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

(Hindi Vidya Prachar Samiti's RAMNIRANJAN JHUNJHUNWALA COLLEGE of Arts, Science & Commerce)
Opposite Ghatkopar Railway Station, Ghatkopar (West), Mumbai 400086, Maharashtra, INDIA.
Website: www.rjcollege.edu.in Email: rjcollege@rjcollege.edu.in Tel No: +91 22 25151763 Fax No: +91 22 25150957

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 Manasi P Jha has completed On Job Training at

Joshi Childrens Hospital.

Date of Commencement	Date of Completion	Total Number of Days	Total Number of Hours completed in OJT
6/1/23	13/2/23	23	69

Name of the Guide/ PI/ Incharge : Vaishali T. Kargutkar dscl

Phone Number of Guide/ PI/ Incharge : 9960378949

Email Address of the Guide/ PI/ Incharge : Jhpathology2002@gmail.com

Ramniranjan Jhunjhunwala College NAAC Re-Accredited "A" Grade Ghatkopar Mumbai-86

JOSHI CHILDRENS HOSPITAL
1st Floor, A - Wing, Manor Appt,
Vartak Road, Virar (West)
Pin - 401 303
VVCMC/C-H-137/2013

Signature of Guide/ PI/ Incharge

Stamp

Dr. Joshi Children's Hospital
Opposite Station, Virar (West) - 401 303.

डॉ. जोशी बाल रुग्णालय
स्टेशनसमोर, विरार (पश्चिम) - ४०१३०३.

Dr. Hemant Joshi M.D. R. No. 40821
Dr. Archana Joshi M.D. R. No. 40822
Dr. Vishal Wadekar M.B.B.S., D.C.H. R. No. 2009/09/3392

डॉ. हेमंत जोशी एम्.डी. रजि.नं. ४०८२१
डॉ. अर्चना जोशी एम्.डी. रजि.नं. ४०८२२
डॉ. विशाल वाडेकर एम.बी.बी.एस., डी.सी.एच. रजि.नं. २००९/०९/३३९२

DATE: 21.02.2024

TO WHOMSOEVER IT MAY CONCERN

This is to certify that MISS, MANASI PRADEEP JHA attended on job training at Joshi Children Hospital for a period from 06.01.2024 to 13.02.2024.

1)She was introduced to routine pathology Techniques like stool routine, urine routine and complete blood count and is reasonably good at it

(2) She was introduced to automated Retina biochemistry analyzers.

She is a hard working individual; honest and sincere in her work. We found her studious, technically sound, and adaptable to variable conditions. She has the ability to handle responsibilities independently.

We wish her success in her future endeavors.

Joshi Hospital

☎(0250) 2502710
Dr. Mrs. Archana Joshi
Reg. No. 40822 M.D., D.C.H.
Child Specialist & Neonatologist
Joshi Children's Hospital & Research Centre
Virar (West), Palghar-401 303.
Mob.: 9960378949

On Job Training Report

Submitted to
Ramniranjan Jhunjhunwala College,
Ghatkopar(W)
In partial fulfillment of the requirements
For the degree of Masters in Biotechnology

Submitted by
Manasi Pradeep Jha
Roll no:- 429

Dr. Joshi Children's Hospital
Opposite Station, Virar (West) - 401 303.

डॉ. जोशी बाल रुग्णालय
स्टेशनसमोर, विरार (पश्चिम) - ४०१३०३.

Dr. Hemant Joshi M.D. R. No. 40821
Dr. Archana Joshi M.D. R. No. 40822
Dr. Vishal Wadekar M.B.B.S., D.C.H. R. No. 2009/09/3392

डॉ. हेमंत जोशी एम्.डी. रजि.नं. ४०८२१
डॉ. अर्चना जोशी एम्.डी. रजि.नं. ४०८२२
डॉ. विशाल वाडेकर एम.बी.बी.एस., डी.सी.एच. रजि.नं. २००९/०९/३३९२

DATE: 21.02.2024

TO WHOMSOEVER IT MAY CONCERN

This is to certify that MISS, MANASI PRADEEP JHA attended on job trainin at Joshi Children Hospital for a period from 06.01.2024 to 13.02.2024.

1)She was introduced to routine pathology Techniques like stool routine, urine routine and complete blood count and is reasonably good at it

(2) She was introduced to automated Retina biochemistry analyzers.

She is a hard working individual; honest and sincere in her work. We found he studious, technically sound, and adaptable to variable conditions. She has the ability to handle responsibilities independently.

We wish her success in her future endeavors.

Joshi Hospital

☎(0250) 2502710
Dr. Mrs. Archana Joshi
Reg. No. 40822 M.D., D.C.H.
Child Specialist & Neonatologis
Joshi Children's Hospital & Research Cent
Virar (West), Palghar-401 303.
Mob.: 9960378949

Summary Of Training Report

This report describes a brief description of the work that has been carried out by me in the laboratory during training at Joshi Children Hospital.It is Paediatric hospital and it has its own pathology laboratory.I have been working in laboratory in this hospital during my training period from 06.01.2024 to 13.02.2024.

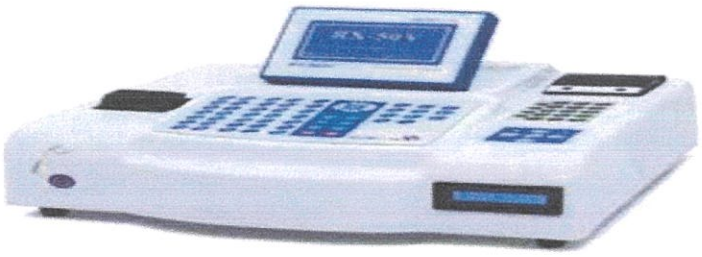
I have learned how to operate **Retina Biochemistry Analyzer** to perform various tests like albumin tests, sugar level tests, or to detect levels of enzymes and creatinine in the blood.

In Hematology, I have learned how to operate **Sysmex XP-300™ Automated Hematology Analyzer** is used to quickly perform full blood counts and reticulocyte counts.I also learned **ABO BLOOD GROUPING BY (Slide Method).**

In Microbiology I have learned about staining, culture of blood, body fluids and urine and stool sample.

I have also learned **Multistix® 10 SG reagent strip** which is used for determination of Glucose, Protein and pH of the urine and stool.

Instrument:- Retina Biochemistry Analyzer



Biochemistry Analyzer is a medical laboratory equipment used extensively in clinical laboratories. It is designed to conduct tests to measure chemicals at various stages of the biological processes on a wide range of test samples from blood, plasma, serum, and cerebrospinal fluid to urine. In other words, it helps to study, analyze, and evaluate the characteristics of different body samples and tackle a broad of medical industry challenges.

NAME OF THE TEST :

- Glucose:-
  1. Fasting blood sugar
  2. Random blood sugar
  3. Postprandial blood sugar
- Renal function test (RFT)
- Liver Function Test (LFT):-
  1. Bilirubin Direct and Total
  2. SGPT(Serum glutamate Pyruvate Transaminase)
  3. SGOT(Serum Glutamate Oxaloacetate Transaminase)

Instrument:- Multistix® 10 SG Reagent Strips

The Multistix® 10 SG reagent strip is the reliable frontline test for detection of a broad range of conditions, from detecting urinary tract infections (UTI1) to diabetes and kidney disorders.

- **Procedure:-**

1. Collect a fresh urine specimen in a clean, dry container.
2. Mix well just before testing, but do not centrifuge.
3. Remove a strip from the bottle and replace the cap.
4. Dip all the test pads of the strip into the urine and immediately remove the strip. If reading the strip visually, start timing.
5. Drag the edge of the strip against the container rim to remove excess urine and blot the edge on a paper towel or tissue if using the CLINITEK 50 or CLINITEK Status Analyzers.
6. If reading visually:• Compare each test pad to the corresponding row of color blocks on the bottle label.

• Read each pad at the time shown on the label, starting with the shortest time.
• Hold the strip close to the color blocks and match carefully.
• Read the pads in good light.

Instrument:- Sysmex XP 300 Hematology Analyzer

It provides a results include histograms for WBC, RBC and PLT.

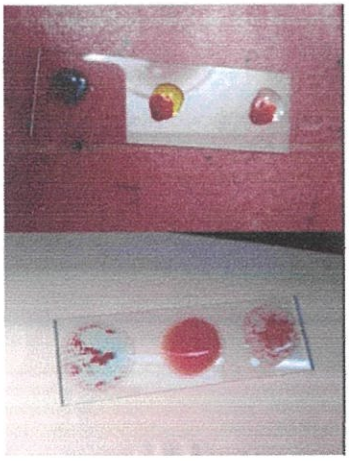
Specimen Collection and Preparation :-

• Blood should be collected following the tube manufacturer's instructions for minimum fill volume and well mixed post draw to distribute the anti-coagulant.
• Good phlebotomy and biohazard safety practices should be observed at all times.
• Operators must label the specimen correctly and/or only accept a properly labeled specimen from another staff member for testing on the XW-100.
• All parts and surfaces of the analyzer as well as QC vials and contents must be regarded as potentially infectious due to its contact with blood.
• Minimum blood fill volumes for use on the analyzer are 1mL for 12-15 mm vacuum tubes. Running tubes with lower than minimum fill volume will result in suppressed values for test parameters.
• Test samples containing various interfering substances or rare cells and samples from patients with various pathological conditions can initially generate compromised results.

NAME OF THE TEST:-

1. Complete blood Count (CBC)
2. Erythrocyte sedimentation rate (ESR)
3. Blood grouping
4. Differential leukocyte count (DLC)
5. G-6-PD-1
6. Coagulation profile

ABO BLOOD GROUPING BY (Slide Method)



Observation of blood grouping slide

PRINCIPLE:- Serum of the specimen submitted is reacted with known a cells and B cells. Agglutination Indicate presence of corresponding antisera in serum.

PROCEDURE:-

1. Place 1 drop of anti-A and I drop of anti-B reagent separately on a labeled slide or tile.
2. Add 1 drop of 20% test red cell suspension to each drop of the typing antiserum(the Suspension may be prepared by adding 20 parts of red cells to 80 part of normal saline).
3. Mix the cells and reagent using a clean stick. Spread each mixture evenly on the slide over an area of 10-15 mm diameter.
4. Tilt the slide and leave the test for 2 minutes at room temperature.Then rock again and Look for agglutination.
5. Record the results.

1.1.1 Physical Examination Of Urine Determination

Determination	Normal Finding
1. Volume of Urine	50 to 200 ml
2. Color of Urine	Pale Yellow
	White
3. Appearance of Urine	Usually clear
4. Reaction	Usually acidic PH 4.88 to 7.5
	PH more than 7.5 Alkaline Urine
5. Odor of Urine	Aromatic
6. Specific gravity of Urine	Varies from 1.003 to 1.060

Table 1.1: Determination & Normal Finding of Urine

Abnormal	Pathologic
>500 ml	Diabetes insipidus, Polyuria
<20 ml	Oliguria, Anuria
Dark yellow	Hepatic and post hepatic condition
Redish	Chyluria Hematuria
Redish	Chyluria Hematuria
Black Urine	Alkaptonuria
Dark yellow	Biliverdin present
Turbid	Presence abnormal Leukocytes.
Milky	Chyle
PH less than 4.8 More, acidic Urine	Fever, Ketosis.
	Sever Vomiting.
Fruity	Acidosis, Ketosis.
Ammonical	Cystitis.
Foul smelling	Urinary tract infection.
Low Sp. Gravity	Chronic nephritis, diabetes insipidus.
High Sp. Gravity	Diabetes insipidus fever, Acute nephritis.

Table 1.2: Abnormal & Pathologic of Urine

Manisha Jha. Rollno:-429.

LEUCOCYTE CULTURE PROCEDURE FOR CHROMOSOME PREPARATION

THEORY:-

Karyotyping is the process of pairing and ordering all the chromosomes of an organism, thus providing a genome-wide snapshot of an individual's chromosomes. Karyotypes are prepared using standardized staining procedures that reveal characteristic structural features for each chromosome. Clinical cytogeneticists analyze human karyotypes to detect gross genetic changes—anomalies involving several megabases or more of DNA. Karyotypes can reveal changes in chromosome number associated with aneuploid conditions, such as trisomy 21 (Down syndrome). Careful analysis of karyotypes can also reveal more subtle structural changes, such as chromosomal deletions, duplications, translocations, or inversions. In fact, as medical genetics becomes increasingly integrated with clinical medicine, karyotypes are becoming a source of diagnostic information for specific birth defects, genetic disorders, and even cancers.

Preparing Karyotypes from Mitotic Cells:-

Karyotypes are prepared from mitotic cells that have been arrested in the metaphase or prometaphase portion of the cell cycle, when chromosomes assume their most condensed conformations. A variety of tissue types can be used as a source of these cells. For cancer diagnoses, typical specimens include tumor biopsies or bone marrow samples. For other diagnoses, karyotypes are often generated from peripheral blood specimens or a skin biopsy. For prenatal diagnosis, amniotic fluid or chorionic villus specimens are used as the source of cells.

G-banding Technique:-

G-banding or Giemsa banding is a DNA staining technique that is used to visually investigate the structure of condensed chromosomes within the nucleus of a cell.
The basic principle of G-banding is that it is specific to the phosphate groups of DNA and attaches itself to regions of DNA where there are high amounts of adenine-thymine bonding, creating dark bands. Conversely, less condensed chromatin, which tends to be rich with guanine and cytosine, incorporates lesser Giemsa stain and appears as light bands. The result of G-banding creates a karyogram (chromosome map), which can be used to identify chromosomal aberrations such as translocations and rearrangements.

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.

Planting

- Remove media, serum and PHA from freezer and keep media and serum in the waterbath at 37° Leave PHA at RT ,clean laminarhood with methanol swab.
- Put on the UV light in the laminar hood for 30 mins.

Follow this procedure for planting the whole blood culture -

Syringe **1 Vial**	**Material** **11 vial**
10 ml 4 ml	RPMI 1640 culture Med. 4ml
5 ml 1 ml	Human Serum/FBS 1 ml
100 ml 0.2 ml	PHA (M) 0 .25 ml
.01 ml	PHA (P) .015 ml
2 ml 8 drops	Blood 8 drops

- Clean the mouth of all the vials with methanol and close the screwcaps tightly.
- Keep the vials in a petridish cleaned with methanol and keep in the incubator at 37° C for 72 hours.
- At the 70th hour add 0.1 ml of colchiciine (10 ug /ml) to each vial.
- Mix properly and incubate for 1 hour.

TERMINATION

- Keep ppts, centrifuge tubes and round bottom tubes for keeping ppts according to the no. of vials and name them properly.
- Remove the vials from the incubator, transfer the contents into graduated glass centrifuge tubes (15ml capacity) with the same labels. Centrifuge at1000 rpm for 10 min.
- Remove the supernatant leaving the pellet behind. Mix and add prewarmed KCL(0.560gms in 100ml) to make the volume to 5ml . mix thoroughly and incubate at 37° C for 20 min

KCL:0.560 gms in 100ml.Adjust pH at 7

FIXATION

Centrifuge at 1000 rpm for 10mins & remove the supernatant. Add fixative (**1:3 glacial acetic acid: methanol**) with simultaneous mixing of the pellet. Make the volume to 5ml and store in refrigerator over- night.

PREPARATION OF SLIDES:

- Next day, the tubes are removed from the refrigerator centrifuged, and 2-3 washes are given with fixative till the pellet becomes white.
- At the end discard the supernatant leaving behind fixative till 0.5 ml. keep the hot plate at 40C, take acidified chilled slides, (slides kept in cold water in refrigerator).
- Mix the pellet thoroughly and drop it on the cold slide from a hight and keep on the hot plate to heat dry.
- Remove the slide, stain with plain Giemsa and see under the microscope.
- If adequate no. of good quality metaphases are achieved the it is a successful culture otherwise it 's a failure.

PREPARATION OF STOCK GIMSA STAIN:

- Take 1gm of giemsa powder & 50ml glycerol.
- Grind it slowly adding glycerol and make it to a paste.
- Then add methanol slowly (50ml) to make a giemsa stock. Keep it in incubator at 37° C over-night.
- Next day filter through filter paper No.41 & preserve it in refrigerator to age for 1 month and then use it.

BANDING TECHNIQUE:

G-Banding:
Prepare 4 slides for each patient and age them for 2-3 days. Keep the slides overnight at 60° C in oven the day before banding is done.

Buffer

			TEDTA		
DW	=	200ml			
Trypsin	=	125mg			
Na2HPO4	=	450mg	EDTA	=	125mg
KH2PO4	=	340mg			

10ml PBS+90ml D/W

WORKING GIEMSA STAIN:

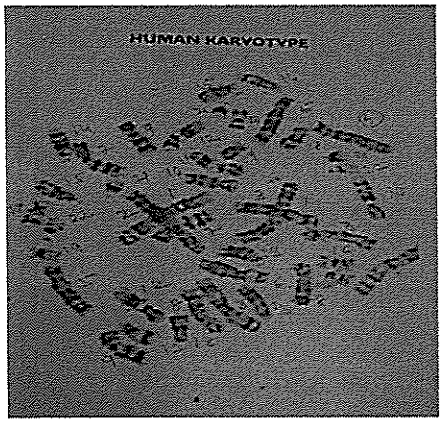
43 ml	S.buffer
5 ml	5 ml methanol
2 ml	2 ml stock giemsa stain.

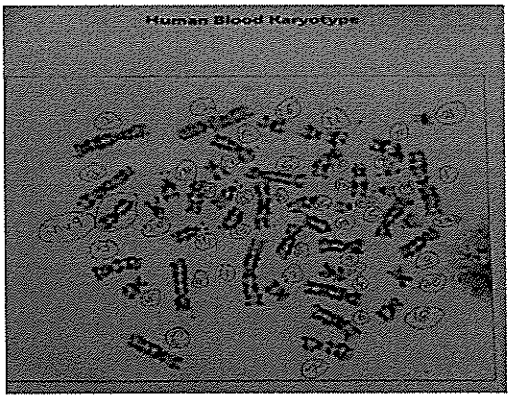
PBS 10X

Nacl	= 8gms
KCL	=0.3gm
Na2HPO4 2h2O	=0.073 gms
KH2PO4	=0.020gm
Glucose	=2gms

Dissolve in 100 ml D/W (10 X)
pH = 6.48

- Prepare TEDTA solution and mix with the help of magnetic stirrer on vortex mixer.
- Keep buffer at 60° C after taking out 43ml for the preparation of working Giemsa stain.
- Take no.1slide of each patient in a petridish and pour S.bffer prewarmed at 60° C.
- Till the slides immerse in the buffer. Keep this petridish at 60° C for 15 min.
- Take trysin EDTA & N. saline in separate petridishes. Remove each slide from the buffer and rock the slide in EDTA mixture for certain fixed time, remove and rinse in normal saline.
- Stain the slide and see under the microscope.
- If good banding is not achieved i.e. over trypsinised, or trypsin treatment was not enough, adjust the time for the second batch of slides accordingly.
- Repeat this procedure for all the 4 slides of each patient.
- Select a good metaphase after screening to check whether it is normal.
- Screen 15-20 good metaphases and select one of them for mapping, take the photograph of the same, and send for printing.
- Stick the photographs.





Karyogram for observing chromosome numbers and abnormalities.