

Dissemination of Education for Knowledge, Science and Culture"
- Dr. Bapuji Salunkhe



Shri Swami Vivekanand Shikshan Sanstha's
VIVEKANAND COLLEGE, KOLHAPUR
(EMPOWERED AUTONOMOUS)

PG - Department of Microbiology




CERTIFICATE OF "ON JOB TRAINING"

This is to certify that Name of student Chavan Sanika Gorakhnath (Exam seat no. 836555) has satisfactorily carried out the required practical work prescribed by the Department of Microbiology, Vivekanand College, Kolhapur (Empowered Autonomous) for M.Sc. - Part- I Semester II course in On Job Training (Sub code - OTT20MIC21) and this report represents her bonafide work in the year 2023-2024.

Place: Kolhapur

Date: 20-5-2024


Examiner


OJT In charge


IC HEAD
DEPARTMENT OF MICROBIOLOGY
VIVEKANAND COLLEGE, KOLHAPUR
(EMPOWERED AUTONOMOUS)

OJT Report, PG Department of Microbiology, Vivekanand College, Kolhapur (Empowered Autonomous)

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VIVEKANAND COLLEGE, KOLHAPUR
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PG - Department of Microbiology




CERTIFICATE OF "ON JOB TRAINING"


This is to certify that Shivani Tanaji Patil_(Exam seat no. 8 3 6 5 6 0)
has

satisfactorily carried out the required practical work prescribed by the BoS
Department of Microbiology, Vivekanand College, Kolhapur (Empowered
Autonomous) for M.Sc. - Part- I Semester II course in On Job Training (Sub code
- OJT20MIC21) and this report represents his/her bonafide work in the year
2023-2024.

Place: Kolhapur

Date: 20/5/2024


Examiner


OJT In charge


DEPARTMENT HEAD
DEPARTMENT OF MICROBIOLOGY
VIVEKANAND COLLEGE, KOLHAPUR
(EMPOWERED AUTONOMOUS)

A
On Job Training report

In Collaboration with

**STEM PLUS CRYOPRESERVATION PVT.LTD
SANGLI MIRAJ KUPWAD, COMMERCIAL COMPLEX,
GAON BHAG SANGLI**

And

**PG Department of Microbiology
Vivekanand College, Kolhapur (Empowered Autonomous)**

By

SANIKA GORAKHNATH CHAVAN

M. Sc. Microbiology

Part I Semester II

Under the Guidance of

Dr. S. D. Mali

PG Department of Microbiology

OJT Report, PG Department of Microbiology, Vivekanand College, Kolhapur (Empowered Autonomous)

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VIVEKANAND COLLEGE, KOLHAPUR
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Place: Kolhapur

Date: 20-5-2024


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VIVEKANAND COLLEGE, KOLHAPUR
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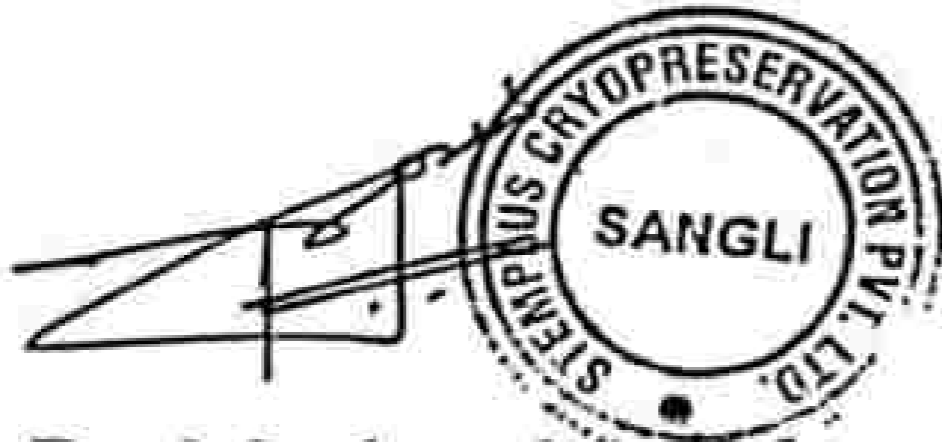
OJT Report, PG Department of Microbiology, Vivekanand College, Kolhapur (Empowered Autonomous)

Date: 15/01/2024

Internship Certificate

This is to certify that Miss. Sanika Gorakhnath Chavan is trained in our organization. She has completed her internship of 10 Days successfully from date 03/01/2024 to 13/01/2024. She got training of basic techniques of Stem Cells, Sterility Testing, Blood Grouping, Instruments handling, Cell Counting, Surgicals Autoclaving, Cord blood collection Kit preparation, Physical examination of cord blood sample. During her tenure she was found to be hard working and committed to work.

Stem Plus Cryopreservation Pvt. Ltd.



Dr. Meghnad Jōshī (Ph.D, PDD)

(CMD)

STEM PLUS CRYOPRESERVATION PVT. LTD.

Sangli Miraj Kupwad Commercial Complex,
C/s No. 1317/2, Near Shivaji Maharaj Putla,
Bus Stand Road, Gaon Bhag, Sangli, MS - 416416, India.
Office : +91 233 2373727
E-mail : info@stempluscryopreservation.com
www.stempluscryopreservation.com

DECLARATION

I the undersigned hereby declare that the On Job Training Report in Collaboration with **STEM PLUS CRYOPRESERVATION PVT. LTD. SANGLI** and PG Department of Microbiology, Vivekanand College, Kolhapur (Empowered Autonomous) is an original work done by me under the guidance of Dr. S. D. Mali PG Department of Microbiology, Vivekanand College, Kolhapur (Empowered Autonomous). The matter included in this report is not a reproduction from any other sources.

I also hereby declare that this project has not been submitted to any time to any other university or institution for the award of any degree or diploma.

Date: 20-5-2024

Place: Kolhapur



Sanika Gorakhnath Chavan
Name of student

ACKNOWLEDGEMENT

At this juncture where the herculean task is nearing its pinnacle, science deems it a pleasure to look back and acknowledge efforts and support of all kith and kin that helped with zeal to turn a distant dream of an industrial training into reality.

We are extremely thankful to Dr. S. D. Mali, Assistant Professor, PG Department of Microbiology, Vivekanand College, Kolhapur (Empowered Autonomous), project guide for her valuable guidance and mentorship throughout this project work given to us during the study.

We are indeed grateful to Faculty Coordinator (OJT) Dr. G. K. Sontakke, PG Department of Microbiology, Vivekanand College, Kolhapur (Empowered Autonomous) for his kind co-operation and valuable support and we are also thankful to all the staff members of our department for their direct and indirect support.

We are thankful to Principal Dr. R. R. Kumbhar, for his kind co-operation and valuable support.

Also, we sincerely thank our parents for helping us in all aspects to complete the project work. Finally, we would like to appreciate our friends, colleagues for their direct and indirect contribution.

Date: 20-5-2024

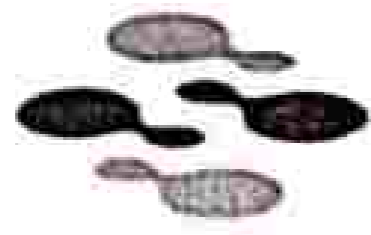
Place: Kolhapur

Sanika Gorakhnath Chavan
Name of student

INTERNSHIP UNDERTAKING

1. Student Name	Sanika Gorakhnath Chavan
2. Current Address	Aaptewada, Bramhanpuri, Miraj Dist- Sangli 416410
1. Residence Address	A/p Kharshing Tal- Kavthemahankal Dist- Sangli
Email id	sc3096947@gmail.com
2. Mobile Nos.	8669582923
3. Aadhar	499332515245
4. PAN	BUPPC3982B
5. Overall GPA	-
6. Internship /Area (Company/Institute)	STEM PLUS CRYOPRESERVATION PVT.LTD.,SANGLI
I confirm that I agree with the terms, conditions, and requirements of the Internship Policy	
Student Signature:- Sanika Gorakhnath Chavan <i>Chavan</i>	
Date:- 20-5-2024	
I confirm that the student has attended the internship orientation and has met all paperwork and process requirements to participate in the internship program, and has received approval from his/her mentor.	
Sign of Department Faculty Coordinator	
Date: 20-5-2024	

About Company



Stem Plus
CRYOPRESERVATION
transforming waste into clinical gold

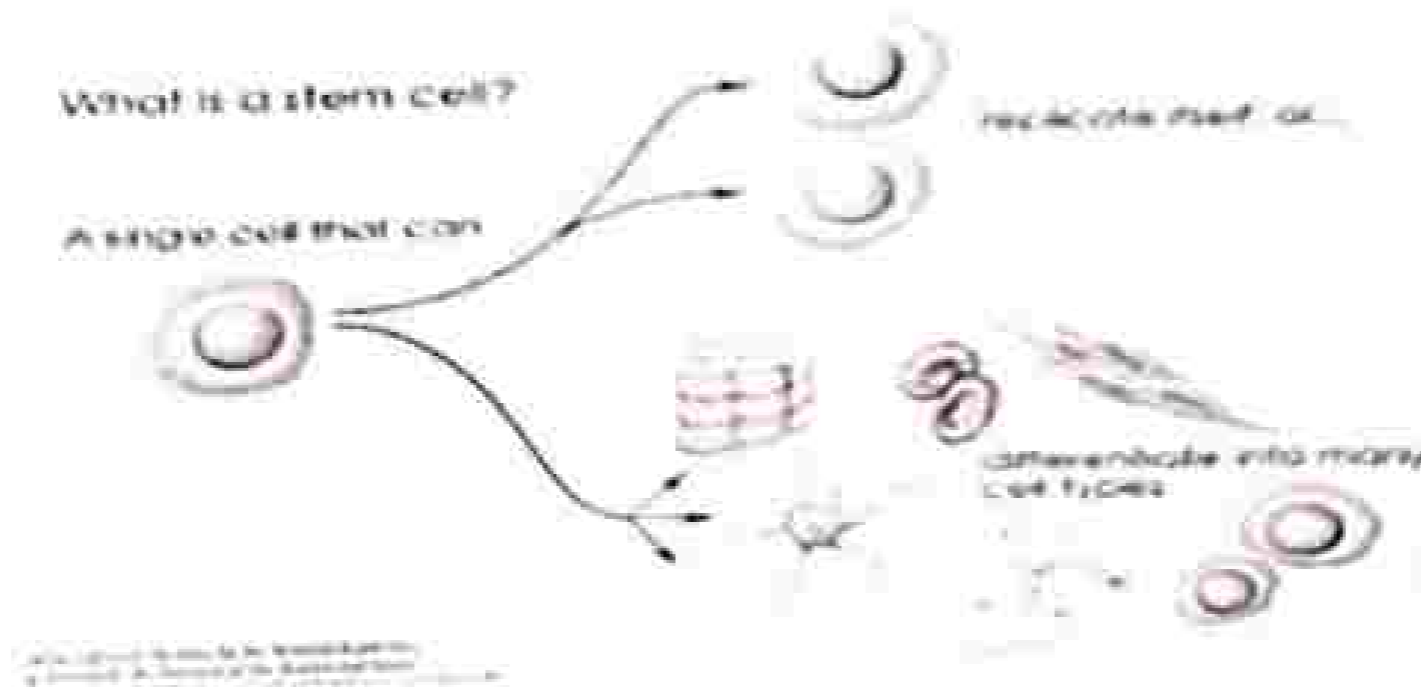
Stem Plus Cryopreservation Pvt. Ltd. is situated in Sangli Miraj Kupwad commercial complex, Bus stand road, gaon bhag Sangli Stem Plus Cryopreservation Pvt. Ltd.. Established in the year 2012, Stem Plus Cryopreservation Pvt. Ltd. in gaon bhag, Sangli a top player in the category stem cell Banks in the Sangli. Over the course of its journey, this bussiness has established a firm foothold in its industry.

Stem Plus Cryopreservation Pvt. Ltd. maintains the highest possible cord blood standards in the industry. They are processed according to FDA and DCGI standards. The lab of Stem Plus Cryopreservation Pvt. Ltd. is run by one MD Pathologist.. This broad range of experts involved in cord blood bank ensures that every aspect of the collection, processing and preservation.

As we know that an internship gives a student the opportunity for career exploration and development and to learn new skills. An internship can give you first hand industry experience . As a part of my achievement and to enhance my skills I had joined an internship program at Stem Plus Cryopreservation Pvt. Ltd., for 10 days from 3 January 2024 to 13 January 2024. I decided to join this internship to gain practical knowledge.

Introduction to Stem cells

Stem cells are a class of undifferentiated cells that are able to differentiate into specialized cells. Stem cells have the remarkable potential to renew themselves. They can develop into many different cell types in the body during early life and growth. Researchers study many different types of stem cells. There are several main categories: the “pluripotent” stem cells (embryonic stem cells and induced pluripotent stem cells) and nonembryonic or somatic stem cells (commonly called “adult” stem cells). Pluripotent stem cells have the ability to differentiate into all of the cells of the adult body. Adult stem cells are found in a tissue or organ and can differentiate to yield the specialized cell types of that tissue or organ.



Adult Stem Cells (ASCs):

Adult stem cells. These stem cells are found in small numbers in most adult tissues, such as bone marrow or fat. Compared with embryonic stem cells, adult stem cells have a more limited ability to give rise to various cells of the body. Adult cells altered to have properties of embryonic stem cells. Scientists have successfully transformed regular adult cells into stem cells using genetic reprogramming. By altering the genes in the adult cells, researchers can reprogram the cells to act similarly to embryonic stem cells.

Types of Adult Stem Cells:

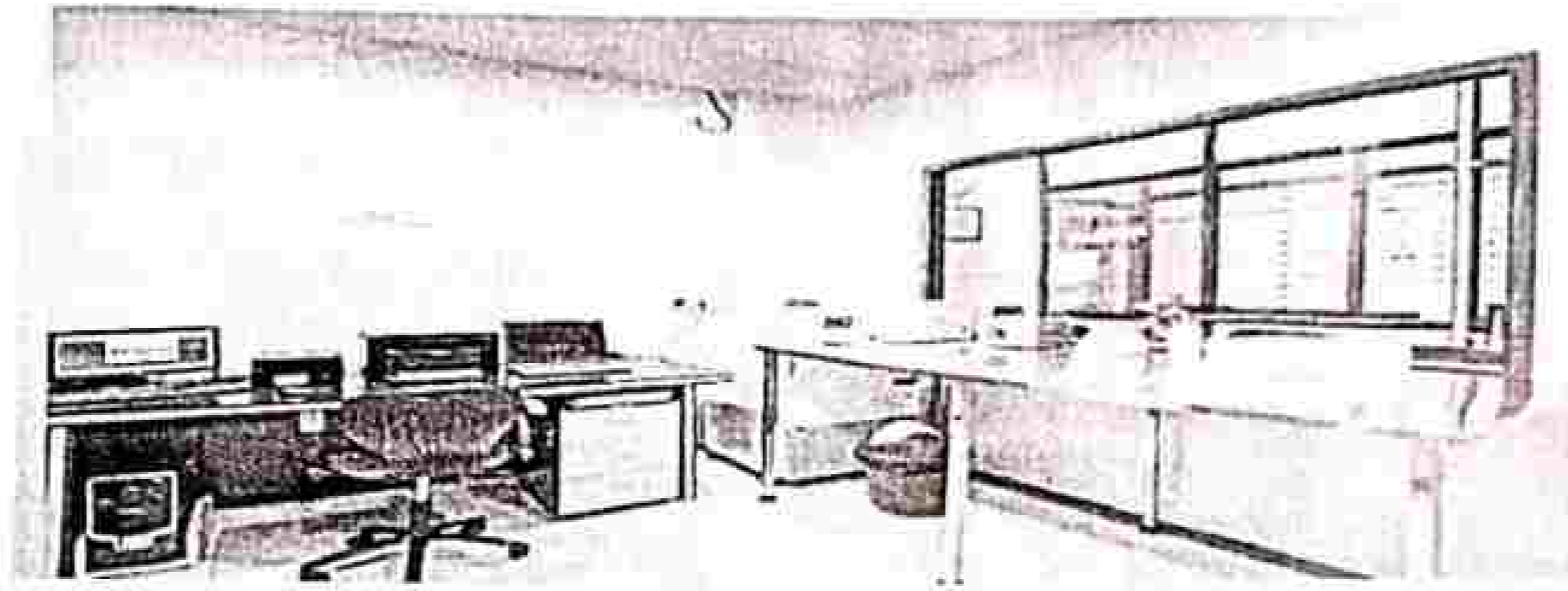
1. Hematopoietic Stem Cells (Blood Stem Cells)
2. Mesenchymal Stem Cells
3. Neural Stem Cells
4. Epithelial Stem Cells
5. Skin Stem Cells

Embryonic Stem Cells (ESCs)

During days 3-5 following fertilization and prior to the embryo (at this stage, called a blastocyst), contains an inner cell mass that is capable of generating all the specialized tissues that make up the human body. These are pluripotent stem cells, meaning they can divide into more stem cells or can become any type of cell in the body. This versatility allows embryonic stem cells to be used to regenerate or repair diseased tissue and organs.

- **Induced Pluripotent Stem Cells** :Induced pluripotent stem cells are stem cells that are created in the laboratory.

a) Physical Examination laboratory



Types of Equipments in the laboratory:

- 1) **Thermometer**- It is used to check temperature of cord blood.
- 2) **Weighing balance**- To check actual volume of cord blood.
- 3) **Rh view box**- To check coagulation of cord blood sample.

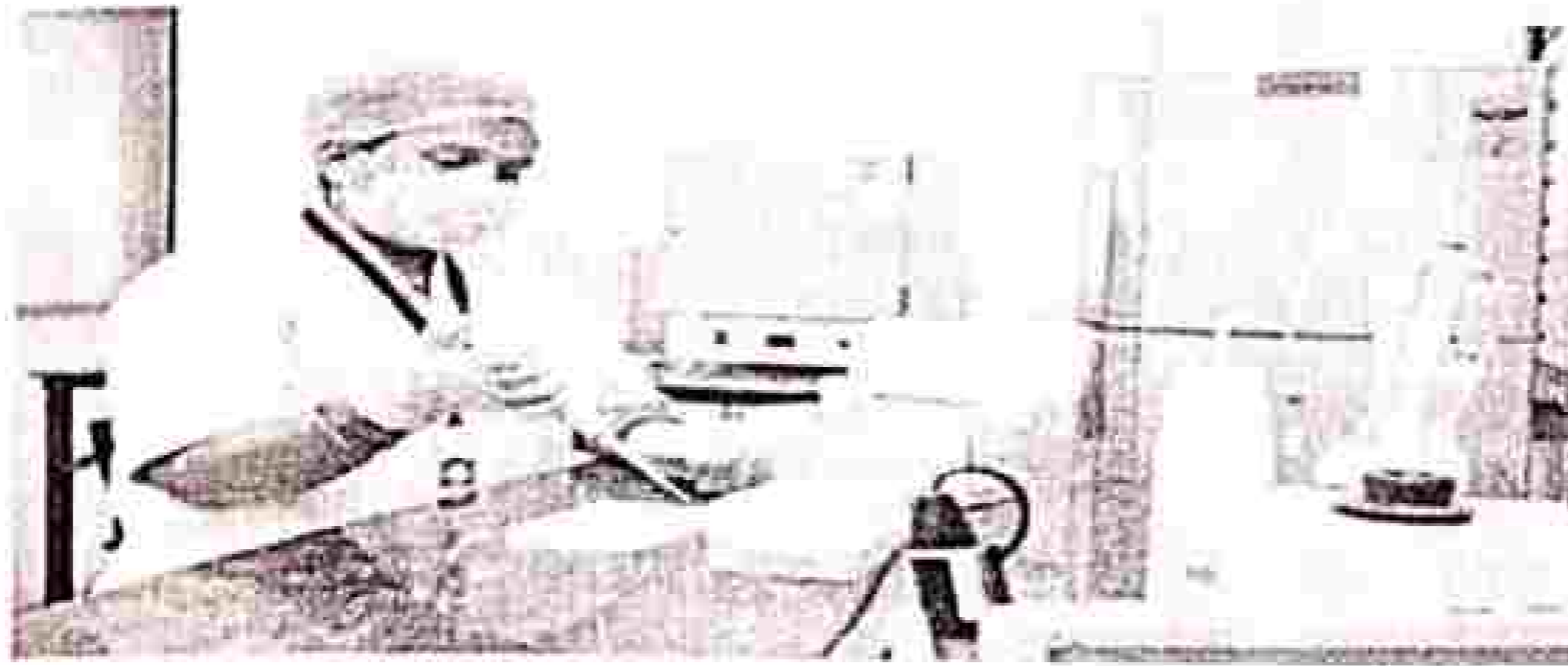
Objective:

To ensure the physical condition and other parameters of cord blood sample received at laboratory fulfills acceptance criteria.

Procedure:

1. Check inside temperature
2. Check coagulation in cord blood bag
3. Check haemolysis of cord blood sample
4. Tube sealing of cord blood bag for transient storage of cord blood sample. Check the weight .

Transduction transmissible disease screening laboratory



Types of equipments in Laboratory:

1. Centrifuge: Separation of serum from maternal blood sample.
2. ELISA reader: to read absorbance of micro well ELISA plate for screening of infectious diseases.
3. ELISA washer: To wash micro well ELISA plate for the screening of infectious diseases.
4. Central Monitoring System: Monitors temperature for all channels.
5. PH meter

Procedure:

1. Separation of serum sample from maternal blood sample
2. Detection of HIV Ag in maternal serum sample
3. Detection of HBs antigen in maternal serum sample
4. Detection of HCV antibodies in maternal serum sample
5. Detection of Anti CMV IgG antibodies in maternal serum sample
6. Detection of Malaria specific antigen
7. Detection of VDRL(syphilis)
8. Detection of Hepatitis C virus

b) Haematology and serology laboratory Equipments:



1. Fluorescent assisted cell sorter
2. Haematology analyser
3. Microscope
4. Water bath
5. Refrizerator
6. Automated cell counter

Cell count and viability:

The number of MNCs and VSELSc are calculated by observing under light microscope. The Neubauer's chamber slide and cover slipe are cleaned with 70% alcohol.10 microlitre Standard dilution method was followed for making final concentration of cells. Then it was mixed with 0.4% Trypan blue stain .

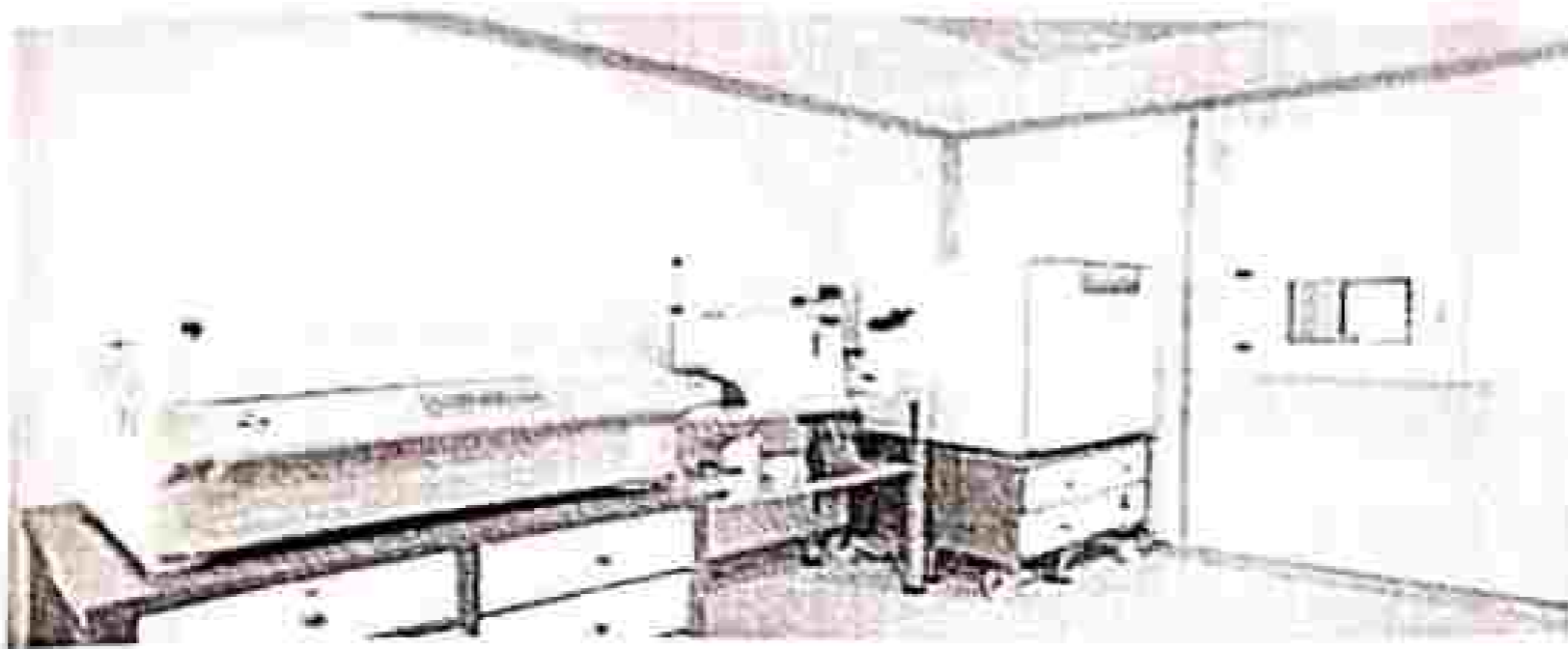
Formula

Total no.of cells = Number of live cells + Number of dead cells

Viability= Live cells÷ Live cells + dead cells ×100

Cells count was taken on Neubauer's chamber using standard protocol.

c) Cord blood Processing Lab



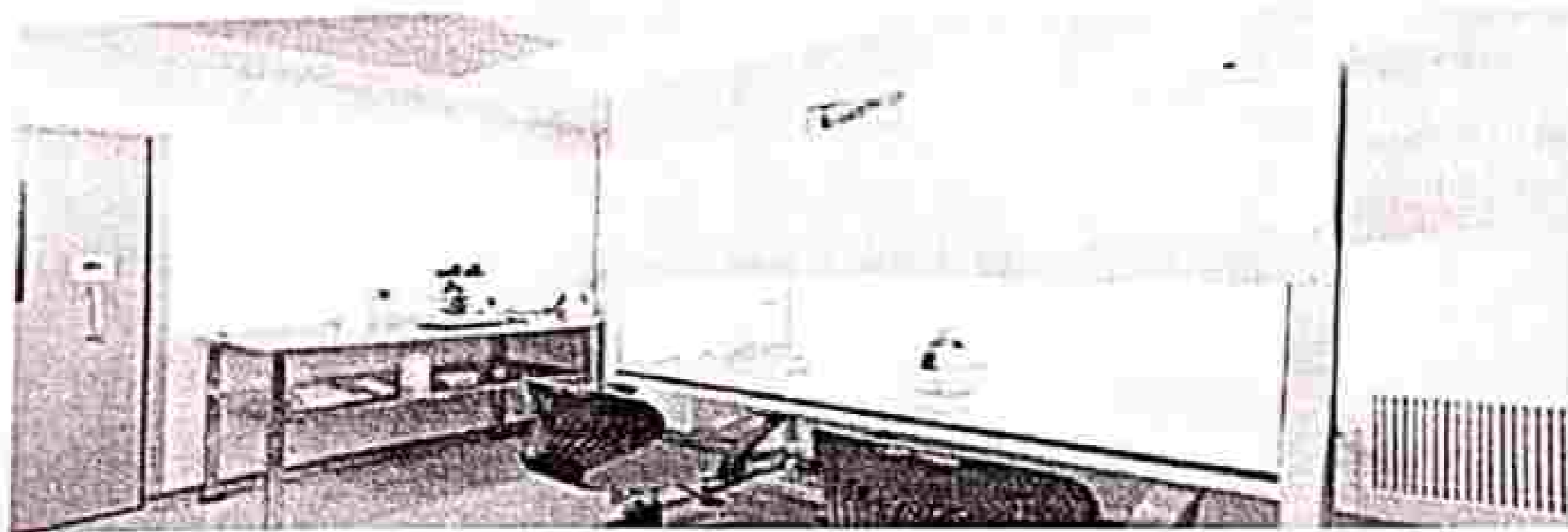
Equipments :

1. Biosafety cabinet
2. Refrigerated centrifuge
3. Shaker
4. Sepax (automated processing)
5. Co₂ incubator

Isolation of Mononuclear cells

1. Distribute the amount of blood equally in each 50ml centrifuge tube.
2. Take out 2ml of Cord blood for Sterility testing
3. Density gradient centrifugation was carried out using Ficoll's reagent (HiMedia)
4. Centrifuge for 2300 for 15mins
5. Remove the Buffy coat .Add 1ml of PBS to the Buffy coat .
6. Centrifuge for 1500rpm for 10mins
7. Take the supernatant.
8. Add 10%DMSO in cryovials and then add MNCs(Every addition should be carried out in cold condition)
9. Then the sample were taken to the CRF for cryopreservation.
10. After checking Sterility reports the sample was stored to main tank from quarantine tank.

d) Sterility and media preparation laboratory



Equipments

1. LAF
2. BOD incubator
3. Incubator(37°C)
- 4: Magnetic stirrer
5. Weighing balance

STERILITY TESTING:

Sterility testing lab contains biosafety cabinet, incubator, BOD & CO₂ Incubator, pass box static etc. The Cord blood sample is tested for fungal and bacterial infection. The first media used is Fluid Thioglycolate to detect aerobic & anaerobic fungal growth and the other media used is Soya Casein to detect fungal growth. Three test tubes are labeled namely +ve control, -ve control & sample. In positive control, inoculation of Staphylococcus aureus is done and in negative control sterile water is used and in the sample test tube we take the cord blood

sample. After 14 days of incubation we examine the sample and is compared with +ve and -ve controls.

Positive control.	Negative control.	Test
<u>Staphylococcus</u>	Sterile water.	Sample
<u>aureus</u> colony		

Cryostorage room



Equipments:

1. CRF (controlled rate freezer)
2. MVE tanks
3. Quarantine tank's
- G4. Cryoshipper's

Cryogenic Storage

After completing all the above steps the cryopreservation is done in the cryogenic storage room. First of all the cryovials that contain the cord blood sample are transferred into Control Rate freezer (CRF). This machine decreases temperature per degree per minute till -196 degree Celsius is obtained. This process takes 3hrs 30 mins. Then transfer this sample into quarantine tank that contains liquid nitrogen till we get all sterility & ELISA test reports after getting all the test reports transfer the sample into the cryogenic freezer for final preservation.

Marketing of Stem cells

As we know that stem cells are the main cells of the body. Those stem cells are helps to cure various types of diseases i.e 80+ diseases are cured by stem cell therapy. Marketing of the stem cells is the main task in that we have explain information about the stem cell, purpose of their preservation ,stem cells applications in medicine,why cord blood is used? And how stem cells are beneficial to the recipient? to the client s. All of these terms which are included in Marketing.

What I learned?

During this internship I learned many more information about stem cells. There types and sources. How stem cells are preserved? In that for preservation of stem cells which procedures are carried out in the laboratory? Along with this I learned which diseases are cured by stem cells therapy also.

For the isolation of stem cells there are main compartments are present. 1) Physical Examination area 2) Sterility testing area 3) Transduction Transmissible Disease. In physical examination to ensure physical condition and other parameters of cord blood sample received at laboratory fulfill acceptance criteria. I observed the temperature of cord blood sample and to check sample for hemolysis and coagulation and determining the value of cord blood. I learned about the generation of bar code for proper Identification of cord blood unit. Separation of serum sample from maternal blood sample for the infectious diseases testing. After the negative result of that testing isolation of MNCs performed. During this internship I understood the instruments used for the stem cells cryopreservation and stem cells application in the Medicine.

This internship has increased my interest in pursuing a career in industry. I truly appreciate all of the time and effort that you and other staff spent training me. This has been a valuable learning experience. Thank you again for all your support, and for all of opportunities you have given me. It was great experience in these stem plus cryopreservation internship.