COLLEGE OF VETERINARY MEDICINE AND BIOMEDICAL SCIENCES

DEPARTMENT OF MICROBIOLOGY, PARASITOLOGY AND BIOTECHNOLOGY

BLS 201: TISSUE AND CELL CULTURE TECHNIQUES

BLS Y2 2025/2026

GROUP ASSIGNMENT – MEDIA AND CELL CULTURE

Instructions

1. Each group is assigned to two (2) 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 1st December 2025 and will be scored.

Question 1: Cell culture media

A. You are tasked with culturing three different cell types: primary chicken fibroblasts, HeLa cells (a continuous human cell line), and a proprietary insect cell line for recombinant protein production.

a) Compare and contrast the key components you would expect to find in the culture media for these three cell types, justifying your choices based on their biological origins and needs.

b) For the primary chicken fibroblasts, the media unexpectedly turns yellow (acidic) within 24 hours of a media change, but no bacterial contamination is visible. Diagnose two potential causes and propose a solution for each.

B. Your lab is culturing a new, fastidious cell type that is not thriving in standard DMEM with 10% FBS. The cells are attaching poorly and dying within 48 hours.

a) Propose a systematic plan to optimize the culture conditions. Your plan should include investigating at least three different components of the culture system (e.g., media formulation, substrate, gaseous environment).

b) Design an experiment to test the effect of different concentrations of FBS (1%, 5%, 10%) on cell attachment and proliferation.

Question 2: Cells and Cell culture techniques

A. A biotech company wants to produce a monoclonal antibody and is deciding between using adherent CHO cells or a suspension-adapted variant of the same cell line.

a) Compare the two culture methods in terms of the specialized equipment, culture vessels, and passaging techniques required for each.

b) As a consultant, advise the company on which method is more scalable for large-volume production. List two major advantages and one significant challenge associated with your recommended method.

B. A field veterinarian has sent you a fresh kidney sample from a rare wild animal suspected of a viral infection. You need to establish a primary cell culture to try and isolate the virus.

a) Describe the step-by-step procedure you would use to obtain renal fibroblast cells from this tissue sample.

b) Explain the major challenges and critical precautions you must take when working with primary cells compared to a continuous cell line like Vero.

Question 3: Cell Splitting, Viability, Volume and Concentration

A. After trypsinizing a confluent T-75 flask of Vero cells, you resuspend the cell pellet in 10 mL of complete medium to create a "stock suspension." You then mix 20 µL of this stock with 80 µL of Trypan Blue. Loading the mixture into a

hemocytometer, you count an average of 120 viable (unstained) cells and 30 dead (blue) cells across the four large corner squares.

a) Calculate the concentration of viable cells (cells/mL) in the original "stock suspension."
b) Calculate the percentage of cell viability.
c) What volume of this stock suspension is required to seed a new 6-well plate, where each well needs 2 mL of medium containing 5×10^4 cells/mL?

B. A T-75 flask yields 6.0×10^6 total cells at 100% confluence. You must seed six 25-cm^2 flasks to start each at 30% confluence. If each 25-cm^2 flask at 100% confluence contains 6.0×10^5 cells, and your available cell suspension concentration after counting is 2.0×10^6 cells/mL, calculate:

a) cells needed per 25-cm^2 flask to achieve 30% confluence;
b) total cells required for six flasks and the split ratio from the original T-75;
c) volume (mL) of cell suspension to add per flask.
(Show formulas and steps — hemocytometer counting rules apply.)

Question 4: The Dilution Dilemma

A. A lab technician diluted a cell suspension 1:5 (1 part cells + 4 parts medium) before counting. The average count in the four large squares of the hemocytometer was 90 cells. The technician then calculated the original cell concentration as 4.5×10^5 cells/mL.

a) Show the correct calculation for the original cell concentration (in cells/mL). Remember each large square has a volume of 0.1 mm^3.

b) Identify the likely mistake the technician made in their calculation and explain how it led to the error.

B. You are testing the effect of a novel anti-cancer drug on cell viability. The drug comes as a 50 mM stock solution in DMSO. You want to treat cells in a 96-well plate with final drug concentrations of 100 µM, 50 µM, 10 µM, and 1 µM.

The final volume in each well is 200 µL, and the concentration of DMSO must not exceed 0.1% to avoid toxicity.

a) Design a serial dilution scheme to achieve these concentrations.

b) Calculate the volume of the stock solution and the volume of medium you would need to add to each well for the 100 µM and 1 µM treatments.

Question 5: Cell Seeding and Culturing

A. You are setting up a 96-well plate to test the effect of a plant extract on cell proliferation. Your target seeding density is 8,000 cells per well in a final volume of 100 µL. Your initial cell count reveals a concentration of 1.2×10^6 cells/mL.

a) Calculate the volume of your initial cell suspension needed to seed one well.

b) Calculate the total volume of cell suspension required to seed all 96 wells, assuming a 10% excess to account for pipetting error.

c) Describe how you would practically prepare this large volume of cell suspension at the correct concentration.

B. You need to prepare 500 mL of complete growth medium for primary cell culture. The recipe is: 89% Basal Medium (e.g., RPMI), 10% Fetal Bovine Serum (FBS), and 1% Penicillin-Streptomycin solution (Pen-Strep).

a) Calculate the volumes (in mL) of each component you need to measure.

b) Your lab manager informs you that due to a budget cut, you must reduce the FBS concentration to 5% for this batch. Recalculate the volumes, keeping the final volume at 500 mL.

Question 6: The Subculture and Cryopreservation

A. You have a T-25 flask of BHK-21 cells at 90% confluence containing approximately 2.0×10^6 cells. Your experiment requires six T-75 flasks, each seeded with 4.0×10^5 cells.

a) Calculate the total number of cells needed for the experiment.

b) Determine the minimum number of subculturing steps (splits) required to go from your single T-25 to having enough cells, assuming you can only split cells 1:3 each time they reach 90% confluence. Justify your answer.

B. You have successfully grown a precious batch of BHK-21 cells to 100% confluence in a T-75 flask. Standard protocol requires you to freeze cells at a density of 1 x 10^6 cells/mL in a freezing medium containing 10% DMSO. After trypsinization and resuspension in 10 mL of media, you take a 10 µL sample and dilute it 1:1 with Trypan blue. You count an average of 120 live cells in one major grid of the Neubauer chamber.

a) Calculate the total number of cells you have harvested.

b) Determine the volume of freezing medium you need to prepare to freeze all your cells at the correct density in 1 mL cryovials.

Question 7: Viral infection I – The Multiplicity of Infection (MOI)

A. You need to infect a culture of 1.5×10^6 cells with the Newcastle Disease Virus (NDV) at a Multiplicity of Infection (MOI) of 2.

a) Calculate the number of plaque-forming units (PFU) required.

b) If your viral stock has a concentration of 5×10^7 PFU/mL, calculate the volume of virus stock to add to your cell culture.

B. You want to infect a 6-well plate of MDCK cells at an MOI of 0.1. Each well contains 2.5 × 10^5 cells. Your virus stock concentration is 8 × 10^7 PFU/mL. Calculate:

a) Amount of virus particles needed per well

b) Volume of virus stock to add per well

c) Final dilution factor used

Question 8: Viral Infection II – Volume Calculation for MOI

A. You have two viral stocks: Stock A (Rift Valley Fever Virus) at 1×10^8 PFU/mL and Stock B (a different isolate) at 2×10^6 PFU/mL. You need to infect 2×10^5 cells in a 24-well plate at an MOI of 0.5 for both viruses.

a) Calculate the volume of each stock required for the infection.
b) The calculated volume for Stock B is impractically small for accurate pipetting. Propose a logical dilution of the stock you would make to overcome this issue and calculate the volume you would use from this new dilution.

B. You need to infect each well of a 6-well plate at MOI 0.1. Each well contains 2.5×10^5 cells. Your virus stock titer is 8.0×10^7 PFU/mL. Calculate:

a) PFU required per well;

b) volume of stock to add per well (µL);

c) how to prepare the inoculum if you need to infect 6 wells and want a total infection volume of 1 mL/well.

(Show formulae (MOI × cell number = PFU) and any dilutions done.)

Question 9: Virus Quantification Using Plaque Assay

A. You infect 5×10^5 cells with a virus at an MOI of 1. After 48 hours, you harvest 2 mL of culture supernatant. You perform a plaque assay by plating 0.2 mL of a 10^{-5} dilution of this supernatant and count 40 plaques.

a) Calculate the viral titer in the harvested supernatant in PFU/mL.

b) Calculate the total viral yield from the culture.

c) What does this yield tell you about the average number of virus particles produced per infected cell (the burst size)?

B. A plaque assay yields the following counts on a 10^{-5} dilution plate: 45 plaques, using 0.2 mL of inoculum.
Calculate:

a) PFU/mL of original stock

b) How much virus you must dilute to obtain 100 plaques on a plate

c) Impact of counting errors and how to improve accuracy

Question 10: Viral Kinetics - Interpretation

A. A student infected Vero cells with a virus and collected the supernatant at different times. The viral titer was determined by plaque assay:

12 hours: 1×10^3 PFU/mL
24 hours: 1×10^6 PFU/mL
36 hours: 5×10^7 PFU/mL
48 hours: 2×10^7 PFU/mL
a) Plot a simple graph of these results (sketch by hand is acceptable for the presentation).
b) Identify the phases of the viral replication cycle (e.g., eclipse, burst, decline) on your graph and explain the biological reasons for the trend observed, including the drop in titer at 48 hours.

B. You are a research assistant characterizing a new, fast-replicating animal virus. You set up a one-step growth curve experiment by infecting 2×10^6 cells at a high MOI (Multiplicity of Infection of 5) to ensure all cells are infected simultaneously. After a 1-hour adsorption period, you remove the viral inoculum and replace the medium. You then collect the culture supernatant at various time points post-infection and titrate the virus using a plaque assay.

Your results are as follows:

Time Post-Infection (hours)	Viral Titer in Supernatant (PFU/mL)
0 (after removal of inoculum)	1.0×10^2
2	1.5×10^2
4	2.0×10^2
6	5.0×10^3
8	5.0×10^6
10	2.5×10^7
12	2.5×10^7
24	1.0×10^7

Tasks:

a) Plot the hypothetical graph of these results (Log_{10} PFU/mL vs. Time).

b) Identify and explain the phases of the viral replication cycle corresponding to the following time windows:

* 0 - 4 hours
* 4 - 8 hours
* 8 - 12 hours
* 12 - 24 hours

c) The viral titer at the 0-hour time point is low but not zero. What does this likely represent?

d) Based on the data, what is the eclipse period for this virus, and what occurs during this phase?

e) A colleague suggests that the drop in titer at 24 hours could be due to the virus becoming unstable. Propose an alternative, biologically relevant explanation for this observation.

Question 11: The Biosafety Blueprint

A. Sokoine University of Agriculture (SUA) is planning to build a new research cell culture laboratory to work with pathogens like *Mycobacterium tuberculosis* and the Rift Valley Fever virus. You are assigned to design that laboratory.

a) What is the minimum Biosafety Level (BSL) required for this work? Justify your answer based on pathogen characteristics.

b) List the three most critical physical design features (e.g., airflow, surfaces) this lab must have and explain why each is non-negotiable.

c) Design the key architectural and operational features of this laboratory, focusing on air handling systems, waste decontamination, and personnel safety protocols.

d) Create a simple workflow diagram showing the path of

i) lab personnel, and

ii) sterile vs. contaminated materials, to minimize the risk of cross-contamination.

B. SUA wants to convert an existing microbiology teaching lab into a BSL-2 cell culture facility that will support routine virology assays.

a) Produce a simple floor/room workflow diagram (sketch acceptable) and a SOP checklist that shows: sample receiving, clean cell room, infectivity/virology room, BSC placement, waste decontamination (autoclave), and unidirectional flow.
b) Explain how your design reduces cross-contamination and protects staff.

Question 12: Cell Culture Contamination

A. You are the senior student in the lab. A new student rushes to you in a panic; all their cell culture flasks have turned cloudy overnight. Under the microscope, you see rapid, jittery movement of small particles.

a) Identify the most likely type of contaminant.
b) Outline the immediate 3-step action plan you would advise the student to take to contain the issue.
c) What are the two most likely causes of this contamination that you would discuss with the student to prevent a recurrence?

B. You are the head of a new BSL-2 cell culture lab. Design a comprehensive "Aseptic Technique and Sterility" Standard Operating Procedure (SOP) for all new students. Your SOP must cover:

a) Personal preparation (PPE, hygiene),
b) Rules for working inside the Biosafety Cabinet,
c) Handling of sterile reagents and equipment, and
d) A step-by-step decontamination protocol for the BSC after working with a potentially infectious sample.

Question 13: 2D and 3D Cell Culture Technologies

A. A cancer research group wants to decide on using 2D or 3D in their research on Cancer.

a) Explain why a cancer research group might choose to use 3D tumor spheroids instead of traditional 2D monolayers of the same cancer cells for drug screening.

b) Describe one key technical method for creating 3D spheroids.

c) List two ways in which the biology of cells in a 3D spheroid is more representative of a real tumor than cells in a 2D monolayer.

B. A pharmaceutical company is tired of the poor predictive power of 2D cell cultures for drug testing. They hire your group to transition to 3D cell culture models.

a) Explain the fundamental differences between 2D and 3D cell cultures and why 3D models are better for drug screening.

b) Choose one 3D culture technique (e.g., spheroids, organoids, or scaffold-based) and describe a detailed protocol for creating a 3D liver model for toxicity testing

Question 14: Sterm Cell Technology

A. Discuss the potential application of stem cell technology in veterinary medicine, specifically for treating tendon injuries in racehorses.

a) What type of stem cell (e.g., embryonic, adult mesenchymal) would be most appropriate and why?

b) Outline the basic steps, from biopsy to treatment, of how you would use this technology.

B. A pharmaceutical company wants to explore using stem cells for regenerative medicine to treat liver cirrhosis.

- Compare and contrast the potential of using Embryonic Stem Cells versus Adult Stem Cells for this application. Your answer must include the advantages, disadvantages, and major technical and ethical challenges associated with each cell type.

Question 15: Quality Control in the Laboratory

A. Your lab uses a continuous cell line for all its experiments. Explain the importance of regularly checking these cells for the following:

a) Mycoplasma contamination.
b) Cell line identity (cross-contamination).
c) Describe one technique used to detect each of the above.

B. You are the Quality Control Officer in a diagnostic laboratory that routinely uses Vero cells for virus isolation. Over the past month, several experiments have shown inconsistent results:

- Cell growth rates have become variable between flasks
- Some cultures show unexpected cell death after 48 hours
- Virus isolation rates from clinical samples have dropped significantly
- One researcher reported possible microbial contamination, but it wasn't confirmed

  a) Create a systematic quality control investigation plan to identify the root cause(s) of these problems (Use the root cause analysis: % whys approach).

  b) The laboratory manager suggests that simply increasing the antibiotic concentration in the media will solve the problem. Explain why this is NOT an appropriate long-term solution and what risks it might introduce.

  c) Design a simple "Cell Culture Health Monitoring" sheet that technicians could use for daily documentation of key quality indicators for each cell culture batch.

  d) Based on the scenario, what is the most likely category of problem (equipment failure, contamination, technician error, or reagent quality) and justify your reasoning.

Question 16: The Ethical Consideration in Cell Culture

A. A research project proposes to create a "chimera" by introducing cells from an endangered African wild dog into a developing mouse embryo to study conservation biology.

a) Identify one major scientific challenge and one ethical concern raised by this research.

b) As a member of the university's ethics review board, what is one key question you would ask the researchers before approving this study?

B. SUA is asked to collaborate on a regenerative-medicine pilot using embryonic stem cells. Prepare a 10-minute group debate pro/con and a one-page policy brief recommending whether to pursue embryonic stem cells or induced pluripotent stem cells (iPSCs), addressing ethical, legal/regulatory, cost, and scientific reproducibility concerns.

****************************ALL THE BEST********************************