

Course description for Postgraduates ,School of Basic Medicine

Course Title: Modern histochemical technique		Course Code: 510.514		
Course category: <input type="checkbox"/> High-level course <input type="checkbox"/> International course <input type="checkbox"/> Advanced international courses <input checked="" type="checkbox"/> Common course				
Course Type: <input checked="" type="checkbox"/> 1st-level discipline basic courses <input type="checkbox"/> 2nd-level discipline basic courses <input type="checkbox"/> Optional professional courses				
The Methods of Assessment: open book exam and closed-book exam				
Teaching Method: Theory & Practice		Applicable Educational Level: Master <input checked="" type="checkbox"/> Doctor <input type="checkbox"/>		
The Beginning of the Term: 1 st Semester	Total Hours/Teaching Hours: 36		Credits: 2	
Applicable Specialty: for all medical majors				
Name of the Teachers of the Course Group	Professional Title	Major	Age	Academic Direction
He Li	professor	Histology and embryology	56	Histology and embryology and neurobiology
Xiaoli Wang	Associate prof.	Histology and embryology	58	Histology and embryology and neurobiology
Honglian Li	Associate prof.	Histology and embryology	51	Histology and embryology and neurobiology
Jun He	Associate prof.	Histology and embryology	47	Histology and embryology and neurobiology
Xiangqian Liu	lecturer	Histology and embryology	46	Histology and embryology and neurobiology
Jianying Shen	lecturer	Histology and embryology	45	Histology and embryology and neurobiology
Cuifang Ye	lecturer	Histology and embryology	43	Histology and embryology and

				neurobiology
Lin Zhou	lecturer	Histology and embryology	42	Histology and embryology and immunology
Ting Peng	lecturer	Histology and embryology	42	Histology and embryology and neurobiology

Course Outline:

The contents in this course contain the classic histochemistry, immunohistochemistry, and in situ hybridization histochemistry. Scientists and their graduate students got glorious research results in life science with these techniques. Immunohistochemistry and in situ hybridization histochemistry are suitable and excellent methods to study the specific genes' expression and localization to reveal their functions.

The main feature of this course is to introduce the specimen preparation and experimental procedures in histochemistry, immunohistochemistry and in situ hybridization histochemistry in detail, to make the students and researchers easily use these methods in their scientific research program.

Part one Histochemistry

Chapter one Overview

Objective and requirement:

1. Master the basic concepts of histochemistry
2. Master the basic demands of histochemical technology

Teaching contents:

1. the definition of histochemistry, the relationship of histochemistry and cytohistochemistry, the relationship of histology and biochemistry, the basic principle of histochemistry.
2. The brief history of development of histochemistry.
3. The basic requirements of histochemical technology.

Chapter Two Specimen preparation of histochemistry

Objective and requirement:

1. Master the aim of fixation
2. Master the effect of fixatives in common use
3. Master the paraffin method and cryostat section
4. Master the quantitative methods of common histochemical technology

Teaching contents:

1. The purpose of fixation; the effects of following commonly used fixations: formaldehyde, glutaraldehyde and ethanol; selection of fixations
2. Preparation of specimen sections: paraffin section, cryostat section, freeze-drying method and freeze substitution method
3. Commonly used histochemical quantitative methods

Chapter Three some histochemical techniques

Objective and requirement:

1. Master the technique of PAS reaction (Periodic acid Schiff reaction)
2. Master the histochemical techniques to show following enzymes: alkaline phosphatase, acid phosphatase, adenosine triphosphatase, acetylcholine esterase and nitric oxide synthase.

Teaching contents:

1. PAS reaction to show polysaccharide
2. Feulgen reaction to show DNA and methyl green pyronine stain to show DNA and RNA
3. AldehydeFuchsin stain
4. The histochemical techniques to show acid phosphatase, alkaline phosphatase, 5'-nucleotidase, glucose-6-phosphatase, adenosine triphosphatase, Non-specific esterase, acetylcholine esterase, cytochrome oxidase, monoamine oxidase, peroxidase, succinodehydrogenase, lactic dehydrogenase and nitric oxide synthase

Part Two Immunohistochemistry

Chapter one Overview

Objective and requirement:

1. Master the basic principle of immunohistochemistry
2. Master the basic requirements of immunohistochemistry

Teaching contents:

1. The relationship of immunohistochemistry and histochemistry, the relationship of immunohistochemistry and biochemistry, the fundamental of immunohistochemistry
2. The brief developmental history of immunohistochemistry.
3. The fundamental requirements of immunohistochemical technique

Chapter Two Antigen and antibody

Objective and requirement:

1. Master the definitions of antigen and antibody
2. Master the molecular structure of antibody

Teaching contents:

1. The definition of antigen, complete antigen, hapten, specificity of antigen, antigenic determinant and foreign body of antigen
2. The definition, molecular structure and immunological competence of antibody

Chapter Three Principle of immunohistochemical technology

Objective and requirement:

1. Master the principles of direct method and indirect method of immunofluorescence technique
2. Master the principle of immunofluorescence double labeling method

3. Master PAP method, ABC method and double immunoenzymatic labeling
4. Master the principle of immunogold staining technique

Teaching contents:

1. Immunofluorescence techniques: antigen direct localization method, antibody direct localization method, antigen indirect localization method, antibody indirect localization method and immunofluorescence double labeling method
2. The principles of following immunoenzyme technics: antigen indirect localization method, enzyme bridge method, PAP method and double immunoenzymatic labeling
3. The principles of following Avidin-Biotin technics: LAB method, BAB method, ABC method and SABC method
4. The principles of immunogold technics: immunogold method, protein A immunogold method and the colloidal gold double labeled method

Chapter Four Specimen preparation of immunohistochemical techniques

Objective and requirement:

1. Master the objective of fixation.
2. Master the method of perfusion fixation.
3. Master the procedure to make the tissue slices.

Teaching contents:

1. The purpose of fixation, and the method of perfusion fixation
2. Paraffin embedded section method, cryostat section method and vibration section method

Chapter Five Procedures of some immunohistochemical techniques

Objective and requirement:

1. Master immunofluorescence technic
2. Master PAP method, ABC method, SABC method, protein A colloid gold amplification method and immuno-gold silver staining method
3. Master the procedure of PAP method prior embedding in immuno-electron microscope technique
4. Master the immunofluorescence double labeling method, PAP-PAAP double labeling method and colloid gold double labeling method immuno-electron microscopic technique

Teaching contents:

1. Procedure for immuno-single labeling: indirect labeling technic for light microscope, PAP method, ABC method, SABC method, immunogold method, protein A gold method and immuno-gold silver staining method for light microscope, and PAP method prior embedding or post embedding in immuno-electron microscope technique
2. Procedure for immune-double labeling: PAP-PAAP double staining, immunofluorescence double labeling method for light microscope, and DAB-TMB double staining and colloid gold staining for electron microscope

Part Three In situ hybridization immunohistochemistry

Chapter One molecular biological foundation of in situ hybridization

Objective and requirement:

1. Know the molecular structure of nucleic acid.
2. Master the specificity of nucleic acid.

Teaching contents:

1. Molecular structure of nucleic acid: chemical constitution of nucleic acid, primary structure and higher structure of nucleic acid
2. Specificity of nucleic acid: denaturation and renaturation, function of RNA, transmit of hereditary information (replication, transcription and translation)

Chapter Two Overview of nucleic acid molecular hybridization

Objective and requirement:

1. Master the definition and principle of nucleic acid molecular hybridization.
2. Master the types and specificity of commonly used nucleic acid probes.
3. Master the principle of nucleic acid probe-label.

Teaching contents:

1. Definition and basic principle of nucleic acid molecular hybridization
2. The types and specificity of commonly used nucleic acid probes: cDNA probe, cRNA probe, oligonucleotide probe
3. The basic principle of nucleic acid probe labeling: radioisotope labeling (terminal deoxynucleotidyl transferase, Random primer extension method, TdT-mediated dUTP nick end labeling and in vitro transcription method) and non-radioisotope labeling (enzymatic reaction labeling and chemical labeling)
4. Commonly used nucleic acid labeling techniques: oligonucleotide probe 3'-terminal radioactive isotope labeling technique, oligonucleotide non-radioactive tailing labeling, in vitro transcription labeling technique of cRNA probe (plasmid transformation, amplification, extraction and purification, preparation of linear DNA template, preparation of digoxin labeled probe)

Chapter 3 Basic principle and procedure of in situ hybridization

Objective and requirement:

1. Master the preparation of tissue section, treatment before hybridization, hybridization and treatment after hybridization.
2. Master the demonstrative method of nonradioactive probe in in situ hybridization.
3. Master the control setting for demonstrative system of in situ hybridization.
4. Master the procedure of in situ hybridization with biotin-labeled oligonucleotide probe or digoxigenin-labeled cRNA probe.
5. Know the demonstrative method of radioisotope-labeled probe.
6. To know the tissue control, probe control, hybridization reaction control and demonstrative system control.
7. To know double labeled in situ hybridization and electron microscopy in situ

hybridization.

Teaching contents:

1. Sample preparation in in situ hybridization: dissection, fixation and section.
2. Treatment before hybridization: improve the permeability of tissue, improve the accessibility of probe and reduce the nonspecific binding.
3. Hybridization: denaturation of double-strand DNA probe and target DNA, hybridization solution, length of the probe, concentration of the probe, hybridization temperature and hybridization stringency.
4. Treatment after hybridization Demonstration: the demonstrative method for both radioactive and nonradioactive probe.
5. Control experiments
 - (1) tissue control: Southern or Northern immunoblotting, immunohistochemistry.
 - (2) probe control: using well-known positive or negative tissue or sense RNA probe as control.
 - (3) hybridization reaction control: blank assay and pretreatment with nuclease.
 - (4) demonstrative system control: autoradiographic system control and nonradioactive system control.
6. The procedure of in situ hybridization
 - (1) use radioisotope-labeled oligonucleotide probe .
 - (2) use biotin-labeled oligonucleotide probe.
 - (3) use digoxigenin-labeled cRNA probe.
2. Double-labeled in situ hybridization
 - (1) in situ hybridization combined with both radioactive and nonradioactive labeling.
 - (2) double-labeled in situ hybridization with nonradioactive probe.
 - (3) double-labeled in situ hybridization with radioactive probe.
3. Electron microscopy in situ hybridization.
 - (1) characteristics of electron microscopy in situ hybridization.
 - (2) the main procedure of electron microscopy in situ hybridization.

Chapter 4 Double-labeling method combined in situ hybridization and immunohistochemistry

Objective and requirement:

1. Know the significance of double-labeling method combined in situ hybridization and immunohistochemistry.
2. Know the procedure of double-labeling method combined in situ hybridization and immunohistochemistry.

Teaching contents:

1. The significance of double-labeling method combined in situ hybridization and immunohistochemistry.
2. in situ hybridization combined with immunohistochemistry.

Guide Books:

组织化学与细胞化学技术（第二版），李和、周莉主编，人民卫生出版社，2014年

Main Reference Books:

1. 组织学与胚胎学实验技术，李继承主编，人民卫生出版社，2010年
2. Fan Lin, et al. *Handbook of Practical Immunohistochemistry: Frequently Asked Questions*. Springer; 2nd ed. 2015 edition (February 21, 2015).
3. Igor B. Buchwalow, et al. *Immunohistochemistry: Basics and Methods*. Springer; 2010 edition.
4. Peiguo Chu, et al. *Modern Immunohistochemistry with DVD-ROM (Cambridge Illustrated Surgical Pathology)*. Cambridge University Press; 2 edition (April 14, 2014)
5. Giselbert Hauptmann. *In Situ Hybridization Methods (Neuromethods)*. Humana Press; 2015 edition (February 19, 2015).
6. Boye Nielsen. *In Situ Hybridization Protocols (Methods in Molecular Biology)*. Humana Press; 4th ed. 2014 edition (September 13, 2014).