

Faculty of Medicine in Rijeka

**Curriculum
2023/2024**

For course

Histological Techniques

Study program:	Medical Studies in English (R) (elective) University integrated undergraduate and graduate study
Department:	Department of Histology and Embryology
Course coordinator:	Doc. dr. sc. Lenartić Maja, dipl. ing.
Year of study:	2
ECTS:	1.5
Incentive ECTS:	0 (0.00%)
Foreign language:	Possibility of teaching in a foreign language

Course information:

Course Description: The primary objective of this course is to familiarize students with the process of preparing tissue samples for histological examination, which holds significant importance in contemporary medical diagnosis. Additionally, the course aims to provide students with hands-on experience in working with human tissues in a laboratory setting, teaching them the necessary procedures for accurate diagnosis and effective treatment approaches.

Requirements for enrollment of courses:

The mandatory prerequisites for enrolling in the course include having attended the classes for Histology and Embryology Courses.

Anticipated Learning Outcomes for the Subject:

Upon successful completion of the course, students are expected to grasp the significance of specific histological techniques used in contemporary laboratory diagnostics, essential for the functioning of clinical medicine. Their prior knowledge of the microscopic structure of the human body and expertise in presenting individual substances and antigens will foster a deeper comprehension of pathomorphological alterations.

Acquiring knowledge of histological laboratory procedures, tissue preparation techniques for analysis, and contemporary methods of displaying individual tissues, cells, and antigens, including molecules on both the cell surface and inside cells, will enhance the ability to identify diverse diagnostic requirements encountered in clinical medicine.

Subject Contents: The course covers the following topics:

1. Preparation and Processing of Samples in the Histological Laboratory:
 - Detailed procedures involving materials, chemicals, and laboratory utensils.
1. Paraffin Preparation of Histological Preparations:
 - Techniques for embedding tissue samples in paraffin for sectioning and staining.
1. Preparation of Frozen Preparations:
 - Techniques for freezing tissue samples for immediate sectioning and examination.
1. Classic Histological Staining:
 - Overview of traditional staining methods to visualize tissue structures.
1. Histochemical Techniques:
 - Methods for demonstrating specific chemical substances in tissues.
1. Immunohistochemical Techniques:
 - Various immunofluorescent and immunoenzymatic techniques for detecting antigens in tissues.
 - Direct and indirect staining methods.
 - Signal amplification techniques.
 - Antigen retrieval techniques.

By the end of the course, students will have a comprehensive understanding of these subject contents, enabling them to proficiently work in a histological laboratory and effectively utilize these techniques in clinical medicine for diagnostic purposes.

List of assigned reading:

1. Anthony L. Mescher: Junqueira's Basic Histology (Text and Atlas), 14th Edition, 2016 (selected chapters)

List of optional reading:

Supplement Literature:

- 2) Shafie Abdulkadir Hassan: Practical Histopathology (Made easy for laboratory professionals), 1st Edition
- 3) Thomas Boenisch et al. : Immunochemical Staining Methods, 3rd Edition, 2001.
- 4) S. Anil and R. Rajendran: Routine Histotechniques, Staining and Notes on Immunohistochemistry

In addition to the textbooks, students will be provided with relevant scientific papers related to the topics covered in the course. These papers will further enhance their understanding of histological techniques and their application in modern laboratory diagnostics and clinical medicine.

Curriculum:

Lectures list (with titles and explanation):

Introduction to Histological Techniques

The lecture on Introduction to Histological Techniques provides a comprehensive overview of fundamental concepts, emphasizing the significance of histology in research and diagnostics. Students gain familiarity with basic histological terminology and explore various methods of tissue preparation for microscopic analysis. The session includes a detailed examination of obtaining paraffin-embedded tissue sections and highlights key differences between frozen and paraffin preparations.

Basics of Histochemistry

The lecture on Basics of Histochemistry provides a foundational understanding of chemical analyses in biological tissues. Students are introduced to key histochemical staining methods, gaining practical insights into the coloration of tissues for microscopic examination. Emphasis is placed on the significance of histochemistry in various scientific fields, elucidating its role in understanding cellular structures and functions.

Basics of Histological Staining

This lecture focuses on fundamental histological staining techniques, aiming to familiarize students with various methods of staining histological preparations. Through the curriculum, students will acquire knowledge about the different approaches to basic histological staining.

Basics of Immunohistochemical Staining

The lecture on immunohistochemical staining covers the principles and techniques involved in utilizing antibodies to visualize specific antigens within tissue samples. Topics include the selection and application of antibodies, understanding antigen-antibody interactions, and the interpretation of staining patterns. The lecture also explores the significance of immunohistochemistry in diagnostic pathology, medical research, and the identification of cellular markers. Practical aspects, such as sample preparation and troubleshooting common issues, are addressed to ensure a comprehensive understanding of immunohistochemical staining methods.

Practicals list (with titles and explanation):

Obtaining Formalin-Fixed Paraffin-Embedded (FFPE) preparations

In this practical lesson on obtaining Formalin-Fixed Paraffin-Embedded (FFPE) preparations, students will gain comprehensive knowledge of the entire process, starting from tissue collection to the final preparation. The lesson covers formalin preparation procedures, emphasizing the importance of proper fixation in preserving tissue integrity. Students will learn hands-on techniques for collecting tissue samples, followed by immersion in formalin to ensure effective fixation. The critical steps of dehydration and embedding in paraffin are explored in detail, highlighting their significance in preparing tissues for microscopic analysis. By the end of the lesson, students will have a practical understanding of the FFPE preparation process, ready to apply this technique in research or diagnostic settings.

Performance of different histological and histochemical stainings

In this practical lesson on the performance of basic histological and histochemical staining, students will engage in hands-on procedures for preparing tissue preparations and executing staining techniques. The focus will be on mastering the sequential steps involved in staining tissues with hematoxylin and eosin (H&E), cresyl violet, and Schiff's periodic acid for histochemical analyses. Students will learn the significance of each staining method in highlighting specific cellular structures and molecules within tissues. The practical session will involve step-by-step guidance on slide preparation, staining reagents, and optimal staining durations. By the end of the lesson, students will have acquired practical skills in basic histological and histochemical staining, essential for microscopic examination and tissue analysis.

Viral antigen detection by IHC in livers of infected mice

In this practical lesson focused on the proof of viral antigens in the liver of infected mice through

immunohistochemistry, students will learn the step-by-step process of preparing tissue specimens for immunohistochemical staining. The lesson emphasizes the meticulous preparation of slides, ensuring optimal conditions for accurate antigen detection. Students will engage in the performance of immunohistochemical staining using specific monoclonal primary antibodies designed to target viral antigens. Additionally, the practical session includes the application of secondary antibodies conjugated with enzymes, a critical step in visualizing the antigen-antibody reactions. By the conclusion of the lesson, students will have acquired practical expertise in employing immunohistochemistry to detect viral antigens, a valuable skill in the field of pathological and virological research.

Seminars list (with titles and explanation):

Paraffinization procedure

In this seminar on the paraffin procedure, participants will delve into a detailed description of all stages involved in obtaining histological preparations through the paraffin method. The seminar will comprehensively cover each component of the paraffin process, from tissue collection to embedding and sectioning, providing a holistic understanding of the procedure. Special emphasis will be placed on elucidating the differences between the advantages and disadvantages of frozen preparations/cuts compared to Formalin-Fixed Paraffin-Embedded (FFPE) specimens. Through discussions and comparisons, participants will gain insights into the practical considerations and applications of both techniques, fostering a nuanced understanding of their respective strengths and limitations in histological preparation. By the end of the seminar, attendees will be equipped with valuable knowledge for informed decision-making in histopathological studies.

Classical histological and histochemical stainings

In this seminar on classical histological and histochemical stainings, participants will explore the foundational techniques employed in visualizing tissue structures and chemical components. The seminar will cover classical staining methods such as hematoxylin and eosin (H&E), cresyl violet, and Schiff's reagent. Detailed discussions will address the specificities and applications of each staining technique, providing participants with a comprehensive understanding of their respective roles in tissue analysis. Practical demonstrations will guide attendees through the step-by-step execution of these classical stains, enhancing their hands-on proficiency. By the seminar's conclusion, participants will have gained valuable insights into the traditional methods crucial for histological and histochemical investigations in biological sciences.

Stoichiometry

In this seminar on stoichiometry, participants will delve into the fundamental principles governing the quantitative relationships in chemical reactions. The content will cover stoichiometric calculations, balancing chemical equations, and understanding the mole concept. Practical applications of stoichiometry in determining reactant quantities, product yields, and limiting reactants will be explored, providing participants with problem-solving skills in various chemical scenarios. The seminar will also discuss the role of stoichiometry in analytical chemistry and its importance in experimental design. By the end of the seminar, participants will have a solid grasp of stoichiometric principles, enabling them to apply quantitative reasoning to chemical processes and reactions.

Immunohistochemical stainings and examples

In this seminar on immunohistochemical stainings, participants will gain a comprehensive understanding of the principles and techniques involved in visualizing specific antigens within tissue samples. The seminar will cover the selection and application of antibodies, elucidating the antigen-antibody interactions critical for precise staining. Detailed discussions will delve into the significance of immunohistochemistry in both diagnostic pathology and research, showcasing its applications in identifying cellular markers and unraveling complex biological processes. Practical sessions will guide participants through the step-by-step process of immunohistochemical staining, fostering hands-on proficiency. By the seminar's conclusion, attendees will possess practical skills and theoretical knowledge necessary for effective immunohistochemical analysis, empowering them in various scientific and medical contexts.

Student obligations:

Students' Obligations:

1. **Active Participation in Practical Sessions:** Students are expected to actively engage in the production of histological preparations under the guidance of teachers. They will learn to apply advanced histochemical and immunohistological staining techniques, gaining hands-on experience in modern medical diagnostics.
2. **Seminar Paper:** With the guidance of teachers, students will be required to prepare a seminar paper on a specific topic within the scope of the course. They should utilize information from literature and reliable online sources to present a well-researched and comprehensive paper.

By fulfilling these obligations, students will develop practical skills in histological techniques and gain a deeper understanding of the challenges and advancements in the field of modern medical diagnostics.

Exam (exam taking, description of the written/oral/practical part of the exam, point distribution, grading criteria):

Exam Conditions: In order to be eligible to take the exam, students must fulfill the following obligations:

1. **Attendance:** Regular attendance in classes is required.
2. **Active Participation:** Students are expected to actively participate in seminars and exercises.
3. **Seminar Paper:** Each student must prepare and present a seminar paper on a topic related to the course.

Evaluation: The student's performance will be assessed and evaluated based on the following criteria:

1. **Class Performance:** The evaluation of student's work during classes, including participation in exercises and the quality of the presented seminar paper, will contribute to up to 70% of the final grade.
2. **Final Exam:** The final exam will be a written examination and will account for 30% of the final grade.

By fulfilling the above requirements and performing well in both class activities and the final exam, students will have the opportunity to achieve a favorable overall grade in the course.

Other notes (related to the course) important for students:

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COURSE HOURS 2023/2024

Histological Techniques

Lectures (Place and time or group)	Practicals (Place and time or group)	Seminars (Place and time or group)
07.05.2024		
Introduction to Histological Techniques: <ul style="list-style-type: none">• P06 (16:15 - 17:00) [182]<ul style="list-style-type: none">◦ HiTe		Paraffinization procedure: <ul style="list-style-type: none">• P06 (17:00 - 18:30) [182]<ul style="list-style-type: none">◦ HiTe
Doc. dr. sc. Lenartić Maja, dipl. ing. [182]		
08.05.2024		
Basics of Histochemistry: <ul style="list-style-type: none">• P13 - ENT (otolaryngology) (08:30 - 09:15) [182]<ul style="list-style-type: none">◦ HiTe		Classical histological and histochemical stainings: <ul style="list-style-type: none">• P13 - ENT (otolaryngology) (09:15 - 10:45) [182]<ul style="list-style-type: none">◦ HiTe
Doc. dr. sc. Lenartić Maja, dipl. ing. [182]		
14.05.2024		
Basics of Histological Staining: <ul style="list-style-type: none">• v (09:15 - 10:00) [182]<ul style="list-style-type: none">◦ HiTe• P13 - ENT (otolaryngology) (10:00 - 10:45) [182]<ul style="list-style-type: none">◦ HiTe		Classical histological and histochemical stainings: <ul style="list-style-type: none">• v (08:30 - 09:15) [182]<ul style="list-style-type: none">◦ HiTe
Doc. dr. sc. Lenartić Maja, dipl. ing. [182]		
15.05.2024		
	Obtaining Formalin-Fixed Paraffin-Embedded (FFPE) preparations: <ul style="list-style-type: none">• Department of Histology and Embryology - Exercise room (16:15 - 19:00) [182]<ul style="list-style-type: none">◦ HiTe	
Doc. dr. sc. Lenartić Maja, dipl. ing. [182]		
04.06.2024		
Basics of Immunohistochemical Staining: <ul style="list-style-type: none">• v (11:00 - 11:45) [180]<ul style="list-style-type: none">◦ HiTe		Immunohistochemical stainings and examples: <ul style="list-style-type: none">• v (11:45 - 12:30) [180]<ul style="list-style-type: none">◦ HiTe
prof. dr. sc. Lenac Roviš Tihana [180]		
05.06.2024		
		Immunohistochemical stainings and examples: <ul style="list-style-type: none">• v (09:00 - 10:30) [180]<ul style="list-style-type: none">◦ HiTe
prof. dr. sc. Lenac Roviš Tihana [180]		
11.06.2024		

	Performance of different histological and histochemical stainings: <ul style="list-style-type: none"> • Department of Histology and Embryology - Exercise room (09:30 - 12:30) ^[180] ^[1647] <ul style="list-style-type: none"> ◦ HiTe 	
prof. dr. sc. Lenac Roviš Tihana ^[180] · Rudančić Tina ^[1647]		
12.06.2024		
	Viral antigen detection by IHC in livers of infected mice: <ul style="list-style-type: none"> • Department of Histology and Embryology - Exercise room (08:00 - 11:00) ^[180] ^[1647] <ul style="list-style-type: none"> ◦ HiTe 	
prof. dr. sc. Lenac Roviš Tihana ^[180] · Rudančić Tina ^[1647]		

List of lectures, seminars and practicals:

LECTURES (TOPIC)	Number of hours	Location
Introduction to Histological Techniques	1	P06
Basics of Histochemistry	1	P13 - ENT (otolaryngology)
Basics of Histological Staining	1	P13 - ENT (otolaryngology) v
Basics of Immunohistochemical Staining	1	v

PRACTICALS (TOPIC)	Number of hours	Location
Obtaining Formalin-Fixed Paraffin-Embedded (FFPE) preparations	4	Department of Histology and Embryology - Exercise room
Performance of different histological and histochemical stainings	4	Department of Histology and Embryology - Exercise room
Viral antigen detection by IHC in livers of infected mice	4	Department of Histology and Embryology - Exercise room

SEMINARS (TOPIC)	Number of hours	Location
Paraffinization procedure	2	P06
Classical histological and histochemical stainings	3	P13 - ENT (otolaryngology) v
Stoichiometry	1	
Immunohistochemical stainings and examples	3	v

EXAM DATES (final exam):
