SOP 018-01-01
October 19, 2018

Washington State University – Spokane

Formalin Fixing and Grossing Tissue for H and E staining

I. Purpose: The purpose of this SOP is to provide instructions for properly preparing tissues for processing and microtomy

II. Materials
- A. Tissue
- B. 10% Neutral Buffered Formalin (or appropriate fixative)
- C. 70% Ethanol
- D. Scalpel
- E. Cassettes
- F. Sponges
- G. Pencil

III. Methods
- A. Fix tissue.
  1. Place fresh mouse or rat tissue in 10% Neutral Buffered Formalin for at least 48 hours. Use a minimum of 20:1 fixative:tissue volume ratio.
  2. If planning on IHC at any point, move the tissue to 70% Ethanol after 48 hours to prevent epitope masking
- B. Gross tissue.
  1. Identify cassettes in pencil. If placing multiple tissues in 1 block, ensure that tissues are compatible for cutting.
  2. Using a scalpel, trim tissue to the thickness of a nickle. Special cutting instructions:
     a. **Liver**: Slice liver across the lobe lengthwise into nickle-thick sections, transecting portal veins.
     b. **Kidney**: Bisect kidney lengthwise, creating a flat cutting face. If kidneys are too bulky, remove tissue opposite the cutting face. Make sure to remove stray fat around the kidney margins.
     c. **Heart**: Bisect the heart lengthwise, transecting the atria and ventricles, creating a flat cutting face.
     d. **Lungs**: Remove one lung lobe. Lungs should not require further grossing.
     e. **Intestines**: Flush the unfixed tissue with extra fixative to remove food. You may either cut the intestines lengthwise and place between filter paper to ensure the tissue does not curl, or cut the intestines into <0.5 cm segments.
     f. **Stomach**: Prior to fixation, butterfly the stomach open from esophagus to duodenum and remove food particles. Place the organ butterflied onto filter paper and staple a second piece to create a flat envelope. This will allow the curved tissue to fix flat.
     g. **Skin**: If at all possible, shave the animal prior to fixation. Hair can cause problems later in the histology process. Cut the tissue into <.5 cm wide and 2 cm long strips. The strips will be stood on their side during embedding to allow a long cutting surface.

   h. **Brain**: Consider which area of the brain is of importance to your research, whether it is best viewed DV or laterally, and whether both hemispheres of the brain are necessary. Subsection through the region of interest at the thickness of a nickle.
   i. Small organs such as adrenals or eyes may not require additional grossing in after fixation.
3. Place grossed in tissue in the corresponding cassette. If tissue needs help lying flat or is small enough to fit through the cassette slats, consider adding a sponge. If tissue is exceptionally large, be aware that pressing too hard against the sponge can cause artifacts that will be exposed during microtomy.
4. Place loaded cassettes into a vessel filled with fixative and proceed to Processing SOP.