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Recommendations for Tissue Prep and Fixation (Quick List):

The overall goal is to capture and preserve tissues in the same morphological and molecular states they were in before removal from the body. Unfortunately, the instant tissues are deprived of their blood supply molecular changes begin to occur – changes in protein phosphorylation occur within minutes whereas others (DNA breakdown) may not begin for hours. Factors that impact on tissue quality include: time to fixation, mode of fixation, size of tissue sample, type of fixative, volume of fixative, temperature of fixative, and total time in fixative.

Recommendations :

1. **Time to fixation: fast** (ideally less than 20 minutes, but better if shorter)**;** pre-fixation, keep tissues at 4° (in neutral, isotonic solutions, w/ physiologic Ca++, Mg++ concentrations) if possible.
2. **Type of fixative**: depends on studies; 10% neutral buffered formalin (NBF) or 4% paraformaldehyde (PFA) most common. (See Fixative Options).
3. **Mode**:
    a. **immersion** (practical and usually adequate).
    b. **cardiac perfusion** (quicker and more effective, but skill-dependent; perfusion fixation *strongly* recommended for animal brain).
4. **Volume of fixative: 10-20 x volume of tissue**
5. **Size of tissue: must be less than 5 mm in one dimension for adequate fixation** (thickness of a nickel)**;** recommended maximum sample size—1.5x1.5x0.4 cm. If fixing tissues in cassettes, make sure the tissue can move freely. Gently shake the cassette to confirm the tissue can move from side to side.
6. **Time in fixative:** 12-18 hrs for small biopsies; 24-72 hrs for tissues (1.5x.1.5x0.4 cm). Depends on temp as well as size and type of tissue. If at 4°, would recommend 48-72 hrs.
7. **Temperature**: the temp of choice is controversial; we recommend 4° to slow down metabolic processes, such as hypoxic responses. Clinical specimens are typically fixed at room temp. Higher temps speed fixation, but they also speed up post-surgical metabolic changes.
8. **Mixing**: gentle mixing (e.g. nutation) to facilitate replenishment of fresh fixative around tissue.

Also see Detailed Discussion of Tissue Prep and Fixation Recommendations for more information.

Stringent adherence to the above recommendations may not be necessary (e.g. for simple histomorphological evaluations) nor are they "ideal" for all studies. However, if molecular analyses are to be performed using paraffin embedded tissues, then how *all* of the above steps are performed should be recorded every time tissues are prepared for paraffin processing. This information is critical for trouble shooting any difficulties encountered downstream. Consistency is important.