidemiology, clinical syndromes, laboratory diagnostics, treatment, prevention and control of hepatitis viruses: HAV, HBV, HCV, HDV, HEV, VHG. Structure, replication, pathogenesis, epidemiology, clinical syndromes, laboratory diagnostics, treatment, prevention and control of retroviruses: human immunodeficiency virus 1 and 2 (HIV-1 and HIV-2), human T-cell leukemia/lymphoma virus type 1 and 2 (HTLV -1, HTLV-2).

L22: Special virology VII: RNA viruses: families *Arenaviridae*. *Filoviridae*. Prions. Structure, replication, pathogenesis, epidemiology, clinical syndromes, laboratory diagnostics, treatment, prevention and control of arenaviruses and filoviruses. Ebola virus and Marburg virus. Biosafety working conditions in the laboratory BSL 2-4. Prions - structure and physiology, pathology and pathogenesis, epidemiology, clinical syndromes, laboratory diagnostics, treatment, prevention and control of prion diseases.

**Seminars:**

S1: Gram-positive cocci: genus *Staphylococcus*. Description and characteristics of the genus *Staphylococcus*. Cultivation characteristics and nutrients. The most important representatives and the infectious diseases they cause. Samples for microbiological research and microbiological laboratory diagnostics: isolation, description of colonies and identification. Sensitivity to antibiotics. Treatment, prevention and control of infections caused by species of the genus *Staphylococcus*.

S2: Gram-positive cocci: genus *Streptococcus*, genus *Enterococcus*. Description and characteristics of the genera *Streptococcus* and *Enterococcus*. Cultivation characteristics and nutrients. The most important representatives and the infectious diseases they cause. Samples for microbiological research and microbiological laboratory diagnostics: isolation, description of colonies and identification. Sensitivity to antibiotics. Treatment, prevention and control of infections caused by species of the mentioned genera.

S3: Gram-negative cocci: genus *Neisseria*. Description and characteristics of genera: *Neisseria*, *Moraxella*, *Haemophilus*, *Bordetella*, *Brucella*, *Francisella*. Cultivation characteristics and nutrients. The most important representatives and the infectious diseases they cause. Samples for microbiological research and microbiological laboratory diagnostics: isolation, description of colonies and identification. Sensitivity to antibiotics. Treatment, prevention and control of infections caused by species of the mentioned genera.

S4: Gram negative rods (fermenting): *Enterobacteriaceae* family: *Salmonella*, *Shigella*, *Yersinia*. Description and characteristics of the *Enterobacteriaceae* family. Genera: *Salmonella*, *Shigella*, *Yersinia*. Cultivation characteristics and nutrients. The most important representatives and the infectious diseases they cause. Samples for microbiological research and microbiological laboratory diagnostics: isolation, description of colonies and identification. Sensitivity to antibiotics. Treatment, prevention and control of infections caused by species of the mentioned genera.

S5: Gram-negative rods (fermenting): family *Enterobacteriaceae*: genera *Escherichia*, *Klebsiella*, *Proteus* and other enterobacteria. Description and characteristics of the family *Enterobacteriaceae* - genera: *Escherichia*, *Klebsiella*, *Proteus*, *Enterobacter*, *Serratia*, *Citrobacter*, *Providentia*, *Morganella*. Cultivation characteristics and nutrients. The most important representatives and the infectious diseases they cause. Samples for microbiological research and microbiological laboratory diagnostics: isolation, description of colonies and identification. Sensitivity to antibiotics. Treatment, prevention and control of infections caused by species of the mentioned genera.

S6: Spiral bacteria: genera *Treponema*, *Borrelia*, *Leptospira*. Description and characteristics of the genus *Vibrio*, *Campylobacter*, *Helicobacter*. Cultivation characteristics and nutrients. Spiral bacteria - genera *Treponema*, *Borrelia*, *Leptospira*. The most important representatives and the infectious diseases they cause. Samples for microbiological research and microbiological laboratory diagnostics. Sensitivity to antibiotics. Treatment, prevention and control of infections caused by species of the mentioned genera.

S7: Anaerobic bacteria. Description and characteristics of sporogenous and asporogenous anaerobic bacteria. Cultivation characteristics, anaerobic cultivation. Sporogenesis. The most important representatives and the infectious diseases they cause. Genus *Clostridium*. Asporogenous anaerobes: *Bacteroides spp*, *Prevotella*, *Peptostreptococcus*. Genus *Actinomyces*. Micromorphology and macromorphology of asporogenic anaerobes. Laboratory diagnostics and isolation of anaerobes. Testing the sensitivity of anaerobic bacteria. Commercial systems for the identification of anaerobes. Samples for microbiological research and microbiological laboratory diagnostics. Sensitivity to antibiotics. Treatment, prevention and control of infections caused by species of the mentioned genera.

S8: Acid-resistant bacteria: genus *Mycobacterium*. Description and characteristics of acid-resistant bacteria genus *Mycobacterium*. Cultivation characteristics and nutrients. *Mycobacterium tuberculosis* - micromorphology and macromorphology of mycobacteria, laboratory diagnostics (cultivation, sensitivity testing, rapid diagnostic methods, clinical samples, sensitivity testing to antituberculosis drugs. Specific staining: Ziehl Nielsen, Kinyoun, auramin. Treatment, prevention and control of infections caused by *Mycobacterium tuberculosis*.

S9: Intracellular bacteria: *Chlamydia*, *Mycoplasma*. Description and characteristics of intracellular bacteria. *Chlamydia*: *Chlamydia trachomatis*, *Chlamydophila pneumoniae*, *Chlamidophyla psittaci*. *Mycoplasmas*: *M. pneumoniae*, *M. hominis*, *Ureaplasma urealyticum*; laboratory diagnosis of *M. pneumoniae*. Laboratory diagnosis of *M. hominis* and *Ureaplasma urealyticum*, clinical samples, antibiogram. Samples for microbiological research and microbiological laboratory diagnostics. Sensitivity to antibiotics. Treatment, prevention and control of infections caused by species of the mentioned genera.

S10: DNA viruses: families *Papillomaviridae*. Structure, replication, pathogenesis, epidemiology, clinical syndromes, laboratory diagnostics, treatment, prevention and control of human papillomaviruses. Oncogenic potential of HPV. Molecular diagnostic methods for HPV detection.

S11: RNA viruses: family *Orthomyxoviridae*. Structure, replication, pathogenesis, epidemiology, clinical syndromes, laboratory diagnostics, treatment, prevention and control of orthomyxoviruses. Influenza virus - "shift" and "drift". Flu pandemics and epidemics. Bird flu.

S12: RNA viruses: family *Paramyxoviridae*. Viruses causing respiratory infections. Parainfluenza virus. Respiratory syncytial virus and human metapneumovirus. Measles virus. Parotitis virus. Respiratory viruses causing infections of the respiratory system: pathogenesis, epidemiology, clinical syndromes, laboratory diagnostics, treatment, prevention and control of rhinoviruses, adenoviruses, orthomyxoviruses, paramyxoviruses, coronaviruses.

S13: Hepatitis viruses. HIV. Structure, replication, pathogenesis, epidemiology, clinical syndromes, laboratory diagnostics, treatment, prevention and control of hepatitis viruses: HAV, HBV, HCV, HDV, HEV, HGV. Serological and molecular methods in the diagnosis of HBV and HCV hepatitis viruses - testing algorithm and interpretation of findings. Structure, replication, pathogenesis, epidemiology, clinical syndromes, laboratory diagnostics, treatment, prevention and control of human immunodeficiency virus 1 and 2 (HIV-1 and HIV-2). Serological and molecular methods in HIV diagnosis - testing algorithm and interpretation of findings.

S14: Viruses causing gastrointestinal infections. Digestive system viruses and viral gastroenteritis - structure, replication, pathogenesis, epidemiology, clinical syndromes, laboratory diagnostics, treatment, prevention and control of viruses causing gastrointestinal infections: rotavirus, adenovirus, astrovirus, calicivirus - norovirus. Viruses whose entry point into the body is the digestive system: picornaviruses. The importance of viral infections of the digestive system. Methods of rapid microbiological diagnosis of viruses causing gastroenteritis.

S15: Viruses causing congenital infections. TORCH. Pathogenesis, epidemiology, clinical syndromes, laboratory diagnostics, treatment, prevention and control of viruses that cause congenital infections: *Togaviridae* (*Rubivirus*), *Parvoviridae* (parvovirus B-19), *Hepresviridae*, hepatitis viruses and HIV. TORCH – serological testing of pregnant women.

#### **Laboratory exercises:**

P1: Basics of bacterial cultivation and identification. Testing the sensitivity of bacteria to antimicrobial chemotherapeutics. Basic staining and micromorphology of bacteria. Micromorphology of bacteria (basics of microscopy, forms of bacteria). Native preparation. Gram staining. Staining with methylene blue. Basics of cultivation (substrates, incubation) and identification of bacteria in the microbiological laboratory. Pure and mixed bacterial culture. Normal human flora. Microorganisms from the air. Hygienic hand washing. Hygienic hand disinfection. Safety in laboratory work: biosafety working conditions (BSL 2-4). Basics of testing the sensitivity of bacteria to antibiotics - disk diffusion method, determination of the minimum inhibitory concentration (MIC) of antibiotics by microdilution, minimum bactericidal concentration (MBK) of antibiotics, E-test, "break point" method and correlation with disk diffusion. Antibiogram interpretation. Selection of antibiotics for testing individual bacterial genera (EUCAST, CLSI). Bacterial resistance to antibiotics - examples of MRSA, ESBL-producing enterobacteria, resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. Practical work: Microscopy of a native yeast preparation. Staining with methylene blue and Gram staining. Microscopy of stained preparations from the collection. Preparation of antibiogram by disk

diffusion method. Taking fingerprints before and after hygienic hand washing. Installation of the air sampling plate. Interpretation and reading of previously prepared antibiograms (disk diffusion and E-test). Determination of MIK and MBK by dilution method.

P2: Gram-positive cocci: Genus *Staphylococcus*. Genus *Streptococcus* and genus *Enterococcus*. Staphylococci: basic clinical samples, description of micromorphology and macromorphology, basic tests for identification (catalase, coagulase, DNA-za), detection of MRSA. Antibiogram for sensitivity testing. Clinical indication for nasal swab sampling. Serological groups of streptococci. Micro and macromorphology of streptococci and enterococci. Microbiological characteristics and tests for identification: *S. pyogenes*, *S. agalactiae* and *S. pneumoniae*. Cultivation characteristics of enterococci. Normal flora of the nasopharynx and pharynx. Clinical indication for swabbing the nose, nasopharynx and pharynx. Clinically significant bacteria in nasopharyngeal and pharyngeal swabs. Preparation of swabs of the nose, pharynx and nasopharynx. Practical work: Basics of microbiological diagnosis of staphylococci and description of staphylococcal colonies (*S. aureus*, *S. epidermidis*). Preparation of staphylococcal preparations and Gram staining. Performing and reading the catalase and coagulase test. Reading of the DNA test for *S. aureus*. Taking a swab of the nasopharynx and pharynx. Antibiogram reading from the previous exercise (disk-diffusion). Reading of the previously prepared *S. aureus* susceptibility test to vancomycin in the dilution method and determination of MIK and MBK. Basics of microbiological diagnosis of streptococci: description of streptococcal colonies on blood agar, description of pneumococcal and enterococcal colonies on BA, preparation of streptococcal preparations from broth and Gram staining, performance and reading of catalase test, microscopy of Gram-stained streptococcal preparations. Microscopy of *S.pneumoniae* stained by Gram and methylene blue from a biological sample. Bile-esculin test reading and growth in 6.5% NaCl broth for enterococcus. Bacitracin test reading for BHSA. Optochin test reading for pneumococcus. Description of bacterial colonies on blood agar exposed to air in the exercise room from the previous exercise and calculation of the number of bacteria in 1 m<sup>3</sup>. Description of bacterial colonies grown on blood agar with fingerprints of the right hand before and after hand washing and disinfection.

P3: Gram-negative cocci and coccobacilli: genus *Neisseria*, genus *Haemophilus*, genus *Brucella*. Micromorphology and macromorphology of *Haemophilus*, *Neisseria* and *Brucella*. Microbiological characteristics and tests for the identification of *H. influenzae*, *N. meningitidis*, *N. gonorrhoeae*, *Brucella spp.* Nitrocefin test. Serological diagnostics in bacteriology and the principles of agglutination and complement binding reactions. Indirect diagnosis of brucellosis: agglutination according to Wright, CBR. Practical work: Description of colonies of *Haemophilus spp.* and saprophytic series at BA and CA. Observing the satellite phenomenon. Production of preparations in the most series and staining according to Gram. Microscopy of *Haemophilus* preparations stained by Gram. Microscopy of Gram-stained preparations of *N. meningitidis* from cerebrospinal fluid sediment. Microscopy of *N. gonorrhoeae* preparations from urethral swabs stained with methylene blue. Creation of oxidase test and nitrocefin test. Microscopy of *Brucella* preparations stained by Gram. Reading of serological reactions to prove brucellosis: CBR and Wright agglutination.

P4: Gram-negative fermenting bacilli: family *Enterobacteriaceae*. Non-fermenting Gram-negative bacteria: genus *Pseudomonas*, genus *Acinetobacter*. Genus *Campylobacter*. Identification of the most common bacterial causes of urinary and intestinal infections. Biochemical tests for the identification of members of the *Enterobacteriaceae* family: genera *Salmonella*, *Shigella*, *Yersinia*, *Escherichia*, *Klebsiella*, *Proteus*. Micromorphology and macromorphology of enterobacteria - description of colonies and preparations. Antibiogram and resistance to antibiotics: multiresistant enterobacteria (beta lactamase, ESBL). Microbiological processing of urine and stool. Basic selective substrates for seeding stool. Agglutination according to Widal. Genus *Campylobacter*: cultivation, identification and antibiogram. Practical work: Preparation and microscopy of different types of Gram-stained enterobacteria. Microscopy of *Klebsiella* shower preparations. Describe colonies of *E.coli*, *Klebsiellae* on blood agar and differential feeding grounds. Describe *Proteus sp.* on blood agar. Read the biochemical series for bacteria *E.coli*, *Klebsiella*, *Proteus* (demonstration). Urine seeding. Uriselect agar routine urine-seeded reading. Antibiogram reading and double synergistic test for ESBL

detection. Description of colonies of *Salmonella*, *Shigella*, *Escherichia* on XLD and SS agar and *Yersinia* on SS agar. Read the biochemical sequence for salmonella, shigella and yersinia (demonstration). Serotyping of enteropathogenic *E. coli*. Read Widal's reaction. Microscopy of Gram-stained preparations of *Pseudomonas aeruginosa*, description of colonies on blood and plain agar, biochemical identification (demonstration) and disc diffusion reading for *Pseudomonas aeruginosa*. Creation of the oxidase test. *Acinetobacter baumannii* - description of colonies on blood agar and microscopy of the Gram-stained preparation. Description of *Campylobacter* colonies on a selective medium. Microscopy of Gram-stained campylobacter preparations.

P5: Gram-positive rods (aerobic and anaerobic): genus *Corynebacterium*, genus *Listeria*, genus *Bacillus*, genus *Clostridium*. Sporogenic bacteria. Anaerobic asporogenous bacteria. *Corynebacterium diphtheriae*, *Listeria monocytogenes*. clinical specimens, description of micromorphology and macromorphology, tests for identification. Coloring according to Lubinski. Genus *Bacillus* - *B. anthracis*, *B. subtilis* Cultivation and identification of sporogenic anaerobic bacteria. Clinical samples for the isolation of anaerobic bacteria. Mixed infections. Sensitivity testing. Sporogenic bacteria. Fulton staining. Genus *Clostridium* - laboratory diagnostics. Asporogenic anaerobes: *Bacteroides spp*, *Prevotella*, *Peptostreptococcus*, *Fusobacterium*. Micromorphology and macromorphology of asporogenic anaerobes. Laboratory diagnostics and isolation of anaerobes. Testing the sensitivity of anaerobic bacteria. Commercial systems for the identification of anaerobes. Practical part: Description of diphtheroid colonies. Microscopy of *Corynebacterium diphtheriae* and diphtheroid preparations stained by Gram and Lubinski. Microscopy of *L. monocytogenes* preparations and description of colonies on blood agar. *B.anthraxis*: microscopy of a preparation stained with methylene blue of mouse peritoneal exudate. Make, Gram stain, microscope and color draw *B. subtilis* preparations. *Clostridium sp.* - Microscopy of the preparation stained according to Gram and Fulton. Description of colonies of *Bacillus subtilis* on blood agar and *Clostridium spp.* on Columbia agar. Microscopy of Gram-stained preparations of asporogenic anaerobic bacteria. Description of colonies of *Bacteroides sp.* on Columbia agar. Reading of a commercial system for the identification and sensitivity testing of anaerobic bacteria (demonstration).

P6: *Mycobacteria*. *Mycoplasmas*. *Mycobacteria*: *Mycobacterium tuberculosis* - micromorphology and macromorphology of *mycobacteria*, laboratory diagnostics (cultivation, sensitivity testing, rapid diagnostic methods, clinical samples, sensitivity testing to antituberculosis drugs. Specific staining: Ziehl Nielsen, Kynion, auramin. *Mycoplasmas*: laboratory diagnostics of *M. pneumoniae*, clinical samples. Laboratory diagnostics of *M. hominis* and *Ureaplasmaurealyticum*, clinical samples, antibiogram. Practical work: Describe *Mycobacterium tuberculosis* colonies on the Lowenstein-Jensen medium. Microscopy of sputum preparations stained according to Ziehl-Neelsen. Read a previously prepared *M.tuberculosis* resistance test to antituberculosis drugs. Microscopy of mycoplasmas and ureaplasma on PPLO agar, commercial tests for identification and antibiogram CBR reading and cold agglutination for *M. pneumoniae*.

P7: Collection of clinical material and methods of virological diagnostics: cultivation and isolation of viruses. Taking clinical material for virological tests. Virus cultivation in cell culture. Cytopathic effects of viruses in cell culture. Virus growth in fertilized chicken egg and experimental animal. Rapid tests for the proof of virus antigens in a clinical sample - latex and immunochromatogenic tests: Adenolex, Directigen RSV, Rotalex. Practical work: performing rapid latex tests and immunochromatographic tests for the diagnosis of viral pathogens from the respiratory and digestive systems. Microscopy of cell culture preparations infected with viruses and identification of CPE.

P8: Serological methods in the diagnosis of viral infections. Molecular diagnostics of viruses. Serology - paired sera, antibody titer and titer dynamics: complement binding reaction (CBR), neutralization test (NT), Mason's and Paul-Bunell's reaction. Enzyme immunoassay (EIA), indirect immunofluorescence (IFA), Western-Blott (WB) - RIBA and immunoperoxidase test. Polymerase chain reaction - principle and diagnostic application. Hemagglutinins and hemagglutination (HA), hemadsorption (HAD), hemagglutination inhibition (IH) and hemadsorption inhibition for virus detection and identification. Determination (titer) of hemagglutinin. Laboratory diagnostics of



infections: influenza virus, parainfluenza virus, respiratory syncytial virus, human metapneumovirus, measles virus, parotitis virus. Diagnosis of Epstein-Barr virus (EBV) and infectious mononucleosis syndrome. Practical work: Reading tests: hemagglutination test (HA) for parotitis virus, hemagglutination inhibition test (IH) for typing the isolated virus, reading the hemagglutination reaction - HA (titration of the isolated virus), reading the IH reaction (titer of specific hemagglutinating antibodies in paired sera of the patient from the flu) and complement fixation reactions (CRV). Reading of previously prepared complement binding reaction for cytomegalovirus in patients with infectious mononucleosis syndrome.

**Mode of teaching**

Lectures, seminars and exercises.

**Student obligations**

Attending all forms of classes is mandatory, and the student must pass all knowledge tests. A student can excuse himself from 30% of all classes. Unexcused absences, as well as absences outside the percentage of excused absences, must be compensated by colloquium.

**Monitoring student work(Connectivity of learning outcomes, teaching methods and grading)**

Exam method: written exam.

Teaching activity	ECTS	Learning outcome	Student activity	Assessment methods	Grade points	
					Min.	Max.
Class attendance	0.25	1-10	Attendance in class and active participation;	Records	3	5
Seminar paper	0.5	1-10	Preparation of seminar paper	Presentation of seminar paper	1	10
Practical work (exercises)	0.75	1-10	Studying for the practical exam and class attendance	Practical examination	6	15
Final exam	3.5	1-10	Continuous studying during classes	Written exam	16	70
<b>Total</b>	<b>5</b>				<b>50</b>	<b>100</b>

During the course, the student will be able to collect a maximum of 100 grade points. Students can obtain a maximum of 30 points during classes through different forms of activities and a maximum of 70 points on the final exam. The student must achieve more than 60% on the written part of the exam. The final grade represents the sum of the grade points achieved during the class and on the final exam.

*Final colloquium - the practical part of the exam* consists of four practical tasks that include microscopy of preparations (5 pieces) from the fields of bacteriology (4 pieces) and virology (CPE), culture on nutrient agar (one bacterial), antibiogram readings and reading of a prepared serological reaction. The student must achieve at least 60% on the practical part of the exam in order to be able to take the final exam. Students can obtain a maximum of 15 evaluation points on the practical exam (see Table 2), depending on the percentage of correctly solved tasks. The final colloquium is organized after the end of classes and within each exam period as a practical part of the exam.

*Evaluation of the final colloquium - practical exam*

Percentage of correctly solved tasks	Number of correct answers	Grade points (15)
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(%)	(X/16)	
60.00 – 69.99	10	6
70.00 – 74.99	11	8
75.00 – 79.99	12	9
80.00 - 84,99	13	11
85,00 – 89.99	14	13
90,00 - 94,99	15	14
95.00 – 100.00	16	15

*Seminars:* During classes, the student can collect a maximum of 10 grade points by actively preparing and presenting the seminar, which is mandatory according to the following criteria:

1-2 grade points: the seminar meets the minimum criteria; 3-5 grade points: average answer with noticeable errors; 6-8 grade points: very good answer with minor errors; 9-10 grade points: exceptional answer.

*By attending lectures,* a student can achieve 3-5 grade points according to the following scheme: participation in 60-79.99% of lectures is evaluated with 3 grade points, 80-89.99% 4 grade points, 90-100% 5 grade points.

*Final exam:*

A student who has properly completed all forms of teaching has acquired the right to signature and participation in final exam. The final exam is mandatory and consists of a written part. During the final exam, a student can receive a maximum of 70 grade points.

The written part of the final exam consists of 70 questions with five possible answers, of which only one is correct. The minimum criterion for obtaining evaluation points is 60% of correctly solved questions. The points obtained on the written part of the final exam are converted into grade points according to the criteria listed in Table 3. The points obtained on the final exam are added to the points obtained during classes. If student does not meet the minimum criteria on the final exam in the first exam period, he/she takes the final exam again in the next exam period.

*Evaluation of the written part of the final exam*

Percentage of correctly solved tasks (%)	Number of correct answers (X/70)	Grade points (70)
60,00 – 60,99	42	16
61,00 – 61,99	43	18
62,00 – 63,99	44	20
64,00 - 64,99	45	22
65,00 - 66,99	46	24
67,00 - 67,99	47	27
68,00 - 69,99	48	30
70,00 - 70,99	49	32
71,00 - 71,99	50	34
72,00 - 73,99	51	36
74,00 - 74,99	52	38
75,00 - 76,99	53	40
77,00 - 77,99	54	43
78,00 - 79,99	55	46

80,00 - 80,99	56	48
81,00 - 81,99	57	50
82,00 - 83,99	58	52
84,00 - 84,99	59	54
85,00 - 86,99	60	56
87,00 - 87,99	61	59
88,00 - 89,99	62	62
90,00 - 90,99	63	66
91,00 - 91,99	64	67
92,00 - 93,99	65	68
94,00 - 94,99	66	69
95,00 - 100,00	67-70	70

**Formation of the final grade:**

The grade points obtained during the class are joined by the points obtained on the final exam. Grading is done by absolute distribution, i.e. based on the final achievement and is compared with the numerical system as follows:

A — excellent (5): 80-100 grade points; B — very good (4): 70-79.99 grade points; C — good (3): 60-69.99 grade points; D— sufficient (2): 50-59.99 grade points

**Required reading(available in the library and through other media)**

Title	Number of copies in the library	Availability through other media
Kalenići sur. Medicinska mikrobiologija. Drugo, izmijenjeno i obnovljeno izdanje. Medicinska naklada, Zagreb, 2019. (udžbenik)	13	

**Additional reading**

Jawetz, Melnick, &Adelberg's Medical Microbiology, 25nd edition. Brooks GF, Carroll KC, Butel JS, Morse SA, Mietzner TA editors. Lange Medical Books/McGraw-Hill: New York, Chicago, San Francisco, Lisboa, London, Madrid, Mexico City, Milan, New Delhi, San Juan, Seoul, Singapore, Sydney, Toronto, 2019

**Course evaluation procedures**

Anonymous, quantitative, standardised student survey on the course and the teacher's work implemented by the Quality improvement office of the Faculty of Medicine Osijek.