

Laboratory Methods in Research

Course objective:

The objective of the course is to solidify students' skills in basic laboratory methods and teach modern laboratory techniques that can be applied in research projects.

Course outcomes:

As a result of participating in the course, students will be able to:

- conduct laboratory work in accordance with standard laboratory safety rules;
- carry out basic laboratory techniques like pipetting, making solutions, filtrations, centrifugations, acid-base titration and fundamental laboratory mathematics and data management;
- set up the laboratory for cell culture experiments and apply basic cell culture techniques and methods for passaging, freezing, and thawing cultured cells;
- use analytical laboratory methods such as flow cytometry, enzyme-linked immunosorbent assay (ELISA), liquid chromatography, and mass-spectrometry.

Course formats:

- seminar presentations;
- laboratory exercises;
- facilitated discussions.

Semesters I and II

Seminars (10 hours): 4 x 2.5

Seminar 1: „ Introduction to cell culture”

Topics:

- laboratory safety rules and aseptic technique
- fundamentals of cell culture
- cell cultures used in laboratories
- basic cell culture conditions (cell cultures in different oxygen concentrations)
- cryopreservation

- morphology of cells in culture
- applications of cell cultures
- cell culture laboratory equipment
- biological contamination of cell cultures

Seminar 2: „ Cell cultures – continuation”

Topics:

- selecting the appropriate cell line for research purposes
- composition and preparation of growth medium, and how to choose the optimal growth medium for cell culture
- the basic guidelines for maintaining cultured cells (definition of subculture, cell density, dissociation of adherent cells, passaging non-adherent cells)
- guidelines for cryopreservation
- cell transfection (guidelines for transfection of cells with DNA and RNA – general information)

Seminar 3: „ Selected research methods used in scientific laboratories”

Topics:

- protein gel electrophoresis (gel preparation, protein sample and buffer preparation, gel run conditions and gel staining)
- DNA gel electrophoresis (gel preparation, DNA sample and buffer preparation, gel run conditions and gel staining)

Seminar 4: „ Selected research methods used in scientific laboratories - continuation”

Topics:

- Enzyme-linked immunosorbent assay (ELISA) (definition and concept, technical aspect)
- flow cytometry (definition and concept of flow cytometry, technical aspects)

Laboratory classes (2 x 2.5):

Laboratory classes 1: „ Basic laboratory methods”

- pipetting with automatic pipets
- weighing of chemicals and making solution
- preparing dilution of solutions
- rules for using basic laboratory equipment: pH meter, centrifuges, spectrophotometer

Laboratory classes 2: „ *Introduction to mammalian cell culture*”

- resuspension of (human and animal) cells from cold storage
- growth media preparation,
- assessing the pH of fresh versus aged growth medium
- counting cells
- cell passaging and cryopreservation
- cell staining (trypan blue and crystal violet staining)
- microscopic evaluation of confluence and morphology

Semesters III and IV

Seminars 1: „ *The application of flow cytometry in scientific research*”

Topics:

- planning and optimizing a flow cytometry experiment
- flow cytometry-based functional assays used in research and diagnostics:
 - cellular phenotyping
 - cell cycle analysis
 - cell proliferation
 - cell apoptosis
 - assessment of mitochondrial dysfunction
 - intracellular cytokine staining
 - identification of antigen-specific T-cells
 - analysis of cellular uptake
 - multiplex assays

- examples of flow cytometry applications in medical diagnostics

Seminar 2: “ Extracellular vesicles (EVs) – powerful little messengers as a new paradigm in cell biology and medicine”

Topics:

- introduction to EVs: biology and function
- tools and methods for studying EVs: biology and functions
- tools and methods for studying EVs: isolation methods and analysis methods
- EVs as innovative diagnostic/prognostic biomarkers in cancer research - EXAMPLES

Seminars 3: The application of modern chromatographic techniques in scientific research"

- principles of chromatography
- high performance liquid chromatography (HPLC) for scientific applications
- qualitative analysis of samples with complicated matrix
- quantification of chemicals using different detectors

Seminar 4: "Proteomics, Metabolomics and Lipidomics"

Topics:

1. Introduction to "-omics techniques"
2. Basics of Mass Spectrometry and liquid chromatography
3. The use of -omics techniques in science
4. Application aspect of research using proteomics, metabolomics and lipidomics

Laboratory class 1 : „Flow cytometry”

- planning a flow cytometry experiment
- determination types of antibodies
- preparing protocol to prepare sample
- running samples through the flow cytometry with assistance from an instructor

Laboratory class 2: “EV isolation and analysis”

- preparing a biological sample for EV isolation
- EV isolation from serum by size-exclusion chromatography
- preparing the EV sample for nanoparticle tracking analysis (NTA): calculations for staining, dilutions
- NTA-analysis of the EV sample in scatter and fluorescent mode