

MONOCLONAL ANTIBODIES: PRODUCTION AND CHARACTERIZATION

CU characterization:

CU name: Monoclonal Antibodies: Production and Characterization Scientific area acronym: BM Duration: Semestral Working hours: 78 Contact hours: 36 ECTS: 3 Observations: N/A

Teacher in charge and respective teaching load in the CU:

In alternate editions of this course, its coordination will be ensured by either Ana Domingos or Sandra Antunes.

Other teachers and respective teaching load in the CU: Ana Domingos – 31 hours Sandra Antunes – 29,5 hours Fernando Cardoso – 8 hours

Intended learning outcomes (knowledge, skills and competences to be developed by the students):

This course aims to transmit concepts related to the production and characterization of monoclonal antibodies. By the end of this course, students should be able to:

- 1. Understand concepts related to the structure, types, and classes of antibodies.
- 2. Acquire knowledge and perform techniques for obtaining hybridomas.
- 3. Execute protocols for obtaining and detecting clones.



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- 4. Compare the differences between monoclonal and polyclonal antibodies and their applications.
- 5. Evaluate the relevance of the application of recombinant antibodies and phage libraries in human health.

Syllabus:

In the theoretical training component, the following topics will be addressed:

- 1. Antibodies: Antibody structure, types, and classes.
- 2. Polyclonal and monoclonal antibodies: Differences, advantages, and applications.
- 3. Monoclonal antibody production: Techniques for producing monoclonal antibodies, including cell fusion, myeloma cells, and fusogenic agents.
- 4. Hybridoma cloning: Limiting dilution method. ELISA technique.
- 5. Applications of antibodies in health.
- 6. Phage libraries: Production and applicability.

In the practical laboratory component, the students will discuss and perform the following techniques or procedures:

- 1. Cell fusion: Extraction and preparation of spleen cells. Preparation of myeloma cell cultures.
- 2. Hybridoma cloning by limiting dilution.
- 3. Identification of positive clones using the ELISA technique.

Teaching methodologies (including assessment):

The teaching methodologies employed will include expository and demonstrative approaches, as well as tutorial support. Practical classes will take place in a Biosafety Level 2 (BSL2) laboratory environment, encouraging discussion among students. Students will have access to an interactive digital protocol covering specific topics of the practical-demonstrative classes to enhance learning consolidation.

The assessment of learning will promote autonomous study and group project. The evaluation will consider the level of success achieved in participating in theoretical (T) and laboratory practical (PL) classes, as well as the completion, presentation, and discussion of the group project related to the application of monoclonal antibodies in health.

Students who achieve a grade equal to or higher than 9.50 on a scale of 0-20 will be approved. The presentation and discussion of the group project are mandatory. The assessment will be weighted as follows: Performance in classes (T, TP, and PL) (10%), group work/written document (40%), and presentation and discussion (50%).

References for consultation / mandatory existence:

- Lu, RM, Hwang, YC., Liu, IJ et al. Development of therapeutic antibodies for the treatment of diseases. J Biomed Sci 27, 1 (2020). <u>https://doi.org/10.1186/s12929-019-0592-z</u>
- Hnasko RM, Larry H Stanker LH. Hybridoma Technology. *Methods Mol Biol* 1318:15-28 (2015). doi: 10.1007/978-1-4939-2742-5_2.



APPENDIX

Organization of Contents by Session and Corresponding Schedule:

Day 1	Lecturer	Contents
13:30h-15:00h (T)	Sandra Antunes	Presentation of the Course and Assessment System. Work Groups. Introduction to the Immune System.
15:00h-16:30h (TP)	Sandra Antunes	Preparation for Laboratory Classes: Good Practices of Manipulation in a Sterile Environment. Disposable Materials; Autoclavable Materials.
17:00h-20:00h (PL)	Ana Domingos Sandra Antunes	Filtration. Cell Counting.
Day 2		
13:30-16:00h (T)	Ana Domingos	 Antibodies. Antibody Structure. Types and Classes of Antibodies. Polyclonal and Monoclonal Antibodies: Differences, Advantages, and Applications. Production of Monoclonal Antibodies. Techniques for Monoclonal Antibody Production. Cell Fusion, Myeloma Cells, and Fusion Agents.
16:3020:00h (PL)	Ana Domingos Sandra Antunes	Cell Fusion. Extraction and Preparation of Spleen Cells. Preparation of Myeloma Cell Cultures. Fusion between Spleen Cells and Myeloma Cells.
Day 3		
13:30h-15:30h (T)	Ana Domingos	Hybridoma Cloning. Limiting Dilution. ELISA Technique. Applications of Antibodies in Health.
16:00-20:00h (PL)	Ana Domingos Sandra Antunes	Observation of Culture Plates. Hybridoma Cloning by Limiting Dilution.
Day 4		•
13:30h-15:30h (PL)	Ana Domingos Sandra Antunes	Observation of Culture Plates. Preparation of Reagents for ELISA Technique.
15:30h-17:30h (PL)	Ana Domingos Sandra Antunes	Identification of Positive Clones using the ELISA Technique.
18:00-20h00 (AT)	Ana Domingos Sandra Antunes	Tutorial Support



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Day 5		
13:30h-15:30h (T)	Fernando Cardoso	Recombinant Antibodies. Phage Libraries.
15:30h-17:00h (TP)	Ana Domingos Sandra Antunes	Analysis of Results obtained in the Laboratory Practical Classes.
17:30-20h00 (AT)	Ana Domingos Sandra Antunes	Tutorial Support
Day 6		
13:30h-16:00h (TP)	Ana Domingos Sandra Antunes Fernando Cardoso	Presentation and Evaluation of Group Projects.
16:30-20h00 (TP)	Ana Domingos Sandra Antunes Fernando Cardoso	Presentation and Evaluation of Group Projects.