Cytogenetic:

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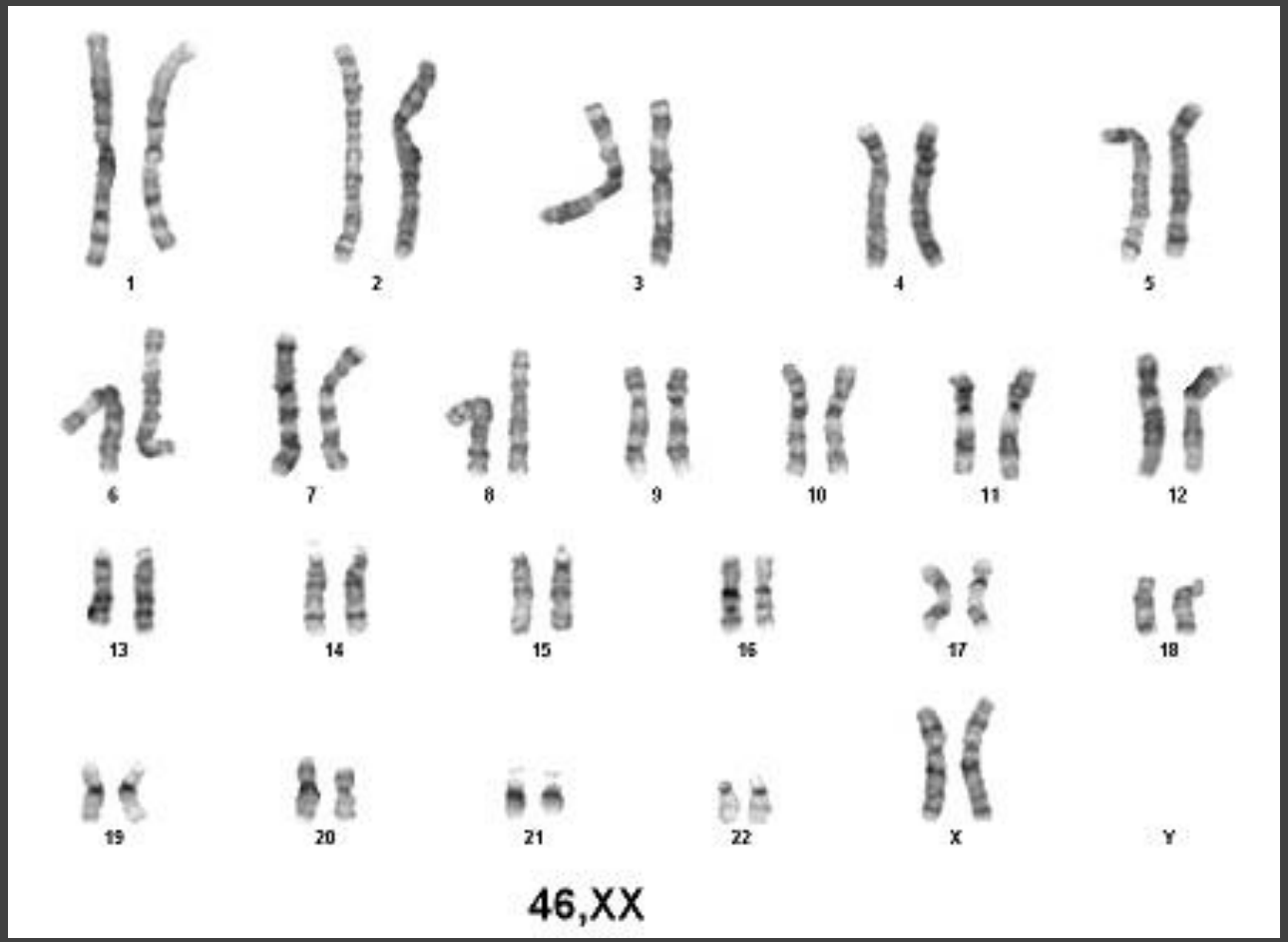
Cytogenetic:

Cytogenetic determines the chromosomal make up of an individual by using Karyotype

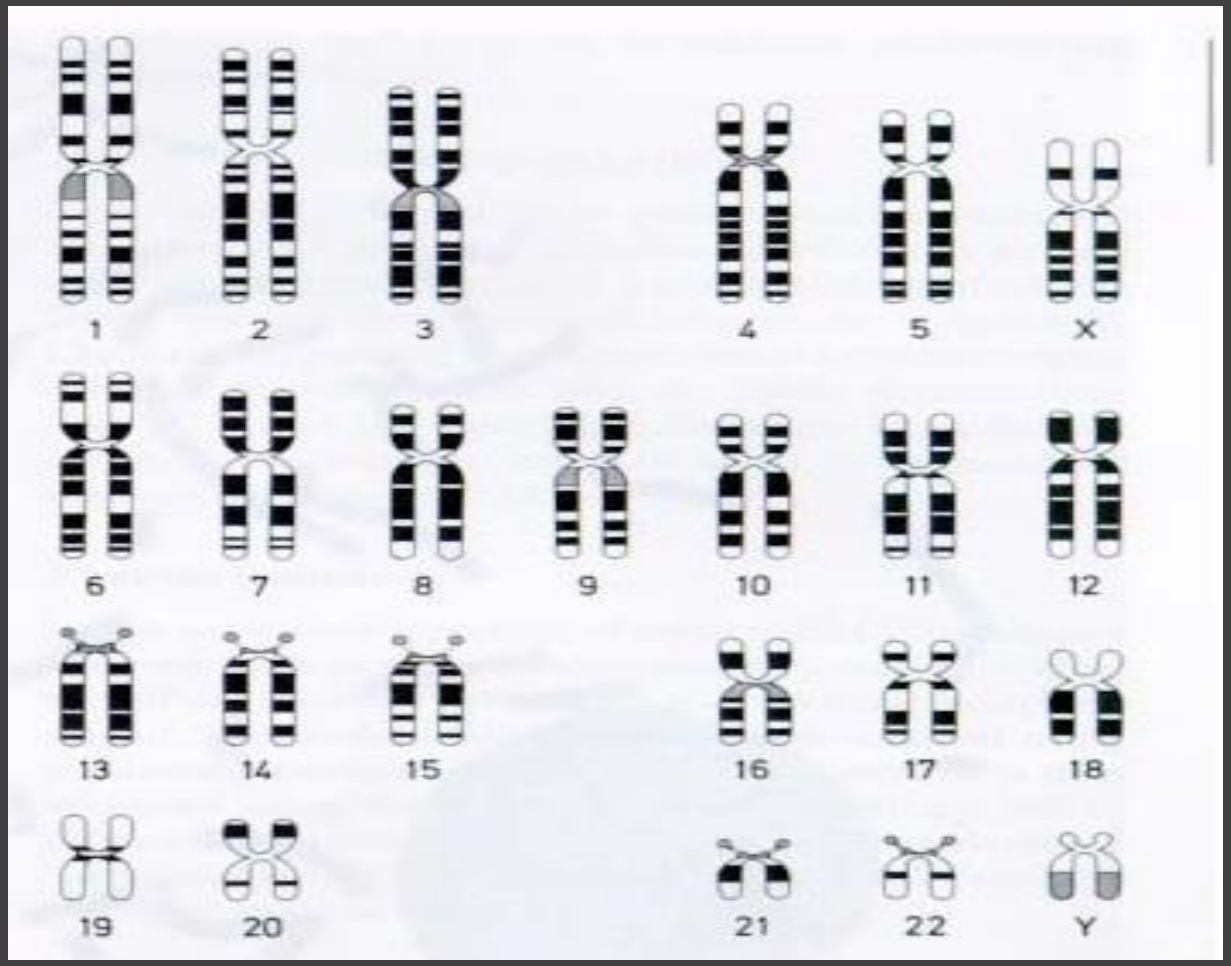
Karyotype:

The chromosomes of a cell, usually displayed as a systematized arrangement of chromosome pairs in descending order of size.

Normal Human Karyotype:



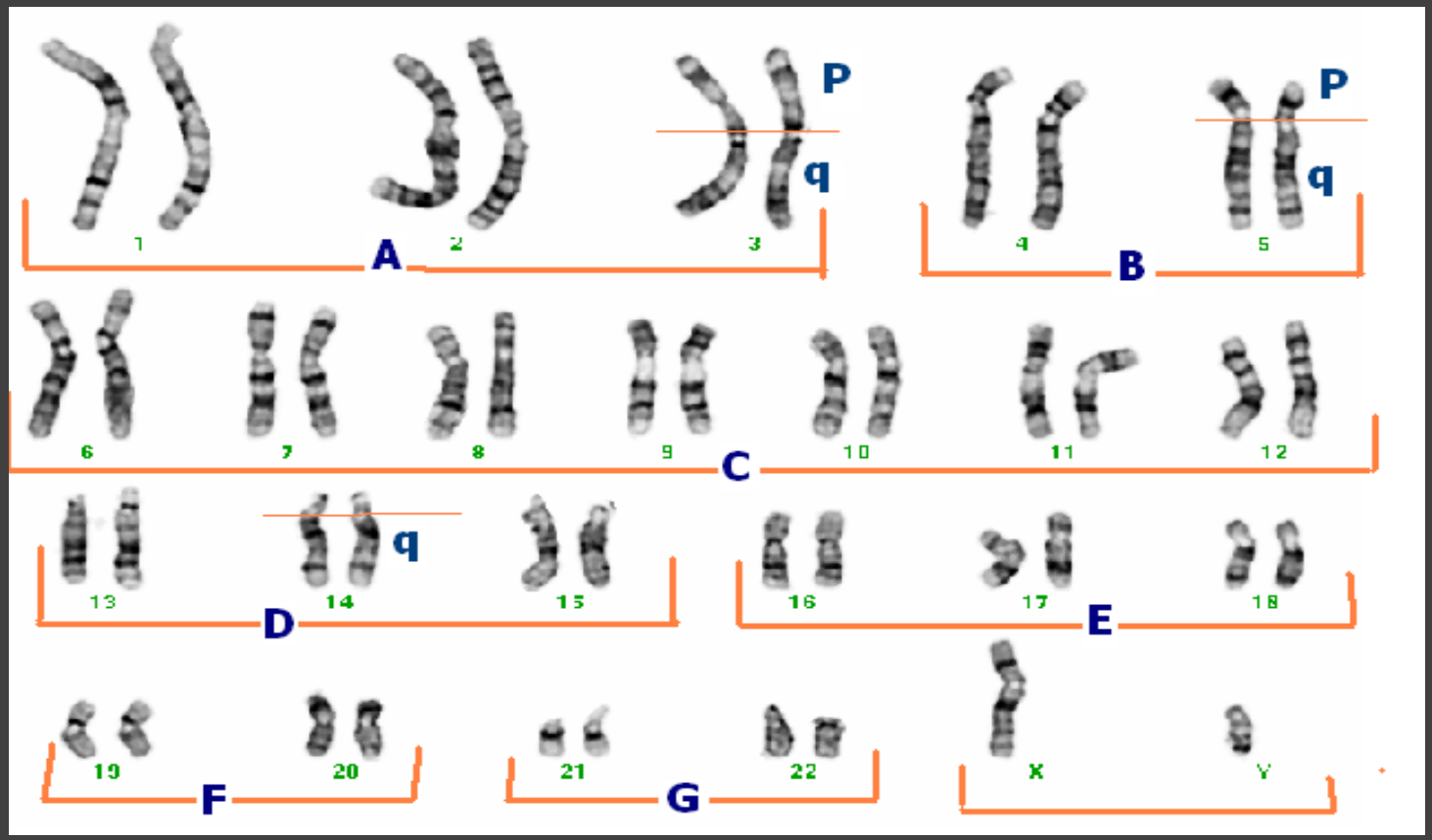
Ideogram :



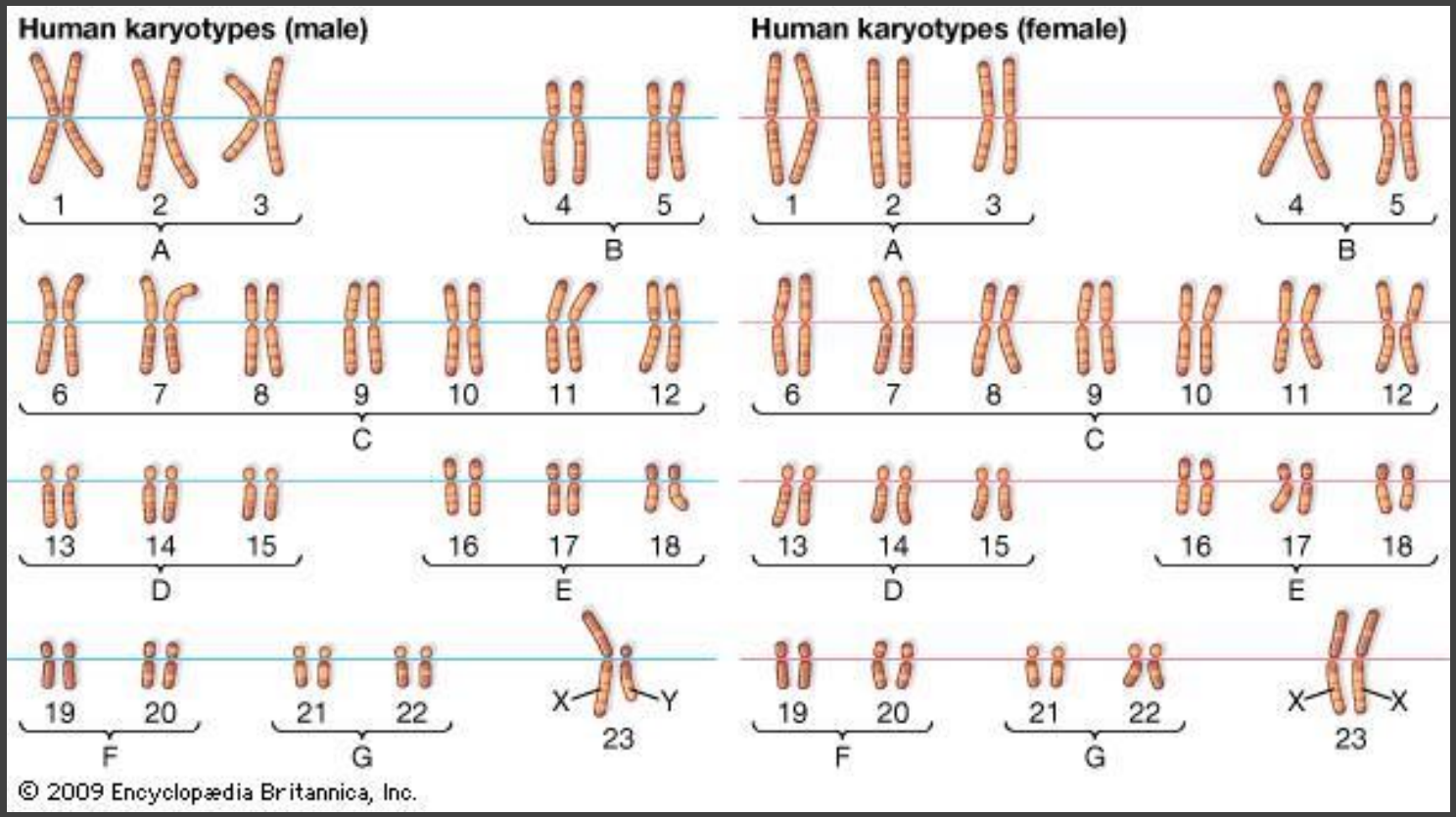
Chromosome groups :

- Chromosomes are categorized into seven groups A to G
- According to the size and centromere position.

Chromosome groups :



Chromosome groups :



Chromosome groups :

Group A (chromosomes 1 to 3)

largest chromosomes with the centromeres in middle

Group B (chromosomes 4 and 5)

large chromosomes with the sub-terminal centromeres.

Group C (chromosomes 6 to 12, and X)

middle-sized chromosomes with middle or sub-terminal centromeres .

Chromosome groups :

Group E (chromosomes 16 to 18)

somewhat smaller than group-D chromosomes with middle or distal somewhat but not terminal.

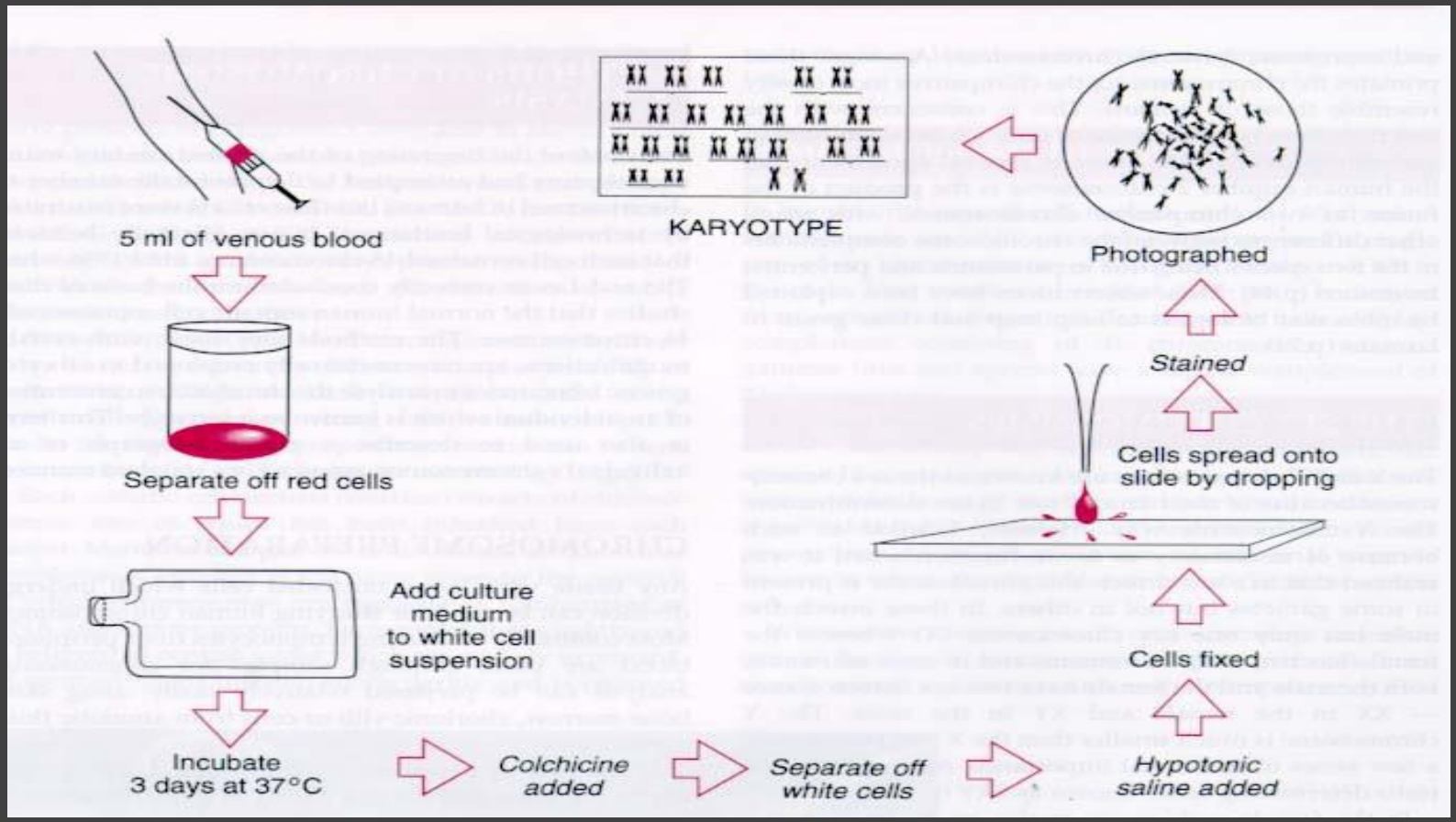
Group F (chromosomes 19 to 20)

short chromosomes with the centromeres in the middle.

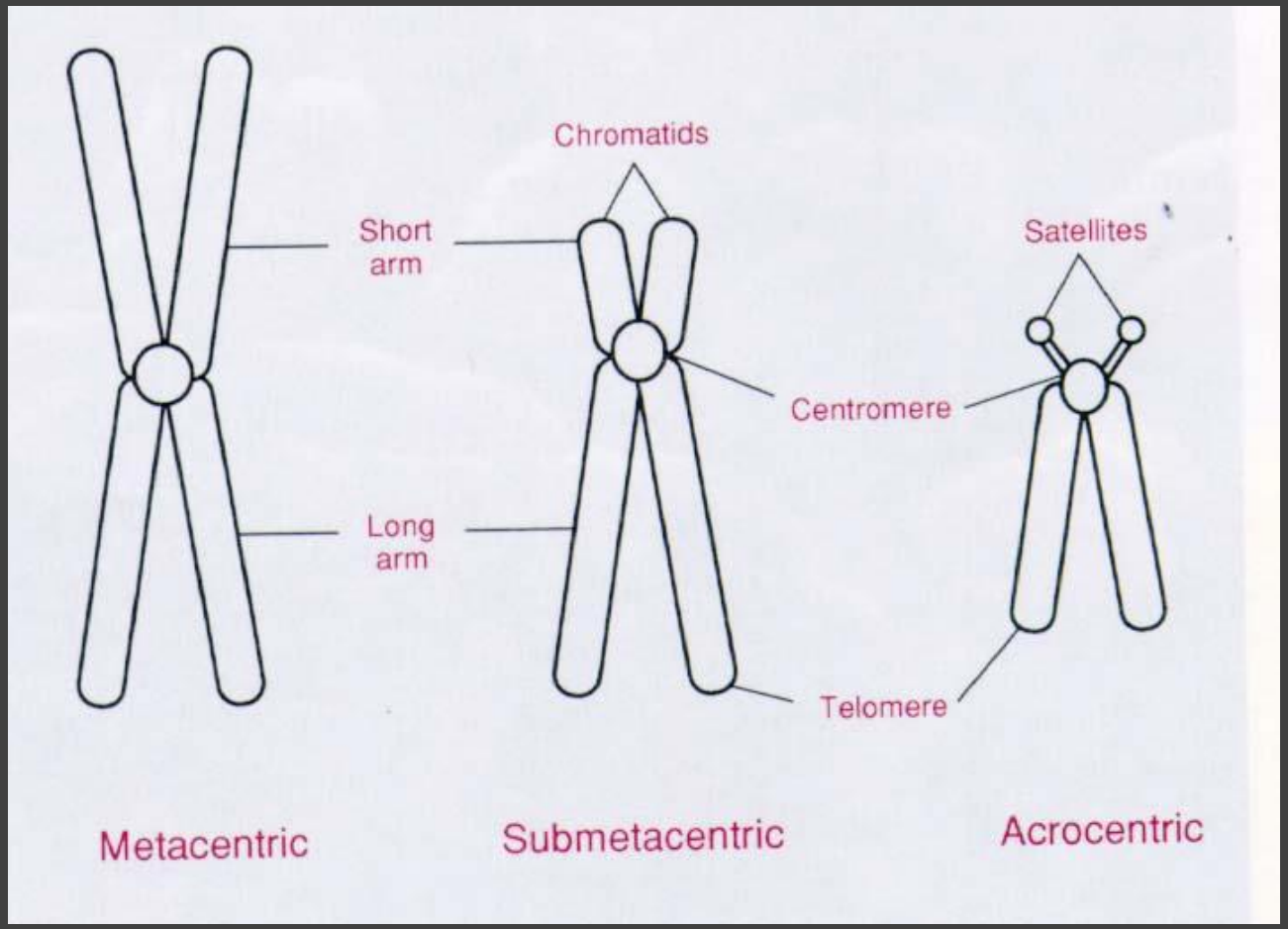
Group G (chromosomes 21, 22, and Y)

the smallest two pairs of autosomes with satellites

Karyotyping:



Chromosomes types



Samples:

- Postnatal (blood samples)
- Prenatal (amniotic fluid, Chorionic Villus Tissues (CVS) and bone marrow biopsy)
- Cancer (solid tumors)

Referral Reasons:

- Dysmorphic features.
- Developmental delay.
- Short stature.
- Failure to develop secondary sex characteristics

Referral Reasons:

- Infertility.
- Recurrent miscarriages.
- Family history of Down syndrome.

Referral Reasons:

- Indeterminate gender at birth.
- New born babies with suspected chromosome abnormality.
- Parents of abnormality found in PND(prenatal diagnosis) .

Cell culture Techniques:

Cell culture Techniques:

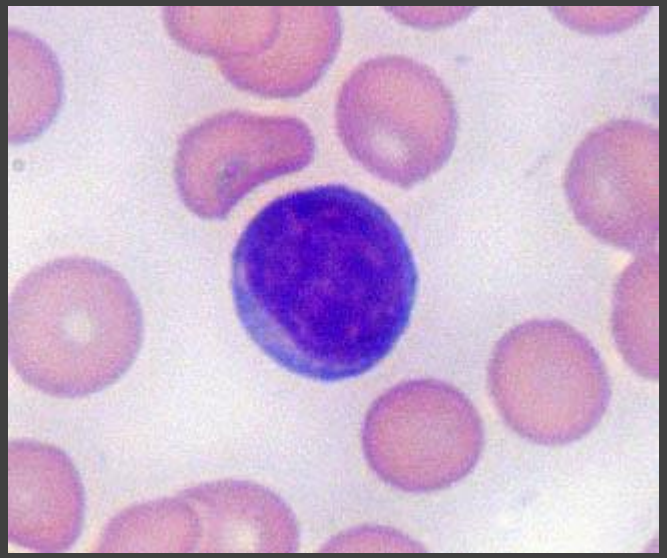
- growth of certain strains.
- experiments in controlling diseases.
- study of the reaction to certain drugs.

Criteria to choose the cells:

- Sample tissue should be representative to all cell lines (especially in case of mosaicism).
- Easy to obtain (noninvasive manner).
- Inexpensive to culture.
- Require short culture duration.
- Yield high-quality metaphase chromosomes in abundance

Cell of choice:

Lymphocytes.



why to choose Lymphocytes?

Lymphocytes are chosen because:

- They accommodate the five criteria .
- They are nucleated (unlike the RBC)

Lymphocytes are chosen because:

- In vitro mitogens mimic the effect of foreign Antigen that gives mitotically active cells.
- They can be obtained from peripheral blood or cord blood .

Lymphocytes are chosen because:

- Phytohemagglutinin (PHA) is principally a T lymphocyte mitogen.

PHA IS A lectin extracted from red beans and stimulates T lymphocyte division.

N.B. T cells making up about 70% of all lymphocytes in healthy persons

Cell Culture Conditions:

- Lymphocytes remain suspended in the culture media, so they don't need culture flasks
- they are cultured in plastic screw capped centrifugation tubes

Cell Culture Conditions:

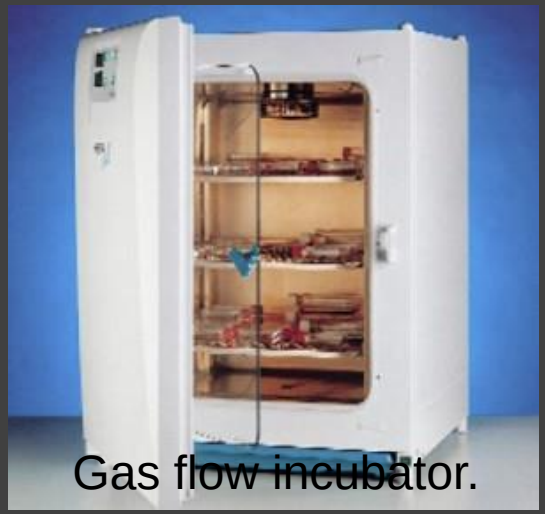
- Temperature always between 37-37.5 C,
- PH of 7.2-7.4 ; controlled via:

  Bicarbonate buffer in the media.

  Providing the incubator with a constant, controlled flow, 5% CO_2.

Cell Culture Conditions:

Humidified, gas flow incubator is recommended.



Gas flow incubator.

Cell Culture Conditions:

strictly aseptic sterile conditions under vertical laminar flow hood

Vertical laminar flow hood.

Sample collection :

Collect samples aseptically in green-top lithium or sodium tubes.

The sample must be accompanied with a proper request form indicating :

- The name of the patient
- The status of the patient.
- Medical history.
- The date.
- The signature of the referring doctor.

Sample volume:

- The inoculum of blood to be added to a culture is varying depending on age and patient status.
- 0.8 ml of PB in case of normal adult
- 0.5ml PB in case of newborns

Sample storage :

- The specimen must never be frozen, because live cells required for culture.
- Peak of mitotic activity at approximately 72 hrs.

Major Media component:

- 15% serum (bovin, human, horse).
- amino acids (L-glutamine), Vitamins.
- Salts.
- Glucose.
- growth factors.
- antibiotics (penicillin, streptomycin).
- Buffers.
- PHA.

N.B.media can be in several forms, powdered or liquid.

Procedure:

- Mix the sample well and transfer 1 ml to 10 ml screw-capped plastic centrifugation tube containing 5ml of PB-Max culture media
- Add 70 µl of PHA .
- Close the tube not tightly ,mix and invert it gently and incubated at 37C for 72 hours in slant position.

Problems in cell culture:

- The cells are growing poorly or not at all.
- The cells have an abnormal morphology.

Sources of the problems in cell culture:

- Materials: They were poor quality, inappropriate, or contaminated.
- Equipment: It may need to be calibrated.
- Environment: The cultures were exposed to the wrong type of environment.
- Cells: They were exposed to toxicity, contamination, or nutritional deficiency.
- Technique: The procedures or protocols were not correct for the cell type.

References:

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- Some landmarks in the development of tissue and cell culture. Retrieved 2006-04-19.
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- Dunham, J.H. and Guthmiller, P. (2008) Doing good science: Authenticating cell line identity. Cell Notes 22, 15–17.