Caris Life Sciences
Tissue Fixation Instructions for Molecular Profiling

The information provided below is for informational purposes and does not guarantee testing can be performed on the specimen submitted.

Fresh Tissue

1. Fix the specimen as soon as possible; keep time to fixation to a minimum. Never exceed one hour at room temperature. Degeneration commences as soon as cells are deprived from blood supply. Record time to fixation for later reference.
2. If fixation is not immediately performed refrigerate the specimen. Refrigeration is not a substitute for fixation. Do not freeze the specimen as slow freezing of tissue will produce ice crystals and damage the specimen.

Proper Fixative Penetration

1. Fixative should penetrate from all sides of the specimen; always place specimens into containers that already contain fixative. This will prevent adhesion of the specimen to the container.
2. Cavities should be opened, where possible hollow organs or open specimens with natural cavities to allow immediate access of fixative.
3. Thickness is important; 3 mm is optimal for fixation. Cut larger specimens into slices or cut small pieces of tumor out before fixation.
4. Some agitation during the first few minutes is useful and will aid penetration.
5. An adequate volume is vital (at least 20:1). Excess fixative is required due to consumption during the fixation process.
6. Allow sufficient time for the fixation process. The fixative needs to penetrate the center of the densest part of the specimen, and then the chemical reactions of fixation need to occur.
7. Room temperature is best. Initial fixation is best carried out at room temperature (20°C).
8. Fixation time: 12 – 24 hours, do not exceed 72 hours.

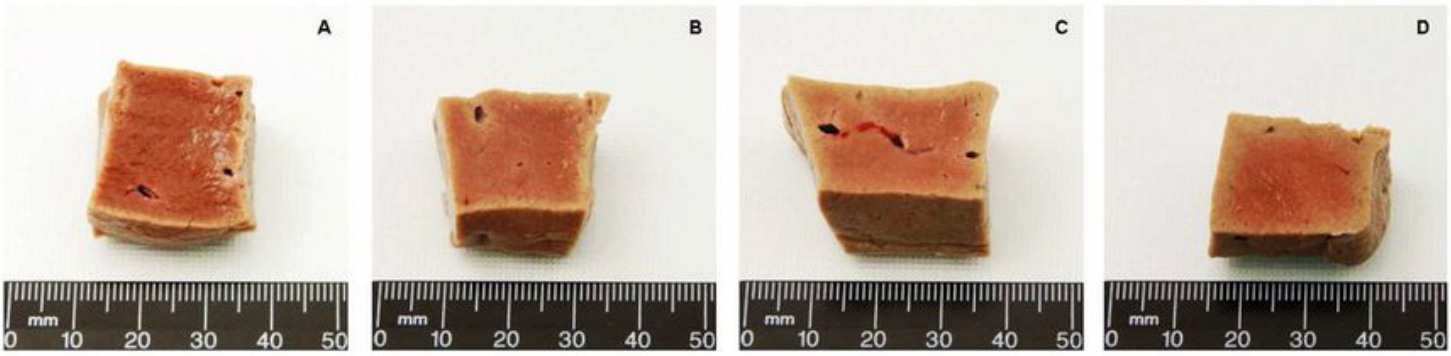

Photo: Leica Biosystems

A composite photograph showing the rate at which 10% neutral buffered formalin penetrates into 25 mm cubes of liver. At the end of each time period a cube has been sliced to reveal the advancing fixation front.

A: one hour (approximately 0.8 mm penetration)

B: two hours (approximately 1.2 mm penetration)

C: four hours (approximately 1.6 mm penetration)

D: eight hours (approximately 2.2 mm penetration).

Note: After eight hours the center of the specimen still remains unfixed. *Extending fixation time will not compensate for thickness.*

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Photo: Leica Biosystems

A whole heart specimen has been sliced to allow proper penetration of fixative.

Photo: Leica Biosystems

A specimen of fatty breast tissue has been squeezed into a small container holding an inadequate volume of fixative. In this case it is likely that the specimen will be poorly fixed and mechanically distorted.

Proper Choice of Fixative

The solution described below is commercially available through several suppliers.

1. Only use Phosphate buffered formalin of this formulation:
    a. 40% formaldehyde: 100 ml
    b. Distilled water: 900 ml
    c. Sodium dihydrogen phosphate monohydrate: 4 g
    d. Disodium hydrogen phosphate anhydrous 6.5 g
    e. Solution pH of 6.8
2. Phosphate buffered formalin is the most widely used formaldehyde-based fixative for routine histopathology. The buffer tends to prevent the formation of formalin pigment. Many epitopes require antigen retrieval for successful IHC following its use. Most pathologists feel comfortable interpreting the morphology produced with this type of fixative.
3. It is most important that *NO* divalent cation salts are added to the formalin based fixatives (e.g. avoid the common variation of Zinc formalin where zinc sulphate is added). Divalent cations inhibit many steps of the PCR reaction, which is necessary for molecular diagnostic workup. Equally important, do *NOT* use other non-formalin fixatives (e.g. alcohol-based Clarke's, Carnoy's, methacarn, Gendre's solution). Also, do *NOT* use Bouin's solution, Zenker's fixative, Helly's fixative, Hollande's solution, because strong acids (acetic and picric) and divalent metal salts (e.g. Mercuric chloride, Calcium chloride or Zinc sulphate) in these fixatives inhibit PCR reactions needed to analyze nucleic acids.
3. If the specimen is a primary bone malignancy or metastasis to the bone, decalcify (if necessary) using EDTA based methods.

Specimen Requirements

Please refer to the Caris Life Sciences Tumor Profiling Requisition for specimen requirements: www.CarisMolecularIntelligence.com/ordering_information.

- **Slides**

  The number of slides needed depends on the service ordered (panel or individual assay(s)) and the amount of tissue on each single slide: 2 slides with 50 mm^2 of tissue contain the same amount as 10 slides with 10 mm^2 each. Yield also depends on

the tumor cell fraction; some tissue infiltrating tumors have a higher content of stromal/epithelial cells, whereas other tumors consist almost entirely of malignant cells.

- **Blocks**

  FFPE (formalin fixed paraffin emebdded) tissue blocks are the preferred specimen type and should contain representative sample of the patient's cancer. FFPE blocks from open biopsy, tumor resection or needle biopsy are all equally good providing that they contain properly fixed cancer sample and will yield sufficient number of sections (see description of Slides above). FFPE blocks from fluid aspirations are frequently insufficient due to the low percentage of neoplastic cells in the block. If a block from an aspirate is provided it must contain at least 20% malignant cells (10% neoplastic cells may be sufficient for RFLP testing of EGFR mutations status).

- **Pathology assessment/review**

  A biopsy may be sent directly to Caris. In this case, put the biopsy material immediately into phosphate buffered formalin and initiate shipment to Caris. Laboratory staff will embed the biopsy and provide a pathology report in addition to the MI Profile tumor profiling report. Formalin shipper kits are available; please contact Client Services for more information.

- **Limited tissue**

  In the event not enough material was submitted, Caris medical staff will recommend a priority list of tests that can be performed on the specimen. The ordering physician will have an opportunity to review and edit the list before testing begins.

Bone Specimen Decalcification

1. If the specimen is soft and without a need for decalcification then process without the use of decalcifying solution. Notate in the gross description and grossing log.
2. Proper 10% NBF fixation of bone specimens should be performed prior to decalcification.
3. Rinse the fixed bone specimen briefly (1-5mins) and several changes with distilled or deionized H2O before immersing in the decal solution in a volume of at least 20 times.
4. Fill the disposable specimen cup with Formical 2000(decal solution).
5. Place the bone specimen into the specimen cup. Wrap the specimen in a biopsy paper as necessary.
6. Label the specimen cup with patient case number, "decal", and the time the specimen was placed into decal.
7. Set timer for amount of time in decal, according to the table: Guidelines for Decalcification End Point.

Specimen Type	Size (diameter in greatest length)	Time in Decal	Additional Time in Decal
Bone marrow core biopsy	Less than 0.2 cm	Start with 20 minutes increments, check pliability	Up to 60 and if bone is more than 0.3 cm, add 10 minute increments, check pliability or end point
Cancellous or spongy bone	Greater than 0.2 cm	Start with 30 minute increments, check pliability	Up to 90 minutes, if more than 0.3 cm thickness, add 15 minute increments, check pliability or end point; consult pathologist
Bone chips	Greater than 0.2 cm	Start with 20 minutes increments, check pliability or end point	Up to 90 minutes, add 10 minute increments, check for pliability or end point, consult pathologist

** Formical 2000 is categorized as a weak acid/EDTA. This has been tested at Caris showing the best results for molecular testing (i.e. sequencing).*

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End Point Test

1. Testing for appropriate decalcification: (choose one from the following)
    a. Touch the specimen with the pad of your gloved finger, it should be pliable, yet firm to the touch.
    b. Pick up the bone and using thumb and index finger; gently bend to check for flexibility.
    c. When tissue begins to float in the solution, decalcification is usually complete; however, some tissues do not float when it is decalcified.
    d. Using a dissecting needle, gently probe into the bone, if it penetrates, it is decalcified.
2. Place the specimen into the cassette for processing. Label the sides for piece count and patient initials and DOB
3. Rinse cassette with distilled or deionized H2O water for 5 minutes with several changes. Do not rinse with tap water, to eliminate contamination of chemicals from municipal tap water.
4. Place cassette in formalin bath for the large processing run.

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