SAWA UNIVERSITY

# اسم المادة و الكورس

## LAB 5 – SAMPLING, CULTURE, AND HARVESTING


جامعـــــــة ســـــــــاوة الاهلية — Sawa University

كليـــــة التقنيـــات الصحيـة والطبيـة — College of health and medical techniques

قسم تقنيات المختبرات الطبية — Department of Medical Laboratories

المرحلـــــــــــــة الثالثة — Third Stage



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محاضرة رقم 4 — الجانب العملي:الوراثة الطبية

Lecture No. 4 — Medical genetics-Practical

## Establish/maintain culture and mitotic arrest


Flask


Petri dish

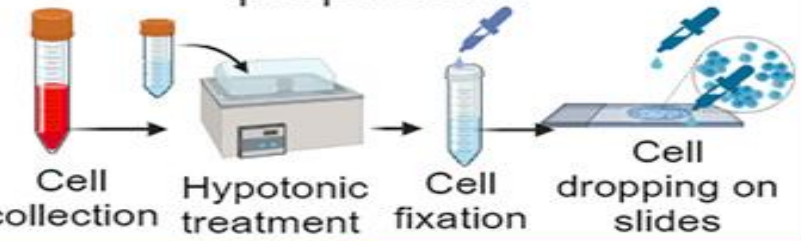

Tube

**Timing: 1 h to 5 days**

**Step 1**

**Step 2**

## Chromosome harvest and slide preparation

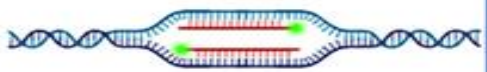



**Timing: 3 h to 8 h**

**Step 3**

## Chromosome staining/ hybridization)


Giemsa staining for conventional cytogenetics

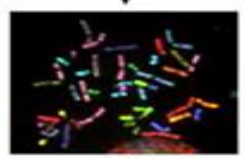

DNA hybridization for molecular cytogenetics

**Timing: 10 min to 3 days**

**Step 4**

## Microscopic visualization


G-band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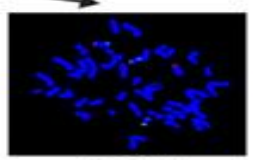
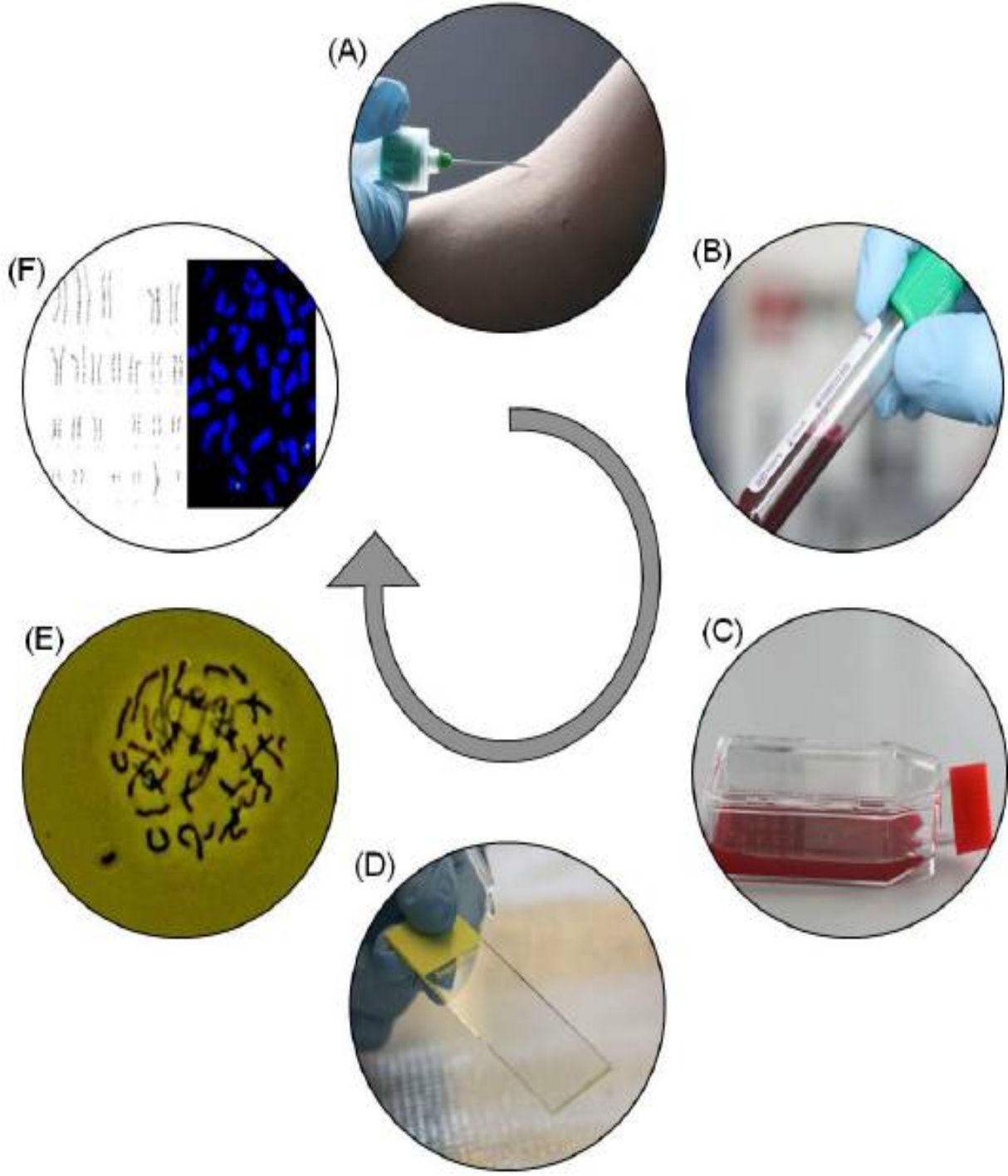

FISH

**Timing: 2 h to 5 h**

# Learning Objectives

**By the end of this laboratory session, students will be able to:**

1. **Define the basic principles of cytogenetic techniques used for chromosomal analysis.**
2. **Describe the correct procedures for sample collection, handling, and preservation in cytogenetic studies.**
3. **Explain the steps of cell culture, including medium composition, incubation conditions, and mitotic stimulation.**
4. **Demonstrate understanding of the harvesting process, including mitotic arrest, hypotonic treatment, and fixation.**



# Introduction

- **Cytogenetic analysis is a specialized laboratory method used to study human chromosomes, aiming to detect numerical and structural abnormalities that cause genetic diseases, developmental defects, or malignancies.**

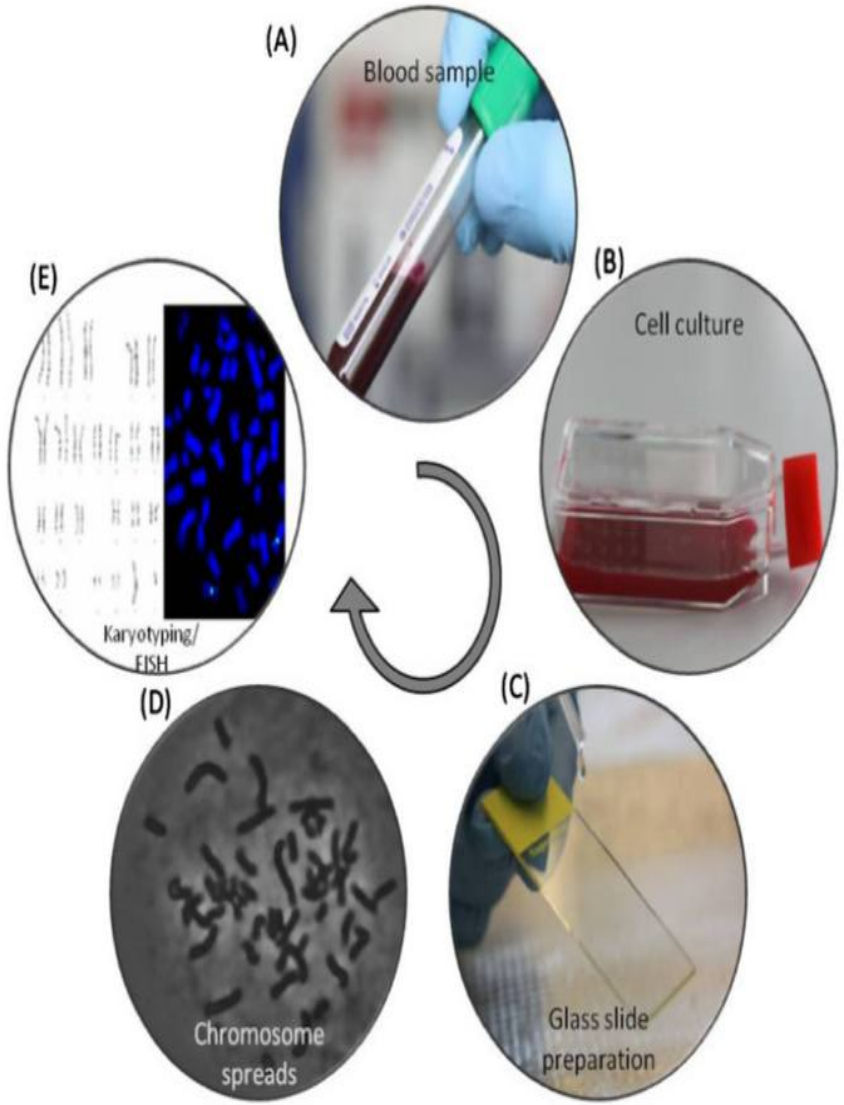
**This process involves three fundamental stages:**

1. **Sampling – obtaining viable cells from the patient.**
2. **Culture – stimulating and maintaining cell growth under controlled conditions.**
3. **Harvesting – arresting, dividing cells at metaphase to visualize chromosomes.**

**Accuracy in these steps determines the success of the karyotype and the quality of chromosomal banding.**



**Fig. 1** The cytogenetics analysis protocol. Blood sample is collected from the patient (**A**) and cultured with growth stimulation for 3 days at 37° C (**B**). Colchicine is added at the end of the culture period to arrest the cells in metaphase. Followed by hypotonic treatment, the cells are dropped on a glass slide (**C**) to obtain metaphase chromosome spreads (**D**). At this point the chromosome spreads can either be analyzed by karyotyping or FISH (**E**)

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# Sampling Techniques



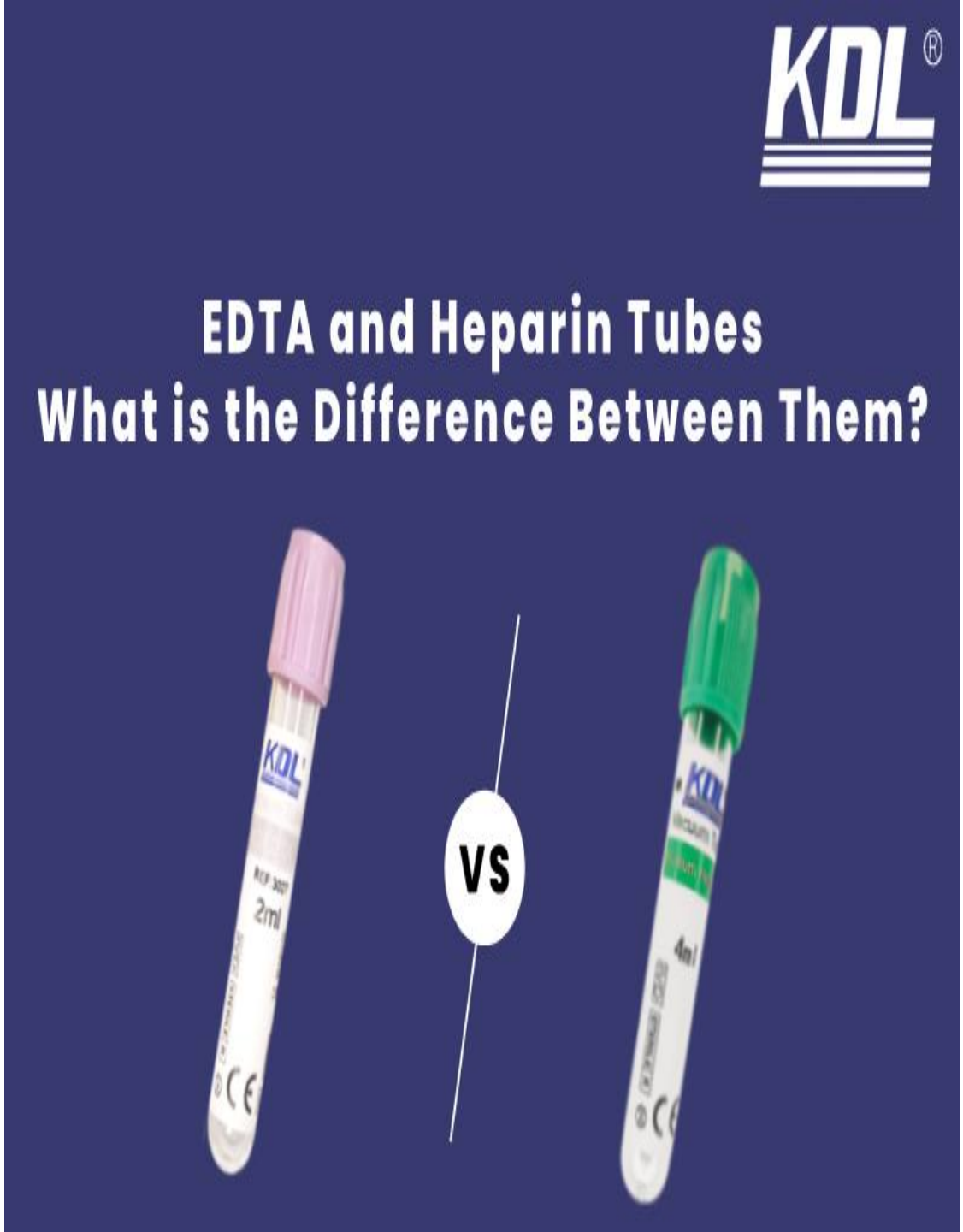
## Samples for cytogenetic testing must contain viable and dividing cells. The type of sample depends on the clinical purpose:

| Sample Type | Source | Clinical Application |
|---|---|---|
| Peripheral Blood | Lymphocytes | Postnatal diagnosis, congenital anomalies |
| Bone Marrow | Hematopoietic cells | Leukemia and lymphoma |
| Amniotic Fluid | Fetal epithelial cells | Prenatal diagnosis |
| Chorionic Villi | Placental tissue | Early fetal testing |
| Skin Biopsy | Fibroblasts | Mosaicism or metabolic disorders |
| Tumor Tissue | Malignant cells | Oncology cytogenetics |

Proper handling ensures the cells remain viable for culture and metaphase preparation.

# Sampling Guidelines

- Collect all samples aseptically to prevent contamination.
- Use sodium heparin tubes as anticoagulant (avoid EDTA).
- Maintain temperature at 18–25°C during transport. Avoid refrigeration, as cold temperatures may reduce mitotic index.
- Deliver to the lab within 24–48 hours.
- Label clearly with patient data and date of collection.
- Use sodium heparin, not EDTA, as an anticoagulant. Heparin preserves cell integrity and viability, making it ideal for cytogenetic cultures. It inhibits clotting by activating antithrombin, with minimal effect on cell function. In contrast, EDTA chelates calcium, disrupting enzyme activity, gene expression, and PCR reactions—thus unsuitable for chromosome studies.



# Cell Culture Techniques

- The goal of cell culture is to induce mitosis and produce actively dividing cells suitable for chromosomal analysis.
- For blood samples, T-lymphocytes are stimulated by phytohemagglutinin (PHA) in a nutrient-rich medium (RPMI 1640 + Fetal Bovine Serum + antibiotics).
- Cultures are incubated at 37°C for 72 hours in a $CO_2$ incubator (5% $CO_2$, 95% humidity).


# Culture Media and Conditions

- **Basal Medium:** RPMI-1640 (blood), MEM (fibroblast).
- **Serum:** Fetal Bovine Serum (10–20%).
- **Mitogen:** PHA for blood lymphocytes.
- **Antibiotics:** Penicillin/Streptomycin.
- **pH indicator: Phenol red (maintained near 7.2–7.4)**
  The pH, sterility, and incubation time are critical for obtaining high mitotic index cultures.
- "**Typical incubation**: 72 h for blood / 24–48 h for bone marrow / 7–10 days for fibroblast cultures."



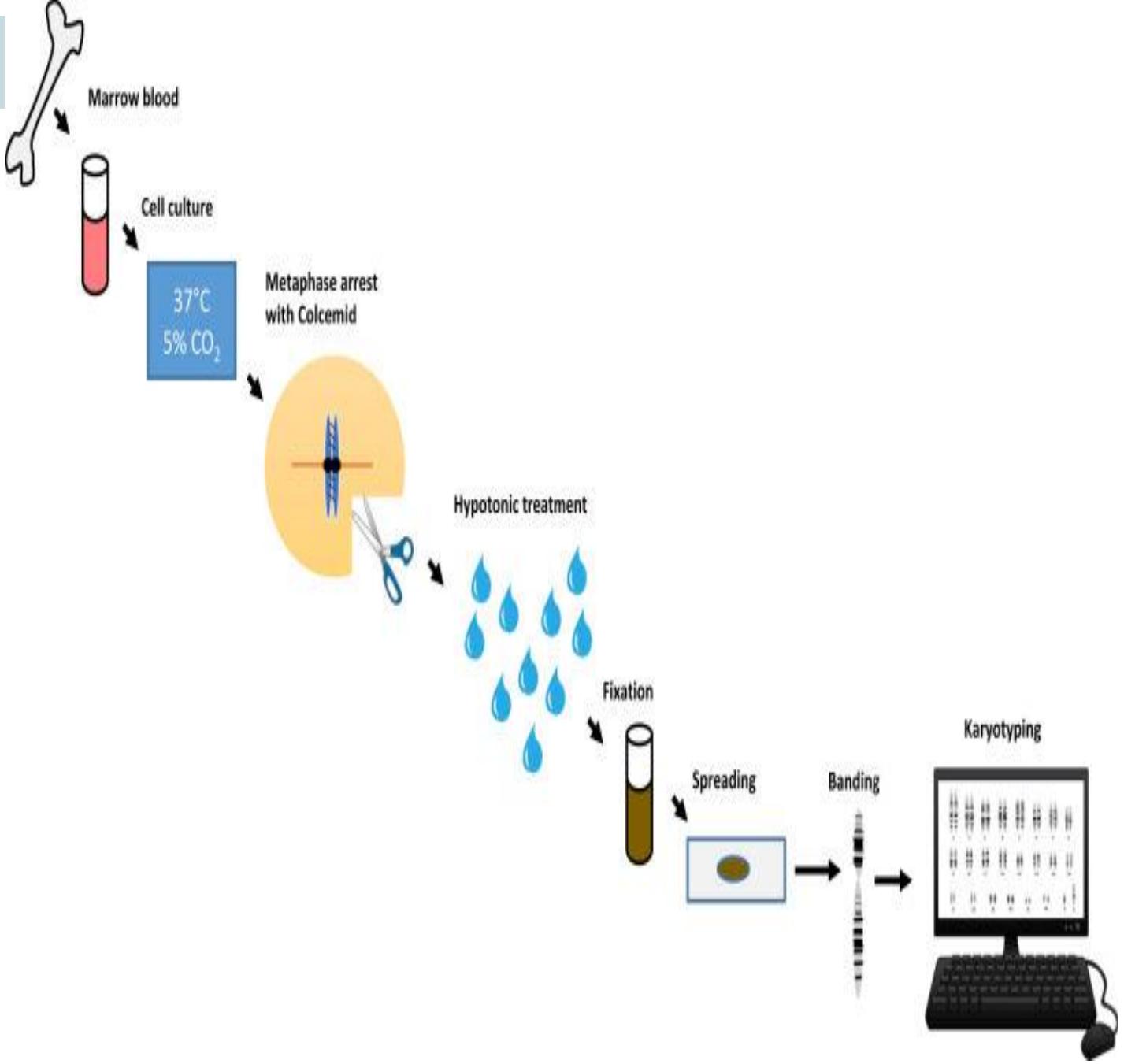
# Harvesting Process



The purpose of harvesting is to obtain cells arrested in **metaphase**, where chromosomes are condensed and visible.

**Steps:**

1. **Mitotic Arrest:** Add Colchicine (0.1 µg/mL) one hour before harvesting.
2. **Hypotonic Treatment:** Add 0.075 M KCl at 37°C for 15–20 minutes to swell the cells.
3. **Fixation:** Use cold Methanol: Acetic Acid (3:1), repeat 2–3 times.
4. **Slide Preparation:** Drop fixed suspension onto cold slides from ~30 cm height, air-dry gently.
   - **Well-spread metaphases depend on proper humidity (45–55 %) and clean slides.**



# Quality Control and Troubleshooting



| Problem | Possible Cause | Solution |
| --- | --- | --- |
| Poor metaphase spread | Incomplete hypotonic treatment | Increase KCl exposure time |
| Chromosome clumping | Humidity or improper drying | Control temperature & humidity |
| Low mitotic index | Old sample or poor PHA activity | Use fresh blood and reagents |
| Contamination | Non-sterile technique | Work aseptically, add antibiotics |

**“Broken chromosomes – may occur due to over-fixation or overheating during drying.”**

# Safety Considerations



- Handle all human materials as potentially infectious (BSL-2).
- Use a biosafety cabinet, Class II, for all sample manipulations.
- Wear a lab coat, gloves, and protective eyewear.
- Dispose of waste following biohazard protocols (autoclave or incineration).

# Summary



**Cytogenetic techniques rely on three essential steps:**

1. **Sampling – correct collection and preservation of viable cells.**
2. **Culture – induction of mitosis under controlled laboratory conditions.**
3. **Harvesting – precise metaphase preparation for chromosomal analysis.**

**Mastering these steps ensures the production of high-quality metaphase spreads, which are essential for karyotyping and chromosomal banding in clinical diagnostics.**

# QUIZ

**Q1. Which anticoagulant is preferred for cytogenetic blood culture?**

**A. EDTA**

**B. Sodium citrate**

**C. Sodium heparin**

**D. Potassium oxalate**

**Q2. Why is EDTA not recommended for cytogenetic cultures?**

# Any questions?