R. J. COLLEGE of Arts, Science & Commerce (AUTONOMOUS)

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Affiliated to UNIVERSITY OF MUMBAI || NAAC Re-Accredited 'A' Grade (CGPA: 3.50)

Department of Biotechnology

On Job Training Completion Report

This is to certify that Aadarsh P. Gupta has completed On Job Training at

Ostic Pharma Pvt Ltd, SINE, IIT-Bombay, Powai, Mumbai-400

Date of Commencement	Date of Completion	Total Number of Days	Total Number of Hours completed in OJT
27/12/23	6/2/24	32 days	64 hrs

Name of the Guide/ PI/ Incharge : Dr. Vinay Saini

Phone Number of Guide/ PI/ Incharge : 8657104859

Email Address of the Guide/ PI/ Incharge : info.ostic@gmail.com

Vinay Saini

Signature of Guide/ PI/ Incharge

OSTIC PHARMA PRIVATE LIMITED SINE, IIT BOMBAY, POWAI, MUMBAI 400076.

Gupta 22-03-24

Stamp

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On Job Training (OJT) at Ostic Pharma Pvt Ltd, Powai-76.

27th December, 2023- 6th February, 2024

The beginning of Sem-Ⅱ of MScBT-1 started with the search for a place to complete the On Job Training (OJT) of 60+ hours in the institution, pharmaceutical company, pathological labs, or any place where work related to life sciences is being done. The search for such a place ended when at Ostic Pharma I got an approval to do the OJT for 1 month.

Ostic Pharma which is a new startup pharmaceutical company in IIT Bombay manufactures a rapid detection kit for various diseases like Coronavirus, Malaria, HIV, Hepatitis, Dengue, etc. On 27 December 2023, I started my OJT here in the Quality Assurance (QA) department. Dr. Vinay Saini the head of the company had observed and guided me in the work that I had done there.

Medical Devices 2017 was the pdf that I had been told to read and understand on the very first day. This pdf consisted of various rules about the manufacturing of medical devices and terms related to medical devices. Since the beginning was good the later days also went amazing where I was told to read and understand the manuals of various rapid detection kits based on the principle of lateral flow immunoassay which they manufacture. I also get a chance to observe and understand how various tools and machines like the U.V spectrophotometer, Cold centrifugal machine, pH meter work. Apart from these machines, there were several other machines which I observed how they had been used for making rapid antigen detection kits for COVID under the brand name **Cov-Ant** kit. I also observed how these kits were tested for their efficacy and specificity at different titter of gold conjugated antibodies (IgM/IgG).

In between the OJT, I also got a chance to visit the IIT Tech Fest event held in January 2024 from the company. I also get a chance to visit and represent Ostic Pharma at CiiA 2024 along with one co-worker. Since the company is involved in the manufacturing of rapid detection kits, they do the business of selling these kits in a B2B way on the Government e-Marketplace (GeM) portal which is a portal where bidding for various kits and various other medical tools is done by various medical institution and hospital. I observed how this portal works, acceptance of the order was done and an invoice for the accepted order was made.

I had also been involved in the packaging of CoV-Ant kits where labelling, keeping the reagents and test kit in boxes, and then packing of boxes were done. I also got an opportunity to formulate a rough SOP for rodent and pest control at storehouse of company.

This was all about the work which I had observed and done some of them practically for little more than a month. The whole period of OJT made me confident about the practical usage of various theoretical knowledge that I had gained about Biotechnology. The lessons and memories got from these experiences are for life time.

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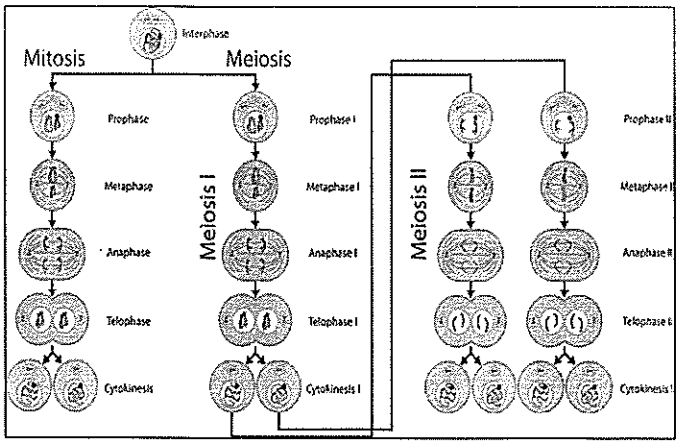
Karyotyping of Human Leukocyte Blood Cells

Karyotyping is the process of pairing and ordering all the chromosomes of organism, thus providing a genome-wide snapshot of an individual's chromosomes.

I. Theoretical Part:

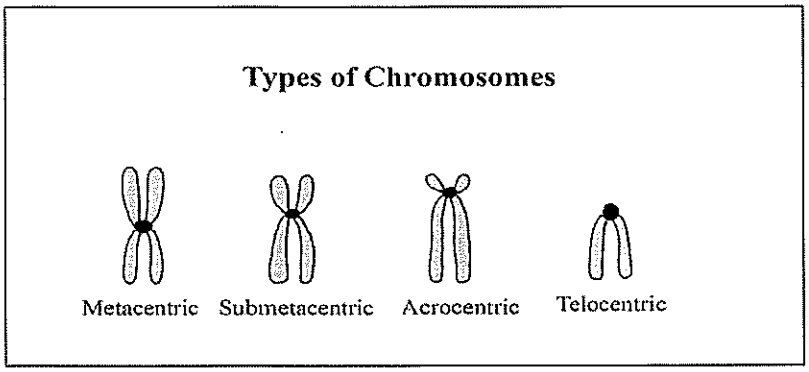
The process of karyotyping of human leukocyte cells had started from the theoretical lecture of human chromosome as genetic material of human which carries all the information requires for the proper growth and function of the body.

- This genetic material is known as Deoxyribonucleic acid (DNA) which gives different traits to different individuals and is passed on from one generation to other during reproduction.
- During non-dividing stages the DNA is uncoiled and not in condense form present as chromatin.
- During mitosis (equational cell division) or meiosis (reductional cell division) phase of cell cycle chromatin condenses and gives chromosomes like structure.
- Both mitotic and meiotic phase consist of various phases which cell follows they are:

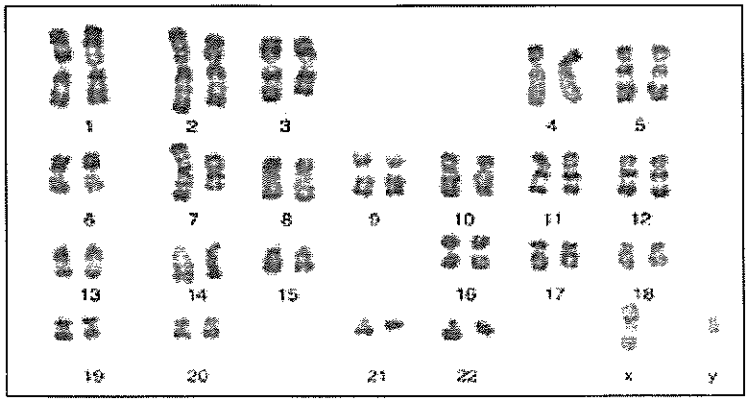


- The human has 23 pairs of chromosomes of which 22 pairs are autosomes and 2 pairs are sex chromosomes.
- The sex chromosomes decides the gender of baby born, X and Y are the two sex chromosomes.

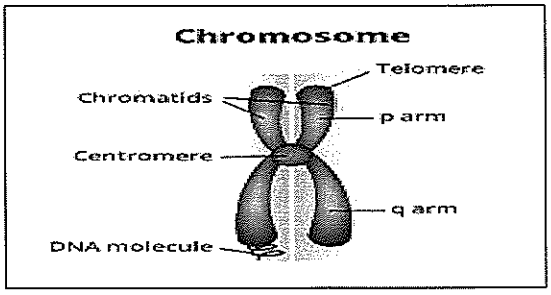
- In pairs if XX is there it means baby born is female, and if XY is there baby born is male.
- Chromosomes are of different types based on the position of their centromere, they are:



- Based on the position of their centromere all 23 pairs are divided into groups they are:



- The centromere divides the chromosome into two part upper part is p- arm (short arm) and below part is q-arm (long arm).



In theoretical lecture abnormalities in chromosome based on their number, structure and associated disorder respectively were also taught, some of them are:

- **Based on Structure:**

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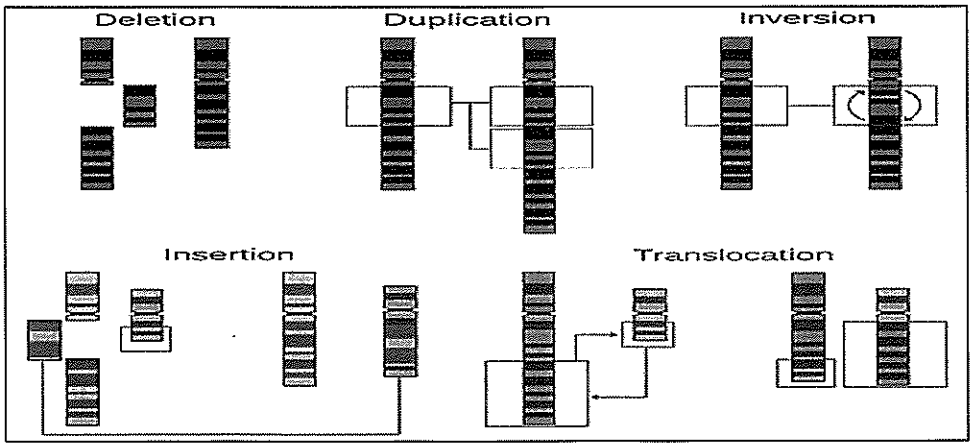

- **Based on Number:**

Disorder	Description
Down's syndrome (Trisomy 21)	Extra chromosome 21
Edward's syndrome (Trisomy 18)	Extra chromosome 18
Patau's syndrome (Trisomy 13)	Extra chromosome 13
Turner's syndrome (Monosomy X)	Single X chromosome in females
Klinefelter's syndrome	Two X chromosomes in males (XXY)
Triple X syndrome (super females)	Three X chromosomes in females
XYY syndrome	Two Y chromosomes in males

II. Practical Part:

In practical part chromosome from human blood leucocyte were arrested and observed at metaphase stage of cell division.

The principle on which the whole practical of karyotyping was based on:

Principle: T lymphocytes from peripheral blood are induced to divide using a plant lectin, phytohemagglutinin. The maximum mitotic index is reached at 72 hours of culture. The culture is treated with colcemid to arrest cells at metaphase. The cells are harvested using the standard hypotonic treatment and fixation. Clinical indications include diagnosis of congenital abnormalities and genetic counseling of parents with congenitally abnormal infants, sex chromosome abnormalities, and habitual abortion studies.

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The first step of karyotyping was the

1. **Planting:** This step was done to induce uncontrollable proliferation of T lymphocytes in number and size.
   Where reagents like RPMI1640 culture media, human serum/FBS(Fetal Bovine Serum) was helping to give nutrition to growing cell, PHA was inducing the uncontrollable proliferation.

- In this step media, serum and phytohemaglutnin (PHA) from freezer were removed.
- Media and serum were kept in the water bath at 37°C .
- PHA was kept at RT, laminar hood was cleaned with methanol swab.
- UV light in the laminar was kept on for 30 mins prior.
- After 30 mins step wise addition of reagents were added in vials:

Material	Syringe of volume	Vial
RPMI1640 culture media	10ml	4ml
Human Serum/FBS	5ml	1ml
PHA(M)	100ul	0.2ml
Blood	2ml	8 drops

- After adding all the reagents the vial was kept in a petridish which was cleaned with methanol and keep in the incubator at 37°C for 72 hours.
- At the 70th hour 0.1 ml of colchicine was added to the vial and was mixed properly and incubated for 1 hour.

After planting, termination step was carried out:

2. **Termination:**

- After 1 hour of incubation vial was removed from incubator it was centrifuge at 1000rpm for 10min.
- After centrifuge the supernatant was removed and pellet was kept in vial.
- Later this pellet was mixed with prewarmed KCl (pH=7) solution and volume up to 5ml was made in vial.
- After mixing properly it was again kept for incubation at 37°C for 20 minutes.

After termination, fixation step was carried out:

3. Fixation:

- After 20 mins of incubation vials was removed from incubator after fixation step and again centrifuge at 1000rpm for 10 mins.
- Once centrifugation was completed supernatant was removed and pellet was resuspended and mixed properly with the fixative.
- Fixative consisted of solution having glacial acetic acid and methanol in ratio of 1:3.
- After mixing the vial was kept in refrigerator for over-night.

After fixation, preparation of slides for observing metaphasic chromosomes was carried out:

4. Preparation of Slides:

- After over-night refrigeration of vial in fixation step the pellet mixed with fixative solution is again washed with same solution for 2 to 3 times until the pellet becomes white after supernatant is discarded.
- Since pellet is left with some fixative (0.5ml) it is mixed well and was drop by dropper from little height on chilled slides.
- After dropping the drops of suspended pellets on slides in it kept on hot plates machine kept on 40°C for some seconds.
- Once done it was stained with Giemsa stain and was observed under con-focal microscope.