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 12(B) of the UGC Act, 1956

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

Best College UNIVERSITY OF MUMBAI

Department of Biotechnology

On Job Training Completion Report

This is to certify that BLESSY PAUL SANAMANDRA has completed On Job Training at

MAXIM CLINICAL LABORATORIES & RESEARCH CENTRE

Date of Commencement	Date of Completion	Total Number of Days	Total Number of Hours completed in OJT
14-01-2024	15-02-2024	26 DAYS	60 HOURS

Name of the Guide/ PI/ Incharge : Dilip Pandey

Phone Number of Guide/ PI/ Incharge : 0251-2325101

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

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

Gupta 22-04-23

MAXIM CLINICAL LABORATORIES & RESEARCH CENTRE Basement Riddhi-Siddhi Apt., Opp. ICICI Bank, Murbad Road, Kalyan (W) TEL.: 0251-2325101/102

Signature of Guide/ PI/ Incharge

Stamp

Date: 15th February 2024

TRAINING CERTIFICATE

This is to certify that MISS. BLESSY PAUL SANAMANDRA was doing her training in MAXIM CLINICAL LABORATORIES & RESEARCH CENTRE as a medical laboratory trainee. She has completed duration of 60 hours and during this period she had been working in all the department of laboratory.

She was found cordial, hard working and punctual.

Wishing her good prospect in future.

MAXIM CLINICAL LABORATORIES & RESEARCH CENTRE
Basement
Riddhi-Siddhi Apt.,
Opp. ICICI Bank,
Murbad Road,
Kalyan (W)
TEL.: 0251-2325101/102

Authorized Signatory

Haris Shaikh
(CEO)

Name: Blessy Paul Sanamandra
Roll No.: 421
Class: M.Sc. Biotechnology Part 1

Maxim Labs, Kalyan was where I gained insight during my On Job Training duration. There were various departments where I gained experience from and as they are mentioned below:
Microbiology:
This is the department where in the reports were sent after 2 days as the incubation time permits. The media preparation of BHI, Blood Agar, MacConkey Agar, Nutrient Agar was also done. The reporting process and the protocols are taken from CLSI guidelines. These test are performed to check certain bacterial infection and to find out the correct antibiotic for the same.

Test	Collection	Procedure	Instrument
Pus Culture	Collection done in a sterile container.	• Wet Mount is prepared and checked for pus cells, epithelial cells. • The sample is streaked on Blood Agar and MacConkey Agar. • Then kept in incubation for 24 hrs. • If growth of certain colonies are seen then a colony is picked up and diluted in BHI (Brain Heart Infusion Broth). • Then the loopful of culture is taken and spread on Nutrient agar plate for Antibiotic test is performed, and kept in incubation for 24 hrs. • Gram staining of the organisms from the colonies is also done.	Incubator
Urine Culture	Collection done in a sterile container. First urine of the day is taken and the midstream urine is preferred.	• The sample is streaked on Blood Agar and MacConkey Agar. • Then kept in incubation for 24 hrs. • If growth of certain colonies are seen then a colony is picked up and diluted in BHI (Brain Heart Infusion Broth). • Then the loopful of culture is taken and spread on Nutrient agar plate for Antibiotic test is performed, and kept in incubation for 24 hrs. • Gram staining of the organisms from the colonies is also done.	Incubator

Sputum Culture	Collection done in a sterile container.	• Wet Mount is prepared and checked for pus cells, epithelial cells. • The sample is streaked on Blood Agar and MacConkey Agar. • Then kept in incubation for 24 hrs. • If growth of certain colonies are seen then a colony is picked up and diluted in BHI (Brain Heart Infusion Broth). • Then the loopful of culture is taken and spread on Nutrient agar plate for Antibiotic test is performed, and kept in incubation for 24 hrs. • Gram staining of the organisms from the colonies is also done.	Incubator
Blood Culture	Collected in Sodium Heparin Tubes	• 5 to 7mL of sample is taken and inoculated or injected in the anaerobic culture vials. • Then kept for incubation for 24 hrs. • After incubation it is streaked on Blood Agar and MacConkey agar. • If growth of certain colonies are seen then a colony is picked up and diluted in BHI (Brain Heart Infusion Broth). • Then the loopful of culture is taken and spread on Nutrient agar plate for Antibiotic test is performed, and kept in incubation for 24 hrs. • Gram staining of the organisms from the colonies is also done.	Incubator
Tuberculosis	Collection done in a sterile container. For 3 Consecutive days from the patient.	• Sample is taken on a slide and heat fixed. • Ziehl Neelsen staining is performed. • The slide is checked under the microscope of 40X magnification for the presence of Acid fast bacilli	None

Serology:

Test	Collection	Procedure	Instrument
3H (HIV, HCV, HbsAg)	Blood sample is taken in clot activator or the plain red top tube.	Serum is then taken and a kit based method is used for the same.	Centrifuge: After the serum is separated to ensure that no RBCs interfere with the test.

Widal	Blood sample is taken in EDTA (Levram's Tube) or the purple top tube.	Whole blood from a patient is mixed with an antiserum containing antibodies against *Salmonella typhi*. If there are antibodies present in the blood sample from the patient, they will react with the antiserum and cause clumping or agglutination of red blood cells.	None
CRP (C Reactive Protein)	Blood sample is taken in EDTA (Levram's Tube) or the purple top tube.	It is a kit based method.	None
ICT (Indirect Coombs Test)	Blood sample is taken in clot activator or the plain red top tube.	• Serum is used as a sample and it is washed three times with saline. • Then the recipient's one drop of blood is added. • Then anti globulin is added to check the agglutination. • If the agglutination is seen then the test is positive and if not clots are then the test is negative.	Centrifuge: After the serum is separated to ensure that no RBCs interfere with the test.
DCT (Direct Coombs Test)	Blood sample is taken in EDTA (Levram's Tube) or the purple top tube.	• Blood is taken and diluted with the saline and then washed 3 times with saline. • Anti human Globulin is added. • Agglutination is checked.	Centrifuge is used for cell washing
Dengue NS1	Blood sample is taken in clot activator or the plain red top tube.	Serum is then taken and a kit based method is used for the same.	Centrifuge: After the serum is separated to ensure that no RBCs interfere with the test.
Typhoid (IgG, IgM)	Blood sample is taken in clot activator or the plain red top tube.	Serum is then taken and a kit based method is used for the same.	Centrifuge: After the serum is separated to ensure that no RBCs interfere with the test.

Hormones:

Chemiluminescence is a chemical reaction that emits energy in the for of light. When used in combination with immunoassay technology, the light produced by the reaction indicates the amount of analyse in a sample. Direct chemiluminescent reactions directly measure the light energy without the use of added steps or amplifying molecules. The ADVIA Centaur CP assays use acridinium

ester as the chemiluminescent label, since AE does not require the addition od a catalyst or substrate. The ADVUA Centaur CP system applies the immunoassay binding principles of antibodies using several formats.
Sandwich Format.
Competitive Format.
Antibody Capture Format.

Test	Collection	Procedure	Instrument
FSH	Blood sample is taken in clot activator or the plain red top tube.	Serum is taken from the tube and centrifuged and the serum is taken into vials to load in the machine.	Advia Centaur CP Immunoassay
LH	Blood sample is taken in clot activator or the plain red top tube.	Serum is taken from the tube and centrifuged and the serum is taken into vials to load in the machine.	Advia Centaur CP Immunoassay
T3, T4, TSH	Blood sample is taken in clot activator or the plain red top tube.	Serum is taken from the tube and centrifuged and the serum is taken into vials to load in the machine.	Advia Centaur CP Immunoassay
FT3, FT4, TSH	Blood sample is taken in clot activator or the plain red top tube.	Serum is taken from the tube and centrifuged and the serum is taken into vials to load in the machine.	Advia Centaur CP Immunoassay
Insulin	Blood sample is taken in clot activator or the plain red top tube.	Serum is taken from the tube and centrifuged and the serum is taken into vials to load in the machine.	Advia Centaur CP Immunoassay

Biochemistry:
Mispa Nano Plus Biochemistry analyser has a colorimetric based analysis with fully automated. Biochemistry analyser; utilising horizontal photometry in either single or bi-chromatic modes.

Tosoh Glycohemoglobin analyser: This instrument is intended to assay HbA1c based on the principle of high performance liquid chromatography, the analyser uses a cation exchange column to separate haemoglobin components by different ionic charge. The various fraction of haemoglobin, including HbA1c are quickly separated into 6 fractions and assayed. A step gradient with 3 different salt concentrations. The absorbances of the various haemoglobin components, separated in the column, are then continuously monitored by the detector. After the assay is complete, results for the various hemoglobin fractions are output to the printers as percentages along with the chromatogram.

Test	Collection	Procedure	Instrument
Blood sugar	Blood sample is taken in blue top tube.	The sample is taken and loaded in the machine.	Mispa Nano Plus Biochemistry Analyser
HbA1c	Blood sample is taken in EDTA (Levram's Tube) or the purple top tube.	The sample is taken and loaded in the machine.	Tosoh Glycohemoglobin Analyser
Urea	Blood sample is taken in clot activator or the plain red top tube.	The sample is taken and loaded in the machine.	Mispa Nano Plus Biochemistry Analyser
Bilirubin	Blood sample is taken in clot activator or the plain red top tube.	The sample is taken and loaded in the machine.	Mispa Nano Plus Biochemistry Analyser
Amylase	Blood sample is taken in clot activator or the plain red top tube.	The sample is taken and loaded in the machine.	Mispa Nano Plus Biochemistry Analyser
Total Protein	Blood sample is taken in clot activator or the plain red top tube.	The sample is taken and loaded in the machine.	Mispa Nano Plus Biochemistry Analyser

Others:

Test	Collection	Procedure	Instrument used
Vitamin D3	Blood sample is taken in clot activator or the plain red top tube.	Serum is taken from the tube and centrifuged and the serum is taken into vials to load in the machine.	Advia Centaur CP Immunoassay
Vitamin B12	Blood sample is taken in clot activator or the plain red top tube.	Serum is taken from the tube and centrifuged and the serum is taken into vials to load in the machine.	Advia Centaur CP Immunoassay

Mantoux test	None	• The area of injection that is on the forearm skin, it is first cleaned and dried. • 0.1mL to 0.2mL of tuberculosis antigen is injected in to the subcutaneous region. • After that the area is marked and the patient is told to come after 48hrs without touching the region marked.	None

Hematology:

Test Type	Collection	Procedure	Instrument used
Hb	Blood sample is taken in EDTA (Levram's Tube) or the purple top tube.	The sample is kept on rotator for two mins to ensure that the anticoagulant is properly mixed in with the sample and then it is loaded on to the machine.	Hematology Analyser
CBC	Blood sample is taken in EDTA (Levram's Tube) or the purple top tube.	The sample is kept on rotator for two mins to ensure that the anticoagulant is properly mixed in with the sample and then it is loaded on to the machine.	Hematology Analyser
ESR (Erythrocyte Sedimentation Rate)	Blood sample is taken in clot activator or the plain red top tube.	Westergren method: measures the distance (in millimeters) at which red blood cells in anticoagulated whole blood fall to the bottom of a standardized, upright, elongated tube over one hour due to the influence of gravity.	None
Malarial Parasite (Antigen Test)	Blood sample is taken in clot activator or the plain red top tube.	It is determined using a kit based method.	None
PTINR (prothrombin time/ international normalized ratio)	Blood sample is taken in blue top tube.	First the sample list taken and incubated with a reagent for 5 mins. Then another reagent is added and the time is noted from the clots formation starts.	Incubator: For 5mins

G6PD (glucose-6-phosphate dehydrogenase)	Blood sample is taken in EDTA (Levram's Tube) or the purple top tube.	Sample is loaded into the machine.	Mispa Nano Plus Biochemistry Analyser
Blood Group	Blood sample is taken in EDTA (Levram's Tube) or the purple top tube.	Anti A, Anti B, Anti D is added to each drop of blood on the slide and the coagulation or the agglutination of the same is checked.	None
Ammonia	Blood sample is taken in clot activator or the plain red top tube.		Mispa Nano Plus Biochemistry Analyser

Name: Blessy Paul Sanamandra

Roll No.: 421

Class: M.Sc. Biotechnology Part I

Background given during the Theoretical Lecture:

What is karyotyping?

Karyotyping, or blood chromosome analysis, is a highly useful test in the diagnosis and management of fertility issues. However, most people who undergo the test don't have a good understanding of why it is done or what the results reveal.The prefix "karyo" refers to the fact that the nucleus of the cell is studied, and the base word "type" refers to the fact that the test is a characterisation, or analysis of the character. Put together, the word karyotype means a characterisation of the cell nucleus.

How to identify Chromosomes?

The isolation and microscopic observation of chromosomes forms the basis of cytogenetics and is the primary method by which clinicians detect chromosomal abnormalities in humans. A karyotype is the number and appearance of chromosomes. To obtain a view of an individual's karyotype, cytologists photograph the chromosomes and then cut and paste each chromosome into a chart, or karyogram, also known as an ideogram.In a given species, chromosomes can be identified by their number, size, centromere position, and banding pattern. In a human karyotype, autosomes or "body chromosomes" (all of the non–sex chromosomes) are generally organized in approximate order of size from largest (chromosome 1) to smallest (chromosome 22). However, chromosome 21 is actually shorter than chromosome 22. This was discovered after the naming of Down syndrome as trisomy 21, reflecting how this disease results from possessing one extra chromosome 21 (three total). Not wanting to change the name of this important disease, chromosome 21 retained its numbering, despite describing the shortest set of chromosomes. The X and Y chromosomes are not autosomes and are referred to as the sex chromosomes.The chromosome "arms" projecting from either end of the centromere may be designated as short or long, depending on their relative lengths. The short arm is abbreviated p (for "petite"), whereas the long arm is abbreviated q (because it follows "p" alphabetically). Each arm is further subdivided and denoted by a number. Using this naming system, locations on chromosomes can be described consistently in the scientific literature.

Although Mendel is referred to as the "father of modern genetics," he performed his experiments with none of the tools that the geneticists of today routinely employ. One such powerful cytological technique is karyotyping, a method in which traits characterized by chromosomal abnormalities can be identified from a single cell. To observe an individual's karyotype, a person's cells (like white blood cells) are first collected from a blood sample or other tissue. In the laboratory, the isolated cells are stimulated to begin actively dividing. A chemical called colchicine is then applied to cells to arrest condensed chromosomes in metaphase. Cells are then made to swell using a hypotonic solution so the chromosomes spread apart. Finally, the sample is preserved in a fixative and applied to a slide.The geneticist then stains chromosomes with one of several dyes to better visualize the distinct and reproducible banding patterns of each chromosome pair. Following staining, the

chromosomes are viewed using bright-field microscopy. A common stain choice is the Giemsa stain. Giemsa staining results in approximately 400–800 bands (of tightly coiled DNA and condensed proteins) arranged along all of the 23 chromosome pairs. An experienced geneticist can identify each chromosome based on its characteristic banding pattern. In addition to the banding patterns, chromosomes are further identified on the basis of size and centromere location. To obtain the classic depiction of the karyotype in which homologous pairs of chromosomes are aligned in numerical order from longest to shortest, the geneticist obtains a digital image, identifies each chromosome, and manually arranges the chromosomes into this pattern.

At its most basic, the karyotype may reveal genetic abnormalities in which an individual has too many or too few chromosomes per cell. Examples of this are Down Syndrome, which is identified by a third copy of chromosome 21, and Turner Syndrome, which is characterized by the presence of only one X chromosome in women instead of the normal two. Geneticists can also identify large deletions or insertions of DNA. For instance, Jacobsen Syndrome, which involves distinctive facial features as well as heart and bleeding defects, is identified by a deletion on chromosome 11. Finally, the karyotype can pinpoint translocations, which occur when a segment of genetic material breaks from one chromosome and reattaches to another chromosome or to a different part of the same chromosome. Translocations are implicated in certain cancers, including chronic myelogenous leukemia. By observing a karyotype, today's geneticists can actually visualize the chromosomal composition of an individual to confirm or predict genetic abnormalities in offspring, even before birth.

Practical:

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 then treated with colcemid to arrest cells at metaphase stage. The cells are then harvested using the standard hypotonic treatment and fixation. Clinical indications include diagnosis of congenital abnormalities and for the genetic counselling of parents with congenitally abnormal infants, sex chromosome abnormalities, and habitual abortion studies. This procedure consists of mainly Planting, Termination, Fixation, Preparation of Slides, Banding Technique.

PROTOCOL:

I. Planting:

Principle:

This step is done to get the leukocytes to the log phase or the dividing phase wherein the chromosomes are clearly visible in the metaphase stage then the cells are arrested in the sam phase with the help of colchicine which is an antimitotic agent that inhibits mitosis by preventing the polymerization of tubulin, formed during the separation of the chromosomes towards the poles by the centrioles.

Instrumentation:

- Laminar Air Flow: The principle of laminar airflow involves the movement of air in parallel layers with uniform velocity and direction, typically in a horizontal or vertical direction. This creates a steady, unidirectional flow of air that helps to prevent the introduction of contaminants into sensitive areas or processes.
- CO_2 Incubator: A carbon dioxide (CO2) incubator is a specialized piece of equipment used in laboratories to provide a controlled environment for cell culture growth. The principle behind a

CO2 incubator involves creating an environment that mimics the conditions necessary for optimal cell growth, including temperature, humidity, and gas composition.

Protocol:

1. Remove Roswell Park Memorial Institute 1640 media, human serum and Phytohemagglutinin (PHA) from freezer.
2. Keep media and serum in the waterbath at 37^{o}_{C}
3. Leave PHA at Room Temperature
4. Clean the laminar hood with methanol swab.
5. Put on the UV light in the laminar hood for 30 mins.
6. Addition to be done in the laminar hood in the vial:

Syringe used (mL)	Material	For 1 Vial (mL)	Function
10mL	RPMI 1640 Media	4mL	Roswell Park Memorial Institute (RPMI) medium or RPMI 1640 is a form of medium used in cell culture and tissue culture. It has been used for growing a variety of mammalian cell lines and growth of human lymphocytes.
5mL	Human Serum	1mL	Cultivation of sensitive human cell types.
100uL	Phytohemagglutinin	0.2mL	Phytohemagglutinin may be used to stimulate mitotic division of lymphocytes maintained in a cell culture system and facilitate cytogenetic studies of the chromosomes.Phytohemagglutinin is an analyte specific reagent.
2mL	Blood (Collected in green top sodium heparin tube)	8 drops	Used as a Sample to be analysed.

7. Clean the mouth of all the vial with methanol and close the screw caps tightly, add a paraffin film to seal the vials.
8. Keep the vials in a petridish cleaned with methanol and keep in the incubator at 37 ° C for 72 hours.
9. At the 70^{th} hour add 0.1 ml of colchicine (10ug /ml) to each vial.
10. Mix properly and incubate for 1 hour.

II. TERMINATION

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

KCl: 0.560 gm in 100ml.Adjust pH at 7
Function of KCl: Potassium chloride solution is a hypotonic solution used to enlarge cells for the adequate spreading of metaphase chromosomes for karyotyping.

III. FIXATION

Principle: Fixative kills the cells and also preserves the structure of cell and its components. These fixed cells are then dropped on a chilled microscope slide, wherein the swollen cells burst and the chromosomes spread. The chromosome spreads are then dried and stained.

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

IV. PREPARATION OF SLIDES:

Instrumentation: Visible Light Microscope
The actual observation of the prepared karyotyping slides is usually done using a bright-field light microscope. This type of microscope allows for the visualization of the stained chromosomes and their banding patterns, which are used to identify and analyze the structure and number of chromosomes present in the sample.

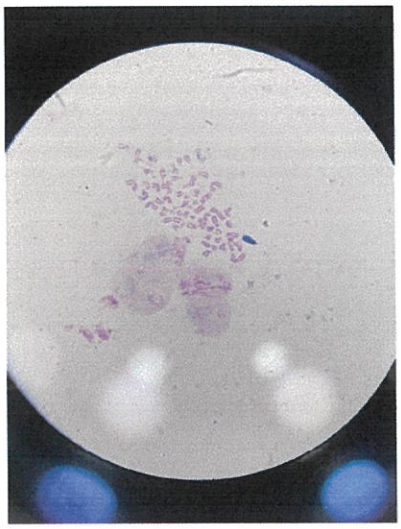
PREPARATION OF STOCK GIEMSA STAIN:

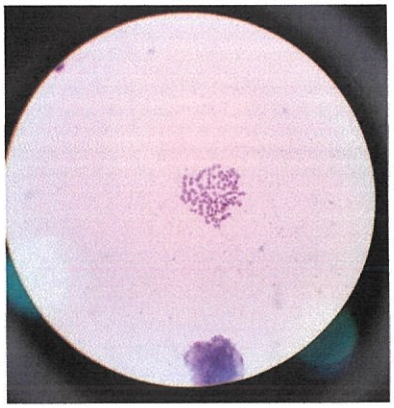
1. Take 1gm of giemsa powder & 50ml glycerol.
2. Grind it slowly adding glycerol and make it to a paste.
3. Then add methanol slowly (50ml) to make a giemsa stock.
4. Keep it in incubator at 37° C over-night.
5. Next day filter through filter paper No.41 and preserve it in refrigerator to age for 1 month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

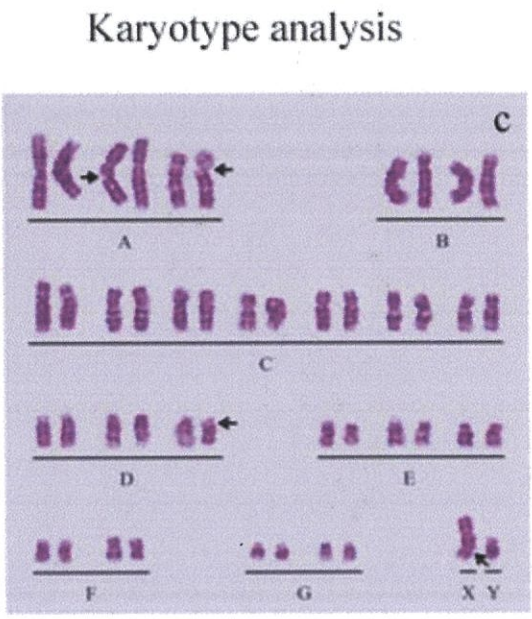
Protocol:

1. Next day, the tubes are removed from the refrigerator centrifuged, and 2-3 washes are given with fixative till the pellet becomes white.
2. At the end discard the supernatant leaving behind fixative till 0.5 ml.
3. Keep the hot plate at 40°C, take acidified chilled slides, (slides kept in cold water in refrigerator).
4. Mix the pellet thoroughly and drop it on the cold slide from a height and keep on the hot plate to heat dry.
5. Remove the slide, stain with plain Giemsa, then clean the slides after staining for about 20 mins and see the slides under the microscope.

Observation:





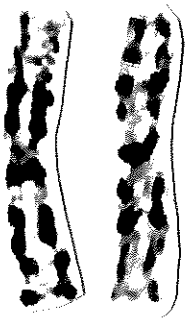


Result:
The chromosomes were arranged accordingly and the analysis was that there were no genetic abnormalities seen in the karyotype of the chromosomes. Using this method of culturing the cells and then analysing them and forming the karyotype for the same can be utilised for the further research purposes and clinical studies or for the diagnosis of various genetic abnormalities.

Conclusion: Chromosome analysis is a conventional technique utilised worldwide to diagnose chromosome instability and rearrangements leading to genetic disorders and malignancy. In addition, a higher resolution for the diagnosis and research of constitutional and cancer-acquired genetic abnormalities can be achieved with the combination of the classical cytogenetic procedures and molecular cytogenetic methodologies such as fluorescence in situ hybridisation (FISH), comparative genomic hybridisation (CGH), and spectral karyotyping (SKY). More recently, these

techniques have been utilised for the evaluation of chromosome instability associated with stem cell research. Karyotypic abnormalities such as aneuploidy of long-term cultured embryonic cells (ES) and adult stem cells of various organisms have been reported by multiple laboratories. Recent evidence supports that some cell lines are inherently more inclined to chromosome instability regardless of culture conditions. For this reason, when establishing and/or maintaining human, mouse, or Rhesus stem cell lines, chromosome analysis is recommended as part of the quality control process. Many reports describe the increasing interest in the use of routine and molecular cytogenetics to monitor the chromosomal stability of stem cells and malignant cells or various organisms in culture. These protocols are increasingly being used by non-genetic laboratories for rapid chromosomal assessment of their cultured cells. These basic procedures for chromosome preparation from leukocytes, which can be applied for both clinical and research purposes.

Human Karyotype



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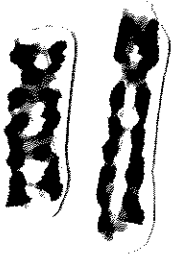

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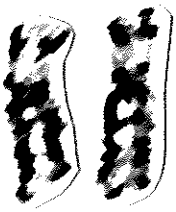

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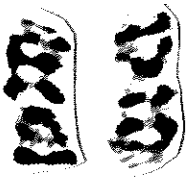

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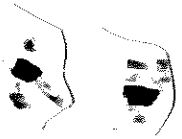

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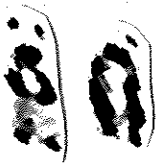

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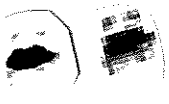

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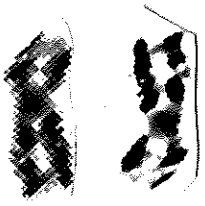

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