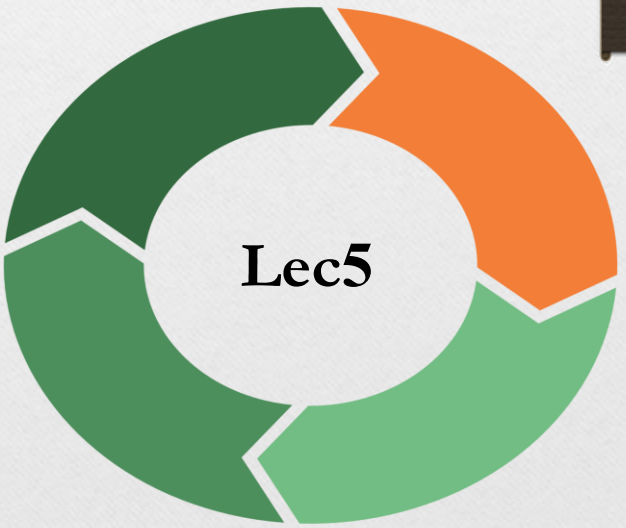
Characteristics of cell cultures, culture conditions, cryopreservation, growth cycle.

Msc. Sarah Raheem



Cell toxicity

- **Cytotoxicity** causes inhibition of cell growth

- **Observed effect** on the morphological alteration in the cell layer or cell shape

- **Characteristics of abnormal morphology** is the giant cells, **multinucleated** cells, a **granular bumpy** appearance, vacuoles in the cytoplasm or nucleus

Working with cryopreserved cells

- **Vial from liquid nitrogen is placed into 37 C water bath, agitate vial continuously until medium is thawed.**

- **Centrifuge the vial for 10 mts at 1000 rpm at RT, wipe top of vial with 70% ethanol and discard the supernatant.**

Working with cryopreserved cells

- Resuspend the cell pellet in 1 ml of complete medium with 20% FBS and transfer to properly labeled culture plate containing the appropriate amount of medium
- Check the cultures after 24 hrs to ensure that they are attached to the plate
- Change medium as the colour changes, use 20% FBS until the cells are established

Freezing cells for storage

- Remove the growth medium, wash the cells
- Separate the cells by trypsin-versene
- Transfer the cell suspension to a 15 ml conical tube, centrifuge at 200g for 5 mts at RT and remove the growth medium by Separate

Freezing cells for storage

- Resuspend the cells in 1-2ml of freezing medium
- Transfer the cells to cryovials, incubate the cryovials at -80 C overnight
- Next day transfer the cryovials to Liquid nitrogen

Cell viability

- Cell viability is determined by staining the cells with trypan blue
- As trypan blue dye is permeable to non-viable cells or death cells whereas it is impermeable to this dye
- Stain the cells with trypan dye and load to Hemocytometer and calculate % of viable cells- % of viable cells=

(Nu. of unstained cells ÷total nu. of cell)x 100

Common cell lines

- **Human cell lines**
- **MCF-7** breast cancer
- **HL 60** Leukemia
- **HEK-293** Human embryonic kidney
- **HeLa** Henrietta lacks

Common cell lines

- **Primate cell lines**
  - **Vero** African green monkey kidney epithelial cells
  - **Cos-7** African green monkey kidney cells

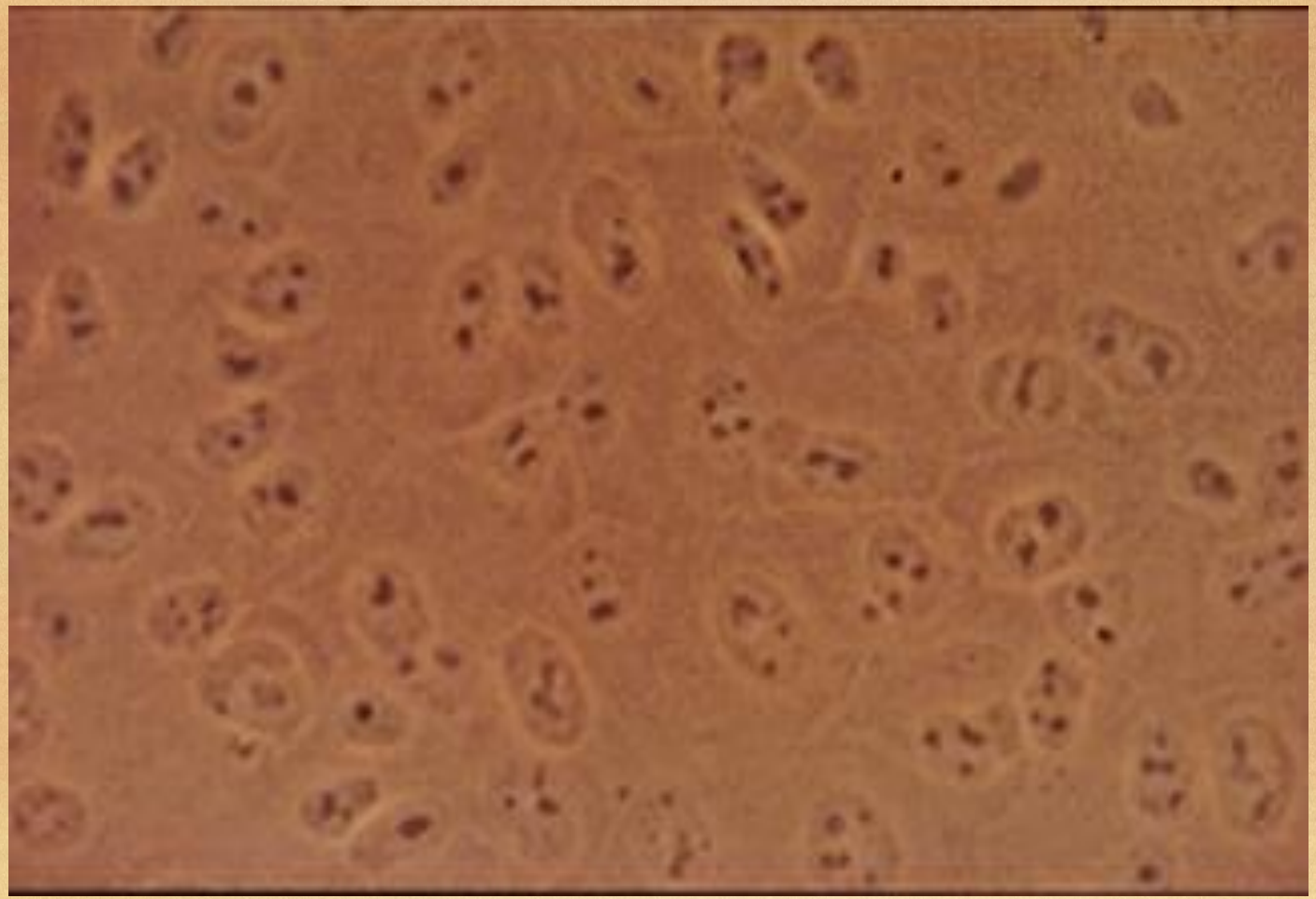
And others such as CHO from hamster, sf9 & sf21 from insect cells

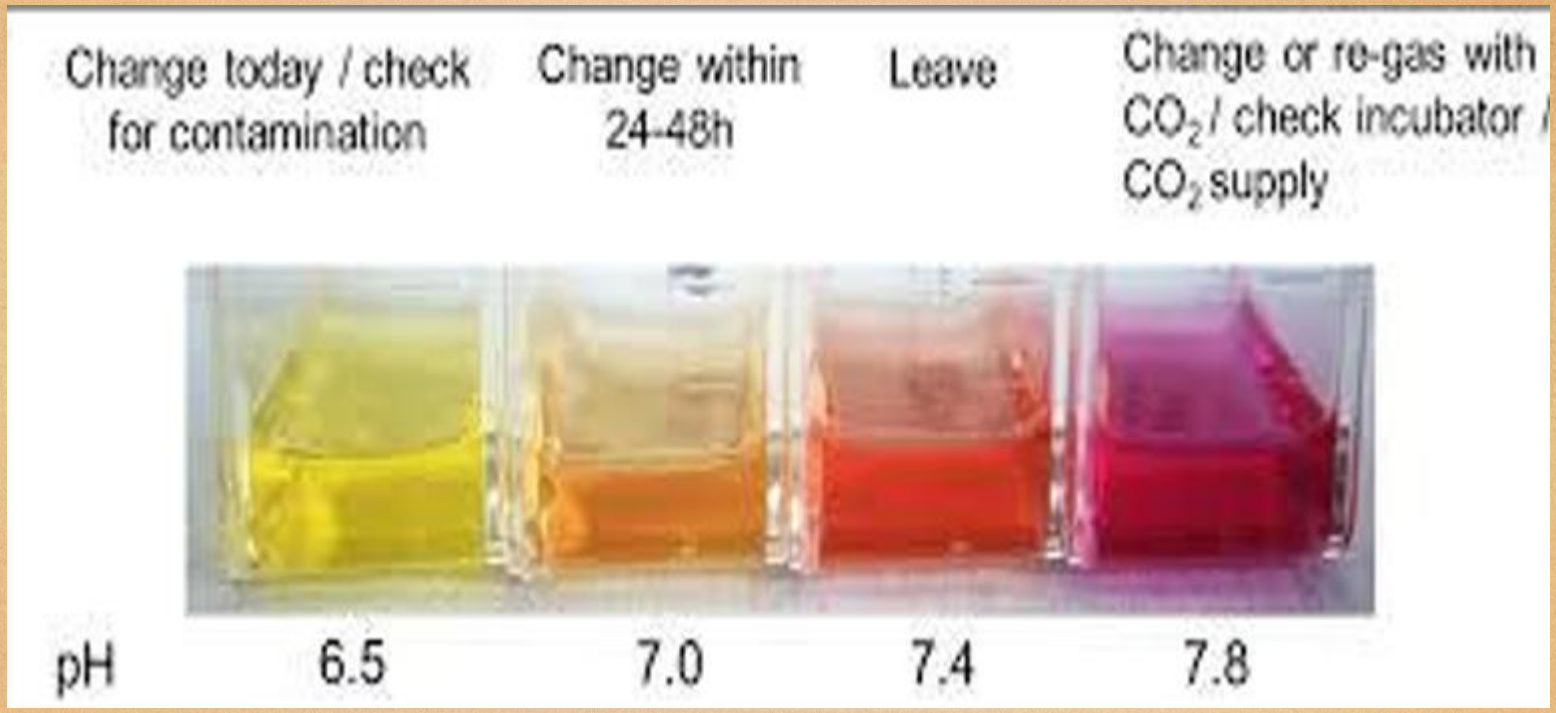
Contaminants of cell culture

Cell culture contaminants of two types

- Chemical-difficult to detect caused by **endotoxins, plasticizers, metal ions or traces of disinfectants that are invisible**

- Biological-cause visible effects on the culture they are **mycoplasma**, **yeast**, **bacteria** or **fungus** or also from cross-contamination of cells from other cell lines




Change today / check for contamination
Change within 24-48h
Leave
Change or re-gas with CO_2 / check incubator / CO_2 supply
pH
6.5
7.0
7.4
7.8

Effects of Biological Contamination's

1. They competes for nutrients with host cells

2◦ Secreted acidic or alkaline by-products ceses the growth of the host cells

3◦ Analyze arginine & purine inhibits the synthesis of histone and nucleic acid

4 ◦ They also produces H2O2 which is directly toxic to cells

5 Slow growth and stoped of cell division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