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
College is recognized under Section 2(f) & 12(B) of the UGC Act 1956
Affiliated to UNIVERSITY OF MUMBAI II NAAC Re-Accredited 'A' Grade (CGPA: 3.50)

Department of Biotechnology

On Job Training Completion Report

This is to certify that Anchal Bradeep Gupta has completed On Job Training at

Agilus Diagnostics Dr. Phadke labs (Mahim).

Date of Commencement	Date of Completion	Total Number of Days	Total Number of Hours completed in OJT
27/12/2023	28/1/2024	33 Days	99 hrs

Name of the Guide/ PI/ Incharge : Dr. Sonal Dhawan

Phone Number of Guide/ PI/ Incharge : 8291097313

Email Address of the Guide/ PI/ Incharge : Sonal.Dhawan@agilus.in

RGadhyay

Dhupta
22-03-24

Signature of Guide/ PI/ Incharge

Stamp

INTERNSHIP REPORT

Submitted By

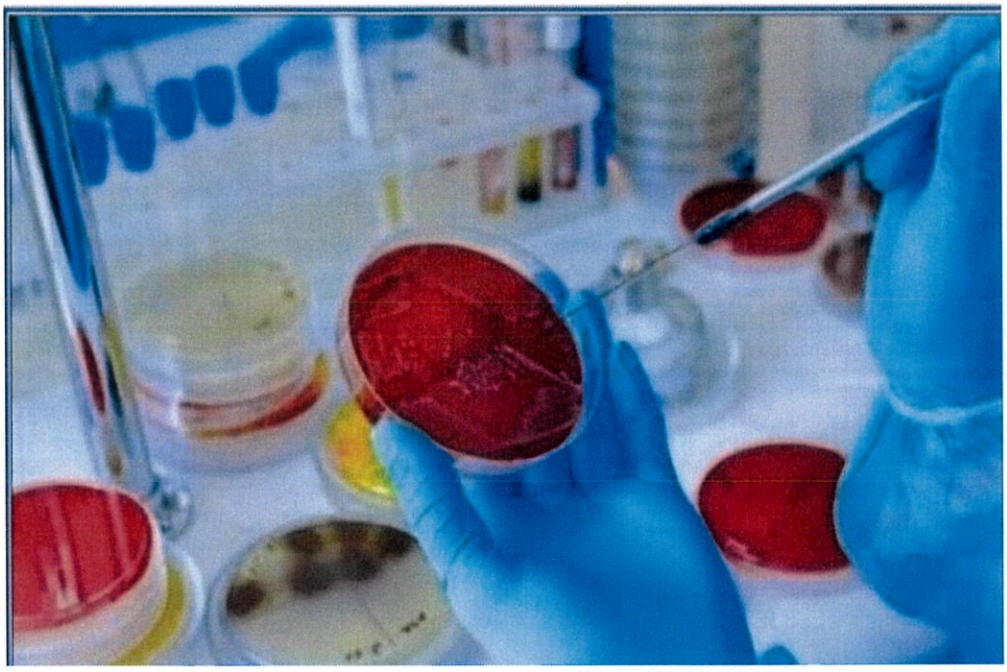
GUPTA ANCHAL PRADEEP

Roll no: 410

MSC Part1

DEPARTMENT OF MICROBIOLOGY

SRL DIAGNOSTICS & Dr. AVINASH PHADKE LAB, MAHIM



INTRODUCTION

Medical microbiology is the study of parasites, fungi, bacteria, and viruses that are the agents of infectious diseases in humans. Advanced pharmaceutical depends on the control of microorganisms in human well-being and quality of life. The divisions of therapeutic microbiology incorporate **Bacteriology** (study of bacteria), **Mycology** (study of fungi), **Parasitology** (study of parasites), and **Virology** (study of viruses). Sizes for the pathogen include the smallest, viruses (50-100nm), bacteria (0.1μm 10μm), and fungi (8μm).

Medical Microbiology as a discipline requires knowledge of human anatomy, histology, and comprehension of pathologies associated with the infectious disease process. The human response to infectious disease is the key consideration. Microbiologists should have enough information about pathogenic organisms and their special characteristics in the context of host disease.

SRL DIAGNOSTICS

In 1963, Dr. Achyut Phadke started the andrology center (medical branch dealing with male fertility). However, with the proliferation of illegal pathology labs, there was a need to set up doctor-led diagnostic centers. Thus in 1980 Dr. Avinash Phadke and his wife Dr. Vandana Phadke started several pathology tests and services, leading to the establishment of Dr. Avinash Phadke Labs. Avinash Phadke Labs offers up to 3,000 different tests to patients, performing more than 30,000 tests a day. It has a cobranding partnership with SRL Diagnostics. Highly qualified and experienced doctors and technicians study various diseases in-depth and perform screening and testing of patient samples, received from across the country and accordingly guide the clinicians to reach a diagnosis.

My Internship at SRL Diagnostics & Dr. Avinash Phadke Lab was in **Microbiology Department**

DEPARTMENT OF MICROBIOLOGY

OVERALL WORKFLOW OF THE LABORATORY

Patient Registration on CLIMS (Centralized Lab Information Management) System

↓

Sample Collection / Sample Acceptance

↓

Sample Segregation

(separating samples into different departments based on the test to be performed)

↓

Sample received by the Microbiology department

↓

Sample Processing

↓

Reporting

↓

Verification by Dr. (MD Microbiology)

↓

Report Typing

↓

The report released to the concerned patient

Different Types Of Tests Performed:

SR. NO	TEST CODE	TEST NAME	METHOD USED	SAMPLE INSTRUCTIONS
1	CSURI	Urine Culture & Susceptibility	Culture/ID by Vitek System/ MALDI-TOF & Convectional Method	Mid-Stream Urine sample to be collected in a sterile container (Urine from catheter bag not allowed)
2	CSBLD	Aerobic Blood Culture & Susceptibility	Automated BACT/ALERT 3D System	Sample should be collected in BACT/ALERT Bottle (Adults:10ml, Children: 5ml, Infants: 2ml)
RESPIRATORY SAMPLE PROCESSING				
3	CSSP	Sputum Culture & Susceptibility	Culture/ID by Vitek System/ MALDI-TOF & Convectional Method	The sputum sample should be collected in a sterile screw-cap container
4	CSUPR	Throat Swab Culture & Susceptibility	Culture/ID by Vitek System/ MALDI-TOF & Convectional Method	Sample in Sterile Container

5	MCULT	Aerobic Culture & Susceptibility (Endotheleal secreation/ Trachaestomy Secreation)	Culture/ID by Vitek System/ MALDI-TOF & Convectional Method	Sample in Sterile Container
MISCELLANEOUS SAMPLE PROCESSING				
6	CSPUS	Pus Culture & Susceptibility	Culture/ID by Vitek System/ MALDI-TOF & Convectional Method	Sample in Sterile Container
7	CSCSF	CSF (Cerebrospinal Fluid) Culture & Susceptibility	Culture/ID by Vitek System/ MALDI-TOF & Convectional Method	Sample in Sterile Container
8	CSFLD	Fluid (All Body Fluids) Culture & Susceptibility	Culture/ID by Vitek System/ MALDI-TOF & Convectional Method	Sample in Sterile Container
9	CSSEM	Semen Culture & Susceptibility	Culture/ID by Vitek System/ MALDI-TOF & Convectional Method	Sample in Sterile Container
10	MCULT	Aerobic Culture & Susceptibility (Tissue, Catheter tip, Cervical swab, Nasal Swab)	Culture/ID by Vitek System/ MALDI-TOF & Convectional Method	Sample in Sterile Container (Tissue sample in Sterile saline)
11	CSVAG	Vaginal Swab Culture & Susceptibility	Culture/ID by Vitek System/ MALDI-TOF & Convectional Method	Vaginal swab for diagnosis of Vaginosis

FUNGAL SAMPLE PROCESSING				
12	MFUN	Fungal Culture	Culture/ID by Vitek System/ MALDI-TOF & Convectional Method	Sample in Sterile Container
13	SUSF	Fungal Susceptibility Test	Vitek System	-
BIOFIRE PROCESSING				
14	BIORESPI	Biofire Respiratory Panel	Multiplex RT-PCR	Nasopharyngeal, Throat Swabs in Viram Transport Medium
15	BIOCSF	Biofire CSF Panel	Multiplex RT- PCR (FDA approved)	CSF Sample in Sterile Container
16	BIOGI	Biofire GI (Gastrointestinal) Panel	Multiplex RT- PCR (FDA approved)	Stool Sample in Sterile Container
17	BIOBCID	Biofire Blood Panel	Multiplex RT- PCR (FDA approved)	Blood/ Body Fluids in Sreile Container
OTHER SAMPLE PROCESSING				
18	NSMRS	Nasal Swab for MRSA (Methylene Resistant S.aureus)	Culture/ID by Vitek System/ MALDI-TOF & Convectional Method	Sample in Sterile Container
19	CLODA	Clostridium Difficile Immunoassay (GDH+ Toxin A & B)	Immunochromatography	Stool Sample in Sterile Container
20	ROTA	Rota Virus Immunoassay	Immunochromatography	Stool Sample in Sterile Container

21	FOOD	Microbiological Evaluation of Food	Conventional Culture & ID as per BIS Standard by MALDI-TOF	Food in Sterile Container
22	WATER	Microbiological Evaluation of Water	Conventional Millipore Filteration & ID as per BIS Standard by MALDI-TOF	Water in Sterile Container
23	HDEX	Stool for Hanging drop Examination	Microscopy	Stool in Sterile Container (solid stool not recommended)
24	ANAEC	Anaerobic Culture	Conventional Method	Sample in Sterile Closed Container
25	MBACC	Rapid Aerobic Culture & Susceptibility- BACT/ALERT 3D System	BACT/ALERT 3D System, ID by MALDITOF	Fluids in BACT/ALERT Bottle
MYCOBACTERIUM TUBERCULOSIS PROCESSING				
26	XPERTTB	GeneXpert MTB/ RIF Assay (DNA- PCR)	Multiplex PCR	All samples were used other than Blood, Urine & Stool
27	MRAPIT	MBC- BACT/ALERT/MGIT System	MGIT/ BACT-ALERT 3D	Sample in Sterile Container (Tissue in St. Saline)
28	MRAPIS	MBC- BACT/ALERT/MGIT System	MGIT/ BACT-ALERT 3D	Sputum sample in Sterile Container
29	HAIN	HAIN'S PANEL 1st & 2nd Line Drug Susceptibility	Line Probe Assay	Pure Culture of Isolate BACT/ LJ Medium

SUMMARY

In this current study, I learned about the terms and codes used for various tests. I learned about the protocols that lab technicians follow to diagnose patients. In detail, I studied sample processing (type of sample, media, and tests based on sample type, characterization of sample, data entry and documentation, patient's history).

I learned about the principles, instrumentation, and operation of various instruments such as MALDI-TOF, GeneXpert, Vitek, BioFire, BACT/ALERT. I had firsthand experience with each instrument and learned a lot.

This experience in the diagnostic field-assisted me in clarifying my concepts and improving my skills. It will also undoubtedly help me in my future studies.

Name: Anchal Gupta

Roll no:410

MSC Part 1

Karyotyping Report

Introduction

The hands-on training for karyotyping was conducted in RJ College. The training aimed to provide us with practical skills and theoretical knowledge required to perform karyotype analysis effectively in a laboratory setting.

Karyotyping is a laboratory technique used to visualize and analyze an individual's chromosomes. Chromosomes are thread-like structures made of DNA and proteins that carry genetic information. Karyotyping can reveal abnormalities in chromosome number or structure, which can be associated with genetic disorders or conditions.

Objectives:

- To familiarize with the principles and techniques of karyotyping.
- To provide hands-on experience in sample collection, cell culturing, chromosome preparation, staining, microscopic analysis, and result interpretation.

Theoretical Sessions:

Lectures and presentations covering the fundamentals of karyotyping, chromosome structure, chromosomal abnormalities, and clinical applications.

Discussions on laboratory safety protocols, quality assurance, and ethical considerations in cytogenetic testing.

Practical Demonstrations:

Procedure:

Sample Collection: Typically, a blood sample is collected from the patient. In some cases, other tissues such as bone marrow or fetal cells may be used.

Cell Culturing: The collected cells are cultured in a laboratory to stimulate cell division and chromosome replication.

Chromosome Preparation: Cells are treated with a chemical to stop cell division at a stage where chromosomes are condensed and visible under a microscope.

Chromosome Staining: The chromosomes are stained with a dye, such as Giemsa stain, to create a banding pattern that helps in identifying individual chromosomes.

Microscopic Analysis: The stained chromosomes are examined under a microscope, and images are captured for analysis.

LEUCOCYTE CULTURE PROCEDURE FOR CHROMOSOME PREPARATION

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	**Material**	**1**
Vial	**11 Vial**	

10ml	RPMI 1640 culture Med.	
4ml		4ml
5 ml	Human Serum/FBS	
1 ml		1 ml
100 ul	PHA (M)	
0 .2 ml		0 .25 ml
	PHA (P)	
.01 ml		.015 ml
2 ml	Blood	
8 drops		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		
DW	=	200ml
Na2HPO4	=	450mg
KH2PO4	=	340mg

TEDTA		
Trypsin	=	125mg
EDTA	=	125mg
10ml PBS+90ml D/W		

WORKING GIEMSA STAIN:

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

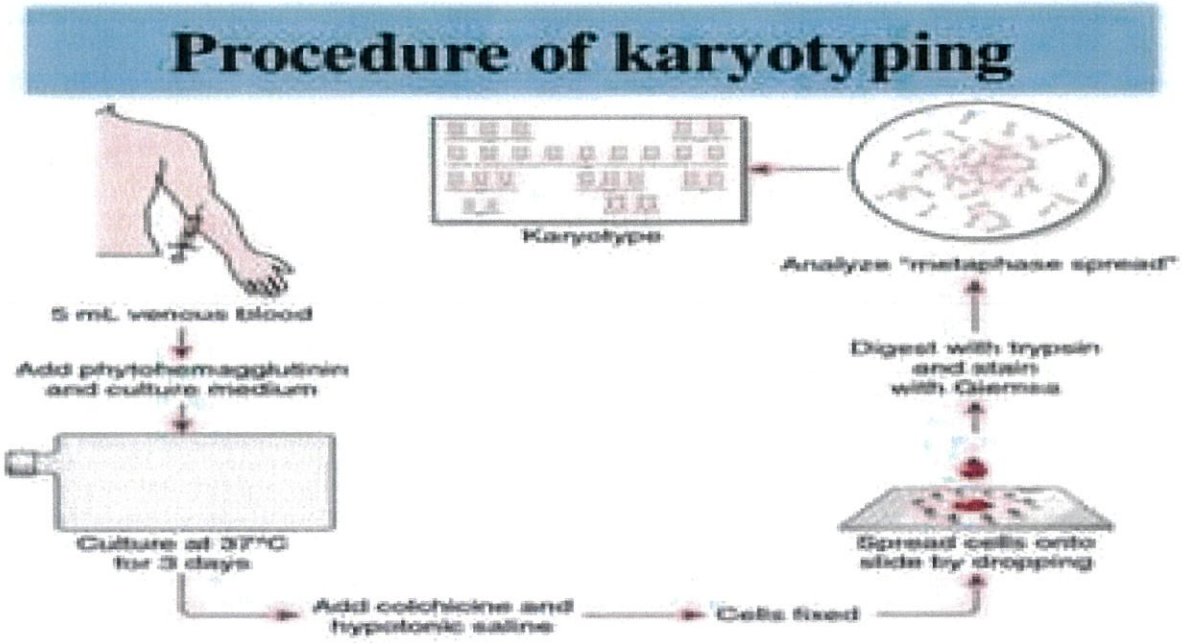
PBS 10X

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. The images of the chromosomes are then arranged and paired according to their size, shape, and banding patterns to create a karyotype. In a typical human karyotype, the 23 pairs of chromosomes are arranged in homologous pairs, with the sex chromosomes (XX for females and XY for males) often separated out.



By examining the karyotype, healthcare professionals can identify several types of genetic abnormalities, including:

1. Aneuploidy: This is the presence of an abnormal number of chromosomes, such as trisomy (three copies of a chromosome) or monosomy (one copy of a chromosome) in place of the usual two copies.

2. Translocations: These occur when a segment of one chromosome breaks off and becomes attached to another chromosome. This can lead to changes in gene expression and potential health implications.

3. Deletions and duplications: Portions of chromosomes can be missing (deletions) or present in excess (duplications), leading to disruptions in the normal genetic balance.

Karyotyping is an important diagnostic tool in prenatal testing, as it can identify chromosomal abnormalities in a developing fetus that may lead to conditions such as Down syndrome (Trisomy 21), Turner syndrome (45,XO), Edward syndrome (Trisomy 18), Klinefelter syndrome (47,XXY), Patau syndrome (Trisomy 13), Cri du chat syndrome and other genetic disorders. It is also used in the evaluation of infertility, recurrent pregnancy loss, and certain types of cancer, as well as in understanding the genetic basis of developmental and intellectual disabilities.

In addition to its clinical applications, karyotyping of cancer cells can help in identifying chromosomal abnormalities associated with specific types of cancer, aiding in diagnosis and treatment planning. It is also used in research to study genetic disorders and understand the role of chromosomes in human health and disease. Advances in technology have led to the development of more sophisticated methods for analyzing chromosomes, such as comparative genomic hybridization (CGH) and fluorescence in situ hybridization (FISH), which can provide more detailed information about specific chromosomal regions and genetic abnormalities.

Overall, karyotyping plays a crucial role in the diagnosis and understanding of genetic disorders, helping to guide patient management and inform genetic counseling. Its ability to visualize and analyze the structure and number of chromosomes provides valuable insights into the genetic basis of health and disease.

Conclusion:

The hands-on training for karyotyping provided us with a comprehensive understanding of the karyotyping process and equipped us with the necessary skills to perform chromosome analysis by combining theoretical knowledge with practical experience.

We extend our gratitude to **Dr. Posam ma'am** who contributed their expertise to the training program.