SUBJECT: Tissue-Tek Tec 5 Embedding Center Procedure

PURPOSE: Tissue fixation provides for preservation of the tissue but leaves the tissue too pliable and without a means of support to allow for thin sections to be cut from the specimen. Infiltration and embedding with paraffin is a very widely practiced, partially automated technique which preserves orientation of cellular structures and provides a supporting mechanism to allow sufficiently thin sections to be cut for most routine Histologic purposes. Embedding orientation is the realm of the Histologist and is a process demanding a considerable degree of skill to prevent the loss of irreplaceable tissue and/or time wasted in re-embedding, re-cutting, and re-staining. Proper recognition of tissue(s) and structures at embedding is critical to the production of diagnostically useful microscope slides for the Pathologists.

SPECIMEN HANDLING:

A. Any well fixed tissue, dehydrated, cleared of alcohol, and completely impregnated with paraffin.

REAGENTS, MATERIALS, EQUIPMENT:

A. Paraplast Extra, Leica Biosystems.
B. Precision Incubator, Precision Scientific, Chicago, IL.
C. Tissue-Tek Tec 5 Tissue Embedding Console System, Sakura Finetek U.S.A., Torrance, CA

PROCEDURE (EQUIPMENT OPERATION):

A. Paraffin is received in solid form and is melted in the large Precision Incubator.

B. Operation of Tissue-Tek Tec 5 Dispensing Unit

1. The reservoir on the dispensing console holds 4 liters and is filled from the top using melted paraffin.
2. The power is left ON at all times due to the long time required to re-melt paraffin in this unit.
3. Temperature is kept at 56º - 65ºC, (preset at 62ºC). Temperature can be changed from the control panel. (See operator manual 3.5).
4. Paraffin is dispensed through the spout on the dispensing arm, by pressing the touch plate on the upright. The dispensing rate is controlled by an adjustment knob on top of the dispenser.
5. A work light is available on the console but it is preferable to use the halogen, gooseneck lamp above the console.
6. Forceps are kept in preheated wells on the side of the dispenser unit. When dispenser wells are used several pairs must be used and constantly rotated into and out of the preheated wells.
7. Surfaces of the Dispensing console are heated except the small "cold spot" in front of

the dispensing arm. These are both useful aids in the embedding process.

8. There are 2 waste drawers. These drawers collect paraffin runoff for later disposal. Drawers are located under the embedding area (to the right and left of the "cold spot"). Empty both drawers when done embedding.

C. Operation of Tissue-Tek Tec 5 Cryo Module

1. Power is turned on just prior to embedding and turned off immediately when done Turn power on at control panel. Press "Cryo" button..
2. Temperature display will be from -10º to 0º. Factory set temperature is -5ºC. It will take 10-20 minutes to reach this temperature.
3. Frost forms on the cold plate during operation. Once power is shut off the frost melts. Wipe slush/water/ice off every day.

PROCEDURE (EMBEDDING):

A. Cassettes to be embedded are removed from the processor and placed on the dispenser console to prevent solidification of the paraffin.

B. Select a cassette for embedding.

C. Choose the appropriate mold for the size tissue and fill with paraffin until the bottom is just covered.

D. Using preheated forceps, remove the tissue from the cassette and place into mold.

E. Orient the tissue properly depending upon type of tissue, recognizable pathological features, or instructions on the Master Sheet.

F. There are four frequently employed means of assuring correct embedding orientation; small filter paper circles, inking, biopsy sponges, and special instructions written on the Master Sheet

G. Once the tissue is oriented correctly move the mold to the "cold spot" on the dispensing console and press tissue gently to the bottom of the mold. If the paraffin has started to harden before all of the pieces have been positioned then move the mold back to a warm area and press all tissue to the bottom of the mold. Proper embedding technique is a skill learned only through practice. An experienced Histologist will be able to rapidly orient and embed tissue specimens with a minimum amount of mold reheating.

H. After the tissue has been placed correctly and the paraffin has started to solidify, then the cassette base, with accession number to the right, is placed on the top of the mold. Paraffin is then dispensed into the mold through the cassette until the cassette is half filled.
Correct placement of the cassette on the mold facilitates correct orientation of the block on the microtome. The cassettes are clamped into the microtome with numbers to the right. Maintaining proper orientation in the microtome helps to reduce section compression which

can occur if the blade cuts into harder layers before softer layers in tissue such as skin. When correctly oriented, the blade will contact softer underlying tissues first and the harder epidermis last.

I. After filling the cassette/mold scan barcoded cassette if it was not scanned before opening. Place the cassette/mold on the cold plate of the Cryo Console. Rapid hardening of the paraffin causes the crystal structure of the solid paraffin to be very fine, thus facilitating the cutting of thinner, smoother sections.

J. Once hardened the paraffin blocks should pop out of the base molds. Base molds are returned to the proper storage bins and the paraffin blocks have the excess wax scraped or melted off with the Para-trimmer of the cassette edges.

REFERENCES:

1. Sheehan, Dezna C., & Hrapchak, Barbara B., Theory and Practice of Histotechnology, 2nd ed., Battelle Press, Columbus, OH., pp. 66-68, 72-73, 1980.
2. Culling, C.F.A., Allison, R.T., Barr, W.T., Cellular Pathology Technique, 4th ed., Butterworths, Boston, MA., pp. 55-67, 1985.