SUBCULTURE OF SUSPENSION CELL LINES

Cells should be cultured in the exponential growth phase when they appear bright and rounded under the microscope, and refractory to vital dye. If phenol red is added to the medium and turns yellowish, the pH is on the acid side of neutral, indicating that the population has passed the exponential phase and is not in the best condition for subculture or storage.

Equipment and Materials

- Laminar flow hood
- CO_2 incubator
- Growth medium
- Flasks
- Inverted microscope

Procedure

If the pH of the medium is acid:

1. Transfer the cell suspension to a centrifuge tube and centrifuge for 5 minutes at 2000 rpm.
2. Remove the supernatant but do not discard it; suspend the pellet in 5 ml of fresh medium and add 10-20% of the supernatant as conditioning agent.
3. After a few hours or the day after, count the cells and, depending on the number, either let the cells grow in the same medium or subdivide them in further flasks with fresh proliferating medium according to the type of cell.

If the pH is neutral:

1. Gently pipette the cell suspension several times to break up any clumps of cells and take an aliquot for cell counting and cell viability determination (see ROUTINE CELL COUNTING AND ASSESSMENT OF VIABILITY).
2. Calculate the cells/ml and re-seed the desired number of cells into new flasks without centrifugation, just by diluting the cells.