MEDICAL BIOLOGY

CYTOGENETIC METHODS

GROUP- 1

- Members:

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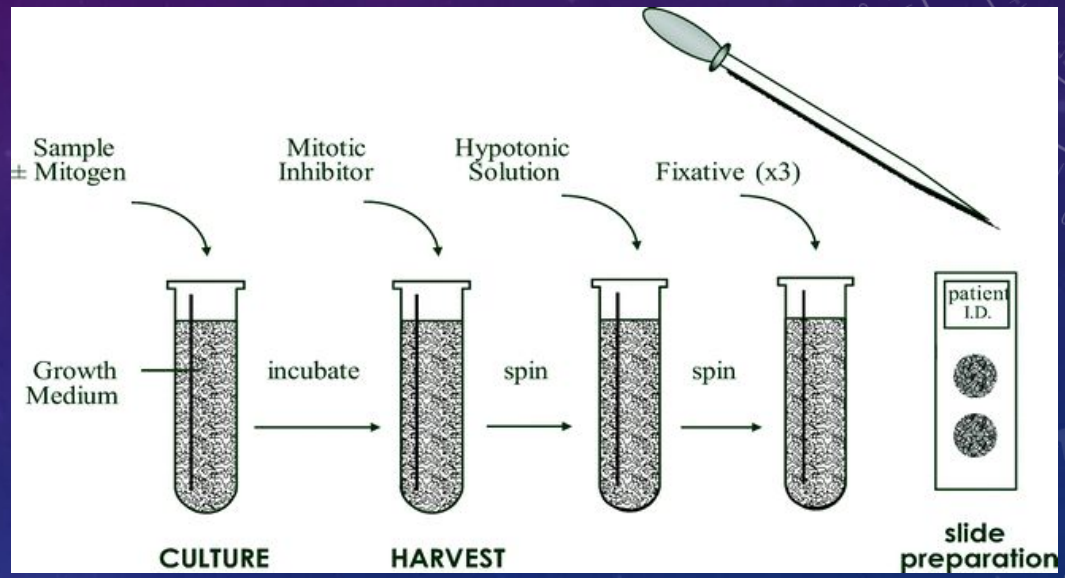
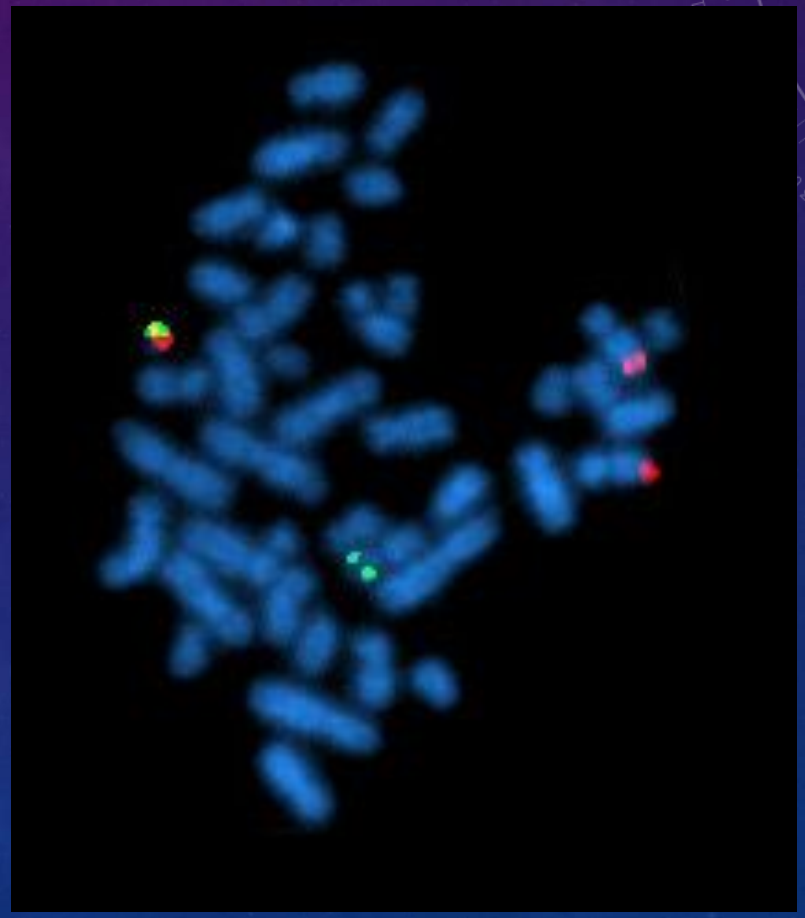
- **Slide preparation :**
- *This section refers to preparation of standard cytogenetic preparations*
- The slide is aged using a salt solution usually consisting of 2X SSC (salt, sodium citrate). The slides are then dehydrated in ethanol, and the probe mixture is added. The sample DNA and the probe DNA are then co-denatured using a heated plate and allowed to re-anneal for at least 4 hours. The slides are then washed to remove excess unbound probe, and counterstained with 4',6-Diamidino-2-phenylindole (DAPI) or propidium iodide.



- **Analysis:**
- Analysis of FISH specimens is done by fluorescence microscopy by a clinical laboratory specialist in cytogenetics. For oncology generally a large number of interphase cells are scored in order to rule out low-level residual disease, generally between 200 and 1,000 cells are counted and scored. For congenital problems usually 20 metaphase cells are scored.

CYTOGENETIC METHODS

- Cytogenetics is the branch of genetics that studies the structure of DNA within the cell nucleus. This DNA is condensed during cell division and form chromosomes. ... Using chromosome banding techniques (classical cytogenetics) or hybridization fluorescently labeled probes (molecular cytogenetics).
- Cytogenetics plays a key role in the detection of chromosomal abnormalities associated with malignancies, as well as the characterization of new alterations that allow more research and increase knowledge about the genetic aspects of these diseases.
- Walther FlemmingWalther Flemming, (born April 21, 1843, Sachsenberg, Mecklenburg [now in Germany]—died Aug. 4, 1905, Kiel, Ger.), German anatomist, a founder of the science of cytogenetics (the study of the cell's hereditary material, the chromosomes

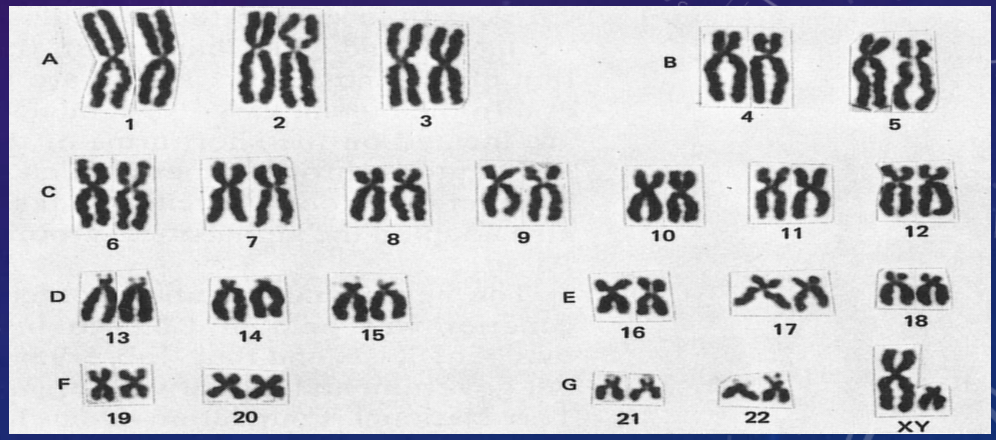


HISTORY OF CYTOGENETIC

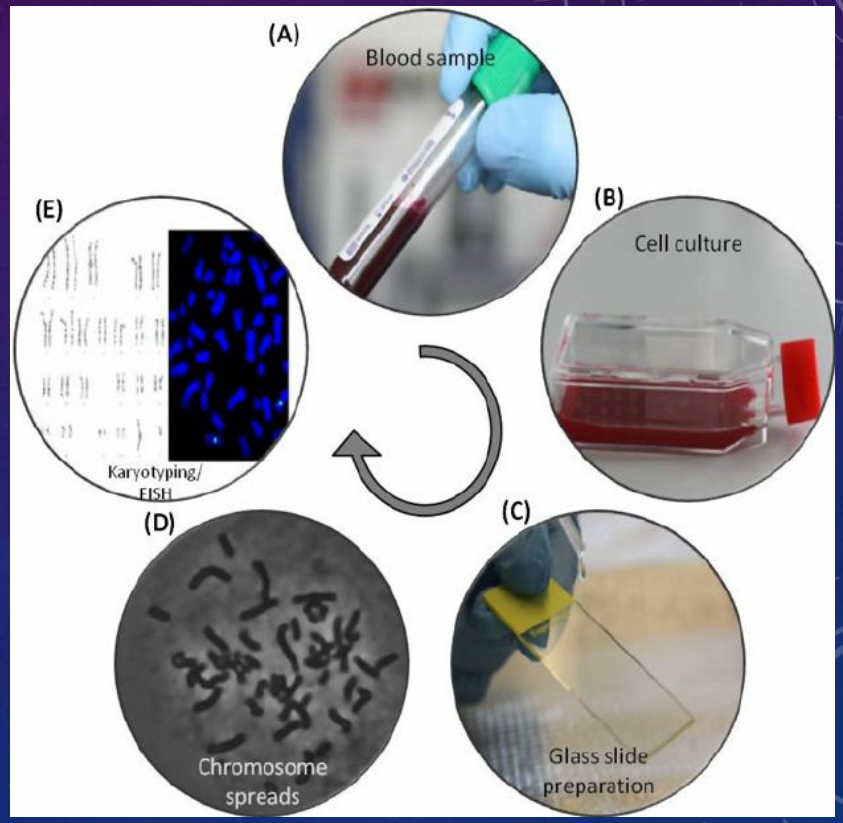
- **Walther Flemming**, (born April 21, 1843, Sachsenberg, Mecklenburg [now in Germany]—died Aug. 4, 1905, Kiel, Ger.), German anatomist, a founder of the science of cytogenetics (the study of the cell's hereditary material, the chromosomes).
- The next stage took place after the development of genetics in the early 20th century, when it was appreciated that the set of chromosomes (the karyotype) was the carrier of the genes.
- Levitsky seems to have been the first to define the karyotype as the phenotypic appearance of the Somatic Chromosomes , in contrast to their genetic contents.
- Investigation into the human karyotype took many years to settle the most basic question: how many chromosomes does a normal diploid human cell contain? In 1912, Hans von Winiwater reported 47 chromosomes in Spermatogonia and 48 in Oogonia, concluding an XX/XO Sex determination mechanism.
- In 1922 was not certain whether the diploid number of humans was 46 or 48, at first favoring 46.

METHODS OF CYTOGENETIC

- **Karyotyping**
- The routine chromosome analysis (Karyotyping) refers to analysis of metaphasechromosomes which have been banded using trypsin followed by Giemsa, Leishmanns, or a mixture of the two.
- This creates unique banding patterns on the chromosomes.
- The molecular mechanism and reason for these patterns is unknown, although it likely related to replication timing and chromatin packing.
- used in cytogenetics laboratories. Quinacrinebanding (Q-banding) was the first staining method used to produce specific banding patterns.
- This method requires a fluorescence microscope and is no longer as widely used as Giemsa banding (G-banding).
- Reverse banding, or R-banding, requires heat treatment and reverses the usual black-and-white pattern that is seen in G-bands and Q-bands. This method is particularly helpful for staining the distal ends of chromosomes.



- **Slide preparation**
- Cells from bone marrow, blood, amniotic fluid, cord blood, tumor, and tissues (including skin, umbilical cord, chorionic villi, liver, and many other organs) can be cultured using standard cell culture techniques in order to increase their number.
- A mitotic inhibitor (colchicine, colcemid) is then added to the culture. This stops cell division at mitosis which allows an increased yield of mitotic cells for analysis.
- The cells are then centrifuged and media and mitotic inhibitor are removed, and replaced with a hypotonic solution. This causes the white blood cells or fibroblasts to swell so that the chromosomes will spread when added to a slide as well as lyses the red blood cells.



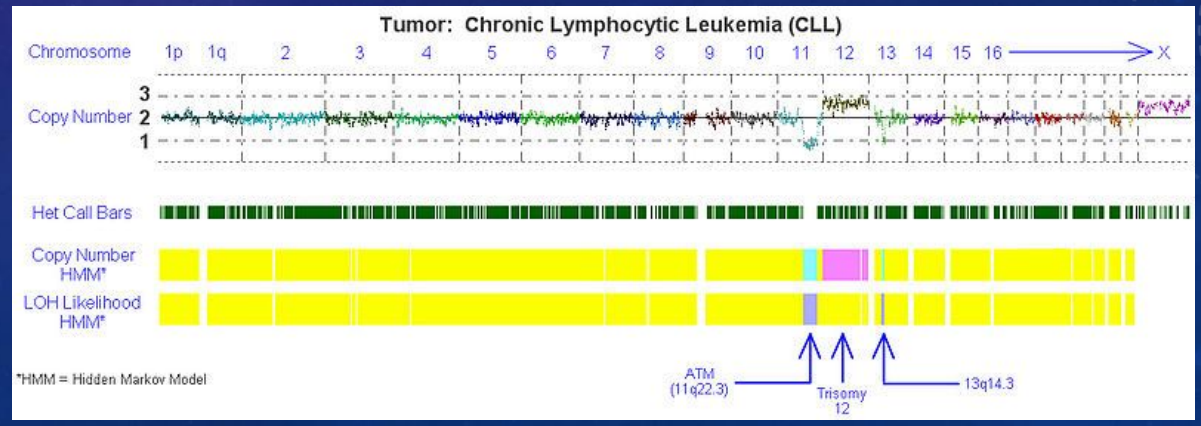
- **Analysis**
- Analysis of banded chromosomes is done at a microscope by a clinical laboratory specialist in cytogenetics (CLSp(CG)).
- Generally 20 cells are analyzed which is enough to rule out mosaicism to an acceptable level.
- The results are summarized and given to a board-certified cytogeneticist for review, and to write an interpretation taking into account the patient's previous history and other clinical findings. The results are then given out reported in an *International System for Human Cytogenetic Nomenclature 2009* (ISCN2009).

- **Fluorescent in situ hybridization**

- Fluorescent in situ hybridization (FISH) refers to using fluorescently labeled probe to hybridize to cytogenetic cell preparations.
- In addition to standard preparations FISH can also be performed on:
- bone marrow smears.
- blood smears.
- paraffin embedded tissue preparations.
- enzymatically dissociated tissue samples.
- uncultured bone marrow.
- uncultured amniocytes.
- Cytospin preparations.

FUTURE OF CYTOGENETICS

- Advances now focus on molecular cytogenetics including automated systems for counting the results of standard FISH preparations and techniques for virtual karyotyping, such as comparative genomic hybridization arrays, CGH and Single nucleotide polymorphism arrays.



QUESTIONS FOR OTHER MEMBERS:

- Cytogenetic method ?
- 2.Genealogical diagnostic methods ?
- 3. Biochemical method. PCR and DNA diagnostics ?
- 4.Population-statistical method ?
- 5. Twin diagnostic methods ?
- 6. Prenatal diagnosis.Medical genetic counseling ?
- 7. Dermatoglyphic method of medical genetics ?

THANK YOU ON BEHALF OF OUT TEAM MEMBERS

- Members (group-1)

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