Overview of Module 4

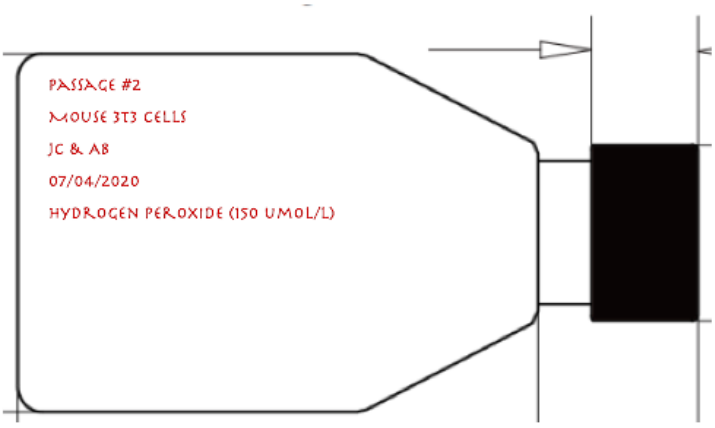
1. What is one application of cell culture?
   Research (to study cell behavior), Therapeutics (to produce drugs, tissue grafts, cell-based therapies), Diagnostics (culturing patient cells to make a diagnosis/ prescribe treatment)
2. What is the difference between a Biological Safety Cabinet (BSC) and an Incubator?
   The BSC is where you WORK with cells (ex: change media, passage cells) and the Incubator is where you store cells so that they can grow
3. List three things that you should NOT do while working in a BSC.
   Move quickly, not spray your hands with ethanol then end the BSC, pour reagents directly from their container into the culture flask, work with the shield up
4. Which solvent do we use to sterilize everything entering the BSC or incubator?
   Ethanol
5. Why do we passage cells?
   To prevent cultured cells from overcrowding and maintain the ideal confluency for our cells
6. Why do you need to keep track of the passage number?
   Cells can only be passaged a certain number of times, as over time they change in morphology and thus experience behavioral changes
7. Why is our cell culture media red?
   Phenol red is added to DMEM, allowing us to visually determine the pH of the cell culture media
8. What does it mean if the cell culture media is yellow?
   Too acidic, cells are too confluent and need to be passaged
9. What does it mean if the cell culture media is purple?
   Too basic, incubator CO_2 is not at 5% and the incubator tubing must be examined
10. Why is it important to not leave active trypsin in the cell culture flask for too long?
    Overexposure to trypsin can damage surface proteins and lyse our cultured cells
11. What deactivates trypsin?
    Calcium and magnesium ions provided by Fetal Bovine Serum in DMEM

Practice with Cell Culture Techniques

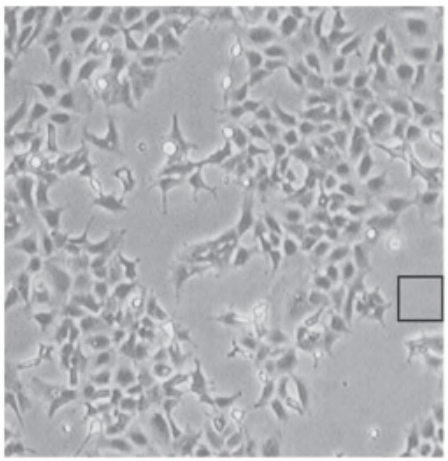
1. Match the key term to its role in cell passaging:

A. Ethanol	E	Separates cells from liquid and debris
B. CO2	F	Its calcium and magnesium ions deactivate trypsin
C. Phenol red	G	Added to prevent to help prevent contamination
D. PBS	H	Breaks down proteins involved in cell adhesion
E. Centrifuge	I	Removes excess liquid from flasks and conical tubes
F. FBS	B	5% inside incubator to simulate biological conditions
G. Antibiotics	J	Our cell culture media
H. Trypsin	C	Visualizes pH changes
I. Aspirator	A	Sterilization solvent
J. DMEM	D	Rinses excess media and cell waste from flask

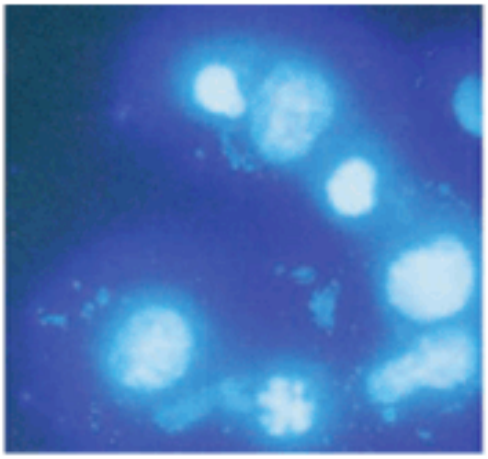
2. If I do a 1:9 Split on one T-25 flask, the cells will be reseeded in 9 T-25 flasks or 3 T-75 flasks.
3. Practice properly labeling your T-25 flask!



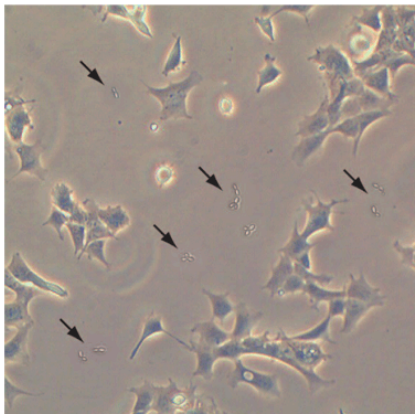
4. Identify the contamination present in the cultures below as bacterial, fungal, yeast, or mycoplasma:



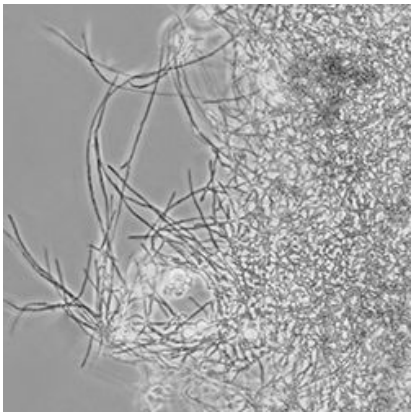
(A)



(B)



(C)



(D)

(a) Picture A contains bacterial contamination.

(b) Picture B contains mycoplasma contamination..

(c) Picture C contains yeast contamination.

(d) Picture D contains fungal contamination.