COLLEGE OF VETERINARY MEDICINE AND BIOMEDICAL SCIENCES

DEPARTMENT OF MICROBIOLOGY, PARASITOLOGY AND BIOTECHNOLOGY

BLS 201: TISSUE AND CELL CULTURE TECHNIQUES

BVM Y4 2025/2026

GROUP ASSIGNMENT – MEDIA AND CELL CULTURE

Instructions

1. Each group is assigned to two (2) or three (3) questions to work on and present during class hours. However, each student must work on all the questions to clearly understand them.
2. Each group must clearly define the problem and all-important terminologies and outline the principles and procedures where relevant.
3. For calculation questions, shows all working out.
4. Prepare a 10-minutes presentation and the presenter will be selected randomly during the day of presentation.
5. The presentations will start on Monday 10th December 2025 and will be scored.

QUESTION 1: Cell Culture Media in Veterinary Research

A. You are setting up cultures for three veterinary cell systems:

1. Primary bovine turbinate (BT) cells
2. MDBK (Madin-Darby bovine kidney) continuous cell line
3. Equine endothelial cells used for African Horse Sickness virus research

a) Compare the required media composition, including serum levels, attachment factors, buffering systems, and supplements.
b) Your BT primary cells detach and round up 12 hours after seeding. Give three likely causes and corrections.

B. You are asked to rescue a slow-growing caprine endothelial cell line that fails to reach confluence.
Design a systematic troubleshooting and optimization strategy targeting:

- Substrate/coating
- Growth factors
- pH and CO_2 conditions
- Serum optimization experiment (FBS: 2%, 5%, 15%)

Prepare an experimental layout table with controls.

QUESTION 2: Veterinary Cell Culture Techniques and Diagnostic Applications

A. You are advising a veterinary biotech choosing between:

- Adherent Vero cells for virus isolation
- Suspension-adapted CHO cells for recombinant veterinary vaccine production

a) Compare the culture systems, equipment, scalability, and biosafety considerations.
b) Recommend one system for mass vaccine production and justify two advantages and one challenge.

B. A veterinarian sends lung tissue from a suspected infectious bronchitis outbreak in poultry.
Describe:

- The complete procedure to establish primary avian lung epithelial cells
- Three major challenges unique to avian primary cell culture
- Critical biosafety precautions for handling avian respiratory samples

QUESTION 3: Cell Counting, Seeding & Viability (Veterinary Applications)

A. After trypsinizing a T-75 flask of Feline CRFK cells, you resuspend in 8 mL medium. You dilute 10 µL cells + 10 µL Trypan Blue and count an average of 95 live and 20 dead cells.

a) Calculate the viable cell concentration (cells/mL).
b) Compute viability percentage.
c) You want to seed 12-well plate wells at 3×10^5 cells/well in 1 mL.
Calculate total volume of stock cell suspension needed.

B. A 175-cm^2 flask of PK-15 cells yields 1.2×10^7 cells at confluence.
Each 75-cm^2 flask contains 2.5×10^6 cells at 100% confluence.
You want to start five 75-cm^2 flasks at 40% confluence.
Your counted suspension = 3×10^6 cells/mL.

Calculate:
a) Cells needed per flask
b) Total cells required

c) Volume of suspension per flask
d) Split ratio from original flask

QUESTION 4: Dilution & Drug Treatment Calculations (Veterinary Antivirals)

A. A technician diluted a cell suspension 1:3 and counted 110 cells/square.
Show the correct formula and calculate the original concentration.
Identify the most common calculation mistake in veterinary diagnostics labs.

B. You are testing a new antiviral drug for FMDV.
Stock = 80 mM.
Target working concentrations for bovine cells = 200 µM, 50 µM, 5 µM, 0.5 µM in 500 µL/well.
DMSO must remain ≤ 0.2%.

Design:

- A complete dilution scheme
- Calculate stock volumes for 200 µM and 0.5 µM wells

QUESTION 5: Seeding and Media Preparation in Veterinary Virology

A. You want to seed goat endothelial cells at 1.2×10^4 cells/well in a 48-well plate.
Your cell stock = 3×10^5 cells/mL.

a) Volume of stock needed per well
b) Total volume for 48 wells + 10% excess
c) How to prepare a uniform working suspension

B. Prepare 1 L of complete medium for porcine cell culture:

- 85% Basal medium
- 12% FBS
- 1% L-Glutamine
- 2% Pen-Strep

Then redesign the recipe for reduced FBS at 6%.

QUESTION 6: Subculture Strategy & Cryopreservation

A. Your T-25 flask of equine endothelial cells contains 3×10^6 cells.
Your experiment requires three T-175 flasks, each seeded at 5×10^6 cells.

a) Total cells required
b) Number of subculture rounds needed if maximum split ratio per passage is 1:4
c) Show calculations

B. You freeze canine MDCK cells at 1×10^6 cells/mL.
After harvesting 12 mL, your Trypan Blue count (diluted 1:1) is 140 live cells/square.

a) Calculate total cells
b) Determine the total cryovial volume you need
c) Volume of freezing medium required (10% DMSO)

QUESTION 7: MOI – Veterinary Virus Infection I

A. You want to infect avian DF-1 cells (4×10^6) with IBDV at MOI 1.5.
Your stock titer = 5×10^7 PFU/mL.

a) PFU required
b) Volume of virus stock to add

B. For infecting a 6-well plate of bovine cells:
Cells/well = 3×10^5
MOI = 0.2
Virus stock = 9×10^7 PFU/mL.

Calculate:

- PFU per well
- Volume per well
- Final dilution factor

QUESTION 8: MOI – Veterinary Virus Infection II

A. You have two viral stocks for Rift Valley Fever research:

- Stock A = 1.2×10^8 PFU/mL

- Stock B = 7×10^9 PFU/mL
  You need to infect 1×10^6 ovine cells at MOI 0.3.

a) Volume required for each stock
b) Propose a practical dilution for Stock B and recalculate volume

B. You will infect 12 wells (24-well plate).
Cells/well = 8×10^5
MOI = 0.1
Stock = 5×10^8 PFU/mL.
Target infection volume = 300 µL/well.

Compute:
a) PFU needed per well
b) Stock volume per well (µL)
c) How to prepare a master inoculum for 12 wells

QUESTION 9: Veterinary Virus Quantification – Plaque Assay

A. You infect goat fibroblasts at MOI = 0.5.
After 72 hours you harvest 3 mL supernatant.
You plate 0.1 mL of a 10^{-4} dilution and count 55 plaques.

Calculate:
a) Viral titer (PFU/mL)
b) Total viral yield
c) Burst size if initial cells = 2×10^6

B. A plaque assay of a canine parvovirus stock gives:
10^{-6} dilution → 38 plaques (0.2 mL plated)

Calculate:

- PFU/mL
- Volume needed to dilute to obtain 100 plaques
- Discuss two major errors that affect plaque assay reliability

QUESTION 10: Veterinary Viral Kinetics

A. You perform a growth curve of a swine influenza strain with titers:
12 h = 2×10^4
24 h = 7×10^5
36 h = 3×10^6
48 h = 1.2×10^6

Tasks:
a) Interpret the viral replication phases
b) Explain biological reasons for the drop at 48 h

B. You infect bovine cells at high MOI to study a new pestivirus.
You are given sample titers at 0, 2, 4, 6, 10, 16, and 24 hours.

Identify:
a) Eclipse phase
b) Exponential phase
c) Plateau/decline phase
d) Biological significance of the non-zero titer at 0 h
e) Suggest one alternative cause (besides instability) for decline

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QUESTION 11: Veterinary Biosafety and Lab Design

A. SUA requires a facility handling:

- Brucella abortus
- Rift Valley Fever virus
- ASFV DNA extraction work

a) State minimum BSL level(s)
b) List and justify four essential design features
c) Create a workflow diagram for personnel & sample movement

B. Convert a standard pathology teaching lab into a BSL-2 virology unit.
Provide:

- Room layout
- Placement of BSC
- Waste flow
- SOP for sample handling
  Explain how cross-contamination risks are minimized.

QUESTION 12: Contamination in Veterinary Cell Culture

A. Your MSc student culturing bovine cells reports turbidity and "fast sparkling movement."
a) Identify the contaminant
b) Recommend an immediate containment protocol
c) List two root causes commonly seen in veterinary labs

B. Write a full SOP for aseptic technique specific to veterinary virology labs including:

- PPE
- BSC workflow
- Handling contaminated materials
- Daily cleaning & decontamination steps

QUESTION 13: 2D vs 3D Veterinary Cell Models

A. A livestock drug company wants better prediction of hepatotoxicity in cattle.
a) Why choose 3D liver organoids over 2D hepatocytes?
b) Describe one method for generating bovine hepatic organoids
c) List two tumor-like biological advantages of 3D over 2D

B. Choose one 3D culture model and design a protocol suitable for ruminant toxicity testing.

QUESTION 14: Stem Cell Technology in Veterinary Medicine

A. Explain how mesenchymal stem cells could be used to treat canine osteoarthritis. Include:

- The best stem cell source
- Steps from harvesting to intra-articular administration

B. Compare embryonic vs adult stem cells in veterinary regenerative liver therapy. Highlight ethical, technical, regulatory, and reproducibility issues.

QUESTION 15: Quality Control in Veterinary Cell Culture Labs

A. Explain importance of checking:
a) Mycoplasma in bovine cell lines
b) Cell line authentication
Provide one detection technique for each.

B. Your lab has inconsistent results from MDBK cells for bovine herpesvirus isolation.
a) Use root-cause analysis (5 whys)
b) Explain why increasing antibiotics is a bad idea
c) Design a daily QC checklist
d) Identify the most likely cause category and justify

QUESTION 16: Ethical Issues in Veterinary Cell Culture

A. A wildlife conservation researcher proposes generating buffalo–goat chimeric embryos to study disease tolerance.
State:

- One scientific challenge
- One ethical concern
- One key question for ethics approval

B. Write a debate outline + 1-page brief comparing embryonic stem cells vs iPSCs for veterinary regenerative medicine.

QUESTION 17: Application of Cell and Tissue Culture

Cell and tissue culture techniques have become essential tools in veterinary research and diagnostics due to their versatility, cost-effectiveness, and ability to mimic in vivo biology. Using your understanding of the applications of cell and tissue culture, answer the following:

a) Explain three major applications of cell and tissue culture in veterinary science, providing one specific example for each application.
(Examples may include physiology/pathology studies, production of biological materials, therapy/regenerative medicine, virology research, diagnosis, and virus quantification.)

b) Describe how cell and tissue culture can be used to study viral replication, outlining the key stages of the virus life cycle that can be investigated in vitro.

c) Discuss the role of cell culture in vaccine production, highlighting how live attenuated vaccines are generated using this technique and why cell culture is preferred over other systems such as embryonated eggs or live animals.

QUESTION 18: Laboratory Design

A well-designed laboratory ensures safe, efficient, and contamination-free handling of biological materials. Using your knowledge of laboratory design:

a) Describe four essential structural components of a laboratory (e.g., rooms, windows, doors, ceilings, walls) and explain how each contributes to safety and effective workflow.

b) Explain the importance of reliable electricity and clean water supply in a biological laboratory. Include at least two examples of equipment or procedures that depend on each resource.

c) Discuss the significance of emergency equipment (such as fire extinguishers and eye-wash stations) and explain why staff training is critical for laboratory safety.

QUESTION 19: Biosafety Levels and Laboratory Classification

Biological laboratories are classified into four biosafety levels to ensure personnel and environmental protection. Answer the following:

a) Compare Biosafety Level 1 (BSL1) and Biosafety Level 2 (BSL2) in terms of:

- Types of organisms handled
- Required safety practices
- Key laboratory design features

b) Describe three major features that distinguish BSL3 laboratories from BSL2 laboratories, explaining why they are necessary for handling high-risk pathogens.

c) BSL4 laboratories represent the highest level of containment. Explain:

- The type of pathogens handled in BSL4
- Two unique containment or personnel protection features not found in BSL3

- Why such extreme measures are required

QUESTION 20: Sterility and Aseptic Techniques

Successful cell and tissue culture depends on maintaining strict sterility and practicing proper aseptic techniques. Using your understanding of sterility control:

a) Describe three major sources of contamination in cell and tissue culture and explain how each can compromise cell viability.

b) Explain the role of HEPA filtration, CO_2 inlet filters, and biological safety cabinets (BSCs) in maintaining sterility during cell culture work.

c) Outline the key aseptic practices that laboratory personnel must follow before and during cell manipulation, and explain how these practices protect both the worker and the cultured cells.

QUESTION 21: Sterile Equipment and Reagents

Cell culture requires sterile equipment and reagents to prevent microbial contamination. Answer the following:

a) List five examples of sterile equipment used in cell manipulation and explain the importance of sterilization by autoclaving or other methods.

b) Discuss the role of three sterile reagents (e.g., FBS, antibiotics, trypsin, basal media) and explain how each contributes to sustaining or assessing cell growth in culture.

c) Describe the three classes of biological safety cabinets, highlighting the differences in airflow, personnel protection, and types of work suitable for each class.

QUESTION 21: Optimum Conditions for Cell Growth

Successful maintenance of cell cultures depends on the ability to provide and regulate specific environmental conditions. Using your knowledge of cell culture requirements, answer the following:

a) Explain the importance of maintaining optimal temperature and humidity in a cell culture incubator. In your answer, describe what happens to cells when these conditions fluctuate beyond acceptable ranges.

b) Discuss the role of light in plant cell culture and describe why light–dark cycles are necessary for successful growth and differentiation.

c) Describe how carbon dioxide (CO_2) contributes to pH regulation in cell culture systems. Include in your explanation the chemical reaction involved and how abnormal CO_2 levels affect cell health.

QUESTION 22: Recovery of Frozen Cells

Recovery of cryopreserved cells is a critical step in maintaining viable and healthy cell cultures. Using your understanding of the thawing and recovery process, answer the following:

a) Explain why frozen cells must be thawed rapidly at 37°C and immediately diluted in growth medium after thawing. In your answer, describe the effect of DMSO on cells when left at room temperature for too long.

b) Describe the purpose of centrifugation during recovery of cells. Why is it necessary to discard the supernatant after the first spin?

c) After resuspending the cell pellet in fresh growth medium, explain why the recovered cells should be incubated for 24 hours before replacing the medium. What cellular processes occur during this period?

QUESTION 23: Splitting and Passaging of Cells

Splitting and passaging are essential procedures for maintaining cell viability and preventing overgrowth. Using your knowledge of the process, answer the following:

a) Compare mechanical splitting using a cell scraper and chemical splitting using trypsin–EDTA. In your answer, discuss the advantages and disadvantages of each method.

b) Explain the importance of confluence in determining when cells should be split. What problems arise when cells exceed 100% confluence, and how does this affect experimental results?

c) Describe how trypsin and EDTA work together to detach cells from a culture flask. What are the consequences of over-trypsinization, and how is the action of trypsin stopped after detachment?

QUESTION 24: Inoculation of Cell Cultures with Viruses

Successful virus propagation in cell culture depends on proper preparation of cells, accurate inoculation procedures, and appropriate post-infection handling. Using your understanding of virus–cell interactions and laboratory techniques, answer the following:

a) Explain why cells must be at approximately 50% confluence before virus inoculation. In your answer, describe what happens when viruses are inoculated onto fully confluent (100%) monolayers.

b) Discuss the purpose of washing cells with basal medium or PBS before adding the virus inoculum. Why is it important to remove serum-containing media during this step?

c) Define multiplicity of infection (MOI) and explain its importance in virus inoculation. How does the chosen MOI influence the outcome of the infection?

QUESTION 25: Staining Viruses in Cell Cultures

Detection of viral antigens in infected cells relies on antibody-based staining techniques that allow visualization under light or fluorescence microscopy. Using your understanding of immunostaining principles, answer the following:

a) Distinguish between primary and secondary antibodies used in viral staining. In your answer, explain how each type is generated and how they interact during the staining process.

b) Compare direct and indirect immunostaining techniques. Describe one advantage and one limitation of each method in the detection of viral proteins.

c) Explain how immunoperoxidase and immunofluorescence staining methods allow visualization of infected cells. In your response, include the role of enzyme–substrate reactions or fluorescent dye excitation in producing detectable signals.

QUESTION 26: Stem Cells

Stem cells are unique cells capable of self-renewal and differentiation into specialized cell types, making them essential in development, disease study, and regenerative medicine. Using your knowledge of stem cells, answer the following:

a) Differentiate between embryonic stem cells and adult stem cells in terms of origin, differentiation potential, and advantages or limitations in research applications.

b) Describe two major sources of stem cells for research and therapy, explaining how stem cells are obtained from each source.

c) Discuss at least three uses of stem cells in medicine and research, giving specific examples of diseases or applications where stem cells can be applied.

d) Identify and explain two major limitations associated with the use of stem cells in research or clinical applications.