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In partial Fulfillment 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

INSTITUTE OF SCIENCE AND TECHNOLOGY

(DEEMED TO BE UNIVERSITY)

Accredited with Grade "A++" by NAAC

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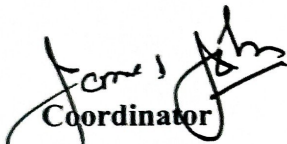
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BONAFIDE CERTIFICATE

This is to certify that this internship report is the bonafide work of Mr. AJITH.C who have done the project work as a team and carried out the training program at "CHENGALPATTU MEDICAL COLLEGE - CHENGALPATTU" and "LIONS BLOOD BANK AND RESEARCH FOUNDATION - EGMORE" under our supervision November 2022 and November 2023.


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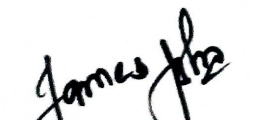
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DECLARATION

I, Mr. AJITH .C (41737001), hereby declare that the internship trainings done by me at "CHENGALPATTU MEDICAL COLLEGE at CHENGALPATTU " under the guidance of Dr. JAMES JOHN and "LIONS BLOOD BANK AND RESEARCH FOUNDATION at EGMORE" under the guidance of Ms. D. JASMINE PRIYA at Sathyabama Institute of Science and Technology is submitted in partialfulfilment of the requirements for the award of Bachelor of science degree in MEDICAL LABORATORY TECHNOLOGY.

Date:

Place: CHENNAI


Mr. AJITH .C

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HISTOPATHOLOGY

Histopathology is the diagnosis and study of diseases of the tissues, and involves examining tissues and/or cells under a microscope.

CHAPTER 1 : RECEPTION

RECEPTION:

Sample collection and report issuing work is done at reception section.

SAMPLE COLLECTION:

- When the collect the samples, we want to check: Is the patient's name and age correct on the requisition form and on the label of the specimen? Is specimen have fixative?
- Mostly 10% formalin using as fixative.

SAMPLE TYPES:

- Biopsy sample (taken from a living body)
- Autopsy sample (taken from a dead body)

SAMPLES LABELING:

Samples labeling based on following categories and also using different colors. Its helping to easily identify:

- General sample (G) - Except reproductive system samples
- Gynaecology sample (Gy) - Reproductive system samples
 - ◆ E.g.: Uterus, Endometrium, Ovaries, Fallopian tube.
- Fluid cytology sample (FCy) - Body fluids
 - ◆ E.g.: Cerebrospinal fluid, Pericardium fluid, Pleural fluid
- Paying (P) - Samples from the outside hospital

And also mentioned the ID number on the label. E.g.: If a general sample is comes as 1956th sample of 2023rd year, it will label as G1956/23.

CHAPTER 2 : TISSUE GROSSING

TISSUE CROSSING & PROCESSING:

Tissue crossing is the process of cutting the tissue like a piece and preparing it to make the section, and it consists of three sequential steps:

- Dehydration
- Clearing
- Impregnation



Fig:2.1: Grossing area

Dehydration:

It is the process of removing water from tissues. It is important because paraffin is not miscible with water. Dehydration is usually complete when less than 3-4% of water remains in the tissues. Time required for this depends on:

1. Permeability of tissues
2. Continuous rotation of fluid to prevent stagnation of fluid around tissues
3. Temperature
4. Vacuum applied

Why want to do dehydration?

Wet fixed tissues (in aqueous solutions) cannot be directly infiltrated with paraffin. First, the water from the tissues must be removed by dehydration. This is usually done with a series of alcohols, say 70% to 95% to 100%.

Examples Of Dehydrants: Ethyl alcohol, Methyl alcohol, Butyl alcohol and Isopropyl alcohol. The most commonly used dehydrant is ethyl alcohol.

Clearing:

Clearing is a process which leaves the tissues clear and transparent. This term relates to the appearance of the tissues after the dehydrating agent has been removed. If the refractive index of the clearing agent is similar to the protein of tissue the tissue becomes transparent. The end point of clearing can be noted by the transparent appearance of the tissue. Thus clearing serves two purposes,

1. Removes alcohol to make paraffin impregnation complete
2. Acts as solvent for the mounting media which renders the tissues transparent and improves the refractive index, making microscopic examination easier.

Clearing Agents: Xylene

It is colourless and most commonly used. Two changes of one hour each are given to get the end point. Prolonged treatment hardens the tissues. It is not preferred for brain tissue.

Other Clearing Agents:

1. Toluene
2. Dioxane
3. Cedarwood oil
4. Chloroform
5. Benzene
6. Carbol xylene - clears rapidly, it is kept reserved for material difficult to clear.

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.

Automatic tissue processor:

Automatic tissue processor is a machine, it can process the tissues automatically. It has 12 jars and they are filled with required reagents. We want to program how long the tissue should be in which reagent (or it may be already programmed). After loading the tissues automatically it will process the tissue by the program. [Fig:2.2]

Automatic tissue processor has 12 containers/jars:

1. 10% formalin
2. 70% Alcohol
3. 80% Alcohol
4. 90% Alcohol
5. 95% Alcohol
6. 100% Alcohol I
7. 100% Alcohol II
8. Acetone

9. Xylene I
10. Xylene II
11. Paraffin wax I
12. Paraffin wax II



Fig:2.2: Automatic tissue processor

CHAPTER 3: EMBEDDING

EMBEDDING:

Embedding in tissue processing is done to provide support, facilitate sectioning, preserve tissue integrity, enable staining, and ease storage and transportation of tissue samples for microscopic examination and analysis.

Procedure:

1. Open the tissue cassette and check whether correct number of tissue pieces is present.
2. Select the mold. There should be sufficient room for the tissue with at least a 2 mm surrounding margin of wax.
3. Fill the mold with paraffin wax.
4. Place the selected tissue firmly into the wax with a warmed forceps. It is important to ensure that the correct orientation is maintained and the tissue surface to be sectioned is kept flat, facing downwards.
5. Insert the identifying label or place the labeled embedding ring or cassette base onto the mold.
6. Cool the block on the cold plate or carefully submerge it under water when a thin layer has solidified over the wax surface.
7. Remove the block from the mold. .



Fig:3.1: Embedding Machine and embedded blocks

CHAPTER 4 : SECTION CUTTING

Section cutting is the process of preparing thin sections of the tissue specimens for microscopic examinations. It's also called as microtomy. This is a crucial step in studying the structure and pathology of tissues. A instrument is using for the section cutting that's called as microtome.

SPECIFIC OBJECTIVE:

- To ensure good section cutting
- To overcome troubleshooters during section cutting.
- To familiarize the staff with the equipment used for section cutting.

MICROTOME:

Microtome is an equipment for cutting section. There are five types of microtomes, □

1. Rotary microtome
2. Sliding microtome
3. Freezing microtome
4. Rocking microtome □
5. Base sledge microtome

a) Rotary Microtome

- The Rotary microtome is so-called because of a Rotary action of the handwheel responsible for the cutting moment.
- It is most commonly used microtome in this microtome knife is fixed while the tissue block is movable.
- Two types of cutting device are used.

- Fixed knife or disposable blades.
 - Fixed knife is made up of stainless steel.
 - The knife is sharpened by a process known as honing stropping.
 - Honing is done manually on stone or an electrically operated automated hone.
 - After honing stropping is done over a level strop.
 - The process of sharpening of microtome knives 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:4.2: Automated Rotary Microtome

Parts of the rotary microtomes:

- Block holder
- Knife clamp screw
- Knife clamps
- Block adjustment
- Thickness gauge
- The angle of tilt adjustment
- Operating handle

b) Sliding Microtome:

In this the tissue block is fixed while the knife is movable so, these microtomes are used as freezing microtome.

c) Freezing Microtome:

In this technique fresh tissue is rapidly frozen and the tissue become firm owing to ice acting as embedding medium thus sections are produced without the use of dehydration solution clearing agent or wax embedding

d) Rocking Microtome:

This is a simple microtome which is not used these days. The knife is immovable while the tissue block is held in a spring baring arm. The rocking microtome was designed primarily for cutting paraffin wax sections but in an emergency use frozen section by inserting a wooden block in which the tissue is frozen.

e) Base Sledge Microtome:

□ This type of microtome is used for very hot tissues or large blocks. Example: Pieces of brain and heart.

SOME NOTES FOR BEST SECTION CUTTING:

- High quality, sharp blades are always used for cutting.
- Knife tilt angle is always optimized for each microtome and blade type.
- Blocks are carefully trimmed to expose tissue. Blocks are roughly trimmed to save time. The surface is not polished before taking final sections. This often produces a “moth-eaten” appearance in the final section, which is full of small, ragged holes.
- Blocks are chilled on a cold wet surface and are always cold when cut (the surface of melting ice is excellent).
- The final sections from each block are cut gently with a uniform, slow rotation.
- Ensure slides and water are clean
- Avoid Cross-contamination: The water surface is always skimmed between specimens to avoid contamination of one section with cells from another.
- Don't Float from Multiple Blocks: Sections from more than one block are never simultaneously floated on the water bath.
- Maintain the tissue flotation both temperature 4–5°C below the melting point of the wax is optimal. Sections should readily flatten but the wax should not melt.
- If sections are left on the flotation bath for more than 15 seconds, the wax melts.
- Avoid wrinkles in sections:
- Don't damage floating sections

- Care is taken to avoid the formation of air bubbles in the flotation bath. Any visible bubbles are dislodged before the sections are laid on the water.

CHAPTER 5 : STAINING

Staining is a crucial process used to enhance the visibility of tissues and cells under a microscope. It involves applying various dyes or stains to biological tissues to highlight specific structures or components, making them easier to observe and analyze. There are several types of staining techniques used in histopathology, including:

- Hematoxylin and eosin (H&E) staining
- Immunohistochemistry (IHC)
- Special stains

HEMATOXYLIN AND EOSIN (H&E) STAINING:

Hematoxylin and Eosin (H&E) staining is one of the most widely used histological staining methods. It provides a general overview of tissue morphology and is commonly used for routine histopathological analysis.

Purpose :

To Stain tissue Sections of Histopathology.

Principle :

Acidic dye - Eosin binds with basic part of cell - Cytoplasm.

Basic Dye - Hematoxylin binds with acidic part & cell - Nucleus.

Required Materials :

- Prepared tissue slides
- Hematoxylin solution
- 1% Eosin solution
- Distilled water
- Bluing agent (e.g., Scott's tap water substitute)
- Different concentrations of alcohol (e.g., 70%, 95%, and 100% ethanol)
- Xylene or xylene substitute
- Mounting medium (DPX)
- Cover slips

Solution preparation:**a) Preparation Of Hematoxylin :**

Hematoxylin - 25g

Absolute Alcohol - 1200ml

Glycerin -1200ml

Saturated Potash alum - Excess

Distilled Water -1200ml

Glacial Acetic Acid - 120ml

b) Preparation Of 1% Eosin :

Eosin Powder - 1g

Distilled Water -100ml

c) Preparation Of 1% Acid Alcohol :

Concentrated Hcl - 1 Ml

ISO Propyl Alcohol - 100ml

d) Preparation Of 10% HCl :

Hydrochloric Acid - 1ml

Distilled Water - 99ml

e) Preparation Of 10% Formalin :

Formaldehyde [40%] - 10ml

Distilled Water - 90ml

Procedure :

1. Keep Sections/Slides in Hot air oven 50-55°C to 15minnes.
2. Drain xylene and hydrate the tissue section by passing through decreasing concentration of alcohol baths (100%, 90%, 80%, 70%) and water.
3. Stain in hematoxylin for 25 minutes.
4. Wash in tap water.
5. Decolorize in 1% acid alcohol - just dip
6. Bluing by running tap water-10 minutes.
7. Dip in 1% eosin - 3 dips
8. Wash in Tap Water.

9. Air dry.

10. Mount the section with DPX



Fig: 5.1: Staining reagents

Reporting Interval: 2 hours

PAP STAINING:

Purpose of the PAP stain:

The purpose of the Pap stain is to detect abnormal cervical cells, aiding in the early detection of cervical cancer during routine Pap smear screening.

Principle:

The principle of pap stain is to clearly distinguish between basophilic and acidophilic cell components and obtain a detailed chromatin pattern.

Materials required:

1. Specimen slides
2. Acid Alcohol
3. Xylene 1&2
4. Hematoxylin
5. EA 50 solution
6. OG 6 Solution
7. Alcohol 1, 2&3 (70%, 95%, 100%)
8. Acetic acid
9. Eosin
10. Distilled water
11. Cover slips

12. Staining dish

13. Timer

Procedure of PAP staining:

1. 80% Alcohol : 1min
2. 70% Alcohol : 1min
3. 50% Alcohol : 1min
4. Tap Water : 10min
5. Harris haematoxylin : 5-8 min
6. Rinse in tap water gently & briefly: 3-4mins
7. 1% Acid Alcohol for differentiation 1 dip.
8. Tap water -10 mins (bluing)
9. 70% alcohol - 5 mins
10. 90% alcohol - 5 mins
11. OG-6 - 2-5 mins
12. 95% alcohol I - 1 min
13. 95% alcohol II - 1 min
14. 95% alcohol III - 1 min
15. EA 50 - 6-10 mins
16. 95% alcohol IV - 1 min
17. 95% alcohol V- 1 min
18. 95% alcohol VI- 1 min
19. Dry and mount
20. Examine the section

IMMUNOHISTOCHEMISTRY (IHC):

Immunohistochemistry (IHC) uses antibodies to detect antigens in a tissue sample. IHC is commonly used to diagnose cancer, predict treatment response and determine likely outcomes (prognosis) of the disease.

Procedure:

1. Arrange the section loaded slides in truf
2. Dip into Xylene I- 10 mints
3. Dip into Xylene II - 10 mints
4. Dip into Iso propyl Alcohol I - 10 mints

5. Dip into Iso propyl Alcohol II - 10 mints
6. Dip into distilled water I - 2 mints
7. Dip into distilled water II - 2 mints
8. Heat the slides with cooker (980ml distilled water - 20ml tris buffer , Cooker 2 whistle)
9. Dip into 4% Immuno wash buffer I - 5 mints
10. Tissue paper cool
11. Mark the slides with blocker marker
12. Add Hydrogen peroxide I drop - 10 mints truf close
13. Dip into immuno wash buffer II - 5 mints
14. Add antibody - 40 mnts - PRIMARY ANTIBODY
15. Dip into immuno wash buffer I - 5 mints
16. Dip into immuno wash buffer II - 5 mints
17. Add target binder (yellow color) - 12 mints - SECONDARY ANTIBODY
18. Dip into immuno wash buffer I - 5 mints
19. Dip into immuno wash buffer II - 5 mints
20. Add pink color antigen - 12 mints - SECONDARY ANTIBODY
21. Dip into immuno wash buffer I - 5 mints
22. Dip into immuno wash buffer II - 5 mints
23. DAB buffer 2ml + DAB chromogen 2 drops mixed
24. To add 2 drops sections 7-10 mints
25. Dip into distilled water I - 2 mints
26. Dip into distilled water II - 2 mints
27. Dip into hematoxylin - 30 sec - 1 mint
28. Dry mount & label

CLINICAL PATHOLOGY

CHAPTER 6 : PERIPHERAL SMEAR

SMEAR PREPARATION:

1. Clean and label the slide
2. Place a small drop of blood on the slide.
3. Bring another slide as spreader at a 30°-45° angle up to the drop, allowing the drop to spread along the contact line of the 2 sides of slide.
4. Quickly push that the spreader slide toward the unfrosted end of the lower slide.

5. Make sure that the smears have a good feathered edge. This is achieved by using the correct amount of blood and spreading technique.
6. Allow the thin smears to dry for fixed.

LEISHMAN STAIN:

Leishman stain is used in microscopy for staining blood smears. It provides excellent stain quality. It is generally used to differentiate and identify leucocytes, malaria parasites, and trypanosoma. It is based on a methanolic mixture of "polychromed" methylene blue.

Principle:

Leishman stain contain methylene blue dye, a basic dye, which gives colour to an acidic component and eosin dye, an acidic dye, which gives colour to a basic component. These dyes differentiate the different component of blood. Leishman Stain belongs to the group of Romanowsky stains. For the detection of malaria parasite Leishman staining seems more sensitive than e.g. Field's stain.

Reagents / Contents:

- Leishman stain
- Leishman stain buffer

Materials required:

- Clean grease-free glass slide
- Loops
- Staining rack
- Blotting paper
- Immersion oil
- Microscope

Type of Specimen:

Clinical specimen - Blood samples with anticoagulant or without anticoagulant (use before clotted)

Procedure:

1. Use blood smears that are as thin as possible and air-dried.
2. Fully cover the smear with Leishman stain

3. Keep for 2-3 minutes.
4. Fully cover the smear with phosphate buffer provided with the kit.
5. Keep for 15 minutes.
6. Rinse gently with distilled water for 1 to 2 minutes.
7. Dry the slides using blotting paper and air-dry.
8. Observe under microscope, 40X and 100X under oil immersion lens.

Interpretation of results :

Erythrocytes	: Light pink to brown
Cores of lymphocytes	: Deep, dark blue to blue-violet
Cytoplasm of lymphocytes	: Light blue
Nuclei of neutrophil	: Deep blue to blue-violet
Granules of neutrophilic	: Red
Cores of eosinophil leukocytes	: Blue violet
Granules of eosinophilic leukocytes	: Deep red

CHAPTER 7 : RETICULOCYTE COUNT

RETICULOCYTES:

Reticulocytes are young, premature, non nucleated red blood cells, contain reticular material (RNA) that stain gray blue. Reticulum is present in newly released blood cells for 1-2 days before the cell reach its full mature state.

A reticulocyte count is a blood test used to measure the number of reticulocytes.

Procedure :

1. Put 2 drops of new methylene blue in a test tube.
2. Add 2 drops of well mixed EDTA blood to the tube.
3. Mix well.
4. Incubate the mixture for 20 minutes at the temperature of 37°C in the incubator.
5. Mix the solution again.
6. Prepare 2-4 good thin smears.
7. Label and let dry.

Formula:

$$\text{Reticulocyte count} = \frac{\text{Reticulocytes counted} \times 100}{\text{Number of red cells}}$$

MICROBIOLOGY

CHAPTER 8 : LIST OF SAMPLES IN MICROBIOLOGY LAB

1. Urine
2. Stool
3. Blood
4. Sputum
5. Throat swab
6. Wound swab
7. Pus
8. Surface sample

CHAPTER 9 : CULTURE MEDIA

Media are used to support the growth of microorganisms, enabling their isolation, identification, and study under controlled laboratory conditions. They provide essential nutrients, vitamins, minerals, and growth factors necessary for microbial growth.

TYPE OF MEDIA:

1. Blood Agar
2. Cled Agar
3. Macconkey Agar
4. Chocolate Agar
5. Muller Hinton Agar

MEDIA PREPARATION:

a) Blood Agar Preparation:

5-10% defibrinated blood is mixed with melted agar after cooling and the media is poured into the plates.

b) L.E.D Agar Preparation:

10 grams of agar powder were combined with 250 ml of distilled water. After 15 minutes of autoclaving at 121 °C and cooling, the media was put into the plates.

c) Macconkey Agar Preparation:

12.88 grams of agar powder were combined with 250 ml of distilled water. After 15 minutes of autoclaving at 121 °C and cooling, the media was put into the plates.

d) Chocolate Agar Preparation:

250 ml of distilled water was added to 7 gm of nutrient agar powder. Autoclaving was done at 121 °C for 15 minutes and cooled till 45C then 5% of defibrinated blood was added. Heated slowly and evenly.

e) Muller Hinton Agar Preparation:

250 ml of distilled water was added to 9.5 gm of Agar powder. Autoclaving was done at 121 °C for 15 minutes and cooled after cooling the media is poured into the plates.



Fig : 9.1: Picture of media before autoclave

SEROLOGY

CHAPTER 10 : HCV TEST

HCV test refers to a test used to detect the presence of hepatitis C virus (HCV) in a person's blood. Hepatitis C is a viral infection that primarily affects the liver and can lead to both acute and chronic forms of hepatitis. The test is crucial for diagnosing hepatitis C infection, monitoring its progression, and assessing the effectiveness of treatment.

PROCEDURE:

1. 100 µl sample Diluent + 10µl sample or NC, PC
2. Incubate 45 mins at 37°C
3. Wash 6 times
4. Add 50µl Assay Buffer
5. Add 50µl conjugate
6. Incubate 30 mins at 37°C
7. Wash 6 times

8. Add 50 µl substrate
9. Incubate 10 mins in Dark
10. 100 µl stop solution
11. Read the microtiter plate

CHAPTER 11 : HBsAg TEST

The HBsAg test, also known as the hepatitis B surface antigen test, is a blood test used to detect the presence of hepatitis B surface antigen (HBsAg) in a person's blood. HBsAg is a protein present on the surface of the hepatitis B virus (HBV), and its detection indicates either acute or chronic hepatitis B infection.

PROCEDURE:

1. Add 25 µl sample Diluent + 75 µl samples or NC, PC + 50µl conjugate in Each well.
2. Incubate 1 hr at 37°C
3. Wash 6 times
4. Add 50µl substrate
5. Incubate 10 min in Dark
6. 100 µl stop solution
7. Read the microtiter plate

CHAPTER 12 : IgM SCRUB TEST

The "IgM SCRUB TEST" is likely a reference to an IgM antibody test used to diagnose scrub typhus, a bacterial infection. IgM antibodies are produced by the immune system in response to acute infection. This test detects IgM antibodies specific to scrub typhus in a blood sample, indicating recent infection. It's essential for diagnosis and treatment planning.

PROCEDURE:

1. 500µl sample Diluent + 5µl control (or) samples
2. Add 100µl of Diluted samples & controls
3. Incubate 30 mins at 37°C
4. Wash 6 times
5. Add 100 µl Enzyme HRP conjugate
6. Incubate 30 mins at 37°C

7. Wash 6 times
8. Add 150µl En wash
9. Incubate 5 mins at RT
10. Wash 6 times
11. Add 100 µl TMB substrate
12. Incubate 10 mins at RT in Dark
13. Add soul stop solution
14. Read the microtiter plate

CHAPTER 13: DENGUE IgM

The Dengue IgM test detects specific antibodies produced by the immune system in response to Dengue virus infection. It helps diagnose acute Dengue fever by identifying recent infections. A positive result indicates recent infection, while a negative result may require further testing or consideration of other factors.

Procedure:

1. Sample diluent 990µl + Samples controls 10µl [controls NC, PC]
2. Add 100 µl Diluted controls & samples in respective wells
3. Incubate 1 hr at 37°C
4. Wash 6 times
5. Add 100 µl freshly prepared conjugate in all wells
6. Incubate 1 hr at 37°C
7. Wash 6 times
8. Add 100µl Tmb substrate in all wells
9. Incubate 10 mins at RT under dark
10. Add 100µl stop solution in all wells
11. Read with in 10 mins



Fig : 13.1: ELISA plate washer

CHAPTER 14 :WIDAL TEST

The Widal test is primarily used to diagnose typhoid fever and other salmonella infections. It helps distinguish between recent and past infections by detecting antibodies specific to Salmonella bacteria in the patient's blood serum.

PRINCIPLES OF WIDAL TEST:

Particulate antigen reacts with its specific antibodies at optimum temperature, pH and electrolyte to produce visible clumps. Killed suspension of salmonella antigens react with specific antibodies in patients, serum to produce agglutination.

Antigens used in WIDAL test are 'o' antigen (somatic antigen) and 'H' antigen (flagella antigen).

1. 'H' antigen of salmonella typhi [STH]
2. 'O' antigen of salmonella typhi (STO)
3. 'H' antigen of salmonella paratyphi A (SPAH)
4. 'H' antigen of salmonella paratyphi B (SPBH)

The paratyphoid 'o' antigens are not employed as they cross react with typhoid 'o' antigen.

SPECIMEN COLLECTION:

Fresh serum should be used Hemolysed lipemic or contaminated serum must be discarded. Specimen stable 8 hours at room temperature and 2 weeks at 2-8°C and 6 months in freeze.

PROCEDURE:

1. Take four sets of test tube and label them 1-8 in each row.
2. Pipette into tube no.1 of all the sets 1.9 ml of normal saline
3. To each serum of the remaining tubes add 1 ml of saline.
4. To tube no.1 of all the sets add 0.1ml of serum to be tested and mix well.
5. Transfer 1 ml of diluted serum sample from tube no.1 to tube no.2 and mix well
6. Transfer 1 ml of serum sample from tube no.2 to tube no.3 and continue this till the test tube no.7 in all test.
7. Discard 1 ml of diluted serum sample achieved from tube no 1-7 respectively in each set is as follows - 1:20, 1:40, 1:80, 1:160, 1:640, 1:1280
8. Tube no.8 in all the sets serum as control 1
9. To all the tube from 1-8 of each set add 1 drop STH antigen SPAH antigen SPBH antigen and mix well.

10. cover on incubate at 37°C over night (18 hours).

11. Dislodge the sedimented bottom gently and observe for agglutination.

BLOOD BANK

CHAPTER 15 : INTRODUCTION

A blood donation is a process whereby a person voluntarily has blood drawn to be used for future transfusions when in need at hospitals for treatment procedures that require them. Donation may be of whole blood (blood drawn directly from the body) or of specific components of the blood; such as red blood cells, white blood cells, plasma, and platelets. Blood banks often participate in the process of collecting blood and other procedures such as managing stocks, approving blood requests and updating donation information.

ROLE OF BLOOD BANK:

A blood bank collects, separates, tests and stores blood until a patient needs it. If you've donated blood before, you've been part of the blood banking process. The first blood bank in the United States was established in 1937 by Dr. Bernard Fantus at Cook County Hospital in Chicago.

IMPORTANCE OF BLOOD DANATION:

Safe blood saves lives. Blood is needed for women with complications in pregnancy and childbirth, children with severe anemia, often as a result of malaria or malnutrition, accident victims, surgery and cancer patients.

Since blood can only be stored for a limited period of time before use, a regular blood supply is always required. Regular blood donations by sufficient numbers of healthy people are necessary to ensure that blood is available everywhere at all times.

Blood is the most valuable gift you can give to others - the gift of life. The decision to donate blood can save lives if the blood is divided into components (red blood cells, platelets, plasma) that can be used individually for patients with certain medical conditions.

STRUCTURE OF BLOOD BANK:

- Reception Room
- Donation Room
- Donor Rest/ Refreshment Room
- Screening Unit
- The Laboratory

- Preservation and Storage
- Blood Bank Records

CHAPTER 16 : BLOOD DONOR

BENEFITS:

Donating blood has benefits for your emotional and physical health. According to a report by the Mental Health Foundation, helping others can:

- Reduce stress
- Improve your emotional well-being
- Benefit your physical health
- Help get rid of negative feelings
- Provide a sense of belonging and reduce isolation

DONOR SELECTION CRITERIA (Eligible to Donate Blood):

Any healthy adult, both male and female, can donate every three months. Good health of the donor must be fully ensured. The universally accepted criteria for donor selection are:

- **Age between** : 18 and 65 years
- **Hemoglobin** : not less than 12.5 gms%
- **Pulse** : between 60 - 100/minute with no irregularities
- **Blood Pressure** : Systolic 100-140 mm Hg and Diastolic 60 - 90 mm Hg
- **Body weight** : not less than 45 Kg

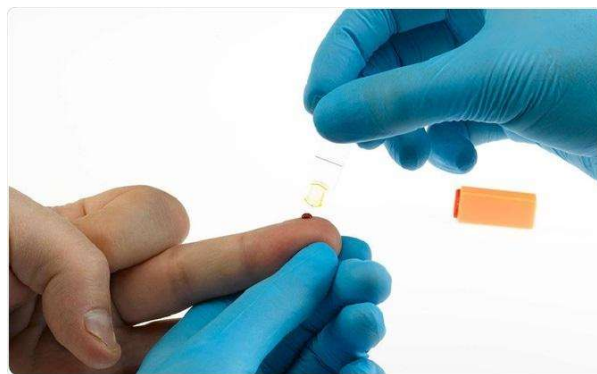


Fig : 16.1 Hemoglobin testing

DONOR REJECTION CRITERIA:

- When Tattooed
- Cancer patient
- Affected with HIV Viruses or Aids
- High blood pressure
- Anemic
- Allergic
- Alcohol
- Smoker
- Kidney disorder
- Low hemoglobin
- Any fever
- Diabetes patient

PERMANENT DEFERRAL:

- Uncontrolled Blood Pressure?
- Heart Disease or Anti Arrhythmic?
- Epilepsy or Anticonvulsants or Cytotoxic drugs?
- Abnormal bleeding tendencies/Enzyme Deficiencies with known history of Hemolysis?
- Diabetes mellitus or treatment with Insulin or Hypoglycemic drugs?
- Chronic liver disease or endocrine disorders/ Chronic kidney disease?
- Parkinsons diseases?
- Psoriasis or treatment for the same?
- Psychiatric disorders / Schizophrenia?
- Major surgeries for Kidney, Heart, Liver or Brain?
- Severe allergic disorders or Asthmatic on steroid therapy?
- Injection Drug users / Transgender / Female Sex Workers / Persons with Multiple Sex Partners / Homosexual / STD / AIDS?
- Gonorrhea Disease/Syphilis?
- Leishmaniasis disease / Leprosy
- Cancer disease Surgery for cancer
- Autoimmune diseases the SLE, RA, Ankylosing Spondylitis, Dermatomyositis, Scleroderma
- 2 Consecutive faints following a blood donation?
- Recipients of organ / System Cells / Tissue Transplant?

தீர்மானம் / PERMANENT DEFERRAL	
1. உயர் இரத்த அழுத்தம் / கட்டுப்பாட்டில் இல்லாதது? H/O Uncontrolled Blood Pressure / Stroke? 2. இதய நோய் / இதய நோய் / இதய நோய் / இதய நோய்? H/O Heart Disease or Anti Arrhythmic? 3. கனியம் / கனியம் / கனியம் / கனியம்? H/O Epilepsy or Anticonvulsants or Cytotoxic drugs? 4. அமில இரத்தம் / அமில இரத்தம் / அமில இரத்தம் / அமில இரத்தம்? H/O Abnormal bleeding tendencies / Enzyme Deficiencies with known History of Hemolysis? 5. சர்க்கரை நோய் / சர்க்கரை நோய் / சர்க்கரை நோய் / சர்க்கரை நோய்? H/O Diabetes mellitus or treatment with Insulin or Hypoglycemic drugs? 6. கல்லீரல் நோய் / கல்லீரல் நோய் / கல்லீரல் நோய் / கல்லீரல் நோய்? H/O Chronic liver Disease or endocrine disorders / Chronic Kidney disease? 7. நரம்பு தளர்ச்சி / நரம்பு தளர்ச்சி / நரம்பு தளர்ச்சி / நரம்பு தளர்ச்சி? H/O Parkinson's diseases? 8. கெர்டி சிஸ்டிக் நோய் / கெர்டி சிஸ்டிக் நோய் / கெர்டி சிஸ்டிக் நோய் / கெர்டி சிஸ்டிக் நோய்? H/O Psoriasis or treatment for the same? 9. மனநலம் / மனநலம் / மனநலம் / மனநலம்? H/O Psychiatric disorders / Schizophrenia?	10. சிறுநீரகம் / சிறுநீரகம் / சிறுநீரகம் / சிறுநீரகம்? H/O Major surgeries for Kidney, Heart, Liver or Brain? 11. அசௌகரியம் / அசௌகரியம் / அசௌகரியம் / அசௌகரியம்? H/O severe allergic disorders or Asthmatic on steroid therapy? 12. நோய் / நோய் / நோய் / நோய்? H/O Injection Drug users / Transgender / Female Sex Workers / Persons with Multiple Sex Partners / Homosexual / STD / AIDS? 13. காலரா / காலரா / காலரா / காலரா? H/O Gonorrhea Disease / Syphilis? 14. காலரா / காலரா / காலரா / காலரா? H/O Leishmaniasis disease / Leprosy 15. புற்றுநோய் / புற்றுநோய் / புற்றுநோய் / புற்றுநோய்? H/O Cancer disease / Surgery for cancer 16. புற்றுநோய் / புற்றுநோய் / புற்றுநோய் / புற்றுநோய்? H/O Autoimmune diseases like SLE, RA, Ankylosing Spondylitis, Dermatomyositis, Scleroderma 17. குந்தம் / குந்தம் / குந்தம் / குந்தம்? H/O 2 Consecutive faints following a blood donation? 18. மருந்து / மருந்து / மருந்து / மருந்து? H/O Recipients of organ / System Cells / Tissue Transplant?

Fig : 16.2 : Permanent deferral for blood donation

CHAPTER 17 : BLOOD DONOR REQUEST FORM

A donation request letter or form is a Formal Document that individuals or groups use to seek support or raise funds for their organization, event or cause. It is also sometimes referred to as a solicitation letter.

CONTENT OF THE FORM:

CONTENT TO BE FILLED		
By Donor:	By Medical Officer:	By Laboratory Technician:
• Type of donor: Voluntary / Replacement • Blood donation date • Name • Address • E mail ID • Mobile No. • College or company address • Gender • Weight • Date of Birth • Age • Blood group • Last Donation Date • Total No. Of Donation	• Pulse • BP • Temperature • CVS • RS • Fit / Unfit	• Blood group • HB • Type of blood bag: ✓ Single ✓ Double ✓ Triple ✓ Quadruple • Blood bag number • Segment number • Blood donation time: ✓ Starting time: ✓ Ending time:

Madras Egmore Lions Blood Bank Research Foundation

EGMORE : 130, Marisale Road, Egmore, Chennai - 600 034. Mob: 9941917925 Tel: 29414949 / 4859 / 6959 Web: www.lionsbloodbank.net

AMBATTUR : C-15, A.T.C. Road, Ambattur Industrial Estate, Chennai - 600 058 Tel: 48594959 Mob: 99419 17500 E-mail: info@lionsbloodbank.net

BLOOD DONOR CONSENT FORM

LOCATION: _____ DATE: _____

குருதி தானகொடுப்பதில் பங்குபெறும் செயல்பாடு
TO BE FILLED BY DONOR

குருதி வங்கி ஊழியர் பூர்த்தி செயல்பாடு
TO BE FILLED BY BLOOD BANK STAFF

TYPE OF DONOR: VOLUNTARY REPLACEMENT

குருதி / Name: _____

முகவரி / Address: _____

மின்னஞ்சல் முகவரி / E-mail ID: _____

மொபைல் எண் / Mobile No.: _____

கல்வி (அ) தொழில் முகவரி / College or Company Address: _____

பாலினம் / Gender: Male Female

வயது / Age: _____

நிறம் / Blood Group: _____

கடைசி தானகொடுத்த நாள் / Last Donation Date: _____

மொத்த தானகொடுத்த எண்ணிக்கை / Total No. of Donation: _____

கடைசி உணவு உண்டாக்கிய நாள் / When did you eat your last meal? _____

குருதி தானகொடுத்த பராமிதிகள் / Donor Eligibility Criteria

வயது / Age: 18 - 65 Years

எடை / Weight: >45 Kgs

குருதியின் அளவு / Hemoglobin: 12.5 gm/dl

தடிப்பு / Pulse: 60-100 / minute

குருதி அழுத்தம் / B.P.: 100/60 - 160/90 mm Hg

பரிந்துரை / Permanent Deferral

1. உயர் குருதி அழுத்தம் / Uncontrolled Blood Pressure / Stroke?

2. குருதி நோய் / Blood Disease / HIV?

3. இதய நோய் / Heart Disease / Anti-Arhythmic?

4. மூளை நோய் / Brain Disease / Anti-Epileptic or Anticonvulsant or Cytotoxic drugs?

5. தடிப்பு / Fungus / HIV?

6. தடிப்பு / Fungus / HIV?

7. தடிப்பு / Fungus / HIV?

8. தடிப்பு / Fungus / HIV?

9. தடிப்பு / Fungus / HIV?

10. தடிப்பு / Fungus / HIV?

11. தடிப்பு / Fungus / HIV?

12. தடிப்பு / Fungus / HIV?

13. தடிப்பு / Fungus / HIV?

14. தடிப்பு / Fungus / HIV?

15. தடிப்பு / Fungus / HIV?

16. தடிப்பு / Fungus / HIV?

17. தடிப்பு / Fungus / HIV?

18. தடிப்பு / Fungus / HIV?

BLOOD DONOR QUESTIONNAIRE

குருதி தானகொடுப்பதில் பங்குபெறும் செயல்பாடு
TO BE FILLED BY DONOR

குருதி வங்கி ஊழியர் பூர்த்தி செயல்பாடு
TO BE FILLED BY BLOOD BANK STAFF

TYPE OF DONOR: VOLUNTARY REPLACEMENT

குருதி / Name: _____

முகவரி / Address: _____

மின்னஞ்சல் முகவரி / E-mail ID: _____

மொபைல் எண் / Mobile No.: _____

கல்வி (அ) தொழில் முகவரி / College or Company Address: _____

பாலினம் / Gender: Male Female

வயது / Age: _____

நிறம் / Blood Group: _____

கடைசி தானகொடுத்த நாள் / Last Donation Date: _____

மொத்த தானகொடுத்த எண்ணிக்கை / Total No. of Donation: _____

கடைசி உணவு உண்டாக்கிய நாள் / When did you eat your last meal? _____

குருதி தானகொடுத்த பராமிதிகள் / Donor Eligibility Criteria

வயது / Age: 18 - 65 Years

எடை / Weight: >45 Kgs

குருதியின் அளவு / Hemoglobin: 12.5 gm/dl

தடிப்பு / Pulse: 60-100 / minute

குருதி அழுத்தம் / B.P.: 100/60 - 160/90 mm Hg

பரிந்துரை / Permanent Deferral

1. உயர் குருதி அழுத்தம் / Uncontrolled Blood Pressure / Stroke?

2. குருதி நோய் / Blood Disease / HIV?

3. இதய நோய் / Heart Disease / Anti-Arhythmic?

4. மூளை நோய் / Brain Disease / Anti-Epileptic or Anticonvulsant or Cytotoxic drugs?

5. தடிப்பு / Fungus / HIV?

6. தடிப்பு / Fungus / HIV?

7. தடிப்பு / Fungus / HIV?

8. தடிப்பு / Fungus / HIV?

9. தடிப்பு / Fungus / HIV?

10. தடிப்பு / Fungus / HIV?

11. தடிப்பு / Fungus / HIV?

12. தடிப்பு / Fungus / HIV?

13. தடிப்பு / Fungus / HIV?

14. தடிப்பு / Fungus / HIV?

15. தடிப்பு / Fungus / HIV?

16. தடிப்பு / Fungus / HIV?

17. தடிப்பு / Fungus / HIV?

18. தடிப்பு / Fungus / HIV?

Fig : 17.1 : Blood Donor Request Form

CHAPTER 18 : BLOOD BAG

These are the disposable biomedical transparent flexible poly vinyl chloride (PVC) containers, designed to collect, process and store the whole blood and blood components. CPDA: Citrate Phosphate Dextrose Adenine is the most commonly used anticoagulant in blood bags.

- Citrate - acts as anticoagulant by chelating calcium.
- Phosphate - lower acidity and have a higher concentration of 2,3 DPG and red cell phosphate
- Dextrose - needed for metabolism of stored red blood cells.
- Adenine - improves the viability of red blood cells.

The bags are available in 350 and 450 ml capacity.

PARTS OF BLOOD BAG:

The blood bag system consists of blood donor bag, donor tube (consists of needle with needle cover), puncturable and nonsealable transfusion pot and clamp.

TYPES OF BAG: [Fig:18.1]

- Single bag : Used for whole blood collection
- Double bag : Used to separate packed cells and Plasma
- Triple bag : Used to separate packed cells, plasma and platelets
- Quadruple bag : Used to separate packed cells, plasma, platelets and cryoprecipitate



Fig:18.1 : Blood bag

CHAPTER 19 : BLOOD DONATION

PROCEDURE OF BLOOD COLLECTION:

Step1: Identify donor and label blood collection bag and test tubes.

Ensure that:

- The blood collection bag is of the correct type.
- The labels on the blood collection bag and all its satellite bags, sample tubes and donor records have the correct patient name and number.

Step 2: Select the vein.

- Select a large, firm vein, preferably in the antecubital fossa, from an area free from skin lesions or scars.

Step 3: Disinfect the skin.

- If the site selected for venepuncture is visibly dirty, wash the area with soap and water, and then wipe it dry with single-use towels.

Step 4: Perform the venipuncture.

- In general, use a 16-gauge needle, which is usually attached to the blood collection bag. Use of a retractable needle or safety needle with a needle cover is preferred if available.

Step 5: Monitor the donor and the donated unit.

- Closely monitor the donor and the injection site throughout the donation process.
- About every 30 seconds during the donation, mix the collected blood gently with the anticoagulant, either manually or by continuous mechanical mixing.

Step 6: Remove the needle and collect samples.



Fig:19.1 : Blood donating patient

CHAPTER 20 : DONOR COUNSELLING

A blood transfusion service is an essential element of a health-care system and individuals who donate their blood provide a unique contribution to saving lives and improving patient health.

Blood donors should be provided with high standards of care and assurance of their health and safety.

Blood donor counselling is a confidential dialogue between a blood donor and a trained counsellor about issues related to the donor's health and the donation process; it may be provided before, during and after blood donation.

NEED FOR DONOR COUNSELLING PROGRAMME:

Absence of a programme on counselling of blood donors, deprives them

- of their right to know their health status and plan behaviour modifications
- an opportunity to the donor to self-exclude from donation
- to clarify myths and misconceptions
- to understand the consequences of Transfusion Transmitted Infections

TWO TYPES OF COUNSELING:

- 1) Pre donation counseling
- 2) Post donation counseling

Pre Donation Counseling:

- Verify the identification of the donor and ensure that you have the correct Medical History Form.
- Review the donor information part of the Medical History Form with the donor.
- Assure the donor that his/her information will be kept confidential.
- Discuss with the donor risk behavior for HIV and other sexually transmitted diseases.
- Explain to the donor the consequences of not being honest when answering the questions.
- Encourage feedback and questions from the blood donor.
- Explain to the donor the quantity of blood to be drawn, tests to be carried out the donated blood and inform him/her that he/she will be notified of the results if any of the results are irregular
- Inform the donor about the importance of self-exclusion from the donation process if he/she believes his/her blood is not suitable for transfusion.
- Discourage blood donation in order to obtain tests results.
- Review the questionnaire with the donor and ask further questions where the responses are not clear.

Post donation counseling:

- In the case of a blood donor who has a confirmed TTI reactive result:
- Inform the donor of the result simply and clearly.
- Give the donor time to consider the information.
- Ensure that the donor understands the result.
- Allow the donor to ask questions.
- Help the donor cope with emotions arising from the test result
- Discuss any immediate concerns and assist the donor to suggest a person among their close family and friends who may be available and acceptable to offer immediate support.
- Describe follow-up services that are available in the health facility and in the community, with special attention to available services for treatment, care and support
- Provide information on other relevant preventive health measures, such as healthy lifestyles and good nutrition.

CHAPTER 21 : BLOOD GROUPING

PRINCIPLE:

ABO and Rh blood grouping system is as based on agglutination reaction. When RBC bringing one or both the antigens are exposed to the corresponding antibodies they interact with one another to produce visible agglutination or clumping. The ABO blood group antigens are O-linked glycoproteins whereat the terminal sugar residues exposed at the cell surface of the RBC decide in-case the antigen is A or B.

PROCEDURE:

1. A blood sample is needed. The test to determine your blood group is called ABO typing. Your blood sample is mixed with antibodies against type A and B blood.
2. Then, the sample is checked to see whether or not the blood cells stick together. If blood cells stick together, it means the blood reacted with one of the antibodies.
3. The second step is called back typing. The liquid part of your blood without cells (serum) is mixed with blood that is known to be type A and type B.
4. People with type A blood have anti-B antibodies. People with type B blood have anti-A antibodies. Type O blood contains both types of antibodies.
5. The 2 steps above can accurately determine your blood type.
6. Rh typing uses a method similar to ABO typing. When blood typing is done to see if you have Rh factor on the surface of your red blood cells, the results will be one of these:
 - Rh+ (positive), if you have this cell surface protein
 - Rh- (negative), if you do not have this cell surface protein



Fig : 21.1 : Blood grouping tile

CHAPTER 22 : SCREENING

The objective of blood screening is to detect markers of infection in order to prevent the release of infected blood and blood components for clinical use. Screening for current or past infections, including:

- Human immunodeficiency virus (HIV)
- Hepatitis B viruses (HBV)
- Hepatitis C viruses (HCV)
- VDRL
- Malaria

HUMAN IMMUNO DEFICIENCY VIRUS (HIV):

HIV is a fourth generation solid phase Enzyme Linked Immunosorbent Assay (ELISA) which employs Agglutinating sera for p24 antigen and highly purified recombinant antigens representing envelope glycoprotein gp 41 of HIV 1 and envelope glycoprotein gp 36 of HIV 2. The use of HIV antigens to sandwich specific antibodies enables detection of both IgG and IgM antibodies. Detection of p24 antigen also reduces window period.

Principle:

Present evidence indicates that Acquired Immunodeficiency Syndrome is caused by HIV-1 and HIV-2. The viruses are transmitted by sexual contact, exposure to blood (including sharing contaminated needles and syringes) or certain blood products, or transmitted from an infected mother to her fetus or child during the prenatal period. Presence of antigen/antibodies to the virus in the serum of a patient indicates viral infection. Microwell strips are coated with Agglutinating sera for p24 antigen along with the recombinant antigens, gp41 and gp36 representing both HIV 1 and HIV 2. Samples along with positive and negative controls are simultaneously incubated with biotinylated Agglutinating sera for p24 antigen. The wells are washed to remove unbound components. The presence of bound antigen/Agglutinating sera is detected by adding antigen-HRP/streptavidin-HRP conjugate. After washing wells to remove unbound enzyme, substrate is added. The reaction is stopped after specified time with acid and absorbance is determined for each well at 450 nm with an ELISA reader. The cutoff value is calculated by the given formula and absorbance of all the wells are compared with the cutoff value. Any sample having absorbance value equal to or more than the cutoff value is considered reactive.

Test Procedure:

1. Bring all the reagents and specimen to room temperature before use.
2. Take out required number of strips and immediately close the pouch.
3. Prepare data sheet indicating the location of controls and specimen.
4. Use controls in duplicate.
5. Add 50 µl activated antibody reagent in each well.
6. Add 100 µl control or specimen in separate wells
7. Apply plate sealer and incubate for 60 minutes at 37°C .
8. Wash each well by filling approximately 350µl diluted wash buffer, giving 30 seconds soak time for each wash and aspirating/flicking off six times. Blot dry.
9. Add 100 µl diluted conjugate in each well and incubate for 30 minutes at 37°C.
10. Wash each well by filling approximately 350µl diluted wash buffer, giving 30 seconds soak time for each and aspirating/flicking off six times. Blot dry.
11. Add 100µl substrate in each well and incubate for 30 minutes at room temperature (22-28°C) away from direct light.
12. Stop reaction by adding 100µl stop solution. The stop solution should be added in the same sequence as substrate addition.
13. Read the absorbance at 450 nm with 600-700 nm as reference within 30 minutes of stopping the reaction.

HEPATITIS B VIRUSES (HBV):

HBV is a solid phase Enzyme Linked Immunosorbent Assay (ELISA) that employs highly purified, high affinity Agglutinating sera for HBsAg having reactivity for both ad and ay subtypes.

Principle:

Microwell strips are coated with Agglutinating sera for HBsAg. Another Agglutinating sera for HBsAg is conjugated to horseradish peroxidase (HRPO). The sample and the conjugate are added in the coated wells and incubated simultaneously. The wells are washed to remove unbound components. Bound enzyme is detected by adding substrate. The reaction is stopped after specified time with acid and absorbance is determined for each well at 450 nm with an ELISA reader. The cutoff value is calculated by the given formula and absorbances of all the wells are compared with the cutoff value. Any sample having absorbance more than the cutoff value is considered reactive.

Test Procedure:

1. Bring all the reagents and specimen to room temperature before use.
2. Take out required number of strips and immediately close the pouch.
3. Prepare ELISA protocol sheet indicating the location of controls and specimen.
4. Use controls in duplicate.
5. Add 100 µl undiluted control or specimen in separate wells.
6. Add 50 µl activated conjugate to all the wells. Mix by gentle tapping.
7. Apply plate sealer and incubate according to the procedure chosen.
8. Wash each well by filling 350 µl diluted wash buffer and aspirating / flicking off six times with 1 minute soak time between each wash. Blot dry.

Procedure	Incubation Time
Short Procedure	60 min. at 37°C
Standard Procedure	120 min. at 37°C
Overnight Procedure	Overnight at 22-28°C

9. Add 100µl substrate and incubate at 22-28°C away from light for 30 minutes.
10. Stop reaction by adding 100µl stop solution. The stop solution should be added in the same sequence as substrate addition.
11. Read the absorbance at 450 nm with 600-700 nm as reference within 30 minutes of stopping the reaction.

HEPATITIS C VIRUSES (HCV):

Hepatitis C Virus (HCV) is now known to be the major cause of parenterally transmitted non-A, non-B hepatitis. Until the virus was characterized, diagnosis was made by exclusion of all other known causes of hepatitis. Antibody to HCV is found in over 80% of patients with well documented non-A, non-B hepatitis. The worldwide prevalence of HCV is 0.2 to 2% in blood donors and up to 80% in intravenous-drug users. Seroepidemiologic studies show that the seroprevalence of HCV infection in India varies between 0.3% to 11.3%. In a large percentage of HCV cases, transmission is by transfusion and other parenteral means such as sharing of needles, occupational exposure to blood and hemodialysis. However, in case of half of HCV infections, the route of transmission is unknown. HCV establishes a chronic infection in 50 to 80% of cases. Chronic infection is often asymptomatic even in the presence of liver damage discernible on biopsy. Chronic HCV is characterized by fluctuating alanine aminotransferase (ALT or SGPT) levels and

recognizable changes in liver histology. Chronic infection can lead to cirrhosis and hepatocellular carcinoma.

Principle:

HCV is an Enzyme Linked Immunosorbent Assay (ELISA) that employs highly purified recombinant antigens representing most conserved antigenic segments of Core, NS3, NS4 and NS5 antigens from multiple genotypes. These antigens are so selected that they recognize all six major genotypes of HCV prevalent worldwide.

Microwell strips are coated with recombinant antigens representing Core, NS3, NS4 and NS5 antigens. Samples along with positive and negative controls are added in the coated wells and incubated. The wells are washed to remove unbound components and anti-human IgG conjugated to horseradish peroxidase (HRPO) is added. After a short incubation the wells are washed again and bound enzyme is detected by adding substrate. The reaction is stopped after specified time with acid and absorbance is determined for each well at 450 nm with an ELISA reader. The cutoff value (COV) is calculated by the given formula and absorbance of all the wells are compared with the COV. Any sample having absorbance more than the COV is considered reactive.

Test Procedure:

1. Bring all the reagents and specimen to room temperature before use.
2. Take out required number of strips and immediately close the pouch.
3. Prepare ELISA protocol sheet indicating the location of controls and specimen.
4. Use controls in duplicate.
5. Add 200 µl sample diluent to separate wells.
6. Add 10 µl of control or specimen in the wells.
7. Gently shake the plate to mix thoroughly. Care should be taken to avoid spillage.
8. Apply plate sealer and incubate for 30 minutes at 22-28°C.
9. Wash each well by filling approximately 350 µl diluted wash buffer and aspirating/flicking off six times. Blot dry.
10. Add 100 µl diluted conjugate in each well and incubate for 30 minutes at 22-28°C.
11. Wash six times as in step 9. Blot dry.
12. Add 100 µl substrate and incubate at 22-28°C away from light for 30 minutes.
13. Stop reaction by adding 100 µl stop solution. The stop solution should be added in the same sequence as substrate addition.

14. Read the absorbance at 450 nm with 600-700 nm as reference within 30 minutes of stopping the reaction.

THE VENEREAL DISEASE RESEARCH LABORATORY TEST(VDRL):

The Venereal Disease Research Laboratory (VDRL) tests are slide microflocculation test that detect antibodies produced against antigens released by damaged host cells in patients suffering from syphilis.

It uses antigen (referred to as VDRL antigen) containing 0.03% cardiolipin, 0.21% lecithin, and 0.9% cholesterol. The antigen, suspended in a buffered saline solution, forms flocculates when combined with lipoidal antibodies in serum or cerebrospinal fluid from syphilis patients.

Test Procedure:

1. Patients' serum is inactivated by heating at 56°C for 30 minutes in a water bath to remove non-specific inhibitors (such as complement
2. VDRL antigen suspension (colloidal suspension of tissue cardiolipid or chemically synthesized cardiolipin), controls and samples are brought to room temperature.
3. One drop (50 µl) of the test specimen, positive and negative controls is pipetted onto separate reaction circles of the disposable slide.
4. A drop of diluted antigen suspension is added to the measured volume of specimen, positive and negative controls.
5. Using a mixing stick the test specimen and the VDRL reagent is mixed such that it thoroughly spreads uniformly over the entire reaction circle.
6. The slide gently rotated and continuously either manually or on a mechanical rotor at 180 r.p.m.
7. Flocculation is checked microscopically using 10X objective and eye piece at about 8 minutes.

MALARIA:

Five species of Plasmodium parasite are responsible for malaria infection in humans viz P. falciparum, P. vivax, P. ovale, P. malariae and P. knowlesi. This test detects the presence of malaria genus specific pLDH released by parasitised blood cells. Since pLDH is produced by viable TM parasites, the assay can also be used to monitor success of anti-malarial therapy.

Test Procedure:

1. Add 25 µl control/whole blood specimen in separate wells.
2. Add 100 µl of antibody reagent diluted with sample diluent in each wells.
3. Gently shake the plate to mix contents.
4. Apply plate sealer & incubate for 30 minutes at 37°C
5. Wash six times with soak time of 30 sec for each wash. Blot dry
6. Add 100 µl of diluted conjugate in each wells and incubate for 30 minutes at 22-28°C
7. Wash six times with soak time of 30 sec for each wash. Blot dry
8. Add 100 µl of ready-to-use substrate. Incubate in dark for 30 minutes at 22-28°C.
9. Add 100 µl of stop solution to stop the reaction in each wells
10. Read the results at 450 nm (ref: 600 – 700 nm) within 30 minutes

CHAPTER 23 : COMPONENT SEPARATION

BLOOD COMPONENTS:

- Whole blood
- Packed red cells
- Fresh frozen plasma
- Cryoprecipitate

Preparation Of Red Blood Cell:

Red cells have a higher specific gravity than plasma, thus settle in the lower portion of the bag.

Procedure:

1. Store the bag at 2-6°C till processed
2. Place bag in buckets of refrigerated centrifuge and balance the opposite bags Centrifuge at 5000 x g for 5 minutes at 2-6°C
3. Express approx 3/4th of the plasma in the satellite bag
4. Keep red cell at 2-6°C and plasma at -30°C

Preparation Of Fresh Frozen Plasma:

Plasma removed from cells within 6-8 hours of collection is rapidly frozen to below.

Procedure:

1. Collect in triple pack system and store at 4°C or in air-conditioned room till processed but not for more than 6-8 hours.
2. Place bag in buckets of refrigerated centrifuge and balance the opposite bags Centrifuge at 5000g for 5 minutes at 4°C
3. Express approx $\frac{4}{5}$ of the plasma in the satellite bag
4. Label the plasma bag and is rapidly frozen within 6-8 hours and the freezing process should not be more than 1 hour at – 70°C
5. Can be stored at – 30°C or below for 1 year.
6. Thaw it in a plasma defroster (Microwave) or place in a plastic over wrap and put it in a 37°C circulating water bath.

Preparation Of Platelets:

- Random donor platelet - prepared from 450ml of whole blood
- Single donor platelet - prepared by apheresis

Procedure:

1. Collect in triple pack system and store at room temperature at 20-22°C for less than 6 hours.
2. Place bag in buckets of refrigerated centrifuge and balance the opposite bags Centrifuge at $2000 \times g$ for 3 minutes at 20-24°C
3. Express approx $\frac{4}{5}$ th of the platelet rich plasma in the satellite bag.
4. Centrifuge the bag with PRP and another satellite bag at 20-40°C at 5000 xg for 5 minutes.
5. Express supernatant platelet poor plasma into another empty bag and leave approx 50 ml of plasma.

Purpose Of Blood Separation:

Each blood cell type has its own unique purpose and function. Separating the various blood components allows scientists to analyze specific cell types. Some blood components can be used to detect diseases, while others can provide a more comprehensive understanding of the human immune response. Blood separation allows researchers to study only the target cell population without any interference from other cell types.



Fig:23.1 : Blood component separation process

CHAPTER 24 : COMPONENTS STORAGE

WHOLE BLOOD:

- The whole blood refrigerated cold for up to thirty five days (depending on the kind of medicinal drug used).
- Whole blood is employed to treat patients who want all the components of blood, like people who have sustained significant blood loss because of trauma or surgery.

PACKED RED CELL:

- PRBCs have a life time of 21–49 days depending on the additive solution used and jurisdiction. During in vitro storage, PRBCs accumulate cellular and organic chemistry changes jointly known as the RBC storage lesion.
- Typically one unit of PRBCs is close to 350 ml in volume, of which erythrocyte volume is 200 to 250 ml., leukocytes, platelets, and anticoagulants. The most normally used anticoagulant is CPDA-1 (citrate, phosphate, dextrose, adenine), which permits for 35 days storage at 1° to 6°C. The hematocrit of such units is a smaller amount than 80% (range 80% to 80%).

FRESH FROZEN PLASMA:

- It may be ready either from blood or plasmapheresis at intervals eight hours of donation, and it should be keep at -18°C or colder. The shelf life of FFP is twelve months, however it may be extended to seven years if keep at -65°C. FFP

contains all stable and labile coagulation factors, such as factor V (FV) and Factor VIII (FVIII).

PLATELETS:

Platelets are normally stored for up to five days before transfusion, though in some blood services the storage amount is extended to seven days.

APPROPRIATE STORAGE TEMPERATURES:

- WBC : 20° to 24°C
- PRBC : 2° to 6°C
- Platelet : 20° to 24°C
- FFP : Below -18°C
- Cryoprecipitated AHF : Below -18°C



Fig:24.1: Refrigerators & Separated plasma

CHAPTER 25 : BLOOD ISSUING

The safe transfusion of blood and blood products requires strict adherence to patient identification processes during all steps in the transfusion chain, including collecting the product from blood bank.

Blood components and blood products should be issued to one recipient at a time. Issue only one unit of red cells at a time from the Transfusion Medicine Laboratory. Multiple units may be issued to a single patient in an emergency or in a transfusion area that has a monitored storage area.

PROCEDURE FOR BLOOD ISSUING:

1. Provide the Transfusion Medicine Laboratory with a written or computer generated request for blood components or blood products to be issued.
2. Verify the following prior to retrieving the blood component or blood product from the Transfusion Medicine Laboratory:
3. Retrieve the requested blood component or blood product from the appropriate controlled storage area within the Transfusion Medicine Laboratory as per facility policy.

4. Visually inspect blood components and/or blood products and document the inspection prior to releasing the blood component or blood product from inventory, If the blood component or blood product does not pass the visual inspection, it must not be issued.
5. Resolve any identified discrepancies prior to the release of blood components or blood products.
6. Ensure a compatibility label/tag is securely attached to the blood component or blood product prior to issue

BLOOD GROUPING:

Blood grouping is done thus you'll safely give your blood or receive a blood transfusion. It's also done to visualize if you've a substance called Rh factor on the surface of your RBC. Your blood group is based on whether or not certain proteins are on your RBC.

CROSS MATCHING:

Cross matching test is a check performed before a blood transfusion as a part of blood compatibility testing. Normally, this involves adding the recipient's plasma to a sample of the donor's RBC. If the blood is incompatible, the antibodies within the recipient's plasma can bind to antigens on the donor RBC.

CHAPTER 26 : CROSS MATCHING

CROSS MATCHING:

- Also known as compatibility testing.
- Cross matching is the most important test before a blood transfusion is given.
- The primary purpose of cross matching is to detect ABO incompatibilities between donor and recipient.
- This is carried out to prevent transfusion reactions by detecting Abs in recipient's serum.

FUNCTIONS OF CROSS MATCHING:

- It is a confirm ABO compatibility between donor and recipient.
- It may detect presence of irregular Ab in patient's serum that will react with donor RBCs.
- Cross match test is divided into two parts:
 - ✓ Major cross match
 - ✓ Minor cross

PROCEDURE:

- Compatibility at Room Temperature -37°C
- Take 1 drop of recipient's serum/plasma in a small test tube.
- Add 5% saline suspension of donor's RBC
- Mix the two.
- Incubate at room temp.
- Centrifuge.
- Check for agglutination or hemolysis.

RESULT:

- Agglutination or hemolysis absent-match
- Agglutination or hemolysis present-mismatch



Fig : 26.1 : Cross matching card centrifuge

CHAPTER 27: BLOOD BANKING RECORDS

Need to be able to trace a unit of blood from its source to its final disposition. Need a process to introduce to the computer system new hardware, software or databases. Risk analysis, training, validation.

Record

Unique unit identification
Medical director approval for exceptions to donation
Donors placed on indefinite deferral
Investigation of transfusion-transmitted disease

Retention Time

- 10 years
- 5 years
- Indefinite
- 10 years

Donor's ABO & Rh type	- 5 years
Donor testing for unexplained antibodies	- 10 years
HCV or HIV look back	- 10 years
Recipient's ABO & Rh type	- 5 years
Patient's transfusion medical record (unit, date, vital signs)	- 10 years
Suspected transfusion complication	- 5 years
Archival of obsolete documents	- 5 years
Orders for tissue	- 10 years

USES:

- Ensures hospitals have good supply or inventories of blood bags.
- List the availability of blood bags at any given time.
- Ability to manage the information of its blood donor.
- Alerts for blood requirement from registered donors.
- Auto-check if the person donated blood in the last 3 months.
- Allows good documentation about the donor and their blood donation activities.
- Support fast searching to find match blood bags for the right person
- Effectively manage blood camps.

CONCLUSION

My internships at both **CHENGALPATTU MEDICAL COLLEGE – CHENGALPATTU** and **EGMORE LIONS BLOOD BANK AND RESEARCH FOUNDATION - EGMORE** has been great learning experience. During this internship I learned about many procedures and analyzers in the laboratories. Overall, 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.

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CERTIFICATES





CHENGALPATTU MEDICAL COLLEGE, CHENGALPATTU - 603001
(AFFILIATED TO THE TAMILNADU DR.M.G.R.MEDICAL UNIVERSITY,
CHENNAI-600032.)



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

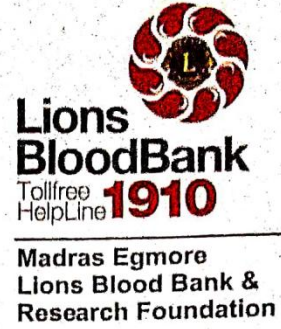
Chengalpattu Medical College,
Chengalpattu.

சென்னை
21/12/23

Date:21.12.2023

Place:Chengalpattu.

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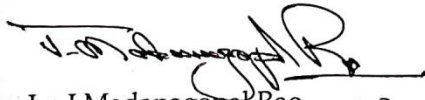
Date: 12th Dec 2022

TO WHOMSOEVER IT MAY CONCERN

This is to certify that Mr. Ajith. C, BSc, 2nd year of Sathyabama Institute of Science and Technology, Chennai -119, undergone Internship training in Madras Egmore Lions Blood Bank & Research Foundation, Egmore, Chennai – 08. He 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 him internship, performance was good and he was able to complete the project on time.

We wish him all the best for his 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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