

Histology Service

1. The histotechnologist receives the tissue samples and processes them through An automatic tissue processor, which prepares the tissue by:
 - a. Fixing the tissue specimens in 10% Neutral Buffered Formalin
 - b. Dehydrating the tissue specimens in graded ethyl or isopropyl alcohol from lowest concentration to highest concentration.
 - c. Clearing is done universally by the use of Xylene and rinses the alcohol away and prepares the tissue for infiltration.
 - d. Infiltration is done by using paraffin (wax) to solidify and harden the tissue specimens to allow sectioning to take place.
2. The histotechnologist must then embed the tissue samples which entails Surrounding the tissue specimen with paraffin to facilitate the cutting of thin sections. The tissue is placed in a mold and melted paraffin is placed overtop the tissue in the mold and is then cooled at -5 degrees. The tissue is then popped out of the mold and is ready for microtomy/sectioning.
3. Microtomy (sectioning) is the next step and involves cutting the tissue into Extremely thin slices using a special instrument called a microtome. The tissue is cut into ribbons and then floated out on a water bath and mounted on a microscope slide. The slide/tissue is then dried in order to allow adhesion to the slide, removal of bubbles and wrinkles, and melts away excess paraffin, which surrounds the tissue to prepare it for staining.
4. The histotechnologist then is ready to stain the sections using the hematoxylin And eosin (H&E) stain, which is routinely used on all tissue, samples. This gives a differential blue (nuclear) and red (cytoplasmic) color to the basic and acidic structures within the tissue. The chemical process is listed below:
 - a. Deparaffinize slides through 3 changes of xylene
 - b. Hydrate slides in 3 changes of 100% ethanol followed by 2 changes in 95% ethanol
 - c. Rinse the slides in water
 - d. Stain the slides in Hematoxylin
 - e. Rinse the slides in water
 - f. Decolorize the slides in 10% acid alcohol
 - g. Rinse the slides in water
 - h. Differentiate the slides in a bluing reagent
 - i. Rinse the slides in water
 - j. Place the slides in 95% ethanol
 - k. Counterstain the slides in Eosin
 - l. Place the slides in 95% ethanol
 - m. Dehydrate the slides in 3 changes of 100% ethanol
 - n. Clear the slides in 3 changes of xylen
 - o. Coverslip the slides

5. Once the slides are completed, the histotechnologist does the following:
 - a. Sends an email message to the primary investigator/staff to notify them that the slides are ready to be picked up.
 - b. Completes the billing form and makes 2 copies. One for self, and one for investigator and then puts the original in Julie Giordano's box for billing purposes.
6. The primary investigator/staff then picks up the slides and is to follow up with The Pathologist for reading/diagnosing the slides. The pathologist then microscopically examines the slides so that he may diagnose all histological observations. This histology observation is the foundation of subsequent diagnosis, prognosis, treatment, and reevaluation.

Depending on what the findings of the Pathologist are, special stains, immunohistochemistry stains, or frozen sections may be required.

***These will be discussed later.**

SPECIAL STAINS

If the slide diagnosis is inconclusive then the pathologist will then order additional special stains to be done. Below are some special stains, which could be used.

1. CONNECTIVE TISSUE STAINS
 - a. Masson's Trichrome(MTS) – stains for muscle fibers and collagen
 - b. Verhoeff's Elastic – stains for elastic fibers and collagen
 - c. Snook's Reticulum – stains for reticulum fibers
 - d. Giemsa – stains for mast cells
2. CARBOHYDRATE TISSUE STAINS
 - a. Periodic Acid Schiff(PAS) – stains for glycogen, mucin, fungi, and Basement membranes.
 - b. Mayer's Mucicarmine – stains for mucin, and capsules of cryptococci
 - c. Alcian Blue 2.5 – stains for sulfated mucosubstances, hyaluronic acid, and sialomucins
 - d. Mowry's Colloidal Iron – stains for acidic mucopolysaccharides
 - e. Congo Red – stains for amyloid
3. PIGMENTS & MINERALS STAINS
 - a. Warthin Starry – stains for melanin
 - b. Perl's Iron (FE)– stains for hemosiderin and some oxides and salts of iron
 - c. Von Kossa – stains for bone and mineral salts of calcium, and iron
4. BACTERIA, FUNGI, AND OTHER MICROORGANISMS STAINS
 - a. Brown & Brenn (B&B)– stains for gram+ and gram- bacteria
 - b. Warthin Starry (W&S)– stains for spirochetes
 - c. Ziehl Neelsen (AFB)– stains for acid fast bacilli
 - d. Grocott's Methenamine Silver(GMS) – stains for fungi, mucin, mycelia, and hyphae

IMMUNOHISTOCHEMISTRY(IHC)

IHC is the identification of specific substances and makes use of precisely selected antibodies to identify (mark) specific antigens. This is usually used for further identification of diagnosis. There are two primary methods used in histology and they are the PAP or peroxidase antiperoxidase method, and the ABC or avidin biotin complex.

The PAP method uses a primary antibody, a secondary antibody, and an antibody that is produced against and linked with peroxidase enzyme(PAP COMPLEX). The secondary antibody is produced in a species different from the primary antibody and PAP Complex, and the primary antibody and PAP Complex are produced from the same species. The secondary antibody therefore acts as a “bridge” or “link” antibody.

The ABC method also uses three reagents; a primary antibody, a secondary antibody that is chemically bound to the vitamin biotin, and a complex of glycoprotein avidin that is bound to biotin and peroxidase. Avidin has the ability to bind nonimmunologically 4 molecules of biotin. This strong affinity gives this method excellent sensitivity.

FROZEN SECTIONS

This is a process by which a fresh piece of tissue or a previously frozen and stored piece of tissue is frozen and cut in lieu of being routinely processed. Using an embedding media and cold temperatures, the tissue is frozen and thus sectioning can be accomplished on a mechanically refrigerated cryostat producing slides similar to those produced during microtomy.