Dissection of Laboratory Animal and Sample Collection for Histology

Rathnamali KGA

Master of Cellular and Molecular Immunology

University of Colombo, Sri Lanka

ABSTRACT

The starting point for the laboratory investigation of a dissection of laboratory animal for experiment is the taking of samples. This review considers some general principles involved in sample collection for histology (Liu et al., 2016). *For disease diagnosis, the tissues sampled should be representative of the condition being investigated and the lesions observed. Samples should be taken with care, to avoid undue stress or injury to the animal or danger to the operator. Where appropriate, samples should be collected aseptically, and care should be taken to avoid crosscontamination between samples* (Lapage, 1958).

Mice and rats are the most used animals in experimental researches, the anatomical, histological and genetic differences between species should be carefully evaluated, to better apply the study model and avoid unnecessary waste avoid (Corte et al., 2021). *Currently, the implantation of defined genetically and sanitarily laboratory animals has aided in new discoveries, through experimental models, contributing to the prevention of uncured diseases such as cancers, AIDS and multiple sclerosis, and also for the development of new surgical treatment techniques. Other applications correspond to the vaccines development, monoclonal antibodies, evaluation and control of biological products, pharmacology, toxicology, bacteriology, virology and parasitology and basic immunology studies, immunopathology, organ transplants and the immunosuppressive drugs development. However, with technological advances, it is now possible to obtain satisfactory results through alternative methods in vitro, using cell culture and other methods, allowing the 84 replacement of laboratory animals* (Gunatilake, 2018).

Key Words: Histology, Rat, Mice, Tissue, Staining.

1. INTRODUCTION

A laboratory animal science is a multidisciplinary branch of science, contributing to the humane use of animals in biomedical research and to the collection of informative, unbiased and reproducible data. The development of the use of animals as a model for man runs almost parallel to the development of medical science ("Guidance for the Description of Animal Research in Scientific Publications," 2014). Laboratory animals are becoming increasingly important for biomedical research. It is said that approximately 70% of biomedical research is associated with the use of experimental animals. Laboratory animal research not only expands our knowledge of science, but also greatly improves human and animal health. The field of laboratory animal science is ever-growing and changing as new experimental techniques are developed and new animal models are created (Hau & Van Hoosier, 2002).

It is essential to know not only the biological features of each laboratory animal but also how to use and care for them responsibly in order to perform high-quality experiments. There are more objectives of using laboratory animals for experiments (Vaught & Henderson, 2011). Some of they are,

- Provides information on how to select and create an animal model to study specific human diseases
- Summarizes the organization and management of animal experiments, in which readers will know the entire process and protocols involved
- Outlines key elements closely related to animal experimentation such as animal genetics, health, facilities, and nutrition
- Discusses the ethical uses of animals in research and teaching including the 3Rs (Reduction, Replacement, and Refinement)
- Presents essential information on the primary laboratory animals, including the newest genetically modified animals (Morawietz et al., 2004), (Liu et al., 2016)

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1. BIOLOGICAL SIMILARITY OF HUMANS AND RATS/MICE

Humans and animals may look different, at a physiological and anatomical level they are remarkably similar. Animals, from mice to monkeys, have the same organs (heart, lungs, brain etc.) and organ systems (respiratory, cardiovascular, nervous systems etc.) which perform the same functions. The similarity means that nearly 90% of the veterinary medicines that are used to treat animals are the same as, or very similar to, those developed to treat human patients (*Canadian Veterinary Journal*, n.d.) . The differences can give important clues about diseases and how they might be treated for instance, if we knew why the mouse with muscular dystrophy suffers less muscle wasting than human patients, this might lead to a treatment for this debilitating and fatal disorder(Course, 2019) .

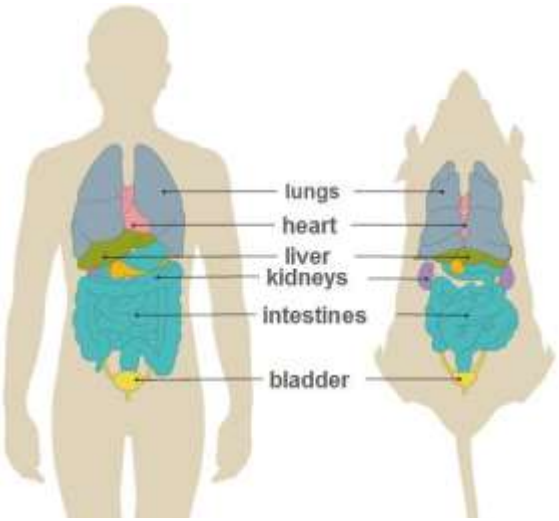

Figure 1: Similarity of human and rat/mouse (The Animal Model, 2022)

1.1 ANATOMY

The figure 2 represented the scientific study of the morphology of the adults. The study of anatomical structures that can be seen by unaided vision is called gross anatomy and also known as topographical anatomy, regional anatomy, or anthropotomy. The study of minute anatomical structures assisted with microscopes is called microscopic anatomy. Cytology is the study of cells and the study of the organization of the tissue is called histology (Parkinson et al., 2011) .

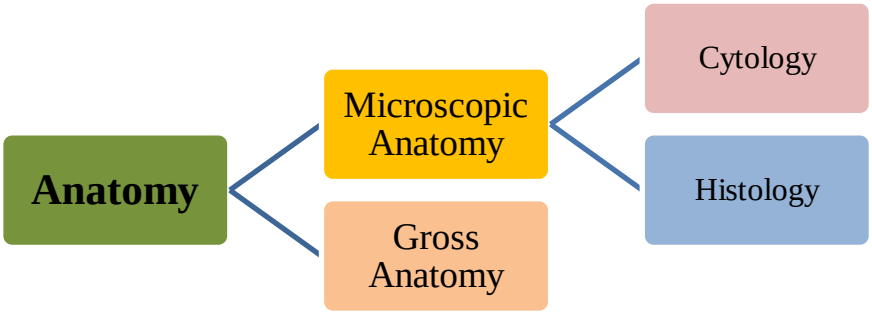

Figure 2: The scientific study of the morphology of the adults (Lapage, 1958)

2. IMPORTANCE OF HISTOLOGY

The knowledge of histology shows how different cells are organized to form tissues and how each cell and tissue show modification according to their functional demands. Although the knowledge of histology is gaining importance in diagnosis of certain diseases like cancers and also helps in figuring out whether the treatment has worked in different diseases (KEMP, 2000).

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2.1 NORMAL AND CANCER CELLS STRUCTURE

There are some of the major differences between normal cells and cancer cells which described below.

2.1.1GROWTH

Normal cells stop growing when enough cells are present. In contrast, cancer cells don't stop growing when there are enough cells present. This continued growth often results in a tumor (a cluster of cancer cells) being formed. There are some genes in the body which carries a blueprint that codes different proteins. There are some proteins acts as growth factors which is chemicals that tell cells to grow and divide. If the gene that codes for one of these proteins is stuck in the "on" position by a mutation the growth factor proteins continue to be produced (Cashman, 2008).

2.1.2 COMMUNICATION

Normal cells respond to signals sent from other nearby cells. When normal cells get these signals they stop growing. Cancer cells do not respond to these signals.

2.1.3 CELL REPAIR AND CELL DEATH

Normal cells are either repaired or die when they are damaged or get old but cancer cells either not repaired or do not undergo apoptosis (Sengupta, 2013).

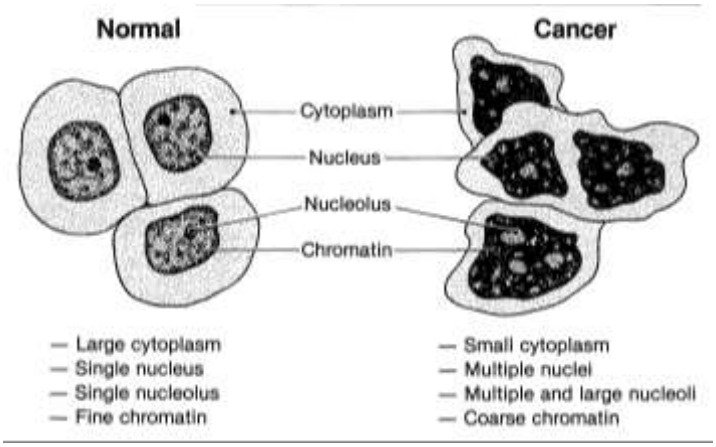

Figure 3: Difference between normal cells and cancer cells

2.1.4 STICKINESS

Normal cells secrete substances that make them stick together in a group. Cancer cells fail to make these substances, and can "float away" to locations nearby, or through the bloodstream or system of lymph channels to distant regions in the body (Sengupta, 2013).

2.1.5 APPEARANCE

Under a microscope, normal cells and cancer cells may look quite different. Furthermore, the normal cells, cancer cells often exhibit much more variability in cell size. In addition, cancer cells often have an abnormal shape, both of the cell and the nucleus. The nucleus appears both larger and darker than normal cells (Morawietz et al., 2004).

3. HISTOLOGY PROCEDURE

Microscopic analysis of cells and tissues requires the preparation of very thin; high quality sections (slices) mounted on glass slides and appropriately stained to demonstrate normal and abnormal structures.

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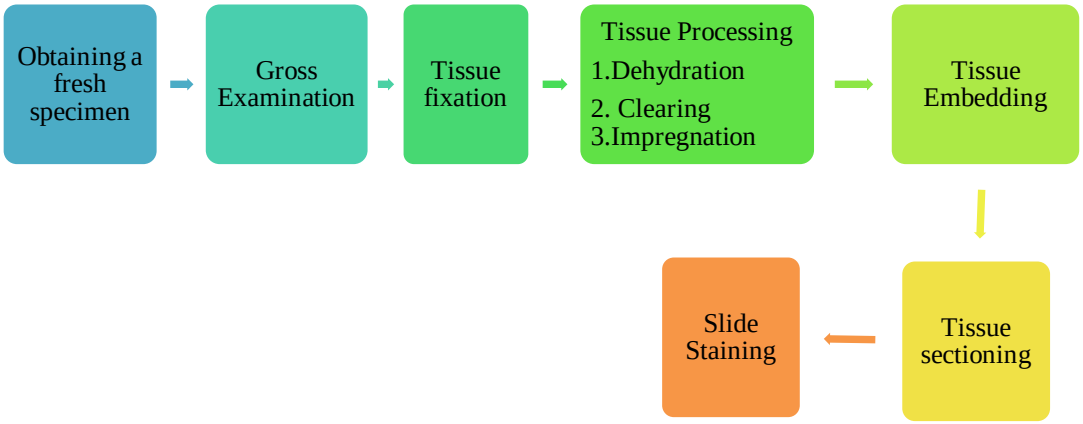

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Figure 4: Histology procedure of analyzing a laboratory animal for experiment

3.1 OBTANING A FRESH SPECIMEN

Materials/ Apparatus which used are for this process as follow:

- Dissecting tray
- Pins
- Dissecting scissors
- Dissecting needles
- Scalpel
- Forceps
- Mask
- Gloves
- Cotton wool/ paper towel
- Surgical spirit

3.1.1 PROCEDURE

First the animals were sacrificed under deep anesthesia and pin the rat down by placing the rat ventral side up. After that wipe the rat ventral side from a cotton swab with surgical spirit. Then lift the abdominal skin with a forceps and cut along ventral midline with the scissors in a cephalic direction. (Be careful not too cut to deeply. Keep the tip of your scissors pointed upwards. Do not damage the underlying structures.) Then make four lateral incisions (OIE, 2008). Then freeing the skin with the scissor tips and pin the flaps to the tray and wash the specimen with normal saline. Finally remove fat layer (Ziser, 2017).

3.1.1.1 BLOOD COLLECTION FROM CARDIAC PUNCHTURE

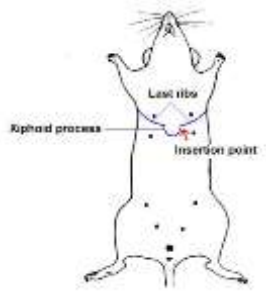

Figure 5: Blood collection from cardiac puncture (Kalani, 2022)

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According to the figure 5 blood sample will be taken from the heart, preferably from the ventricle slowly to avoid collapsing of heart. Sterile, 23 gauge needle is inserted to the angle between Xiphoid cartilage and the last rib and holding the syringe at an angle of about 300 to the horizontal , it is pushed slowly foreword until a small show of blood in the syringe (Morawietz et al., 2004).

Notice that the muscles are packed in sheets of pearly white connective tissue called fascia, which protect the muscle and bind them together. Carefully cut through the muscles of the abdominal wall in the pubic region, avoiding the underlying organs (Ibrahim et al., n.d.). To do this, hold and lift the muscle layer with a forceps and cut through the muscle layer from the pubic region to the bottom of the rib cage, in a similar way you did with the skin.

Figure 6: Internal abdominal regional anatomy of mouse and rat (Parkinson et al., 2011)

This figure 6 represented the internal abdominal regional anatomy of mouse and rat. The abdominal contents are exposed after dissection of the abdominal musculature and splitting of the pubic symphysis in the female mouse (A) and in the male rat (B). Salivary glands (SG), sternum (St), xiphoid (X), small intestine (SI), stomach (S), cecum (C), liver (L), and testes (T) are indicated. Note that the rat does not have a gallbladder.

Figure 7 represented the ventral aspect of rodent liver. The ventral aspect of the rodent liver demonstrates the four liver lobes. (A) The mouse liver median lobe is divided into right and left portions by a bifurcation containing the gallbladder. (B) The rat liver median lobe has an obvious central cleft, but the rat does not have a gallbladder.

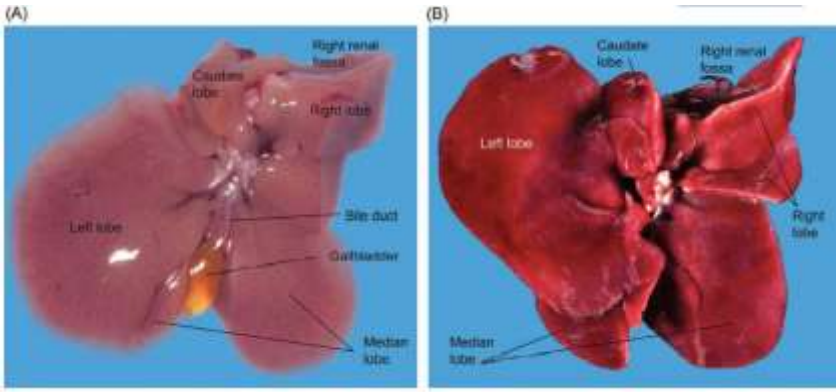

Figure 7: The ventral aspect of rodent liver (Risselada et al., 2010)

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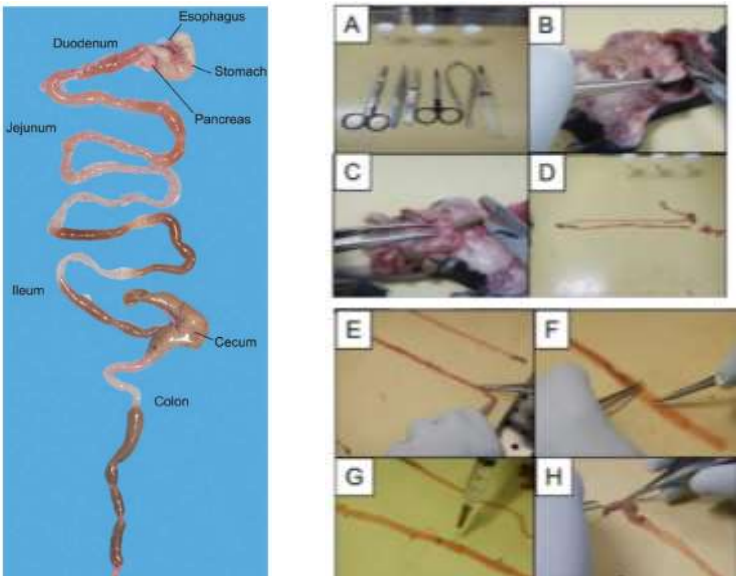

Figure 8: Dissection of alimentary tract and preparation of an intestine (Sensation, 2002)

3.2 GROSS EXAMINATION

In this method, following points are considered before doing the experiment. They are,

- Colour
- Texture
- Shape
- Size / length
- Weight
- + / - lesions(Ziser, 2017)

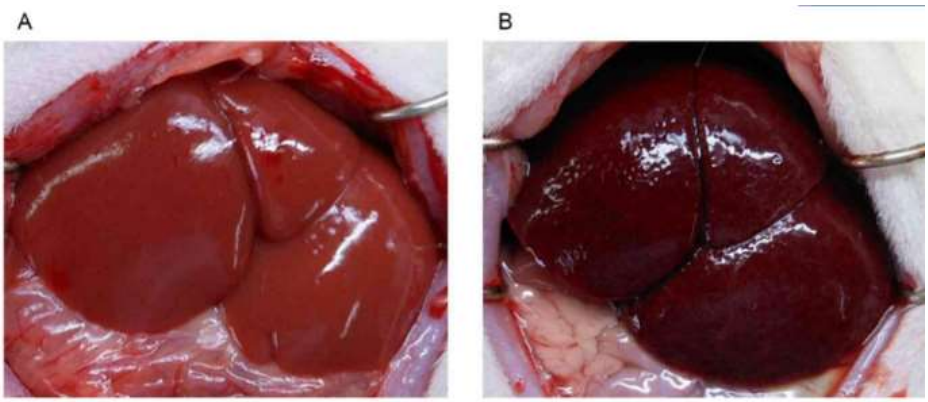

Figure 9: The macroscopic findings in (A) the normal liver of a control rat and (B) the liver of a rat with MCT-induced SOS.

Figure 9 represented the macroscopic findings in (A) the normal liver of a control rat and (B) the liver of a rat with MCT-induced SOS. The latter showed accumulation of bloody ascites. In addition, the liver surface was dark red in color (Peer-reviewed, 2019).

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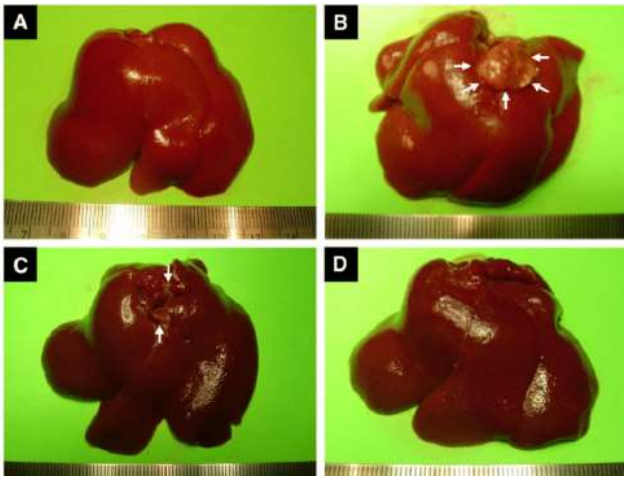

Figure 10: Morphological examination of rat liver tissue at the end of the study (Sengupta, 2013)

In this figure 10 macroscopically visible hepatic nodules are shown by arrows. Representative livers were excised from several groups: (A) normal (Group A) showing absence of nodules; (B) showing a large nodule; (C) showing small nodules; (D) with no visible nodules.

3.2.1 SELECTION OF SPECIMEN

Take specimen from more than one areas of affected organs. The cut must be made

- Quickly
- Sharply
- Accurately

There is no,

- Pitching
- Squeezing
- Bending
- Crushing on the specimen

3.2.2 IMPORATANT FACTORS IN SECTIONING

3.2.2.1 LOCALIZATION

An anatomical site or part of an organ from which a sample should be taken (i.e. lobe).

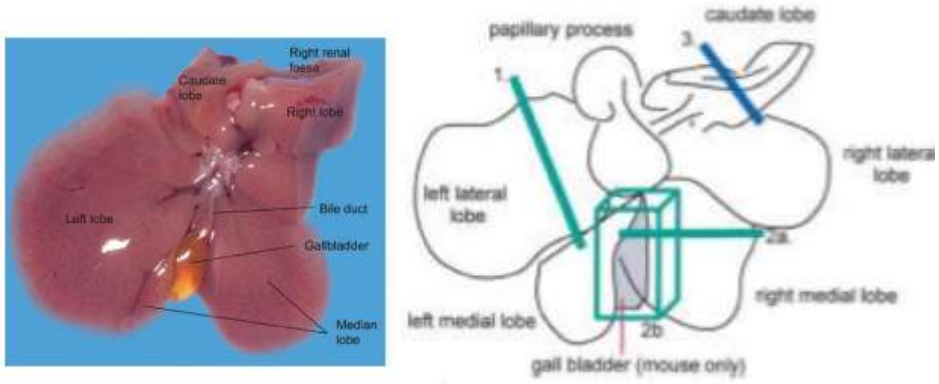

Figure 11: Liver, visceral aspect, indicating the cut levels for mice (Revised guides for organ sampling and trimming in rats and mice, 2022)

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3.2.2.2 NUMBER OF SAMPLES:

The number of organs (i.e. both for bilateral organs) and organ pieces prepared for evaluation (not necessarily identical with the number of slides/blocks) are considered in this step.

3.2.2.3 DETERMINATION OF THE TRIMMING DIRECTIONS

- Transverse: - perpendicular to the long axis of an organ or part of an organ.
- Llongitudinal vertical: - in the direction of the long axis of the body, an organ or part of an organ in the dorsoventral axis or parallel to it (in the text also referred to in short as "longitudinal").
- Longitudinal horizontal: - in the direction of the long axis of the body, an organ or part of an organ, perpendicular to the dorsoventral axis (in the text also referred to in short as "horizontal") (Morawietz et al., 2004).

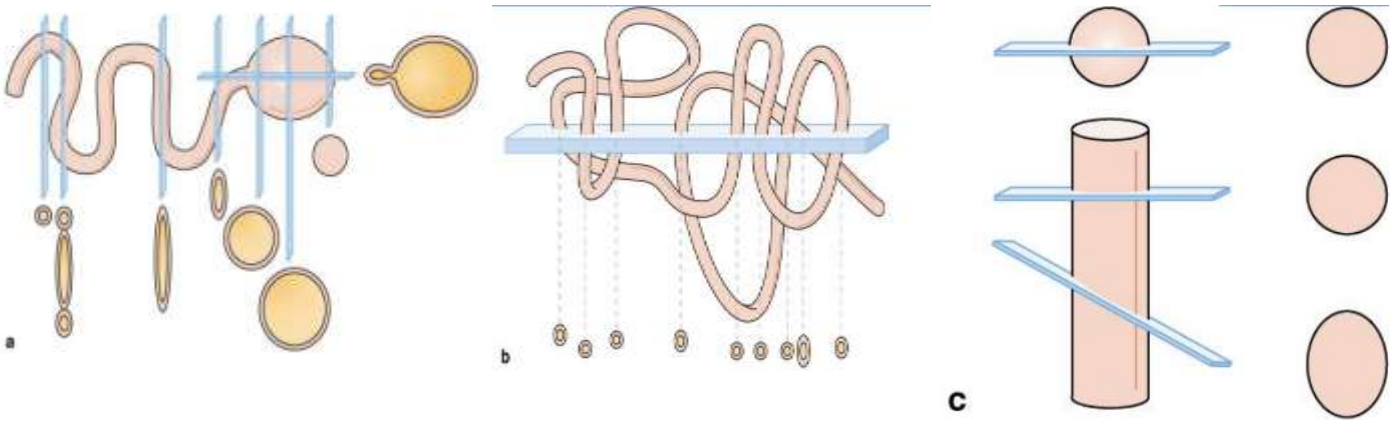

Figure 12: Three-dimensional structures appear to have only two dimensions in thin sections (Morawietz et al., 2004)

Figure 12 showed the, (a): Sections through a hollow swelling on a tube produce large and small circles, oblique sections through bent regions of the tube produce ovals of various dimensions. b): A single section through a highly coiled tube shows many small, separate round or oval sections. On first observation it may be difficult to realize that these represent a coiled tube, but it is important to develop such interpretive skill in understanding histological preparations. (c): Round structures in sections may be portions of either spheres or cylinders. Additional sections or the appearance of similar nearby structures help reveal a more complete picture (Vaught & Henderson, 2011).

3.3 FIXATION

Fixation is used to maintain the natural state of specimen. Fixation facilitates proper staining of tissue. The fixative should be 20-25 times of volume of specimen.

3.3.1 Characters of a good fixative

- Penetrate quickly
- Prevent tissue from shrinkage
- The specimen container must be,
- Leak proof
- Wide mouth
- Made with a nonreactive material
- Be a suitable size to contain appropriate volume of fixative (10 times the specimen)
- Container should carry a detailed label of the specimen included

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Figure 13: Specimen container (Laboratory - Mediray, 2022)

Formalin is the most commonly used fixative. It is prepared by mixing 40 % formaldehyde gas in 100 % w/v of distilled water. The resultant mixture is 100 % Formalin. Routinely, 10 % formalin is used which is prepared by mixing 10 mL of 100 % formalin in 90 mL of distilled water.

3.4 TISSUE PROCESSING

Aim of tissue processing is to embed the tissue in a solid medium firm enough to support the tissue and give it sufficient rigidity to enable thin sections to be cut , and yet soft enough not to damage the knife or tissue.

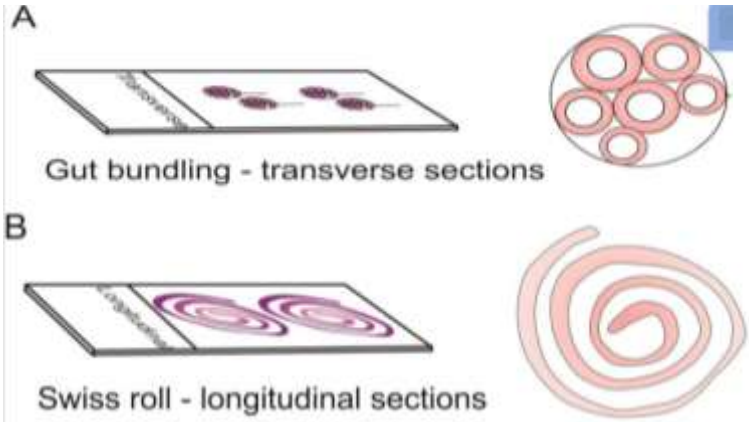

Figure 14: Diagram of orientation of tissue for intestinal histology including cross sections (Williams et al., 2016)

Figure 14 represented the A; achieved through gut bundling technique) and longitudinal sections, B; achieved through Swiss roll method.

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3.4.1 STAGING ON TISSUE PROCESSING

Figure 15: Tissue processing steps

For dehydration first used 70% ethanol and then 80%, 90% and finally 100% ethanol and for clearing xylene are used and paraffin used for wax impregnation.

3.5 TISSUE EMBEDDING

Embedding is the process in which the tissues or the specimens are enclosed in a mass of the embedding medium using a mould. Paraffin is the most commonly used medium for this.

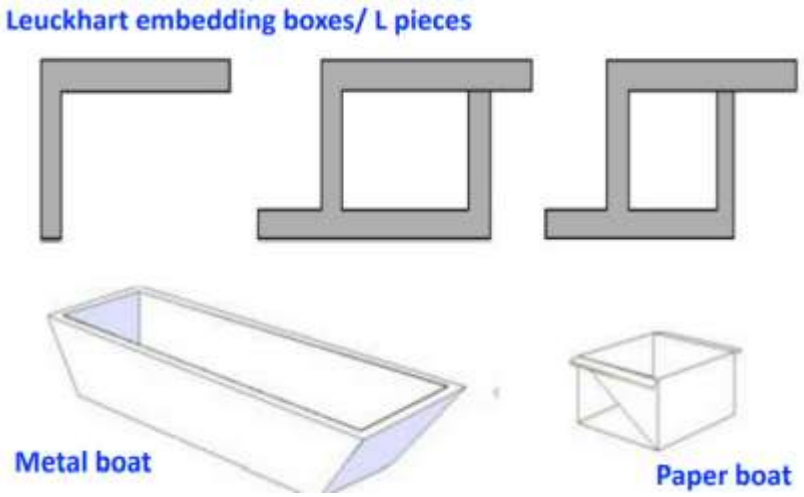

Figure 16: Different embedding moulds

3.5.1 PROPER ORIENTATION IN BLOCKING/ EMBEDDING BLOCKING TIPS

- Place the tissue in the centre & deepest position
- Always place the cut surface downwards
- Avoid contamination with the previously taken samples

3.6 TISSUE SECTIONING

Place tissue block in microtome with wide edge of trapezoid lowest, and parallel to knife and trimmed block with excess paraffin removed and block face in a trapezoid shape.

3.7 STAINING

Staining is employed to give both; -contrast to the tissue and highlighting particular features of interest. There are main two types of staining. They are,

1. **Routine stains**: for this method hematoxylin and eosin stain (H&E) are used "routinely" with all tissue specimens to reveal the underlying tissue structures and conditions. With this H and E method cell nuclei are stained blue and

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cytoplasm and many extra-cellular components in shades of pink. In histopathology many conditions can be diagnosed by examining an H&E alone (Manuscript & Studies, 2010).

2. **Special stains**: this is an alternative stains used when the H&E does not provide all the information the pathologist or researcher needs.
   Steps followed in staining (Bartlett et al., 1988).

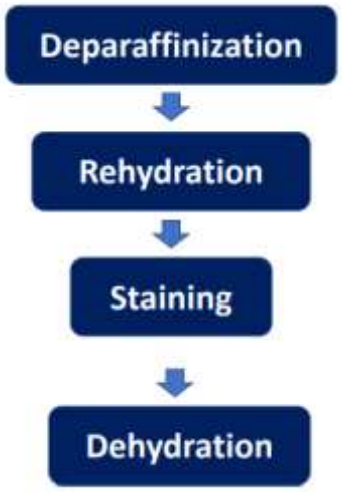

Figure 17: Steps of staining

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C. Author: E-mail: aarathnamali@gmail.com

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