Cytogenetic

Cytogenetic: **-** is a branch of genetics that is concerned with the study of the structure and function of the cell, especially the chromosomes. In the routine laboratory environment human cytogenetic is always concerned with light microscope studies of chromosomes.

Almost all human cytogenetic studies involve the examination of dividing cell population by blocking cell division at metaphase with an inhibitor of spindle formation. The nuclear membrane breaks down and chromosome condensation takes place as usual, but the chromosomes fail to organize themselves into a metaphase plate.

❖ **Cytogenetic Sample Collection:-**

1. Peripheral Blood: Collect 10ml for adults and 1-3ml for infants of whole blood in preservative-free sodium heparin (green top vacutainer) OR in a sterile heparinized syringe. Keep blood samples *at room temperature***.**

Figure (1). Green top vacutainer contain Lithium Heparin.

2. Bone marrow Aspirate: Collect up to 5ml in preservative-free sodium heparin (green top vacutainer) OR in a sterile heparinized syringe.
3.Tissue Samples (Skin Biopsy, Solid Tumors, Lymph Nodes): Collect a 2mm punch or about 2-4mm2 tissue section and immediately place in a sterile vial with 3-5ml cytogenetic tissue transport media (or sterile saline if media is not available).
4. Amniotic Fluid: Collect 15-30ml of fluid obtained under sterile conditions into tissue culture tubes or sterile syringes.
5. Chorionic Villi, placental tissue, umbilical tissue, or fetal organ tissue: Collect about 2-4mm2 of villi, placental tissue, umbilical tissue, or fetal organ tissue and place in a sterile vial with 3-5ml cytogenetic tissue transport media (or sterile saline if media is not available).

❖ Blood culture:-

Blood is one of the most accessible human tissues and growth potential after mitogen stimulation is excellent. It is also one of the easiest tissues to study because the cells have a cell cycle which is well characterized, the cell can be synchronized for the preparation of long chromosomes with high resolution banding, and rapid results can be obtained as sufficient mitotic figure are available for analysis after 2-3 days in culture.

➢ Blood cells involved and how can be stimulated?

Blood contains a number of different cell types. In normal blood neither the red cells nor the platelets contain nuclei, so that chromosome and DNA studies are only possible on nucleated WBCs.
Spontaneous divisions among unstimulated white cells either in vivo or in vitro are rare unless the individual from whom they derived had blast cells in their peripheral blood. The challenge which arises in samples for cytogenetic analysis is to induce some of the white cells presents in blood samples to divide, as well known the types of WBCs present in peripheral blood are: Granulocytes , Monocytes. Lymphocytes.

Lymphocytes (T- lymphocytes) are concerned with cell mediated immunity are of three types; T-helper, T-suppressor, and T-cytotoxic cells with different surface markers. In normal adult, about 70% of the 1.5-4.5 X 109 lymphocytes per liter of blood are T-lymphocytes; the remainder is B-lymphocytes, So T- lymphocyte becomes the cell of choice. Short term blood culture depends on the ability to find cellular growth factors (mitogens) which will induce resting T- lymphocytes to divide by activating complex pathways which involve:

1. T-cell antigen receptor complex.
2. Tyrosine kinesis.
3. Lymphokines. There are several lymphokines but the most important is the PHA (Phytohemagglutinin), Interlukin-2.

Mitogens: - They are number of agents which will cause blood cell to become mitotically active in cell culture, by activating complex pathways which involve a number of specific intermediates. They include substances such as PHA (Phytohemagglutinin), concanavalin-A and pokeweed mitogen (PWM).

Mutagens: - They are natural or human made agents (physical or chemicals) which can alter the structure or sequence of DNA and cause higher rate of mutation.

Mutation: - is a sudden and permanent, transmissible change of DNA backbone of the gene, when the chemical structure of the gene undergoes random modification. The altered gene may continue to replicate in their changed form during cell division.