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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

INSTITUTE OF SCIENCE AND TECHNOLOGY

(DEEMED TO BE UNIVERSITY)

Accredited with Grade "A++" by NAAC

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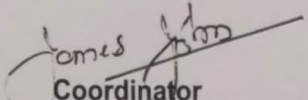
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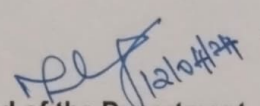
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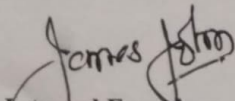
This is to certify that this internship report is the bonafide work of STEPHEN XAVIER. M who have done the Internship work as a team and carried out the training program at "CHENGALPATTU MEDICAL COLLEGE, CHENGALPATTU", "EGMORE LIONS BLOOD BANK AND RESEARCH FOUNDATION, EGMORE", under our supervision from August 2023 to April 2024.

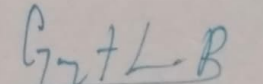

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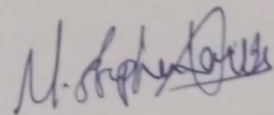
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DECLARATION

I, M. STEPHEN XAVIER (41737017), hereby declare that the internship trainings done by me at LIONS BLOOD BANK AND RESEARCH FOUNDATION at EGMORE under the guidance of Ms. D. JASMINE PRIYA and "CHENGALPATTU MEDICAL COLLEGE" under the guidance of Dr. JAMES JOHN Nat 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: 12/04/24

PLACE : CHENNAI



(M. STEPHEN XAVIER)

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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.

DATE:

PLACE: CHENNAI

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1.INTRODUCTION

I had the opportunity to do my internship at institutions, **CHENGALPATTU MEDICAL COLLEGE AND LIONS BLOOD BANK AND RESEARCH FOUNDATION, EGMORE**. During the period from 01.12.22 to 08.12.22, I completed my internship at Lions blood bank and research foundation in egmore followed by an internship at Chengalpattu Medical College from 01.11.23 to 30.11.23. These internships provided me with valuable experience observing a wide range of tests conducted in both blood banks and diagnostic laboratories. This report summarizes my learning and observations from these internships.

2. HISTOPATHOLOGY

Histopathology is the branch of medicine that involves the examination of tissues, organs, and cells under a microscope to study the changes associated with disease. It helps in diagnosing diseases, determining the extent of disease progression, and guiding treatment decisions.

2.1 SPECIMEN ACCESSIONING:

Receiving, recording, and labelling medical specimens—such as tissue samples, blood samples, or other body fluids—in a laboratory setting is known as specimen accessioning. This procedure entails giving each specimen a special identification number, documenting pertinent patient data, and making sure that handling and storage are done correctly to preserve sample integrity. For the purpose of tracing specimens throughout the laboratory process and guaranteeing correct test findings, precise accessioning is essential.

Receiving Of Specimens: Hospitals, clinics, and other healthcare facilities provide specimens to the laboratory when they arrive.

Documentation: Accurate records are kept of all pertinent data pertaining to the patient and the specimen, including the patient's name, identification number, date and time of collection, and kind of specimen.

Labeling: In order to keep track of each specimen container during the testing procedure, each one is tagged with a special identification, frequently a barcode.

Verification: To guarantee correctness and completeness of information, the supplied specimens are cross-checked with the related documentation.

Appropriate Handling: In order to preserve specimen integrity and avoid contamination, proper practices are followed when handling them.

Storage: Depending on the type of specimen and testing requirements

2.2 GROSS EXAMINATION

Specimens are physically inspected before undergoing additional testing or processing. Usually carried out by pathologists or laboratory technicians, this evaluation entails:

1. **Visual Inspection:** Examining the specimen's color, size, shape, and any obvious abnormalities or lesions from the outside.
2. **Measurement:** If applicable, note measurements of the specimen's size, weight, and volume.
3. **Description:** Documenting the characteristics of the specimen, including any notable features, such as presence of tumors, inflammation, or abnormal growths.
4. **Dissection:** To study the specimen's interior anatomy and pinpoint particular regions of interest, it may be sectioned or dissected if necessary.
5. **Photography:** Taking pictures of the specimen to record its appearance and help with additional research or advice.

A thorough gross inspection yields important preliminary data about the specimen that informs and directs further diagnostic procedures and tests, including molecular testing, microbiological cultures, and histopathological investigation. It is essential for the precise diagnosis and treatment of illnesses.



Fig no. 1 - Grossing

2.3 TISSUE FIXATION

In a medical laboratory, tissue fixation is an essential step in the histopathological processing of tissue samples. It entails preventing degradation and conserving the tissue's cellular makeup and structure so that microscopic inspection can be performed. Tissue fixation's primary objectives are to:

Prevent Putrefaction and Autolysis: Fixation preserves the morphology and cellular architecture of the tissue by stopping enzymatic and cellular processes that would otherwise cause the tissue to decompose.

Preserve Cellular Structures: Fixation aids in the preservation of cellular structures, such as membranes and organelles, which are crucial for precise histological interpretation.

Enable Staining: Hematoxylin and eosin staining, which provides contrast and highlights particular cellular components, is one of the staining procedures that can be applied to fixed tissues in histology.

Enable Long-Term Storage: Tissues that have been properly treated can be kept for a long time without suffering serious degeneration, making them accessible for use in research, consultation, and future reference.

Formalin (formaldehyde solution), alcohol-based fixatives (such as ethanol), and other chemical solutions are frequently used fixatives in medical laboratories. The kind of tissue, planned downstream analyses, and preservation needs all influence the fixative selection.

Following fixation, tissues are usually subjected to additional processing procedures such drying, embedding, sectioning, staining, and mounting onto slides so that pathologists or lab workers can examine them under a microscope. An important part of histopathological examination is tissue fixation, which yields vital data for illness diagnosis and patient treatment planning.

2.4 TISSUE PROCESSING

In a medical laboratory, the term "tissue processing" describes the set of procedures carried out on tissue samples following their fixation. These procedures are meant to get the tissue ready for pathologists' microscopic inspection and analysis. The following steps are commonly included in the tissue processing workflow:

Dehydration: Alcohol or similar organic solvent is progressively added to the water to dehydrate the fixed tissue. Dehydration causes the tissue to lose water, which makes it more susceptible to embedding medium penetration.

Cleaning: After the tissue has been dehydrated, it is cleaned using xylene or another clearing agent. By making the tissue transparent and eliminating the alcohol or solvent, clearing makes embedding and sectioning easier.

Infiltration and Embedding: A molten embedding material, like paraffin wax, is infused into the tissue to saturate it and provide structure for sectioning. After that, the tissue is placed within an embedding medium-filled mold and let to solidify.

Sectioning: A microtome is used to trim and cut the implanted tissue block into thin slices, or sections. The slices are placed onto glass slides for additional processing; they are normally 4-5 micrometers thick.

Staining: To bring attention to particular cellular components and structures, tissue sections are stained using a variety of histological stains. Hematoxylin and eosin (H&E) is a common stain that contrasts the color of the cytoplasm (pink) with the nuclei (blue).

Cover Slipping: Following staining, the tissue slices are cleaned, dried off, and then mounted using a mounting media and a coverslip. Cover sliding keeps the staining intact and shields the tissue for microscopic inspection.

Microscopic Examination: Pathologists or lab technicians examine the prepared slides under a microscope. Identification of cellular anomalies, tissue architectures, and pathological alterations suggestive of illness is possible through microscopic analysis.

In histopathology, tissue processing is an essential step that helps with accurate disease diagnosis as well as patient management and therapy choices. To guarantee the integrity and quality of the tissue specimens as well as the precision of the diagnostic findings, each step of the tissue processing procedure must be carried out with great care.

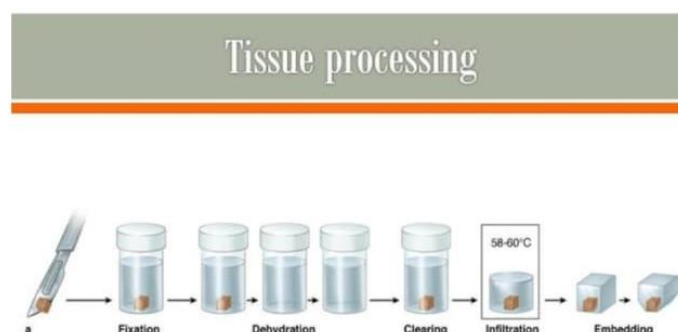


Fig no. 2 – Tissue processing

2.5 TISSUE EMBEDDING

In order to give structural support for thin sectioning, tissue samples are immersed in a solid media, usually paraffin wax, in a process known as tissue embedding in medical laboratories. This procedure maintains tissue integrity, enabling precise microscopic analysis and disease identification.

TECHNIQUE OF EMBEDDING

1. Open the tissue cassette and check whether correct number of tissue pieces is present.
2. Select the mould. There should be sufficient room for the tissue with at least a 2 mm surrounding margin of wax.
3. Fill the mould 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 labelled embedding ring or cassette base onto the mould
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 mould.

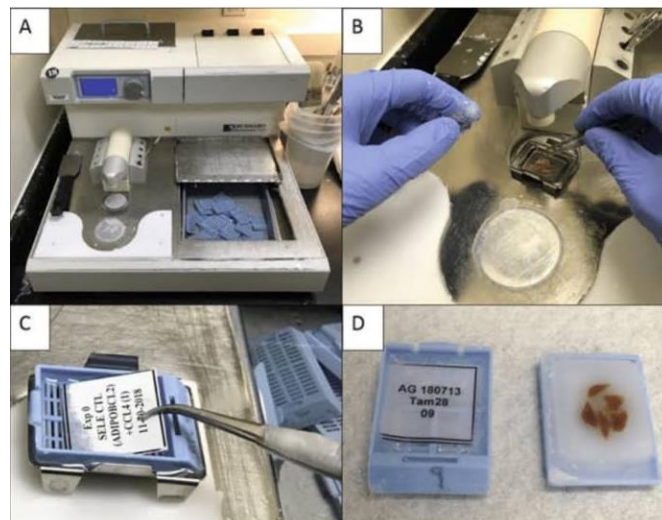


Fig no. 3- Tissue embedding.

2.6 TISSUE SECTIONING

Sectioning of tissues in a medical laboratory entails slicing thin sections of tissue samples embedded in a microtome. Subsequently, these thin slices are placed on slides for additional processing, staining, and examination under a microscope. This procedure enables pathologists to analyze the cellular structure and morphology of tissues in order to provide precise diagnoses and determine appropriate treatments.

2.7 SLIDE STAINING

One of the most important steps in the histological analysis of tissue samples in a medical laboratory is slide staining. It entails dotting tissue slices on slides with different dyes or stains in order to increase contrast and make particular cellular structures or components visible under a microscope. This procedure is essential for helping pathologists and researchers carry out research, comprehend tissue morphology, and diagnose illnesses.

The staining process typically involves several steps:

Fixation: Tissue samples need to be fixed in order to maintain their structural integrity and stop decaying before staining. Typically, formalin or other fixatives are used to cross-link proteins and stop them from degrading.

Embedding: To maintain uniform thickness of the sections and to provide support for sectioning, tissue samples are immersed in a solid media, such as paraffin wax.

Sectioning: Using a microtome, thin slices of the implanted tissue are cut and placed on glass slides. These sections are usually exceedingly thin, with a thickness of 5–10 micrometers

Deparaffinization and hydration: Prior to staining, the tissue sections must be deparaffinized and rehydrated because they are encased in wax. To do this, the slides are submerged in a variety of solvents, including alcohol and xylene, in order to dissolve the wax and replenish tissue hydration.

Staining: To draw attention to particular cellular components or structures, a variety of staining procedures are employed. Typical staining techniques consist of:

Hematoxylin and eosin (H&E) staining: Nuclei are stained blue-purple by hematoxylin, and the cytoplasm and extracellular matrix are stained pink by eosin. In regular histology, this staining is extensively employed to visualize the overall architecture of the tissue.

Special stains: These stains are intended to target certain cellular structures or components, like muscle fibers, connective tissue, or bacteria. Gram stain for bacteria, Masson's trichrome stain for collagen, and periodic acid-Schiff (PAS) stain for glycogen are a few examples.

MICROTOMY

Microtomy is defined as the means by which tissue can be sectioned and attached to a surface for microscopic examination. Microtomes are defined as precision instruments designed for cutting material into sections, thin enough for examination with a microscope.

CLASSES OF MICROTOMES

- Rotary
- Rocking
- Sliding
- Sledge
- Microtomes for frozen section
 - Freezing microtome
 - Cryostat
- Ultramicrotome



Fig no. 4 - Microtomy

ROTARY MICROTOME

- It is most commonly used microtome in this the 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.

SLIDING MICROTOME

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

FREEZING MICROTOME

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

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 bearing arm.

BASE SLEDGE MICROTOME

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

MICROTOME KNIVES

Microtome knives are used in microtomes to cut thin slices, or sections, of tissues samples for microscope examination. The slides are typically less than 50 micrometres thick and are used for a variety of purposes, including histological analysis, tissue culture, and electron microscopy.

DIFFERENT TYPES OF KNIVES

METAL KNIVES

1. Biconcave: used for rocking, rotary on freezing microtome. It has concavity on the both sides
2. Planoconcave: it is flat on one side and concave on the other side with degree of concavities, used in sledge, rotary and rocking microtomes
3. Wedge-shaped knife: it plain on both sides.it used in routine paraffin section, frozen section and all types of microtomes.
4. Chisel-shaped knife: It has a blunt edge, hard objects (undecalcified bone) have detached handle.

NON-METAL KNIVES

Glass knives: The knives are made from specially and glass strips. They are used in an ultramicrotome to cut ultrathin slices of samples for electron and light microscope for resin and for cryosection.

Diamond knives: They are used in electron microscopy for sectioning epoxy resin block.

DISPOSABLE KNIVES:

Nowadays, disposable knives are commonly used. They are made of steel and coated with special Poly Tetra Fluoro Ethylene (PTFE), which allows the ribbons to be easily sectioned.

Types of disposable knives

- Low profile knives
- High profile knives

ADVANTAGES

- Honing not required Section quality improved

DISADVANTAGE

- Relatively expensive

3. MICROBIOLOGY

3.1 CULTURE:

Bacterial culture was the first method developed to study the human microbiota, using an artificial medium that allows growth and isolation of bacteria. The first to have cultured a bacterium in a reproducible way was Louis Pasteur in 1860. A population of bacteria grown in the laboratory is referred to as a culture. A pure culture contains only one single type; a mixed culture contains two or more different bacteria.

Types of culture methods:

- Streak culture or surface plating
- Lawn or carpet culture
- Stroke culture
- Stab culture
- Pour plate method
- Liquid culture
- Anaerobic methods of culturing bacteria



Fig no. 5 - Culture

STREAK CULTURE:

Using this method, individual cells are spaced enough away from one another by spreading a loopful of culture on an agar plate. By progressively diluting the inoculum, the streaking method allows the bacterial cells to be counted as colony forming units (CFUs).

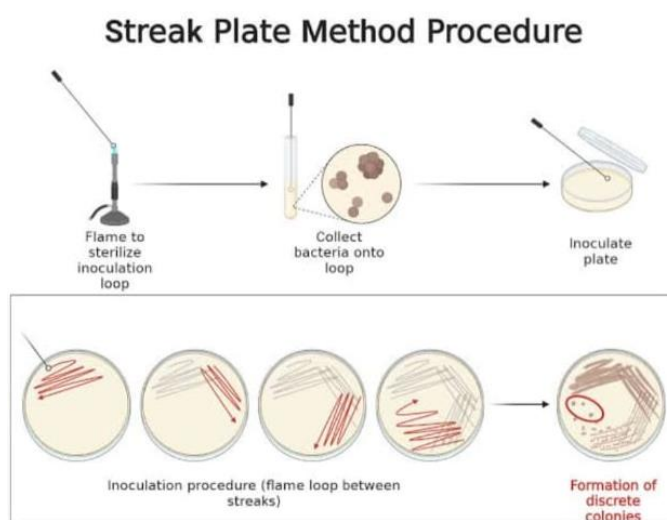


Fig no. 6 – Streak plate culture

LAWN OR CARPET CULTURE:

In order to prepare lawn cultures, the plate's surface is flooded with a liquid culture or suspension of the bacteria, the surplus inoculum is pipetted off, and the plate is then incubated. An alternative method of inoculating the plate surface is to use a brush soaked in the bacterial suspension or culture



Fig no. 7 – Lawn culture

STROKE CULTURE:

Agar slope or slant tubes are used to make stroke cultures. In order to seed slopes, lightly spread the agar's surface in a zigzag pattern, being careful not to cut the agar. It is used to supply pure bacterial growth for slide agglutination and other diagnostic procedures.

STAB CULTURE:

By puncturing a suitable medium with a long, straight charged wire. For gelatin liquefaction, stock cultures & motility.

POUR PLATE METHOD:

When molten agar medium is injected, either before or after it has solidified, the pour plate method is a microbiological laboratory technique used to separate and count the live bacteria contained in a liquid sample.

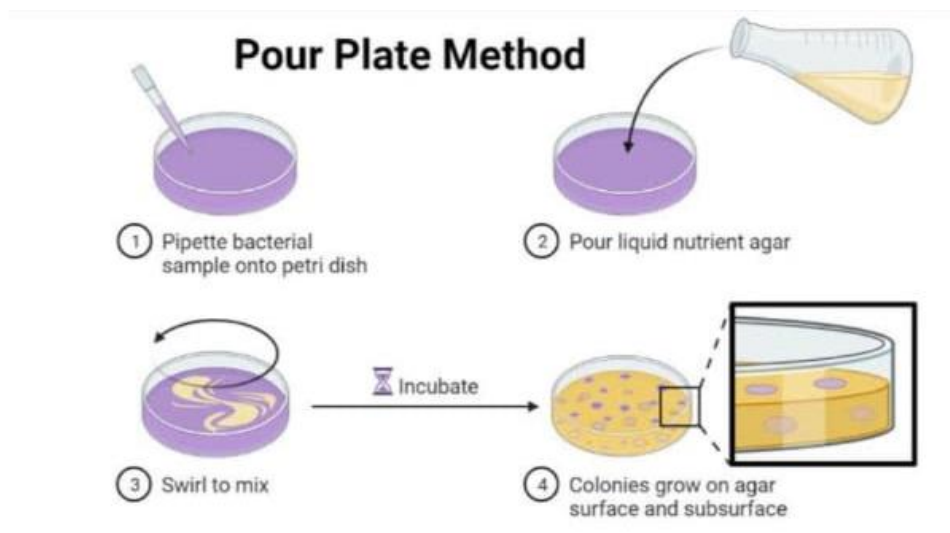


Fig no. 8 – Pour plate method

LIQUID CULTURE:

Inoculated by a charged loop, pipette or syringes. For blood cultures & sterility testing.



Fig no. 9 – liquid culture.

ANAEROBIC CULTURE METHODS:

Anaerobic condition can be achieved by:

- Cultivation in vacuum
- Displacement of oxygen with other gases
- Chemical or biological methods
- By displacement and combustion of oxygen
- By reducing agents
- Anaerobic chamber.

3.2 MEDIA

CHOCOLATE AGAR

Chocolate agar is essentially the same as blood agar except that during preparation the red blood cells are lysed when added to molten agar base. As a result, the cell lysis releases intracellular nutrients such as hemoglobin, hemin (“X” factor), and the coenzyme nicotinamide adenine dinucleotide (NAD or “V” factor) into the agar which is utilized by fastidious bacteria. Red blood cell lysis gives the medium a chocolate-brown coloration when prepared from which the agar gets its name. The most common bacterial pathogens that require this enriched medium for growth include *Neisseria gonorrhoeae* and *Haemophilus* species.

PREPARATION OF CHOCOLATE AGAR:

Composition of Chocolate Agar

The composition of chocolate agar is the same as that of blood agar. The only difference is blood agar is heated in a water bath to prepare chocolate agar. Commercially available chocolate agar medium, however, has a different composition and requires no addition of blood

Ingredients	Gm/L
Casein/animal tissue digest	15.0
Cornstarch	1.0
Potassium phosphate, dibasic	4.0
Potassium phosphate, monobasic	1.0
Sodium chloride	5.0
<u>Agar</u>	10.0
Hemoglobin solution (2%)	500.0 ml
Isovitox enrichment	10.0 ml

Preparation of Chocolate Agar

1. Prepare the blood agar base as instructed by the manufacturer.
2. Sterilize by autoclaving at 121°C for 15 minutes.
3. Add 5-7% v/v of defibrinated blood (horse or sheep blood). Then place the media in a water bath of 75 -80°C. Keep swirling gently until the color changes to dark brown.
4. Pour into sterile Petri plates under aseptic conditions after the media has cooled to 50-55°C.
5. Label the plates with the name of the media, and date of preparation, and store them invertedly at 2-8°C until use.

Chocolate agar slants

1. Dispense 4 mL of the medium into 16X125 mm screw-cap tubes
2. Keep the tubes in a slanted position and let them solidify.

This agar slants appear brown to brownish-red color. Store these at 4°C when not in use and warmed to room temperature (25°C) before use.



Fig no. 10 – chocolate agar

BLOOD AGAR:

Blood Agar is a multi-nutrient enhanced medium that is typically used as a base media for the manufacture of blood agar through blood supplementation.

COMPOSITION OF BLOOD AGAR:

Like the majority of other nutritional media, blood agar contains salt, beef extract for vitamins and minerals, and one or more protein sources. In addition to these ingredients, the medium also contains 5% of defibrinated mammalian blood. A variety of suppliers sell the blood agar base commercially, or if the materials are available, it can be made in a lab.

Final pH at 25°C: 7.3 ±0.2

S.NO	INGREDIENTS	GRAM/LITRE
1.	Peptone	10.0
2.	Tryptose	10.0
3.	Sodium chloride	5.0
4.	Agar	15.0

PREPARATION OF AGAR:

- One kilogram of the prepared medium plus one thousand milliliters of deionized or distilled water is added.
- To fully dissolve the medium, the suspension is brought to a boil.
- After that, it is autoclaved for roughly 15 minutes at 121°C and 15 pounds of pressure to sanitize it.
- 5% v/v sterile defibrinated blood is aseptically added to this and thoroughly mixed.
- After that, the media is transferred into sterile Petri dishes in an aseptic environment.
- Before used, the plates can be dried out by placing them in the hot air oven for a short while at a lower heat setting once the media has solidified.

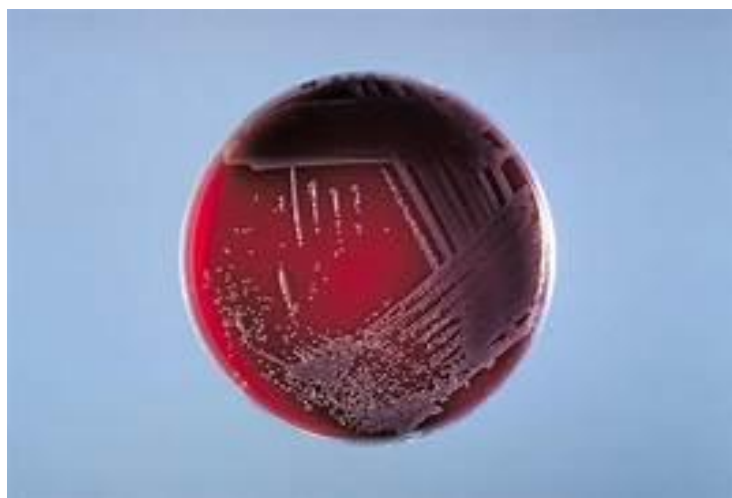


Fig no. 11 – Blood agar

BLOOD AGAR STORAGE

The prepared medium should be stored between 20–30°C, while the media in powder form should be kept in a firmly covered container between 10 and 30°C.

Because the medium is hygroscopic and absorbs moisture very fast, it is important to store the product appropriately while it is dry and to tightly close the bottle to avoid lump formation.

The medium-containing container needs to be kept dry, well-ventilated, and shielded from sources of fire and extremely high or low temperatures. Use the product before the labeled expiration date.

MACONKEY AGAR:

MacConkey agar is a selective and differential media used for the isolation and differentiation of non-fastidious gram-negative rods, particularly members of the family Enterobacteriaceae and the genus *Pseudomonas*. The inclusion of crystal violet and bile salts in the media prevent the growth of gram-positive bacteria and fastidious gram-negative bacteria, such as *Neisseria* and *Pasteurella*. The tolerance of gram-negative enteric bacteria to bile is partly a result of the relatively bile-resistant outer membrane, which hides the bile-sensitive cytoplasmic membrane. Other species specific bile-resistance mechanisms have also been identified.

PROTOCOL:

Streak a plate of MacConkey's agar with the desired pure culture or mixed culture. If using a mixed culture use a streak plate or spread plate to achieve colony isolation. Good colony separation will ensure the best differentiation of lactose fermenting and non-fermenting colonies of bacteria.

Streak plate of *Escherichia coli* and *Serratia marcescens* on MacConkey agar. Both microorganisms grow on this selective media because they are gram-negative non-fastidious rods. Growth of *E. coli*, which ferments lactose, appears red/pink on the agar. Growth of *S. marcescens*, which does not ferment lactose, appears colorless and translucent.

COMPOSITION OF MAC CONKEY AGAR:

INGREDIENTS	AMOUNT
Peptone (pancreatic digest of gelatine)	17gm
Proteose peptone (meat and caesine)	3gm
Lactose monohydrate	10gm
Bile salts	1.5gm
Crystal violet	0.001gm
Sodium chloride	5gm
Neutal red	0.03gm
Agar	13.5gm

PREPARATION OF MAC CONKEY AGAR:

- Suspend 49.53 grams of dehydrated medium in 1000 ml of distilled water.
- Heat to boiling to dissolve the medium completely.
- Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.
- Cool to 45°C -50°C.
- Mix well before pouring into sterile Petri plates.

MacConkey Agar



Fig no. 12 – Mac conkey agar

CLED AGAR:

The Cystine-Lactose-Electrolyte-Deficient (CLED) medium is a non-selective medium that can sustain the development of most urinary pathogens. It was initially developed by Sandys and subsequently modified by Mackey and Sandys. It is typically used for routine diagnostic urine bacteriology. A differential medium called CLED Agar is used to separate and count the bacteria in urine. All urinary potential pathogens can thrive in the medium, which also gives different colony morphology. Because it lacks electrolytes, it promotes the growth of pollutants and urinary infections while providing good colonial differentiation and preventing the expansion of *Proteus* species.

PRINCIPLE:

The CLED Agar principle The CLED is made up of beef extract, an enzymatic digest of gelatin, and an enzymatic digest of casein. These ingredients supply the nitrogen, vitamins, minerals, and amino acids needed for growth. The growth of the cysteine-dependent dwarf coliform colony is supported by L-cystine. Carbon and energy are produced by the fermentable carbohydrate lactose. Lactose-fermenting organisms will reduce the pH and turn the medium's green color to yellow. The presence of the pH indicator Bromthymol Blue indicates a shift in color. The solidifying ingredient in the medium is agar.

CONSTITUTION	GRAM/LITRE
Lactose	10.0
Enzymatic digest of gelatin	4.0
Enzymatic Digest of Casein	4.0
Beef extract	3.0
L-Cystine	0.128
Bromothymol Blue	0.02
Agar	15.0

PREPARATION OF CLED AGAR:

- Suspend 36 gram of the medium in one liter of distilled water.
- Mix well and heat with frequent agitation and boil for one minute until complete to dissolution.
- Autoclave at 121°C for 15 minutes.
- Cool to 50°C, mix well and dispense into plates. When the medium is solidified, invert the plates to avoid excess moisture. The prepared medium should be stored at 8-15°C.

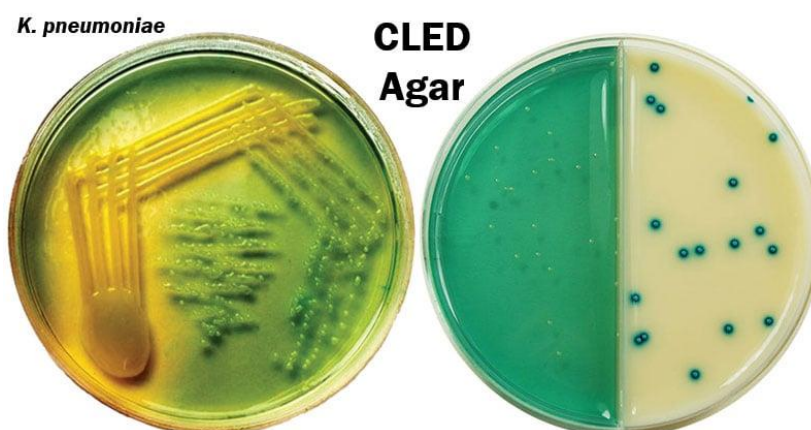


Fig no. 13 – Cled agar

USES OF CLED AGAR:

- It is used for routine urine culture and uses for growth and enumeration of urinary tract organisms.
- It helps in the differentiation of lactose fermenter and lactose non-fermenter.
- It is suitable for the isolation and counting of many aerobically growing microorganisms, such as Enterobacteriaceae, Pseudomonas, and other non-fermenting Gram negative rods, enterococci, staphylococci, Candida species, and many others from urine specimens

3.3 GRAM STAINING:

Gram staining is a differential bacterial staining technique used to differentiate bacteria into Gram Positive and Gram Negative types according to their cell wall composition.

It is the most widely used and the most important staining technique in bacteriology, especially in medical bacteriology. It is generally the first test performed on bacteria during their identification and observation process.

PREPARATION:

Prepare a bacterial smear: Take a small amount of bacterial culture and spread it thinly on a clean microscope slide. Let it air dry.

Fix the smear: Pass the slide over a flame a few times to heat-fix the bacteria. This kills the bacteria and adheres them to the slide.

Primary stain - Crystal Violet: Flood the smear with crystal violet stain for about 1 minute. This stains all cells purple.

Rinse with water: Rinse the slide with water to remove excess stain.

Gram's iodine (Mordant): Flood the smear with Gram's iodine solution for about 1 minute. This forms a complex with the crystal violet, enhancing its retention in Gram-positive bacteria.

Decolorization: Rinse the slide with alcohol or acetone for a few seconds until the runoff is colorless. This removes the stain from Gram-negative bacteria but not from Gram-positive ones.**Counterstain - Safranin:** Flood the smear with safranin for about 30 seconds. This stains Gram-negative bacteria pink/red, as they have lost the crystal violet-iodine complex.

Rinse and dry: Rinse the slide with water, blot dry with bibulous paper, and observe under a microscope.

Gram-positive bacteria will appear purple, while Gram-negative bacteria will appear pink/red. Proper interpretation involves observing the morphology and arrangement of the stained bacteria.

4.SEROLOGY

4.1 WIDAL TEST:

Widal test is an agglutination test used for diagnosis of enteric fever (Typhoidal or Paratyphoidal fever) serologically in clinical/diagnostic laboratories. This tests measure agglutinating serum antibodies level produced against the O (somatic) and H (flagellar) antigen of *Salmonella* Typhi and Paratyphi A, B, and C.

This test is widely used throughout the world as a major diagnostic tool for detecting enteric fever.

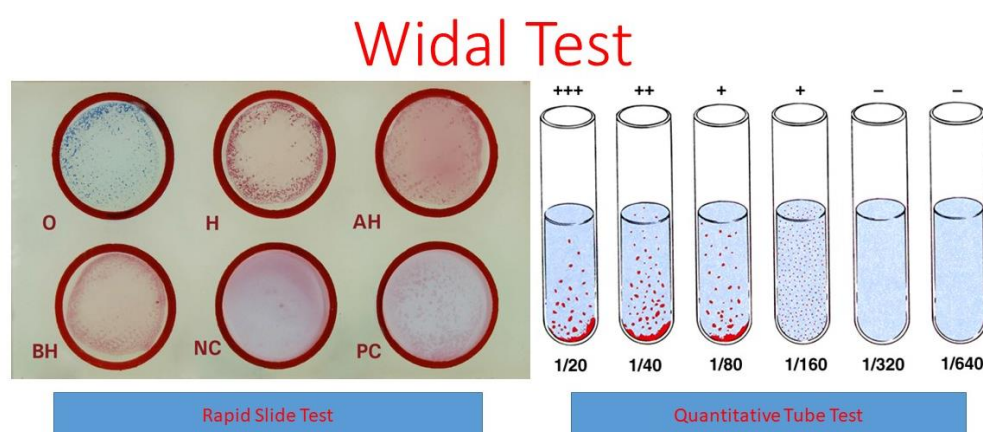


Fig no. 14 – widal test

PREPARATION WIDAL ANTIGENS

- H suspension of bacteria is prepared by adding 0.1 per cent formalin to a 24 hours broth culture or saline suspension of an agar culture.
- For preparation of O suspensions of bacteria, the organisms is cultured on phenol agar (1:800) to inhibit flagella.
- Standard smooth strains of the organism are used; *S* Typhi 901, O and H strains are employed for this purpose.
- The growth is then emulsified in small volume of saline, mixed with 20 times its volume of alcohol, heated at 40° C to 50° C for 30 minutes and centrifuged.
- The antigens are treated with chloroform (preservative) and appropriate dyes are added for easy identification of antigens.

PROCEDURE OF WIDAL TEST

SLIDE TEST

1. Place one drop of positive control on one reaction circles of the slide.
2. Pipette one drop of Isotonic saline on the next reaction circle. (-ve Control).
3. Pipette one drop of the patient serum to be tested onto the remaining four reaction circles.
4. Add one drop of Widal TEST antigen suspension 'H' to the first two reaction circles. (PC & NC).
5. Add one drop each of 'O', 'H', 'AH' and 'BH' antigens to the remaining four reaction circles.
6. Mix contents of each circle uniformly over the entire circle with separate mixing sticks.
7. Rock the slide, gently back and forth and observe for agglutination macroscopically within one minute.

4.2 HIV SCREENING TEST:

A test to check for human immunodeficiency virus (HIV) infection. HIV is the virus that causes acquired immunodeficiency syndrome (AIDS). The most common type of HIV test is called the HIV antibody test, which checks for antibodies against HIV in a sample of blood, urine, or fluid from the mouth. It can take from 2 weeks to 6 months after a person is infected with HIV before the antibodies are found with an HIV antibody test. Another type of HIV test checks for RNA or DNA from HIV in a sample of blood. This test can find HIV in a person's blood about 9-11 days after the person is infected with the virus.

TYPES OF HIV TESTS :

- Standard HIV Blood Test
- Antigen/Antibody Test
- Nucleic Acid Tests (NAT)
- Standard HIV Blood Test
- The HIV blood test is also conducted to examine the antibodies against the virus present in the host.

The tests are more accurate in the case of 23 to 90 days from the initial infection.

PROCEDURE OF HIV BLOOD TESTS

Standard HIV Blood Test

This HIV blood test is conducted at the labs. Here, the technician will draw some blood from your vein. The blood is then sent to the lab for the determination of infection. The blood from the vein allows better detection of infection as compared to rapid testing through a finger prick or any other bodily fluid.

Antigen/Antibody Test

The test is conducted using a similar method as the standard HIV blood test. The blood is drawn from the veins of the person taking the test. The combination test is now the standard for HIV blood tests in most labs, such as metropolis.

Nucleic Acid Tests (NAT)

This HIV test is conducted using blood drawn from your vein. The labs then isolate the copies of HIV-2 RNA from the plasma sample to provide a quick and accurate result.

4.3 CRP TEST:

C-Reactive Protein (CRP), also known as Pentraxin 1, is a non-glycosylated protein in the Pentraxin family that also includes Pentraxin 2/SAP and Pentraxin 3/TSG-14. CRP is an acute phase reactant, a protein made by the liver and released into the blood within a few hours after tissue injury, the start of an infection, or other cause of inflammation.

PROCEDURE OF CRP TEST

Qualitative Test

- Bring all reagents and serum sample to Room Temperature and mix latex reagent gently prior to use. Do not dilute the controls and serum.
- Place 1 drop of Serum, Positive control and Negative control on separate reaction circle on glass slide.
- Then add 1 drop of CRP latex reagent to each of the circles.
- Mix with separate mixing sticks and spread the fluid over the entire area of the cell.
- Tilt the slide back and forth slowly for 2 minutes observing preferably under artificial light.
- Observe for visible agglutination.

4.4 RA FACTOR TEST:

INTRODUCTION An antibody called rheumatoid factor can be found in the blood through a standard blood test. In reality, rheumatoid factor is an antibody that binds to other antibodies. Normal blood proteins called antibodies play a crucial role in our immune system. An antibody called rheumatoid factor is typically absent from a healthy individual. Rheumatoid factor antibody is commonly known as an autoantibody since it binds to normal antibodies. To aid with the diagnosis of rheumatoid arthritis, medical professionals use the rheumatoid factor test.

PRINCIPLE

Detection of Rheumatoid factor by using Latex Agglutination Purpose RF-latex is slide agglutination test for the qualitative and semiquantitative detection of RF in human serum. Latex particles coated with human gammaglobulin are agglutinated when mixed with samples containing RF.

PROCEDURE

1. The reagents and samples are reached to room temperature. The sensitivity of the test may be reduced at low temperatures.
2. Place 50 µl of the sample and one drop of each Positive and Negative controls into separate circles on the slide test.
3. Swirl the RF-latex reagent gently before using and add one drop(50 µL) next to the sample to be tested.
4. Mix the drops with a stirrer, spreading them over the entire surface of the circle.

5.BLOOD BANK

5.1 INTRODUCTION:

A blood bank is a center where blood gathered as a result of blood donation is stored and preserved for later use in blood transfusion.

What are the function of blood banking?

Blood banking, the process of collecting, testing, processing, and storing blood for later use, is a cornerstone of emergency and surgical medicine and is dependent on the clinical laboratory for ensuring the safe use of blood and its components.

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 daycare service

BLOOD DONOR SELECTION AND REJECTION CRITERIA

5.2 SELECTION CRITERIA:

The criteria for the donor are:

- 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
- Pulse – between 50 and 100/ minutes with no irregularities
- Women should not be pregnant or breast feeding her child
- Women donor should not donate during menstrual cycle

5.3 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
- Low hemoglobin or anemic
- When tattoo
- Allergic
- High blood pressure
- Alcoholic
- Smoker
- Organ transplantation

5.4 BLOOD BAG AND ITS TYPE

Blood bags are used for the reliable collection, separation, storage and transport of blood

SINGLE BLOOD BAG:

- Used for collection preservation, transfusion, of human blood
- This single blood bag system with primary bag contains anticoagulant
- Packed RBCs are made by removing 200-250ml of plasma from whole blood.
- 1ml blood weighs **1.06gm**.



Fig no. 15 – Single bag blood

DOUBLE BLOOD BAG:

- For whole blood collection.
- Separation of 2 different blood components (red blood cell and plasma)
- This is obtained through the process of centrifugation and extraction.
- The primary bag contains CPDA-1 anticoagulant solution.



Fig no. 16 – double blood bag

TRIPLE BLOOD BAG:

It includes a primary bag and satellite bag

This system separate RBC, plasma puffy coat (includes leucocytes and platelets)



Fig no. 17 – triple blood bank

5.5 BLOOD DONOR CONSENT FORM

[illegible][illegible]

Fig no. 18

Blood donor consent form

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.

5.6 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.

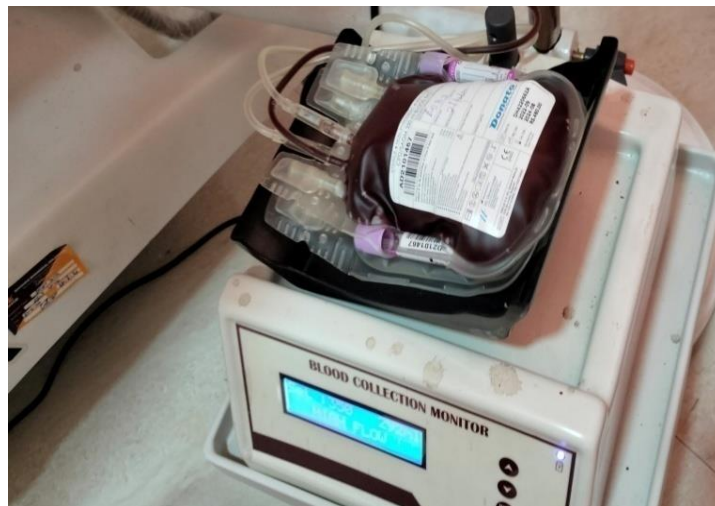


Fig no. 19 - Donation

5.7 DONOTION

- You'll go into a donor room where you'll lie down on a cot.
- A phlebotomist (an employee who draws blood) will clean your arm and insert a new, sterile needle into your vein. This takes just a few seconds, and it can feel like a quick pinch.
- You'll donate about 1 pint (one unit) of blood. The process should take less than 10 minutes. But if you're donating platelets, red cells, or plasma by apheresis, the process can take much longer: up to 2 hours.
- When you're done, you'll raise your donation arm and put a little bit of pressure on it, which helps your blood clot. Then they'll put an adhesive strip on your arm.

5.8 BLOOD DONOR CONSELING:

Blood donor counseling 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

COUNSELLING IN BLOOD BANKS

- Motivate the donor at high risk to test for HIV
- Help the donor to take the right decision
- Create awareness among the donors on HIV AIDS and TTIs
- Encourage voluntary blood donor to donate regularly
- Donor consent for testing of blood for all tests
- Provide an opportunity to ask question and refuse donations
- Risk history assessment
- Donation and test information
- Getting the reports after the tests

TWO OF COUNSELING:

1. PRE DONATION COUNSELING

This is an opportunity to explain to the blood donor about the donation process, the value of honest responses and the benefit of blood donation.

2. POST DONATION COUNSELING

Donors be notified in a confidential manner of test results for TTI markers and any medically significant finding identified during per-donation evaluation.

PRE-DONATION COUNSELING

- Donor 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 infection
- Donation and test information
- Getting the report after the tests
- Result seeking donors should be given post donation information and counseling

POST DONATION COUNSELING

- Donors be notified in a confidential manner of test results for TTI markers and any medically significant finding identified during the per-donation evaluation.
- Counseling should be conducted in an environment that is conducive to a confidential, one-to-one discussion between the counselor and the donor.
- Whether it is provided in fixed location or mobile setting, the venue for donor counseling should provide adequate audio and visual privacy and confidentiality.
- 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 question.
- Help the donor cope with emotions arising from the test result

5.9 BLOOD GROUPING:

As you are probably aware, the blood of human beings varies in specific viewpoints, however it has all the aspects of being comparable. Different types of collection of blood have been done. Two such groupings—the ABO and Rh—are broadly utilized all around the world.

ABO SYSTEM:

ABO grouping depends on the presence or absence of two surface antigens on the RBCs to be specific A also, B. Like, the plasma of various people contains two normal antibodies. The distribution of antigens and antibodies in the four gatherings of blood, A, B, AB, and O are given in Table. You likely realize that during blood transfusion, any kind of blood can't be utilized; the blood of a donor has to be carefully coordinated with the blood of a beneficiary before any blood transfusion to stay away from extreme issues of clumping.

RH TYPING:

Another antigen, the Rh antigen similar to one present in Rhesus monkeys (hence Rh), is likewise seen on the outer layer of RBCs of a larger part (almost 80 percent) of people. Such people are called Rh positive (Rh+ve) furthermore, those in whom this antigen is missing are called Rh negative (Rh-ve). An Rh-ve individual, whenever presented with Rh+ve blood, will form specific antibodies against the Rh antigens. Subsequently, the Rh group should also be matched prior to transfusions. A special case of Rh incompatibility (mismatching) has been seen between the Rh-ve blood of a pregnant mother and with Rh+ve blood of the fetus. Rh's antigens of the fetus don't get presented to the Rh-ve blood of the mother in the first pregnancy, as the two types of blood are well separated by the placenta.

The ABO blood typing system groups your blood into one of four categories:

- Type A has the A antigen.
- Type B has the B antigen.
- Type AB has both A and B antigens.
- Type O has neither A nor B antigens.



Fig no. 20 – RH typing

PROCEDURE

- First mix the patient blood with three different reagents including either the three different antibodies. A B or Rh antibodies.
- Look for agglutination, then you take a look at what has happened.
- Figure out the ABO blood group
- Figure out the Rh blood group
- Figure out the blood type
- Label the two different clean slides with the unique ID of the patient.
- Pipette 50/50 μL (one-one drop) of the blood and deliver it to the first slide in two corners and a drop in the remaining slide at the center.
- Put 1 drop of A, B, and Rh antisera onto each slide respectively, and mix it thoroughly with the help of the clean sticks or using a clean glass slide.
- Tilt the glass slide back and forth for up to 2 minutes.
- Look for the agglutination in each slide.
- In the case of Rh, if agglutination is seen it is reported as Rh positive and if no agglutination is seen, it is reported as Rh negative as shown table of result-interpretation

5.10 SCREENING TEST

HIV:

An antigen and antibody test performed by a lab on blood from a vein can usually detect HIV 18 to 45 days after exposure. There is also a rapid antigen and antibody test available test is done with a finger stick. Antigen and antibody test means you are protected against the hepatitis B virus.

Blood test can be detect signs of the hepatitis B virus in your body and tell your provider whether it acute or chronic

A simple blood test can also determine If your immune to the condition Liver ultrasound.

HCV:

A blood test called an HCV antibody test is used to find out if someone has ever been infected with the hepatitis C virus. The HCV antibody test, sometimes called the anti- HCV test, looks for antibodies to the hepatitis C virus in blood

HPD:

This histrionic personality disorder (HPD) Test is deigned to see if you might have the symptoms of histrionic personality disorder. About 3% of adult will have histrionic personality disorder in their lifetime.

MALARIA:

Ensure specimen and test kits are brought to room temperature before testing.open the foil wrapped pouch and remove the cassette. Transfer the whole blood specimen to the blood collection tube using a pipette. Read the result 10 minutes once the coloured lines have appeared.

5.11 COMPONENTS SEPARATION

NEED FOR SEPARATION:

- Optimal survival of each constituents
- In whole blood stored at 2-6°C, platelets stay viable for 1 day factor V and VII decrease.
- While after separation, platelets stay upto 5 days and factor V&VII can be stored as FFP for 1year at -30°C
- Transfusion of only required component thus avoid the use of unnecessary component which could be contraindicated in a patient.
- One donor can save several patients.

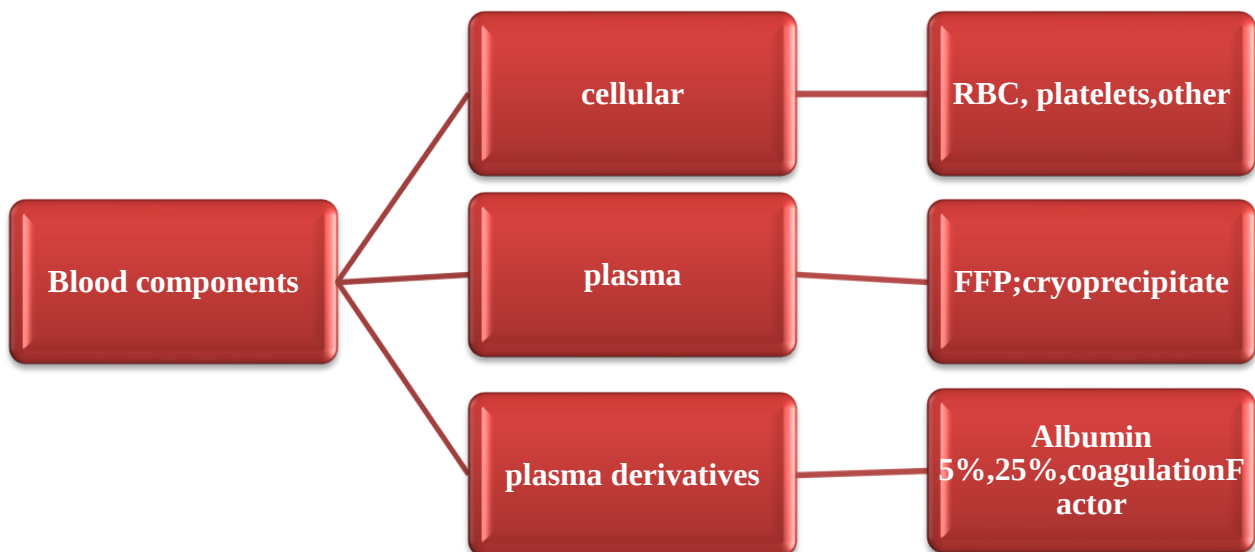


FIG NO. 21 – Components of blood

EQUIPMENTS:

- Freezer
- Blood bank refrigerator 2-6°C
- Refrigerated centrifuge with swing out head and oval cups
- Laminar flow
- Weighing scale
- Dielectric sealer
- Water bath 37°C or plasma defroster
- Platelet agitator
- Sterile docking device

PRECATION IN COLLECTION OF BLOOD:

1. Proper selection of donor
2. Clean aseptic site of vein puncture
3. Uninterrupted flow of blood
4. Collect correct amount of blood
5. Automatic mixing of blood during collection
6. Separate platelets within 6-8 hours
7. Triple pack and quad pack system

PRECATIONS IN CENTRIFUGATION:

1. Balance & use of rubber discs
2. Broad side of the bag faces the outside wall of the cup
3. Correct speed and time to be maintained
4. Look for any abnormal vibration

PACKED RED BLOOD CELL:

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

Store the bag at 2-6°C till processed. place bag in buckets of refrigerated centrifuge and balance the opposite bags centerifuge at 5000*g for 5min at 2-6°C



Fig no. 22 – PACKED RED BLOOD CELL

5.12 STORAGE OF BLOOD COMPONENT:

After the processing of blood into blood components, safe and adequate storage of these components is of immense value for two main reasons:

- To maintain therapeutic efficacy and life span of the constituents.
- To prevent bacterial over growth.

Whole blood:

Whole blood and red cell concentrate must always be stored between 2°C and 6°C. A fall in temperature less than 2°C can cause freezing injury to the red cells leading to haemolysis. If haemolysed blood is transfused to a patient, it can lead to fatal consequences.

Temperatures more than 6°C can lead to overgrowth of non specific bacteria which may have entered the blood unit during collections or component preparation.

As red cells consume glucose for their continued metabolism, storing a blood unit at 2-6°C will also decrease the rate of glycolysis

Fresh frozen plasma (FFP)

FFP is usually transfused to restore or to maintain the clotting mechanism or as a volume replacement when crystalloids and colloids are not used. Fresh frozen plasma is prepared by removing plasma from a unit of blood within 6 hours of collection and snap freezing at -70°C and storing at -30°C.

The speed of freezing is very important for maintaining the coagulation factors such as F VIII and F V.



Fig no. 23 – FRESH FROZEN PLASMA

Liquid/frozen plasma

Liquid plasma can be stored frozen at - 30°C or lower as plasma which has adequate amount of plasma proteins lacks the labile coagulation factors FVIII and FV. The shelf life of fresh frozen plasma and frozen plasma is one year.

Platelets:

Platelets are usually stored as concentrates. They are harvested from a single unit of blood either from PRP (platelet rich plasma) or from buffy coat and resuspended in 50 ml of plasma. Platelets should be stored at 22°C in plasma under conditions in which the pH is maintained at values above 6.8. Temperatures below 20°C during platelet preparation and storage cause a striking increase in the number of large aggregates in the platelet-rich plasma. A fall of pH of platelet concentrate due to lactate production from platelet glycolysis leads to loss of viability. Shelf life of platelet concentrate is only 5 days.

Platelet concentrates must be gently and constantly agitated either on a flat bed agitator for better viability. Without agitation, there is a rapid fall in pH, due to collection of lactic acid metabolites.

5.13 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:

- Cross matching while tube 2 will be the auto control. Tube receives all the treatments of tube 1, except that it does not contain donor's cells.
- Add 2 drops of patient's serum in both the tubes.
- Add 1 drop of 5% saline suspension of donor's cells in tube 1 and 1 drop of patient's cell suspension (5% in saline) in tube 2.
- Mix and centrifuge at 1500 rpm for 1 minute.
- Gently dislodge the cell bottom and examine for agglutination and heterolysis. Record the results. If agglutination is noted in tube 1, ABO incompatibility is suspected.
- Incubate both tubes at 37°C for 15 minutes then add a drop of 22% albumin along the side of each tube. Mix and re-incubate for another 20 to 30 minutes.
- Centrifuge at 1500 rpm for 1 minute. Examine for agglutination and haemolysis. Record the results of agglutination with grading.
- Wash the cells 3 to 4 times with saline, decant completely after each wash and add 2 drops of antihuman globulin serum to the sediment cells. Shake the tubes to mix the contents and then centrifuge at 1500 rpm for 1 minute.
- Examine for agglutination and grade the agglutination reaction. Also look for hemolysis. Some of the antibodies bind with the complement and bring hemolysis which should be considered as an evidence of immunologic reaction (positive).

- If there is no agglutination reaction (negative), add a drop of already sensitized cells. The sensitized or check cells must agglutinate, if the cells are adequately washed and AHG is reactive.



Fig no. 24 – CROSS MATCHING

INTERPRETATION AND RESULTS:

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's own plasma, making the antibodies very dilute and ineffective.



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TAMILNADU

CERTIFICATE OF OBSERVERSHIP

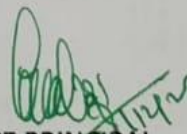
This is to certify that the following students studying in Sathyabama Institute of Science and Technology, Chennai has undergone observership for a period of One Month From 01.11.2023 & 30.11.2023 in the Department of Microbiology & Histopathology at Chengalpattu Medical College, Chengalpattu-603001.

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

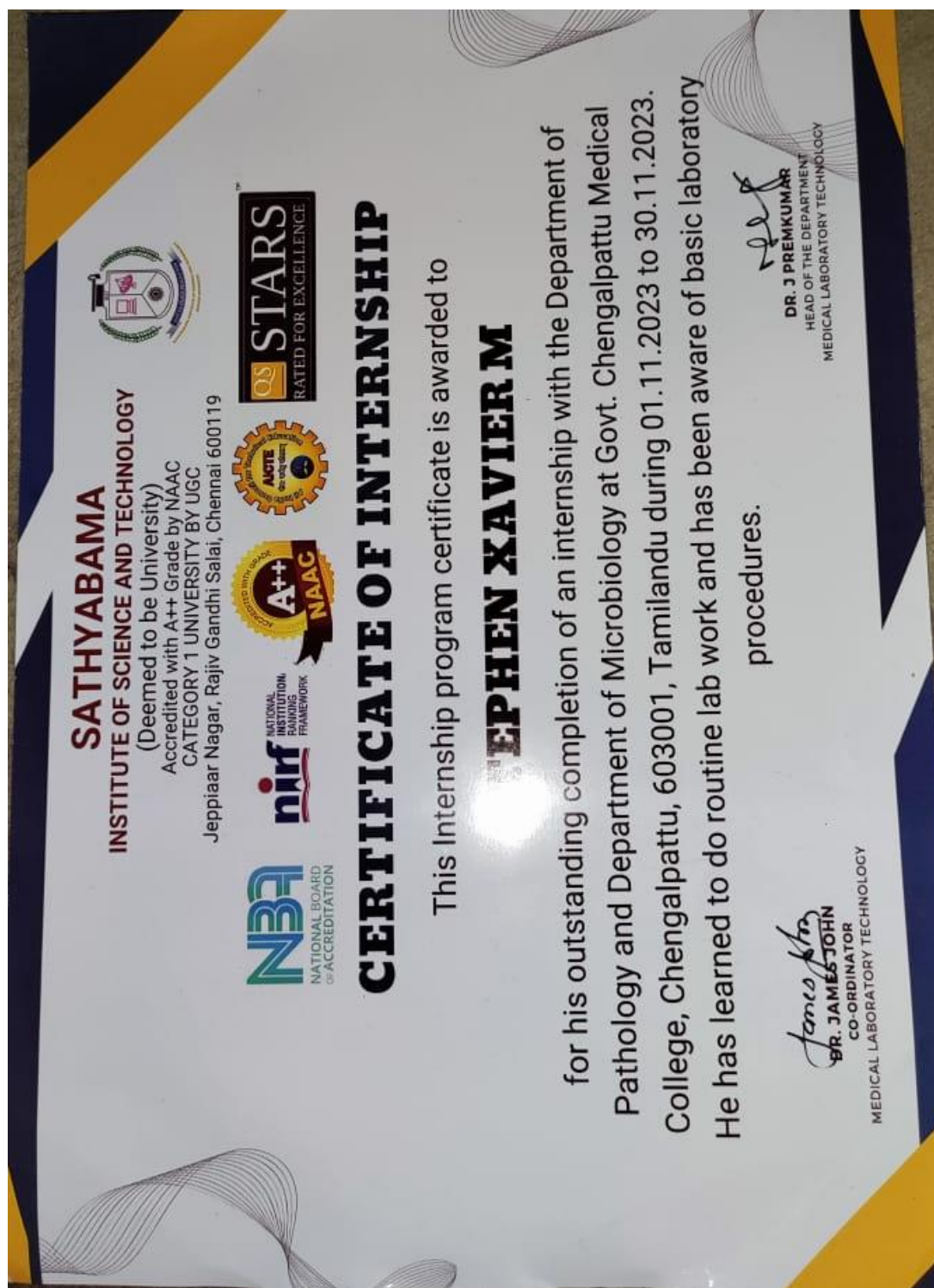
Chengalpattu Medical College,
Chengalpattu.

Date:21.12.2023

Place:Chengalpattu.

21/12/23

6.



7.

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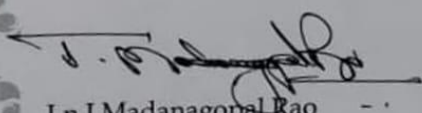
Date: 12th Dec 2022

TO WHOMSOEVER IT MAY CONCERN

This is to certify that Mr. Stephen Xavier. M, 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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Tel : 28414949 / 4959 / 5959
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

8. CONCLUSION

Had a great experience and learned a lot new things about blood banking and its maintainance donor selection, handling blood unit, components separation storage, detecting groups, cross matching, compatability tests, and to record and register data of individual donor and recipient information. By observing all the give thing.

- Blood transfusion or its product is an invaluable therapeutic should be with good reason because of its potential hazards
- Blood loss during a surgical procedure should be minimized.
- RBC booster should be give pre and post to patients.
- This report the blood bank management system related issues
- In future we must overcome this drawback by using modern technologies.
- This report the blood bank management system related issues
- Problem to calculate amount of blood in stock.
- In future we must overcome this drawback by using modern technologies.