EXERCISE - 7

- **Growth and maintenance of BHK-21 cell line / Sub-culturing of BHK-21 cell lines**

REQUIREMENT –

- Laminar flow cabinet
- Inverted microscope
- BHK-21 cells in cell culture flask
- New cell culture flasks / bottles
- EMEM (Minimum essential medium)
- FCS (Fetal Calf Serum)
- EMEM with 10% FCS
- TPVG (Trypsin phosphate versene glucose)
- TPB (Tryptose phosphate broth)
- PBS (Phosphate buffer saline)
- 37°C water bath / incubator
- Micro pipettes
- Micro tips

PROCEDURE –

1. Clean the laminar hood with cotton / tissue paper soaked with 70% ethanol and sterilize the hood for about 30 minutes by putting on the UV light.
2. Observe the cultures under the inverted microscope to observe the confluent cell monolayer. Cells should be evenly distributed.
3. Prewarm TPVG, Medium at 37°C.
4. Decant the culture medium from the flask.
5. Wash the cells with PBS to remove the remaining dead cells and the remaining media.
6. Add TPVG with pipette and rinse the cell sheet. Remove TPVG immediately as per the flask volume.
7. Add about 0.5 ml (As per flask volume) of fresh TPVG and distribute it evenly on the cell sheet and incubate the flask at 37°C for 2 minutes.
8. Gently tap the flasks to dislodge cells. Continue incubation till cell starts detaching. (Cell sheet would turn translucent when the action of trypsin is complete)
9. Add 5ml of EMEM with 10% FCS and detach the cells by uniform pipetting.
10. Add sufficient volume of the growth medium and distribute into 3 cell culture bottles.

11. Cap the flasks and tilt gently to distribute the cells uniformly.
12. Label the flasks and keep inside the CO_2 incubator at 37°C for cell multiplication and monolayer formation.