

INTERNSHIP REPORT

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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

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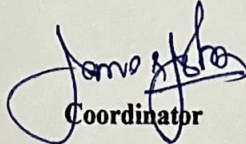
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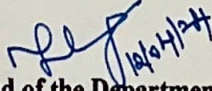
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DEPARTMENT OF MEDICAL LABORATORY TECHNOLOGY
BONAFIDE CERTIFICATE

This is to certify that this internship report is the bonafide work of **Monika G** who have done the internship as a team and carried out the training internship at “**Lions Blood Bank And Research Foundation At Ambattur and Chengalpattu Medical College At Chengalpattu** under our supervision from August 2023 to April 2024.



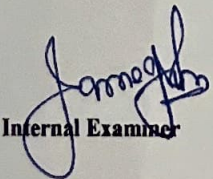
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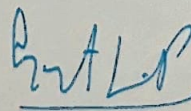
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Submitted for Viva voce examination held on 12th April 2024



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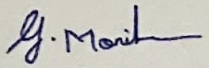
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DECLARATION

I, Ms Monika .G (41737031), hereby declare that the internship trainings done by me at Lion Blood Bank Research Foundation at Ambattur under the guidance of Ms. D. Jasmine Priya and Chengalpattu Medical College at Chengalpattu " under the guidance of Dr. James John at Sathyabama Institute of Science and Technology is submitted in partial fulfilment of the requirements for the award of Bachelor of science degree in Medical Laboratory Technology.

DATE: 12/04/2024

PLACE: Chennai


(Ms. MONIKA. G)

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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.

CONTENT

S.NO	Topics	Page No
1	Introduction of Histopathology	1
2	Fixation	2
3	Decalcification	3
4	Tissue processing	3
5	Tissue Embedding	9
6	Microtomy	11
7	Section Cutting	14
8	Staining	15
9	Preparation of haematoxylin and eosin	16
10	Procedure of haematoxylin and eosin stain	19
11	Mounting media	21
12	Papanicolaou staining	21
13	Microbiology	24
14	Media Preparation	25
15	Bac Tec	26
16	Gram stain	27
17	CRP	28
18	Dengue	29
	Blood banking Selection criteria	30
20	Blood donation	31
21	Blood grouping	32
22	Screening test	33
23	Cross matching	35
24	List of figure	37
25	CMC certificate	38
26	Sathyabama general hospital certificate	39
27	Blood banking	40
28	Conclusion	41

I had the opportunity to do my internship at institutions **CHENGALPATTU MEDICAL COLLEGE, CHENGALPATTU** and **LIONS BLOOD BANK AND RESEARCH FOUNDATION, AMBATTUR**. During the period from 28.11.22 to 03.12.22, I completed my internship at Lions blood bank and research foundation in Ambattur followed by an internship at Voluntary health services 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.

INTRODUCTION OF HISTOPATHOLOGY:

Histopathology- Definition it is a branch of pathology which deals with the study of disease in a tissue section. The tissue undergoes a series of steps before it reaches the examiners desk to be thoroughly examined microscopically to arrive at a particular diagnosis. To achieve this, it is important that the tissue must be prepared in such a manner that it is sufficiently thick or thin to be examined microscopically the structures in a tissue may be differentiated. The objective of the subsequent discussions will be to acquaint the staff with-their responsibility; the basic details of tissue handling, processing and staining

TISSUE PROCESSING:

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

SPECIMEN ACCESSIONING:

Tissue specimen received in the surgical pathology have a request form that list the patient information and history along with a description of the site of origin. The specimen is accessioned by giving them a number that will identify each specimen for each patient and maintaining a registrar book. Tissue like bones or cartilage into small plastic cassette which hold the tissue. Initially the cassette is placed in a fixative.

GROSS EXAMINATION:

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



Fig :1.1 grossing station

FIXATION:

Fixation is the process by which the constituents of the cells are fixed in a physical and partly chemical state so that they will withstand subsequent treatment with various reagents with minimal loss of significant distortion or decomposition and keep the tissue in as life-like manner as possible.

AIMS

1. Should stop bacterial degeneration, autolysis and putrefaction.
2. Should not distort the cellular constituents by swelling or shrinkage, and maintain as close a resemblance as possible to the natural structure of tissue components.
3. The tissue should withstand the chemicals used at various stages of processing
4. Allow clear staining of the sections.
5. To increase tissue consistency to permit the cutting of thin slices of tissue at varying microns.
6. To increase optical differentiation of cellular structures.

❖ BASED ON COMPONENTS

❖ BASED ON THE ACTION ON TISSUES

❖ BASED ON THEIR MODE OF ACTION



Fig: 1:2 specimen

DECALSIFICATION:

Decalcification is the process of removal of calcium from decalcified tissue and making suitable for section cutting. In presence of calcium salts makes the tissue hard and brittle, which will cause difficulty in section cutting and damage to the microtome knife

SELECTION OF TISSUE

Thin sizes of bone and other calcified hard tissues are obtained by using fine forceps. Have to take of tissue at 4-5mm

FIXATION

Adequate fixation should be needed before decalcification otherwise tissue will be damaged in acid.

Bony tissue is fixed in 10% buffered neutral formalin for 2-4 days, for bone marrow zenkers formalin use.

PROCEDURE

1. Insert a pipette into the decalcifying solution containing the specimen.
2. Withdraw approximately 5 mL of the decalcifying solution from the specimen and place it in a test tube. Add a piece of litmus paper.
3. Add 5 mL of the ammonium hydroxide/oxalate working solution, mix well till the litmus paper turns blue (alkaline solution)



Fig:1.3 Decalcification system

TISSUE PROCESSING:

Tissue processing is the technique by which fixed tissues are made suitable for embedding within a supportive medium such as paraffin, and consists of

- Fixation
- Dehydration
- Clearing
- Impregnation in wax
- Embedding

- Casting

TISSUE SAMPLING

Tissue sampling generally follows standard protocols established by each laboratory for various categories of specimens. Tissue blocks for processing should be thin; usually 1–2 mm thick for urgent processing of specimens and 3–5 mm for routine material to be processed overnight. Specimens should not be tightly packed into processing cassettes or containers, but should have sufficient free space to facilitate fluid exchange. Small specimens and tissue fragments, e.g. endometrial biopsies, endoscopic biopsies, etc. are processed, wrapped in lens tissue and placed in fine mesh containers.

PRINCIPLES OF TISSUE PROCESSING

Tissue processing occurs due to diffusion of various substances/fluids into and out of stabilized porous tissues. The diffusion process results from the thermodynamic tendency of processing reagents to equalize concentrations inside and outside the bits of tissue, thus generally conforming to Fick's law. This law states that rate of solution diffusion through tissues is proportional to the concentration gradient (the difference between concentrations of the fluids inside and outside the tissue) as a multiple of temperature-dependent constants for specific substances.

LABELING OF TISSUES

Specimens are generally identified by a numbering system that is not bleached by subsequent fluid and solvent treatment. Examples include:

1. A numbered card label generated by computer-printer (self-adhesive bar code label) or handwritten in soft lead pencil or waterproof ink.
2. Color-coded plastic cassettes (Tissue-Tek system), machine or manually labelled (the label should include a serial number for the specimen and that particular year, e.g. 07/20204 for the seventh specimen received in the year 2024)

STAGES OF TISSUE PROCESSING

- Dehydration

Clearing

- Impregnation in wax (infiltration)
 - Embedding
 - Casting or blocking.

Each stage should be of sufficient length to ensure completeness. The tissues are transferred from reagent to reagent in tissue processing cassettes. These are metal or plastic containers with perforations. The cassettes are transported through various reagents in machines called 'tissue

processors. 'Manual processing' may also be done, where cassettes are transported manually from reagent to reagent and is done in small scale laboratories with a smaller number of specimens. Factors affecting the rate of processing are:

1. **Specimen size:** The thicker the specimen, longer is the filtration time.
2. **Agitation:** Effective agitation reduces the overall processing time by 25–50% with improved impregnation of the tissues.
3. **Heat:** Increases the rate of penetration and chilling decreases it.
4. **Viscosity:** The higher the viscosity of the fluid, the slower the rate of penetration.
5. **Vacuum impregnation:** Vacuum considerably reduces the impregnation time.
6. **Dehydration**

The first step in processing is dehydration. Water is present in tissues in free and bound (molecular) forms. The water needs to be removed before it is replaced by wax during impregnation and embedding. Wet-fixed tissues (in aqueous solutions) cannot be directly infiltrated with paraffin. The first stage of processing is the removal of aqueous fixatives from the tissues by various dehydrating compounds such as alcohols or a substitute. The dehydrating agents are hydrophilic and attract water molecules from the tissue or affect dehydration by repeated dilution of the aqueous tissue fluids.

Procedure

Dehydration consists of passing the tissue through series of progressively more concentrated alcohol baths. It is best accomplished by the use of graded alcohol, say 70–95% or to 100%. The transfer of tissue directly to a higher grade, i.e. 95% alcohol is risky, since it is liable to cause tissue shrinkage. Tissues are carefully transferred from one container to another at proper intervals (allowing the tissue to drain for few seconds) between each change. The volume of a dehydrating agent in each stage should be at least 10 times the volume of tissue to be dehydrated. The concentration of the first alcohol depends on the fixative and size and type of the tissue, e.g. delicate tissue such as embryo and brain need lower concentration (50% alcohol to start with) and smaller intervals between two strengths of alcohol. Tissues immersed in alcoholic fixatives such as Carnoy's fluid may be placed directly in 100% alcohol. Duration of dehydration should be kept to the minimum; consistent with the tissues being processed. Tissue blocks 1 mm thick should receive up to 30 minutes in each alcohol, blocks 4–5 mm thick requires up to 90 minutes or longer in each change. Tissues may be held and stored indefinitely in 70% ethanol without harm. Automated programs are also available in tissue processors

Dehydrating Agents

1. Ethanol
2. Isopropyl alcohol
3. Butyl alcohol

Clearing

Clearing consists of replacing dehydrant with a substance that will be miscible with the embedding medium (paraffin) with which the tissue must be impregnated. The essential requirement of clearing agent is that it should be miscible with both dehydrating agents and impregnating agents. The clearing agents often have the same refractive index as tissues, as a result, when the anhydrous tissue is completely infiltrated with the clearing agent, it becomes translucent. This property is used to ascertain the endpoint and duration of the clearing step. The presence of opaque areas indicates incomplete dehydration. Transition solvents extract certain tissue substances such as lipids, but otherwise do not alter tissue reactivity nor behave as secondary fixatives during processing. The boiling point of the clearing agent gives an indication of its speed of replacement by paraffin wax. Fluids with low boiling point are easily replaced. Viscosity also influences the speed of penetration.

Criteria for Choosing Suitable Clearing Agents

- Ability to rapidly remove dehydrating agents
- Easily removed by melted paraffin
- It should not produce excessive shrinkage and hardening
- It should not dissolve out aniline dyes used in staining
- Minimal tissue damage
- Least flammable
- Least toxicity
- Cost-effective.

Procedure

After dehydration, the tissue is transferred to a clearing agent. After an appropriate time, interval, the tissue is transferred to a second change of clearing agent. The volume of clearing agent is optimally 30–40 times the volume of the specimen. The amount should not be less than 10 times the volume of tissue. The smaller pieces of tissue are cleared in 30 minutes to 1 hour, whereas larger tissues (> 5 mm thick) are cleared in 2–4 hours. The endpoint of clearing can be noted by transparent

appearance of the tissue against light. Prolonged exposure to most clearing agents causes the tissue to become brittle and difficult to section.

Clearing Agents

1. **Xylene:** The most common clearing agent in the laboratory is xylene. It is reasonably cost-effective. It clears rapidly and the tissues are rendered transparent, facilitating endpoint determination. Long-term immersion of tissue in xylene results in tissue distortions therefore tissues should not be left in it for more than 3 hours.
2. **Toluene:** It is better at preserving tissue structure and causes less hardening of tissues than xylene. It is a suitable clearing agent for automatic tissue processing. It is preferable for processing brain sections. However, toluene is more expensive than xylene, more toxic and a possible carcinogen, so toluene is less commonly used.
3. **Chloroform:** It is an expensive, heavy, highly volatile, slowly penetrating transition solvent. It causes less brittleness than xylene and is often used on dense tissues such as uterus and muscle. The tissues can be cleared overnight without rendering them brittle because of its tolerance. It is also good for nervous tissues, lymph nodes and embryos. But it causes severe health hazard, acts slowly and may lead to sectioning difficulties.

Impregnating in Wax

Impregnating is the process in which the clearing agent is replaced by paraffin or its substitute that completely fills all the tissue cavities, giving a firm consistency to the specimen and allowing easier handling and cutting of thin sections without any damage to the tissue or its cellular constituents. This is done at the melting point temperature of wax in use, i.e. 54–64°C, in case of paraffin wax. The volume of the wax should be 25–30 times the volume of the tissue.

Criteria for Ideal Infiltrating Medium

- Soluble in processing fluids
- Suitable for sectioning and ribboning
- Capable of flattening after ribbon cutting
- Molten between 30 and 60°C
- Translucent or transparent; colorless at its melting point

Ideally an infiltrating and embedding medium should be:

AUTOMATED TISSUE PROCESSING METHODS

Tissues are usually more rapidly processed by machine than by manual methods, although the latter can be accelerated by using microwave or ultrasonic stimulation. For routine purposes, tissues are most conveniently automatically processed through dehydration, clearing and infiltration stages by machine. There are two broad principles of automatic tissue processors, tissue-transfer fluid types

A tissue transfer tissue processor with integrated fume. Tissue cassettes are loaded into basket on the rotating head, which transfer tissues around the series of reagent containers.

STEP OF PROCESSING	DURATION	
	DAYTIME	OVERNIGHT
Fixative		120min
Fixative		120min
70% ethanol		60min
90%ethanol		60min
Absolute ethanol	30min	60min
Absolute ethanol	30min	60min
Absolute ethanol	30min	60min
Toluene or substitute	30min	60min
Toluene or substitute	30min	60min
Paraffin wax	30min	90min
Paraffin wax	30min	90min
Paraffin wax	30min	90min
Paraffin wax (under vacuum)	30min	30min
Total time	4.5hrs	16hrs

In day schedule for urgent specimens, tissues 2 mm and fixed in Carnoy's fluid. In overnight schedule for routine processing, tissue blocks 2–3 mm and single load. In weekend processing, tissues are held in fixative or preferably 70% ethanol until Sunday.



Fig:1.4 automated tissue processor

TISSUE EMBEDDING:

Embedding is the process by which processed tissues are surrounded by a support medium such as agar, gelatine or wax, which on solidification will provide sufficient support during sectioning. The choice of the embedding media depends upon the microscope, the type of microtome and also the type of tissue used. Paraffin wax is a suitable embedding media for most tissues from which sections taken of 4–6 μ thickness are satisfactory for most diagnostic purposes. In instances where paraffin wax is unsuitable for embedding (e.g. as in a requirement for better support) and there is a need for very thin sections or preservation of enzymes then plastic may be used in place of paraffin.



Fig:1.5 embedding station

EMBEDDING TISSUES IN PARAFFIN WAX

Tissues are embedded by placing them in a mould filled with molten embedding medium, which is then allowed to solidify. Embedding requirements and procedures are essentially the same for all waxes. The technique for paraffin wax is provided here in details. At the completion process tissues are held in clean molten paraffin wax, which is free of solvent and particulate.

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:1.6 paraffin tissue blocks

ALTERNATIVE EMBEDDING MEDIA

Alternative embedding media may provide optimum support for tissues in applications for which paraffin waxes are unsuited, for example when:

- Tissue components are heat or reagent labile
- Hard or dense tissues are inadequately supported
- Adhesion between specimen and wax is poor
- Very thick or very thin sections are required
- Sectioning whole organs such as lung or brain.

Aqueous Media Agar

Agar is not used alone, but as a double embedding media. The high melting point and low gelling temperature of agar make it ideal for double embedding the multiple small tissue fragments. Agar gel does not provide sufficient support for sectioning the tissue when employed alone. Its main use is in acting as a cohesive agent for small friable pieces of tissue before embedding.

Gelatine

Gelatine is used primarily in the production of sections of whole organs in Gough-Wentworth's whole-organ sectioning method. The low melting point of gelatine (35–40°C) makes it unsuitable for double embedding. Gelatine embedding supports large tissue blocks for sectioning. It may be used for frozen sections of friable or partially necrotic tissue or small fragments of tissue such as uterine curetting and also in phospholipid and enzyme studies for histochemistry with a freezing

microtome. This technique has now largely been superseded by other media; the optimum compound temperature (OCT) used for cryotomy.

Plastic (Resin)

Resin was introduced as an embedding media in the preparation of ultrathin sections required for electron microscopy. The plastic media is a pale liquid and it polymerizes to a solid in blocking. The change in the physical state of an embedding media from liquid to solid is called polymerization and is brought about by joining molecules together to produce a complex macromolecule made of repeating units (poly means many). These agents are readily available, relatively inexpensive, easy to prepare and handle, of low viscosity and allow short infiltration time. Consistently thin sections can be cut and enable a wide range of staining methods to be used. Plastic is classified according to their chemical composition.

Acrylic Media Butyl Methacrylate/Glycol Methacrylate

Methacrylate was introduced into micro techniques in 1949. The principal use of methacrylate monomers is its ability to polymerize in the presence of a catalyst, such as heat ultraviolet light or artificial catalysts such as benzoyl peroxide (1.5–2.0%), which results in radical formation with subsequent polymerization. Methacrylate is readily miscible in ethanol and gives a clear hard block when polymerized. Methacrylate rapidly infiltrates and dehydrates tissue at room temperature. However, damage to the tissue is common during polymerization because of marked shrinkage of tissue components. Mixes can be devised to produce plastics with a wide range of properties. These resins are more suited for embodiment of hard materials such as decalcified bone and other hard tissues. Glycol methacrylate has been used for high-resolution autoradiography.

Celloidin

Celloidin is the purified form of nitrocellulose obtained by treating cellulose with sulfuric and nitric acid. It is supplied as pulpy, cotton-like material and is referred to as gun cotton. In the past, celloidin was preferred over paraffin as an embedding medium because it provided better support for sectioning hard tissues such as bone, uterus as well as for delicate specimens such as eyes, central nervous system tissues and embryonic tissues. The working strength is 2%, 4% and 8%, the solvent being equal parts of ether and alcohol.

MICROTOMES

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

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

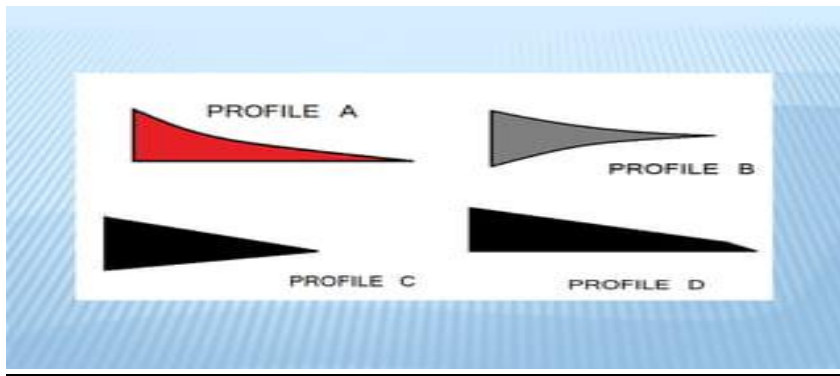


Fig:1.7 microtome knife

SECTION CUTTING

Theory of Sectioning

It is believed that a sharp knife with a narrow edge probably wedges off sections by splitting and tearing rather than shearing. Successful sections require:

1. **Proper material:** Properly prepared material with the supporting medium matching the specimen.
2. **A sharp knife:** Poorly prepared material can sometimes be cut by a good knife, but a well-prepared block may be totally ruined by a poor knife.
3. **A proper microtome:** The choice of microtome should depend on the application. Unless very old, misused, damaged or of poor quality a microtome is rarely the cause of poor sections.
4. **A skilled operator:** Practice is the word in the acquiring of skills. A skilled operator must be able to recognize and correct difficulties as they arise.

PARAFFIN SECTION CUTTING

Trimming of Paraffin Blocks:

Once tissues have been blocked, excess paraffin on all aspects of the block of tissues should be removed to leave approximately 3 mm of paraffin around it. Somewhat more paraffin should be available at the back of the block. Once this is done the block is fixed to a metal block object holder or a wooden chuck by means of a hot wooden handled spatula. Both knife and block are then cooled by means of a solid block of ice. In order to arrive at the optimum plane of cutting where adequate sampling of the tissue block is done, coarse trimming is resorted to with the microtome setting at 20–25 μ . A different ‘coarse’ knife may be used for the purpose or alternatively a different portion of the knife may be used.

PROCEDURE

1. Place the microtome on a table
2. Insert knife & screw tight

3. Mount the block within the clamp of the holder
4. Adjust tilt of knife =2-6degree
5. Adjust the block holder-wax block almost touching the level of the knife
6. Tighten all the adjusting screws
7. Bevel of the knife face is parallel to the cutting motion
8. Section thickness=10-15 microns
9. Operate until complete sections are cut
10. Reset section thickness 4-5 microns
11. Ice can apply to the surface block->wiped->cut
12. Right hand operates the microtome
13. Left hand hold section away from the knife
14. Gentle breathing on block removes creases



Fig:1.8 microtome

STAINING

Staining is the process of applying dyes on paraffin cut sections to study the architectural and cellular pattern of the tissues. Different tissues display varying affinities for most dyes so that they become easily identifiable, thus enabling and establishing presence or absence of disease.

1. Routine staining
2. Special staining
3. Vital staining
4. Supravital staining
5. Metachromatic staining

BASE ON TISSUE AFFINITIES

1. Basic stain: It is an active colouring substance found in a base combined with a colourless inorganic acid radical. Any substance stained by the basic dye is considered to be basophilic
2. Acid stain: It is active colouring substance found in an acid combined with a substance that is stained by an acid dye is referred to as acidophilic.

BASE ON SOURCE

1. Natural dye: They are obtained from the plants and animals
E.g. Haematoxylin, carmine

2. Synthetic: They are derived from coal tar and hydrocarbon benzene

E.g. Aniline, azo dyes

METHODS OF STAINING

Direct Staining

Direct staining is the process of giving colour to the sections by using aqueous or alcoholic dye solutions, e.g. methylene blue and eosin.

Indirect Staining

The process by which action of the dye is intensified by adding another agent, i.e. a mordant, which serves as link or bridge between tissue and dye.

THEORIES OF STAINING

The nature of dye-tissue inter-reaction involves either a chemical reaction or physical phenomenon:

1. Physical phenomenon defines the differential solubility and is the basis of staining lipids with alcoholic solutions of such dyes as oil red O, where the dye is more soluble in the fat than the solvent in which it is employed; and adsorption involves the attraction and surface fixation of small molecules by large molecules, e.g. staining of glycogen by iodine.
2. The chemical reaction may be nonspecific; it gives color to the different tissue components and therefore increases the contrast of the cells. Staining of acid nucleoproteins by basic dyes or basic cytoplasmic constituents by acid dyes and dyes staining bacteria are also examples of chemical reactions.

FACTOR AFFECTING STAINING

Staining is a complex process and influenced by both chemical and physical processes and also by the process of fixation

- Solvent-solvent interaction
- Dye-dye interaction
- Dye-tissue interaction

PREPARATION OF HEMATOXYLIN AND EOSIN

Haematoxylin and eosin are the combination of two histological stain. The haematoxylin stain cell nuclei purplish blue, and eosin stains the extracellular matrix and cytoplasm pink, with other structure taking on different shade, hues, and combination of these colour.

- **Alum haematoxylin:** Using alum, which is a double salt-like potash alum, e.g. Delafield's, Ehrlich's, Mayer's, Harris and Cole's haematoxylin
- **Iron haematoxylin:** Using ferric chloride and ferric ammonium sulphate, e.g. Weigert's iron haematoxylin and Heidenheim's haematoxylin, respectively.

Formula of Common Useful Haematoxylin

Alum Haematoxylin

Harris alum haematoxylin

- Hematoxylin crystals: 5.0 g
- Absolute ethyl alcohol: 50 mL
- Ammonium or potash alum: 100 g
- Distilled water: 1,000 mL
- Red mercuric oxide: 2.50 g.

Method

- Dissolve hematoxylin in alcohol
- Dissolve alum salts in water by heat
- Remove from heat and mix both solutions
- Bring to boil rapidly
- Remove from heat and add mercuric oxide slowly
- Reheat until dark purple
- Remove and plunge the vessel into cold water until cool
- The stain is ready to use, as soon as possible.

Note: 2–4 ml of glacial acetic acid to every 100 ml stain increases the nuclear stain precision. Always filter before use. Harris alum haematoxylin used as a regressive stain.

Expected results

- Nuclei: Blue
- Background: As counterstain or unstained.

Ehrlich's alum haematoxylin

- Hematoxylin: 2 g
- Absolute ethyl alcohol: 100 ml
- Glycerol: 100 ml
- Distilled water: 1,000 ml
- Glacial acetic acid: 100 m

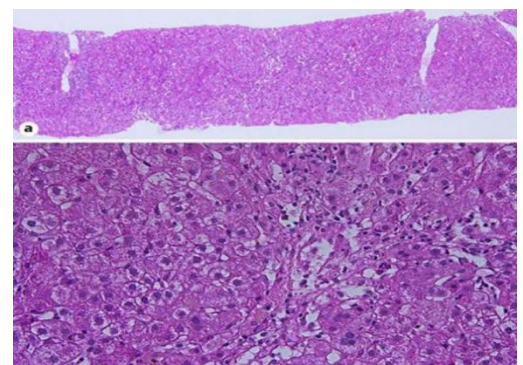


fig :1.9 H&E stain

- Potash alum: 15 g.

It is an excellent nuclear stain and used as a regressive stain.

Mayer's haematoxylin

- Hematoxylin: 1 g
- Distilled water: 1,000 ml
- Potash or ammonium alum: 15 g
- Sodium iodate: 0.2 g
- Citric acid: 1 g
- Chloral hydrate SLR: 50 g

This is used as both progressive and regressive stain. It is used as a progressive nuclear counter stain for the demonstration of glycogen.

Harris haematoxylin

Harris haematoxylin is similar to Mayer's haematoxylin, but uses absolute alcohol instead of water as a solvent.

Gill's haematoxylin

- Distilled water: 730 ml
- Ethylene glycol: 250 ml
- Hematoxylin: 2 g
- Sodium iodate: 20 g
- Aluminum sulfate: 17.6 g
- Glacial acetic acid: 20 ml

Combine reagents in the order given above, mix and keep for 1 hour at room temperature. The stain can be used immediately



Fig:1.10 H&E

Eosins

The eosins are the most suitable stain to combine with haematoxylin to demonstrate the cell cytoplasm and general histological architecture of a tissue. Their ability to differentiate between cytoplasm of different types of cells; between different types of connective tissue fibres and matrices, with different shades of pink and red, makes them invaluable as a regular counter stain. The eosins are acid xanthene or phthalein dyes. The name 'eosin' is derived from the peculiar pale pink colour

resembling dawn pink. The most frequently used stain among this group is eosin Y. The common members of this group are:

- Eosin Y (eosin yellow water soluble)
- Eosin R (eosin red spirit or alcohol soluble)
- Eosin B (eosin blue)

Watery eosin solution (used more commonly than eosin R):

- Eosin Y: 10 g
- Distilled water: 1,000 ml
- 0.5 ml acetic acid may be added to shorten staining
- Thymol may be added to prevent fungus formation.

Alcoholic eosin solution:

- Eosin R (spirit soluble): 2g
- Distilled water: 160 ml
- 95% ethyl alcohol: 640 ml.

Dissolve eosin R in distilled water and add 95% ethyl alcohol. If a darker pink shade is required add a drop of acetic acid to each 100 mL of solution.

Although, eosin is the most widely employed cytoplasmic stain, substitutes are available such as phloxine, erythrosine, azophloxin, Beirich scarlet, picric acid, orange G (yellow), light green SF (green), all in concentrations

PROCEDURE OF H&E STAINING

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

14. 80% ethyl alcohol: 2 minutes.
15. 95% ethyl alcohol.
16. Absolute alcohol: 2 changes.
17. Xylene: 2 changes.
18. Mount with Entellan [dibutyl phthalate in xylene (DPX)/Canada balsam].

Results

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

Advantages

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

Disadvantages

1. Too much cannot be read into the hematoxylin-eosin stain and with experience ratification with regard to cell structures or products with special stains must be done.
2. Deparaffinization is important and should be adequate. Air bubbles on the section at this stage can cause nonstaining and misinterpretation and should be removed by repeated dipping in the solution.

Slides

For normal routine work, 76 × 4 mm slides are available with a variety of thicknesses; those specified as of 1.0–1.2 mm in thickness are preferred, because they do not break easily. Slides of larger size should be used for sectioning of eyes and CNS when such sections do not fit onto the regular size. Special equipment such as slide racks are available for holding slides at any stage of staining

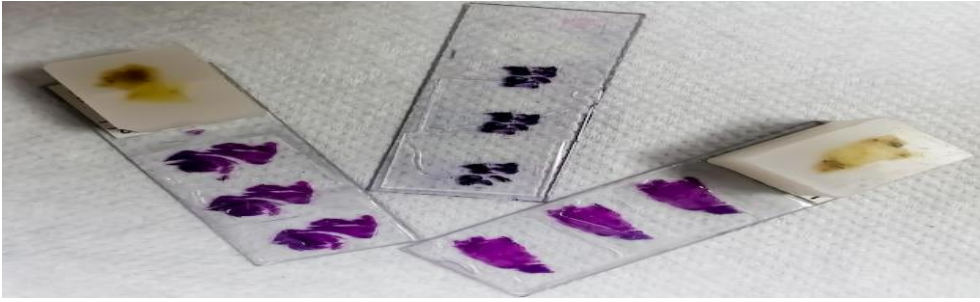


Fig:1.11 slide

Mounting Media

The final step of preparation of stained section is to cover the slide surface containing tissue with a thin coverslip. This makes the slide permanent and permits microscopic examination, whenever possible. The refractive index of the mounting medium must be approximately equal to that of dried protein. This is in order to provide the maximum degree of transparency to the stained tissue sections. To be effective, a mountant should possess certain characteristics. These include the following:

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

Papanicolaou Staining Method

Papanicolaou staining method is the routine staining procedure used in any cytopathology laboratory. This technique is named after George N Papanicolaou, the father of exfoliative cytology and is devised for the optimal visualization of cells exfoliated from epithelial surfaces of the body. It is a polychrome staining reaction designed to display many variations of cellular. The pap stain has been most frequently used for screening of cervical cancer

Step	Stain	Duration
1	90%ethanol(fixation)	15min
2	80%ethanol	2min
3	50%ethanol	2min
4	Distilled water	5dips
5	Haematoxylin stain	2min
6	0.05%HCL solution	10 second
7	Running tap water	10min
8	50% ethanol	2min
9	80%ethanol	2min
10	95%ethanol	2min
11	OG-6 stain	2min
12	95%ethanol	2min
13	95%ethanol	2min
14	EA-36 stain	2min
15	95%ethanol	2min
16	Absolute ethanol	2min
17	Absolute ethanol	2min
18	Absolute ethanol + xylene	2min
19	Xylene	5min
20	Xylene	Till clear
21	Mount in dibutyl phthalate in xylene (DPX)	

RESULT

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

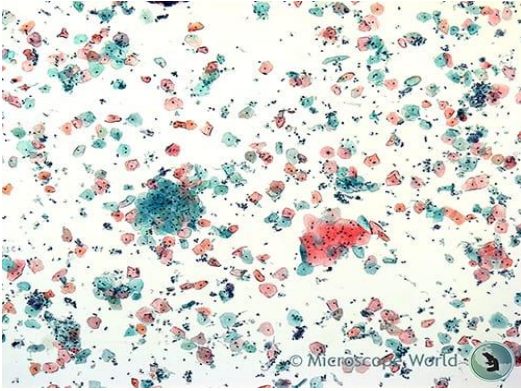


Fig:1.12 pap stain under microscope

Purpose of Pap smear test

- To detect early cancer of the cervix
- To determine estrogen activity related to menopause or endocrine abnormalities

MICROBIOLOGY

Microbiology is the study of all living organism that are too small to be visible with the naked eye. This includes bacteria archaea, viruses, fungi, protozoa and algae collectively known as microbes.

TYPES OF SAMPLES RECEIVED IN LABORATORY

- a) Urine sample
- b) Sputum sample
- c) Blood sample
- d) Blood sample
- e) Throat swab
- f) Water
- g) FNAC smear

INSTRUMENTS

- ✓ Bactec system
- ✓ Microscope
- ✓ Micro scan
- ✓ Hot air oven

DIFFERENT TYPES OF MEDIA PEPARATION

BLOOD AGAR:

- It is used most commonly. For blood agar preparation 5-10% defibrinated sheep blood is mixed with melted agar at 45-50°C blood work as an enrichment material and also act as an indicator. Bacteria when grown in blood agar produce hemolysis around their growing colonies. Some bacteria produce no hemolysis.
- Beta hemolysis, there is clear zone around bacterial colony of complete hemolysis, e.g. streptococcus pyogenes is a beta hemolytic streptococcus.
- Alpha hemolysis, when there is greenish discoloration around bacterial colony due to formation of biliverdin, e.g. viridians streptococci.

CLED agar

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

PEPARATION:

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

MacConkey Agar

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

SELECTIVE:

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

INDICATOR:

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

PREPARATION:

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

CHOCOLATE AGAR:

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

PREPARATION:

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



Fig:2.1 weighing machine

PREPARATION AND STERILIZATION CULTURE MEDIA

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



PROCEDURE

- ✓ Appropriate amount of broth powder is weight into Scott bottle and dissolve with distilled water
- ✓ The bottles are loosely recap and aside for sterilization
- ✓ All the media are sterilized at 121°C for 15 minutes.
- ✓ After autoclaving the media is removed. The broth preparation is allowed to cool and the cap of each bottle is tightened.

BacTec



PRINCIPLE

fig:2.2 bactec machine

The sample to tested is inoculated into the vial which is entered into the BACTEC instrument for incubation and periodic reading. Each vial contain a sensor which responds to the concentration or the consumption of oxygen needed growth of microorganisms

PROCEDURE

- ✓ Introduce the blood about 5ml into the both bottle
- ✓ Put the bottle for and 24 and 48 and 72hrs
- ✓ Red light shows the positive negative shows green
- ✓ Positive culture can take to grow on media



fig:2.3 blood culture

GRAM STAINING

PRINCIPLE:

The basic principle of gram staining involves the ability of the bacteria crystal violet dye solvent treatment.

REAGENT:

1. Crystal violet, the primary stain
2. Iodine, the mordant
3. A decolourizer made of acetone and alcohol (95%)
4. Safranin the counterstain

PROCEDURE

1. Smear air dry and heat fixation
2. Crystal violet (1-2 minutes)
3. Gently washing in flowing water
4. Iodine violet (1-2 minutes)
5. Gently washing in flowing water
6. Acetone or alcohol (2 to 5 seconds)
7. Gently washing in flowing water
8. Safranin (1-2 minutes)
9. Gently washing in flowing water
10. Gently blot dry

INTERPRETATION

Gram positive - blue or purple

Gram negative – pink

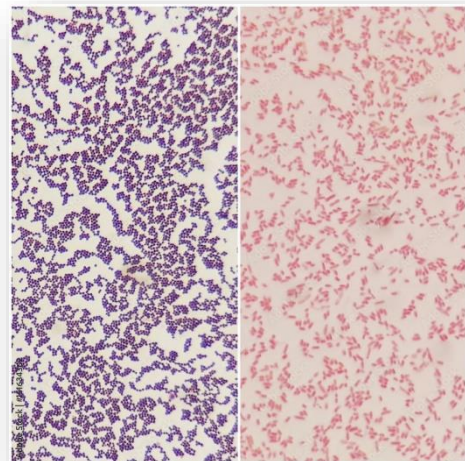


Fig:2.4 microscopic view of gram stain

SEROLOGY

Serology is the scientific study of serum. In practice, the term usually refers to the diagnostic identification of antibodies in the serum. Such antibodies are typically formed in response to an infection (against a given microorganism), against other foreign proteins (in response, for example, to a mismatched blood transfusion), or to one's own proteins (in instances of autoimmune disease)

CRP

Rapid latex agglutination test

PRINCIPLE:

The test is based on the reaction between patient serum containing CRP as the antigen & corresponding antibody coated to the treated surface of latex particle. The coated particles enhance the detection of an agglutinate reaction when antigen is present in the serum being tested.

MATERIALS REQUIRED

- ❖ CRP latex reagent
- ❖ Glycine - saline buffer
- ❖ Capillary pipette
- ❖ Applicator sticks
- ❖ Glass slide
- ❖ Serologic pipettes & rubber bulb

PROCEDURE (QUALITATIVE TEST)

1. Gently resuspend the crp latex reagent and add one drop to each test field
2. Mix well with the provided sticks'
3. Rotate the slide for 2 minutes and read immediately under an oblique indirect light



Fig:2.5 CRP

Dengue IgM METHOD: ELISA method

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

PROCEDURE

1. 1000µl sample diluent + 10ul samples
2. 100µl in each well
3. 1 hour incubation at 37°C
4. Wash 6 times
5. 50µl dengue antigen
6. 1 hour incubation at 37°C
7. Wash 6 times
8. 50µl anti dengue monoclonal antibody
9. 1 hour incubation at 37°C
10. Wash 6 times
11. 50µl AVIDIN-HRP
12. Incubate 1\2 hour at 37°C
13. Wash 6 times
14. 100µl TMB substrate
15. Incubate 10mins in dark
16. 100µl stop solution
17. Read



Fig:2.6 ELISA PLATE WASHER

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 day care service

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

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

BLOOD DONATION (PROCEDURE)

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

REGISTRATION:

1. We will sign in and go over basic eligibility
2. You will ask to show ID such as driver license
3. Well, ask you for your complete address

HEALTH HISTORY:

1. You will answer a few questions about health history and place you have travelled, during a private and confidential interview
2. We will check your temperature, pulse, blood pressure, and hemoglobin

YOUR DONATION:

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

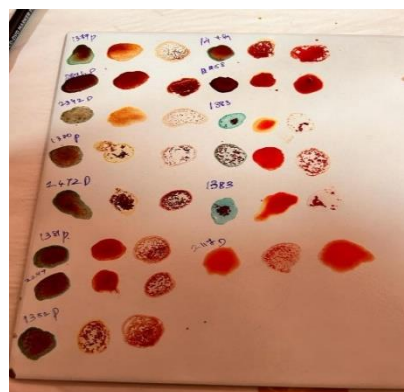
BLOOD GROUPING

PRINCIPLE:

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



Fig:3.1 blood grouping antigens



PROCEDURE:

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

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.

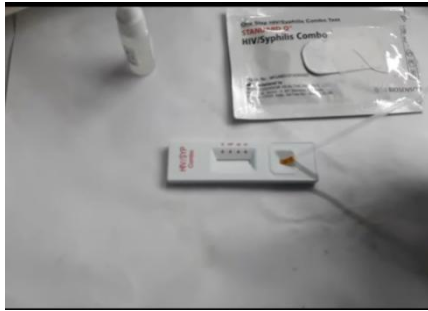


Fig:3.3 card test

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.

VDRL:

Screening test for syphilis. it measures substances (protein) called antibody, which your body may produces if you come in contact with the bacteria that cause syphilis.

MALARIA:

The ELISA malaria antibody test is based on binding of anti-plasmodium antibodies present in a serum sample to antigens immobilized on 96 well plates. The antigens are a mixture of a total extract of cultured plasmodium falciparum and recombinant plasmodium vivax

COMPONENT SEPARATION

PACKED RED BLOOD CELL:

Packed red blood cell from a donation of 450 ml of whole blood are obtained after centrifugation and plasma removal.



Fig:3.4 packed red blood cell

FRESH FROZEN PLASMA:

Fresh frozen plasma obtained after centrifugation of whole blood and red cell removal or through direct separation. The plasma must be frozen within 8 hours after collecting in order to preserve coagulation factors and other plasma protein.



Fig:3.5 FFP

PLATELETS:

Platelets can be prepared by using a centrifuge to separate the platelets rich in plasma from donated whole blood. platelets from several different donors are then combined to make one transfusable unit

STORAGE OF BLOOD COMPONENT:

Packed red blood cells: 2-6 degree Celsius up to 42 days

Fresh frozen plasma :30 degree Celsius for up to 2 years

Platelets: 20 -24 degree Celsius for 5 days, under constant mixing of the bag platelets aggregation occurs in the cold and in absence of antigen

CROSS MATCHING:

- a. 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.
- b. Cross matching play's important role to detect:
- c. Most recipient antibodies directed against donor's red blood cell antigen
- d. Most donor antibodies directed against recipient's red blood cell antigens.
- e. 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:

1. 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.
2. Add 2 drops of patient's serum in both the tubes.
3. 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.
4. Mix and centrifuge at 1500 rpm for 1 minute.
5. 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.
6. 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.
7. Centrifuge at 1500 rpm for 1 minute. Examine for agglutination and hemolysis. Record the results of agglutination with grading.
8. 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.
9. Examine for agglutination and grade the agglutination reaction. Also look for hemolysis.
10. Some of the antibodies bind with the complement and bring hemolysis which should be considered as evidence of immunologic reaction (positive).

11. 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:3.6 cross matching card

INTERPRETATION AND RESULT:

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



Fig:3.7 cross matching result

LIST OF FIGURES

FIGURE NO.	DESCRIPTION	PAGE NO.
1.1	Grossing station	2
1.2	Specimen	2
1.3	Automated tissue processor	8
1.4	Embedding station	9
1.5	Paraffin tissue block	10
1.6	Microtome knife	14
1.7	Microtome	15
1.8	H&E stain under microscopic view	17
1.9	Haematoxylin and eosin	18
1.10	Slide	21
1.11	PAP smear under microscopic view	23
2.1	Weighing machine	25
2.2	Bactec machine	26
2.3	Blood culture	26
2.4	Microscope view of gram stain	27
2.5	CRP	28
2.6	ELISA plate washer	29
3.1	Blood grouping antigens	32
3.2	Blood grouping tile	32
3.3	Card test	33
3.4	Packed red blood cells	34
3.5	FFP	34
3.6	Cross matching card	36
3.7	Cross matching result	36



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(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



[Signature]

VICE PRINCIPAL

Chengalpattu Medical College,
Chengalpattu.

Date:21.12.2023

Place:Chengalpattu.

8.12.2023

Sharing Life. Saving Lives..since 1984



**Madras Egmore
Lions Blood Bank &
Research Foundation**

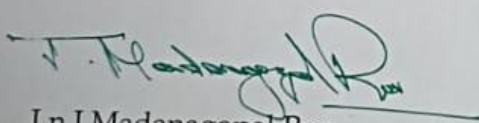
Date: 12th Dec 2022

TO WHOMSOEVER IT MAY CONCERN

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

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

We wish him all the best for her upcoming career.


Ln.J.Madanagopal Rao
Chairman



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

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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

CONCLUSION:

During my internship at Lions blood bank and Chengalpattu Medical College, Chengalpattu, I have gained extensive knowledge in compatibility testing, blood grouping, screening, plasma grouping, microbiology, different staining techniques and more. I'm grateful for the guidance and have gained practical knowledge.



Lions Blood bank, Ambthur



**Chengalpattu Medical College,
Chengalpattu**

