SOPS for General Surgery Labs:

Title: Research Histology Services

Address: E1515 Thomas Starzl Transplantation Institute
200 Lothrop Street, Pittsburgh, PA

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Research Histology Services is located in the Thomas E. Starzl Biomedical Science Tower at the University of Pittsburgh. Our goal is to provide research histology services to primary investigators and researchers throughout the various departments of the University, and in the external biomedical community. The RHS lab serves as the Core Liver Laboratory for the Immune Tolerance Network, providing histology services for ongoing clinical patient research studies throughout the United States and Canada.

The lab provides a comprehensive range of histological research techniques that include decalcification, standard tissue processing, biopsy processing, paraffin embedding, OCT embedding of frozen tissues, serial sectioning, step/level sectioning, and cryostat sectioning. A wide spectrum of assays is available in addition to the routine hematoxylin and eosin (H&E) stain. Special histochemical stains routinely provided include; Masson Trichrome, PAS (periodic acid Schiff), Prussian Blue (Iron), Verhoff's Elastin Stain, Cresyl Violet, Toluidine Blue, and Luxol Fast Blue; additional histochemical staining is done upon request. Wide arrays of Immunoperoxidase and Immunofluorescent staining and apoptosis/tunel stains are also available.

Standard Turnaround Times

- One day
  - Processing and embedding
  - H&E on pre-cut glass slide
  - Special stain on pre-cut glass slide
- Three days
  - Processing, embedding and H&E
  - Frozen slides from OCT block
  - H&E stained slide from paraffin block
  - Special stain from paraffin block
  - Immunohistochemistry on pre-cut slide
- One week:
  - Processing, embedding, H&E, immunohistochemistry and special stain
  - Levels or step sectioning

- Turnaround times may vary depending on workload. Lab personnel will do their best to accommodate your specific turnaround time needs.

Submission/Requisition Form

- Fill out the submission form and bring with samples to E1515. List researcher name, PI name, tissue type, species, type of fixative used and solution tissue is presently in, services requested, and any specific handling instructions. Please include a phone number where you can be reached for questions and to be notified when services are completed. A separate sheet is also provided to list the specimen identification.

Submitting Tissue

Samples for Paraffin Embedding

- We accept tissue which has been fixed in a variety of fixatives. Our standard processing uses 10% NBF (Neutral Buffered Formalin). When using other reagents, once fixation is complete please transfer samples to 70% Ethanol.
- 10% NBF, cassettes, biopsy wraps /cassettes, and transport containers are available for our customers use. Pick up in E1515.
- When tissue is placed into cassettes, the tissue surface that is placed down in the cassette will be placed down for embedding; this is the surface that is sectioned first. Please list any special embedding requests on requisition form.
- Use only a reagent resistive marker (e.g. Shurmark) or a #2 hard lead pencil for labeling slides or cassettes, never a pen**.** Solvents used in processing can remove the ink from many "permanent" Sharpie markers.
- To prevent small tissues from being lost during processing, place in biopsy cassettes or wrap in filter paper. The tissue processor uses vacuum to facilitate infiltration which can remove tissue from the cassette.
- Refrain from overcrowding. Tissue that is compressed in the cassette will not adequately fix, infiltrate, section, or stain. Specimens should be cut thin enough to allow adequate fixative penetration. Do not allow tissue to touch all sides of the cassette or become smashed in the lid. A thickness of between 0.2 and 0.5 cm (approximately the size of a nickel is a good guide) works best when trimming samples. If batches of tissues are being submitted at the same time that vary significantly in size (whole organs vs. biopsy samples), sort by size and submit in separate cassettes.

Allow ~1mm/hour for the fixative to penetrate your tissues and a volume of 10-20 times fixative volume to tissue.

- Place specimens in spill-proof container with a tight-fitting lid. Be sure that all tissue and or cassettes are completely submerged so samples do not dry out.

- Label the transport container with the PI and Researcher (submitter), solution, and date.

Frozen Samples

- Rapid rather than slowly freezing reduces ice crystal formation in tissue. Tissue should be gently blotted free of extraneous fluid prior to being frozen.
- A pre-labeled tissue mold is filled one third full with OCT embedding compound tissue specimen is oriented in tissue mold (clearance between the edge of tissue and side of mold should be maintained); the remainder of tissue mold is filled with OCT embedding medium (ensure that specimen is completely surrounded and covered by OCT). Tissue mold is floated on liquid nitrogen bath until completely frozen (OCT will be firm and opaque/white). Remove mold from liquid nitrogen and tightly wrap in foil, place foil wrapped specimen in plastic bag that is properly labeled with specimen identification and date. Tissue should be held in -80° C. Do not let frozen tissue thaw until after sectioning.