

APPLICATIONS OF MAMMALIAN CELL MANIPULATION IN VETERINARY SCIENCES

Study Programme: MIMV **Curricular Year:** 3rd **Semester:** 1st **Optional** **Credits:** 2.5 ECTS

Lecturer(s): Fernando A. C. Ferreira (CCP, R), José Prates, Graça Dias, Graça Pires, António Duarte, Manuela Oliveira, Solange Gil, Frederico Silva, Alexandre Leitão

1. Contact hours: Lectures - 20h; Practicals – 8h; Total - 28h

2. Objectives: Consolidation of knowledge and skills on cell culture techniques and cell analysis.

3. Programme: Theoretical - advantages and limitations of using cell cultures, primary cell cultures versus cell lines, cell cycle and in vitro metabolism. Compositions of culture media, supplements, saline solutions and matrix for cell growth. Routine culture techniques: seeding, subculturing and cryopreservation. Strategies for synchronization and induction of senescence. Identification and elimination of bacterial contamination and Mycoplasma spp.. Equipment and environment in culture room. Aseptic techniques and sterilization of material. Creating and maintaining a cell bank. Cell viability tests, of, adhesion, invasion, motility and angiogenesis methods/techniques. Transient and stable transfections. Transformation and immortalization of cell lines. Direct and indirect immunofluorescence. Principles and applications of in situ Hybridization, protein silencing by RNAi and Flow Cytometry. Immunoblotting techniques (Western blot and ELISA). Applications of cell biology in veterinary research, in diagnostic/prognostics of diseases. Practical - Thawing, seeding, subculturing and cryopreservation of continuous cell lines. Cell counts and calculation of dilutions. Determination of cellular viability in different cell lines by MTT assay. Observation of fusion proteins by fluorescence microscopy. Visualization of intracellular protozoa and interpretation of viral titrations.

4. Bibliography:

Ferreira, F. & Martins, C. (2021). Theoretical and Practical Notebooks - curricular unit lessons support.

Mani, S., Singh, M., Kumar, A. (2023). *Animal Cell Culture: Principles and Practice*. Springer Springer Eds. <https://doi.org/10.1007/978-3-031-19485-6>

Capes-Davis, A., Freshney, R. I. (2021). *Freshney's Culture of Animal Cells: A Manual of Basic Technique and Specialized Applications*. 8th ed., Wiley-Blackwell.

4. Assessment:

Examination in 40 multiple-choice and short questions.