Cell Culturing
Course Outcome Summary

Course Information

Organization	Madison Area Technical College
Developers	Joe Lowndes, Ph.D.
Development Date	9/1/1998
Course Number	10-007-123
Instructional Level	Associate Degree
Potential Hours of Instruction	126
Total Credits	3

Description
Covers the basic techniques of plant and animal cell culture. Plant unit includes media preparation isolation of explants and establishment of callus from suspension cultures, growth factor bioassays, regeneration of whole plants from tissue and plant genetic engineering techniques. Mammalian cell unit includes media preparation, maintenance of cultured cells, transfection of cultured cells, cloning, monoclonal antibody production, and ELISA assays.

Types of Instruction

Instruction Type	Contact Hours	Credits
Classroom Presentation	18	3
On-Campus Laboratory and Clinicals	108	

Textbooks

M. Butler. *Mammalian Cell Biotechnology*.

Donald F. Wetherell. *Plant Tissue Culture*.

Carl Tant. *Awesome Green*.

Carl Tant. *Plant Biotech Lab Manual*.

Learner Supplies

Lab coat.

Safety glasses/goggles.

Calculator.

Laboratory notebook from Scientific Notebook Co..

Prerequisites
Industrial and Applied Microbiology (10-806-174) or consent of instructor
General Cell Biology (10-007-115)
OR consent of instructor

Competencies

Unit 1. Introduction to Plant Cell and Tissue Culture

A. Examine the classification of plants

You will demonstrate your competence:

1. through the completion of written assignments
2. through written examinations

Your performance will be successful when:

1. you outline the characteristics of vascular and nonvascular plants
2. you examine the basic plant life cycle
3. you explore mitosis and meiosis
4. you outline the characteristics of gymnosperms and angiosperms
5. you compare and contrast dicots and monocots

B. Summarize the basic plant structures

You will demonstrate your competence:

1. through the completion of written assignments
2. through written examinations

Your performance will be successful when:

1. you identify parts of the whole plant structure
2. you identify reproductive parts of plant
3. you explain the basic structure and function of leaves, stems, and roots

C. Examine the basic elements of plant culturing

You will demonstrate your competence:

1. through the completion of written assignments
2. through written examinations

Your performance will be successful when:

1. you outline the time line for successful plant cell culturing
2. you outline the different methods of plant propagation
3. you explain the different types of plant growth
4. you explain the basic causes of explant loss

Unit 2. Plant Cell and Tissue Culture Conditions

A. Examine components needed for media.

You will demonstrate your competence:

1. through the completion of written assignments
2. through written examinations

Your performance will be successful when:

1. you summarize components needed for media
2. you explain the function of each media component
3. you summarize the plant hormones used to stimulate plant growth
4. you explore how plant hormones affect plant growth and organogenesis
5. you examine how to prepare media

B. Demonstrate the effects of hormones and chemicals on plant cultures.

You will demonstrate your competence:

1. through the completion of laboratory activities
2. through the completion of written explanations of results and observations of

laboratory activities

Your performance will be successful when:

1. you produce root and stem cultures from callus cultures with no contamination
2. you perform a bioassay for cytokinin activity
3. you calculate and plot plant cell growth by determining cell density using a hemacytometer

Unit 3. Plant Culturing Methods

A. Establish and maintain plant cultures with no contamination.

You will demonstrate your competence:

1. through the completion of laboratory activities
2. through the completion of written explanations of results and observations of laboratory activities

Your performance will be successful when:

1. you produce callus growth with two or more types of explants
2. you establish and maintain plant cell suspension culture
3. you establish and maintain a meristem culture

B. Manipulate plant protoplasts.

You will demonstrate your competence:

1. through the completion of laboratory activities
2. through the completion of written explanations of results and observations of laboratory activities

Your performance will be successful when:

1. you isolate plant protoplasts from flower petals
2. you determine protoplast viability
3. you perform protoplast fusions to produce hybrid cells
4. you examine applications of plant protoplasts

Unit 4. Plant Modification

A. Examine genetic transformation of plants.

You will demonstrate your competence:

1. through the completion of written assignments
2. through the completion of laboratory activities
3. through written examinations
4. through the completion of written explanations of results and observations of laboratory activities

Your performance will be successful when:

1. you explain the methods used for recombinant DNA transfer with plans including the use of Agrobacterium, electroporation and microprojectile bombardment
2. you examine a basic technique for isolating DNA from plant material
3. you demonstrate Agrobacterium-mediated transformation of plant tissue cultures

B. Examine somaclonal variation and mutagenesis.

You will demonstrate your competence:

1. through the completion of written assignments
2. through the completion of laboratory activities
3. through written examinations
4. through the completion of written explanations of results and observations of

laboratory activities

Your performance will be successful when:

1. you examine the problems and advantages of genetic variation
2. you demonstrate the mutagenic properties of sodium azide on plant cells
3. you examine traits of crop plants studied for modification
4. you examine novel trait which could improve a plant variety

Unit 5. Products from Plant Cultures

A. Examine products made by plant culture methods.

You will demonstrate your competence:

1. through the completion of written assignments
2. through the completion of laboratory activities
3. through the completion of written explanations of results and observations of laboratory activities

Your performance will be successful when:

1. you explain the relationship between secondary plant metabolites and products of economic importance
2. you summarize the types of cultures used to facilitate production
3. you analyze the relationship between growth rate and production of secondary metabolites

B. Demonstrate the production and release of secondary matabolites by plant cells.

You will demonstrate your competence:

1. through the completion of laboratory activities
2. through the completion of written explanations of results and observations of laboratory activities

Your performance will be successful when:

1. you use a cell culture to produce a secondary metabolite that inhibits the growth of algae or fungus
2. you demonstrate the use of a plant-derived biocatalyst

Unit 6. Introduction to Animal Cell Culture

A. Examine the history of animal cell culture.

You will demonstrate your competence:

1. through the completion of written assignments
2. through written examinations

Your performance will be successful when:

1. you identify the major historic advances in cell culture
2. you explain the advantages and disadvantages of cell culture
3. you summarize the different types of cultures

B. Examine the biology of the cultured cell.

You will demonstrate your competence:

1. through the completion of written assignments
2. through written examinations

Your performance will be successful when:

1. you examine the anatomy and function of subcellular organelles
2. you examine the elements of selection in the origin and evolution of cell lines
3. you explain the cell cycle and the signals from the environment that regulate it

C. Examine laboratory safety and biohazard issues.

You will demonstrate your competence:

1. through the completion of written assignments
2. through written examinations

Your performance will be successful when:

1. you outline the elements of risk assessment
2. you summarize the typical general safety hazards in a tissue culture laboratory
3. you explain the proper level of protection for various biohazard procedures
4. you explain proper disposal procedures for tissue culture materials

Unit 7. The Culture Environment

A. Examine the equipment needs of a tissue culture lab.

You will demonstrate your competence:

1. through the completion of written assignments
2. through written examinations
3. through the completion of written explanations of results and observations of laboratory activities

Your performance will be successful when:

1. you examine essential and beneficial equipment for a tissue culture facility
2. you examine other types of equipment that may be found in a tissue culture facility
3. you examine the necessary consumable items used in a tissue culture facility

B. Examine aseptic technique.

You will demonstrate your competence:

1. through the completion of written assignments
2. through the completion of laboratory activities
3. through written examinations
4. through the completion of written explanations of results and observations of laboratory activities

Your performance will be successful when:

1. you examine the objectives of aseptic technique
2. you examine good technique for work surface, personal hygiene, pipetting and sterile handling
3. you explain the mechanism of laminar flow hoods

C. Examine the factors that influence the growth of cells during incubation.

You will demonstrate your competence:

1. through the completion of written assignments
2. through written examinations

Your performance will be successful when:

1. you compare the surfaces and the dishes, plates, and vessels that cells will grow on
2. you explain the relationship between CO, temperature, buffering, and pH
3. you explain the basic constituents of media
4. you contrast the advantages and disadvantages of serum-free media

D. Prepare media for culturing cells.

You will demonstrate your competence:

1. through the completion of laboratory activities

2. through the completion of written explanations of results and observations of laboratory activities

Your performance will be successful when:

1. you clean and sterilize equipment
2. you prepare media and sterilize by filtration
3. you test media for sterility

Unit 8. Basic Techniques of Cell Culture

A. Aseptically maintain and passage cultured cells.

You will demonstrate your competence:

1. through the completion of laboratory activities
2. through the completion of written explanations of results and observations of laboratory activities

Your performance will be successful when:

1. you culture CHO-KI, NIH3T3, and NS-I cell lines without contamination
2. you establish a primary cell culture
3. you count cells using a hemocytometer
4. you demonstrate cryopreservation techniques by freezing and thawing CHO-KI cells

B. Manipulate transfected and transformed cultured cells.

You will demonstrate your competence:

1. through the completion of laboratory activities
2. through the completion of written explanations of results and observations of laboratory activities

Your performance will be successful when:

1. you transfect CHO-KI cells with plasmid DNA
2. you select and clone transfected cells
3. you transform NIH3T3 cells and stains foci with Giemsa stain

C. Examine methods for analysis of cultured cells.

You will demonstrate your competence:

1. through the completion of written assignments
2. through written examinations
3. through the completion of written explanations of results and observations of laboratory activities

Your performance will be successful when:

1. you examine methods for DNA, RNA and protein analysis of harvested cells
2. you explain how cell cultures can be used to assay viability and cytotoxicity

D. Examine industrial aspects of cell culturing.

You will demonstrate your competence:

1. through the completion of written assignments
2. through written examinations

Your performance will be successful when:

1. you examine the basic components of a bioreactor
2. you summarize the concept of downstream processing
3. you examine various products from cultured animal cells

Unit 9. Immunological Techniques

A. Examine basic immunology

You will demonstrate your competence:

1. through the completion of written assignments
2. through written examinations
3. through the completion of written explanations of results and observations of laboratory activities

Your performance will be successful when:

1. you examine the basic biology of B cells and T cells
2. you diagram an antibody molecule
3. you compare polyclonal and monoclonal antibodies
4. you examine the methods and uses for antibodies, ELISA, Western Blot, and hybridoma production

B. Examine the use of animals in cell cultures

You will demonstrate your competence:

1. through the completion of written assignments
2. through written examinations
3. through the completion of written explanation of results and observations of laboratory activities

Your performance will be successful when:

1. you examine the ethical considerations of animal use
2. you examine the regulations regarding animal care and use
3. you demonstrate proper handling and care of laboratory animals
4. you examine the proper injection technique for immunization of mice

C. Produce hybridomas with spleen from immunized mice and NS-I cells

You will demonstrate your competence:

1. through the completion of laboratory activities
2. through the completion of written explanations of results and observations of laboratory activities

Your performance will be successful when:

1. you perform the fusion process to create hybridoma cells
2. you perform ELISA to screen hybridoma cells for antibody production