Penn Center for Musculoskeletal Disorders Histology Core

Protocol: Sample fixation, decalcification and processing

Materials

- 10% neutral buffered formalin
- Formical-2000 decalcification solution (Statlab)
    - This solution is a mix of formic acid and EDTA for rapid but gentle decalcification. It is suitable for both routine histology and immunohistochemistry
- Ethanol

Fixation

1. Submerge sample in 10% neutral buffered formalin. Ensure there is at least 10 times fixative volume to tissue volume.
2. Cap container and keep at 4°C. Fixation times vary based on tissue thickness:
    - Rodent tissues (e.g. bones, joints, spine segments): ~3 days
    - Large animal tissues (e.g. joints, spine segments): at least 7 days
    - It is critical not to under-fix tissues
3. Rinse thoroughly in running tap water

Decalcification (optional for mineralized tissues)

1. Submerge sample in Formical-2000. Ensure there is at least 10 times solution volume to tissue volume.
2. Cap container and gently agitate on orbital shaker
3. Replace solution every 3 days until completely decalcified
    - Decalcification time will vary depending on sample size and mineral content. When decalcification is compete, the sample should appear completely clear on x-ray or fluoroscopy. Alternatively, physical inspection can be used (e.g. inserting a small needle). Complete decalcification is essential for effective sectioning. Rodent tissues will typically take less than 1 week. Large animal tissues may take 1 week to 1 month.
4. Rinse thoroughly in running tap water

Storage and Paraffin Processing

- Submerge sample in 70% ethanol, cap container and submit to the core for paraffin processing. Select the processing cycle (based on sample type and thickness) in consultation with core staff.