

INTERNSHIP REPORT

Submitted to

SATHYABAMA INSTITUTE OF SCIENCE AND TECHNOLOGY

In partial Fulfilment for the Award of The Degree Of

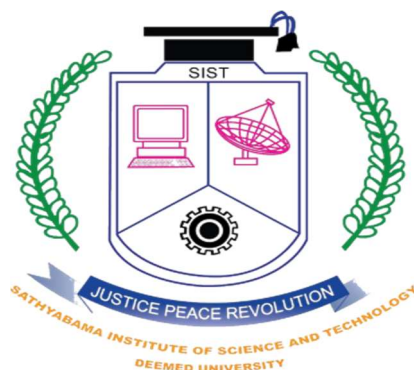
BACHELOR OF SCIENCE

In

MEDICAL LABORATORY TECHNOLOGY

By

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DEPARTMENT OF MEDICAL LABORATORY TECHNOLOGY

SCHOOL OF ALLIED HEALTH SCIENCES

SATHYABAMA

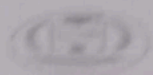
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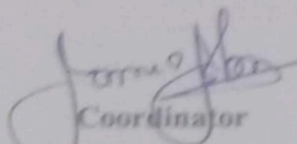
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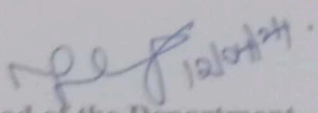
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BONAFIDE CERTIFICATE

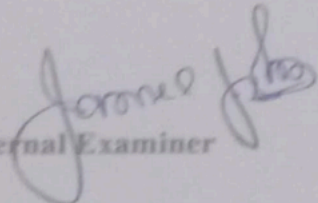
This is to certify that this internship report is the Bonafide work of Shakeera banu . k who have done the Internship work as a team and carried out the training Internship at Chengalpattu Medical College in Chengalpattu and lions blood bank, at Ambattur under our supervision from August 2023 to April 2024.

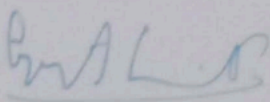

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DECLARATION

I, Ms Shakeera banu . k (41737030), hereby declare that the internship trainings done by me at Lion Blood Bank Research Foundation at Ambattur under the guidance of Ms. D. Jasmine Priya and Chengalpattu Medical college at Chengalpattu under the guidance of Dr. James John at Sathyabama Institute of Science and Technology is submitted in partial fulfilment of the requirements for the award of Bachelor of science degree in Medical Laboratory Technology.

Date: 12.04.2024

Place: CHENNAI

Kg shakeera Banu

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ACKNOWLEDGEMENT

We are pleased to acknowledge our sincere thanks to Board of Management of **Sathyabama Institute Of Science And Technology** for their kind encouragement in doing this internship program and for completing it successfully. We are grateful to them.

We would like to express our sincere and deep sense of gratitude to our Head of the Department **Dr. J. Premkumar** and Course Coordinator **Dr. James John** and the for their valuable guidance, suggestions and constant encouragement paved way for the successful completion of our internship report.

We wish to express our thanks to all Teaching and Non-teaching staff members of the Department of Biomedical Engineering who were helpful in many ways for the completion of the internship.

We would like to express our thanks to our parents for their unwavering support to us during these years.

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I am worked in **HISTOPATHOLOGY LABORATORY** - Chengalpattu Medical college, laboratory during my internship training period on 01st November 2024 and 31st November 2024 from 09am to 04pm

HISTOPATHOLOGY

INTRODUCTION:

A study of normal tissue by microscope is histology.

Histology is the science of examination of normal tissues at microscopic level .Histopathology is examination of disease tissue for presence or absence of change in the structure due to disease process

Both are done by examining thin section of tissues which are colour differently by dice and strains

1. TISSUE PROCESSING :

A procedure which need to take place after gross examination between tissue fixation and the embedding and then sectioning of paraffin blocks is tissue processing.

SPECIMEN ACCESSIONING:

Tissue specimen received in the surgical pathology have a request form that list the patient information and history along with a description of the site of origin. The specimen are accessioned by giving them a number that ill identify each specimen for each patient and maintaining a registrar book.

GROSS EXAMINATION:

Tissue removed from the body for diagnosis arrive in pathology department and are examined by a pathologist, pathology assistant. When a malignancy is suspected, then the specimen is covered with ink in order to mark the margins of specimen. different coloured inks can be used to identify the different area if needed. when section are made and processes ,the ink will mark the actual margin on the slide. Gross examination consists of describing the specimen and placing all or parts of it by cutting a sharp knife in case of soft tissue or by a saw in case of hard tissue like bones or cartilage into small plastic cassette which hold the tissue . the cassette are placed in a fixative



Fig : 1.1 Gross station

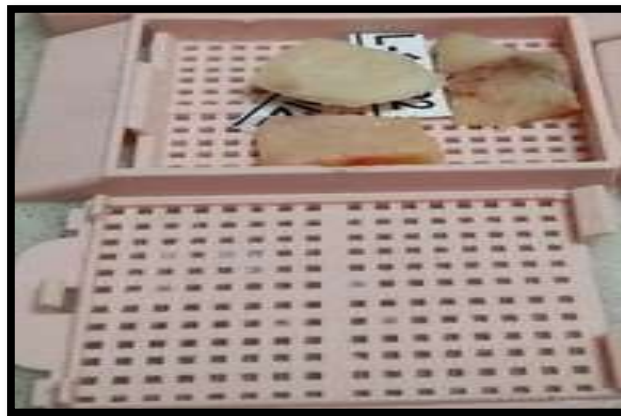


Fig : 1.2 Tissue cassette

TISSUE PROCESSING :

In modern laboratory the process of dehydration, clearing and impregnation are carried out in a composite equipment in which is known as automated tissue process.

AUTOMATED PROCESSING SCHEDULE:

1	80% alcohol(holding point)	1 hour
2	95% alcohol	2 hour
3	95% alcohol	1 hour
4	100% alcohol	1 hour
5	100% alcohol	1 hour
6	100% alcohol	1 hour
7	Chloroform	1 hour
8	Chloroform	1 hour
9	Chloroform	1 hour
10	Paraffin wax	2 hour
11	Paraffin wax	2 hour
12	Paraffin wax	2 hour



Fig : 1.3 Tissue processor

FIXATION:

- i. Any tissue removed from the body starts decomposing immediately because of loss of blood supply and oxygen accommodation of product of metabolism autolize enzyme.
- ii. This process of decomposition is prevented by fixation.
- iii. Fixation is the method of preserving cells and tissue in life like condition as far as possible.
- iv. During fixation tissues are fixed in complete physical and chemical state most fixative act by denaturation or precipitation of cells protein or by making soluble components of cell insoluble.

TYPES OF FIXATIVE:

- ❖ Simple - one formalin
- ❖ Compound- Two or more subs-bouins+zenkers

GROUPS:

- ❖ Microanatomical -anatomy
- ❖ Cytological- intracellular constituents
- ❖ Histochemical-enzymes

EXAMPLE FOR FIXATION

- ❖ Formalin
- ❖ Gultaraldehyde
- ❖ Picric acid
- ❖ Alcohol
- ❖ Osmium tetroxide

FORMALIN

- i. It is a most commonly used fixative in routine practice

- ii. It is commercially available as saturated solution of formaldehyde gas in water 40% by weight for all practical purposes
- iii. For been examination 10% of formalin is used.
- iv. It is prepared by dissolving 10ml of commercially available formalin in 90ml of water.
- v. Duration of fixation depends upon the size and thickness of the tissues, type of tissues and density of tissues.
- vi. It takes 6 to 8 hours for fixation of thin piece of tissue 4mm at room temperature
- vii. Formalin acts by polymerization of cellular proteins by forming methylene bridges between molecule.

GLUTARALDEHYDE

This is used fixative electron microscopic is used as 4% solution stored at 4`c and the tissue should remain immersed in it for 4 hours for fixation.

PICRIC ACID (BOUIN'S FLUID)

- 1. This is uses a fixative renal and testicular biopsies
- 2. Picric acid stains at tissues yellow. It also a good fixative for demonstration of glycogen.
- 3. It is prepared as saturated picric acid 375ml,40% formaldehyde and 125ml, glacial acetic acid 125ml.

DISADVANTAGE:

- ✧ It makes the tissues harder and brittle
- ✧ Causes of lysis RBC

CARNOY'S FIXATIVE (ALCOHOL)

- 1. Alcohol is mainly endometrium ,it acts bind denaturation of cell protein both ethyl ,methyl alcohol can be used
- 2. Methyl alcohol as used 100%solution for 20 to 30 mins.
- 3. Ethyl alcohol can either used as 95% solution as carnoy's fixative for tissue.
- 4. Absolute alcohol
- 5. Chloroform 150ml
- 6. Glacial acetic acid 50 ml
- 7. Carnoy's is good fixative for glycogen and dissolve fat.

OSMIUM TETROXIDE

- 1. It is used as fixative for neurological tissues and for electromicroscopic.
- 2. Osmium tetroxide is best fixative for lipids.

2.DEHYDRATION:

First the water from the tissue from the tissue must be removed by dehydration. This is usually done by 1 hour 30 minutes each series of alcohol from 70% to 80% to 95%.

3. CLEARING:

1. This is a process in which alcohol from the tissue and cell is removed at it is replaced fluid in which wax is soluble.
2. It also makes the tissue transparent
3. Xylene is the most commonly used clearing agent.
4. Toulene
5. Benzene (carcinogenic)
6. Chloroform (poison)
7. Cedarwood oil (more expensive & very viscous which can be also used as clearing).

IMPREGNATION :

- This is a process in which empty spaces in the tissues and cells after removal of clearing agent or taken up by molten paraffin wax.
- This hardens the tissue which helps in section cutting. impregnation is done in molten paraffin wax .which has a melting point ranging from 54 to 62°C.

EMBEDDING

- Embedding of tissue molten wax, blocks can be conventionally prepared using metallic moulds.
- Now a days plastic moulds of different colours for blocking are also available.
- The moulds are placed over a smooth surface so molten wax is poured into the cavity in the moulds.
- The processed tissue pieces are carefully placed into wax with number tags and the examining surface interest facing downwards.
- Wax is allowed to solidified, after solidified the L moulds are removed.



Fig : 1.4 Embedding processor



Fig : 1.5 Moulding processor



Fig : 1.6 Tissue blocks

SECTION CUTTING

MICROTOMY:

Microtome is an equipment for cutting section and the technique of section cutting is called microtomy.

Paraffin section: A large proportion of histopathological examination makes use of paraffin wax processing for the production of tissue sections.

ROTARY MICROTOME:

1. It is most commonly used microtome in this the microtome knife is fixed while the tissue block is movable.
2. Two types of cutting device are used.

Fixed knife or disposable blades.

3. Fixed knife is made up of stainless steel. The knife is sharpened by a process known as honing stropping.
 4. Honing is done manually on stone or an electrically operated automated hone. after honing stropping is done over a level strop.
 5. The process of sharpening of microtome knife can also be done by automatic knife sharpener.
- Now a days disposable blades for microtome are also available which may be high profile or low profile.



Fig : 1.7 Section cutting

TISSUE FLOTATION BATH

Tissue flotation bath is required after the step of cutting paraffin section and before these are placed on slides. it lets tissues relax and smoothen before being mounted on slides as well as makes paraffin stick to the slide. this ensures removal of wrinkles and folds before sections are placed on the slide.



Fig : 1.8 Tissue floating bath



Fig : 1.9 Tissue slide

MAKING SLIDE AND NUMBERING:

Sections tissue pick up on a glass microscopic slides from water bath and placed in a hot air oven for 15 minutes to help the sections adhere to the slides. is then numbered by non removable ink.

STAINING

CLEARING:

Rinse in xylene for 10 dips to remove wax. Then rinse in graded alcohol (70%,80%,95%) for dips each to remove xylene.

PROCEDURES OF HEAMATOXYLIN AND EOSIN STAIN

NUCLER STAINS

HEMATOXYLIN EHRLICH'S:

- 2% haematoxylin in alcohol 100ml
- 3% ammonium or potassium alum in distilled water 100ml
- Mix the two above and add the following in order
- Glycerol 100ml
- Acetic acid 10ml
- Keep the bottle loosely plugged
- Let it ripper for 1-3 months
- Hematoxylin harris

WEIGERT'S IRON HEMATOXYLIN

- Haematoxylin 1 gm
- Alcohol 100ml
- Let it ripen for a week
- 30% solution of ferric chloride 4ml
- Distilled water 100ml
- Hydrochroloric acid conc.1ml

MAYER HEMATOXYLIN

- Hematoxylin 1 gm
- Distilled water 1000ml
- Heat distilled water to 55 to 60.c and add hematoxylin rotate till dissolved
- Ammonium or potassium alum 50 gms
- Sodium iodate 0.20gm (to be weighed exactly)

- Add the above in order given
- Chloral hydrate 50.0gm

EOSIN

- Most suitable stain to combine with alum hematoxylin.
- Eosins are **xanthine dyes (tetrabromofluorescein)**
- TYPES OF EOSIN - commercially available

1. Ethyl eosin
2. Eosin B
3. Eosin Y

EOSIN Y:

- Eosin yellowish
- Most widely used
- It is water & alcohol soluble
- Used as a cytoplasmic stain-0.5-1% solution in distilled water with a crystal of thymol - prevent fungal growth.
- Addition of acetic acid sharpens the staining.

ETHYL EOSIN (eosin alcohol - soluble)

EOSIN B (eosin bluish, erythrosine)

PROCEDURE OF H&E STAINING

1. Deparaffinize with xylol: 5–10 minutes.
2. Absolute ethyl alcohol: 5 minutes.
3. 95% ethyl alcohol: 5 minutes.
4. 80% ethyl alcohol: 5 minutes.
5. 60% ethyl alcohol: 5 minutes.
6. Bring sections to water.
7. Harris hematoxylin: 15 minutes.
8. Rinse with tap water.
9. Differentiate with 1% hydrochloric acid in 70% ethyl alcohol (check with microscope to see background is colorless or pale gray; nuclei should show chromatin).
10. Wash with tap water.
11. 'Blue' with running tap water: 10–20 minutes.
12. Stain with watery eosin: 15 seconds.
13. Depending on the color required, rinse in water: 2 minutes.
14. 80% ethyl alcohol: 2 minutes.
15. 95% ethyl alcohol.
16. Absolute alcohol: 2 changes.
17. Xylene: 2 changes.
18. Mount with Entellan [dibutyl phthalate in xylene (DPX)/Canada balsam].

Results

- Nuclei: Blue to blue-black
- Cytoplasm and other substances: Pink
- Muscle fibers: Deep pink/red
- Red blood cells: Orange
- Fibrin: Deep pink.

ADVANTAGES

- Simple procedure
- Cost-effective
- Pleasing colors
- Most histological structures or by products can be located and studied

DISADVANTAGES :

Dehydration after staining is very important:

- ❖ To increase capability of viewing of the material to be examined (one may not be able to get 'close' to the slide with a high dry or oil immersion lens with improper dehydration and one may think the section is 'thick').
- ❖ To help preserve the stains in the slide. Remember all stains used are water soluble and any retained water in the final stages of dehydration will lead to 'fading' of the stain after a few weeks or month



Fig: 1.10 Staining processor



Fig : 1.11 staining slides

Mounting Media

The final step of preparation of stained section is to cover the slide surface containing tissue with a thin coverslip. This makes the slide permanent and permits microscopic

examination, whenever possible. The refractive index of the mounting medium must be approximately equal to that of dried protein, i.e. between 1.53 and 1.54. This is in order to provide the maximum degree of transparency to the stained tissue sections. To be effective, a mountant should possess certain characteristics. **These include the following:**

- It should be colorless and transparent
- It should have no adverse effect on tissue components
- It should be able to completely permeate and fill tissue interstices
- It should protect the section from physical damage and chemical activity (oxidation and changes in pH)
- It should be resistant to contamination
- It should be completely miscible with the dehydrant or clearing agent
- It should set without crystallizing, cracking or shrinking (or otherwise deform the material being mounted) and not react with leach or induce fading of stains
- Once finally set, the mountant should remain stable.



Fig : 1.12 DPX mounting

Papanicolaou (PAP) Staining

The objective of this practical is to demonstrate the Papanicolaou staining technique for the cytological examination of cellular samples.

INTRODUCTION: PAP staining is a commonly used staining technique in cytology to differentiate and visualize various cellular components. It is particularly useful for the examination of cervical smears and other body fluid specimens. Here's a lab protocol for performing PAP staining:

MATERIALS:

- Glass slides with cytological smears
- Papanicolaou staining kit (PAP stain kit)
- Hematoxylin solution
- Orange G solution
- EA-36 (Eosin Y) solution
- OG-6 (Light Green SF Yellowish) solution
- Ethanol (70% and 95%)
- Distilled water
- Microscope
- Coverslips

PROCEDURE:

1. Preparation of Smears: Prepare the cytological smears on clean glass slides. The type of specimen will depend on the purpose of the examination (e.g., cervical smear, body fluid smear).
2. Fixation: Fix the smears in 95% ethanol for about 10-15 minutes. Fixation helps preserve the cellular morphology and prevents cell loss during subsequent staining steps.
3. Hematoxylin Staining: a. Immerse the slides in Hematoxylin solution for 3-5 minutes. This step will stain the nuclei and other acidic structures within the cells. b. Rinse the slides with running tap water to remove excess Hematoxylin.
4. Differentiation: a. Place the slides in 70% ethanol for a few seconds for differentiation. b. Rinse the slides with distilled water to remove excess ethanol.

5. Orange G Staining: a. Cover the slides with Orange G solution and let them stand for 3-5 minutes. Orange G stains the cytoplasm and other basic structures in the cells. b. Rinse the slides with distilled water to remove excess Orange G.
6. EA-36 (Eosin Y) Staining: a. Cover the slides with EA-36 solution for 1-2 minutes. EA-36 is a counterstain that highlights the red blood cells and other structures. b. Rinse the slides with distilled water to remove excess EA-36.
7. OG-6 (Light Green SF Yellowish) Staining: a. Cover the slides with OG-6 solution for 1-2 minutes. OG-6 stains the background, aiding in the visualization of cellular details. b. Rinse the slides with distilled water to remove excess OG-6.
8. Dehydration: a. Gradually dehydrate the slides by placing them in increasing concentrations of ethanol (95% and 100% ethanol). This step removes water from the slides.
9. Clearing: a. Clear the slides by placing them in xylene or a xylene substitute. Xylene makes the slides transparent, which is necessary for mounting.
10. Mounting: a. Place a small drop of mounting medium on the smear. b. Carefully place a coverslip over the smear, avoiding air bubbles.
11. Drying: Allow the slides to dry completely at room temperature before examining them under a microscope.
12. Microscopic Examination: Examine the stained smears under a microscope using various magnifications. Observe and document the cellular details, such as nuclear morphology, cytoplasmic characteristics, and any abnormal cellular features.



Fig : 1.13 PAP staining

FIXATION

Choice of fixative and technique is depended on specimen types and analyzed in the laboratory .The stain offered are suitable for both Gyn (EA-36,EA-50) and non Gyn(EA-65,EA-EA50) specimen as determined by the user

RESULT

- Hematoxylin-nuclei-blue/black
- Light green SF-cytoplasm-blue/green
- OG-6-Keratinizing cells-pink/orange
- EosinY-Squamous cell,nucleoli, RBC's-red/pink

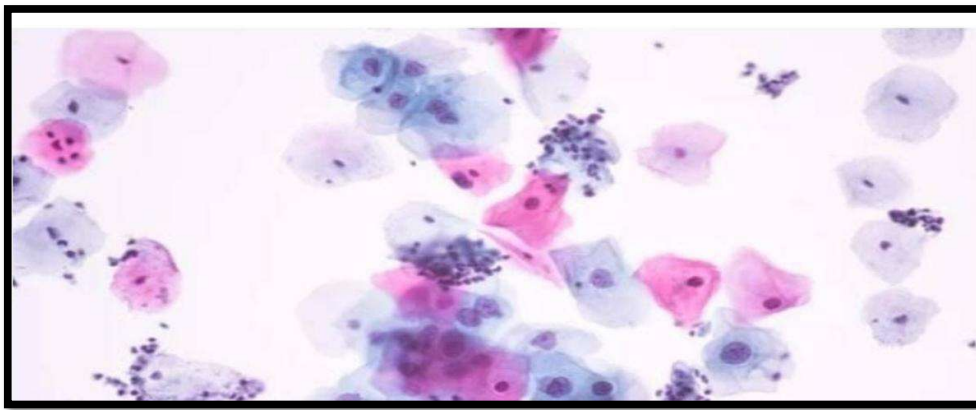


Fig :1.14 fixation results

MICROBIOLOGY;

Microbiology is the study of the biology of microscopic organism-viruses, bacteria algae, fungi, slime molds, and protozoa. The methods used to study and manipulate these minute and mostly unicellular organism differ from those used in most other biological investigation.

Microbiology encompasses numerous branches including virology, bacteriology, protistology, mycology and parasitology.

Eukaryotic microorganism possess membrane bound organelles and include fungi and protists. Where prokaryotic organism, all which are microorganism are conventionally classified as lacking membrane-bound organelles and include bacteria and Archaea.

These micro-organisms may be harmful or beneficial.

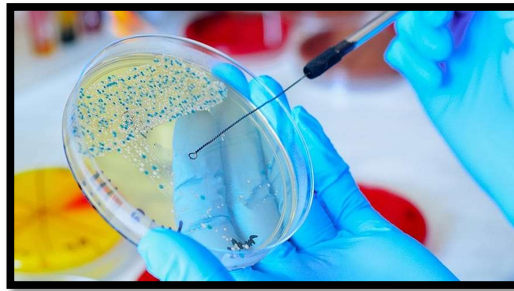


Fig :2.1 streaking plates

DIAGNOSTIC TESTS

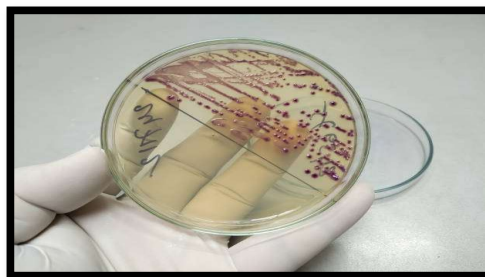


Fig : 2.2 UTI

UTI

- Chronic pyelonephritis
- Cystitis
- Urethritis
- Prostatitis
- Urine culture

FAECAL CULTURE TEST:

- Diarrhoea
- Typhoid
- Food poisoning



Fig :2.3 Faecal culture test

SPUTUM CULTURE TEST

- Tuberculosis
- Pneumonia
- Bronchitis
- Cellulitis



Fig : 2.4 blood culture plate

BLOOD CULTURE TEST

- Bacteraemia
- Sepsis
- Septic shock

MEDIA PREPARATION

CLED agar (cysteine lactose electrolyte deficient)

It is a non-selective differential plating medium for the growth and enumeration of urinary tract microorganism.

PREPARATION:

36.0g of medium is suspended in one liter of distilled water, slowly heated and frequently stirred. Boiled for a minute and sterilized at 121 °C for 15 minutes and poured into petri dishes. Plates were inverted when solidified for storage purposes and to avoid moisture.

MACCONKEY AGAR

Most commonly used for enterobacteriaceae. It contains agar, peptone, sodium chloride, bile salt, lactose and neutral red. It is a selective medium.

SELECTIVE :

As bile salt does not inhibit the growth of enterobacteriaceae but inhibits growth of many other bacteria.

INDICATOR ;

Bacteria that ferment lactose take a pink colour due to production of acid. Acid turns the indicator neutral red to pink. These bacteria are called 'lactose fermented', e.g. *Escherichia coli*. Colorless colony indicates that lactose is not fermented.

PREPARATION:

50g of the agar was suspended and measured into one litre of purified water and mixed thoroughly and was heated with frequent agitation, then boiled for one minute to completely dissolve the powder. The agar was autoclaved at 121 °C for 15min.

CHOCOLATE AGAR:

It is prepared by heating blood agar. It is used for culture of *meningococcus*, *pneumococcus*, *gonococcus*, and *haemophilus*.

PREPARATION:

2 liter of distilled water was added to 144g of agar powder. Autoclaving was done at 121 celcius for 15minutes and cooled till 45°C then 5% of defibrinated blood was add. Heated slowly and evenly to 65°C ,cooled till 45°C and poured into plates

BLOOD AGAR:

It use most commonly. For blood agar preparation 5-10% defibrinated sheep blood is mixed with melted agar at 45-50°C blood work as an enrichment material and also act as an indicator. Bacteria when grown in blood agar produce hemolysis around their growing colonies. Some bacteria produce no hemolysis.types of changes.

A) Beta haemolysis, there is clear zone around bacterial colony of complete hemolysis, e.g. streptococcus pyogenes is a beta hemolytic streptococci.

B) Alpha haemolysis, when there is greenish discolouration around bacterial colony colony due to formation of biliverdin, e.g. viridans streptococci

C) Gamma haemolysis,or,no hemolysis. There is no change in the medium near the bacterial colony.

PEPARATION AND STERILIZATION CULTURE MEDIA

Culture media are available commercially as powder, they require only the addition of water, nutrient medium is a general purpose preparation for culturing microorganism which are not nutritionally fastidiou. The agar has the same composition.

AUTOCLAVING



Fig : 2.5 Autoclave

Autoclaving is a process that use moist heat and pressure so the all part of the material to be sterilized reach 121 °C for 15 minutes. An autoclave is in essence, a large pressure cooker a chamber which may sealed off against surrounding air

OBJECTIVE

To prepare sterile nutrient agar for culturing microorganism.

MATERIAL AND REAGENTS

- Commercial nutrient agar
- Balance
- Distilled water
- Scott bottles
- Measuring cylinder beaker
- Forceps
- Universal bottles

PROCEDURE

Appropriate amount of broth (with agar) powder is weighed into scott bottles and dissolve with distilled water. They are mixed well.



Fig : 2.6 media preparation

- The bottle are loosely recap and set aside for sterilization
- All the media are sterilized at 121 °C for 15minutes
- After autoclaving he media is removed. The broth preparation is allowed to cool and the cap of each bottle is tightened

TSI (TRIPLE SUGAR IRON) TEST

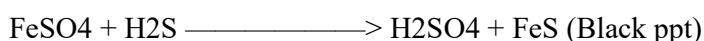
OBJECTIVE

- To check the ability of organism to ferment sugar (lactose, sucrose) and their ability to produce H₂S gas.

PRINCIPLE OF TSI TEST:

- Triple Sugar Iron Agar (TSIA) contains 3 sugar glucose (dextrose) sucrose, lactose in the ratio of 1:1:10 as the source of carbon as its name suggest. Besides this the media also contains yeast and beef extract, peptic digest of animal tissue and casein as the source of protein.
- This test is used to differentiate gram negative enteric. Enter bacteria and other glucose fermenter first begin to metabolize glucose as glucose utilizing enzymes are present constitutively and bacteria can gain the most energy by using simplest sugar.
- All other sugar must be converted to glucose before they enter the EMP. Glucose utilization occur both aerobically on the slant where O₂ is available and on the butt where there is anaerobic condition. Once the glucose fermenting bacterium has reduced all of the available glucose to pyruvate, it will further metabolize pyruvate via TCA cycle to produce acid end products. The acid in the medium cause the pH indicator phenol red, to change its colour to yellow. Thus, after 6 hours of incubation both the slant and butt of TSIA that has been inoculated with a glucose fermenter will appear yellow.
- After the depletion of limited glucose in the medium, the organism that has enzymes to degrade lactose or sucrose starts to utilize them as they are present 10 times more concentration than glucose and the organism continue to make acid end products.
- Hence, the butt and slant of TSIA will still remain yellow for lactose and sucrose fermentors (degrades) after 18 hours of incubation and the reaction is called acid/acid.
- If the organism being tested cannot use the lactose or sucrose in the medium, it must shift to protein utilization as there is no other source or energy in the media.

- As the protein breakdown occurs only in aerobic condition, the byproduct of protein and amino acid metabolism i.e NH_3 changes the pH environment of the medium from the neutral to alkaline in the slant region where the condition is aerobic. Hence, the colour of slant appears red and the reaction is called alkali/acid while the colour of the butt is still yellow due to anaerobic glucose breakdown.
- Gas production can be detected when holes are formed or the medium is broken into several fragments. H_2S production by organism results in the blackening of the medium. This colour is due to the production of H_2S from an ingredient of the medium, sodium thiosulphate, which then combines with another component of the medium, ferrous ammonium sulphate resulting in the formation of black, insoluble compound, ferrous sulphide.



REQUIREMENTS

- TSIA slant
- Given sample of bacterial cultures (E.coli and Klebsiella)
- Inoculating wire
- Burner
- Incubator.

PROCEDURE OF TSI TEST

1. Take 5-7 ml of the autoclaved TSIA media in a sterile test tube and slant was made by tilting the media till it solidified.
2. Using a flamed inoculating loop, pick a colony from the bacterial culture.
3. Inoculate the organism in TSIA slant by stabbing in the butt and then with the same loop streaking on the whole slant surface of the medium
4. Incubate at 37°C for 18-24 hours.
5. Observe for the color change in the slant and butt, gas production and H_2S production.

RESULT INTERPRETATION

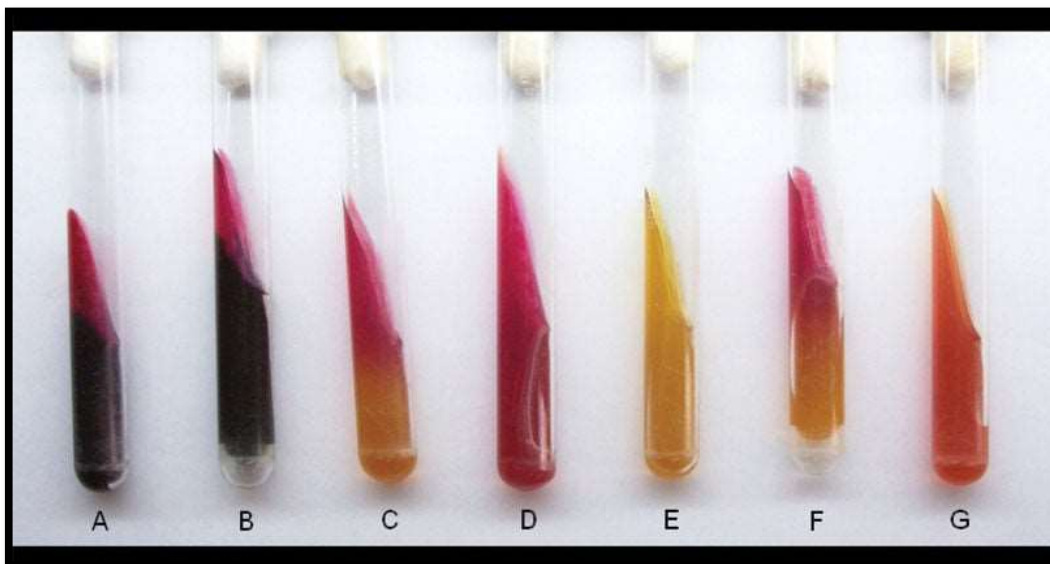


Fig : 2.7 TSI

Color observed	Ph (slant/butt)	Utilization
Red /yellow	Alkali/acid	Glucose only peptone
Yellow/yellow	Acid/ acid	Glucose, lactose and sucrose
Red/red	Alkali/alkali	No sugar are utilized, utilize peptone

H₂S gas production is indicated by blackening of media

Gas production is indicated by bubbles

GRAM STAINING

Gram stain is a method of staining used to classify bacterial species into two large groups: gram - positive and gram negative bacteria. It may also be used to diagnose a fungal infection . gram staining differentiates bacteria by the chemical and physical properties of their cell wall.

PRINCIPLE

The basic principle of gram staining involves the ability of the bacterial cell wall to retain the crystal violet dye during solvent treatment.

PROCEDURE

1. Make a smear & dry thoroughly in cool air fix the dried film by passing it briefly through a bunsen flame.
2. Flood the slide with crystal violet . for upto 1 min wash off briefly with tap water & drain
3. Flood the slide with gram's iodine & allow to act as a mordant for about 1 min wash off tap water & drain
4. Decolourise the smear with acetone for 10-30 sec & immediately wash off with water
5. Flood the slide with safranin & counterstain for about 30 sec wash off with tap water, drain and blot dry with paper
6. Examine under oil immersion objective

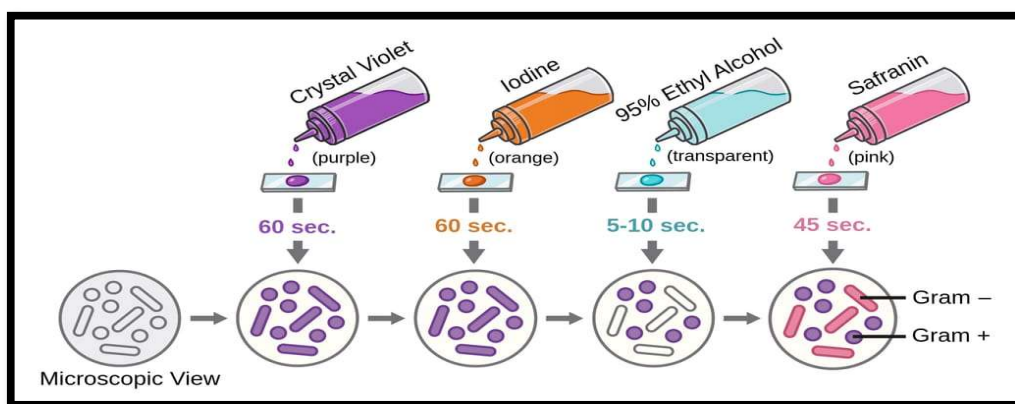


Fig : 2.8 Gram staining

SEROLOGY

Antibody serology test check for the presence or level of specific antibodies in the blood. Antibodies are proteins that your immune system makes to fight foreign substances. These substance are often pathogens such as virus bacteria

ELISA METHOD

DENGUE NS1 Ag:

- Add 25ml sample diluent DN to all well
- Add 75 μ of controls & sample in the well as marked
- Add 50 μ of conjugate to well
- Incubate 60min at 37°C
- Wash 6 times
(350 μ / well & 30 sec soak time diluted wash buffer (1:20))
- Add 50 μ i color reagent to all well
- Incubate 1hrs at 37°C
- Wash 6 time
- Add 100 μ Tmb substrate in all wells
- Incubate 10min at under dark
- Add 100 μ stop solution in all wells
- Read within 10min.

HBs Ag

- 25 μ l sample diluent
- 75 μ samples & NC, PC
- 50 μ l conjugate in each well
- Incubate 1hrs at 37°C
- Wash 6 time
- 50 μ l substrate
- Incubate 10 min in dark
- 100 μ l stop solution
- Read

$$\text{Cov}=\text{NC}=0.15$$

WIDAL TEST

The widal test is an advanced way to check for antibodies that your body makes against the salmonella bacteria that causes typhoid fever. It look for O and H antibodies in a patient's sample. This test help detect life-threatening illnesses like typhoid fever

PROCEDURE (SLIDE METHOD)

- Collect a blood sample from the patient and let it clot
- After the clot has formed, centrifuge the sample to separate to serum from cells
- Place a drop of the serum on a clean glass slide and label it with the patient's name and the date
- Add a drop of antigen solution to the serum drop
- Mix the two drops using a clean toothpick or glass rod, making sure they are thoroughly combined.
- Rotate the slide slowly for 4 minutes to allow agglutination to occur. Agglutination is the clumping of bacteria caused by the interaction between the antibodies in the serum and the antigens in the serum and the antigens in the solution.
- Examine the slide under a microscope at 40x magnification to look for agglunation. The clumping of the bacteria indicates a positive results.

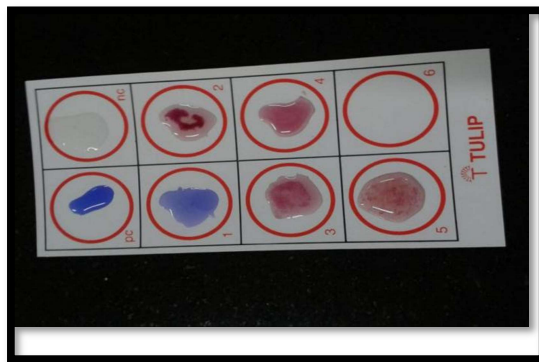


Fig : 2.9Widal test

PURPOSE

- A very high fever is up to 104 degrees fahrenheit.
- Headaches
- Usually, abdominal pain,constipation, and perhaps diarrhea later.
- Small red spots over the abdomen or on the chest (rose-colored spots)
- Loss of appetite and weakness other symptoms of widel (typhoid fever) include:
- Body pains
- Blood in the stools
- Chills
- Severe fatigue
- Difficulty in paying attention
- Thee agitation,confusion, and hallucinations

INTRODUCTION OF BLOOD BANK

WHAT IS BLOOD BANKING?

A blood bank is a centre where blood gathered as a result of blood donation is stored and preserved for late use in blood transfusion. The term “Blood Bank” typically refers to a division of a hospital where the storage of blood product occurs and where proper testing is performed (to reduce the risk of transfusion related adverse events). However, it sometimes refers to a collection centre, and some hospital also perform collection. Blood banking includes tasks related to blood banking collection, processing, testing, separation and storage.

WHAT ARE THE FUNCTION OF BLOOD BANKING?

The First blood bank in the United States was established in 1937 by **DR. Bernard Fantuz** at Cook County Hospital in Chicago. At the time, it was not possible to store blood for more than a few days. Most transfusions were person-to-person, a dangerous process which made it hard to find donors.

This article provides a glimpse into five key aspects of blood banking:

- Recruitment of donors and maintenance of donor's record
- Collection, preservation, and distribution of the blood and blood component
- Teaching, Training and research
- Clinical and therapeutic function like transfusion and day care service

SELECTION CRITERIA:

- Weight should not be less than 45 kg
- Age should be between 18 and 65.
- Blood pressure should be within normal limits for the age group.
- Pulse and temperature should be normal.
- Hemoglobin should be not less than 12.5 gram
- Women should not be pregnant or breast feeding her child Pulse – between 50 and 100/minutes with no irregularities
- Women donor should not donate during menstrual cycle Have donated blood in past 3 months.

REJECTION CRITERIA:

- Affected with HIV virus or AIDS
- If the patient had undergone any open surgery

- When lady is pregnant
- Cancer patient ,kidney patient, TB Patient are not eligible
- When tattoo
- Allergic
- High blood pressure
- Alcoholic

BLOOD DONATION (PROCEDURE)

THE BLOOD DONATION PROCESS: The blood donation process from the time you arrive until the time you leave take about an hour . the donation itself is only about 8 – 10 minutes on average

REGISTRATION:

- We will sign in and go over basic eligibility
- You will be ask to show ID such as your driver license
- Well ask you for your complete address

HEALTH HISTORY:

- You will answer a few question about health history and place you have traveled, during a private and confidential interview
- We will check your temperature ,pulse, blood pressure, and hemoglobin

YOUR DONATION:

- 1.If you are donating whole blood , well cleanse an area on your arm and insert a brand new sterile needle for the blood draw .this feel like a quick pinch and is over in second.
- 2.Other type of donation such as are made using an aphersris machine which will be connected to both arms.
- 3.A whole blood donation takes 8 -10 minutes , during which you will be seated comfortably or lying down .
4. When approximately a pint of whole blood has been collected , the donation is completed and a staff person will place a bandage on your arm.

BLOOD GROUPING

PRINCIPLE:

- The human blood consists of 4 main components , the red blood cell ,the white blood cell, the plasma, the platelets.
- The red blood cells are the components tat help in determining person's blood type.
- The ABO system is main blood grouping system behind the principle the helps classify people into one of the following four groups,i .e ., A, B, AB, OR O
- Antibodies for the antigens A and B exist naturally in the plasma and these are referred to as they form antigen.
- ABO blood grouping is based on the principle of an agglutination reaction. It is the popular method for blood group identification to determine the presence and absence of cellular antigens and their relative antibodies in the blood



Fig : 3.1 blood grouping reagent

PROCEDURE:

1. Label the tile with lead pencil A, B and O circle.
2. Place one drop of test serum in each circle.
3. Add one drop of corresponding 3% red cell suspension in each circle.
4. Mix well with glass rod , gently till and take the tile backward and forward for two minutes.
5. And figure out the ABO blood group.
6. Figure out the R h blood group.
7. Figure out the blood type.

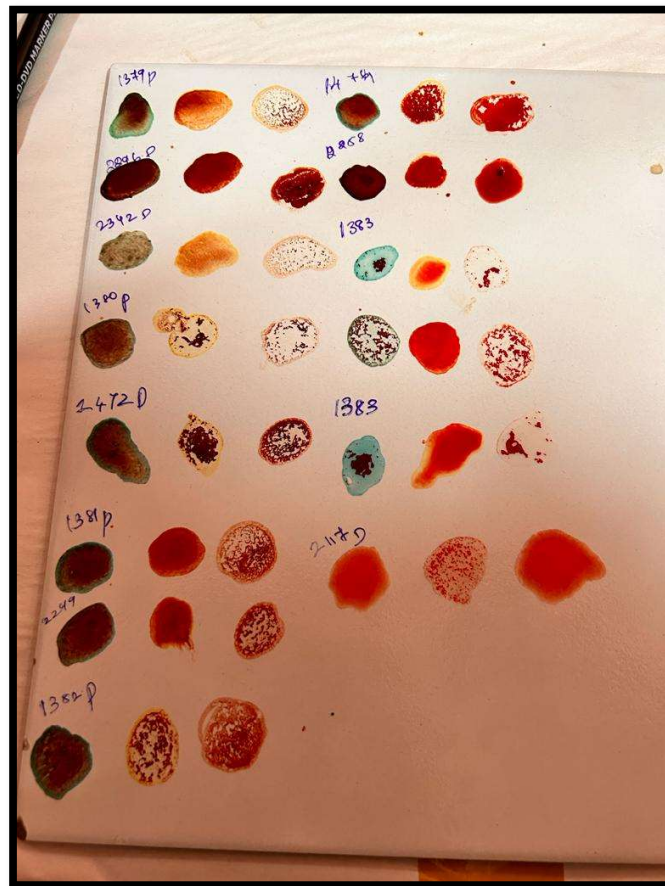


Fig : 3.2 blood grouping tile

BLOOD DONATION PROCEDURE:

❖ **REGISTRATION:**

The Medical team goes over basic eligibility and donation information, you will show you donor card and driver's license.

❖ **MEDICAL HISTROY :**

You will answer some question about your health history and places you have traveled.

❖ **MINI PHYSICAL :**

We will check your temperature , pulse , blood pressure and hemoglobin level present in a sample of blood.

❖ **BLOOD DONATION :**

The actual donation takes about 8-10 minutes , during which you will be seated comfortably.

❖ **REFRESHMENTS :**

Done! Time to have a snack and something to drink

BLOOD DONOR COUNSELLING:

Blood donor counselling is a confidential dialogue between a blood donor and a trained counselor about issues related to the donor's health and the donation process; it may be provided before, during and after blood donation. There are benefits for both the BTS and the wider health system in implementing blood donor counseling.

- ✓ Donors consent for testing of blood for all tests.
- ✓ Provide an opportunity to ask question and refuse donation.
- ✓ Risk history assessment.
- ✓ Transfer knowledge of HIV and blood –borne infections.
- ✓ Donation and test information.
- ✓ Getting the reports after the tests.

COMPONENTS SEPARATION:

BLOOD COMPONENT:

- Whole blood
- Platelets
- Plasma
- Cryoprecipitate
- Packed red blood cell

PACKED RED BLOOD CELL:

- Packed red blood cell from a donation of 450 ml of whole blood are obtained after centrifugation and plasma removal.
- Thalassemia / anemia.
- Storage 2–6-degree celcius up to 42 days.



Fig : 3.3 Packed red blood cell

FRESH FROZEN PLASMA:

- Fresh Frozen plasma (FFP) is used for patients with a coagulopathy who are bleeding or at risk of bleeding, and where a specific therapy or factor concentrate is not appropriate or unavailable.
- Fresh frozen plasma is obtained after centrifugation of whole blood and red cell removal, or through direct separation.
- The plasma must be frozen within 8 hours after collecting in order to preserve coagulation factors and other plasma protein.

STORAGE : 30 degree celcius for up to 2 years



Fig : 3.4 Fresh frozen plasma

PLATELETS:

Platelets are mainly obtained through thrombapheresis .platelets product formerly obtained from a mix of more than 10 buffy coat can now be obtained from a single thrombapheresis of about 40 minutes.

STORAGE :20-24 degree celcius for 5 days,under constant mixing of the bags (platelets aggregation occurs in the cold and in absecnce of agitation.

CROSS MATCHING

CROSS MATCHING:

Cross matching is a procedure performed prior to transfusion of blood or blood products to detect any serological incompatibilities in the blood of donor and recipient. Before a donor's blood is transfused into a recipient, there should be no antigens or antibodies in both, that would react with each other resulting in transfusion reaction. Cross matching is designed to prevent such transfusion reactions which may occur after transfusion.

- Cross matching plays important role to detect:
- Most recipient antibodies directed against donor's red blood cell antigen
- Most donor antibodies directed against recipient's red blood cell antigens.
- Major errors in ABO grouping, labeling and identification of donor and recipients.

PRINCIPLE:

Cross-matching will detect incompatibilities between the donor and recipient that will not be evident on blood typing. There are two types of cross-matches: Major cross-match and Minor cross-match.

PROCEDURE

- Prepare donor and recipient blood sample for major cross match donor red blood cells and recipient serum or plasma
- For minor cross match : Recipient red cells and donor 's serum or plasma .
- Prepare 3-5% cell suspension of red cells.
- Major Cross match Match :: label a test tube add two drops of the patient serum and one drop of the appropriate donor cell suspension.
- Minor Cross Match :: Label a test tube add two drops of the appropriate donor serum and one drop of the patient cell suspension.
- Mix the tube and incubate at 37 C for about 45 mintues. Add two drops of AHG (Antihuman globulin) and mix well
- Centrifuge at 1500 rpm for 1 minute.



Fig : 3.5 cross matching kit

INTERPRETATION AND RESULT

Compatible donor and recipient blood should show no agglutination in both major and minor cross match. Blood which shows incompatibility in major cross should never be transfused, because the large plasma volume of the recipient blood containing antibodies can destroy the donor's red cells easily. The minor incompatibility is less important because the donor serum which contains the antibodies is diluted in the recipient own plasma, making the antibodies very dilute and ineffective.

BLOOD BANKING RECORD

RECORD:

- Systematic procedure by which the records of an organization are created, captured, maintained and disposed off.
- Records involve every aspect of blood banking ie, donor recruitment donor selection, donor recall, blood collection, blood components, screening of blood for.

TYPES OF RECORDS

- Technician record
- Donor record
- Reception record
- Sample storage record
- Test record
- Equipment maintenance record
- Transfusion record

TECHNICIAN RECORD:

- Attendance
- Cont.No
- Address
- In time & Out time
- Personal information
- Name
- DOB
- In time & out time
- Age

DONOR RECORD:

- Donor Details
- Name
- M/F
- Age
- Weight/Height
- Blood grouping
- Blood donation date (volume)
- Use Donor identification

RECEPIENT RECORD:

- Name
- M/F, Age
- In patient or out patient
- Receipient date
- Whole blood, RBC, Plasma, platelets

SAMPLE STORAGE RECORD

- Blood donation date
- Temprature & shelflife

TYPE OF SAMPLE:

- Whole blood
- RBC
- plasma
- platelets

DISCARDING RECORD

- Date of collection
- Discarding date
- Discarding technician(name&sign)

PURPOSE OF RECORD:

- To create statistic
- To retain blood donors
- To maintain panel donors
- To recognize & to give awards.
- To undertaken any research activity
- To help during emergency
- To protect the interest of the patients

LIST OF THE FIGURES

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CHENGALPATTU MEDICAL COLLEGE, CHENGALPATTU - 603001
(AFFILIATED TO THE TAMILNADU DR.M.G.R.MEDICAL UNIVERSITY,
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GOVT. CHENGALPATTU MEDICAL COLLEGE, CHENGALPATTU 603001
TAMILNADU
CERTIFICATE OF OBSERVERSHIP

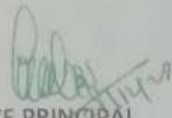
This is to certify that the following students studying in Sathyabama Institute of Science and Technology, Chennai has undergone observership for a period of One Month From 01.11.2023 & 30.11.2023 in the Department of Microbiology & Histopathology at Chengalpattu Medical College, Chengalpattu-603001.

Sl.No.	Name of the Student	Department	Period of posting
1	Mary G	Microbiology & Histopathology	01.11.2023 to 30.11.2023
2	Stephen Xavier M		
3	Oviya M.S		
4	Monika G		
5	Sowmiya S		
6	Shakeera Banu K		
7	Beruthose Banu S		
8	Ajith C		
9	Santhosh Kumar M.S		

They attend the training during the above period has been

Good




VICE PRINCIPAL

Chengalpattu Medical College,
Chengalpattu.

Date:21.12.2023

Place:Chengalpattu.

5.9
21/12/23

SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY
(Deemed to be University)

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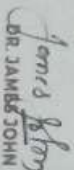
CERTIFICATE OF INTERNSHIP

This Internship program certificate is awarded to

SHAKEERA BANU K

for her outstanding completion of an internship with the Department of Pathology and Department of Microbiology at Govt. Chengalpattu Medical College, Chengalpattu, 603001, Tamilandu during 01.11.2023 to 30.11.2023.

She has learned to do routine lab work and has been aware of basic laboratory procedures.


DR. JAMBATHOHIN
CO ORDINATOR
MEDICAL LABORATORY TECHNOLOGY


DR. J PREMKUMAR
HEAD OF THE DEPARTMENT
MEDICAL LABORATORY TECHNOLOGY

Sharing Life. Saving Lives...since 1984



Date: 12th Dec 2022

TO WHOMSOEVER IT MAY CONCERN

This is to certify that Ms. Shakeera Banu. K, BSc in MLT, 2nd year of Sathyabama Institute of Science and Technology, Chennai - 119, undergone Internship training in Madras Egmore Lions Blood Bank & Research Foundation(Ambattur Unit), Chennai - 58. She has been observed blood bank procedures including Bleeding the Donors, Bloodgrouping, Cross-matching, Component separation, TTI screening and attend Blood donation camp for the period from 28th Nov 2022 to 3rd Dec 2022.

During her internship, performance was good and she was able to complete the project on time.

We wish him all the best for her upcoming career.

Ln.J.Madanagopal Rao -
Chairman



This project made possible through the co-operation of Lions Club International Foundation, Clubs in the District 324 - A1, A5 & A6 and well wishers - sponsored by Lions Club of Madras Egmore

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Web : www.lionsbloodbank.net
AMBATTUR : C-15, A.T.C. Road,
Ambattur Industrial Estate,
Chennai - 600 058 Tel : 48504959
Mob : 99419 17505
E-mail : info@lionsbloodbank.net

CONCLUSION

The internship was really a great experience. I'd recommend to my fellow juniors. The place is really good for developing skills. I gained experience .and technician knowledge.

1. I learn about blood collection
2. Blood banking works
3. In, I have learn about staining and tissue processing techniques.
4. In microbiology, I learn about staining and blood culture