CYTOGENETICS '1'

Lab 2: Cell culture technique

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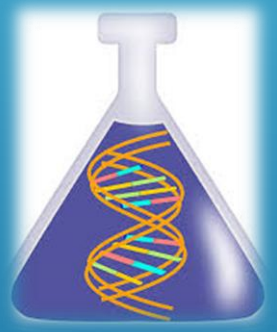
Definitions

- **Cytogenetics;** visual study of chromosomes at microscopic level
- **Karyotype:**
  Chromosome complement
  Also pictures of chromosomes
- **Idiogram:**
  stylised form of karyotype

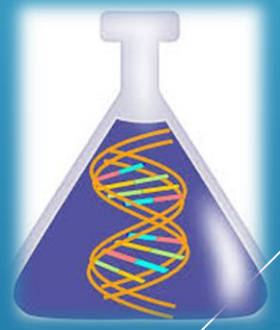


Sample types:

Postnatal: blood sample

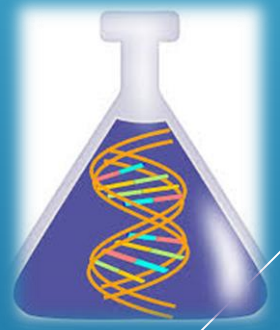
Prenatal: amniotic fluid, chronic villus sampling CVS

Cancer: solid tumours



Referral reasons

- Dysmorphic features
- Developmental delay
- Short stature
- Failure to develop secondary sex characteristics
- Infertility
- Recurrent miscarriage
- Family history of Down syndrome
- Indeterminate gender at birth
- New born babies with suspected chromosome abnormality
- Parents of abnormality found in PND



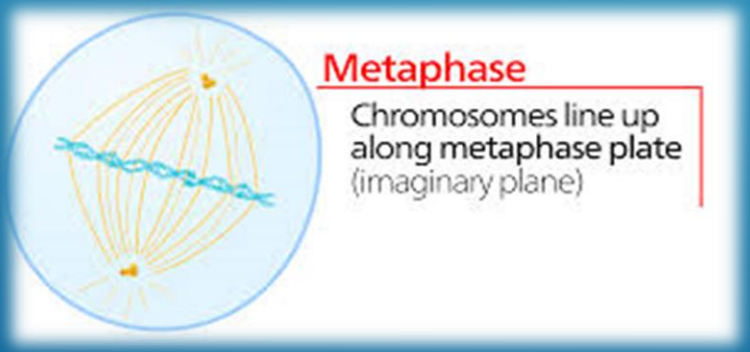
Cell culture technique

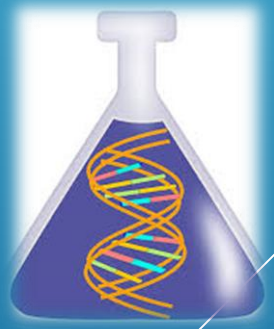
- **Cells from different sample types can be cultured in order to;**

Increase their number

- **Required cell stage is;**

‘Metaphase’

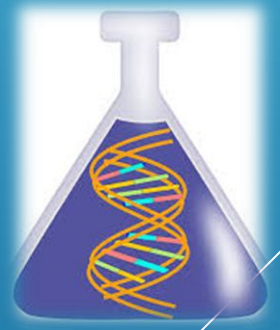


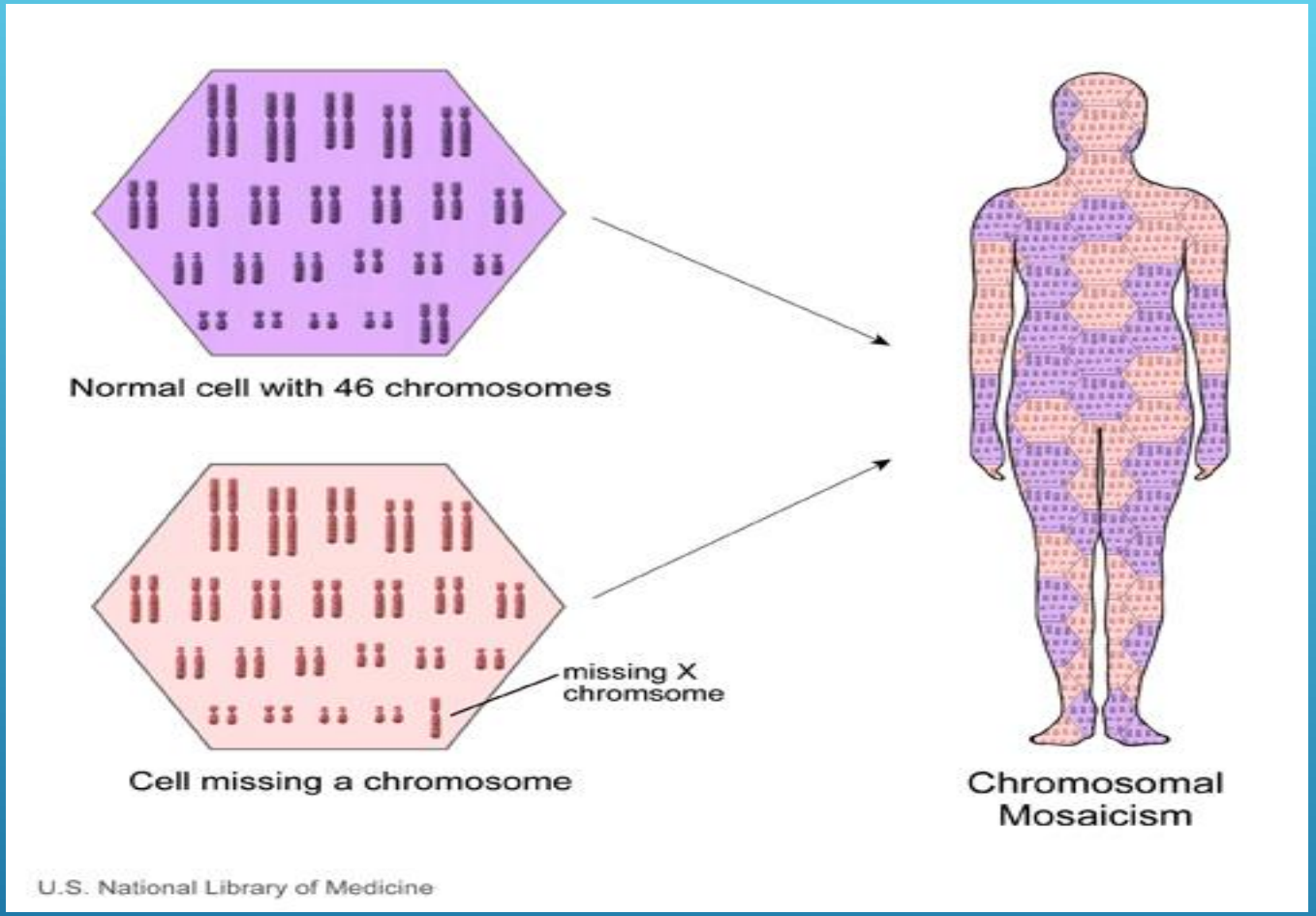


Sample selection criteria

- **Representative to all cell lines (mosaicism is suspected)**
- **easy to obtain**
- **Inexpensive** (amniotic fluid take 1 week)
- **Yield high-quality metaphase in abundance**

  eg; Lymphocytes

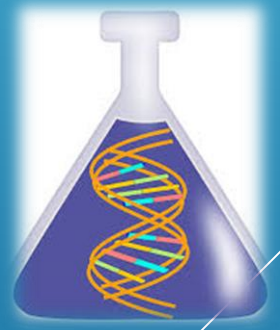




Mosaicism
(2 different cell lines)

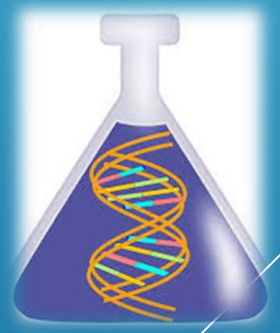
Lymphocytes

- Nucleated
- T - lymphocytes make up ¬ 70% of all lymphocytes in healthy persons
- They obtained from pb or cord blood in newborns
- Peak mitotic activity 72hrs



Sample collection

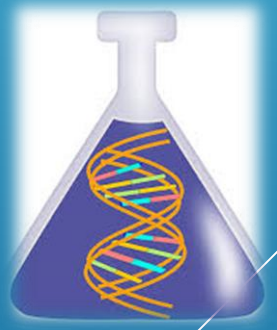
- Green-top lithium or sodium tubes
- proper request
  (patient's name, status, history, date of collection)
- Adults: 0.8 ml PB
- Newborns: 0.5 ml PB or cord blood
- Live fresh cells required – never frozen



Cell culture conditions

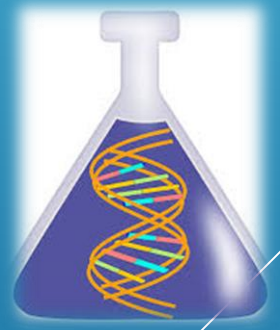
Media components:

- 15% serum (bovine, human, horse)
- Amino acids (L-glutamine)
- Vitamins
- Salts
- Glucose
- Growth factors
- Antibiotics .. Why?
- Buffers
- PHA; Phytohemagglutinin – T lymphocyte mitogen ??



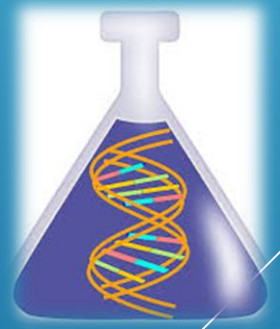
PHA

- **Extracted from plants**
- **Used in medicine as a mitogen to trigger T lymphocyte cell division**



Cell culture conditions

- Powdered or liquid
- 37-37.5 C ??
- PH 7.2-7.4 controlled via:
  - bicarbonate buffer
  - controlled flow of 5% CO2
- Humidified, gas flow (recommended)



Sterile conditions

Double doors gas flow incubator

Vertical laminar flow hood

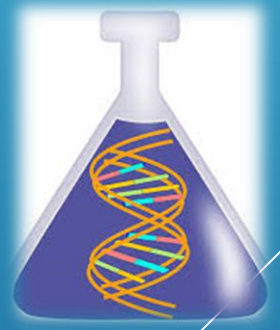
Procedure

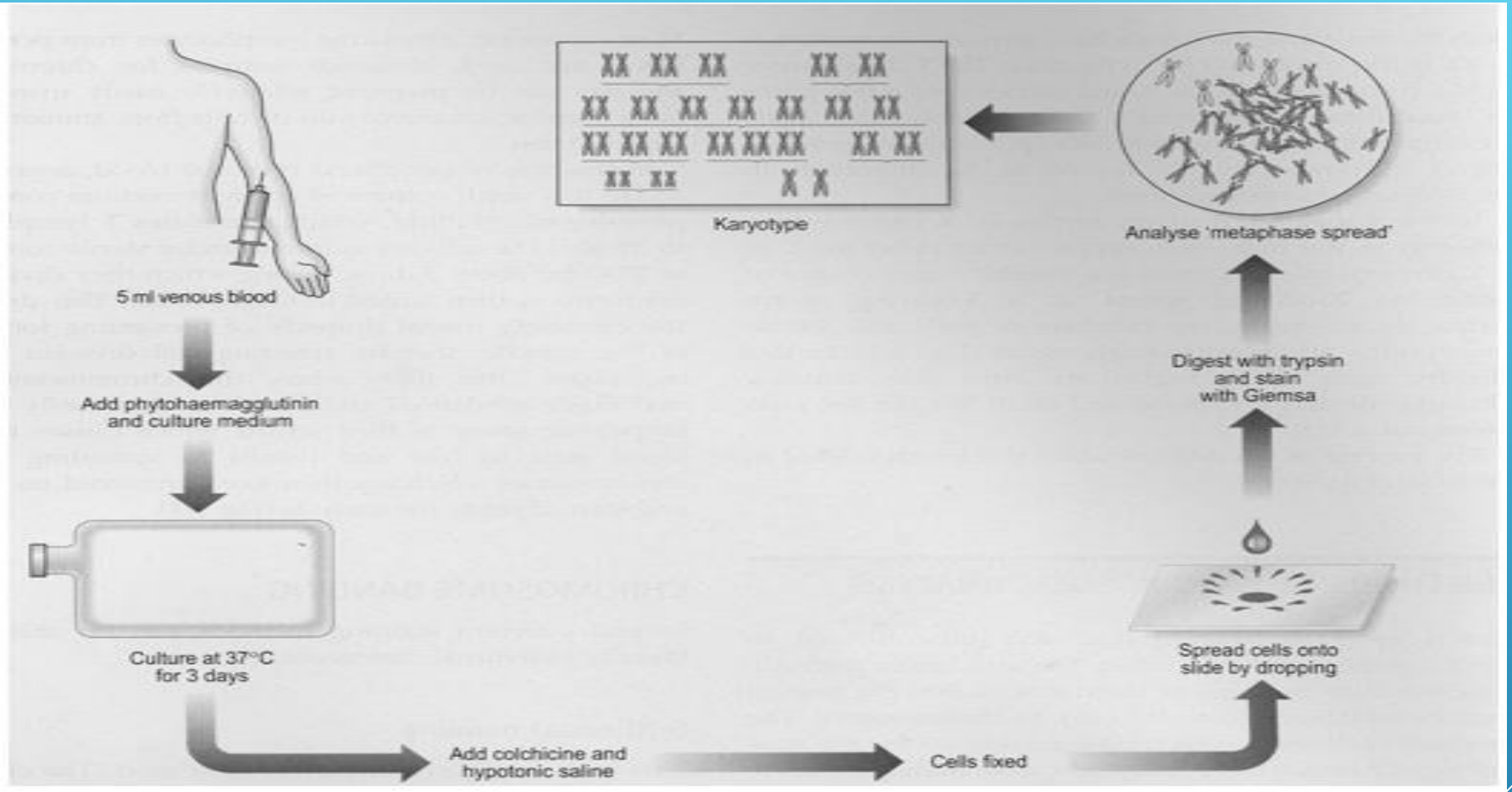
Mix the sample well

Transfer 0.6 ml to 10ml screw-capped plastic centrifugation tube containing PB-Max culture media

Close the tube, invert it gently

Incubate at 37C for 72hrs in slant position





Peripheral blood karyotyping steps

Thanks for listening

Questions ?