Department of Microbiology, Parasitology and Biotechnology
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Group Assignment Questions: Cell and Tissue Culture Techniques (BBL 211) -2026/2027

Instructions:

1. Your presentation should be clear, structured, and demonstrate a deep understanding of the principles behind your calculations and explanations.
2. Your slides must use visual aids (diagrams/figures, flowcharts) where appropriate to enhance your presentation. **Not a copy-pasted text**
3. Maximum length: **10 pages (Word format)** + **PowerPoint slides** (10-minute presentation).
4. Each student must present a part – **divide sections within** the group.

Group 1: Laboratory Safety and Aseptic Technique

Scenario: Your laboratory is preparing to introduce a new, potentially hazardous cell line (e.g., a virally transformed cell line) into the shared cell culture facility. Your group is tasked with creating a standard operating procedure (SOP) for handling this new line to ensure the safety of all personnel and prevent cross-contamination.

Tasks:
a) Outline the key principles of aseptic technique that must be strictly adhered to when working with this new cell line. Explain the specific roles of the Class II Biosafety Cabinet (BSC), Personal Protective Equipment (PPE), and the correct sequence of movements to prevent contamination.
b) You need to prepare 2.5 L of a 70% (v/v) ethanol solution for surface decontamination from a stock of 95% (v/v) ethanol. Using the $C_1V_1 = C_2V_2$ formula, calculate the exact volumes of 95% ethanol and sterile distilled water required. Explain why this specific concentration is effective.

Group 2: Cryopreservation and Cell Banking

Scenario: A research group has successfully established a new primary cell line. To ensure a long-term, reliable supply, they need to create a master cell bank. Your group is responsible for designing the cryopreservation protocol and planning the recovery of the cells for a future experiment.

Tasks:
a) Describe the step-by-step process of cryopreserving an adherent cell line. Your explanation should cover the role of the cryoprotectant (e.g., DMSO), the importance of a controlled-rate freezing process, and the correct procedure for thawing cells to maximize viability.
b) After thawing one vial from the master bank, you perform a viability test using Trypan Blue. You mix 50 µL of the cell suspension with 50 µL of Trypan Blue. After counting 4 large squares on a hemocytometer, you record 180 viable (unstained) cells and 45 non-viable (stained) cells.

i) Calculate the dilution factor.
ii) Calculate the total number of cells counted.
iii) Determine the percentage viability of the thawed cells.
iv) If the total volume of the thawed cell suspension was 10 mL, calculate the total number of viable cells available for your experiment.

Group 3: Primary Cell Culture and Tissue Isolation

Scenario: Your lab is initiating a new project to study the effects of a drug on liver cells. You have received a small tissue sample from a biopsy and need to establish a primary culture. The cells will be cultured in a T-25 flask.

Tasks:
a) Discuss the major challenges associated with establishing a primary cell culture compared to using an established, continuous cell line. What are the key considerations for the enzymatic digestion (e.g., with trypsin or collagenase) of the tissue and the initial seeding of the cells to ensure successful attachment and growth?
b) You want to seed the cells at a high density of 5.0×10^4 cells/cm^2 in a T-25 flask (which has a growth area of 25 cm^2). Your initial cell suspension after tissue digestion has a concentration of 3.5×10^5 cells/mL.

i) Calculate the total number of cells required to seed the T-25 flask.
ii) Calculate the volume of cell suspension needed to achieve this seeding density.

iii) If you prepare 10% extra volume to account for pipetting error, what is the final volume of the cell suspension you will prepare?

Group 4: Viral Isolation and Identification

Scenario: A clinical sample is suspected of containing a novel virus. Your lab is using a cell culture-based approach for viral isolation. You will inoculate a susceptible monolayer of cells to see if cytopathic effects (CPE) develop.

Tasks:
a) Describe the principles of viral isolation using cell culture. What are the signs of viral infection in a cell culture (cytopathic effects)? Explain the difference between a primary cell line, a diploid cell line, and a continuous cell line in the context of viral susceptibility and their practical applications in diagnostic virology.
b) A sample is received and must be prepared for inoculation. You perform a serial dilution of the virus sample to determine its titre. You inoculate 4 wells of a 24-well plate for each dilution. After 5 days, you observe CPE. You want to calculate the 50% Tissue Culture Infective Dose ($TCID_{50}$) using the Reed-Muench method.

- The following data is obtained:
    - Dilution 10^{-4}: 4 wells positive for CPE (out of 4)
    - Dilution 10^{-5}: 2 wells positive for CPE (out of 4)
    - Dilution 10^{-6}: 1 well positive for CPE (out of 4)
    - Dilution 10^{-7}: 0 wells positive for CPE (out of 4)
- Calculate the $TCID_{50}$ of the original sample based on these results.

Group 5: Three-Dimensional (3D) Cell Culture and Organoids

Scenario: Your research group is moving from traditional 2D monolayer cultures to 3D cell culture models to better mimic the *in vivo* tumour microenvironment. You plan to culture cancer cells in a Matrigel matrix to form spheroids or organoids.

Tasks:
a) Explain the fundamental differences between 2D and 3D cell culture. Discuss the advantages of 3D models (like organoids) for applications such as drug screening, disease modelling, and tissue engineering. What are the key challenges in maintaining and analyzing 3D cultures?
b) You need to prepare a 3D culture in a 24-well plate. The protocol requires a final

concentration of 2 mg/mL of Matrigel in a final volume of 500 µL per well. You have a stock of Matrigel at 10 mg/mL.

i) Using $C_1V_1 = C_2V_2$, calculate the volume of Matrigel stock required per well.
ii) Calculate the volume of cell suspension (in culture medium) that will be added to the Matrigel to make the final 500 µL volume.

Group 6: Drug Discovery and Development

Scenario: Your group is tasked with designing an *in vitro* assay to test the efficacy of three novel anti-cancer drug candidates. You will use a 96-well plate format and a colorimetric assay (e.g., MTT assay) to measure cell viability.

Tasks:
a) Describe the workflow for a typical *in vitro* cytotoxicity assay. Explain the purpose of including a vehicle control (e.g., DMSO alone), a negative control (medium only), and a positive control (e.g., a known cytotoxic agent). What are the advantages of using a 96-well plate for high-throughput screening?
b) You have a 50 mM stock solution of one of the drugs dissolved in 100% DMSO. The assay requires a final drug concentration of 25 µM in each well, with a final volume of 200 µL. The final DMSO concentration in the well must not exceed 0.5% (v/v). Design a two-step dilution scheme:

i) First, create an intermediate dilution in culture medium.
ii) Then, calculate the volume of this intermediate to add to each well to achieve the final 25 µM concentration while staying within the DMSO limit. Show all calculations.

Group 7: Stem Cell Culture and Applications

Scenario: A biotechnology company is developing a new therapy based on mesenchymal stem cells (MSCs). Your group is responsible for the expansion and quality control of these cells before they are formulated for clinical use.

Tasks:
a) Discuss the unique characteristics of stem cells (self-renewal and differentiation potential). What are the critical quality control measures that must be performed on a stem cell culture intended for therapeutic use? Include factors like cell identity, purity, viability, and sterility.

b) You have a T-75 flask (growth area 75 cm^2) of MSCs at 80% confluence. At 100% confluence, a T-75 flask of these cells contains 6.0×10^6 cells.

i) Calculate the number of cells in the flask at 80% confluence.
ii) If you want to subculture these cells into five new T-75 flasks at a seeding density of 5.0×10^3 cells/cm^2, do you have enough cells? Show your calculations.
iii) Based on your calculation, what split ratio (e.g., 1:2, 1:3) would you use to ensure you have enough cells to seed all five flasks?

Group 8: Addressing Contamination in Cell Culture

Scenario: After a long weekend, you arrive at the lab to find that one of your T-25 flasks containing an important adherent cell line has become cloudy. Under the microscope, you observe numerous small, rod-shaped particles moving rapidly, independent of the cells.

Tasks:
a) Based on these observations, what is the most likely type of contaminant? Justify your conclusion. What are the potential sources of this contamination, and what steps would you take to recover your culture and prevent the spread of contamination to other cultures in the lab?
b) You suspect the contamination is bacterial. To confirm, you perform a viable count on an agar plate. You serially dilute the contaminated medium: 1 mL of culture into 9 mL of saline (Dilution A), then 1 mL of Dilution A into 9 mL of saline (Dilution B). You then plate 100 µL of Dilution B onto an agar plate and incubate it. After 24 hours, you count 80 colonies.

i) What is the total dilution factor of Dilution B?
ii) Calculate the original concentration of bacteria in the contaminated medium in Colony Forming Units per milliliter (CFU/mL).

Group 9: Bioprocessing and Scale-Up

Scenario: Your lab has successfully produced a monoclonal antibody (mAb) in a 1 L spinner flask using a suspension cell line. The project is moving to the pilot scale, and you need to scale up the process to a 10 L stirred-tank bioreactor.

Tasks:
a) Discuss the key parameters that must be considered and controlled when scaling up a cell culture process from a spinner flask to a stirred-tank bioreactor. Include factors such

as agitation/mixing, aeration, pH, and temperature.
b) Your 1 L culture had a final viable cell density of 2.0×10^6 cells/mL. You want to inoculate a 10 L bioreactor with a starting viable cell density of 2.5×10^5 cells/mL. The working volume of the bioreactor is 8 L.

i) Calculate the total number of viable cells in your 1 L culture.
ii) Calculate the total number of cells required to inoculate the 8 L bioreactor at the target density.
iii) Calculate the volume of your 1 L culture that you need to use as the inoculum for the bioreactor.
iv) Calculate the volume of fresh culture medium needed to bring the final volume to 8 L.

Group 10: Cell-Based Assays (Proliferation)

Scenario: Your group is testing the effect of a new growth factor on the proliferation of a specific cell line. You will use a colorimetric assay to quantify cell number over a 3-day period.

Tasks:
a) Describe the principles of a common cell proliferation assay (e.g., MTT, XTT, or Alamar Blue). Explain how the assay works, what it measures, and how you would use it to generate a dose-response curve for your growth factor.
b) You set up a 96-well plate with cells at 1,000 cells/well. After 3 days, you perform a hemocytometer count from a control well. You mix 10 µL of the cell suspension with 90 µL of Trypan Blue. You count the four large corner squares and find 60 viable cells and 10 non-viable cells.

i) Calculate the dilution factor.
ii) Calculate the viable cell concentration in cells/mL.
iii) Calculate the total number of viable cells in the well (assume the total volume in the well was 200 µL).
iv) If the cells doubled twice in the 3-day period (from 1,000 to 4,000 cells), and the count only shows a fraction of this, what could this discrepancy indicate about your experimental setup or the cell's behavior?

Group 11: Quality Control and Equipment

Scenario: Your lab is implementing a new quality control (QC) program. As part of this program, you must ensure that your cell cultures are free from mycoplasma, a common and insidious contaminant that can affect cell growth and function.

Tasks:
a) What are the key characteristics of mycoplasma that make it so problematic in cell culture? Describe the potential sources of mycoplasma contamination and the methods used for its detection (e.g., PCR, Hoechst DNA staining). How is a mycoplasma-contaminated culture treated or eliminated?
b) You receive an equipment maintenance log for your lab's centrifuge. The log shows a rotor with a radius of 10 cm. The protocol you are using requires a centrifugation step at 300 × g for 5 minutes.

i) What does the "g" in 300 × g represent? Why is it preferable to use g-force instead of RPM for protocols?
ii) Calculate the necessary RPM for the centrifuge to achieve this force. (The formula is: $RCF = 1.118 \times 10^{-5} \times r \times RPM^2$, where RCF is the relative centrifugal force in g, r is the radius in cm, and RPM is revolutions per minute).

ALL THE BEST!