Embedding Paraffin Processed Tissue

Purpose/Policy Statement:
Paraffin impregnated tissue specimens are oriented in a base-mold, overlaid with molten paraffin wax and cooled to create tissue-containing solidified paraffin blocks for microtomy. Accurate specimen identification is maintained throughout the embedding process.

Definitions:
- en face sections provide two dimensional approaches which is sufficient in skin cancer diagnosis and margins. To submit en face margin, the cut surface (non-inked) is placed face down on the cassette. This surface will approach the microtome blade first. This circumstance allows the pathologist request deeper levels of the block to reach the true margin.

STEPS / KEY POINTS

PROCEDURE:
1. Transfer the embedding cassettes from the automatic tissue processor processing chamber containing molten paraffin wax into the embedding center auxiliary holding chamber containing molten paraffin wax, arranged in an upright position for visualization of the accession number.
2. By color-coded priority, place tissue cassette onto the heated work surface of the embedding center.
3. Visualize PA variance documentation written on the side of the cassette that will contain the number of sample pieces represented and dictated in the report.
4. Remove the metal lid from a single cassette and carefully inspect the inside surface of the lid for any portion of the sample that may have become attached; return the specimen to its original tissue cassette.
5. VALIDATE THAT THE TISSUE CASSETTE CONTAINS THE NUMBER OF PIECES DOCUMENTED. Discrepancies are additionally viewed by a second histotechnologist; record the variance on the BIOPSY SAMPLE VARIANCE DOCUMENTATION log. If a second histotechnologist is not present, the cassette may be put aside until a second opinion can be obtained. Some circumstances the physician assistant will need to be notified to guide the histotechnologist for orientation.
6. Using a mold which corresponds closely to the tissue dimensions, dispense molten wax to fill the base area of the mold.
7. Using CLEAN warm forceps or scalpel blade, quickly orient the submitted tissue from the cassette into the base of the mold, taking care to center and correctly align the entire tissue sample (see Procedure Notes). The underside of the base mold may be briefly touched to the cold-plate to create a semi-solid wax base in which the tissue may be positioned and held onto the bottom surface of the mold. The surface of tissue section to be cut must be placed parallel to the mold bottom.
8. Place the embedding cassette (number-side up) onto the top of the base mold and dispense additional molten paraffin into the mold through the embedding cassette holes until the empty portion of the cassette is full.

9. Quickly transfer the base mold onto the cooling plate.

10. Following complete low-temperature crystallization, the resulting wax block may be easily lifted from the base mold.

QUALITY CONTROL:
1. The daily paraffin temperature monitoring and embedding center work area maintenance is recorded in the Performance Improvement Recordbook.
2. Tissue sample variances are recorded on the BIOPSY SAMPLE VARIANCE DOCUMENTATION log.

PROFICIENCY TESTING: College of American Pathologists HistoQip.

PROCEDURE NOTES:
1. Select a sized base mold (Step 6) which will provide 3-5 mm space around the edge of the tissue sample.

2. The dictated number of biopsy pieces is recorded on the side of the cassette to aid the embedder in visualization and retrieval of the entire sample. The Specimen Master Log and the gross pathologic description may also be utilized to fully understand the sample type and necessary orientation. The PA Variance List is a system that the physician assistant uses to communicate to the histologist the number of pieces and type of tissue in the cassette. In the event of a discrepancy between sample number recorded vs. sample number visualized, the embedding histotechnologist will consult with another histotechnologist for assistance in retrieval of the entire sample and when this cannot be successfully accomplished the variance will be documented on the BIOPSY SAMPLE VARIANCE DOCUMENTATION log.

3. During handling of minute samples, black embedding sponges, embedding bags or embedding tissue paper may be retained in the top of the embedding cassette to aid in both future investigation and as a signal to the microtomist of necessary special handling.

4. During tissue orientations (Step 7) do not over-cool the wax base; this may result in block inconsistencies, create hairline cracks and/or result in difficult microtomy. Don’t use a mold that is too cold or has hardened wax around the edges. The difference in wax vs. mold temperature will cause the block to separate at the edges.

5. Each histotechnologist will achieve task accountability by marking the cassette by either inking with a color-coded marker or placing name/color confetti in the molten paraffin. Each block must be accountable and easily identify who embedded it.

TECHNIQUE NOTES: Embedding

1. The molds cool faster if the wax was cleaned off the bottom before using.

2. Straighten out VAS, FTS and TAR before cutting- it makes it easier to divide.

3. Consider how many levels you need to pick-up before choosing a mold.

4. Fill the mold up completely with molten wax before placing the cassette on top- otherwise air can be trapped and a bubble in the block will result.

5. If you question the embedding of a block, reembed it before it is soaked or remove all water droplets from the block. Water will expand and infiltrate the tissue causing it to peel or chuck out of the new block.

6. Avoid under filling the cassette as this can allow unstable clamping in the microtome and lead to cutting “thick then thin” sections and other problems.

7. Chose a mold that will minimize trimming to prevent paraffin waste.
PROCEDURE VISUALS:

- Orient biopsy specimens submitted **EN TOTO ON END OR ON EDGE** Use consistency and aligning.

- Orient biopsy specimens submitted **BISECTED or DISSECTED on the CUT EDGE**. If you have issues cutting tissue embedded like #1, turn the block on your microtome to get a better section like #2. Leave it up to the histotech and their technique.

- Orient large dissected specimens in the block as they are submitted in the embedding cassette, with the cut side down. **See biopsy pad technique**.

- Orient “breadloaf” dissected specimens with the largest cut edge down.

- Use consistency when orienting and aligning dermatology specimen epidermis and tissue marked with colored ink.

- When possible, arrange multiple sample pieces in the block to permit maximum representation on the resulting microslide. **Note: prefers to have pair for each ribbon on a slide. Max of 8 total sections on 1 slide**.

- Do not layer specimens; all tissue pieces must be embedded firmly to the mold bottom for viewing by the microtomist.

- Orient fragile tissue placed in; paper, nylon bag or pads by tampering up side down in a mold with semi-cooled wax. The wax will act like glue and the specimen will adhere to the mold. Gently peel off and place cassette on top of mold then set aside to melt then arrange specimen close together. To be cut parallel to the mold, tamp specimen. (CB, ECC, EMB, lung bits, liver needle cores)
• Orient needle core pieces; 1. without overlapping or layering, 2. arrange to permit maximum representation on the slide and 3. cut parallel to the mold bottom by pressing lightly with the tamper. Some tissue can be straightened in warm wax before placing on cold plate.

• Cut and orient VAS, FTS, and TAR lumen down. Sterilization- submitted whole, the histotech cuts. Hysterectomy- the PA cuts at the bench, places tubes between biopsy pads. FTS- means the histotech is responsible for correct cut and orientation in block.

• Orient layered tissues consisting of epidermis or mucosa on the cut edge. Use consistency and aligning.

Equipment/Supplies (If Applicable):

**SPECIMEN:** Previously dissected, fixed, and cassetted tissue samples which have been dehydrated, cleared, and infiltrated with paraffin as an embedding medium.

**MATERIALS, REAGENTS:**

- Embedding Molds – Miles Tissue-Tek #7301
- Paraffin
- Forceps, scalpel blade, magnifying lamp

**BIOPSY SAMPLE VARIANCE DOCUMENTATION** log

**INSTRUMENTATION OR EQUIPMENT:**

- Embedding Center

**CAUTION – HEATED WORK SURFACES**

Wear appropriate protective equipment.

Use extreme caution to avoid hazards of exposure to high-temperature work surfaces.