University in Zagreb School od Dental Medicine

PhD program at School of Dental Medicine

Academic year 2021./2022.

Course title Laboratory methods in immunology: polymerase chain reaction and flow cytometry

Department Department of Immunology

Adress University hospital Center Sestre milosrdnice, Vinogradska cesta 29, 10000 Zagreb

Total ECTS points 4.0

Course leader Ivan Šamija

Course associates Blaženka Ladika Davidović, PhD

Teaching plan

	No. classes
Lecture	0
Seminar	7
Practical	13
Total	20

1 class = 45 minutes

Course description

Within the course students will learn about work in experimental and clinical immunology laboratory. Principles and applications of different methods for analyzing the number and function of immune cells, methods for analyzing other components of immune reaction, laboratory methods based on use of monoclonal antibodies, different applications of polymerase chain reaction in immunology laboratory, and some other methods will be explained. Students will learn in details which is the purpose for use of different methods described, especially what are clinical indications, and how to interpret the results, especially how the results could influence the course of treatment for patients. Flow cytometry and polymerase chain reaction methods will be described in details: different applications and clinical indications for application of these two methods; physical, chemical, and biological principles, instruments and procedures; interpretation of results and potential difficulties and problems in use of these methods. In the practical part of the course students will go in details through whole procedure of gene expression analysis by reverse transcription – polymerase chain reaction: RNA isolation, reverse transcription, real-time polymerase chain reaction, and analysis and interpretation of results. In the practical part of the course students will also go in details through whole procedure of flow cytometry immunophenotyping of peripheral blood lymphocytes: isolation of cells, labeling of cells with fluorescently labeled monoclonal antibodies, sample processing in flow cytometer, analysis and interpretation of results.

Learning outcomes

1. explain the principles and applications of most important methods used in immunology laboratory
2. evaluate and choose for which clinical and experimental problems to apply methods of polymerase chain reaction and flow cytometry
3. understand, interpret and apply results of polymerase chain reaction and flow cytometry analysis

4. explain the whole procedure of gene expression analysis by reverse transcription - polymerase chain reaction and flow cytometry immunophenotyping of peripheral blood lymphocytes

Course contet

Lecture

	Lecture topics	Number of classes/hours
1.	-	-
2.	-	-
3.	-	-
4.	-	-
5.	-	-
6.	-	-
7.	-	-
8.	-	-
9.	-	-
10.	-	-

1 sat = 45 minuta

Seminari

	Seminar topics	Number of classes/hours
1.	Work in experimental and clinical immunology laboratory, principles and applications of different methods	1
2.	Clinical indications for use and clinical interpretation and significance of results of different methods used in immunology laboratory with emphasis on flow cytometry and polymerase chain reaction	1
3.	Physical, chemical and biological principles of polymerase chain reaction and instruments (thermocycler, real-time PCR machine)	1
4.	Different applications of polymerase chain reaction; interpretation of results and potential difficulties and problems in use of polymerase chain reaction	1
5.	Physical, chemical and biological principles of flow cytometry and flow cytometer	1
6.	Different applications of flow cytometry and interpretation of results and potential difficulties and problems in use of flow cytometry	1
7.	Discussion and evaluation of what's been learned in seminars 1-6 and discussion about students' ongoing research projects (PhD and others)	1
8.	-	-

9.	-	-
10.	-	-

1 sat = 45 minuta

Vježbe

	practicals topics	Number of classes/hours
1.	RNA isolation	2
2.	Reverse transcription	1
3.	Real-time polymerase chain reaction setup	1
4.	Analysis of polymerase chain reaction results	1
5.	Interpretation of polymerase chain reaction results and discussion	1
6.	Isolation of cells for flow cytometry immunophenotyping	1
7.	Labeling of cells with fluorescently labeled monoclonal antibodies	2
8.	Sample processing in flow cytometer	1
9.	Analysis of flow cytometry immunophenotyping results	2
10.	Interpretation of flow cytometry results and discussion	1

1 class = 45 minutes

Literature

Imunologija. J. Lukač. Stomatološki fakultet Sveučilišta u Zagrebu. Skripta za studente Stomatološkog fakulteta Sveučilišta u Zagrebu.

Stanična i molekularna imunologija, 8th edition. AK Abbas, AH Lichtman i S Pillai. Medicinska naklada, Zagreb, 2018. pp. 503-516.

Murphy KM. Janeway's Immunobiology. 9th edition. New York: Garland Science, 2017. pp. 749-790. (ISBN: 978-0-8153-4243-4)

Shapiro HM. Practical flow cytometry. 4th edition. Hoboken: Wiley-Liss, 2003.

Mc Pherson MJ, Moller SG. PCR. 2nd edition. New York: Taylor & Francis, 2006.

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CV (*curriculum vitae*) and bibliography of course leader

Ivan Šamija was born in Zagreb, Croatia in 1976. He studied molecular biology at the University of Zagreb, Faculty of Science and graduated in 2000 obtaining a degree of graduate engineer in biology, field of molecular biology. In 2006 he finished a postgraduate study of biology, field of study immunobiology and physiology at the same University and obtained a Ph.D. degree in the scientific area of natural sciences. Since 2017 he is scientific adviser in the scientific area of biomedicine and health, field of basic medical sciences. Since 2000 he has been employed at the Clinical Hospital

Centre Sestre milosrdnice, Department of Oncology and Nuclear Medicine, Zagreb, Croatia, first as junior researcher, since 2008 as staff scientist, molecular biologist, and since 2018. as head of laboratory department. From 2008 till 2009 (12 months) he performed postdoctoral research at National Institutes of Health, National Cancer Institute, Bethesda, USA. He participated as a researcher in several competitive scientific projects. He was awarded Rector's Award of the University of Zagreb in 1997 and Postdoc stipend from the Croatian Science Foundation in 2008. He has participated at teaching several courses on undergraduate, graduate and postgraduate level at the University of Zagreb School of Medicine, School of Dental Medicine and Faculty of Science. He is a mentor of three defended doctoral dissertations. Since 2015 he is employed as Assistant Professor and since 2021 as Associate Professor and Head of Chair of Immunology at University of Zagreb School of Dental Medicine. He is an author on 83 scientific papers including abstracts in conference books of abstracts, of which 16 full scientific papers published in Current Contents and Science Citation Index listed journals. He is a collaborator or chapter author in 15 scientific books of which 8 university textbooks. He is co-author of one accepted patent. His main scientific interests are application of molecular biology and immunology in cancer diagnostics, monoclonal antibodies as drugs for cancer and cancer immunology.

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